Use of Biochemical Markers to Study and Follow Patients with Osteoarthritis

Patrick Garnero, PhD, DSc

Corresponding author
Patrick Garnero, PhD, DSc
Synarc, Molecular Markers, Le Buroparc Batîment T4,
16 rue Montbrillant, 69003 Lyon, France.
E-mail: patrick.garnero@synarc.com

Current Rheumatology Reports 2006, **8**:37–44
Current Science Inc. ISSN 1523-3774

Osteoarthritis is characterized by progressive destruction of articular cartilage and subchondral bone and synovial reaction. Radiologic findings that form the basis of the diagnosis of osteoarthritis are poorly sensitive to detect early disease and for monitoring progression of joint damage. Blood-based proteomic analyses suggest that biochemical alterations can be observed well before radiologic damage is evidenced. New cartilage-specific markers, including assays for type II collagen synthesis and degradation, have been developed. Recent prospective studies indicate that blood and urine levels of these new markers are associated with progression of joint damage. Biological markers respond rapidly to treatment and therefore will certainly play an important role in the development and the monitoring of disease-modifying therapies. Because osteoarthritis involves different tissues and complex biologic processes, a combination of different biochemical markers appears to be the most promising diagnostic strategy.

Introduction

Osteoarthritis is a prevalent age-related disease that causes significant morbidity. The major clinical manifestation of osteoarthritis is abnormal and degraded cartilage, inflamed and/or thickened synovial tissue, and altered bone structure, resulting in pain, mobility impairment, and disability. Plain radiography, the reference technique for assessing the severity of joint destruction, provides direct information on bones but only indirect information on cartilage. Furthermore, sensitivity to change is limited, and it is often necessary to wait 1 to 3 years, to obtain reliable information on progression. Consequently, there is an urgent need for new diagnostic tests with improved sensitivity. Such tests are especially awaited for the efficient development of disease-modifying osteoarthritis therapies (DMOADs). MRI which provides direct information on the alteration of the different joint tissues is more sensitive than radiography to detect cartilage loss and is currently being optimized in osteoarthritis [1]. There has recently been a considerable interest in developing specific biological markers which reflect quantitative and dynamic variations in joint tissue remodeling. In this paper we will briefly review the most recent development in biological marker technology and discuss their potential clinical uses in osteoarthritis.

New Methodologies and New Biochemical Markers

A biochemical marker is generally considered as a single molecule or fragment of connective tissue matrices which is released into biological fluids during the process of tissue turnover. Several individual biochemical markers reflecting the synthesis and the degradation of the three main joint tissues (ie, bone, cartilage, and synovium) have been proposed over the years and the list is continuously expanded (Table 1). Recently, new approaches have been proposed for identifying osteoarthritis biochemical markers including genomics, proteomics, and metabolomics. These new methodologies coupled with sophisticated statistical methods allow the simultaneous analyses of multiple markers and are especially promising for early diagnosis of osteoarthritis.

Using a genomic approach based on isolation of mRNA from circulating blood and subsequent reverse transcription polymerase chain reaction, Marshall et al. [2] identified six genes which were significantly downregulated in patients with mild osteoarthritis (according to arthroscopy assessment) compared with healthy controls. Combination of these six genes in a multiple variable model was able to correctly identify 85% with mild osteoarthritis and controls. Proteomic generally involves separation of proteins by two-dimensional (2D) electrophoresis followed-up by their identification using mass spectroscopy. Proteomic analysis has recently been used to identify biochemical markers related to disease development and progression but also autoimmunity in osteoarthritis. Using this approach, it was shown that

Table 1. Biological markers of bone, cartilage, and synovium turnover

	Synthesis	Degradation
Bone		
Type I collagen	N- and C-propeptides (PICP, PINP)	Pyridinoline (PYD); Deoxypyridinoline (DPD); C and N-telopeptide (CTX-I, NTX-I, ICTP); Helical peptide
Non-collagenous proteins	Osteocalcin; Bone alkaline phosphatase	Bone sialoprotein (BSP); Tartrate resistant acid phosphatase (TRAP, 5b isoenzyme); Cathepsin K
Cartilage		
Type II collagen	N- and C-propeptides (PIICP, PIIANP and PIIBNP)	PYD; Type II collagen C-telopeptide (CTX-II); Type II collagen collagenase neoepitope (C2C CI2C, TIINE); Type II collagen helical fragments (Helix-II and Coll 2-1)
Aggrecan	Chondroïtin sulfate (epitopes 846, 3B3, 7D4)	Core protein MMPs and aggrecanase neoepitopes; Keratan sulfate (epitopes 5D4, ANP9)
Non-aggrecan and non-collagen proteins	Glycoprotein 39 (YKL-40); Cartilage-derived retinoic acid sensitive protein (CD-RAP)	COMP
Synovium/synovitis		
Type III collagen	Type III N-propeptide (PIIINP)	PYD; CTX-I, NTX-I; Glucosyl-galactosyl-pyridinoline (Glc-Gal-PYD)
Non-collagenous proteins	Hyaluronan; YKL-40; COMP	
Proteases	MMP-1, 2, 3, 9	
Systemic inflammation	C-reactive protein (CRP)	

C-reactive protein and the molecules S100A8 (calgranulin A), S100A9 (calgranulin B), and S100A12 (calgranulin C) (members of the S100 family of calcium binding proteins) were significantly elevated in the synovial fluid and serum of 15 patients with erosive rheumatoid arthritis (RA) compared with 15 non-erosive RA subjects [3]. The complex S100A8/A9 (also called caltroprotectin) and S100A12 were also reported to be higher in patients with RA compared with osteoarthritis subjects. S100 proteins are a new class of mediators of inflammations involved in the migration of leucocytes and monocytes. Applying 2D electrophoresis to human chondrocyte extracts followed-up by reaction with serum samples from 20 patients with osteoarthritis, 20 patients with RA, and 20 healthy controls, Xiang et al. [4••] identified 19 auto-antigens specific to osteoarthritis, 11 specific to RA, and 22 which were common to the two diseases. Triosephosphate isomerase (TPI) was subsequently identified by mass-spectroscopy as one of the unique osteoarthritis auto-antigens. Indeed, immunoglobulin anti-TPI auto-antibodies were detected in about 25% of osteoarthritis serum and synovial fluid samples but in less than 6% of patients with RA or lupus. Presence of anti-TPI autoantibodies in patients with osteoarthritis was associated with lower radiographic grade. This study underscores the importance of auto-immunity (which is a well recognized etiologic factor in RA) in the physiopathology of osteoarthritis [5•] and the potential for using auto-antibodies as diagnostic biochemical markers of osteoarthritis. Another new approach is metabolomic and consists of the determination of a profile of metabolites specific to patients with osteoarthritis. Using nuclear MR spectroscopy followed-up by statistical principal component analysis it has been reported that urinary hydroxybutyrate, pyruvate, creatine/creatinine, and glycerol were increased in 45 patients with knee or hip osteoarthritis compared with healthy controls, suggesting altered energy utilization in osteoarthritis [5•]. These basic technical developments will ultimately allow identifying a panel of biochemical markers which could then be assessed simultaneously by micro-array platforms. This strategy was recently used in a case control study of the Baltimore Longitudinal Study of Aging to analyze 160 candidate blood proteins implicated in tissue matrix degradation, cellular activation, and inflammation. It was shown that a combination of a few of these proteins were already differently expressed in the 21 patients with no osteoarthritis at the time of investigation who developed osteoarthritis in the following 10 years compared with the 66 individuals who remained free of radiologic disease [6]. Because the outcome of the studies using these novel technologies is highly dependent of sample collection and data processing (for which standardization is still lacking) these findings obtained on a small number of patients will have to be independently replicated in larger samples.

While validation is awaited for these promising powerful, but still complex methods, the use of a combination of

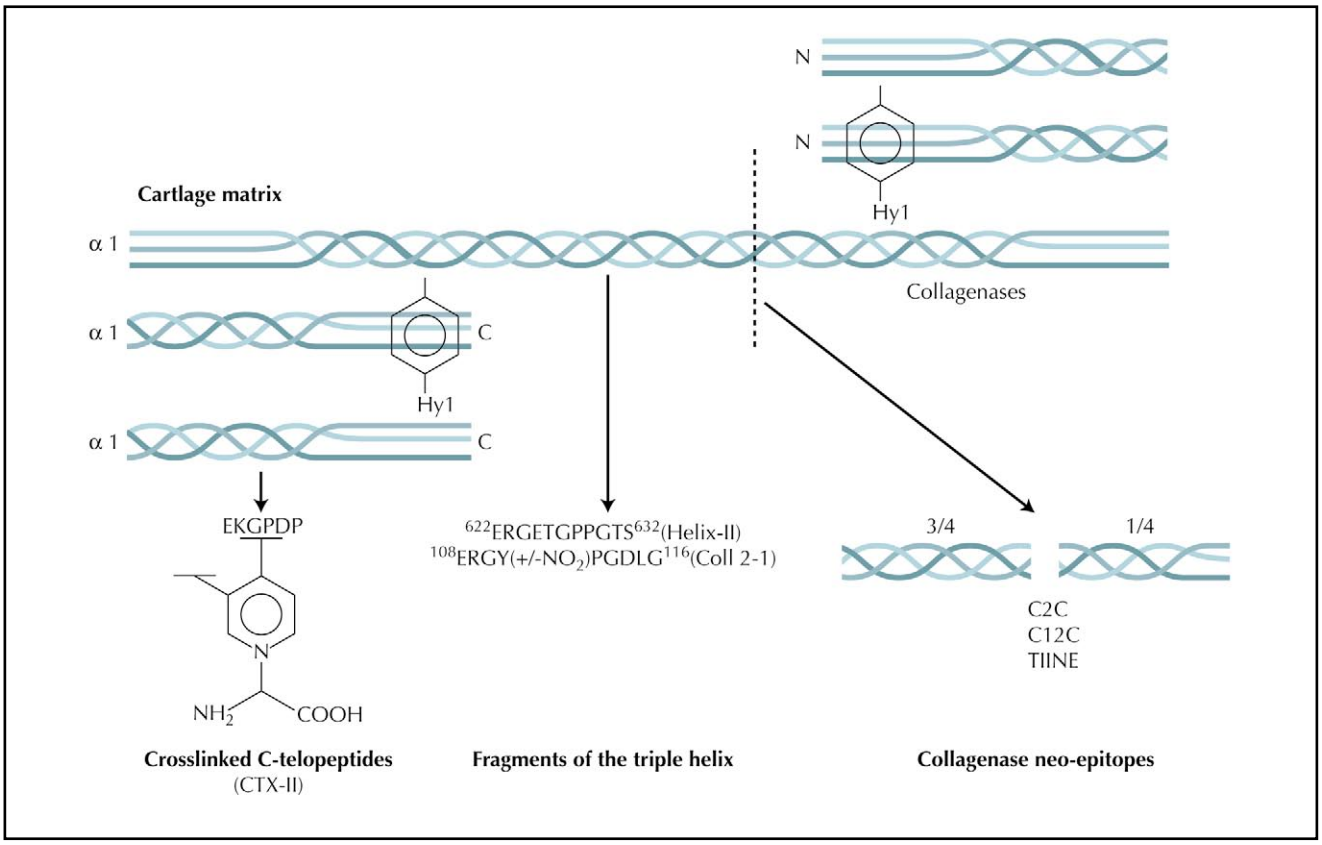

Figure 1. Type II collagen fragments as specific biological markers of cartilage degradation. Type II collagen is formed by the association of three identical α1 chains in triple helix except at the ends (telopeptides). In the extracellular matrix of cartilage, collagen molecules are cross-linked by pyridinoline (PYD) involving the telopeptide regions. During cartilage degradation, different molecules are released in synovial fluid, serum, and urine. These include neoepitopes generated by the collagenases (eg, C2C, C12C and TIINE), fragments of the triple helix (Helix-II and Col 2-1) and C-terminal crosslinking telopeptides (CTX-II).

specific individual biochemical markers seems currently to be a reasonable strategy. Major achievements have been recently obtained, especially with the development of new specific and well characterized biochemical markers of type II collagen, the most abundant protein of cartilage matrix. Type II collagen is synthesized by the chondrocytes as procollagen which is constituted by the collagen molecule itself that forms the framework of cartilage matrix and the N- (PIINP) and C- terminal (PIICP) propeptides at each end. Propeptides are cleaved-off during the maturation of collagen molecules, they are released into the biological fluids and their concentration is believed to directly reflect the rate of type II collagen synthesis. There are two splicing alternative forms of type II procollagen which differ by the presence (IIA) or absence (IIB) of a 69 amino acid sequence coded by exon 2 in the N-propeptide. Procollagen IIA is expressed mainly during development, but can be re-expressed in osteoarthritic cartilage, whereas the IIB variant is the major form of adult cartilage. An enzyme-linked immunoassay for PIIANP was developed using a specific polyclonal antibody raised against recombinant exon-2 protein. When a comparison was made with healthy sex and age-matched controls, increased serum levels of PIIANP were reported in early knee osteoarthritis [7], whereas decreased values [8•] were found in patients with advanced knee osteoarthritis. These biochemical marker data suggest that cartilage repair mechanism is effective early osteoarthritis, but may become deficient with advancing disease.

The recent development of assays specific for type II collagen breakdown represents a breakthrough in the field of biological markers for osteoarthritis, given that degradation of collagen fibers is associated with irreversible cartilage destruction. Antibodies recognizing different type II collagen fragments have been developed (Fig. 1). Among these, Helix-II is a new specific fragment arising from the degradation of the helical domain of type II collagen. Helix-II (which is believed to reflect the degradation of the main part of type II collagen) appears to be released from cartilage degradation by enzymatic pathways which are in part different from those involved in the generation of CTX-II, a fragment of the C-telopeptides region [9]. Urinary Helix-II levels were found to be markedly increased in 90 patients with knee osteoarthritis (+ 56%) and in 89 patients with early RA (+ 123%) compared with 162 healthy sex and age-matched controls [10••]. Interestingly the combined measurement of Helix-II and CTX-II was more effective than one of these

two markers alone to identify patients with a rapidly progressive hip osteoarthritis [11], probably because they reflect different mechanisms of cartilage degradation.

Cartilage matrix molecules including type II collagen can undergo post-translational modifications which can be either mediated by an enzymatic process or be spontaneous and age-related. Measuring post–translational-modified cartilage matrix proteins may lead to the development of biochemical markers which can give valuable information on altered biological processes related to osteoarthritis. Chondrocytes can express high levels of inducible and neuronal forms of nitric oxide synthetase which generated nitric oxide. Nitric oxide can then react with superoxide radical to form peroxynitite, a potent oxidizing radical that can in turn react with tyrosine residues of proteins to form nitrotyrosine. Two different assays recognizing a sequence (which can be either unnitrosylated [Coll 2-1] or nitrosylated [Coll 2-1 NO_2]) of the triple helix of type II collagen have been developed [12•]. Increased serum levels of Coll 2-1 and Coll 2-1 NO_2 have been reported in patients with knee osteoarthritis. One year changes of their urinary levels (but not baseline values) were modestly related to more rapid disease progression of knee osteoarthritis over 3 years [13]. It remains unclear however from these studies whether there is an additive value of investigating the nitrosylated form of type II collagen fragments in osteoarthritis. Another post-translational modification of cartilage matrix molecule is the non-enzymatic glycation which is the spontaneous condensation of reducing sugars like glucose with free amino groups in lysine or arginine residues which gives rise to the formation of advanced glycation end products (AGE). Because cartilage matrix is characterized by a very low turnover in healthy adults, AGEs accumulate in this tissue with aging and they may affect the biochemical, cellular, and biomechanical properties of cartilage [14••]. Pentosidine is one of the most studied AGEs that form a covalent cross-link between adjacent collagen molecules. Serum pentosidine was increased by 37% in 38 patients with advanced knee osteoarthritis compared with 38 healthy controls [15]. Whether pentosidine is a valuable biochemical marker for osteoarthritis remains to be demonstrated as serum and urinary levels were not associated with the extent of radiologic damage [15]. One has to remember that pentosidine is not specific for cartilage because it is also found in other aging tissues including bone. In addition pentosidine is only one of the multiple AGEs (which are for the vast majority not yet characterized) occurring in aged tissues and it is currently unknown which of these AGEs are the most relevant for osteoarthritis.

Emerging biochemical markers include those reflecting the degradation of aggrecan, the major proteoglycan of cartilage, and serum cathepsin K. Antibodies against various aggrecan neo-epitopes generated by the activity of the matrix metalloproteases and agrecanases (including ADAMTS5, which has recently been shown to play a major role in experimental osteoarthritis) [16] have developed, but clinical data with these markers are still lacking. Cathepsin K is a cysteine protease which has been suggested to play a role in cartilage and bone damage in osteoarthritis. An immunoassay for serum cathepsin K has recently been developed and increased levels were reported in patients with RA, levels correlating with radiologic damage [17]. Whether measurement of serum cathespin K will prove to be useful in osteoarthritis remains to be investigated.

Pre-analytical Factors Influencing Biochemical Markers of Osteoarthritis

Levels of biochemical markers measured in blood or urine (because assessment of synovial fluid is often impracticable) provide information on systemic skeletal tissue turnover and are not necessarily specific of the alterations occurring in the signal joint. For example, it has been shown that degenerative disease of the knees, hips, hands, and lumbar discs contributed independently and additively to urinary CTX-II levels, clearly illustrating the total body contribution to systemic levels [18•,19]. The potential contribution of intervertebral discs is of particular relevance because disc degeneration is common with ageing. Adjusting systemic levels by a total body osteoarthritis score based on radiographic damage and cartilage volume estimated by quantitative MRI may in part overcome this limitation [20]. The clearance of the markers from the joint compartment to the bloodstream is complex, varies across individuals, and can increase with inflammation, after joint mobilization and exercise. Recent investigational studies demonstrated that serum hyaluronic acid (HA) increases within the first hour after rising from bed and then remains stable in 20 patients with knee osteoarthritis [21]. Serum cartilage oligomeric matrix protein (COMP) was shown to increase significantly during running exercise [22], but also after moderate walking activity, although changes were modest and transient [23]. Serum and urinary levels of most biochemical markers also vary with sex, age, menopausal status, ethnicity, and osteoarthritis risk factors such as body mass index, as recently reported for serum HA in a large population-based study of 455 individuals [24••]. In a 6-week randomized controlled trial of patients with painful knee osteoarthritis, it has been reported that ibuprofen (2400 mg) prevented the significant elevation of CTX-II and glucosyl-galactosyl-pyridinoline (a specific biochemical marker of synovium tissue turnover) observed in patients receiving placebo [25]. Although it remains to be determined whether nonsteroidal anti-inflammatory drugs have disease-modifying effects, these data underscore that commonly prescribed therapy in osteoarthritis may be a confounding factor in biochemical marker clinical studies. Clearly pre-analytical factors contribute to the intra- and inter-subject variability

of biochemical markers levels and consequently need to be investigated and be controlled as tightly as possible.

Clinical Uses of Biological Markers for Osteoarthritis

The clinical researcher and the rheumatologist dream of a biochemical marker which can be used to diagnose the presence of the disease, preferably before radiologic evidence of joint damage, to identify patients at increased risk for disease progression and to monitor the efficacy of treatments including DMOADs.

Diagnosis

Several cross-sectional studies have found elevated or decreased levels of biological markers in knee and hip osteoarthritis, as compared with healthy sex and age-matched controls. These studies clearly demonstrated, however, that there is a large overlap in marker levels between osteoarthritis patients and controls, indicating that the measurements of a single of the currently available markers are probably insufficiently sensitive to be useful for the diagnosis of osteoarthritis. An important limitation of most of these cross-sectional studies is the lack of radiologic assessment of the controls. Thus, it is likely that a significant proportion of controls have asymptomatic osteoarthritis which would then lead to an under estimation of the true diagnostic accuracy of the markers. Another issue is that these studies included mainly patients with advanced disease as the selection was based on a radiologic Kelgreen and Lawrence (KL) score at or above 2. Because biochemical markers reflect dynamic changes in tissue turnover, their levels are likely to be altered well before radiologic damage can be observed as suggested by the proteomic analyses discussed above. Consequently, for assessing the diagnostic utility of biochemical markers, it may be more appropriate to include patients with early osteoarthritis which could be identified using sensitive imaging modalities such as MRI. In a random sample of 372 women and men from 26 to 61 years of age, it was shown that radiologic damage was present in only 17% of the subjects (mostly KL grade 1) but cartilage defects by MRI could be detected in 44% of the subjects [26]. The severity of cartilage defect of the total knee joint was significantly associated with increased urinary CTX-II levels. In another study of 377 patients with knee osteoarthritis we reported a strong association between urinary CTX-II and the severity of bone marrow abnormalities (BMA) score by MRI [27••], which has been suggested to be an early feature of osteoarthritis. Interestingly patients who showed an improvement in their BMA score during 3 months also demonstrated a significant decrease of urinary CTX-II. In the near future, many more studies relating biochemical markers with the various MRI features of the joint will be undertaken and should bring valuable information for the biological interpretation of both of these two new diagnostic modalities.

Prediction of progression

Progression in osteoarthritis shows considerable variation across individuals and the predictive capacity of clinical indices is poor. Recent longitudinal studies are in this respect encouraging as they suggest that some new biochemical markers may have a role in predicting disease progression. In a large population-based cohort of 1235 men and women, it was found that baseline levels of urinary CTX-II in the highest quartile was associated with a higher risk for radiologic progression of knee and hip osteoarthritis in the subsequent 6.6 years [28••]. Sharif et al. [29••] followed a group of patients with early knee osteoarthritis (40% with a KL score < 2) prospectively for 5 years. They showed that progression was not linear over this period and that serum COMP which was measured every 6 months was associated with this phasic pattern of progression [29••]. One could argue that assessment of progression in these studies is unreliable because based on the measurement of joint space narrowing (JSN) using standard standing anterior-posterior radiographs. This concern is especially relevant for short-term studies in knee osteoarthritis but of less critical importance for the hip and long-term evaluation. Interestingly, higher levels of serum stromelysine have recently been reported to be associated with greater JSN measured over 30 months using state of the art radiography positioning in women with knee osteoarthritis participating in a randomized trail of doxycycline [30•].

Because of the complex involvement of bone, cartilage and synovium tissue in joint damage in osteoarthritis, it is likely that only a combination of several biochemical markers will be necessary to adequately predict disease progression. In a study including 12 patients with rapidly destructive hip osteoarthritis and 28 patients with slowly progressive disease we found that the combination of urinary CTX-II and Helix-II, two biochemical markers of type II collagen breakdown, was more effective than one of these markers alone to identify patients with rapidly progressive hip osteoarthritis [11]. In a longitudinal 5-year study of 84 patients with early knee osteoarthritis, combination of serum PIIANP (a marker of type II collagen synthesis) with urinary CTX-II (reflecting cartilage breakdown) allowed to identify 92% of patients who showed radiologic progression whereas one of these two markers when used alone could identify 40% to 70% of patients who progressed [7]. In the ECHODIAH cohort of patients with hip osteoarthritis followed-up over 3 years we measured 10 different biological markers at baseline. Using principal component analyses, these markers could be segregated into five independent clusters which reflect different physiopathologic processes [31]. The combination of urinary CTX-II with HA which belonged to two of these independent clusters was more predictive than one

of these markers alone. Indeed, the third of patients with highest levels of CTX-II or HA had a risk of progression which was increased by 1.8- to two-fold compared with the rest of the patients, whereas this risk was multiplied by 3.7 in the 13% of the patients that had both markers elevated [32••].

Monitoring Efficacy of Disease-modifying Osteoarthritis Treatment

One of the main issues which currently impair efficient development of structure osteoarthritis-modifying therapies is the low sensitivity of plain radiograph, requiring long-term studies to show a significant difference between placebo and active-drug treated patients. Biological markers may prove capable of providing earlier information compared with demonstration of slowing of JSN by radiograph. The paucity of data on the potential role of biological markers for monitoring the treatment of osteoarthritis is chiefly ascribed to the absence of medications with established chondroprotective activity. In a randomized clinical trial of 137 subjects with knee pain, no significant effect of glucosamine sulfate could be demonstrated on the serum and urinary levels of the type II collagen neopitopes C2C and C12C after 6 months of treatment [33]. These negative findings can be explained by the lack of sensitivity of the particular markers utilized in this study or the lack of efficacy of glucosamine sulfate to decrease cartilage damage as the disease-modifying activity of this compound is still debated. Antiresorptive bone agents currently used for the treatment of postmenopausal osteoporosis have been suggested to play a role as DMOADs mainly because of the importance of subchondral bone remodeling in osteoarthritis initiation and/or progression. Animal models of osteoarthritis have indeed shown that agents such as the bisphosphonates risedronate, alendronate, and zoledronate, calcitonin, estrogens, and selective estrogen-receptor modulators (SERMs) could partially prevent progression of joint damage. A series of recent studies have investigated the effects of these treatments on urinary CTX-II using stored samples from randomized clinical trials in postmenopausal women. In a placebo controlled study of 171 healthy postmenopausal women, all doses of oral and transdermal estradiol produced a significant and similar 25% decrease of urinary CTX-II within 4 weeks after initiation of treatment that was sustained for 2 years [34]. The same group of investigators also reported that the SERM levormeloxifene induced a 50% decrease of urinary CTX-II in 302 postmenopausal women. Again the response was observed within 3 months, sustained for 2 years with no significant dose-dependent relationships [35••]. More recently, a dose-dependent decrease of CTX-II was reported after 3 months of treatment of postmenopausal women with oral salmon calcitonin, the decrease reaching 20% with the 1 mg dose. Finally in large randomized phase II and III trials of the bisphosphonate risedronate in patients with knee osteoarthritis, a dose dependent decrease of urinary CTX-II was also reported [36•,37]. With the exception of levormeloxifene the reduction of CTX-II was about 50% lower than that observed for the type I collagen biochemical markers of bone resorption urinary NTX-I or CTX-I. The biological and clinical interpretation of these findings requires further investigation. Indeed, the decrease of CTX-II could result from indirect effects of these drugs on subchondral bone turnover and/or a direct action on cartilage metabolism which has been suggested for example for calcitonin. Because, it remains to be shown that these therapies have indeed disease-modifying activity in humans with osteoarthritis, the clinical relevance of these changes to predict efficacy on joint damage also remains to be investigated. Interestingly enough, although risedronate did not demonstrate a significant reduction of radiologic progression compared with placebo, there was a significant relationship between the level of CTX-II measured before and 6 months after treatment and JSN independently of treatment allocation [37]. RA may serve as a model to validate biochemical markers as surrogate markers of efficacy because efficient disease-modifying antirheumatic drugs (DMARDs) are available. In a randomized study of the combined sulphasalazine, methotrexate, and prednisone therapy in early RA, we showed that the magnitude of CTX-II decrease at 3 months was associated with the changes in radiologic scores after 5 years independently of the changes in disease activity and inflammation [38••]. These data suggest that early changes of biochemical markers of cartilage turnover may predict long-term structural efficacy of DMARDs.

Conclusions

Biochemical markers of osteoarthritis are increasingly tissue-specific and the influence of the various factors that could obscure their clinical interpretation better characterized. The panel of new markers is likely to expand with the optimization of genomic/proteomic based technologies. An optimal combination of biochemical markers is likely to be useful for identifying osteoarthritis patients at increased risk for disease progression and to speed the development of DMOADs.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Gray ML, Eckstein F, Peterfy C, et al.: **Toward imaging biomarkers for osteoarthritis.** *Clin Orthop Relat Res* 2004, **427:(Suppl)**:S175–S181.
2. Marshall KW, Zhang H, Yager TD, et al.: **Blood-based biomarkers for detecting mild osteoarthritis.** *Osteoarthritis Cartilage* 2005, **13**:861–871.

3. Liao H, Wu J, Kuhn E, et al.: **Use of mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of patients with rheumatoid arthritis.** *Arthritis Rhem* 2005, **50:**3792–3803.

4.•• Xiang Y, Sekine T, Nakamura H, et al.: **Proteomic surveillance of autoimmunity in osteoarthritis: identification of triosephosphate isomerase as an autoantigen in patients with osteoarthritis.** *Arthritis Rheum* 2004, **50:**1511–1521.

An intriguing study that underscores the potential importance of autoimmunity in osteoarthritis and the application of proteomics for discovery of new biochemical markers

5.• Lamers RJAN, van Nessselrooij JHJ, Kraus VB, et al.: **Identification of a urinary profile associated with osteoarthritis.** *Osteoarthritis Cartilage* 2005, **13:**162–168.

The first evidence of a unique urinary metabolite profile in patients with ostoearthritis.

6. Ling QM, Patel D, Zhan M, et al.: **Changes in selected proteins associated with osteoarthritis development in the Baltimore Longitudinal study of aging (BSLA).** *Arthritis Rheum* 2005, **52(Suppl):** S256.

7. Garnero P, Sharif M, Charni N, et al.: **A 5 year longitudinal study of type II collagen synthesis and degradation and their association with disease progression in early knee osteoarthritis.** *Arthritis Rheum* 2005, **52(Suppl):** S74.

8.• Rousseau JC, Sandell LJ, Delmas PD, Garnero P: **Development and clinical application in arthritis of a new immunoassay for serum type IIA procollagen NH2 propeptide. Human Reproduction.** *Methods Mol Med* 2004, **101:**25–38.

This study described in detail the technical development of an immunoassay for a promising marker of cartilage synthesis.

9. Garnero P, Desmarais S, Charni N, Percival MD: **The type II collagen fragments HELIX-II and CTX-II reveal distinct enzymatic pathways of cartilage collagen degradation: Diagnostic and therapeutic implications in rheumatoid arthritis and osteoarthritis.** *Arthritis Rheum* 2005, **44(Suppl):**S56.

10.•• Charni N, Juillet F, Garnero P: **Urinary type II collagen helical peptide (Helix II) as a new biochemical marker of cartilage degradation in patients with osteoarthritis and rheumatoid arthritis.** *Arthritis Rheum* 2005, **52:**1081–1090.

A report of a new type II collagen degradation fragment which provides evidence that combination of markers reflecting different mechanisms of cartilage degradation is more effective than a marker alone to identify patients with RA at high risk for progression.

11. Garnero P, Conrozier T, Juillet F, et al.: **Urinary type II collagen helical peptide (HELIX-II) levels are increased in patients with a rapidly destructive hip osteoarthritis.** *Ann Rheum Dis* 2005, **64(Suppl 3):**OP0117.

12.• Deberg M, Labasse A, Christgau S, et al.: **New serum biochemical markers (Coll 2-1 and Coll 2-1 NO2) for studying oxidative-related type II collagen network degradation in patients with osteoarthrtitis and rheumatoid arthritis.** *Osteoarthritis Cartilage* 2005, **13:**258–265.

An example of using post-translational modifications (nytrosilation of tyrosine residues) of proteins for developing process-specific biochemical markers of osteoarthritis.

13. Deberg M, Labasse AH, Collette J, et al.: **One year increase of Coll 2-1, a new marker of type II collagen is highly predictive of radiological OA progression.** *Osteoarthritis Cartilage* 2005, **13:**258–265.

14.•• Verzijl N, Banlk RA, Tekoppele JM, DeGroot J: **Ageing and osteoarthritis: a different perspective.** *Curr Opin Rheumatol* 2003, **15:**616–622.

An excellent review of the role of non-enzymatic glycation in the physiopathology of ostoearthritis.

15. Senolt L, Braun M, Olejarova M, et al.: **Increased pentosidine, an advanced glycation end product, in serum and synovial fluid from patients with knee osteoarthritis and its relation with cartilage oligomeric matrix protein.** *Ann Rheum Dis,* 2005, **64:**886–890.

16. Glasson SS, Askew R, Sheppard B, et al.: **Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis.** *Nature* 2005, **434:**644–648.

17. Skoumal M, Haberhauer G, Kolarz G, et al.: **Serum cathepsin K levels of patients with lonstanding rheumatoid arthritis: correlation with radiological destruction.** *Arthritis Res Ther* 2005, **7:**565–570.

18.• Garnero P, Sornay-Rendu E, Arlot M, et al.: **Association between spine disc degeneration and type II collagen degradation in postmenopausal women: the OFELY study.** *Arthritis Rheum* 2004, **50:**3137–3144.

One of the first studies investigating the total body contribution to systemic levels of a biochemical marker of cartilage degradation by radiographic assessment of multiple joints.

19. Meulenbelt I, Kloppenburg M, Kroon HM, et al.: **Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis (OA) in hip, knee, and facet joints in subject with familial OA at multiple sites: the GARP study.** *Ann Rheum Dis* 2005, Aug 3 [Epub ahead of print].

20. Moscowitz RW, Holderbaum D, Hooper MM: **Total quantitative osteoarthritis load (TQOL) assessment tool: a proposed methodology for biomarker correlations.** *Arthritis Rheum* 2005, **52(Suppl):**S71.

21. Crisione LG, Elliot AL, Stabler T, et al.: **Variation of serum hyaluronan with activity in individual with knee osteoarthritis.** *Osteoarthritis Cartilage* 2005, **13:**837–840.

22. Kerstin UG, Studbendorff JJ, Schmidt MC, Bruggemann G-P: **Changes in knee cartilage volume and serum COMP concentration after running exercise.** *Osteoarthritis Cartilage* 2005, **13:**925–934.

23. Mundermann A, Dyrby CO, Andriacchi TP, King KB: **Serum concentration of oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults.** *Osteoarthrtis Cartilage* 2005, **13:**34–38.

24.•• Elliot AL, Kraus VB, Luta G, et al.: **Serum hyaluronan levels and radiographic knee and hip osteoarthritis in African Americans and Caucasians in the Johnston County Osteoarthritis Project.** *Arthritis Rheum* 2005, **52:**105–111.

A large population based-study showing the influence of age, sex, and ethnicity on serum HA but still demonstrating the strong independent association of this synovitis marker with radiologic osteoarthritis of knee and hip

25. Gineyts E, Mo JA, Ko A, et al.: **Effects of ibuprofen on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis.** *Ann Rheum Dis* 2004, **63:**857–861.

26. Ding C, Garnero P, Cicuttini F, et al.: **Knee cartilage defects: association with early radiographic osteoarthritis, decreased cartilage volume, increased joint surface area and type II collagen breakdown.** *Osteoarthritis Cartilage* 2005, **13:**198–205.

27.•• Garnero P, Peterfy C, Zaim S, Schoenharting M: **Bone marrow abnormality on magnetic resonance imaging is associated with type II collagen degradation in knee osteoarthritis: a three-month longitudinal study.** *Arthritis Rheum* 2005, **52:**2822–2829.

The first study relating bone marrow edema by MRI with a biochemical marker of type II collagen degradation in knee osteoarthritis.

28.•• Reijman M, Hazes JM, Bierna-Zeinstra SM, et al.: **A new marker for osteoarthritis: cross-sectional and longitudinal approach.** *Arthritis Rheum* 2004, **50:**2471–2476.

The largest and longer study of urinary CTX-II as a prognostic marker for progression of knee and hip osteoarthritis.

29.•• Sharif M, Kirwan JR, Elson CJ, et al.: **Suggestion of nonlinear or phasic progression of knee osteoarthritis based on measurements of serum cartilage oligomeric matrix protein levels over five years.** *Arthritis Rheum* 2004, **50:**2479–2488.

This longitudinal 5 year study shows that radiologic disease progression is non linear in knee osteoarthritis and that serum COMP can track this phasic pattern.

30.• Lohmander LS, Brandt KD, Mazzuca SA, et al.: **Use of plasma stromelysin (matrix metalloproteinase 3) concentration to predict joint space narrowing in knee osteoarthritis.** *Arthritis Rheum* 2005, **52:**3160–3167.

One of the few longitudinal study using state of the art radiography to validate biochemical markers as a predictor of disease progression

31. Garnero P, Mazières B, Guéguen A, et al.: **Cross-sectional association of ten molecular markers of bone, cartilage and synovium with disease activity and radiological joint damage in hip osteoarthritis patients: the ECHODIAH Cohort.** *J Rheumatol* 2005, **32:**697–703.

32.•• Mazieres B, Garnero P, Gueguen A, et al.: **Molecular markers of cartilage breakdown and synovitis are strong independent predictors of structural progression of hip osteoarthritis. The ECHODIAH cohort.** *Ann Rheum Dis,* In press.

The largest longitudinal study in hip osteoarthritis investigating 10 different biochemical markers and showing that the combination of a cartilage degradation marker with a synovitis marker predicts independently and additively disease progression

33. Cibere J, Thorne A, Kopec JA, et al.: **Glucosamine sulfate and cartilage type II collagen degradation in patients with knee osteoarthritis: randomized discontinuation trial employing biomarkers.** *J Rheumatol* 2005, **32:**896–902.

34. Ravn P, Warning L, Christgau S, Christiansen C: **The effect on cartilage of different forms of application of post-menopausal estrogen therapy: comparison of oral and transdermal therapy.** *Bone* 2004, **35:**1216–1221.

35.•• Christgau S, Tanko LB, Cloos PAC, et al.: **Suppression of elevated cartilage turnover in postmenopausal women and in ovariectomized rats by estrogen and a selective estrogen-receptor modular (SERM).** *Menopause* 2004, **11:**508–518.

This study describes a new potential animal model of cartilage degeneration and shows the chondroprotective effects of SERMs in this particular model.

36.• Spector TD, Conaghan PG, Buckland-Wright JC, et al.: **Effect of risedronate on joint structure and symptoms of knee osteoarthritis: results of the brisk: randomized, controlled trial [ISRCTN01928173].** *Arthritis Res Ther* 2005, **7:**R625–R633.

The first clinical study of the bisphosphonate risedronate in knee osteoarthritis showing dose dependent response of a biochemical marker of cartilage degradation.

37. Garnero P, Bingham C, Aronstein W, et al.: **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.** *Arthritis Rheum* 2004, **50(Suppl):**S656.

38.•• Landewé R, Geusens P, Maarten B, et al.: **Markers for type II collagen breakdown predict the effect of disease modifying treatment on long-term radiographic progression in patients with rheumatoid arthritis: the Cobra study.** *Arthritis Rheum* **50:**1390–1399.

In this study the magnitude of the changes of urinary CTX-II after 3 months was predictive of radiologic progression at 5 years in patients with early RA treated with a combined therapy. These data suggest that biological markers may be valid surrogate markers for treatment efficacy in clinical trials of DMARDs.