similar to the patterns observed in the A1 and D1 samples. The pattern of the boiled SF supernatant sample was identical to the pattern observed by the CsCl density gradient purified samples. Without purifying the aggrecan fragments from SF, or using only Blue Sepharose chromatography, no clear Western blot immunobands were detected.

Conclusions: To reliably detect aggrecan fragments by Western blot the SF sample needs a purification level of approximately 80 μg sGAG/mg total protein. Purification of aggrecan fragments using CsCl density centrifugation from SF is the gold standard. Although anion chromatography samples have lower sGAG yields compared to the CsCl density samples, the chromatography method has an advantage in that both low Mw (e.g. G1-IPEN/TEGE) and high Mw (sGAG containing) fragments can be analyzed in the same sample. The same advantage is found for the boiled SF supernatant and in addition this method is quick and easy.

87 BIOMARKERS OF CARTILAGE AND BONE DAMAGE AS A MEASURE OF JOINT DAMAGE IN HAEMOPHILIA

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Purpose: Biomarkers of bone and cartilage turnover have frequently been evaluated for joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). Results have thus fare not been very conclusive. Some biomarkers such as urinary CTXII and serum COMP appear to correlate with severity of joint degeneration, whereas other are less distinctive. Hemophilic arthropathy (HA) is a very progressive joint degeneration as a result of frequent joint bleeds. From clinical practice it is concluded that the rate of degeneration exceeds that of OA and RA joints. This degeneration has characteristics of both inflammation mediated (as seen in RA) and degenerative (as seen in OA) joint disease. Furthermore, the joint damage is largely restricted to 3 major joints (ankle, knees, and elbows). Therefore, it might be that this rapidly progressive, localized joint degeneration can be used for the evaluation and validation of biomarkers of cartilage and bone turnover. In the present study we therefore investigated whether commercially available biomarkers of cartilage and bone in blood and/or urine are associated with severity of joint damage in patients with haemophilic arthropathy.

Methods: Blood and urine were collected from 36 patients suffering from haemophilia. Urine samples were assessed for the amount of CTX-I and CTX-II. Serum samples were assessed for the amount of CTX-I, CTX-II, COMP, C1,2C, C2C, and CS846. Radiographs of ankles, knees and elbows were scored according to Pettersson, a radiographic joint score specific for haemophilic arthropathy based on cartilage and bone changes.

Results: U-CTX-II ($R = 0.39$; $p = 0.01$), C1,2C ($R = 0.31$; $p = 0.04$) and CS846 ($R = 0.31$; $p = 0.03$) showed (marginal) correlations with the Pettersson score. Slightly better correlations were obtained when only narrowing of joint space width (JSW) as one of the items in the Pettersson score was used. The other biomarkers showed no correlation with the Pettersson score. Also the bone biomarkers did not correlate with specific bone changes. Interestingly, combined indexes of different markers, based on linear stepwise regression analysis, increased the correlation significantly up to $R = 0.65$; $p \leqslant 0.001$) for the combination of U-CTX-II, COMP and CS846.

Conclusions: The present results show that even despite this rapidly progressive degeneration of 6 large joints, from the individual biomarkers determined only U-CTX-II, C1,2C and CS846 show correlation with the severity of arthropathy. Importantly, a relation improved when the markers were related to the process they are supposed to describe (cartilage degeneration markers with JSW narrowing). Most important, combination of markers, significantly improve the relation with the radiographically determined joint degeneration. In general however, it may be concluded that these markers alone seem not of sufficient value for evaluation of joint damage yet.

88 URINE PENTOSIDINE LEVELS, WHEN NORMALISED TO KIDNEY FUNCTION, MIGHT BE A USEFUL PREDICTOR OF ADVANCED GLYCATION OF CARTILAGE IN OSTEOARTHRITIS

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Purpose: Age-related changes in articular cartilage are likely to play a role in the aetiology of osteoarthritis (OA). One of the major age-related changes in cartilage is the accumulation of advanced glycation endproducts (AGEs). Pentosidine is considered an adequate marker for the many AGEs formed *in vivo*. The best way to study the effect of pentosidine on the development of OA is by taking cartilage biopsy. However this may interfere with the disease process. Therefore, other sources need to be found to overcome this problem. Skin and cartilage pentosidine are highly correlated. However skin is not easily obtained in daily clinical practice.

We investigated whether urine pentosidine levels can be used as surrogate marker for cartilage AGE level.

Methods: Paired skin and urine samples were obtained from a cohort of 300 patients with knee and/or hip pain being part of the Dutch CHECK cohort. Since urine pentosidine levels can vary in time due to diet variation, urine was collected at 2 different time points with 3 month time interval. Pentosidine levels were measured in digested skin samples and urine by HPLC. As measure of kidney function, an estimation of the glomerular filtration rate was calculated by using the Cockcroft Gault formula: $[(140 - \text{age}) \times \text{weight (kg)}/(0.81 \times \text{serum creatinine } (\mu\text{mol/l})] \times 0.81$ for women only.

Results: Skin and urine pentosidine levels increased linearly with age ($R = 0.407$, and 0.241 respectively; both, $P < 0.001$). In literature a good linear relationship between cartilage and skin pentosidine levels was found ($R = 0.486$). Urine and skin pentosidine levels showed a much lower correlation ($R = 0.229$, $p < 0.01$). However, variation was significantly reduced by using the mean urine pentosidine of 2 time-points and when urine pentosidine was corrected for kidney function (glomerular filtration rate); the correlation between urine and skin pentosidine levels increased significantly ($R = 0.628$; $p < 0.001$).

Conclusions: From this study it appears that urine pentosidine when corrected for kidney function can be used as marker for cartilage AGE. Based on this study it is worthwhile to study the predictive value of urine pentosidine in the development and progression of osteoarthritis.

89 THE IMPORTANCE OF BALANCED CARTILAGE AND BONE TURNOVER

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Purpose: The pathogenesis of osteoarthritis (OA) likely involves interactions between the bone and cartilage compartments of the joint, resulting in a least some degree of coupling of these pathological processes. It is presently debated whether the pathogenesis is initiated in the bone or cartilage sub-compartments. We investigated cross-sectional and longitudinal relationships between markers for cartilage and bone turnover in healthy subjects and subjects with radiographic OA. The focus was primarily correlations between bone and cartilage markers and secondarily cartilage loss.

Methods: The 21-month study included 159 subjects prospectively selected as representative for the general population. Radiographs were acquired in a load-bearing semi-flexed position and the Kellgren and Lawrence index (KL) was assessed. Bone resorption was measured by the biochemical marker serum CTX-I (C-terminal telopeptide of collagen type I) and cartilage degradation by urine CTX-II (collagen type II, sample acquired as second morning void). The total medial tibial and femoral cartilage volume was quantified automatically in a computerized framework from MRI scans acquired using a Turbo 3D T1 sequence on a 0.18T Esaote scanner (40º FA, TR 50 ms, TE 16 ms, scan time 10 minutes, resolution 0.7 mm × 0.7 mm × 0.8 mm). To ensure a homogeneous population, only those over 30 years at were included, resulting in 145 subjects with age 58±13, BMI 26±4, 48% female, and 25% with radiographic OA (defined as $KL > 1$).

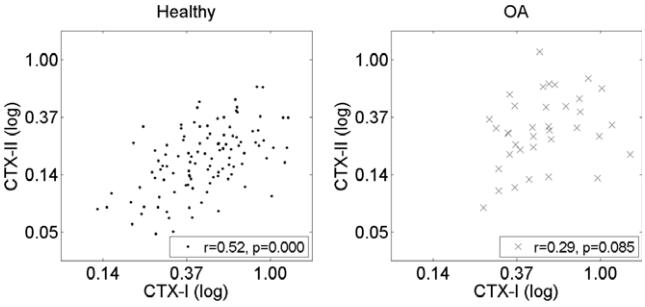

Figure 1.