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.

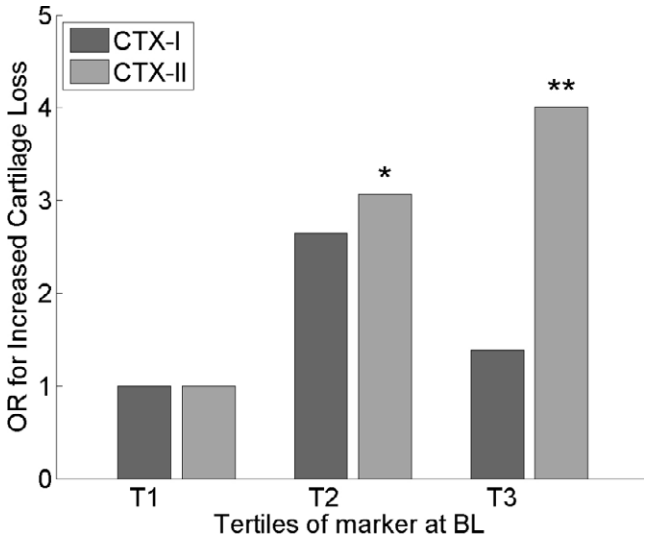

Figure 2.

Conclusions: The results support that a balanced cartilage and bone turnover may be of major importance for joint health, and that distabilization 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 AGGRECAN

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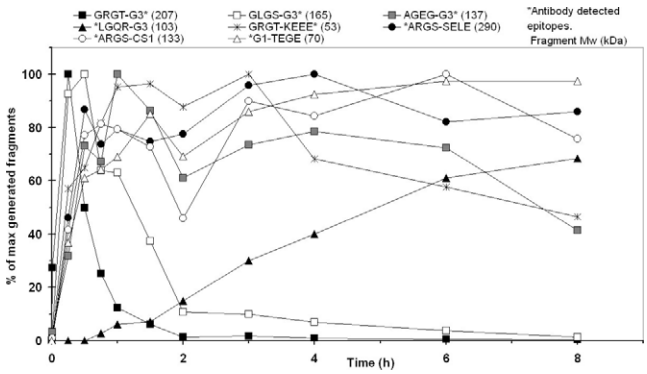
Purpose: In normal adult cartilage there is a balance between synthesis and degradation of extra cellular matrix components. In diseases such as OA this balance is disturbed. MMPs and aggrecanases (i.e. ADAMTS-4, -5) are the major enzymes involved in cartilage degradation. Proteolytic cleavage of aggrecan 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, TAQE↓AGEG 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 (42 nM) was digested by recombinant human ADAMTS-4 (1 nM) up to 24 h at 37ºC. Samples were deglycosylated, separated by SDS-PAGE, transferred to PVDF membranes, and probed by neoepitope 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, AGEG 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 AGEG-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.



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, TEGE↓ARGS and the TAQE↓AGEG 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)

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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 $\geqslant$ 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

significant correlation has been observed between radiological damage index and COMP levels, with a Pearson correlation index of 0.45 (95% CI: 0.32−0.64, p = 0.018).
Conclusions: We observed increased serum COMP levels in patients with symptomatic radiological hand OA. Higher serum COMP levels seems to correlate to more aggressive and severe disease, in terms of pain (VAS values) and radiologic damage (Kellgren and Lawrence index).

92 EVALUATION OF THE USEFULNESS OF BIOMARKERS IN KNEE OSTEOARTHRITIS OR LUMBAR SPONDYLOSIS IN MASS COHORT STUDY OF JAPAN

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Purpose: It is socially required to establish the correlation of some specific biomarkers and the clinical stage of osteoarthritis (OA) or spondylosis (SP). The aim of this study is to evaluate the usefulness of some serum or urine biomarkers in OA or SP in the large population cohort study of Japan.
Methods: Eight hundred and sixty four peoples (319 males and 544 females) over fifty-year-old at the area of Hidakagawa-cho (Wakayama prefecture) and Taichi-cho (Same) were the target of this study. Radiological grade were estimated with Kellgren and Lawrence (K-L) grade. Serum levels of cartilage oligomeric matrix protein (COMP), urinary levels of CTX-I and CTX-II were measured and analysed to evaluate the correlation of knee OA or SP.
Results: Prevalence of knee OA was 56.3% and lumbar SP was 62.6%. Clinical stage of knee OA was that 79 in grade 0, 279 in grade 1, 359 in grade 2, 97 in grade 3, 50 in grade 4. Clinical stage of SP was that 39 in grade 0, 282 in grade 1, 275 in grade 2, 166 in grade 3, 102 in grade 4. Serum levels of COMP and urine levels of CTX-II were both positively correlated with the clinical stage of knee OA or lumbar SP ($p < 0.0001$) in the analysis of covariance (ANCOVA). There was no correlation in urinary levels of CTX-I and the radiological severity of knee OA or lumbar SP. There was no correlation among COMP, CTX-I or CTX-II.
Conclusions: Serum levels of COMP and urinary levels of CTX-II were usuful to evaluate the clinical stage of knee OA or lumbar SP. Biomarkers in serum or urine were easy to collect and simple to estimate the clinical evaluation of OA or SP.

93 SERUM DICKKOPF-1 (DKK-1) LEVELS ARE INCREASED IN PATIENTS WITH ACTIVE RHEUMATOID ARTHRITIS, BUT DECREASED IN PATIENTS WITH KNEE OSTEOARTHRITIS AND ARE ASSOCIATED WITH CARTILAGE AND SYNOVIAL TISSUE TURNOVER

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Purpose: Recent animal studies have suggested that Dkk-1, a secreted inhibitor of Wnt signaling pathway, could play an important role in mediating the alterations of joint tissue turnover associated with rheumatoid arthritis (RA) and osteoarthritis (OA), but the clinical relevance of these findings remain unclear. The aim of this study was to analyze the associations between circulating Dkk-1 and cartilage and synovial tissue turnover in patients with RA and knee OA.
Methods: We measured circulating Dkk-1 by a new sensitive two site ELISA using antibodies raised against synthetic sequences of human Dkk-1 in 55 patients with active RA (65% women mean age: 55.4±14.1 yr, median disease duration 11 yr), 85 patients with painful knee OA (74% women; mean age: 62.8±7.7 yr; radiological Kellgren Lawrence score II-III, median disease duration: 41.5 months) and 93 healthy controls. Cartilage tissue degradation was evaluated by the measurement of circulating levels of the type II collagen helical fragment (Helix-II) and the type II collagenase neoepitope Col2:3/4C$_{longmono}$ (C2C). Synovial tissue metabolism was assessed by circulating levels of the nitrosylated form of type III collagen N-telopeptide (IIINys).
Results: Compared to healthy sex and age matched controls, circulating Dkk-1 levels were on average 83% higher ($p < 0.0001$) in patients with RA, but 37% ($p < 0.0001$) lower in subjects with knee OA. In patients with RA, Dkk-1 were positively associated with Helix-II ($r = 0.48$, $p = 0.0018$) and IIINys ($r = 0.28$, $p = 0.04$) levels. Conversely, Dkk-1 levels negatively correlated with C2C ($r = -0.23$, $p = 0.035$) in patients with knee OA.
Conclusions: Higher circulating Dkk-1 is associated with increased cartilage degradation and synovial tissue turnover in RA, but lower cartilage destruction in patients with knee OA. These clinical data support the role of Dkk-1 in joint damage in both RA and OA, with possible divergent effects.

94 CORRELATIONS BETWEEN ANGIOGENIC BIOMARKERS AND DISEASE ACTIVITY IN THE OSTEARTHRITIC KNEE: MODULATION WITH AN AUTOLOGOUS PREPARATION RICH IN GROWTH FACTORS

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Purpose: To examine the relationship between baseline angiogenic biomarkers and disease activity in knee OA and to determine whether treatment with an autologous Preparation Rich in Growth Factors (PRGF) obtained from the patient's own blood modulates specific angiogenic biomarkers in synovial fluid.
Methods: We performed a prospective longitudinal study comprising a cohort of 81 symptomatic idiopathic or secondary knee OA patients presenting with joint effusion. Patients were treated with a series of three weekly intra-articular injections of autologous PRGF, which is leukocyte-free plasma that is moderately enriched in platelets (2−3 fold the levels found in peripheral blood). Synovial fluid (SF) was aspirated as part of the therapeutic procedure. ELISAs were used to determine the levels of VEGF-A, HGF, and TGF-β1 in synovial fluid before and during the treatment. Response to PRGF treatment was defined as a reduction greater than 20% from baseline to week five post-treatment in WOMAC sub-scales for pain, rigidity, and function. Spearman correlations were computed to determine the relationship between baseline levels of VEGF-A, HGF, and TGF-β1 and clinical variables including age, BMI, Ahlbäck grade, and baseline WOMAC sub-scales. Pearson correlations were also used to examine relationships between baseline biomarkers and clinical variables. Improvement percentages for each of the WOMAC sub-scales were analysed at 5 weeks post-treatment. Multivariate logistic regression was used to examine factors that could affect the response to PRGF treatment. The model contained all variables: demographic, clinical and synovial fluid angiogenic markers. The predictive values are presented as odds ratios (OR) with 95% confidence intervals (CI) and probability values.
Results: The mean age was 65 years (range: 44−80), 54% of the patients were females, and BMI was 28±3.5 kg/m^2 (range: 22−38). The percentage of patients who achieved >20% and >40% reduction in clinical features according to WOMAC subscales was 19.8% and 35.8% for pain, 15.8% and 38.2% for rigidity, and 22.2% and 19.8% for function.
Baseline levels of angiogenic biomarkers were: VEGF-A: 941±483 pg/ml; HGF: 884±589 pg/ml and TGF-β1: 860±1032 pg/ml. Correlations were observed between TGF-β1 and VEGF (0.495, $p < 0.001$), TGF-β1 and HGF (0.628, $p < 0.001$), and VEGF and HGF (0.469, $p = 0.001$). VEGF was associated with Ahlbäck grade (0.295, $p = 0.011$). The levels of TGF-β1 were associated with baseline rigidity and function WOMAC subscales, Pearson coefficients 0.233, $p = 0.038$ (rigidity) and 0.250, $p = 0.030$ (function). Baseline HGF was associated with function 0.287, $p = 0.013$.
ANCOVA analysis showed that VEGF levels, when adjusted for age, were modified in response to PRGF treatment ($p = 0.008$).
After considering demographic, clinical, and biological factors in the regression analyses, the variables significantly involved in pain reduction were changes in VEGF during treatment (OR = 1.040, CI = 1.008 to 1.072; $p = 0.013$) in conjunction with sex (OR = 0.152, CI = 0.03 to 0.767; $p = 0.023$). It is also noteworthy that we observed a weak association of baseline TGF-β1 with pain reduction (OR = 0.999, CI = 0.998 to 1.000; $p = 0.091$). The variables significantly involved in rigidity reduction (>20%) were baseline pain (OR = 0.858, CI = 0.745 to 0.988; $p = 0.033$) in conjunction with baseline HGF (OR = 0.997, CI = 0.994 to 0.999; $p = 0.010$).
Conclusions: The complexity of OA requires novel approaches that identify angiogenic biomarkers and their relationship with joint homeostasis and disease activity.