# Biochemical Markers for Osteo-arthritis: Is There any Promising Candidate?

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## Abstract

Osteo-arthritis (OA) is the most common degenerative joint disease. Progressive destruction of articular cartilage is one of the prominent features of the disease. The diagnosis of the disease is generally based on clinical and radiographic findings, which are insufficient to determine early cases and predict disease course. There is a need for biomarkers that help early diagnosis, assess disease activity, predict prognosis and monitor therapeutic effects in patients with OA. There is a growing number of publication considering candidate markers in this field. Aim of this paper is to review recent assays that study biochemical markers which reflect cartilage, synovium and bone turnover and their clinical uses in patients with OA.


***Key words:*** **Osteo-arthritis, biomarkers, bone, cartilage, synovium.**

## Introduction:

Osteo-arthritis (OA) is the most common joint disorder characterised by progressive cartilage destruction, causing pain and loss of function. OA affects millions of individuals each year and becoming the most important pain cause of geriatric population. Articular cartilage, synovium and bone contribute to the pathogenesis of the disease. The diagnosis of OA is mainly based on clinical observation and radiologic aspects. Bone sclerosis, osteophyte formation and joint narrowing are well known radiological features of OA. Progression of cartilage destruction is evaluated with the measurement of joint space width by radiography. However radiologic evaluation is insufficient to determine early cases, when no significant joint damage has occurred yet. Also it is not possible to evaluate minor changes of cartilage by conventional radiography.

Therefore, there is an urgent need for new assessment tools with high sensitivity. In this respect laboratory markers have drawn great interest in recent years. Such molecular markers are promising for improving diagnosis, assessment of disease activity, prognosis and monitoring therapeutic effects in patients with OA. This report reviews recent assays that study biochemical markers which reflect cartilage, synovium and bone turnover and their clinical uses in patients with OA.


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**Table 1:** *Biochemical Markers for Osteo-arthritis* [1-3]

| Tissue | Synthesis | Degradation |
|---|---|---|
| Bone | PICP, PINP, OC, ALPbone | PYD, DPD, CTX-1, NTX-1, ICTP, TRAP, BSP, Cathepsin K, Helical peptide |
| Cartilage | PIICP, PIIANP, PIIBNP, YKL-40, CS, CD-RAP | PYD, CTX-II, C2C, C12C,TIINE, Helix-II, Coll2-1, COMP, KS, Aggrecanase neopeptides, Coreprotein MMPs |
| Synovium | YKL-40, COMP, MMPs, HA, PIINP | PYD, CTX-I, NTX-I, Glc-Gal-PYD |
| Systemic inflammation | CRP, hsCRP, TGFβ1, TNFα, IL-6, IL-1, RAGE, ECP | |

## Biochemical Markers:

A biochemical marker refers to characteristic that is released from connective tissue matrices and objectively measured in biological fluids. An appropriate marker should be disease specific. In addition a good marker should be able to reflect actual disease activity, monitor changes with therapy and can predict the prognosis. Recently numerous markers have been suggested for identifying and monitoring OA. They can reflect cartilage and synovial breakdown and synthesis, bone turnover and inflammation (Table 1)[1-3]. These products can easily be obtainable from body fluids such as blood, urine or synovial fluids.

## Cartilage Matrix Protein (COMP):

Cartilage matrix protein (COMP) is non-collagenous biochemical marker of cartilage degradation. It is primarily isolated from the extracellular matrix of cartilage[4]. High serum and synovial fluid levels were detected in various disease such as rheumatoid arthritis, OA, juvenile idiopathic arthritis and psoriatic arthritis[5-7]. Studies suggest that serum COMP levels can be used as a marker for cartilage destruction associated with OA. A meta-analysis by Hoch *et al*[8] concluded that serum COMP levels are elevated in patients with radiographic knee OA and higher levels of serum COMP are associated with radiographic OA severity. In a study[9] that examines the relationship between cartilage markers and cartilage loss on MRI in patients with knee OA, only COMP was found to be a predictor of cartilage loss. In another survey[10] femoral cartilage thickness detected by ultrasound was found inversely related to serum COMP levels in patients with early stage knee OA. Authors also reported that for every unit increase in COMP level, there was 33 % higher risk for tibiofemoral osteophyte progression[11]. In a study[12] with two hundred and seventy-two patients with knee OA patients, higher serum COMP levels were correlated with non-symptomatic narrowing of the articular space. Similarly, Conroizer *et al*[13] found that serum COMP level has a positive correlation with joint space narrowing in hip OA. In a recent study Golightly *et al*[14] investigate COMP, hyaluronic asid (HA), keratin sulphate (KS) and high sensitivity C-reactive protein (CRP) as a predictor of radiographic knee OA. Authors have suggested that high levels of COMP and HA may predict incident radiographic knee OA[14]. According to the results of another survey[15], serum levels of COMP have been correlated with rapidly progressing OA and remain significantly high in first 3 years of disease duration. All these findings suggest that serum COMP levels may be a useful assessment tool for OA. On the other hand COMP is particularly abundant in tendons, ligament and meniscus. Therefore increased concentrations can be related to injuries of these structures [16, 17]. Also serum concentrations vary by ethnicity, gender, age and exercise[18-20].

## Type II Collagen Biomarkers:

Type II collagen is the most important protein of human cartilage and it is relatively specific for the hyaline cartilage. Because altering in articular cartilage turnover is the main pathology in OA, type II collagen has been investigated for a potential marker[21]. Type II collagen is composed of a triple helix of three identical alpha chains. It is firstly synthesised as a 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. These propeptides are clevead-off during the subsequent maturation stage and released into the biological fluids. Also there are alternative forms of procollagen that differ by the presence of a 69 aminoasid sequence in the N-propeptide. During the degradation process of type II collagen, different molecules are released in biological fluids. These include fragments of triple helix, collagenase neo-epitopes and C-terminal crosslinking telopeptides. Type II collagen biomarkers are summarised in Table 2.

**Table 2:** *Type II Collagen Biomarkers*[21]

| | |
|---|---|
| Cleavage neoepitopes | C2C, C1,2C, TIINE, Coll2-1/4N1, Coll2-1/4N2 |
| Denaturation epitopes | Coll2-1, Coll2-1/NO2, Helix-II, CB-11 (COL2-3/4m), AH8, AH9, AH12 |
| Telopeptide epitopes | Col2CTx, CTX-II |
| Propeptide epitopes | CPII, PIIANP |

C terminal crosslinking telopeptides (CTX-II) and Helix II are markers of collagen degradation. These two markers are believed to reflect different but complementary parts of cartilage degradation.While CTX-II is a fragment of C-telopeptides region, Helix-II is fragment of the helical domain of Type II collagen. Recent studies[22-24] have shown that urinary levels of CTX-II and Helix-II were significantly higher in patients

with OA compared with healthy controls. CTX-II were found to be associated with radiological progression in patients with knee and hip OA and this association is stronger in participants with joint pain[11,25]. Contrarily in another trial[26] CTX concentrations were correlated with radiologic progression but were not correlated with clinical status. High levels of urinary CTX-II are associated with rapid progressive disease[27,28]. Urinary levels of CTX-II is also reported to be linked to the efficacy of treatment in OA [29]. Levels of CTX-II and Helix-II are influenced from patients body mass index[23,30,] but there are conflicting data about relationship between age and urinary CTX levels [23,30].

N propeptide of type IIA procollagen (PIIANP) is one of the two splice forms of type-II procollagen. It is mainly expressed in embryonic cartilage and believed to re-expressed in osteo-arthritic cartilage[27,31]. Recent studies have shown that its combination with CTX-II could distinguish patients at high risk for rapidly progressive joint damage in OA. Because this two markers represent imbalance between cartilage synthesis (PIIANP) and degradation (CTX-II)[27,28]. Rousseau *et al*[32] found decreased levels of PIIANP in patients with knee OA and RA suggesting that type IIA collagen synthesis may be altered in these arthritic diseases. Sharif *et al*[28] assessed serum concentration of PIIANP and urinary concentration of CTX-II for five years in patients with mild-to-moderate knee OA.The authors observed that over the 5 -year study period average PIIANP and CTX-II levels were higher in patients with progressive disease. The risk of progression was highest in patents with 5 year levels of PIIANP in the highest quartile and/or CTX-II in the two highest quartiles [28]. Kumm *et al*[10] report that tendon calcification is associated with higher levels of PIIANP in men with early stage knee OA. The investigators conclude that males showed a tendency toward synthesis and females showed a tendency toward degradation, during early stages of the disease [10].

There are also promising type II collagen biochemical markers such as Type II collagenase neoepitopes (C2C, C1-2C, TIINE), Coll 2-1, Coll 2-1 NO2, CPII, CPIII which need further human studies. In a recent study Ishijima *et al*[33] suggest that cartilage turnover markers such as CTX-II, C2C, CPIII, bone resorption marker NTX and HA were all significantly increased in subjects with knee pain independent of grade. Coll 2-1 and Coll 2-1 NO2 levels tended to be associated with radiological progression of OA[34]. Deberg *et al*[35] demonstrated Coll 2-1 levels were decreased after total hip or knee arthroplasty. In contrast Coll 2-1 NO2 levels remained elevated. This finding suggest that Coll2-1 can be a useful disease specific marker for monitoring structural changes in a single joint [35]. CPII levels in synovial fluid was elevated in patients with OA compared with healthy subjects [36]. Also CPII levels found to be predictive of radiographic progression in early stage OA [37].

## Glucosyl-Galactosyl-Pyridinoline (Glc-Gal-PYD):

Ürinary Glc-Gal-Pyd is a marker of synovial tissue turnover and reflects synovial matrix degradation. It has been shown to be associated with cartilage loss and radiographic knee OA [26,38]. Gineyts *et al*[39] designed a study that aimed to evaluate the effect of ibuprofen on CTX-II and Glc-Gal-Pyd levels in knee OA. At baseline urinary levels of CTX-II and Glc-Gal-Pyd were higher in patients with knee swelling. After 4-6 weeks of treatment, placebo group patients with knee effusion had significantly higher urinary CTX-II and Glc-Gal-Pyd concentrations, compared with ibuprofen group[39]. A trial which considered relation between markers and disease activity in patients with knee OA concludes that Gly-Gal-Pyd and CTX-II were the most important predictors of the WOMAC index and joint damage, respectively[26].

## Hydroxyproline and Lysylpridinoline:

Hydroxyproline (HP) and lysylpridinoline (LP) are components of collagen. They are both derived from bone. HP is also derived from cartilage. Otterness *et al*[40] carried out a study in 39 patients with knee or hip OA. They investigate 14 molecular markers used to monitor OA. There was a strong correlation between urinary HP levels and baseline clinical status of the patients. However HP levels did not reflect the clinical changes after one year follow-up[40]. Thompson *et al*[41] have reported a correlation between radiological score and collagen crosslinks. In contrast Astbury *et al*[42] have found higher urinary levels of collagen cross-links in patients with OA compared with healthy controls, but no associations with radiological grades. Overall, collagen cross-links may be useful for understanding cartilage and bone destruction in OA.

## Aggrecan Biomarkers:

Aggrecan is the major proteoglycan in the articular cartilage. Aggrecan markers are also studied as

potential molecular markers of cartilage turnover. There are variable reports about keratan sulphate (KS) depending on the antibodies used[43,44]. Interestingly, Nakajima *et al*[45] reported significant reduction in KS levels after arthroscopic surgery in patients with knee OA. Epitope 846 of chondroitin sulphate (CS) reflects proteoglycan synthesis. Studies found that serum levels of epitope 846 decreased in patients with OA [31]. Also serum hyaluronic acid (HA) is considered as a potential biomarker in OA. HA levels were shown to be increased in sera of patients with knee and hip OA and suggested to have a predictive value for further radiographic progression [26,46-49]. Matrix metalloproteinases (MMPs) are endopeptides that are capable of cartilage matrix degradation. MMPs levels reflect inflammation and predict joint erosion in rheumatoid arthritis. Similarly in the OA patients serum levels of MMP3 has been shown to be increased[50]. In a randomised prospective study nimesulide treatment reduced serum levels of MMP-3 and MMP-13 in patients with flare-up of OA[51]. In this study the decrease in levels of MMP-13 correlated significantly with the decrease in levels of CTX-II and HA. Endogenous inhibitors of MMPs are called as tissue inhibitors of matrix proteinases (TIMPs). Among entire types of TIMPs, TIMP-1 has the highest affinity for MMP-3 and MMP-13[52]. Chevalier *et al* [53] investigated serum levels of TIMP-1 and hyaluronic acid in hip OA. The authors found that serum levels of TIMP-1 is beneficial in discriminating slowly progressive disease from rapidly progressive one [53].

## YKL-40:

YKL-40 (human cartilage glycoprotein-39) is a recently discovered human glycoprotein which is related to histopathological changes in synovium and cartilage. High levels of YKL-40 have been measured in serum and synovial fluid of patients with OA especially in later stages[54]. Zivanovic *et al* reported that YKL-40 concentration is correlated with the level of cartilage destruction and can be used for assessment of destruction.

## Osteocalcin:

There have been a number of studies considering osteocalcin (OC) as a biomarker for OA. Joint space narrowing was significantly associated with serum OC level in patients with hand OA[55]. Higher OC levels were significantly correlated with decreased rate of cartilage loss and radiologic progression of knee OA[56,57]. In contrast Naito *et al*[58] demonstrate that OC levels are not elevated in patients with OA. Similarly Jung *et al*[59] found no relationship between serum OC concentrations and ultrasonographic findings of knee OA.

## Inflammatory Biomarkers:

Although OA is commonly known as a non-inflammatory disease, markers that reflect inflammatory process also have been studied. Otterness *et al* investigated 14 serum and urine markers in an attempt to find association with particular clinical end points. Swelling of the joint was correlated with inflammation markers. CRP was the most highly correlated. Elevated levels of high sensitivity CRP predict cartilage loss in OA and poorer outco mes in knee arthroplasty[60, 61]. At the first year assessment the change in patient related clinical variables such as, patient self assessment, pain on weight bearing and stiffness was correlated with TGFβ1. In an animal study higher levels of synovial TGFβ1 predict the later development of more severe OA changes[62]. TNFα, receptor for advanced glycation endproducts (RAGE), IL-6, IL-1 are other assessed markers of inflammation for OA.

## Adipokines:

Adipokines (adiponectin, leptin and nesfatin-1) are cytokines released from adipose tissue. They also secreted from osteoblasts, synoviocytes and chondrocytes and therefore thought to be linked to OA. Elevated levels of adiponectin leptin and nesfatin-1 were shown in synovial fluids of patients with OA. In addition, they found to be correlated with disease progression [63-66].

## Conclusions:

There is an increasing interest in the use of biochemical markers in patients with OA, especially in order to predict disease progression and monitoring the treatment. Also new markers have been investigating to identify healthy individuals at high risk for the development of OA. The ESCEO working group have been identified avenues for future research in this field. According to their recommendation, further studies must be performed in order to reveal mechanisms of OA, development of new biomarkers, assays and technological development, prognosis and patients under risk of OA[3]. Although, there are promising candidate markers, none of them have been specifically recommended for clinical usage yet.

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