summed K&L grades between baseline and 5 years were 0, 1.5–3.0, 3.0–5.5, and 5.5–17.5 for 39.0, 18.3, 22.5, and 20.1% of participants, respectively. pLeptin was positively associated with pResistin (stand beta 0.115, P=0.000) and negatively with pAdiponectin (stand beta -0.080, P=0.012), other associations between adipokines were not statistically significant. pLeptin showed statistically significant associations with sPIINP and sPIINP (stand betas 0.050, P=0.01 and 0.056, P=0.005, adjusted for age, BMI, and gender). pLeptin showed a statistically significant association with OA progression before adjustment (stand beta 0.099, P=0.007), that turned non-significant after adjustment for demographic variables (stand beta -0.006, P=0.917). pAdiponectin was statistically significantly associated with sCOMP only (stand beta 0.105, P=0.001, adjusted) and not associated with OA burden and progression (data not shown).

pResistin showed a statistically significant association with sPIINP, sCOMP, and sC12C (stand betas 0.091, -0.083, and -0.086, respectively, P=0.014) and was associated with radiographic OA burden (stand beta 0.093, P=0.008, adjusted) and progression (in binary logistic regression: (Exp(B) 1.165, P=0.031, adjusted for age, gender, BMI, and baseline summed K&L grade. Multiple linear regression: stand beta 0.011, P=0.769, adjusted).

Conclusions: Leptin and resistin showed the most associations with biomarkers and radiographic OA burden and progression. Leptin may function as a humoral mediator between OA progression and demographics, while resistin also showed an effect on OA burden and progression apart from the studied demographics. The low grade of all associations may be partly due to the limitations of biomarkers and radiographic disease measures in early-stage OA. Nevertheless, these data indicate that systemic adipokines could be involved in early-stage OA burden and progression.

This study was funded by CHECK (Cohort Hip & Cohort Knee), an initiative of the Dutch Arthritis Association.

162 COMPARISON OF HEALTHY AND NORMATIVE AGING REVEALS A METABOLIC COMPONENT IN HAND OA AND OA BIOCHEMICAL MARKER PROFILES.
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Purpose: Epidemiological studies have shown associations between osteoarthritis (OA) and age, as well as between OA and metabolism, which are not completely understood. It can be questioned whether intrinsic metabolic programming affects OA risk and circulating levels of biochemical markers applied in OA research such as COMP and CTX2. These biomarkers measured in serum and urine reflect cartilage turnover and degradation, respectively.

Insight in interactions between age, metabolism and OA will allow for better clinical classification of patients and for a more reliable assessment of disease activity or progression using biochemical markers. Such insights may be obtained from studies focused on aging and metabolism, independent of disease. To such end, we have analyzed levels of serum COMP and urinary CTX2 and hand radiographic OA (ROA) scores in healthy aging subjects from long-lived families, their partners as population controls and OA patients. We analyzed age related aspects of the biochemical markers and hand ROA scores and compared the marker and ROA score profiles in healthy and normative aging and in disease. Next we tested whether glucose levels influenced the levels of biochemical markers and hand ROA scores in the healthy and normative aging subjects.

Methods: The Leiden Longevity Study (LLS) consists of long-lived siblings, their offspring and partners of the offspring as controls. In middle age, the familial longevity trait is reflected by a 30% increased survival, low prevalence of metabolic disease and healthy metabolic profile (such as lower glucose levels). We measured uCTX2 and sCOMP levels in a subset of the females of the LLS (N=151 healthy agers and N=181 controls) and scored hand radiographs (20 joint sites) according to Kelgren and Lawrence (N=107 healthy agers and N=128 controls). The same scores were available for the OA affected females of the CARP study (N=315). Furthermore, these were previously typed for the biochemical marker levels sCOMP and uCTX2. Linear mixed models were applied testing for relationships between age and hand ROA data or marker levels, whilst correcting for BMI, familial dependencies and potential batch effects between the CARP and LLS study.

Results: sCOMP shows a comparable age related increase in healthy and normative agers as well as in OA patients. Healthy agers, however, had a significantly higher mean sCOMP level as compared to the controls. Remarkably, uCTX2 levels showed no increase with age in healthy agers whereas a significant and equal age related increase was observed amongst the controls and OA patients.

Healthy ageing women have the lowest age related increase of hand ROA score, whereas this has a significant linear increase via controls to the OA patients (study groups coded as 0, 1 and 2 for healthy agers, controls and CARP patients respectively, BMI and age adjusted). When we additionally selected the healthy agers with glucose levels below the median within their group, the interaction of study group with hand ROA scores became more pronounced between healthy agers and controls.

Conclusions: When interpreting OA biomarkers, age is a major factor to consider. Furthermore, a healthy aging metabolic profile as reported for the Leiden longevity family members associated to a lower age-related increase in hand ROA scores and uCTX2 levels as compared to controls. This may indicate that metabolic health should also be considered in the assessment of the risk for hand ROA onset and monitoring of progression on the basis of these biomarkers.

Comp as a new potential biomarker for osteoarthritis.

163 20KDa FRAGMENT OF OSTEOGLYCIN: AN INNOVATIVE TOOL FOR OA PATIENTS CHARACTERIZATION.
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Purpose: Osteoarthritis is the most common rheumatologic disease and is characterized by increased protease activity resulting in cartilage degradation. Nowadays, radiographic measures are the traditional study for diagnosis, but the abnormal metabolic process begins in the articular cartilage several years before destruction of the tissue can be detected radiologically. Our aim in this study is the search for new osteoarthritic molecular biomarkers, by the screening of different ECM proteins and their fragments in osteoarthritic (OA) and normal cartilage (CT).

Methods: Normal samples were obtained from femoral cartilage of patients who underwent hip replacement due to osteoporotic fracture. OA cartilage samples came from knee and hip arthritic patients undergoing arthroplasty. ECM proteins from these samples were extracted using Guanidinium chloride (4 mol/l) method. After the extraction, proteins and glycosaminoglycans (GAG’s) were quantified by Bradford and dimethyl-methylene-blue (DMB) assay respectively. Proteoglycans studied from the ECM protein extracts of knee and hip cartilage were normalized by Bradford concentration and analyzed by Western blotting. The bands intensity was quantified by Quantity One software. In order to study the activity of the protease in front of each proteoglycan studied and to find out specific fragments from OA patients, the extracts were digested with recombinant matrix metalloproteinase 13 (MMP-13) at 2, 4, 6, 8, 16 and 24h and analyzed with western blot.

Results: Osteoglycin (OGN), Matrilin3 (MATN3), and Cartilage oligomeric matrix protein (COMP) were identified by Western Blotting. Our results showed that MATN3 expression did not present any difference between CT, OA hip and OA knee cartilage. We could observe digestion of MATN3 by MMP-13 after 6 hours of incubation and it was nearly completely digested at 24h.

In the case of COMP expression, the analysis showed a different band pattern in OA knee samples compared with CT and OA femoral samples. Interestingly, a COMP fragment of approximately 60 KDa was observed at 16h and 24h of incubation in all the samples.

Regarding the OGN results, we surprisingly found that it was more abundant in OA knee samples than in CT ones, contrary to other previously described proteoglycans observations. Concerning to OGN digestion, we could also see differences between samples; while OGN from CT samples was completely digested at 16h, OGN from OA femoral samples showed still a weak band at 16h of digestion that was completely digested at 24h. Finally, in OA knee samples, at 24 hours the digestions was not still
complete. Furthermore, about 30% of OA samples (hip and knee) showed a fragment of approximately 20KDa that it's not present in none of the 18 control samples analyzed. This fragment seems not to be specific of MMP13 since it was present before the incubation with the protease, and the intensity of the band did not increase with the in vitro MMP-13 digestion.

Conclusions: We found three ECM proteins susceptible of MMP13 digestion that could be potential OA biomarkers. Interestingly, results showed that 30% of OA patients had a MMP13 not specific fragment from OGN. The presence or absence of this fragment could be useful as a tool to identify a group of patients with similar characteristics

164 ANALYSIS OF THE SERUM BIOMARKERS IN HUMAN KNEE OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is characterized by a degeneration of cartilage whose main clinical symptoms are pain, deformity and loss of function. With the coming of global aging society, OA has become a leading cause of disability in the elderly despite its poorly understood pathogenesis limits the discovery of targets for pharmaceutical intervention and there are few effective medical treatments beyond pain control and surgery. Effective method of early diagnosis and treatment of OA are desperately needed. To search proteins that might serve as serum biomarkers for OA diagnosis, treatment or pathogenesis, this study preformed technologies to identify differentially expressed proteins from serum obtained from patients with knee osteoarthritis(OA) and controls.

Methods: There are 4 subjects of OA and 4 age- and sex- matched normal adults involved in the study, 4 subjects of OA are randomly selected from 30 patients diagnosed with primary knee osteoarthritis, Serum samples from each subject were treated with ProteoExtract Albumin/IgG Removal Kit and Clean-up Kit, then cross-labeled with different CyDye, followed by two dimensional difference in gel electrophoresis(2D-DIGE), map scanning, and then the maps of protein spots were compared using the software DeCyder v6.0 to find out the different expressed proteins. Treat these proteins with Matrix assisted laser desorption ionization-time of flight mass spectrometry(MALDI-TOF-MS), and get peptide fingerprinting maps(FPM), then search them with MASCOT in Swiss-Prot/Uniprot date base to identify these proteins. Finally, use bioinformatics to analysis biological functions and research value of these proteins.

Results: We found 14 differentially expressed protein spots compared serum samples from patients with OA relative to the controls. The MS revealed 14 proteins that alpha-2-macroglobulin, gelosin, vitamin K-dependent protein S, kinnogenen-1, complement C3 were increased, and haptoglobin, inter-alpha-trypsin inhibitor heavy chain H4(ITIH4), apolipoprotein E were decreased in OA serum samples. Alpha-2-macroglobulin, ITIH4, kinnogenen-1 were related to the body's inflammatory reaction, vitamin K-dependent protein S, apolipoprotein E were involved in cell apoptosis, and alpha-2-macroglobulin, complement C3 participated in immune response. Some studies indicated that alpha-2-macroglobulin is related to the degradation of cartilage extracellular matrix. Some studies found that alpha-2-macroglobulin, apolipoprotein E, complement 3, gelosin, haptoglobin and kinnogenen-1 appeared in synovial [[Unsup-ported Character - II][uid], and apolipoprotein E, alpha- 2-macroglobulin, haptoglobin increased in synovial [[Unsupported Character - II][uid from OA compared with the health controls.

Conclusions: This study successfully settled the serum comparative proteomics technique of OA, combined the 2D-DIGE and MALDI-TOF-MS, and get peptide fingerprinting maps(FPM), then search them with MASCOT in Swiss-Prot/Uniprot date base to identify these proteins. Finally, use bioinformatics to analysis biological functions and research value of these proteins.

165 356-373 ELISA, A MORE SENSITIVE SANDWICH ASSAY FOR DETECTING AGGREGAN FRAGMENTS CLEAVED BY ADAMTS AT 373–374 SITE, IS A POTENTIAL BIOMARKER FOR HUMAN JOINT DISEASES.

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Purpose: Aggrecan degradation is believed to be a crucial process in joint diseases such as OA and RA. The ADAMTs: cleavage site TEGE373-374ARCS is a hot spot for investigation of aggrecan degradation. We have developed a competitive ELISA AGNcx1 for detecting fragments containing neo-epitope NITEGE373, and a sandwich ELISA G1-373 for detecting fragment containing both NITEGE373 and one epitope located in G1/G2 domain of aggrecan. There are disadvantages for the existing ELISAs: lower sensitivity for the fragment in serum samples and unsatisfied technical performance result for G1-373 with higher coefficient of variation (CV) variation.

The aim of this study was to develop a sandwich ELISA 356-373 with higher sensitivity detecting fragments containing NITEGE373. The ELISAs were characterized and compared by using the Full Depth Cartilage (FDC) explant culture model.

Methods: Full depth bovine cartilage (FDC) including all subpopulations of chondrocytes from superficial zone to the deep zone of the cartilage explants was obtained from the joint of one to two-year- old calf. The pieces (20–25mg) were placed in 96-well plate and incubate for 21 days at 37°C with 5% CO2. Serum free medium containing oncostatin M and tumor necrosis factor (TNF) alpha were replaced every second or third day and store at −20°C before analysis by: (1) competitive NITEGE373 ELISA (AGNcx1) detecting aggrecan fragments containing neo-epitope NITEGE373, (2) sandwich G1-373 ELISA detecting aggrecan fragments containing both neo-epitope NITEGE373 and G1 domain, (3) sandwich 356-373 ELISA detecting fragments containing both neo-epitope NITEGE373 and epitope 352TVQTVTW358 in interglobular domain (IGD).

Results: We found that: (1) aggrecanase-derived aggrecan fragments containing NITEGE373 were dose-dependently released in the early (day 2-7 of culturing) and mid phase (day 9-14) into the supernatants from FDC explants stimulated with catabolic cytokines. (2) The fragments released, profiled by the three assays, were similar but different: the NITEGE373 competitive ELISA and the 356-373 sandwich ELISA showed two peak releases. The G1-373 sandwich ELISA only showed one release. (3) The release of NITEGE373 fragments detected by NITEGE373 competitive ELISA or the 356-373 sandwich ELISA was delayed by MMPs inhibitor GM6001, whereas the fragments release detected with G1-373 sandwich ELISA was partially but significantly inhibited by GM6001 (33% inhibition), indicating that MMPs are involved in G1-373 ELISA-detecting fragment derivation. (4) Higher sensitivity and better technical performance data was achieved for the 356-373 ELISA compared to NITEGE373 competitive ELISA and G1-373 ELISA, respectively. (5) 356-373 ELISA could be applied for human samples testing with good dilution recovery, indicating its further potential clinical application for joint diseases as a biomaker.

Conclusions: We developed a new sandwich ELISA detecting the ADAMTSs derived fragments containing neo-epitope NITEGE373 and another epitope 352TVQTVTW358 in IGD. This ELISA is more sensitive than the previous competitive NITEGE373 ELISA which are lack of sensitivity for human samples test, and more technically robust than the G1-373 ELISA which has weak stability. Its clinical application, i.e., patient samples detection, will be investigated in the coming future.

166 THE IDENTIFICATION AND DEVELOPMENT OF COMPETITION ELISA FOR NEUTROPHILELASTASE- DERIVED LUBRICIN FRAGMENT: A POTENTIAL BIOMARKER OF EARLY ARTICULAR CARTILAGEDEGRADATION