sured by the CartiLaps ELISA assay and corrected for urinary creatinine levels. We used log(uCTX-II) to have approximately normally distributed marker values.
The BL distribution of KL scores was (145,88,30,24,1) for KL 0-4. Among the BL healthy knees (KL 0), 101 were non-progressors at FU (KL 0) and 25 were progressors (KL $>$ 0).
The prognostic ability was evaluated on whether the biomarker BL values separated progressors from non-progressors with MANOVA statistical significance and by sample size (n) and odds ratio (OR) for predicting progression.
An aggregate biomarkers was made by combining all biomarkers and the *cartilage longevity* marker by combining uCTX-II with cartilage volume and homogeneity by linear discriminant analysis. They were evaluated by repeated random sampling of disjoint training and evaluation subsets to avoid performance overestimation due to overtraining.
Results: The table lists p value for separating progressors from non-progressors with the corresponding *n* and OR for each biomarker. Only the compartment with best performance is listed for the MRI markers. Also age and BMI predicted progression ($p<0.01$) but not congruity and sex.

Prediction of Progression (evaluated by MANOVA p-value, sample size n, and odds ratio OR)

Biomarker	P-value	*n*	OR
JSW	0.5	–	–
uCTX-II	0.004	71	6.7
Volume (MF.VC)	0.08	218	2.8
Area (MT.AC)	0.07	216	2.8
Thickness (MT.ThCtAB)	0.2	–	–
Smoothness (MT.SmoC)	0.01	118	3.8
Homogeneity (MT.HomC)	0.002	69	4.9
Aggregate: All markers	0.001	50	6.3
Aggregate: Cartilage Longevity (uCTX-II, Homogeneity, Volume)	0.00003	29	14.6

Conclusions: The results demonstrated that both biochemical and MRI-based biomarkers were suitable prognostic biomarkers while JSW appeared unsuited. The best individual biomarker was uCTX-II requiring *n* 71 and offering OR 6.7. However, the cartilage longevity marker combining uCTX-II, homogeneity and volume had *n* 29 and OR 14.6. This prognostic marker may therefore provide much stronger study population selection - allowing clinical studies to show the true effect of potential treatments. Furthermore, the longevity marker is intuitively appealing since it combines aspects of cartilage quantity (volume), quality (homogeneity), and breakdown (uCTX-II) - the natural components in a comprehensive prognosis on expected cartilage life-span.

65

AGGRECAN CONSISTS OF TWO DISTINCT POOLS: ONE HIGH-MOLECULAR-WEIGHT, AGGRECANASE DEGRADABLE REVERSIBLE POOL, AS WELL AS AN IRREVERSIBLE, MMP-SUSCEPTIBLE LOW-MOLECULAR-WEIGHT POOL

E.U. Sumer, S.H. Madsen, B-C. Sondergaard, C. Christiansen, P. Qvist, M.A. Karsdal
Nordic Bioscience, Herlev, Denmark

Purpose: Cartilage turnover consists of both physiological and pathological turnover. Until now, these processes have not been separated due to lack of molecular tools. Today, it is still debated which family of enzymes that are the major players of aggrecan degradation under pathological conditions. Previously, we have shown that early stage aggrecanase-mediated aggrecan degradation was fully reversible after catabolic stimulation with Tumor Necrosis Factor Alpha (TNFα) and Oncostatin M (OSM) in combination, followed by anabolic treatment with Insulin Growth Factor-I (IGF-I), whereas late stage MMP-mediated degradation was irreversible. In this study, we further investigated the time-dependent degradation profile of proteoglycans in cartilage with focus on MMP and aggrecanase activity and characterized these different molecules.
Methods: Cartilage depletion was stimulated in bovine articular cartilage explants by TNFα (20ng/ml) and Oncostatin M (OSM) (10ng/ml) treatment. Cartilage degradation was measured by two independent immunoassays: MMP-mediated aggrecan degradation and aggrecanase-mediated aggrecan degradation, ^{342}FFGVG-G2 and ^{374}ARGSVI-G2 respectively. Glycosaminoglycans (GAGs) were quantified by a colorimetric assay. Cell culture supernatants were further investigated by western blots by monoclonal antibody against the MMP-generated ^{342}FFGVG neoepitope, or the aggrecanase-generated ^{374}ARGSVI sequence. Importantly because of the location of the fragments in aggrecan, in theory, MMP-generated fragments cannot be generated secondary to aggrecanase cleavage, but the aggrecanase site may be generated after MMP cleavage.
Results: Cytokine stimulation resulted in early release of aggrecanase-mediated cartilage degradation fragments at day 7, as quantified by ^{374}ARGSVI-G2, which was increased approximately 723% and GAGs were similarly elevated by 206%. The increases were apparent until day 11 where aggrecanase-mediated aggrecan depletion was exhausted. In contrast, MMP-mediated aggrecan degradation began at day 16 until the end of the study-period, resulting in a final increase of 1598% of ^{342}FFGVG-G2 fragments. Western blot analysis showed high molecular weight GAG-entailing ^{374}ARGSVI fragments at day 7, in contrast to low molecular weight non-glycolysated ^{342}FFGVG analytes at day 21. When digesting OSM/TNFα stimulated explants from day 21 *in vitro* with ADAMTS-4, we observed a 225% increase in the (^{374}ARGSVI-G2/^{342}FFGVG-G2) ratio (P=0.04).
Conclusions: This study demonstrates that two distinct pools of aggrecan molecules exist in the articular cartilage. Secondary, in contrast to previous publications that MMP-processed aggrecan can be further degraded by aggrecanases. The pool of high-molecular weight aggrecan, susceptible for aggrecanase-mediated degradation, may be associated with fully reversible cartilage degradation. In contrast, low molecular weight aggrecan, degraded by MMPs may be associated with irreversible cartilage destruction. These data begin to provide a molecular understanding of reversible and non-reversible cartilage turnover.

66

A NEW SERUM-BASED ASSAY FOR TYPE II COLLAGEN HELICAL PEPTIDE (SERUM HELIX-II) IS ASSOCIATED WITH LONG-TERM RADIOLOGICAL PROGRESSION IN KNEE OSTEOARTHRITIS

P. Garnero[1], N. Charni Ben Tabassi[2], F. Juillet[2], R.C. Whittles[3], M. Sharif[3]
[1]INSERM U664 and Synarc, Lyon, France; [2]Synarc, Lyon, France; [3]3Department of Anatomy, University of Bristol, Bristol, United Kingdom

Purpose: Degradation of type II collagen is one of the hallmarks of cartilage damage in osteoarthritis (OA). Urinary biochemical markers reflecting the degradation of the helical (Helix-II) and C-telopeptide (CTX-II) regions of type II collagen have been developed and levels of both markers have been shown to be associated with more rapid radiological progression in OA. However, urinary markers have limitations including relatively high imprecision due to variable urine dilution and the need for creatinine correction. In addition a precise sampling of urine is often difficult in elderly patients. To overcome these limitations it is useful to obtain serum-based biochemical markers. The aim of our study was to investigate the association between serum

levels of Helix-II measured by a new ELISA and radiological progression in patients with knee OA followed prospectively for 5 years.

Methods: Eighty-three patients with persistent pain (> 3 months) in one or both knees were included [54% female, mean age; 62 yr; Kellgren-Lawrence scores: 0 (13%), I (5%), II (4%), III (77%), IV (1%)]. Serum for measurements of Helix-II and urinary CTX-II were measured at baseline, 2 yr, 3 yr and 5 yr and knee radiographs were obtained at baseline and 5 yr. Disease progression was defined as either a reduction in the tibiofemoral joint space by at least 2 mm or total knee replacement of either knee during the 5-year follow-up.

Results: During the 5-year follow-up, 24 patients had a radiological progression and 59 did not progress. Serum Helix-II increased during follow-up (10%/yr, p=0.0032) and mean 5-year levels were higher in progressors than non-progressors (+30%, p=0.0345). When used as a continuous variable, each SD increase of mean 5 year serum Helix-II levels was associated with an age-sex-BMI adjusted relative risk (RR) of progression of 1.69 (95% confidence interval: 1.01-2.81). In a multiple logistic regression model, both serum Helix-II and urinary CTX-II were independently associated with disease progression. Patients with high serum Helix-II (highest quartile) and high urinary CTX-II (above median) had an 8 fold higher risk of radiological progression [RR: 7.79 (1.77-34.2)].

Conclusions: Increased serum Helix-II levels are associated with a higher risk of radiological progression in knee OA independent of urinary CTX-II, suggesting that the two type II collagen degradation markers reflect complementary biological pathways of cartilage degradation. Overall, our data suggest that the combination of serum Helix-II and urinary CTX-II would be a more powerful predictor of disease outcome in knee OA.

67

OSTEOARTHRITIC AND AGE-RELATED CHANGES IN TYPE II COLLAGEN IN HUMAN HIP CARTILAGE

L. Dahlberg, L. Lindberg, S. Hosseininia
Lund University, Malmö, Sweden

Purpose: To examine type II collagen content and denaturation and their relationship in hip cartilages. The occurrence of similar collagen molecule damage in knee and hip OA cartilage supports the contention that collagen damage is a common feature in joint disease.

Methods: 21 OA (aged 45 to 89 years, 13 women) and 26 reference (aged 55 to 99 years, 20 women) femoral heads collected at total hip replacement surgery or in femoral neck fracture patients were kept frozen at -80°C until analysis.

In OA, cartilage was sampled from areas that had full-thickness cartilage whereas the reference cartilage was taken from the superior mostly loaded area of femoral head. A small amount of the 50 mg from each hip sampled cartilage was used for routine histology to examine degradatavie changes according to Mankin grading system (0-13). Each freeze- dried cartilage sample was treated with 1mg/ml α-Chymotrypsin in 50 mM Tris containing the proteinase inhibitors to extract denatured collagen. The supernatants and residues were hydrolysed in 6N HCl at 110°C overnight, and then freeze-dried. These samples were dissolved in 500μl of distilled water and clarified using equal amount of charcoal and AG-1 X8 anion exchange resin. The amount of hydroxyproline was measured at 550 nm absorbance by colorimetry. The L-4- Hyroxyproline was used as standard.

Results: The reference group was older than OA group ($p<0.001$).

The median Manking grade in OA and reference samples were 4.5 (range 1-8) and 1 (range 0-3), respectively. In OA cartilage, the collagen content was about 9% lower than that of reference cartilages (1830 μg/ml vs. 2009 μg/ml, P=0.015). The proportion of degraded collagen was almost four times higher in OA than that of reference cartilages (3.7 vs. 1.1%) ($P\leq0.001$).

There was no difference between men and women in collagen content or degraded collagen in any of the groups.

There were no relationship between age and total collagen content and no positive relationships between percent of collagen denaturation and age in in reference or OA cartilages.

There was an inverse relationship between the percentages of degraded collagen and collagen content in OA cartilage (r=-0.66, p=0.001). In contrast, no obvious relationship between degraded collagen and collagen content was present in the reference group (p=0.17).

Conclusions: In the present study we extend previous findings of increased amount of collagen degradation in knee OA and show that similar changes also occur in hip OA. We also confirm an inverse relationship between collagen denaturation and content in hip OA. The latter may represent a molecular explanation to OA progression. At a higher rate of type II collagen damage, lesser amount of collagen is maintained in the tissue. A possible mechanism behind this is that released molecular fragments may stimulate further extracellular matrix degradation causing a vicious circle. This seems disease specific since an inverse relationship between collagen degradation and content was not obvious in reference cartilage.

Age is commonly discussed as a risk factor for OA since the frequency of OA increases as population ages. In this study, collagen content and degradation was not significantly affected by age. It suggests that OA may develop independently of age and that OA in elder people may merely depend on long exposure time to risk factors rather than age per se. In conclusion, the present study provides new perspectives of cartilage collagen metabolism in human OA cartilage. Similar molecular changes occur in hip and knee OA cartilage seemingly without relation to age. Therapeutic interventions in order to control joint cartilage collagen content using inhibitors that decrease collagen degradation ould be considered in the management of progressive hip and knee OA.

68

SHIFT IN CHONDROCYTE TGF-BETA RECEPTOR USAGE, ALK5 TO ALK1 SIGNALING, AS A POTENTIAL CAUSE FOR OSTEOARTHRITIS

E.N. Blaney Davidson[1], D.F. Remst[1], E.L. Vitters[1], H.B. van Beuningen[1], A.B. Blom[1], M-J. Goumans[2], W.B. van den Berg[1], P.M. van der Kraan[1]
[1]*Radboud University Nijmegen Medical Centre, Nijmegen,*
[2]*University Medical Centre Utrecht, Utrecht, The Netherlands*

Purpose: Ageing is the primary risk factor for osteoarthritis (OA). Chondrocytes in OA show a deviant behavior that resembles terminal differentiation of growth-plate chondrocytes, characterized by elevated MMP-13 expression. Previously we have demonstrated a reduction in TGF-beta signaling via the ALK5 receptor in aged mice and experimental OA. ALK5 activates the Smad2/3 route, which is known to suppress terminal differentiation. Recently, it has been shown that in certain cell types TGF-beta is also able to activate the Smad1/5/8 route via the ALK1 receptor. This route has been shown to induce chondrocyte terminal differentiation. We investigated whether during ageing and OA, TGF-beta signaling switches from ALK5 to ALK1, favoring progression of chondrocyte differentiation.

Methods: We investigated whether TGF-beta signals via ALK5 or ALK1 in chondrocytes by Western blotting. To assess whether ALK5 and ALK1 were specific for Smad2/3 or Smad1/5/8 respectively, chondrocytes were transfected with Ad-caALK5 or Ad-caALK1 after which a Western Blot was performed. Further-