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REVIEW ARTICLE

Application of biochemical markers in development of drugs for treatment of osteoarthritis

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Abstract

Osteoarthritis is a chronic disease for which no efficacious medical intervention is yet available. Recent disappointments in late-stage clinical development of disease-modifying osteoarthritic drugs (DMOADs) have precipitated efforts in biomarker discovery aimed at developing an analytical tool box with the potential to improve the clinical development process. In this review, we seek to provide an overview of the biochemical marker repertoire currently available with a special focus on data originating from their application in clinical development programmes. Finally, we discuss possible directions in future biomarker research.

Keywords: *Joint diseases; osteoarthritis; biochemical markers*

Introduction

The road to the identification and the clinical validation of both efficacy and safety of the chondroprotective drug is paved with numerous obstacles. Over the last few years the disappointments associated with efforts to develop a disease-modifying osteoarthritis drug (DMOAD) have been numerous and still today the millions of patients suffering from this serious, chronic disease can only be offered treatments targeting signs and symptoms.

This unmet medical need has been the primary driver behind major efforts to provide the pharmaceutical industry with improved analytical techniques allowing better and more efficient clinical trial design and implementation (Krasnokutsky et al. 2007). One aspect of this work includes the search for reliable biomarkers applicable in the drug development process.

The clinically relevant end points in osteoarthritis (OA) trials are sign and symptoms (Abadie et al. 2004), and also time to joint replacement have been used; however, neither of these end points are easy to use in the early and mid-phases of clinical drug development programmes, and this dilemma has nourished efforts to develop biomarkers with higher sensitivity and specificity to reflect the degenerative processes associated with OA progression.

In contrast to X-rays, magnetic resonance imaging (MRI) allows direct visualization of the joint including more or less accurate assessment of cartilage (Pessis et al. 2003) and cartilage measurements from MRI have been shown to be more associated with OA symptoms than joint space narrowing (JSN) (Wluka et al. 2004) and to be predictive of knee arthroplasty (Cicuttini et al. 2004).

In parallel, and also as a consequence of the efforts to identify DMOADs, a series of biochemical markers has been developed over the past few years and a majority of these are related to the turnover of the tissue in the joints, i.e. the bone, the cartilage and the synovium.

In this report, we aim to provide a review of biochemical markers currently applied in OA drug development programmes. In particular, we investigate the application of these analytical markers in clinical studies as these have been reported in the last few years and we seek to provide insight into the markers emerging in the horizon.

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Rationale for application of biochemical markers in OA drug development

A key finding in osteoarthritis is the progressive damage of joint tissue, including cartilage erosion, osteophyte formation, subchondral sclerosis and synovial alterations (Krasnokutsky et al. 2007). Each of these pathological findings and their underlying metabolic processes could in principle serve as targets for biochemical assessment of the joint, and over the past decade a large number of biochemical tests have become available for this purpose. However, before addressing each of these biochemical markers, a few important aspects relating to their application should be considered.

BIPED classification

Biomarkers, including the biochemical markers, may have many applications in the study of OA as well as in the management of the patients. However, appropriate use of the markers is based on compliance to a set of basic requirements and recently the Osteoarthritis Biomarkers Network funded by the National Institutes of Health/National Institute of Arthritis, Musculoskeletal, and Skin Disease (NIH/NIAMS) proposed a classification scheme for biomarkers termed BIPED, an acronym for Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic (Bauer et al. 2006). The objective of the BIPED classification system is to provide specific biomarker definitions for improving development capabilities and analysis of OA biomarkers and of communicating advances within a common framework. Briefly, the five categories are characterized by the following key features:

1. Burden of disease: burden-of-disease markers assess the severity or extent of disease, e.g. severity within a single joint and/or number of joints affected.
2. Investigative: an investigative marker with insufficient information to allow inclusion into one of the existing biomarker categories. The investigative category includes markers for which a relationship to various normal and abnormal parameters of cartilage extracellular matrix turnover has not yet been established in human subjects.
3. Prognostic: the key feature of a prognostic marker is the ability to predict the future onset of OA among persons without OA at baseline or the progression of OA among those with the disease.
4. Efficacy of intervention: an efficacy-of-intervention biomarker provides information about the efficacy of treatment among persons with OA or those at high risk for development of OA.
5. Diagnostic: diagnostic markers are defined by the ability to classify individuals as either having or not having a disease.

The inter-relationship of these distinct categories of biomarkers is depicted in Figure 1. Obviously, a single biomarker can encompass features from more than one category to the extent that the associated requirements are met by the marker and this has been established by appropriate validation. Likewise, the model does not require any OA biomarker to be applicable in all aspects of diagnosing and monitoring. While genetic markers can assist in the prognosis of OA (risk assessment) and prediction of treatment sensitivity they will have little if any potential in the monitoring of treatment efficacy. The model in Figure 1 also suggests that treatment could be considered in subjects without the classical OA diagnosis but with one or more risk factors for disease, accumulating in a poor prognosis.

Burden of disease (BIPED no. 1)

The classification as a burden of disease marker is generally based on investigations of sensitivity and specificity (and derivatives of these), i.e. by comparison of test results in a study population to a generally accepted 'gold standard'.

Clark and co-workers (Clark et al. 1999) reported elevated circulating levels of cartilage oligomeric protein (COMP) in OA patients compared with age- and gender-matched controls. In addition, they found increasing levels with increasing knee K/L grade as well as with number of knee and hip joints involved. In other studies, it has been reported that the total radiographic score in patients with OA at multiple sites (knee, hip, hand, vertebral facet joints, spinal disc degeneration) was significantly associated with the urinary fragments of the C-telopeptide of type II collagen (CTX-II) (Meulenbelt et al. 2006). In addition they found that all the site specific radiological OA (ROA) scores, except for spinal disc degeneration, contributed independently to this association. In other studies CTX-II has been associated with disease severity, i.e. K/L grade, JSN and osteophyte scores (Jordan et al. 2006), with total radiographic scores in the GARP study (Meulenbelt et al. 2006), and was reported to be the marker showing the most consistent association with signs and symptoms of disease severity among ten bone, cartilage and synovium markers (Garnero et al. 2005a).

Investigative (BIPED no. 2)

Markers, for which scientific evidence for their classification into the other categories are still insufficient, are designated investigative.

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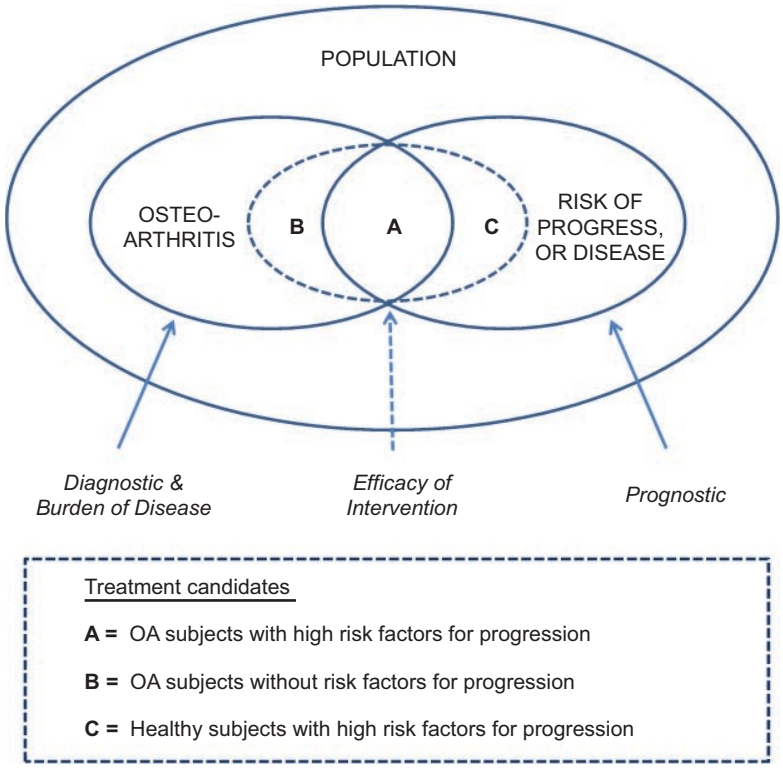

Figure 1. Biomarkers can be classified as markers of 'burden of disease', investigative', 'prognostic', 'efficacy of intervention' or 'diagnostic', i.e. BIPED (Bauer et al. 2006). This figure provides a schematic representation of the inter-relationship of these distinct categories of biomarkers as this relates to osteoarthritis (OA). Markers able to classify individuals (alone or in combination) as either having OA or not having OA are defined as diagnostic. Burden of disease markers will be able to provide information as to the severity or extent of the disease among individuals with OA. Some of the OA patients will present with one or more of a set of risk factors for OA progression and these can be assessed with the prognostic biomarkers. These can also predict the future onset of OA among individuals without current OA. Candidates for treatment can be anyone with OA or with a high risk of developing OA, and markers of efficacy of intervention are able to determine treatment response. The appropriate application of the final group of markers, i.e. the investigative biomarkers, has yet to be determined.

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Prognostic (BIPED no. 3)

Even though cartilage cannot be visualized on X-rays, plain radiographs are widely used for assessment of structural damage in joint diseases, which is an important element in several OA scoring systems, e.g. Kellgren and Lawrence index (Kellgren & Lawrence 1957) and Whole-Organ Magnetic Resonance Imaging Score (WORMS) (Peterfy et al. 2004). However, cartilage damage that is detectable on radiographs is already considered irreversible joint damage (Reijman et al. 2004) and from both a clinical research as well as an individual patient point of view it would be desirable to have techniques available that had sufficient sensitivity to identify healthy or very early-stage individuals with an increased risk of developing disease. Recently, Reijman and coworkers performed a longitudinal, population-based study in 1235 men and women, and found that elevated urinary excretion of type II collagen fragments (CTX-II) was associated an 8.4-fold increased risk of radiographic progression of OA in the hip (Reijman et al. 2004). Similar studies have associated other biochemical markers with risk of progression of structural joint damage in OA, e.g. COMP (Hunter et al. 2007) and serum hyaluronan (Mazieres et al. 2006). These and other studies suggest that biochemical markers of the extracellular matrix (ECM) could potentially assist in the detection of individuals with an elevated risk of accelerated cartilage loss eventually expressed in structural damage as detected by radiographs and/or MRI. At least some studies suggest that these individuals can be detected prior to any radiographic evidence of joint disease, and this offers the opportunity to include study participants in clinical trials at a very early stage of disease development, and, very importantly, individuals with a relatively high risk of progression of disease.

Recent studies have demonstrated that the classical inclusion criteria for intervention trials in OA, i.e. the American College of Rheumatology (ACR) criteria, often recruit subjects with a relatively low risk of progression. In the 2-year, multinational study of risedronate in knee OA structural arthritis (KOSTAR) (Bingham et al. 2006) only 13% of those receiving placebo had structural progression over the 2 years. And in a recent phase III study of a matrix metalloproteinase inhibitor, the placebo group

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had a change in joint space width (JSW) from 3.386 to 3.252 over 12 months (Krzeski et al. 2007), which is consistent with an annual decrease in JSN of 0.13 ± 0.15 mm found in a meta-regression analysis covering 34 studies (Emrani et al. 2008). In both intervention studies the trial drugs failed to meet the primary end points, i.e. no significant beneficial effect over the placebo group.

Efficacy of intervention (BIPED no. 4)

Presently, no medical intervention is available which will arrest the pathological processes underlying the destruction of joint tissue in OA, i.e. the disease-modifying osteoarthritis drug (DMOAD). Therefore the benefit from applying biochemical markers in this important clinical situation can only be speculated, and at present the regulatory agencies require improvement in clinical outcomes for approval of DMOADs (Abadie et al. 2004). In fact it has even been speculated that possible DMOADs might have been missed or not proven because of inadequate biomarker tools to track progression of OA (Samuels et al. 2008).

The use of radiographs for monitoring the structural impact of medical intervention is associated with some difficulties. The rate of change, which can be detected by X-rays, e.g. JSN, is modest. In general, the annual change is in the range 0.13 ± 0.15 mm (Emrani et al. 2008), which is similar to the precision error of the technique. This unfavourable signal-to-noise ratio calls for a large number of study subjects for a long period of time in clinical trials of potential chondroprotective drugs. We know from associated medical areas, e.g. osteoporosis, that biochemical markers are much more dynamic (than bone mass measurements) and partly because of this carry significant potential for monitoring medical intervention. In OA, biochemical markers have also been investigated (see later section), but a few studies should be addressed here. In the knee OA structural arthritis (KOSTAR) study, all doses of risedronate failed to improve above placebo, signs and symptoms measured by the Western Ontario and McMaster Universities (WOMAC) osteoarthritis index and did not slow radiographic progression (Bingham et al. 2006). Interestingly, in a subanalysis, it was demonstrated that in subjects with accelerated cartilage degradation at baseline (quantitatively assessed by urinary CTX-II), a biochemical response after 6 months of risedronate use was associated with a significant reduction in radiological progression compared with subjects with no biochemical response (odds ratio (OR) 0.57; 95% confidence interval (CI) 0.39–0.85) (Garnero et al. 2008a). In a later section, application of markers in clinical trials will be reviewed, but here reference is made to recent reviews on the potential impact of new and better biomarkers on the development of DMOADs (Qvist et al. 2008, Krasnokutsky et al. 2007, Schaller et al. 2005, Sumer et al. 2006).

Diagnostic (BIPED no. 5)

Usually osteoarthritis is localized to a single or a few joints, and even the damage in the affected joints can be condensed to just small, focal lesions, and therefore the sensitivity of the markers to reflect the pathological changes has been questioned. However, a few studies address the capability for systemic detection of localized joint damage. In a rodent model of traumatic OA it was demonstrated that 2 weeks after anterior cruciate ligament transsection in a single joint in the rat, the urinary excretion of CTX-II was elevated by 15.7% ($p < 0.05$) compared with sham operations (Hayami et al. 2004, Hayami et al. 2006). In humans the diagnostic marker will have to be compared with a well-defined and accepted diagnostic definition, and in OA this could be the ACR criteria. Taking the complexity of these requirements into consideration, it is generally reported that a single biochemical test relatively poorly distinguishes individuals with OA from healthy controls. Alternatively, the test under investigation can be evaluated against a 'gold standard' method, and in OA, a radiograph is generally accepted, with a Kellgren–Lawrence score of 2 or more required for assigning the OA diagnosis.

Biochemical markers of joint tissue

As a consequence of the aim to develop DMOADs, the diagnostic industry as well as academic research has been focused on the development of biochemical markers reflecting the turnover, i.e. formation and degradation, of the constituents of the ECM. One of the major obstacles associated with this development approach is the fact that many of the molecular components in the joint are not specific for the joint tissue, but can be found elsewhere in the body.

Type I collagen is the most prevalent organic molecule in bone; however, it can be found in most other connective tissues throughout the body, and therefore tissue specificity has to be attributed by other means. The test for fragments of the C-telopeptide of type I collagen (CTX-I) (Rosenquist et al. 1998) uses monoclonal antibodies recognizing the C-telopeptide of type I collagen, however only after it has been proteolytically cleaved by cathepsin K, which is produced by the osteoclast. The antibodies recognize the amino acid sequence EKAHDGGR with a free C-terminal arginine, i.e. they recognize a neo-epitope lacking in the uncleaved molecule. The test has been used for determining the concentration of type I collagen fragments originating from osteoclast-mediated bone resorption, and it has been reported to be associated with loss of bone mineral density (BMD) (Bjarnason & Christiansen 2000) and increased risk of osteoporotic fractures (Garnero et al. 2000b). In contrast, the biochemical marker cross-linked carboxyterminal

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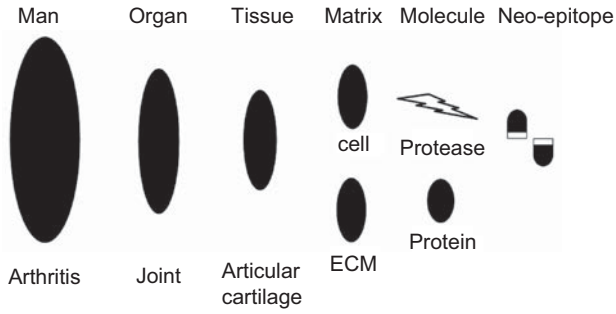

Figure 2. Visualization of the generation of pathology-relevant neo-epitopes. In osteoarthritis (OA), the organs of interest are the joints, and part of the pathology is expressed in the articular cartilage. This tissue contains, among other things, the chondrocytes and the extracellular matrix. The chondrocytes express proteases, in particular the matrix metalloproteinases and aggrecanases. The most abundant cartilage proteins are collagen type II and aggrecan. Protease-generated fragments of collagen type II and aggrecan produced through the action of these important enzymes, which may be relevant molecules in tissue destruction, can be used to monitor tissue turnover. These fragments, such as C-terminal telopeptide of type II collagen, may be used in clinical settings, in preclinical models and in simple *ex vivo* and *in vitro* systems. Therefore, this and other neo-epitopes are candidates for application in translational science.

telopeptide of type I collagen (ICTP) uses polyclonal antibodies to the C-telopeptide of type I collagen and these antibodies recognize an epitope which is destroyed by cathepsin K (Sassi et al. 2000). Consequently, CTX-I and ICTP, while both detecting peptide fragments originating from the C-terminal telopeptide of type I collagen, reflect different collagenolytic pathways (Garnero et al. 2003). ICTP has recently been applied in studies of multiple melanoma and bone metastasis (Lein et al. 2009, Koopmans et al. 2007, Voorzanger-Rousselot et al. 2006, Leeming et al. 2006).

This illustrates, that careful consideration should be given to the inter-relationship between the constituents of the matrix and the sensitivity to the proteases in their proximity. It should be possible to take advantage of this molecular interaction in the design of new biomarkers, which by virtue of their amino acid sequence and subsequent post-translational modifications, including proteolytic cleavage, carry sufficient information to allow its detection to be an accurate reflection of important pathological metabolic processes in the target tissue. Figure 2 is a schematic representation of the association between the systemic detection of the neo-epitopes and the pathogenesis in the target tissue. The action of enzymes on ECM components results in matrix degradation fragments, i.e. the neo-epitopes. The most abundant molecules in the cartilage ECM are collagen type II and aggrecan. These proteins are sequentially degraded when cartilage erosion occurs. In OA, the tissue of interest is the articular cartilage (Figure 2). The enzymes presently receiving the most attention are the matrix metalloproteinases (MMPs) and aggrecanases (ADAM-TS). Protease-generated fragments of collagen type II and aggrecan produced by these important enzymes are potential molecular targets for biomarker discovery. The study of biomarkers in OA has attracted much attention, and the field has been the subject of several reviews over the past few years (Henrotin et al. 2007, Rousseau & Delmas 2007, Sumer et al. 2006, Abramson & Krasnokutsky 2006, Garnero 2000a, 2006a, b, Kraus 2006, Schaller et al. 2005).

Another aspect which needs careful consideration upon interpretation of biochemical data is the possibility of interfering factors, e.g. preanalytical factors and confounding factors (an extra variable that correlates with both the dependent and the independent variable in a statistical model). Examples of preanalytical factors could be food intake (Qvist et al. 2002) and diurnal variation (Kong et al. 2006, Quintana et al. 2008), and many of these markers reflecting turnover of connective tissue components are influenced by body mass index (BMI), age, gender, ethnicity, etc. (Jordan et al. 2003). To control for these confounding factors the inclusion of appropriate control specimens/subjects are required in scientific studies. The quality of these controls will determine the validity of the conclusions made.

The joint has three major compartments, i.e. the bone, the articular cartilage and the synovium, and all three are affected by the disease (Samuels et al. 2008), which manifest as osteophyte formation, subchondral sclerosis, articular cartilage breakdown and alterations of the synovium.

Cartilage

A central hallmark in OA pathogenicity is a gradual destruction of articular cartilage and local denudation leading to loss of joint function (Fosang et al. 2003). The turnover of cartilage is normally maintained by a balance between catabolic and anabolic processes; however, in the case of pathological matrix destruction, the rate of cartilage degradation exceeds the rate of formation, resulting in a net loss of cartilage matrix (Zhen et al. 2008).

Cartilage is a non-vascularized tissue consisting of chondrocytes (Archer & Francis-West 2003) and ECM. The ECM is a composite network of proteins, primarily collagen type II, but also type IX and XI are present in minor amounts (Garnero et al. 2000a), interacting with polysaccharides and proteoglycans. Aggrecan is the predominant proteoglycan in cartilage, and less abundant proteoglycans include decorin, fibromodulin, perlican and biglycan. Other important molecules in articular cartilage include COMP (Saxne & Heinegard 1992), link protein (Ryu et al. 1982) and hyaluronan (or hyaluronic acid (HA)).

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The ECM builds the structural integrity of the joint and serves to protect the chondrocytes from being destroyed by the mechanical load. In addition, the articular cartilage allows smooth, pain-free movement of the skeletal surfaces.

Chondrocytes can respond to mechanical stimulation through receptors, which are also receptors for components of the ECM (Millward-Sadler & Salter 2004) thereby activating a number of intracellular cascades affecting tissue remodelling, partly through direct or indirect upregulation of MMP expression (Pulai et al. 2005, Xu et al. 2007). In particular, the MMPs and the aggrecanases are considered important for degradation of articular cartilage (Zhen et al. 2008, Bondeson et al. 2008, Gendron et al. 2007, Sandy 2006, Nagase & Kashiwagi 2003), but also the cysteine proteases have been associated with degradation of the cartilage matrix (Dejica et al. 2008, Salminen-Mankonen et al. 2007).

As cartilage is a non-vascularized tissue, partly degraded constituents of the ECM have to diffuse through the ECM before it is released into the synovium cavity and will then have to pass the synovial membrane before entering the bloodstream through the lymphatic vessel. Eventually, some of these molecules are secreted into urine. In these body fluids they can serve as biological markers of the metabolic events occurring in the diseased joint(s).

Selected investigations are referenced for each biomarker in Table 1.

Bone

Apart from the articular cartilage, which has attracted much attention in the study of OA pathogenesis, an increasing body of evidence suggest that healthy subchondral bone turnover is a prerequisite for preservation of the structural integrity of the articular cartilage (for recent reviews, please refer to Goldring 2009, Karsdal et al. 2008, Felson & Neogi 2004).

The proliferative abnormalities observed in the skeletal compartment in OA encompass not only the bony sclerosis underneath the eroded cartilage (subchondral sclerosis) and osteophytes, but also ossification at the ligaments and the joint capsule is observed (Felson 2004). During the development of OA, the subchondral cortical and trabecular bone architecture and properties are modified by cellular processes involving both osteoclasts and osteoblasts (Goldring 2009).

These macroscopic alterations, even though being localized by nature, will eventually lead to changes in (systemic) biochemical parameters, and these have been reported in several studies (Hunter & Spector 2003). It should be appreciated, that the skeletal changes occurring during the development of OA are mediated by the same cellular and biochemical mechanisms operating during physiological bone remodelling (Goldring 2009), and therefore many of the biochemical markers used in the study of (generalized) bone turnover can be adapted to the study of skeletal involvement in OA as well. In general, however, bone markers have not been strongly associated with disease progression in OA, and in a recent report Hunter and co-workers conclude that even the association of these with bone marrow lesions is weak (Hunter et al. 2008).

Markers of bone formation include osteocalcin, bone alkaline phosphatases, and the propeptides of type I collagen (N-terminal propeptide of type I procollagen (PINP), C-terminal propeptide of type I procollagen (PICP)), and degradation markers include primarily various fragments of type I collagen (CTX-I, N-terminal telopeptide of type I collagen (NTX-I), ICTP).

Synovium

The vast majority of biomarker research has been focused on the two other compartments, i.e. the bone and the articular cartilage, but recent data suggest that synovial involvement, namely inflammation and proliferation, is a key component of OA (Samuels et al. 2008).

The synovium consists of the intima, which is a layer of cells (mostly macrophages and specialized fibroblasts), a superficial microvasculature net and the subintima, which contains numerous lymphatic vessels draining liquid from the synovial cavity (Garnero et al. 2000a). The ECM of the subintima consists of type I and III collagen, which to some extent carry unusual glycosylations at the hydroxylysine residues, and hyaluronan as well as glycoproteins such as fibronectin, laminin, entactin and tenascin (Garnero et al. 2000a).

Several recent reports find signs of increased inflammatory infiltration and overexpression of mediators of inflammation in the synovial membrane in OA (Benito et al. 2005, Pearle et al. 2007), e.g. interleukin (IL)-15, which seems more elevated in early compared with late OA (Scanzello et al. 2009).

In particular, the urinary concentrations of the glycosylated pyridinium cross-linker, glucosyl-galactosyl -pyridinoline (Glc-Gal-PYD) has been investigated in OA as a marker of synovium tissue destruction (Gineyts et al. 2001). The cross-link has been reported to be elevated by 18% (p <0.05) in knee OA and was significantly associated with total WOMAC index (Garnero et al. 2001b). Not surprisingly, Glc-Gal-PYD has been reported to be elevated in rheumatoid arthritis (RA) as well (Marotte et al. 2008). Recently, increases over 1 year in a type II collagen marker originating from the triple helix, i.e. Coll 2-1 having the amino acid sequence

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Table 1. Biological marker assays for detection of tissue turnover in the human joint.

ID	Target molecule	Short description	Application in human studies
KS/MAb OA-1	Aggrecan	Sandwich ELISA using Mab to keratan sulphate and mAb OA-1 to AGase neo-epitope ARGSVIL (Pratta et al. 2006)	• Detection of fragments in human synovial fluid (Pratta et al. 2006)
CS846	Aggrecan	Monoclonal antibody αHFPG-846 (IgM) recognizing chondroitin sulfate moieties on aggrecan (Glant et al. 1986). (Manufacturer: Ibex, Canada)	• Not predictive for radiographic progression of JSN in knee OA (Mazzuca et al. 2006); however, mean levels related to JSN • Elevated in RA, higher levels in fast progressors, and 1 year level predictive of radiographic progression over 4 years (Verstappen et al. 2006).
342-G2	Aggrecan	Sandwich ELISA using monoclonal antibody AF28 binding to the neo-epitope 342FFGVG and monoclonal antibody F78 binding to G1/G2 for detection of MMP-generated aggrecan fragments (Sumer et al. 2007)	• Elevated, but non-significantly, in RA (Sumer et al. 2007)
G1-G2	Aggrecan	Sandwich ELISA using monoclonal antibody F78 binding to G1/G2 both as capture and detector antibody for detection of intact aggrecan and all aggrecan fragments carrying G1 and/or G2 (Sumer et al. 2007)	• Suppressed 27–31% in RA (Rousseau et al. 2008, Sumer et al. 2007)
Serum CRP	C-reactive protein	C-reactive protein (CRP). Numerous assays available and preferably ultrasensitive formats should be applied	• Acute-phase protein widely used as marker of inflammation • CRP levels decreased significantly in RA patients responding (EULAR) to intervention (etanercept and/or methotrexate) (Sennels et al. 2008) • Single measurements of CRP does not allow detection of OA (Hurter et al. 2005)
COMP	Cartilage oligomeric protein	Competition ELISA using polyclonal antibodies (Saxne & Heinegard 1992). However, sandwich ELISA based on two monoclonal antibodies recognizing different antigenic determinants is described (Vilim et al. 2002). (Manufacturer: AnaMar Medical, Sweden)	• Change in COMP from baseline to 6 years was associated with (1) development of incident radiographic hip OA, (2) stabilization of disease (Chaganti et al. 2008) • Baseline COMP predictive of cartilage loss over 30 months assessed by WORMS (Hunter et al. 2007) • Associated with subjective joint inflammation (Garnero et al. 2005a) • Significant correlation between baseline COMP and JSN over 3 years (Vilim et al. 2002) • Increased 16% in OA (Garnero et al. 2001b) • Baseline COMP predicted cartilage loss determined by MRI (Hunter et al. 2007)
PICP	C-terminus propeptide of type I procollagen	RIA using polyclonal antibodies raised to fibroblast PICP digested with bacterial collagenase (Melkko et al. 1990). (Manufacturer: Orion Diagnostic, Finland)	• PICP concentration in synovial fluid is poorly correlated to stage of OA (Schmidt-Rohlfing et al. 2002) • Postoperative PICP concentrations was not dependent on fixation condition following total knee arthroplasty (Li et al. 2004) • PICP concentrations were not different in patients with benign prostatic hypertrophy with and without osteoarthritis (Akimoto et al. 1997)
PINP	N-terminus propeptide of type I procollagen	A. RIA using polyclonal antibodies recognizing PINP (Melkko et al. 1996) (Manufacturer: Orion Diagnostic, Finland) B. Electrochimiluminecense using MAbs to PINP (Garnero et al. 2008b). (Manufacturer: Roche Diagnostics, Germany)	• A 57% or more increase in PINP had a sensitivity and specificity of 89% and 82%, respectively, for developing heterotopic ossification following total hip arthroplasty (Wilkinson et al. 2003) • PINP levels were unrelated to indexes of joint damage and symptoms in patients with hip OA (Garnero et al. 2005a)

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Table 1. Continued.

ID	Target molecule	Short description	Application in human studies
CTX-I	Type I collagen	A sandwich ELISA using MAb F1103 and F12 both binding to a cathepsin K-derived C-telopeptide neo-epitope, EKAHD-β-GGR, where D-β-G denotes an isomeried linkage between D and G (Rosenquist et al. 1998). (Manufacturer: IDS, UK). Also available in an automated version (Garnero et al. 2001a). (Manufacturer, Roche Diagnostics, Germany)	• Decreased 38% in OA (Garnero et al. 2001b) • CTX-I was unrelated to bone-related radiographic scores in patients with haemophilic arthropathy (Jansen et al. 2009) • Following 24 weeks of chondroitin sulfate therapy, changes in CTX-I was significantly ($p < 0.018$) different in OARSI responders vs non-responders (Mazieres et al. 2007) • No association between severity of radiographic OA and CTX-I levels (Jordan et al. 2006) • CTX-I levels were elevated in patients with progressive OA compared with non-progressive OA (Bettica et al. 2002)
NTX-I	Type I collagen	EIA detecting a fragment of the N-telopeptide of type I collagen (Hanson et al. 1992). (Manufacturer: Inverness, US)	• Not associated with incident radiographic hip OA or progression (Chaganti et al. 2008) • NTX-I levels were elevated in patients with progressive OA compared with non-progressive OA (Bettica et al. 2002)
ICTP	Type I collagen	RIA detecting a fragment of the C-telopeptide of type I collagen (Elomaa et al. 1992). (Manufacturer: Orion Diagnostic, Finland)	• Baseline ICTP correlated with Larsen score in RA (Forsblad et al. 2004), and responded to HRT treatment • Decreasing in RA patients treated with infliximab (anti-TNFα MAb) (Chopin et al. 2008)
PIINP	N-terminus propeptide of type I procollagen	Monoclonal antibody recognizing the amino acid sequence GPQPAGEQGPRGDR located in the N-terminal propeptide of type I procollagen (Olsen et al. 2007)	• Application in humans not reported
PIIANP	N-terminus propeptide of type I procollagen, splice variant A	An ELISA using rabbit polyclonal antibodies raised to recombinant exon-2 of the N-terminal propeptide of type I procollagen (Rousseau et al. 2004a). (Manufacturer: Millipore, USA)	• Decreased 31–53% in OA (Rousseau et al. 2004a, b, Garnero et al. 2002) • However, high 5 year mean levels were associated with increased risk for progression (Sharif et al. 2007)
CPII	C-propeptide of type II collagen	EIA using rabbit polyclonal antibodies binding to the C-propeptides of type II collagen, i.e. a marker of collagen synthesis (Nelson et al. 1998). (Manufacturer: Ibex, Canada)	• Elevated in RA (8.0 ng ml^{-1}), but reduced in OA (4.2 ng ml^{-1}) compared with controls (5.2 ng ml^{-1}) (Nelson et al. 1998) • CPII levels were not associated with structural progression over 30 months (Mazzuca et al. 2006)
9A4/5109	Type II collagen	The collagenase-derived neo-epitope…….. GEGAAGPSGAEGPPGPQG^{775} containing the C-terminus of the long ¾ fragment. MAb5109 detects the first underlined sequence, MAb 9A4 the second (neo-epitope) (Downs et al. 2001)	• Urinary levels in OA was 312 pM compared with less than 123 pM in controls (8/10 control individuals had undetectable concentrations) (Downs et al. 2001)
CTX-II	Type II collagen	A. Competition ELISA using MAb F4601 recognizing the C-telopeptid**e** neo-epitope EKGPDP (Christgau et al. 2001). (Manufacturer: IDS, UK) B. MAb 2B4 recognizing the C-telopeptid**e** neo-epitope EKGPDP (Lohmander et al. 2003)	• Increased 25–177% in OA (Christgau et al. 2001, Garnero et al. 2001b, Jung et al. 2004) • Associated with pain (VAS) (Garnero et al. 2005a) High levels associated with structural progression (radiographic and MRI) (Mazieres et al. 2006, Reijman et al. 2004, Dam et al. 2009)
uTIINE	Type II collagen	An LC-MS/MS assay using MAb 5109 (see above) to affinity purify fragments subjected to MS/MS. Detects a collagenase-derived 45-mer containing the C-terminus of the long ¾ fragment (Hellio Le Graverand et al. 2006)	• OA patients treated with doxycycline and with progression had increased baseline urinary TIINE (47%). No significant association in placebo group (Hellio Le Graverand et al. 2006) • Baseline uTIINE was not predictive of radiographic progression (JSN); however, serial measurements of uTIINE reflected concurrent JSN (Hellio Le Graverand et al. 2006)

Table 1. continued on next page

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Table 1. Continued.

ID	Target molecule	Short description	Application in human studies
HELIX-II	Type II collagen	A competition ELISA using polyclonal rabbit antibodies recognizing the neo-epitope ^{622}ERGETGPP*GTS^{632}, where P* denotes hydroxyproline (Charni et al. 2005)	• uHELIX-II was increased 56% in OA (Charni et al. 2005) • Specificity recently questioned (Eyre & Weis 2009) as the GPPGTS^{532} neo-epitope is found in both α1(III), α5(IV), and α2(XI), but not in type II collagen
C2C	Type II collagen fragment	EIA using a monoclonal antibody recognizing the carboxyl-terminus of the 3/4 piece of the degraded alpha1(II) chain (Poole et al. 2004). (Manufacturer: Ibex, Canada)	• C2C levels were not associated with structural progression (JSN) over 30 months (Mazzuca et al. 2006) or cartilage loss (WORMS) (Hunter et al. 2007)
C1,C2	Type II collagen fragment	EIA using rabbit polyclonal antibodies binding to the carboxy-terminal (COL2-3/4C (short)) neo-epitope generated by cleavage of native human type II collagen by collagenases. Cross-reactivity to type I collagen (Billinghurst et al. 1997). (Manufacturer: Ibex, Canada)	• Baseline levels not associated with structural progression over 30 months (Mazzuca et al. 2006, Hunter et al. 2007)
PIIINP	N-terminus propeptide of type III procollagen	RIA using polyclonal antibodies recognizing PIIINP (Risteli et al. 1988) (manufacturer Orion Diagnostic, Finland)	• Increased 33% in OA (Garnero et al. 2001b) • Associated with joint surface area (Garnero et al. 2001b)
Glc-Gal-PYD	Glucosyl-galactosyl-pyridinoline	HPLC method for determination of the non-reducible collagen cross-linker glucosyl-galactosyl-pyridinium present in synovium and absent in bone cartilage and other soft tissue (Gineyts et al. 2001)	• Increased 18% in OA (Gineyts et al. 2001) • Associated with total WOMAC index (Garnero et al. 2001b)
Serum HA	Hyaluronic acid	Based on HA binding protein isolated from bovine cartilage. (Manufacturer: e.g. Pharmacia, Sweden, and Corgenix, UK)	• Increased 233% in OA (Garnero et al. 2001b) • High levels associated with structural disease progression (JSN ≥ 0.5 mm over 3 years) (Mazieres et al. 2006) • Serum HA was positively associated with all definitions of radiographic OA (p <0.0001) (Elliott et al. 2005)
YKL-40	Human glycoprotein 39	A RIA using polyclonal antibodies to a glycoprotein of MW 40 kDa (Johansen et al. 1993). A combined monoclonal capture and polyclonal (rabbit) detector sandwich assay is available. (Manufacturer: Quidel Corporation, USA)	• Not related to structural disease progression (Mazieres et al. 2006)
OC	Osteocalcin	Numerous assays available	• Decreased 36% in OA (Garnero et al. 2001b) • Associated with total WOMAC index (Garnero et al. 2001b)

ICTP, cross-linked carboxyterminal telopeptide of type I collagen; C1/C2, C-terminus of MMP-cleaved type I/II collagen; C2C, C-terminus of MMP-cleaved helical type II collagen; C6S/C4S, chondroitin 6-sulfate, 4-sulfate; COMP, cartilage oligomeric protein; CPII, C-terminus propeptide of type II procollagen; CRP, C reactive protein; CS846, chondroitin sulfate containing aggrecan; CTX-I, fragment of C-telopeptide of type I collagen; CTX-II, fragment of C-telopeptide of type II collagen; Glc-Gal-PYD, urinary glucosyl galactosyl pyridinoline; HA, hyaluronan; MMP, matrix metalloproteinase; NTX-I, fragment of N-telopeptide of type I collagen; OA, osteoarthritis; OC, osteocalcin; PICP, carboxyterminal propeptide of type I procollagen; PINP, N-terminal propeptide of type I procollagen; PIINP, N-terminal propeptide of type II procollagen; PIIINP, N-terminal propeptide of type III procollagen; RA, rheumatoid arthritis; TNFα, tumour necrosis factor alpha; uTIINE, urinary fragment (45mer) of type II collagen; YKL-40, human glycoprotein 40kDa.

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108HRGYPGLDG116 and its nitrosylated counterpart Coll 2-1 NO2 (108HRGY(NO2)PGLDG116), were both predictive of JSN in patients with knee OA (Henrotin et al. 2004). Subsequent studies demonstrated that both markers were elevated in both RA and OA compared with controls (Deberg et al. 2005); however, neither of the markers were associated with the severity of radiological OA. Finally, Coll 2-1, but not Coll 2-1 NO_2, was reduced following hip replacement in OA patients (Deberg et al. 2008). Another nitrated type III collagen marker, the nitrated N-telopeptide of type III collagen based on the recognition of the sequence QY*DSY*DVKSG (IIINys) (Charni-Ben et al. 2009, Richardot et al. 2009) was reported to be elevated in RA.

Overview of biochemical markers of joint tissue

As described above, the joint contains several compartments each of which has a complex biochemical composition, and therefore the biochemical marker potential of the joint is substantial. Table 1 provides an overview of the biochemical marker repertoire currently available for quantitative assessment of the tissue turnover in the joint.

Application of biochemical markers in clinical studies of OA

Peer-reviewed reports of recent clinical trials investigating biochemical markers in OA was searched for in

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Table 2. Osteoarthritis (OA) biomarkers in clinical trials.

No.	Study design	Intervention	Study subjects	Biological marker	Findings/comments/conclusions	Ref.
1	Prospective, Randomized, 200 days	Anti-inflammatory drugs	433 OA	S-NT-proBNP	Biochemical monitoring related to potential CV adverse events	(Brune et al. 2008)
2	Longitudinal, Randomized, 6 months	Diet and exercise	87 obese OA	S-IL-6, S-TNF-α, S-CRP, S-sTNFR1 and S-sTNFR2	Intensive weight-loss intervention improve inflammatory markers and physical function	(Miller et al. 2008)
3	Longitudinal, 2 weeks	Anti-TNFα blockers (infliximab or etanercept)	63 RA, 10 OA, 34 controls	IL-17 gene expression	IL-17/TNF-α expression ratio could be a suitable marker of response to anti-TNF-α therapy	(Kohno et al. 2008)
4	Longitudinal, Randomized, Placebo-controlled, 24 months (KOSTAR)	Risedronate	1885 OA	U-NTX-I, U-CTX-II	CTX-II levels after 6 months were associated with radiological progression at 24 months. No association of NTX-I with disease progression could be detected	(Garnero et al. 2008a)
5	As study 4		2483 OA	U-CTX-II	Risedronate failed to improve sign and symptoms (WOMAC, PGA). However, risedronate induced a dose-dependant reduction in U-CTX-II.	(Bingham et al. 2006)
6	Longitudinal, Randomized, Placebo-controlled, 30 months	Doxycycline	120 OA	uTIINE	uTIINE was unrelated to change in JSN observed in the treatment group	(Otterness et al. 2007)
7	As study 6				Baseline uTIINE was not a consistent predictor of JSN	(Hellio Le Graverand et al. 2006)
8	As study 6			S-C2C, S-C1/2C, S-CPII, S-CS846	None of the biomarkers was a significant predictor of progression of JSN. Over the interval from baseline to 16 months, the mean and the maximum of the intercurrent CS846 values were significantly associated with JSN	(Mazzuca et al. 2006)
9	As study 6			S-MMP-3	Subjects in the placebo group whose MMP-3 concentration was in the upper tertile of the baseline distribution had an odds ratio of 4.12 ($p < 0.037$) for progression of JSN compared with the lower tertile	(Lohmander et al. 2005)
10	Prospective, Two nested case–control studies, 8.3 years	None	340 OA	S-COMP, S-NTX-I	Measurement of serum COMP at baseline and 6 years may be a method of identifying patients at risk for developing incident RHOA and those with baseline RHOA that will not rapidly progress	(Chaganti et al. 2008)
11	Cross-sectional	None	603 OA, 596 controls	36 SNPs	Additive information from a number of genetic variants can predict a substantial proportion of risk of knee OA	(Valdes et al. 2008)
12	Longitudinal, Randomized, Placebo-controlled, 24 weeks	Chondroitin sulfate	307 OA	S-CTX-I, U-CTX-II, S-HA	Study drug failed to meet clinical end points. However, significant difference in non-responders and responders for 24 weeks changes in CTX-I and CTX-II.	(Mazieres et al. 2007)
13	Longitudinal, Randomized, 24 weeks	Exercise	58 OA	S-COMP	Increase in S-COMP immediately after excise, which returned to baseline after 30 min of rest. No change in S-COMP after 6 weeks of exercise programme	(Andersson et al. 2006)
14	Longitudinal, 1 month	Intra-articular HA injections	32 OA	C6S/C4S and aggrecan in synovial fluid	Baseline concentrations of C6S and aggrecan was associated with improvement in joint score after injection	(Sugimoto et al. 2006)
15	Longitudinal, Randomized, Double-blind Placebo-controlled, 84 days	Oral salmon calcitonin (sCT)	53 OA	CTX-II, C2C, MMP-1, MMP-8, MMP-13, MMP-3, TIMP1, TIMP2, hyaluronan	Calcitonin induced significant improvement in Lequesne's functional score at day 84. Significant decrease in MMP-3 and hyaluronan in 0.5 and 1 mg daily sCT groups, while CTX-II, C2C and MMP-13 decreased in the 1 mg group only.	(Manicourt et al. 2006)

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Table 2. Continued.

No.	Study design	Intervention	Study subjects	Biological marker	Findings/comments/conclusions	Ref.
16	Longitudinal	Basic fibroblast growth factor (bFGF) and soluble E-selectin (sE-sel)	30 RA, 15 OA and 15 controls	bFGF, sE-sel, IL-1β and IL-6 in serum and SF	Serum bFGF and sE-sel were significantly elevated in RA compared with OA patients and controls, and both markers were elevated in SF from RA compared with OA. A positive correlation was found between SF bFGF with grades of joint derangement assessed radiologically	(Sharaki et al. 2004)
17	Longitudinal, Randomized, 3 months	Celecoxib and aceclofenac	30 OA	SF-PGE(2) and COX-2 expression in synovial membrane	Both drugs improved joint pain and function, inhibited SF PGE(2) concentration, and induced a decrease in synovial COX-2 mRNA expression and protein synthesis	(Alvarez-Soria et al. 2008)
18	Longitudinal, Randomized, 3 months	Acetaminophen (paracetamol) and rofecoxib	20 OA	Serotonin, P-SP, P-BEND, KOR in PBMCs.	Plasma SP levels were elevated in both groups compared with baseline. Acetaminophen significantly reduced plasma BEND levels, which was correlated to pain relief by VAS.	(Shen et al. 2006)
19	Cross-sectional (case–control)	NA	(1) 335 OA and 335 controls, (2) 443 OA and 303 controls, (3) 346 OA and 264 controls	>25 000 SNPs located within approximately 14 000 genes	A genetic variant in *LRCH1* was consistently associated with radiographic knee OA.	(Spector et al. 2006)
20	Prospective, Double-blind, Placebo-controlled, 3 years	Diacerein	333 OA (ECODIAH)	S-PINP, S-PIIINP, S-COMP, S-YKL-40, S-HA, S-MMP-1, S-MMP-3, S-CRP, S-CTX-I and U-CTX-II	uCTX-II and S-HA in the upper tertile had a relative risk of progression of 3.73 (95% CI 2.48–5.61) compared with patients with markers in the two lower tertiles	(Mazieres et al. 2006)
21	Cross-sectional	NA	376 OA (ECODIAH)	PINP, PIIINP, COMP, YKL-40, HA, MMP1, MMP3, CRP; U-CTX-I and U-CTX-II	Pain was associated with CTX-II ($p < 0.0095$) and CRP ($p < 0.046$) and joint inflammation with COMP ($p < 0.013$). Radiographic signs of joint damage were associated with CTX-II ($p < 0.001$ for JSW; $p < 0.007$ for bone sclerosis)	(Garnero et al. 2005a)
22	Cross-sectional	NA	119 OA	S-CICP, S-ICTP, DPD, S-PTH, S-estrogen, S-testosterone, S-bAP, S-hydroxy vitamin D	Bone biochemical markers were reported not to be associated with OA	(Drees et al. 2005)
23	Cross-sectional	NA	28 RA and 26 OA	N-acetyl-beta-hexosoaminidase (Hex) in SF and serum	Hex activity in SF from RA was 15.2 nmol ml^{-1} min^{-1}, and in OA SF 6.15 nmol $ml^{-1}min^{-1}$. In serum Hex activity was 4.0–4.7 nml ml^{-1} min^{-1} in both groups and controls	(Popko et al. 2005)
24	Longitudinal, 3 months		377 OA	U-CTX-II, S-CTX-I	Bone marrow abnormality scores by MRI correlated significantly with CTX-II levels ($p < 0.0001$) at baseline. Baseline CTX-II levels in the highest tertile had a relative risk of 2.4 (95% confidence interval 1.1–5.0) of worsening bone marrow abnormalities at 3 months compared with patients with levels in the lowest tertile. CTX-I was unrelated to bone marrow abnormalities	(Garnero et al. 2005b)

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Table 2. Continued.

No.	Study design	Intervention	Study subjects	Biological marker	Findings/comments/conclusions	Ref.
25	Longitudinal, Single blind Randomized, 4 weeks	Nimesulide and ibuprofen	90 OA	U-CTX-II, S-HA, S-MMP-3, S-MMP-1 and S-MMP-13	At 4 weeks nimesulide, not ibuprofen, reduced the CTX-II (p <0.001), HA (p <0.05), MMP-3 (p <0.05) and MMP-13 (p <0.001). Furthermore, in the nimesulide group, the decrease in levels of CTX-II correlated significantly with the decrease in levels of HA and MMP-13	(Manicourt et al. 2005)
26	As study 25			S-MMP-3, S-TIMP-1, S-HA, S-YKL-40	Nimesulide significantly reduced HA and MMP-3, whereas ibuprofen increased significantly MMP-3. None of the NSAIDs were able to change induce changes in TIMP-1 and YKL-40	(Bevilacqua et al. 2004)
27	Longitudinal, Double blind Placebo-controlled, 24 weeks	Glucosamine sulfate	137 OA	S-C2C and S-C1/C2	No significant differences between the placebo and glucosamine group was observed for C2C or C1/C2	(Cibere et al. 2005)
28	Longitudinal, Randomized, 4 weeks	Intra-articular sodium HA or cross-linked hylan G-F-20	40 OA	ICAM-1 and VCAM-1 in SF	Both ICAM-1 and VCAM-1 decreased after HA injections, but no difference in ICAM-1 and VCAM-1 was observed between the treatment groups	(Karatay et al. 2004)
29	Cross-sectional	Nutrition and exercise	274 OA	S-IL-6, S-CRP, S-TNFα, S-IL-6sR, S-IL-2sR, S-TNF-sR1 and S-TNF-sR2	High TNF-sR1 and TNF-sR2 were significantly associated with poor physical function (WOMAC) and more symptoms of pain and stiffness	(Penninx et al. 2004)
30	Longitudinal, Randomized, 18 months	Weight loss and exercise	316 OA	Serum leptin	Serum leptin decreased (p <0.01) in diet groups, but not in controls and exercise only	(Miller et al. 2004)
31	Longitudinal, Randomized, Place bo-controlled, 6 weeks	Ibuprofen	201 OA	U-CTX-II, Glc-Gal-PYD	The drug induced response in CTX-II was different (13%) compared to placebo (p < 0.017)	(Gineyts et al. 2004)
32	Prospective, 24 months	Total knee replacement	40 OA	PICP, OC, ICTP	PICP and OC were higher in the unstable fixation group at 12 and 24 months. No difference observed for ICTP	(Li et al. 2004)
33	Longitudinal, Double blind Randomized, Place bo-controlled, 3 years	Glucosamine sulphate	212 OA	U-CTX-II	Increased baseline levels of CTX-II were associated with a worsening of the WOMAC index (p <0.01). No significant difference in the CTX-II response in the placebo group and the glucosamine-treated group. The 12 months change in CTX-II in OA patients with elevated CTX-II at baseline correlated with the change in average joint space width observed after 36 months (R = 0.43, p <0.05)	(Christgau et al. 2004)

ICTP, cross-linked carboxyterminal telopeptide of type I collagen; BEND, β-endorphin; bAP, bone alkaline phosphotase; bFGF, basic fibroblast growth factor; CICP, C-terminal propeptide of type I collagen; C1/C2, C-terminus of MMP-cleaved type I/II collagen; C2C, C-terminus of MMP-cleaved helical type II collagen; C6S/C4S, chondroitin 6-sulfate, 4-sulfate; COMP, cartilage oligomeric protein; COX-2, cyclooxygenase-2; CPII, C-terminus propeptide of type II procollagen; CRP, C reactive protein; CS846, chondroitin sulfate containing aggrecan; CTX-I, fragment of C-telopeptide of type I collagen; CTX-II, fragment of C-telopeptide of type II collagen; DPD, deoxypyridinoline; Glc-Gal-PYD, urinary glucosyl galactosyl pyridinoline; HA, hyaluronan; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; IL-2sR, soluble receptor for IL-2; IL-6sR, soluble receptor for IL-6; KOR, kappa opoid receptor; MMP, matrix metalloproteinase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NTX-I, fragment of N-telopeptide of type I collagen; OC, osteocalcin; P, plasma; PICP, carboxyterminal propeptide of type I procollagen; PINP, N-terminal propeptide of type I procollagen; PIINP, N-terminal propeptide of type II procollagen; PIIINP, N-terminal propeptide of type III procollagen; PGE(2), prostaglandin E(2); PTH, parathyroid hormone; sE-sel, soluble E-selectin; S, serum; SF, synovial fluid; SP, substance P; TIMP1, tissue inhibitor of MMP 1; TIMP2, tissue inhibitor of MMP 2; TNF, tumour necrosis factor; TNF-sR1, soluble receptor 1 for TNF-α; TNF-sR2, soluble receptor 2 for TNF-α; uTIINE, urinary fragment (45mer) of type II collagen; VCAM-1, vascular cell adhesion molecule-1; YKL-40, human glycoprotein 40 kDa.

PubMed and have been reviewed (Table 2). Using the MESH terms 'biological markers' and 'osteoarthritis' and limiting to clinical trials in humans published within the last 5 years (from December 2003 to December 2008), a list of 33 papers, including two with only an English summary, was identified. Among the 33 papers, 21 applied one or more biochemical markers of joint tissue listed in Table 1, and all five possible applications encompassed

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by the BIPED classification scheme are represented. The wide selection of markers applied in these studies partly reflects the lack of consensus in this emerging area.

The vast majority of studies in Table 2 employ two or more biomarkers to identify disease changes, but only a few, e.g. Maziéres and co-workers (2006) actually combine the markers. In a recent study it was demonstrated that ratios between markers of cartilage degradation and cartilage synthesis provided a better separation of OA stages than any individual biomarker (Cibere et al. 2009). As in other disease areas, e.g. liver fibrosis (Gressner et al. 2007), allocating more efforts into the development of algorithms combining biomarkers of both bone and cartilage and eventually also of the synovium should be considered. Among the 33 studies, positive associations to disease prognosis, primarily structural progression, were reported for COMP (one study), HA (one study) and CTX-II (five studies).

In a longitudinal intervention study of diacerein, Mazieres and co-workers found by multivariate analysis that HA and CTX-II were significantly (both $p < 0.0001$) associated with radiographic progression of knee OA. With HA or CTX-II in the highest tertile, the relative risk for progression was 1.69 (95% CI 1.25–2.27) and 2.00 (95% CI 1.49–2.70), respectively, compared with the two lowest tertiles (Mazieres et al. 2006). This association remained significant after adjustment for baseline clinical, radiological and treatment variables, and as the two biomarkers were independent, patients with both markers in the highest tertile had a relative risk of progression of 3.73 (95% CI 2.48–5.61) compared with the two lowest tertiles.

In a recent study of oral salmon calcitonin, daily doses of 1 mg induced a significant reduction in both function and pain scores above placebo at days 42 and 84 in subjects with knee OA (Manicourt et al. 2006). At day 84, urinary levels of a series of biomarkers, including CTX-II, MMP-3, MMP-13 and HA, were significantly suppressed compared with baseline.

In another recent clinical trial, Spector and colleagues (2005) reported results from the British study of risedronate in structure and symptoms of knee OA (BRISK). It was a 1-year, prospective, double-blinded, placebo-controlled study of risedronate for treatment of mild to moderate knee OA. A total of 285 men and women were randomized to receive daily doses of 5 or 15 mg of risedronate or placebo. While the 15 mg group showed significant improvement in WOMAC index and patient global assessment, the trend towards attenuation in JSN did not reach statistical significance. Only 8% of the OA patients in the placebo group had detectable radiographic progression of the disease. In two parallel and much larger multinational 2-year studies, i.e. the knee OA structural arthritis (KOSTAR) study, all doses of risedronate failed to improve above placebo signs and symptoms measured by WOMAC and did not slow radiographic progression (Bingham et al. 2006).

Two other markers of type II collagen-derived neoepitopes should be addressed: the HELIX-II and uTIINE. The Helix-II test, originally reported by Charni and co-workers (2005) uses rabbit antibodies to the helical peptide ERGETGPhypGTS (hyp is hydroxyproline), which is generated primarily by cathepsin K, L and S, but is destroyed by cathepsin B (Charni et al. 2008), and a 56% elevation of Helix-II concentrations was reported in patients with knee OA (Charni et al. 2005), and elevated baseline concentrations were significantly associated with increased risk of structural progression. It should be noted, however, that the molecular specificity of the Helix II test has recently been questioned (Eyre & Weis 2009). The other marker, uTIINE, is a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay detecting a specific collagenase-derived 45-mer of the helical part of type II collagen (Hellio Le Graverand et al. 2006), which, however, was not found to be predictive of structural progression (JSN).

The data reported until now for the association of aggrecan-derived markers with radiographic outcome or clinical relevant measures of disease activity in OA patients have been fewer and less convincing. Pratta and co-workers reported detection of ARGSV-containing fragments in human OA synovial fluid (Pratta et al. 2006) using the KS/mAb OA-1 sandwich enzyme-linked immunosorbent assay (ELISA); however, measurement of fragments in circulation was not reported. CS846 was not predictive of JSN in 60 radiographic progressors and 60 non-progressors over 30 months, but mean CS846 levels were associated with JSN in the first and second half of the study (Mazzuca et al. 2006), and clearly further studies are needed to determine the benefit of this and other aggrecan-derived markers in clinical trials.

Circulating levels of COMP have been reported to be associated with disease progression as referenced in Table 1. Other studies have reported similar associations (Sharif et al. 1995) (Fex et al. 1997) (Georges et al. 1997); however negative findings have been reported (Conrozier et al. 1998).

These and other data suggest, that biochemical markers are being intensively investigated in clinical settings, as are other types of biomarkers; however, we are still quite far from being able to provide evidence-based recommendations, as to the optimal use of these technologies. For the present biochemical markers to be implemented optimally in clinical settings, a better understanding of the individual marker is needed, and in this context the BIPED classification might provide the needed clarification. Only after the clarification of the clinical usefulness of individual biomarkers in groups of patients are we able to investigate their application in the individual patient.

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Future directions in biomarker research

Clearly, the number of scientific reports associating biochemical markers with clinical relevant end points or indexes of structural damage in OA is not overwhelming, but it definitely reflects an area of intense research. In the near future, we anticipate significant progress in at least two areas of research.

First, major efforts are currently dedicated to the development of molecular markers reflecting specific metabolic processes relevant for the pathogenicity of OA, in particular the degenerative processes in the articular cartilage. An increased amount of evidence suggests that proteases may not only be important for destruction of the articular cartilage, but in addition for repair and maintenance. In particular Murphy and Nagase (2008) reappraised the role of metalloproteinases in cartilage homeostasis, based on an increased amount of evidence that MMP inhibitors in clinical studies have failed to show the promised efficacy, e.g. the report by Krzeski et al. (2007). They suggested that some metalloproteinases may be essential for normal joint physiology, homeostasis and repair. At present much attention is directed towards MMP and aggrecanase activity by the quantification of promiscuous protease sites in aggrecan, which do not distinguish between the enzymes mediating degradation or repair. In the future, specific proteolytic fragments derived from type II collagen, aggrecan or other proteins derived from the joint tissue may be identified and provide yet better tools to understand the molecular processes behind tissue turnover and homeostasis.

Targeting the biological markers to fragments carrying one or more neo-epitopes allows both the amino acid sequence of the 'mother' peptide as well as the tag of the proteases to be included in the determinants of the specificity, and this might provide sufficient selectivity to target the markers to local pathological events. As illustrated in Figure 3, it can be speculated that measurements of type II collagen fragments carrying the cathepsin K-derived neo-epitope will reflect metabolic processing in the calcified cartilage, whereas elevation in both type II collagen and aggrecan fragments simultaneously will reflect proteolytic activity in articular cartilage.

Second, the modest association of single markers measurements with long-term structural changes in the joints is driving efforts to combine biochemical markers as well as different biomarker technologies, including imaging and genetic markers, for better prognostic capabilities. While we are waiting for the first DMOAD, we could consider treatment paradigms based on identification of treatment candidates at a very early stage, prior to classical OA symptoms, and subsequently institute preventive medical intervention. Clearly, such an approach call for a high level of prognostic accuracy (apart from availability of safe therapeutic treatment options), which will allow targeting of the intervention to those individuals, who will benefit the most. Such accuracy is not offered by the current procedures; however we anticipate that adopting a more integrated approach in this important clinical decision-making will carry major potential. It has been reported, that biochemical markers of bone resorption and measurements of bone mineral density are independent predictors of fracture risk and that their combination therefore increases the predictive power of the risk assessment procedure (Garnero et al. 1996, 1998). We anticipate that genetic markers, biochemical markers and imaging technologies in combination will increase prognostic accuracy and thereby potentially could offer the necessary justification for early, preventive intervention in OA.

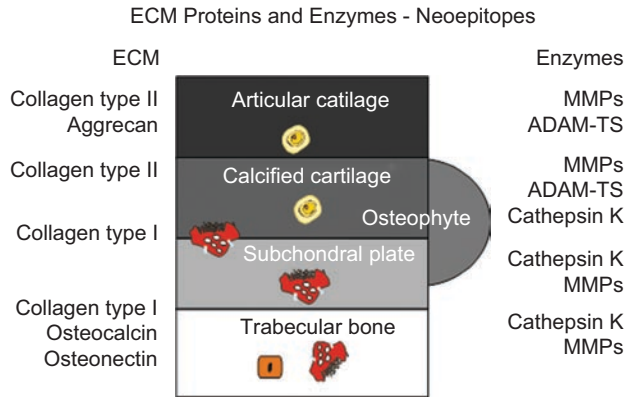

Figure 3. Schematic representation of biomarker candidates based on proteolytic processing of extracellular matrix (ECM) proteins. Some of these proteins have a wide tissue distribution, but targeting the biomarker towards neo-epitopes generated by cleavage by relevant proteases could offer additional selectivity. MMP, matrix metalloproteinase; ADAM-TS, aggrecanases.

Although the biomarker field in OA is still at an immature stage, we anticipate that the significant efforts allocated to marker discovery in this important area will ensure that an improved, integrated OA biomarker tool box will be available upon the emergence of the first DMOAD.

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