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Application of biomarkers in the clinical development of new drugs for chondroprotection in destructive joint diseases: a review

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Abstract
Emerging evidence supports the concept that biochemical markers are clinically useful non-invasive diagnostic tools for the monitoring of changes in cartilage turnover in patients with destructive joint diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA). Epidemiological studies demonstrated that measurements of different degradation products of proteins in the extracellular matrix of hyaline cartilage in urine or serum samples are (1) increased in OA or RA patients compared with healthy individuals, (2) correlate with disease activity, and (3) are predictive for the rate of changes in radiographic measures of cartilage loss. The present review provides an updated list of available biomarkers and summarize the research data arguing for their clinical utility. In addition, it addresses the question whether or not the monitoring of biomarkers during different treatment modalities could be a useful approach to characterize the chondro-protective effects of approved and candidate drugs. Finally, it briefly reviews the *in vitro*/*ex vivo* experimental settings — isolated chondrocyte cultures and articular cartilage explants — that can assist in the verification of novel markers, but also studies assessing direct effects of drug candidates on chondrocytes. Collectively, biomarkers may acquire a function as established efficacy parameters in the clinical development of novel chondro-protective agents.

Keywords: *Extracellular matrix, biomarkers, arthritis, clinical trials, drugs, experimental methods*

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Rational for establishing biomarkers

Cartilage is characterized by being a tissue with low turnover and a subsequent long half-life (Christgau & Cloos 2004). The major part of cartilage is composed of the extracellular matrix (ECM), a composite network of proteins such as collagens type II, IX and XI interacting with negatively charged polysaccharides and proteoglycans, synthesized and secreted by the cells of cartilage known as chondrocytes (Archer & Francis-West 2003). An important role of the ECM is to protect the chondrocytes from the potential damaging forces of mechanical load. During normal cartilage tissue

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turnover, ECM production balances breakdown, thereby ensuring the continuous renewal of this critical tissue component. However under pathological conditions, formation cannot keep up with the degradation processes driven by harming stimuli, and a loss of the structural integrity of the cartilage emerges as a net result (Martin & Buckwalter 2002).

Osteoarthritis (OA) and rheumatoid arthritis (RA) are severe chronic debilitating diseases. Their clinical manifestation is best characterized by destruction of articular cartilage and consequent loss of peripheral joint function (Garnero et al. 2000). However, OA and RA are two different diseases with distinct mechanisms and pathologies. Although the hallmark of both diseases is an erosion of the articular cartilage, RA is an autoimmune disease with a chronic inflammation of the joints, characterized by an influx of inflammatory cells like monocytes, macrophages and fibroblasts into the synovial fluid recruited by CD4+ T-cells, with subsequent release of pro-inflammatory agents like cytokines from these cells, which ultimately induce cartilage degradation through activation of different proteases (Flugge et al. 1999, Goldring & Goldring 1999). Different from RA, OA is suggested to be a disease both involving the cartilage and bone tissue (Felson & Neogi 2004). It is devoid of an inflammatory process, and characterized by a destruction of cartilage (often with a focal appearance), bone abnormalities like sub-chondral bone thickening, deformation, and the formation of cysts and osteophytes (Aakesson 1999). The underlying mechanisms involve accelerated catabolic processes, which are accompanied by an increased release of peptide fragments from the ECM, which can potentially be detected in the synovial fluid, serum or urine, and therefore serve as proxies for the rate of cartilage turnover. Because of the differences in the pathology of OA and RA, different biomarkers would be expected to possess different potentials depending on the disease in question. Neo-epitope degradation markers, which are dependent on the activity of proteases may have more relevance in RA due to increased inflammation, whereas markers released upon activation of the sub-chondral bone might be more prevalent in OA. Each marker needs to be validated for each intended use, including the range of distinct diseases it should cover.

Until now, the clinical diagnosis of patients with OA as well as RA has been based on self-reported symptoms of joints and the more objective imaging techniques such as radiography of the joints (Scott & Houssien 1996). Importantly, by the time the characteristic radiologic signs of joint destruction emerge, the disease reaches an advanced stage, where the only curative intervention is arthroplasty. Thus, there is a need for diagnostic tools that can point out individuals at high rate of cartilage degradation, who are prone to acquiring progressive joint damage and related complications.

Monitoring of cartilage loss is a sine qua non of clinical trials that intend to assess the efficacy of candidate drugs for chondro-protection. Although radiography is currently the golden standard of monitoring changes in cartilage, its use is not without limitations. Since cartilage cannot be visualized by radiography, measurements of joint space width are only a proxy for actual articular thickness (Ory 2003). Additional difficulties arise from the fact that the annual changes in JSW are 0.1–0.2 mm, which underscore the need of a long follow-up period and inclusion of a large number of individuals in clinical trials to ensure sufficient statistical power. Consequently, clinical development of chondro-protective agents is a very time consuming and costly process, which in turn drives high prices for marketed drugs. More dynamic measures

with direct implications to cartilage status thus remain an ongoing wish of clinical investigators of the field.

To date, many biochemical markers have been proposed for the non-invasive monitoring of cartilage degradation and formation, and several new candidates can be expected to come. As the loss of cartilage is a direct indicator of the pathological disease activity, quantification of degradation markers reflects the degree of cartilage erosion (Christgau et al. 2001, Downs et al. 2001, Kojima et al. 2001). In contrast to degradation markers, formation markers reflect the levels of synthesis of cartilage: an attempt of the chondrocytes to compensate for the loss of matrix components during pathology. Newly synthesized molecules are not immediately found in circulation, but have to diffuse through the ECM, pass the synovium, and then the synovial membrane, before entering the bloodstream through the lymphatic vessel. This increases the likelihood of post-translational modifications, such as cleavage by proteases, making them less "true" formation markers. Thus, the distinction between anabolic and catabolic markers may be more complex in this specific avascular tissue. Cartilage biomarkers have also been proposed to reflect the whole turnover of the tissue, involving both formation and degradation (Garnero et al. 2000). In contrast, markers of bone metabolism are more easily separated into formation and degradation markers. The turnover process in the skeletal tissue is different from cartilage and mediated by two different types of cells; the osteoblasts, synthesizing matrix components, and the osteoclasts, degrading matrix molecules (Burger et al. 1986). Accordingly, more bone markers of either formation or degradation have been identified to date, as excellently reviewed elsewhere (Garnero & Delmas 1996, Christenson 1997, Seibel 2005).

Systemic levels of cartilage biomarkers in serum and urine give an indication of the total metabolic activity in cartilage, originating from all the joints in the entire body, and hence is not limited to reflection of abnormalities involving a few joints, which is often the case in arthritic diseases (Dequeker et al. 1993). Therefore, the fact that biomarkers by their nature are systemic, the applicability of these markers for assessment of localized joint diseases has been questioned. The work conducted by Hayami et al. has provided information regarding this issue (Hayami et al. 2004). They used the rat Anterior Cruciate Ligament Transection (ACLT) model of OA, which specifically involves the erosion of just one joint site, and assessed the response of released biomarkers. They observed a significant increase in the release of urinary CTX-II, which is a degradation product of the C-terminal end of type II collagen, compared with SHAM operated rats. This study demonstrates, that an elevation in the level of a biomarker arising from the destruction of just a single joint can indeed be detected in urine. Meulenbelt et al. investigated if cartilage degradation in OA patients affected at multiple prevalent joint sites (knee, hip, hand, spinal facet joints and disc degeneration), represented by a total ROA score, was correlating to CTX-II (Meulenbelt et al. 2005). They observed an increase in the concentrations of CTX-II with increasing ROA score. In other words, the more diseased joints in a patient with OA, the higher excretion of the biomarker in urine. Furthermore, biomarker levels may be suitable tools for the identification of sub-clinical arthritis, before any clinical symptoms are evident, which is obviously not possible by radiological means. This, however, needs to be investigated.

Some variabilities such as age, gender, ethnicity and body mass index (BMI) may influence background levels of some biomarkers (Jordan et al. 2003, Mouritzen et al.

2003). More parameters, adding to heterogeneity between different individuals are the variation of synovial clearance of metabolites into the bloodstream, and structural changes, which sometimes occur on molecules, when entering the bloodstream from the joint compartments. In addition, attaining a steady-state concentration of biomarkers in the circulation after metabolic processing in the liver and/or kidney may also be different from one person to another (Poole & Dieppe 1994). As opposed to bone turnover markers, which have marked circadian variation (Qvist et al. 2002, Garnero & Delmas 2003), cartilage markers in general show little diurnal variability (Christgau et al. 2001, Garnero & Delmas 2003, Andersson et al. 2006), which suggests, that currently, it is not necessary to standardize collection times of blood and urine samples. A list of biomarkers from cartilage, which will further be addressed in this review is shown in Table I.

Biochemical markers of cartilage

PIIPC

When looking at the markers reflecting cartilage synthesis, the amount of PIICP is two- to fourfold times elevated in synovial fluid, and 7.6-fold in cartilage tissue of OA patients compared with healthy individuals (Lohmander et al. 1996, Nelson et al. 1998). However, when measuring the biomarker in serum, decreased levels were detected in the same OA patients (Nelson et al. 1998). Similarly, the serum level of the splice variant IIA form of PIINP was also decreased in OA patients (Rousseau et al. 2004). Explanation to these apparent controversies is currently unknown and requires further investigations.

Oligosaccharides

Specific oligosaccharide epitopes such as chondroitin sulfate (CS) or keratan sulfate (KS) of aggrecan were among the first group of markers used for assessing cartilage erosion (Fawthrop et al. 1997). The aggrecan epitope 846 is a large-size molecule, which is suggested to reflect aggrecan synthesis (Månsson et al. 1995, Garnero et al. 2000). The binding of GAG antibodies is dependent on the length, as well as sulphation pattern of the molecules, suggesting an issue of heterogeneity among different individuals (Mehmet et al. 1986). Aggrecan epitope 846 is highly expressed in OA cartilage (Rizkalla et al. 1992), and is elevated in synovial fluid (Poole et al. 1994). Circulating levels of aggrecan 846 levels are the highest in OA patients with the longest disease period as well as greatest cartilage loss (Poole et al. 1994). But different from OA, there is not a clear tendency in RA, as serum 846 levels seem high in the slowly progressing, but depressed in the rapidly progressing form of the disease (DeGroot et al. 2002). However, it should be stressed that carbohydrate epitopes of CS and KS are found in a number of other proteoglycans of the ECM, as well as in other tissues even though the majority resides in cartilage (Funderburgh et al. 1987, Moller et al. 1994, Garnero et al. 2000, Perrimon & Bernfield 2000, Lemons et al. 2001, DeGroot et al. 2002). Collectively, currently existing clinical data do not demonstrate convincing differences between CS and KS levels in serum between progressive and non-progressive RA or OA individuals, which may diminish the use of these markers in clinical assessment and monitoring of arthritic diseases (DeGroot et al. 2002).

Table I. Biochemical markers of cartilage turnover.

Biomarker identity	Biochemical process	Tissue specificity	Characterization and in vivo function	Mean CV per cent of intra- and interassay	References
Aggrecan	Depends on the epitope	Found in cartilage	Aggrecan is the major proteoglycan in cartilage. It mediates withdrawal of water into cartilage by osmosis, which exerts a swelling pressure on the collagen network, making the tissue ideal for resisting compressive load	5.7%	Kiani et al. (2002) IBEX Pharmaceuticals
Type II collagen: PIINP and PIICP	Formation marker	Found in cartilage	Cross-linked collagen molecules are responsible for the rigid form and tensile properties of articular cartilage	6.8% 3.6%	Eyre (1991), Sugiyama et al. (2003), Rousseau et al. (2004)
Hyaluronan	Marker of turnover	Expressed predominantly in cartilage as well as in cells of the synovial lining such as macrophages and fibroblasts	Hyaluronan is a non-sulfated GAG made up by the repeating disaccharide D-glucuronic acid-N-acetyl-D-glucosamine. It functions as an anchoring component for proteoglycans in cartilage	8.9%	Kongtawelert & Ghosh (1990)
YKL-40	Marker of turnover	Increased levels in RA chondrocytes, synovial fibroblasts, macrophages, neutrophiles, leukocytes, the liver, brain, kidney, placenta and in different tumour cells	YKL-40 is a mammalian proteoglycan with a structure similar to bacterial and fungal chitinases	8.7%	De Ceuninck et al. (2001)
Cartilage oligomeric protein (COMP)	Degradation marker	Predominantly found in cartilage, but also in tendon, ligament, meniscus, osteoclasts, synoviocytes and dermal fibroblasts	COMP is a disulfide-linked, pentameric proteoglycan belonging to the thrombospondin family. COMP is involved in collagen fibril formation in the presence of Zn2+ for the maintenance of the integrity of the collagen network	<5%	Crnkic et al. (2003)

Table I (*Continued*)

Biomarker identity	Biochemical process	Tissue specificity	Characterization and in vivo function	Mean CV per cent of intra- and interassay	References
Type II collagen: Col 2-1 and Col 2-1 NO2	Degradation markers	Found in cartilage	Col 2-1 is an epitope in the triple helical region of type II collagen with the sequence HRGYPGLDG. The tyrosine in the sequence can furthermore undergo nitration resulting in Col 2-1 NO2	8.8% 8.4%	Deberg et al. (2005)
Type II collagen: Glucosyl-galactosyl-pyridinoline (Glc-Gal-PYD)	Degradation marker	Found mostly in the synovium and in very small amounts in cartilage	PYD has an anchoring function linking the triple alpha helices of collagen type II molecules to each other	9.5%	Gineyts et al. (2004)
Type II collagen: C2C and C1,2C	Degradation markers	Found in cartilage	C2C and C1,2C are neo-epitopes created by digestion of type II collagen by collagenases	13.8% 11.3%	Verstappen et al. (2006)
Type II collagen: C-terminal telo-peptide CTX-II	Degradation marker	Found in cartilage	CTX-II is a six amino acid-long neo-epitope with the sequence EKGPDP released from the C-terminal part of type II collagen by collegenases	7.8%	Christgau et al. (2001, 2004), Garnero et al. (2001, 2002)

Hyaluronan

Hyaluronan is a high molecular weight non-sulfated glycosaminoglycan (GAG). Each chain is composed of 8000–16 000 of the repeating disaccharide D-glucuronic acid-N-acetyl-D-glucosamine, and its main function is to work as an anchoring component of the ECM for retaining different proteoglycans namely aggrecan, versican, neurocan and brevican in cartilage. These proteoglycans interact with hyaluronan by a common loop structure referred to as proteoglycan tandem repeat (Knudson & Knudson 2001).

It is predominantly expressed in cartilage, but is also synthesized by the cells of the synovial lining such as macrophages or fibroblasts (Garnero et al. 2000, Knudson & Knudson 2001). Majeed et al. have detected increased levels of hyaluronan in patients with RA compared with age matched healthy controls in a prospective study after 6 and 12 months of disease onset (Majeed et al. 2004). Pavelka et al. observed that patients with knee OA with elevated serum levels of hyaluronan had a faster radiological progression of the knee (Pavelka et al. 2004). Still, other clinical data support the fact that the increase of this GAG in serum of patients with arthritis predicts a more pronounced progression of the disease state (Elliott et al. 2005). Although a number of clinical studies do provide consistency and evidence for the use of hyaluronan as a marker of arthritis, one major drawback for its applicability in clinical practice is its large diurnal variability in serum of RA patients (Manicourt et al. 1999).

YKL-40

Another marker that has undergone clinical investigation is YKL-40. It is also referred to as human cartilage glycoprotein 39 with a structure similar to bacterial and fungal chitinases, and is found in low concentrations in normal cartilage (Henrissat & Bairoch 1993). On the other hand, YKL-40 expression has been detected in high quantities in the liver, brain, kidney, placenta, tumour cells, synovial fibroblasts, macrophages, neutrophiles and leukocytes (Hakala et al. 1993, Johansen et al. 1995, Kirkpatrick et al. 1997, Volck et al. 1998). Though YKL-40 is related in sequence, it does not possess glycosidase activity for substrates of chitinases, as its active site is different from normal chitinases, still making its biological function unknown (Henrissat & Bairoch 1993, Garnero et al. 2000). Increased serum and synovial fluid concentrations of YKL-40 are detected in patients with active RA and late-stage knee OA (Johansen et al. 1993). Furthermore, Conrozier et al. have also detected increased serum YKL-40 levels in patients with hip OA (Conrozier et al. 2000). Elevated concentrations have also been detected in other pathological conditions involving processes of inflammation. Consequently, YKL-40 may be regarded as an inflammation marker (Christgau & Cloos 2004). Large-scale prospective studies are awaited for evaluating the clinical potential of this marker as a reflection of cartilage turnover (Garnero et al. 2000).

COMP

Cartilage oligomeric matrix protein (COMP) is disulfide linked pentameric proteoglycan, which is found mostly in articular cartilage, but detection has also been demonstrated in meniscus, tendon, dermal, synovial fibroblasts and osteoclasts

(Oldberg et al. 1992, Dodge et al. 1998, Muller et al. 1998, Di Cesare et al. 2000). It is a member of the thrombospondin family, which has been shown to stimulate collagen type II fibril formation in the presence of Zn^{2+}, as well as interaction with other matrix components and cells (Rosenberg et al. 1998). A number of cross-sectional studies have shown elevated COMP levels in synovial fluid and serum of patients diagnosed with OA or RA (Sharif et al. 1995, Recklies et al. 1998, Di Cesare et al. 1999, Wislowska & Jablonska 2005). Moreover, there is a positive correlation between serum COMP levels and the disease activity of OA (Sharif et al. 2004, Wislowska & Jablonska 2005) as well as the radiographic progression of the joint disease and the Western Ontarion/McMasters Universities (WOMAC) score; an index for pain, stiffness and physical function (Vilim et al. 2002, Wislowska & Jablonska 2005). Similar findings were obtained in clinical investigations involving RA patients; COMP levels were correlated with the disease activity score (DAS) and damage of cartilage assessed by X-ray (Skoumal et al. 2003). However, one RA study conducted on joints of hands and feet did not show increased levels of COMP in patients with Larsen score progression over 5 years, nor in individuals with non-progressive disease compared with baseline levels (Fex et al. 1997), suggesting that multiple time-points are needed, when evaluating COMP as a disease marker in arthritis in clinical trials. In general, most clinical studies done on COMP demonstrate, that increased COMP levels reflect cartilage degradation in OA as well as in RA patients.

Products of protein nitration

Nitration of proteins is a prominent feature of the pathophysiology in compromising joint diseases like arthritis. This phenomenon is caused by the interaction of particularly aromatic residues (Van der Vliet et al. 1995) with a peroxynitrite anion ($ONOO^-$). This anion is a potent oxidant, formed following the reaction between nitric oxide (NO) and superoxide anion (O_2^+). In fact, it has been demonstrated that type II collagen is sensitive to nitration (Paik et al. 2001). Indeed, chondrocytes themselves are capable of producing O_2^+ and NO (Henrotin et al. 1993), and nitrotyrosine has been observed in the cartilage of individuals with arthritis (Loeser et al. 2002). Deberg et al. have developed immuno-assays against the sequence ^{108}HRGYPGLDG^{116} in the triple helical region of collagen type II (Col 2-1) as well as against its nitrated derivative (Col 2-1 NO_2) for the quantification of type II collagen degradation (Deberg et al. 2005). In one study, they found a marked increase in the serum levels of both Col 2-1 and Col 2-1 NO_2 in OA and RA patients compared with healthy controls. In another 3 year follow up study consisting of 75 OA patients, they monitored the urine levels of Col 2-1 and Col 2-1 NO_2 and mean joint space width (JSW). They found a negative correlation between the levels of these biomarkers after 1 year and the 3 year change of the JSW, indicating that an increase in the levels of Col 2-1 and Col 2-1 NO_2 in the circulation were indicative of Joint Space Narrowing (JSN) and radiological OA progression (Deberg et al. 2005). More longitudinal clinical studies in the future will shed a light on the utility of these fairly new biomarkers, reflecting the oxidative damage to the cartilage in diseased conditions.

Degradation products of collagen type II

Glucosyl-galactosyl pyridinoline (Glc-Gal-PYD). In the ECM, type II collagen fibrils are made up by anchoring of multiple triple alpha helices, and the cross-links between adjacent collagen molecules is mediated by a molecule referred to as pyridinoline (PYD) (Garnero et al. 2000). Urinary secretion of PYD cross-links are commonly used for the monitoring of bone, synovium and cartilage degradation (Delmas & Garnero 1996, Garnero et al. 2000). However, recently, a glycolysated analogue of the molecule: Glucosyl-galactosyl pyridinoline (Glc-Gal-PYD) has been found in abundance in the synovium, while it is absent from the skeletal tissue, and only observed in very low amounts in cartilage (Gineyts et al. 2001). A number of clinical studies in the literature have already indicated its relevance in clinical assessment. One prospective study, involving patients with early RA, conducted by Garnero and co-workers showed 70% increase of this marker compared with healthy controls. Moreover, the marker also correlated with JSN over a period of 1 year, and patients having elevated levels of Glc-Gal-PYD had a higher risk of progression of the disease (Garnero et al. 2002). In another cohort of RA patients, the level of Glc-Gal-PYD was increased 109% compared with controls, and the elevation was even more pronounced in patients with destructive disease compared with non-destructive (Gineyts et al. 2001). Furthermore, an association of the urinary levels of Glc-Gal-PYD with prevalence of knee OA, WOMAC index, JSN and osteophyte score has been described (Garnero et al. 2001, Jordan et al. 2005) and Gineyts et al. measured increased concentrations of Glc-Gal-PYD in OA patients with knee swelling as opposed to those, whose knees were not swollen (Gineyts et al. 2004). In summary, a number of clinical studies in patients with RA or OA have already provided convincing data for the monitoring of Glc-Gal-PYD in joint diseases, and more studies in the future will undoubtedly determine its clinical utility.

C2C and C1,2C

C2C and C1,2C are neo-epitopes created by digestion of type II collagen by specific collagenases (Downs et al. 2001, Kojima et al. 2001), and therefore, these epitopes are believed to give a direct reflection of cartilage destruction. In a human clinical study, the authors concluded that the serum ratio of C1,2C/C2C correlated with cartilage erosion and radiographic progression of OA (Cerejo et al. 2002). However, this ratio could only predict progression for a subset of individuals with knee OA but without OA in the hand, whereas progression was not demonstrated for patients with both knee and hand OA. Yet other studies conducted by Billinghurst et al. and Dahlberg et al. demonstrate that the level of C1,2C is significantly elevated in human OA cartilage compared with control cartilage (Billinghurst et al. 1997, Dahlberg et al. 2000). The above-mentioned clinical studies are supported by several pre-clinical observations also showing increases of these neo-epitopes under diseased conditions (Kojima et al. 2001, Chu et al. 2002). Elevated C2C levels have been detected in serum of transgenic mice, where activation of human MMP-13 gene was done postnatally with the development of early lesions of articular cartilage, as well as in rats induced with inflammatory arthritis (Song et al. 1999, El-Maadawy et al. 2003). Higher C1,2C concentrations were observed following treatment of bovine articular and nasal cartilage samples with the pro-inflammatory cytokine IL-1, which is known to stimulate cartilage catabolism *in vitro* (Fosang

et al. 1996, Billinghurst et al. 2000). Collectively, though it seems like different pre-clinical models support a role for these biomarkers, more cross-sectional clinical studies are awaited to conclude their efficacy for monitoring cartilage catabolism in human arthritis.

CTX-II

The most thoroughly validated marker of collagen type II fragments is CTX-II. Urinary level of CTX-II has been reported to be associated with disease activity in OA and RA (Christgau et al. 2001, Garnero et al. 2004, Jung et al. 2004). CTX-II levels also correlate with the amount of joint erosion in OA and RA patients (Christgau et al. 2001, Garnero et al. 2001, 2002, Christgau & Cloos 2004). In a large population-based prospective study (including 1235 men and women at mean age 66 years, mean follow up 6.6 years), Reijman and co-workers investigated the association of CTX-II levels with the prevalence and progression of hip and knee OA monitored by radiography (Reijman et al. 2004). The investigators observed that individuals with a CTX-II level in the highest quartile had a 6.0- and 8.4-fold increased risk for radiographic progression of knee and hip OA, respectively. Of even more importance, they demonstrated that a high baseline CTX-II level was significantly associated with radiographic progression of disease in both knee and hip (Reijman et al. 2004).

The relationship between baseline levels of CTX-II and long-term radiographic progression was also investigated in a study, where 110 patients were treated for RA (Garnero et al. 2002). This study was a 5-year follow up based on the Combinatietherapie Bij Reumatiode Artritis (COBRA) study (Boers et al. 1997, Landewe et al. 2002). Garnero and co-workers found a significant correlation between CTX-II levels at baseline and long-term radiographic progression (Garnero et al. 2002). Later it was reported that changes in the level of CTX-II after 3 months of therapy were predictive of long-term radiographic progression, and they concluded that the predictive value of CTX-II was independent of changes in other measures of disease activity (Landewe et al. 2004).

In summary, the potential of CTX-II as a predictor of joint destruction has been verified in multiple clinical studies, as these have shown a correlation to radiological progression, as well as to the WOMAC score (Garnero et al. 2001, Christgau et al. 2004, Jordan et al. 2005).

Matrix metallo proteinases (MMPs) and their inhibitors as markers of inflammation

In RA, considerable attention has been given a family of enzymes referred to as Matrix Metallo Proteinases (MMPs). MMPs are known to degrade both aggrecan and type II collagen, and may therefore participate in the destruction of ECM components during pathological conditions (Cawston 1996, Fosang et al. 2003). MMPs are secreted in an inactive pro-form, which are then activated extracellularly. Most MMPs and their inhibitors, termed tissue inhibitor of metalloproteinases (TIMPs), are expressed by both chondrocytes and cells of the synovium (Dean et al. 1989, Walakovits et al. 1991).

MMP-3 expression is increased in early RA, and correlates with JSN and bone erosion, and is known to cleave proteoglycan and activate other MMPs (Garnero et al. 2002, Posthumus et al. 2003). Serum MMP-3, and to a lesser extent MMP-1 levels, are decreased after initiating anti-TNF-alpha therapy furthermore indicating their

relevance in inflammatory related pathologies (Brennan et al. 1997). Other studies show increased SF and serum levels of MMP-1 and MMP-3 in patients with knee or hip OA (Lohmander et al. 1993, Ishiguro et al. 1999). Similarly, TIMP-1 and TIMP-2 are also elevated in SF of patients with knee OA, though not to a similar extent as MMP-1 and MMP-3, still resulting in a markedly increased MMP:TIMP ratio in patients compared with controls (Ishiguro et al. 1999). Konttinen and co-investigators have identified the presence of MMP-13 and MMP-15 exclusively in SF from RA patients, by assaying a large number of MMPs through reverse transcriptase polymerase chain reaction technique (Konttinen et al. 1999). Interestingly, high levels of MMP-1 and MMP-3 have also been detected in other inflammatory diseases, where joint involvement is absent like systemic lupus erythematosus (Keyszer et al. 1999). In summary, many clinical studies provide evidence for the potential of MMPs and TIMPs as markers of inflammation, but more trials are needed to pinpoint the ones with the greatest clinical significance (Garnero et al. 2000, Wollheim 2000, DeGroot et al. 2002).

Changes in biomarkers during pharmacological interventions

There is an ongoing search for medications that could facilitate the prevention and treatment of destructive joint diseases. Table II provides an overview of clinical studies that investigated changes in the level of biomarkers in patients receiving pharmacological agents. In a 1-year prospective study of 135 patients with RA, the response to treatment with Adalimumab, a disease modifying antirheumatic drug (DMARD), composed of a fully humanized antibody to tumour necrosis factor-alpha was investigated (Garnero et al. 2004). It was observed that the level of CTX-II was reduced by 17% compared with baseline after 12 weeks of treatment, and this suppression was maintained until study termination. Conversely, changes in CTX-II could not be detected in subjects treated with placebo for 12 weeks, but levels dropped significantly after switching from placebo to Adalimumab (Garnero et al. 2004). In another study with 212 OA patients, the chondroprotective effects of Glucosamine sulphate, as assessed by scoring the WOMAC index and X-ray analysis was compared with changes in CTX-II (Christgau et al. 2004). They observed a 16% drop in CTX-II levels after a period of 1 year with Glucosamine sulphate treatment in patients with an initial high baseline concentration of CTX-II. In those patients, the change in CTX-II levels over 1 year correlated with JSN detected after 3 years, and moreover baseline CTX-II levels were associated with a worsening of the WOMAC score.

Crnkic and co-investigators monitored the release of COMP in RA patients treated with Infliximab and Etanercept, and observed a decrease of approximately 11 and 10%, respectively, after 3 months of treatment, which remained low after 6 months (Crnkic et al. 2003). The decrease in COMP levels were observed in patients responding, as well as non-responding, according to the ACR20 criteria (Crnkic et al. 2003).

These observations raise the question as to the specificity of CTX-I and CTX-II. Are they simply different markers reflecting common metabolic processes? It has been demonstrated that CTX-I is ninefold elevated in Paget's disease, while CTX-II remained in the normal range (Garnero et al. 2001). In another study, patients with

Table II. Changes in different biomarkers of cartilage in patients with OA or RA during treatment with anti-resorptive or disease-modifying agents.

Drug	Participants	Duration	Biochemical markers (marker response)	References
Biphosphonates: Risedronate	284 with OA	2 years	CTX-II ⇓ 31% (after 6 months)	Garnero et al. (2004)
Non-steroid anti inflammatory drugs (NSAIDS): Adalimumab Ibuprofen Infliximab Etanercept	135 with RA 201 with OA 32 with RA 17 with RA	7 months 4–6 weeks 6 months 6 months	CTX-II ⇓ 17% (after 12 weeks) CTX-II stabilized (4–6 weeks) COMP ⇓ 11% (after 3 months) COMP ⇓ 10% (after 3 months)	Garnero et al. (2004) Gineyts et al. (2004) Crnkic et al. (2003) Crnkic et al. (2003)
Others: BAY 12-9566 Glucosamine sulphate Soy protein	35 with OA 212 with OA 135 with OA	3 weeks 3 years 3 months	846 ⇑ significantly CTX-II ⇓ 16% (after 1 year) YKL-40 ⇓ significantly	Leff et al. (2003) Christgau et al. (2004) Arjmandi et al. (2004)

knee OA had decreased concentrations of CTX-I compared with controls, and increased levels of CTX-II (Garnero et al. 2001).

Oestergaard and co-workers used the collagen induced arthritis (CIA) rat model for RA to enlighten the link between sub-chondral bone and cartilage turnover. At study termination (day 23), they observed elevated CTX-I levels in serum in CIA rats compared with controls (165%), by which time, there was a CTX-II increase of 750% and 3297% in serum and synovial fluid respectively (Oestergaard et al. 2006). Collectively, this study demonstrates a marked diversity in the release pattern of CTX-I and CTX-II, and provides two important pieces of information: (1) articular cartilage degradation proceeds erosion of sub-chondral bone, and does not seem to raise concerns for cross-reactivity of these two markers, and (2) sub-chondral bone turnover also seems to be affected in this animal model of RA.

In summary, it seems reasonable to assume that specific biological markers of cartilage indeed carry noteworthy potential as diagnostic tools in arthritic diseases, as well as for the monitoring of chondro-protective effects.

Validation of biochemical markers of cartilage turnover

Clinically relevant endpoints are often impractical to apply in the early phases of clinical development (proof-of-principle). In trials of new drugs for rheumatoid arthritis, improvement is most often defined by an outcome measure of the American College of Rheumatology (ACR), reflecting a reduction in number of tender and swollen joints plus similar improvement in at least three of five other measures; pain, global assessment by patient and physician, self-assessed physical disability, and level of acute-phase reactant (Felson et al. 1995). Similar, in trials of osteoarthritis endpoints are based on various subjective measures of pain and function (e.g. WOMAC), and the relationship to structural indexes, e.g. radiographic measurement of joint space narrowing (JSN), are not well developed. Obviously, these clinically relevant outcomes, if applied early in clinical drug development, would both delay the development process and at the same time increase the costs to an unacceptable level. Therefore, research is ongoing to identify alternative clinical measurements (surrogate endpoints), which can be assessed with biomarkers associated with the pathophysiology of the disease (Rolan 1997, Lesko & Atkinson 2001). It is important to recognize that the surrogate endpoint has to both correlate with the clinical outcome and at the same time reflect the pathophysiology of the disease. By doing so, medical intervention on the surrogate end point will most likely predict the effect of the drug on the clinical end point, and this is a much stronger condition than merely being correlated (Lesko & Atkinson 2001).

In both RA and OA, a hallmark of the disease is the irreversible loss of articular cartilage. The thickness of the cartilage can indirectly be quantified by measurement of the distance between the skeletal surfaces, i.e. in the medial tibio-femoral compartment, on radiographic images of the knee joint. However, this biomarker is responding very slowly to medical intervention, and therefore it has been investigated if the dynamics of biochemical markers could contribute to the assessment of disease in arthritis.

Appropriate application of any biomarker, including biochemical markers, is dependant on the quality of its validation, and numerous approaches have been adopted for this purpose. Usually, however, the evaluation is initiated by determining

the association of the marker to the clinically relevant end point. Elsewhere in this review, the association of various biochemical cartilage markers with clinical parameters such as ACR20, WOMAC, etc. has been described, and in addition the response on the marker to medical intervention has been provided. However, to address further the position of the marker in the causal chain of pathological events leading to the clinical end point, other investigations are needed. In arthritis, as stated above, a key finding is the destruction of articular cartilage, and therefore much effort has been allocated to the evaluation of biomarkers in models of involving structural damage to articular cartilage.

Articular cartilage *ex vivo* explants model provides the opportunity to investigate both formation and degradation of the extracellular matrix. In this system, the chondrocytes are embedded in their natural matrix, where the nearby environment is preserved, including macromolecules and cell-binding proteins, and it allows a more sensible evaluation of signals transmitted from and to the chondrocytes through the complex architecture of the cartilage (Karsdal et al. 2002). Furthermore, the explant model allows preservation of the chondrocyte phenotype such as their spherical appearance (Hascall et al. 1983). The pioneering work of porcine cartilage explant cultures were performed by Fell & Barratt (1973). Today, the explant cartilage models are extensively used as a degradation assay, in which changes in cartilage metabolism in response to different agents can be followed (Roy-Beaudry et al. 2003). The explant system allows to study the cytokines involved in cartilage degradation (Saklatvala 1986), as well as to investigate the potential chondro-protective effect of proteins or compounds that inhibit cartilage breakdown. Proteoglycan is rapidly lost during cartilage degradation, but is easily replaced by chondrocytes under normal conditions (Hascall et al. 1983). Loss of proteoglycan always occurs before collagen type II degradation can be observed (Billinghurst et al. 2000), the latter being an irreversible step in cartilage degradation, as the lost collagen cannot be replaced (Shingleton 2003). These data verify that the markers are associated with important steps in disease development.

Alternatively, biomarkers can be associated with metabolic activity of the chondrocyte, by studying isolated chondrocytes (Takigawan et al. 1997, Hauselmann & Hedbom 1999, Amirahmadi et al. 2004, Ishikawa et al. 2004), chondrosarcoma cells (Schorle et al. 2005) or differentiated mesenchymal stem cells (Lunstrum et al. 1999), although these models lack the physiological relevance achieved by having the chondrocytes integrated into the ECM as described above. Finally, pellet cultures can be used in a similar approach (Manning & Bonner 1967, Johnstone et al. 1998, Yoo et al. 1998, Barry et al. 2001).

Conclusion

Intensive research in the past decade has provided a strong support for the notion that cartilage degradation can be estimated by measurements of different degradation products of the ECM of articular cartilage. Links between serum or urinary levels of several biomarkers, and the severity or progression of destructive joint diseases have been established. Furthermore, changes in biomarkers to intervention was shown to foresee therapeutic benefits as well. There are now several *in vitro* and *ex vivo* experimental set-ups that can assist in the characterization of biomarkers, and provide information, as to what extent, they reflect catabolic or anabolic processes of cartilage

turnover. When the latter aspects are established, monitoring of the given biomarker may help to clarify the mechanisms of action of novel drug candidates. The use of biomarkers as efficacy parameters in clinical drug development will likely rise in the upcoming years and contribute to the identification of chondroprotective agents for a better clinical management of destructive joint diseases, such as OA and RA.

Finally, levels of CTX-II, Glc-Gal-PYD and COMP have been shown to correlate with clinically relevant endpoints like WOMAC, and in addition, these markers are associated with indexes of structural damage in the joints, i.e. measurements of joint space narrowing, supporting their utility in clinical development.

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References

Aakesson K. 1999. Osteoarthritis and degenerative spine pathologies. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. Orlando, FL: Academic Press. p. 637–648.

Amirahmadi SF, Pho MH, Gray RE, Crombie DE, Whittingham SF, Zuasti BB, Van Damme MP, Rowley MJ. 2004. An arthritogenic monoclonal antibody to type II collagen CII-C1 impairs cartilage formation by cultured chondrocytes. Immunology and Cell Biology 82(4):427–434.

Andersson M, Petersson IF, Karlsson K, Jonsson NE, Månsson B, Heinegård D, Saxne T. 2006. Diurnal variation in serum levels of cartilage oligomeric matrix protein (comp) in individuals with knee osteoarthritis or rheumatoid arthritis. Annals of Rheumatology Disease [Epub ahead of print].

Aoyama T, Liang B, Okamoto T, Matsusaki T, Nishijo K, Ishibe T, Yasura K, Nagayama S, Nakayama T, Nakamura T, Toguchida J. 2005. PGE2 signal through EP2 promotes the growth of articular chondrocytes. Journal of Bone Mineral Research 20:377–389.

Archer CW, Francis-West P. 2003. The chondrocyte. International Journal of Biochemistry and Cell Biology 35(4):401–404.

Arjmandi BH, Khalil DA, Lucas EA, Smith BJ, Sinichy N, Hodges SB, Juma S, Munson ME, Payton ME, Tivis RD, Svanborg A. 2004. Soy protein may alleviate osteoarthritis symptoms. Phytomedicine 11(7–8):567–575.

Barry F, Boynton RE, Liu B, Murphy JM. 2001. Chondrogenic differentiation of mesemchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. Experimental Cell Research 268:189–200.

Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, Mitchell P, Hambor J, Diekmann O, Tschesche H, Chen J, Van Wart H, Poole AR. 1997. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. Journal of Clinical Investigation 99(7):1534–1545.

Billinghurst RC, Wu W, Ionescu M, Reiner A, Dahlberg L, Chen J, Van Wart H, Poole AR. 2000. Comparison of the degradation of type II collagen and proteoglycan in nasal and articular cartilages induced by interleukin-1 and the selective inhibition of type II collagen cleavage by collagenase. Arthritis and Rheumatism 43(3):664–672.

Boers M, Verhoeven AC, Markusse HM, Van De Laar MA, Westhovens R, Van Denderen JC, Van Zeben D, Dijkmans BA, Peeters AJ, Jacobs P, Van Den Brink HR, Schouten HJ, Van der Heijde DM, Boonen A, Van der Linden S. 1997. Randomised comparison of combined step-down prednisolone, methotrexate and sulphasalazine with sulphasalazine alone in early rheumatoid arthritis. Lancet 350(9074):309–318.

Brennan FM, Browne KA, Green PA, Jaspar JM, Maini RN, Feldmann M. 1997. Reduction of serum matrix metalloproteinase 1 and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti-tumour necrosis factor-alpha (cA2) therapy. British Journal of Rheumatology 36(6):643–650.

Burger EH, Boonekamp PM, Nijweide PJ. 1986. Osteoblast and osteoclast precursors in primary cultures of calvarial bone cells. The Anatomical record 214:32–40.

Cawston TE. 1996. Metalloproteinase inhibitors and the prevention of connective tissue breakdown. Pharmacology and Therapy 70:163–182.

Cawston TE, Curry VA, Summers CA, Clark IM, Riley GP, Life PF, Spaull JR, Goldring MB, Koshy PJ, Rowan AD, Shingleton WD. 1998. The role of oncostatin M in animal and human connective tissue collagen turnover and its localization within the rheumatoid joint. Arthritis and Rheumatism 41:1760–1771.

Cerejo R, Dunlop DD, Cahue S, Channin D, Song J, Sharma L. 2002. The influence of alignment on risk of knee osteoarthritis progression according to baseline stage of disease. Arthritis and Rheumatism 46(10):2632–2636.

Christenson RH. 1997. Biochemical markers of bone metabolism: an overview. Clinical Biochemistry 30(8):573–593.

Christgau S, Bitsch-Jensen O, Hanover BN, Gamwell HE, Qvist P, Alexandersen P, Bang HD. 2000. Serum CrossLaps for monitoring the response in individuals undergoing antiresorptive therapy. Bone 26:505–511.

Christgau S, Cloos PA. C. 2004. Cartilage degradation products as markers for evaluation of patients with rheumatic disease. Clinical and Applied Immunology 4(4):277–294.

Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, Gineyts E, Rosenquist C, Qvist P. 2001. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone 29(3):209–215.

Christgau S, Henrotin Y, Tanko LB, Rovati LC, Collette J, Bruyere O, Deroisy R, Reginster JY. 2004. Osteoarthritic patients with high cartilage turnover show increased responsiveness to the cartilage protecting effects of glucosamine sulphate. Clinical Experiments in Rheumatology 22(1):36–42.

Chu Q, Lopez M, Hayashi K, Ionescu M, Billinghurst RC, Johnson KA, Poole AR, Markel MD. 2002. Elevation of a collagenase generated type II collagen neoepitope and proteoglycan epitopes in synovial fluid following induction of joint instability in the dog. Osteoarthritis and Cartilage 10(8):662–669.

Conrozier T, Carlier MC, Mathieu P, Colson F, Debard AL, Richard S, Favret H, Bienvenu J, Vignon E. 2000. Serum levels of YKL-40 and C reactive protein in patients with hip osteoarthritis and healthy subjects: a cross sectional study. Annals of Rheumatology Disease 59(10):828–831.

Crnkic M, Månsson B, Larsson L, Geborek P, Heinegard D, Saxne T. 2003. Serum cartilage oligomeric matrix protein (COMP) decreases in rheumatoid arthritis patients treated with infliximab or etanercept. Arthritis Research and Therapy 5(4):181–185.

Dahlberg L, Billinghurst RC, Manner P, Nelson F, Webb G, Ionescu M, Reiner A, Tanzer M, Zukor D, Chen J, Van Wart HE, Poole AR. 2000. Selective enhancement of collagenase-mediated cleavage of resident type II collagen in cultured osteoarthritic cartilage and arrest with a synthetic inhibitor that spares collagenase 1 (matrix metalloproteinase 1). Arthritis and Rheumatism 43:673–682.

De Ceuninck F, Pastoureau P, Agnellet S, Bonnet J, Vanhoutte PM. 2001. Development of an enzyme-linked immunoassay for the quantification of YKL-40 (cartilage gp-39) in guinea pig serum using hen egg yolk antibodies. Journal of Immunology Methods 252(1–2):153–161.

Dean DD, Martel-Pelletier J, Pelletier JP, Howell DS, Woessner JF. J. 1989. Evidence for metalloproteinase and metalloproteinase inhibitor imbalance in human osteoarthritic cartilage. Journal of Clinical Investigation 84:678–685.

Deberg M, Labasse A, Christgau S, Cloos P, Henriksen DB, Chapelle JP, Zegels B, Reginster JY, Henrotin Y. 2005. New serum biochemical markers (Coll 2-1 and Coll 2-1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. Osteoarthritis and Cartilage 13:258–265.

DeGroot J, Bank RA, Tchetverikov I, Verzijl N, Tekoppele JM. 2002. Molecular markers for osteoarthritis: the road ahead. Current Opinion in Rheumatology 14(5):585–589.

Delmas PD, Garnero P. 1996. Biochemical markers of bone turnover. In: Marcus R, Feldman D, Kelsey J (eds) Osteoporosis. San Diego, CA: Academic Press. p. 1075–1085.

Dequeker J, Mohan S, Finkelman RD, Aerssens J, Baylink DJ. 1993. Generalized osteoarthritis associated with increased insulin-like growth factor types I and II and transforming growth factor beta in cortical bone from the iliac crest. Possible mechanism of increased bone density and protection against osteoporosis. Arthritis and Rheumatism 36(12):1702–1708.

Di Cesare PE, Fang C, Leslie MP, Della Valle CJ, Gold JM, Tulli H, Perris R, Carlson CS. 1999. Localization and expression of cartilage oligomeric matrix protein by human rheumatoid and osteoarthritic synovium and cartilage. Journal of Orthopedic Research 17(3):437–445.

Di Cesare PE, Fang C, Leslie MP, Tulli H, Perris R, Carlson CS. 2000. Expression of cartilage oligomeric matrix protein (COMP) by embryonic and adult osteoblasts. Journal of Orthopedic Research 18(5):713–720.

Dodge GR, Hawkins D, Boesler E, Sakai L, Jimenez SA. 1998. Production of cartilage oligomeric matrix protein (COMP) by cultured human dermal and synovial fibroblasts. Osteoarthritis and Cartilage 6(6):435–440.

Downs JT, Lane CL, Nestor NB, McLellan TJ, Kelly MA, Karam GA, Mezes PS, Pelletier JP, Otterness IG. 2001. Analysis of collagenase-cleavage of type II collagen using a neoepitope ELISA. Journal of Immunology Methods 247(1–2):25–34.

Elliott AL, Kraus VB, Luta G, Stabler T, Renner JB, Woodard J, Dragomir AD, Helmick CG, Hochberg MC, Jordan JM. 2005. Serum hyasluronan levels and radiographic knee and hip osteoarthritis in African Americans and Caucasians in the Johnston County Osteoarthritis Project. Arthritis and Rheumatism 52(1):105–111.

El-Maadawy SS, Wahl CL, Pidoux I, Ionescu M, Hellio Le Graverand-Gastineau MP, Poole AR. 2003. Induction of osteoarthritis by expression of an active human MMP-13 transgene in cartilage. Paper presented at the ACR/ARHP Annual Scientific Meeting, San Antonio, TX, USA. [abstract]

Eyre DR. 1991. The collagens of articular cartilage. Seminars in Arthritis and Rheumatism 21(3):2–11.

Fawthrop F, Yaqub R, Belcher C, Bayliss M, Ledingham J, Doherty M. 1997. Chondroitin and keratan sulphate epitopes, glycosaminoglycans, and hyaluronan in progressive versus non-progressive osteoarthritis. Annals in Rheumatism Disease 56(2):119–122.

Fell HB, Barratt ME. 1973. The role of soft connective tissue in the breakdown of pig articular cartilage cultivated in the presence of complement-sufficient antiserum to pig erythrocytes. I. Histological changes. International Archives in Allergy and Applied Immunology 44:441–468.

Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, Katz LM, Lightfoot Jr R, Paulus H, Strand V, Tugwell P, Weinblatt M, Williams J, Wolfe F, Kieszak S. 1995. Preliminary definition of improvement in rheumatoid arthritis. Arthritis and Rheumatism 38:727–735.

Felson DT, Neogi T. 2004. Osteoarthritis: is it a disease of cartilage or of bone? Arthritis and Rheumatism 50:341–344.

Fex E, Eberhardt K, Saxne T. 1993. Tissue-derived macromolecules and markers of inflammation in serum in early rheumatoid arthritis: relationship to development of joint destruction in hands and feet. British Journal of Rheumatology 36(11):1161–1165.

Flugge LA, Miller-Deist LA, Petillo PA. 1999. Towards a molecular understanding of arthritis. Chemistry and Biology 6:157–166.

Fosang AJ, Last K, Maciewicz RA. 1996. Aggrecan is degraded by matrix metalloproteinases in human arthritis. Journal of Clinical Investigation 98(10):2292–2299.

Fosang AJ, Stanton H, Little CB, Atley LM. 2003. Neoepitopes as biomarkers of cartilage catabolism. Inflammation Research 52:277–282.

Funderburgh JL, Caterson B, Conrad GW. 1987. Distribution of proteoglycans antigenically related to corneal keratan sulphate proteoglycan. Journal of Biology and Chemistry 262(24):11634–11640.

Garnero P, Bingham C, Aronstein W, Cohen S, Conaghan P, Cline G, Beary J, Meyer J. 2004. Treatment with risedronate reduced urinary CTX-II, a specific biochemical marker of cartilage type II collagen degradation in a 24 month study of knee OA. ACR Abstract Book: 656.

Garnero P, Christgau S, Delmas PD. 2001. The biphosphonate zoledronate decreases type II collagen breakdown in patients with Paget's disease of bone. Bone 28(5):461–464.

Garnero P, Delmas PD. 1996. New developments in biochemical markers for osteoporosis. Calciferous Tissue International 59(Suppl. 1):2–9.

Garnero P, Delmas PD. 2003. Biomarkers in osteoarthritis. Current Opinion in Rheumatology 15:641–646.

Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD. 2002. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis and Rheumatism 46(1):21–30.

Garnero P, Landewe R, Boers M, Verhoeven A, Van der Linden, Linden S, Christgau S, Van der Heijde D, Boonen A, Geusens P. 2002. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis: the COBRA study. Arthritis and Rheumatism 46(11): 2847–2856.

Garnero P, Landewe R, Van der Heijde D, Gotlieb L, Kupper H, Geusens P. 2004. Adalimumab monotherapy decreases urinary CTX-II, a specific molecular marker of cartilage type II collagen degradation in patients with active rheumatoid arthritis. American College of Rheumatology Conference 50:5567.

Garnero P, Piperno M, Gineyts E, Christgau S, Delmas PD, Vignon E. 2001. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee

osteoarthritis: relations with disease activity and joint damage. Annals of Rheumatology Disease 60(6):619–626.

Garnero P, Rousseau JC, Delmas PD. 2000. Molecular basis and clinical use of biochemical markers of bone, cartilage and synovium in joint diseases. Arthritis and Rheumatism 43(5):953–968.

Garnero P, Sornay-Rendu E, Arlot M, Christiansen C, Delmas PD. 2004. Association between spine disc degeneration and type II collagen degradation in postmenopausal women: the OFELY study. Arthritis and Rheumatism 50(10):3137–3144.

Gineyts E, Garnero P, Delmas PD. 2001. Urinary excretion of glucosyl-galactosyl pyridinoline: a specific biochemical marker of synovium degradation. Rheumatology 40:315–323.

Gineyts E, Mo JA, Ko A, Henriksen DB, Curtis SP, Gertz BJ, Garnero P, Delmas PD. 2004. Effects of ibuprofen on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis. Annals of Rheumatology Disease 63(7):857–861.

Goldring SR, Goldring MB. 1999. Rheumatoid arthritis and other inflammatory joint pathologies. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. Orlando, FL: Academic Press. p. 623–636.

Hakala BE, White C, Recklies AD. 1993. Human cartilage gp-39 a major secretory product of articular chondrocytes and synovial cells is a mammalian member of a chitinase protein family. Journal of Biology and Chemistry 293:781–788.

Hascall VC, Morales TI, Hascall GK, Handley CJ, McQuillan DJ. 1983. Biosynthesis and turnover of proteoglycans in organ culture of bovine articular cartilage. Journal of Rheumatology (Suppl. 11:45–52.

Hauselmann HJ, Hedbom E. 1999. In-vitro models of cartilage metabolism. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of bone and cartilage metabolism. Orlando, FL: Academic Press. p. 325–338.

Hayami T, Pickarski M, Wesolowski GA, McLane J, Bone A, Destefano J, Rodan GA, Le Duong T. 2004. The role of subchondral bone remodeling in osteoarthritis. Arthritis and Rheumatism 50(4):1193–1206.

Henrissat B, Bairoch A. 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochemistry Journal 293:781–788.

Henrotin YE, Deby-Dupont GP, Deby C, De Bruyn M, Lamy M, Franchimont P. 1993. Production of active oxygen species by isolated human chondrocytes. British Journal of Rheumatology 32:562–567.

Ishiguro N, Ito T, Obata K, Fujimoto N, Iwata H. 1996. Determination of stromelysin-1, 72 and 92 kDa type IV collegenase, tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2 in synovial fluid and serum from patients with rheumatoid arthritis. Journal of Rheumatology 23:1599–1604.

Ishikawa T, Nishigaki F, Christgau S, Noto T, Mo J, From N, Minoura K, Hirayama Y, Ohkubo Y, Mutoh S. 2004. Cartilage destruction in collagen induced arthritis assessed with a new biochemical marker for collagen type II C-telopeptide fragments. Journal of Rheumatology 31:1174–1179.

Johansen JS, Cintin C, Jorgensen M, Kamby C, Price PA. 1995. Serum YKL-40: a new potential marker of prognosis and location of metastases of patients with recurrent breast cancer. European Journal of Cancer 31(9):1437–1442.

Johansen JS, Jensen HS, Price PA. 1993. A new biochemical marker for joint injury. Analysis of YKL-40 in serum and synovial fluid. British Journal of Rheumatology 32(11):949–955.

Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. 1998. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Experimental Cell Research 238:265–272.

Jordan JM, Luta G, Stabler T, Renner JB, Dragomir AD, Vilim V, Hochberg MC, Helmick CG, Kraus VG. 2003. Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: the Johnston County Osteoarthritis Project. Arthritis and Rheumatism 48:675–681.

Jordan KM, Syddall HE, Garnero P, Gineyts E, Dennison E, Aihie Sayer A, Delmas PD, Cooper C, Arden NK. 2005. urinary CTX-II and glucosyl-galactosyl-pyridinoline are associated with the presence and severity of radiographic knee osteoarthritis in men. Annals of Rheumatism Disease [Epub ahead of print].

Jung M, Christgau S, Lukoschek M, Henriksen D, Richter W. 2004. Increased urinary concentration of collagen type II C-telopeptide fragments in patients with osteoarthritis. Pathobiology 71(2):70–76.

Karsdal MA, Larsen L, Engsig MT, Lou H, Ferreras M, Lochter A, Delaisse JM, Foged NT. 2002. Matrix metalloproteinase-dependent activation of latent transforming growth factor-beta controls the conversion of osteoblasts into osteocytes by blocking osteoblast apoptosis. Journal of Biology and Chemistry 277:44061–44067.

Keyszer G, Lambirini I, Nagel R, Keysser C, Keysser M, Gromnica-Ihle E. 1999. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1) and MMP-1/TIMP-1 complex in rheumatic disease: correlation with clinical activity in rheumatoid arthritis versus other surrogate markers. Journal of Rheumatology 26:251–258.

Kiani C, Chen L, Wu YJ, Yee AJ, Yang BB. 2002. Structure and function of aggrecan. Cell Research 12(1):19–32.

Kirkpatrick RB, Emery JG, Connor JR, Dodds R, Lysko PG, Rosenberg M. 1997. Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. Experimental Cell Research 237(1):46–54.

Knudson CB, Knudson W. 2001. Cartilage proteoglycans. Cell and Developmental Biology 12:69–79.

Kojima T, Mwale F, Yasuda T, Girard C, Poole AR, Laverty S. 2001. Early degradation of type IX and type II collagen with the onset of experimental inflammatory arthritis. Arthritis and Rheumatism 44(1):120–127.

Kongtawelert P, Ghosh P. 1990. A new sandwich-ELISA method for the determination of keratan sulphate peptides in biological fluids employing a monoclonal antibody and labelled avidin biotin technique. Clinica et Chimica Acta 195(1–2):17–26.

Konttinen YT, Ainola M, Valleala H, Ma J, Ida H, Mandelin J, Kinne RW, Santavirta S, Sorsa T, Lopez-Otin C, Takagi M. 1999. Analysis of 16 different matrix metalloproteinases (MMP-1 to MMP-20) in the synovial membrane: different profiles in trauma and rheumatoid arthritis. Annals of Rheumatology Disease 58(11):691–697.

Koshy PJ, Henderson N, Logan C, Life PF, Cawston TE, Rowan AD. 2002. Interleukin 17 induces cartilage collagen breakdown: novel synergistic effects in combination with proinflammatory cytokines. Annals of Rheumatology Disease 61:704–713.

Landewe RB, Boers M, Verhoeven AC, Westhovens R, Van De Laar MA, Markusse HM, Van Denderen JC, Weststedt ML, Peeters AJ, Dijkmans BA, Jacobs P, Boonen A, Van der Heijde DM, Van der Linden S. 2002. COBRA combination therapy in patients with early rheumatoid arthritis: long-term structural benefits of a brief intervention. Arthritis and Rheumatism 46(2):347–356.

Landewe R, Geusens P, Boers M, Van der Heijde D, Lems W, Te Koppelle J, Van der Linden S, Garnero P. 2004. Markers for type II collagen breakdown predict the effect of disease-modifying treatment on long-term radiographic progression in patients with rheumatoid arthritis. Arthritis and Rheumatism 50(5):1390–1399.

Lee DA, Reisler T, Bader DL. 2003. Expansion of chondrocytes for tissue engineering in alginate beads enhances chondrocytic phenotype compared with conventional monolayer techniques. Acta Orthopedica Scandinavica 74:6–15.

Leff RL, Elias I, Ionescu M, Reiner A, Poole AR. 2003. Molecular changes in human osteoarthritic cartilage after 3 weeks of oral administration of BAY 12-9566, a matrix metalloproteinase inhibitor. Journal of Rheumatology 30(3):544–549.

Lemons ML, Sandy JD, Anderson DK, Howland DR. 2001. Intact aggrecan and fragments generated by both aggrecanse and metalloproteinase-like activities are present in the developing and adult rat spinal cord and their relative abundance is altered by injury. Journal of Neurosciences 21(13):4772–4781.

Lesko LL, Atkinson AJ. Jr. 2001. Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. Annual Review in Pharmacology and Toxicology 41:347–366.

Loeser RF, Carlson CS, Del Carlo M, Cole A. 2002. Detection of nitrotyrosine in aging and osteoarthritic cartilage: correlation of oxidative damage with the presence of interleukin-1 beta and with chondrocyte resistance to insulin-like growth factor 1. Arthritis and Rheumatism 46:2349–2357.

Lohmander LS, Hoerrner LA, Lark MW. 1993. Metalloproteinases tissue inhibitor and proteoglycan fragments in knee synovial fluid in human osteoarthritis. Arthritis and Rheumatism 36:181–189.

Lohmander LS, Yoshihara Y, Roos, Kobayashi T, Yamada H, Shinmei M. 1996. Procollagen II C-propeptide in joint fluid: changes in concentration with age, time after knee injury, and osteoarthritis. Journal of Rheumatology 23(10):1765–1769.

Lunstrum GP, Keene DR, Weksler NB, Cho YJ, Cornwall M, Horton WA. 1999. Chondrocyte differentiation in a rat mesenchymal cell line. Journal of Histochemistry and Cytochemistry 47:1–6.

Majeed M, McQueen F, Yeoman S, McLean L. 2004. Relationship between serum hyaluronic acid level and disease activity in early rheumatoid arthritis. Annals of Rheumatology Disease 63(9):1166–1168.

Manicourt DH, Poilvache P, Nzeuesseu A, Van Egeren A, Devogelaer JP, Lenz ME. 1999. Serum levels of hyaluronan, antigenic keratan sulfate, matrix metalloproteinase 3, and tissue inhibitor of metalloproteinases 1 change predictability in rheumatoid arthritis patients who have begun activity after a night of bed rest. Arthritis and Rheumatism 42:1861–1869.

Manning WK, Bonner WM. Jr. 1967. Isolation and culture of chondrocytes from human adult articular cartilage. Arthritis and Rheumatism 10:235–239.

Månsson B, Carey D, Alini M, Ionescu M, Rosenberg LC, Poole AR, Heinegård D, Saxne T. 1995. Cartilage and bone metabolism in rheumatoid arthritis. Journal of Clinical Investigation 95:1071–1077.

Martin JA, Buckwalter JA. 2002. Aging, articular cartilage chondrocyte senescence and osteoarthritis. Biogerontology 3:257–264.

Mehmet H, Scudder P, Tang PW, Hounsell EF, Caterson B, Feizi T. 1986. The antigenic determinants recognized by three monoclonal antibodies to keratan sulphate involve sulphated hepta- or larger oligosaccharides of the poly(N-acetyllactosamine) series. European Journal of Biochemistry 157(2):385–391.

Meulenbelt I, Kloppenburg M, Kroon HM, Houwing-Duistermaat JJ, Garnero P, Hellio Le Graverand MP, DeGroot J, Slagboom PE. 2006. Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis in hip, knee, hand and facet joints in subject with familial osteoarthritis at multiple sites: the GARP study. Annals of Rheumatology Disease 65:360–365.

Moller HJ, Larsen FS, Ingemann-Hansen T, Poulsen JH. 1994. ELISA for the core protein of the cartilage large aggregating proteoglycan, aggrecan: comparison with the concentrations of immunogenic keratan sulphate in synovial fluid, serum and urine. Clinica et Chimica Acta 225(1):43–55.

Mouritzen U, Christgau S, Lehmann HJ, Tanko LB, Christiansen C. 2003. Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index. Annals of Rheumatology Disease 62:332–336.

Muller G, Michel A, Altenburg E. 1998. COMP (cartilage oligomeric matrix protein) is synthesized in ligament, tendon, meniscus, and articular cartilage. Connective Tissue Research 39(4):233–244.

Nelson F, Dahlberg L, Laverty S, Reiner A, Pidoux I, Ionescu M, Fraser GL, Brooks E, Tanzer M, Rosenberg LC, Dieppe P, Poole RA. 1998. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. Journal of Clinical Investigation 102(12):2115–2125.

Oestergaard S, Chouinard L, Doyle N, Smith SS, Tanko LB, Qvist P. 2006. Early elevation in circulating levels of C-telopeptides of type II collagen predicts structural damage in articular cartilage in rodent model of collagen induced arthritis (CIA). Arthritis and Rheumatism (submitted).

Oldberg A, Antonsson P, Lindblom K, Heinegard D. 1992. COMP (cartilage oligomeric matrix protein) is structurally related to the thrombospondins. Journal of Biology and Chemistry 267(31):22346–22350.

Ory PA. 2003. Interpreting radiographic data in rheumatoid arthritis. Annals of Rheumatology Disease 62(7):597–604.

Paik DC, Dillon J, Galicia E, Tilson MD. 2001. The nitrite/collagen reaction: non-enzymatic nitration as a model system for age related damage. Connective Tissue Research 42:111–122.

Pavelka K, Forejtova S, Olejarova M, Gatterova J, Senolt L, Spacek P, Braun M, Hulejova M, Stovickova J, Pavelkova A. 2004. Hyaluronic acid levels may have predictive value for the progression of knee osteoarthritis. Osteoarthritis and Cartilage 12(4):277–283.

Perrimon N, Bernfield M. 2000. Cellular functions of proteoglycans — an overview. Cell and Developmental Biology 12:65–67.

Poole AR, Dieppe PA. 1994. Biological markers in rheumatoid arthritis. Seminars in Arthritis and Rheumatism 23(6):17–31.

Poole AR, Ionescu M, Swan A, Dieppe PA. 1994. Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan. Implications for pathogenesis. Journal of Clinical Investigation 94(1):25–33.

Posthumus MD, Limburg PC, Westra J, Van Leeuwen MA, Van Rijswijk MH. 2003. Serum matrix metalloproteinase 3 levels in comparison to C-reactive protein in periods with and without progression of radiological damage in patients with early rheumatoid arthritis. Clinical and Experimental Rheumatology 21(4):465–472.

Qvist P, Christgau S, Pedersen BJ, Schlemmer A. 2002. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (Serum CTX): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. Bone 31:57–61.

Recklies AD, Baillargeon L, White C. 1998. Regulation of cartilage oligomeric matrix protein synthesis in human synovial cells and articular chondrocytes. Arthritis and Rheumatism 41(6):997–1006.

Reijman M, Hazes JM, Bierma-Zeinstra SM, Koes BW, Christgau S, Christiansen C, Uitterlinden AG, Pols HA. 2004. A new marker for osteoarthritis: cross-sectional and longitudinal approach. Arthritis and Rheumatism 50(8):2471–2478.

Rizkalla G, Reiner A, Bogoch E, Poole AR. 1992. Studies of the articular cartilage proteoglycan aggrecan in health and osteoarthritis. Evidence for molecular heterogeneity and extensive molecular changes in disease. Journal of Clinical Investigation 90(6):2268–2277.

Rolan P. 1997. The contribution of clinical pharmacology surrogates and models to drug development — a critical appraisal. British Journal of Clinical Pharmacology 44:219–225.

Rosenberg K, Olsson H, Morgelin M, Heinegard D. 1998. Cartilage oligomeric matrix protein shows high affinity zinc-dependent interaction with triple helical collagen. Journal of Biology and Chemistry 273(32):20397–20403.

Rousseau JC, Zhu Y, Miossec P, Vignon E, Sandell LJ, Garnero P, Delmas PD. 2004. Serum levels of type IIA procollagen amino terminal propeptide (PIIANP) are decreased in patients with knee osteoarthritis and rheumatoid arthritis. Osteoarthritis and Cartilage 12(6):440–447.

Roy-Beaudry M, Martel-Pelletier J, Pelletier JP, M'Barek KN, Christgau S, Shipkolye F, Moldovan F. 2003. Endothelin 1 promotes osteoarthritic cartilage degradation via matrix metalloproteinase 1 and matrix metalloprotease 13 induction. Arthritis and Rheumatism 48:2855–2864.

Saklatvala J. 1986. Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. Nature 322:547–549.

Schorle CM, Finger F, Zien A, Block JA, Gebhard PM, Aigher T. 2005. Phenotypic characterization of chondrosarcoma-derived cell lines. Cancer Letters 226(2):143–154.

Scott DL, Houssien DA. 1996. Clinical and laboratory assessments in rheumatoid arthritis and osteoarthritis. British Journal of Rheumatology 35(3):6–9.

Seibel MJ. 2005. Biochemical markers of bone turnover: part I: biochemistry and variability. Clinical Biochemistry Reviews 26(4):97–122.

Sharif M, Kirwan JR, Elson CJ, Granell R, Clarke S. 2004. Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years. Arthritis and Rheumatism 50(8):2479–2488.

Sharif M, Saxne T, Shepstone L, Kirwan JR, Elaon CJ, Heinegaard D, Dieppe PA. 1995. Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. British Journal of Rheumatology 34(4):306–310.

Shingleton WD. 2003. In vitro model of human articular cartilage degradation. Methods in Molecular Biology 225:99–106.

Skoumal M, Kolarz G, Klingler A. 2003. Serum levels of cartilage oligomeric matrix protein. A predicting factor and a valuable parameter for disease management in rheumatoid arthritis. Scandinavian Journal of Rheumatology 32:156–161.

Song X, Zeng L, Jin W, Thompson J, Mizel DE, Lei K, Billinghurst RC, Poole AR, Wahl SM. 1999. Secretory leukocyte protease inhibitor suppresses the inflammation and joint damage of bacterial cell wall-induced arthritis. Journal of Experimental Medicine 190:535–542.

Sugiyama S, Itokazu M, Shimizu K. 2003. Procollagen II C propeptide level in the synovial fluid as a predictor of radiographic progression in early knee osteoarthritis. Annals of Rheumatology Disease 62:27–32.

Takigawan M, Okawa T, Pan H, Aoki C, Takahashi K, Zue J, Suzuki F, Kinoshita A. 1997. Insulin-like growth factors I and II are autocrine factors in stimulating proteoglycan synthesis a marker of differentiated chondrocytes acting through their respective receptors on a clonal human chondrosarcoma-derived chondrocyte cell line HCS-2/8. Endocrinology 138:4390–4400.

Van der Vliet A, Eiserich JP, O'Neill CA, Halliwell B. 1995. Tyrosine modification by reactive nitrogen species: a closer look. Archives of Biochemistry and Biophysics 319(2):341–349.

Verstappen SM. M, Poole AR, Ionescu M, King LE, Abrahamowicz M, Hofman DM, Bijlsma JW. J, Lafeber F. 2006. Radiographic joint damage in rheumatoid arthritis is associated with differences in cartilage turnover and can be predicted by serum biomarkers: an evaluation from 1 to 4 years after diagnosis. Arthritis Research and Therapy 8(1):R31.

Vilim V, Olejerova M, Machachek S, Gatterova J, Kraus VB, Pavelka K. 2002. Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. Osteoarthritis and Cartilage 10(9):707–713.

Volck B, Price PA, Johansen JS, Sorensen O, Benfeld TL, Nielsen HJ, Calafat J, Borregaard N. 1998. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. Proceedings of the Association of American Physicians 110(4):351–360.

Walakovits LA, Moore VL, Bhardwaj N, Gallick GS, Lark MW. 1991. Detection of high levels of stromelysin and collagenase in synovial fluid from patients with rheumatoid arthritis and post-traumatic knee injury. Arthritis and Rheumatism 35:35–42.

Wislowska M, Jablonska B. 2005. Serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis and knee osteoarthritis. Clinical Rheumatology 24(3):278–284.

Wollheim F. 2000. Markers of disease in rheumatoid arthritis. Current Opinion in Rheumatology 12(3):200–204.

Yoo JU, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM, Johnstone B. 1998. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. Journal of Bone and Joint Surgery A 80:1745–1757.