rated by SDS-PAGE, transferred to PVDF membranes, and probed by FFGV (BC-14), IPEN and TEGE neoepitope antibodies, and by antibodies against aggrecan G1 and G3 domains. Immunodetected aggrecan fragments were quantified using chemiluminescence and digital luminescence imager. N- and C-terminal ends not verified by immunodetection where estimated using a calculation model.
Results: The Western blot results showed that MMP-3 cleaves aggrecan in several sites in six separate regions (a–f, Fig. 1). Time dependent kinetic studies showed that the most preferred MMP cuts in aggrecan were in region a, e and f. The second most preferred cut occurred in region d, while cuts in region b and c were the least preferred. Also, in both young (knee healthy) and adult (OA) cartilage, Western blot quantification of the *in vivo* MMP-generated G1-IPEN fragment was 3 times lower (mol/mol) compared to the *in vivo* aggrecanase generated G1-TEGE fragment. Except for the common G1-IPEN other fragments were detected in cartilage *in vivo* (G1-CS1, 210-245 kDa) and in OA synovial fluid (ARGS-CS1, 130-160 kDa; CS1-KEEE 125 kDa) with suggested N- and C-terminals from MMP cuts in the CS1 region.

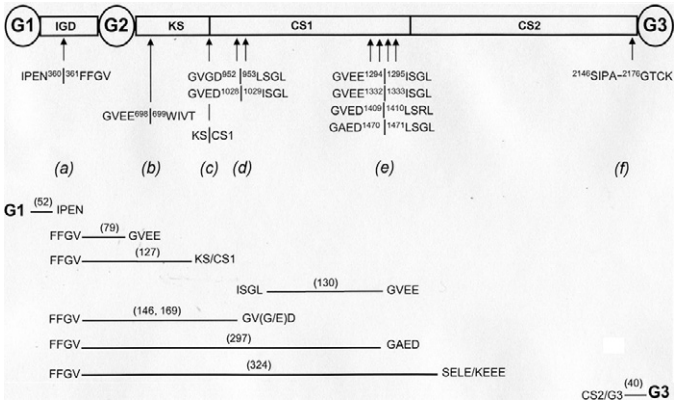

Figure 1. Several MMP cleavage sites were found in six regions (a–f) in human aggrecan. OA cartilage (A1D1) was *in vitro* digested by MMP-3 and fragments were visualized by Western blot. Illustration of human aggrecan, MMP cleavage sites and MMP-3 *in vitro* generated fragments (Mw in kDA).

Conclusions: There are at least 24 potentially MMP cleavage sites in the CS1 domain but our results suggest that only a few sites, in the beginning and in the end of this domain, are available for MMP proteolysis. Two of the MMP cuts, in the IGD and in the KS region, results in loss of most of the glycosaminoglycans from aggrecan and thereby loss of cartilage resilience. Quantification of *in vivo* generated G1-IPEN and G1-TEGE fragments indicate significant contribution of MMP proteolysis against aggrecan in both young (knee healthy) and adult (OA) cartilage.

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SERUM AND URINARY BIOCHEMICAL MARKERS FOR KNEE AND HIP OSTEOARTHRITIS: A SYSTEMATIC REVIEW APPLYING THE CONSENSUS BIPED CRITERIA

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Purpose: Molecules that are released into biological fluids during matrix metabolism and/or cellular activity in articular cartilage, subchondral bone, and synovial tissue could serve as biochemical markers of the process of osteoarthritis (OA). Unfortunately, actual breakthroughs in the biochemical OA marker field are limited so far. Critical evaluation of knowledge on the performance of currently available biochemical OA markers could guide and stimulate further biochemical OA marker research. Therefore, biochemical OA marker performance was systematically reviewed according to the "BIPED" classification, as published in 2006 in O&C.
Methods: PubMed, Scopus and EMBASE were systematically searched for publications on blood and urinary biochemical markers in human primary knee and hip OA. 84 relevant publications were identified. Data were scored and tabulated according to the "BIPED" classification.
Results: 26 biochemical OA markers were scored according to the "BIPED" classification structure. Comparison of individual biochemical marker performance was hampered by an uneven distribution of "BIPED" scores; uCTX-II and sCOMP were investigated most extensively. Also, studies showed heterogenic designs. The majority of studies involved relatively small cohorts and/or a cross-sectional design. Therefore, comparison of categories of biochemical markers was performed instead. Biochemical markers of cartilage degradation were investigated most extensively and performed well in comparison with other categories. Biochemical markers of bone metabolism performed less adequately. Biochemical markers of synovial tissue activity were investigated minimally, but performed relatively well.
Conclusions: This systematic review shows that there is a lack of consistent, high-quality evidence for the vast majority of commercially available biochemical OA markers. This lack of interest for some of the markers is not necessarily supported by publications showing their limited applicability. Additional investigation of biochemical OA markers in large-scale and/or longitudinal cohort studies is crucial to increase our knowledge on the applicability of these markers. Importantly, also the nature, origin and metabolism of biochemical markers need further investigation. International standardization of such future investigations should be pursued to obtain more high-quality, homogenous data on the full spectrum of biochemical markers. Finally, all involved parties should realize that most biochemical markers are still in an explorative stage and publication bias is therefore undesirable and should be prevented.
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SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN, HYALURONAN, HIGH-SENSITIVITY C-REACTIVE PROTEIN, AND KERATAN SULFATE AS PREDICTORS OF INCIDENT RADIOGRAPHIC KNEE OSTEOARTHRITIS: DIFFERENCES BY CHRONIC KNEE SYMPTOMS

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Purpose: To explore prognostication of incident radiographic osteoarthritis (OA) at the knee with OA-related biomarkers: cartilage oligomeric matrix protein (COMP), hyaluronan (HA), high-sensitivity C-reactive protein (hsCRP), and keratan sulfate (KS) in a large, community-based sample.
Methods: Baseline serum biomarkers of COMP,and KS were measured using in-house sandwich enzyme linked immunosorbent assays, HA was measured with the Hylauronic Acid Test kit, and hsCRP was measured with the UBI Magiwel Enzyme Immunoassay for 803 participants recruited from 1991-1997 in the Johnston County Osteoarthritis Project. Follow-up assessments were completed from 1999-2003 (mean = 6.3 years; range= 3.0 - 10.2 years). Linked baseline and follow-up anterior-posterior radiographs with weight-bearing and footmat positioning were read for Kellgren-Lawrence (KL) grade (0-4) and osteophyte (OST) and joint space narrowing (JSN; both 0-3 with Burnett atlas). Three incident radiographic OA outcomes were explored and defined as: 1) a KL grade of 0-1 at baseline and $\geq$2 at follow-up, 2) an OST grade=0 at baseline and $\geq$1 at follow-up, and 3) a JSN grade=0 at baseline and $\geq$1 at follow-up. Five hundred forty two knees were at risk for incident OA by KL grades (KL grade of 0-1 at baseline), 349 knees at risk for incident OST formation (OST=0 at baseline), and 440 knees at risk for incident JSN (JSN=0 at baseline). For each outcome, Cox regression models were used to estimate the hazard ratio (HR) for a 1-unit increase in the natural log of each biomarker, adjusting for age, race, gender, and body mass index and accounting for bilateral clustering of knees using robust variance estimates. History of lower extremity injury (knee or hip injury or fracture) and chronic knee symptoms were explored as potential modifiers of the association.
Results: In adjusted models, the hazard of incident knee OA by KL and OST increased with higher baseline lnCOMP levels (adjusted HR [aHR] = 1.46, 95% confidence interval [CI] = 0.96-2.23, and aHR= 2.26, 95% CI =1.45-3.53, respectively). The hazard of incident knee JSN also increased with higher lnCOMP levels (aHR=2.14, 95% CI= 1.32-3.47) and higher lnHA levels (aHR=1.63, 95% CI= 1.26-2.12). In unstratified analyses, higher levels of lnhsCRP and lnKS did not predict the incident knee outcomes. Neither history of lower extremity injury nor chronic knee symptoms was a strong modifier of these associations ($p > 0.10$). However, a pattern was observed of higher adjusted HRs for lnCOMP, lnHA, and, most notably, for lnKS with