Results: At baseline (BL) CTX-I and CTX-II were linearly correlated in the healthy population ($r = 0.52$, $p < 0.001$) but only borderline for the OA subjects ($r = 0.29$, $p = 0.09$) (Figure 1).

There was a linear correlation between BL CTX-II scores and follow-up (FU) CTX-I ($r = 0.23$, $p < 0.01$). Contrarily, there was no correlation between BL CTX-I and FU CTX-II ($r = 0.08$, $p = 0.4$).

Inspection of the healthy subjects showed that elevated CTX-II scores at BL predicted longitudinal cartilage loss. Specifically, the odds ratio (OR) for increased cartilage loss was 4.0 – comparing highest and lowest tertiles of CTX-II ($p < 0.01$). For CTX-I, the BL scores were not significantly indicative of cartilage loss (Figure 2). All results persisted after correction for gender and age.

Figure 2.

Conclusions: The results support that a balanced cartilage and bone turnover may be of major importance for joint health, and that destabilization of this delicate equilibrium may be a catalyst for initiation and progression of OA. This emphasizes a potential need for OA treatments that restore the bone/cartilage metabolic balance. By comparison of BL and FU biomarker levels, it could be hypothesized that at least a specific stage of cartilage breakdown (elevated CTX-II at BL) is preceding bone remodeling (elevated CTX-I at FU) in OA. This may provide a piece for the complex puzzle of causal relationships between cartilage and bone breakdown.

90 TIME DEPENDENT ADAMTS-4 IN VITRO DIGESTION OF HUMAN AGGREGAN

M. Hansson, M. Svensson, S.L. Lohmander, A. Struglics. Lund University, Clinical Sciences Lund, Department of Orthopaedics, Lund, SWEDEN

Purpose: In normal adult cartilage there is a balance between synthesis and degradation of extracellular matrix components. In diseases such as OA this balance is disturbed. MMPs and aggrecanases (i.e. ADAMTS-4, -5) are the major enzymes involved in cartilage degradation. Proteolytic cleavage of aggrecan is seen at early stage of OA and in knee injuries. Several reports suggest aggrecanases to be the enzymes responsible for this degradation. ADAMTS-4 cleaves aggrecan at specific sites in the interglobular domain (TEGE | ARGS) and in the chondroitinsulphate (CS) rich region 2 (SELE | GRGT, KEEE | GLGS, TAGE | AGEGR and ISQE | LGQR). To understand the process of cartilage degradation it is important to know in which order the enzymes cleaves aggrecan. The present study was undertaken to investigate the time dependent degradation of human aggrecan monomers by ADAMTS-4.

Methods: Human aggrecan was purified from a pool of total knee OA-cartilage (n = 10, from knee replacement surgery) by guanidine extraction and CsCl density gradient centrifugation, collecting the aggrecan in the A1D1 fraction. Aggrecan (42nM) was digested by recombinant human ADAMTS-4 (1 nM) up to 24h at 37°C. Samples were deglycosylated, separated by SDS-PAGE, transferred to PVDF membranes, and probed by neoeptope antibodies (ARGS, SELE, KEEE, and LGQR) and antibodies against aggrecan G3 and G1 domains. Immunodetected aggrecan fragments were quantified using chemiluminescence and digital luminescence imager.

Results: The A1D1 fraction extracted from human joint cartilage lacks ARGS fragments and low molecular weight G3 fragments (GRGT, GLGS, AGEGR and LGQR-G3), but contain G1-G3 monomers, G1-SELE/KEEE and G1-CS1 fragments which serve as substrates for ADAMTS-4 in the beginning of the digestion. After 15 min of digestion all the G1-G3 monomers were degraded and G1-SELE/KEEE and GRGT/GLGS-G3 fragments were produced (see figure). After 2 h these fragments could only be detected at 10% of maximum concentration. The AGEGR-G3 fragments reached maximum concentration after 1 h and then slowly decreased to 10% level at 24 h. The GRGT-KEEE fragment reached a maximum concentration after 1 h, and thereafter decreased slowly. The ARGS-SELE and ARGS-CS1 fragments increased with similar kinetics up to 80% of maximum concentration within 30 min and thereafter slowly increased to a maximum concentration at 4 h digestion. The production of the LGQR-G3 fragment was slow, reaching maximum after 12 h.

Figure 2. Time dependent ADAMTS-4 in vitro digestion of human aggrecan monitored by Western blot.

Conclusions: These results suggest that ADAMTS-4 cleaves aggrecan first in the KEEE | GLGS site, closely followed in time by cleavages in the SELE | GRGT, TAGE | ARGS and the TAGE | AGEGR sites. The cut in the ISQE | LGQR site seems to be the last preferred cleavage site for the enzyme.

The data show that aggrecan monomers, purified from OA cartilage, can be further digested by ADAMTS-4 suggesting it to be a relevant sample for aggrecan degradation studies. The aggrecan fragments detected in this study have also been detected in synovial fluids from patients with arthritis and knee injuries, suggesting that these in vitro digestions reflect conditions found in vivo.

91 SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP) AND HAND OSTEOARTHRITIS (HOA)

M. Scarpellini, A. Lurati, K. Re, M. Marrazza. Magenta Hospital, Busto Arsizio, ITALY

Purpose: Cartilage oligomeric matrix protein (COMP) is a pentameric glycoprotein related to thrombospondin family and found predominantly in cartilage. Increased levels of COMP have been detected in patients with various degenerative and inflammatory joint diseases and have been correlated with accelerated joint damage. Aim of our study was to assess the hypothesis that there’s a significant correlation between COMP serum levels, joint radiological damage and clinical severity in patients with hand osteoarthritis (OA).

Methods: 54 patients with hand OA referring to Rheumatology Unit of our Hospital were enrolled in 2007. COMP serum values were measured by inhibition ELISA with monoclonal antibody. Radiographs were evaluated according to the grading system of Kellgren and Lawrence by an experienced rheumatologist. Patient’s pain was assessed with a visual analogue scale (VAS 0–100 mm).

Results: We enrolled 54 patients (45 with primary OA of the hand and 9 with erosive OA of the hand). In the whole population the COMP, VAS and radiological score mean values were 11.49 (range 5.79–33, SD 5.5), 25.3 (range 0–75 SD 18.3) and 38.8 (range 18–65, SD 9.8) respectively. Patients with symptomatic hand OA (arbitrary cut-off set to VAS ≥ 30) presented significantly higher serum COMP levels compared to those with non-symptomatic narrowing of the articular space (27.4±2.1 vs 11.4±5, p = 0.011), with a Pearson correlation index between VAS and COMP values of 0.31 (95% CI: 0.22–0.67, p = 0.02). Furthermore, a