

## International Journal of Bone and Rheumatology Research (IJBRR)

### Biochemical Markers in Osteoarthritis

Review Article

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#### Abstract

Osteoarthritis (OA) is a chronic disease with a long silent period. The hallmarks of osteoarthritis (OA) include cartilage loss that leads to joint destruction and severe impairment of mobility. Involvement of subchondral bone and synovial tissue is well documented. OA is the most prevalent cause of disability in the aging population of developing countries. The diagnosis is generally based on clinical symptoms and radiographic changes. However, X-ray has a poor sensitivity that does not allow an early detection of OA or the monitoring of joint damage progression.

Another imaging technique is the magnetic resonance imaging (MRI). Although this medical test is more sensitive than plain radiography, it is more expensive and can't be routinely applied to many patients. The limitations offered by such tools have cleared the need to identify more specific biological markers, which evaluate quantitative variations in joint remodeling, diagnostic, prognostic and efficacy of intervention.

OA affects cartilage, subchondral bone, and synovium. Thus, molecules derived from these tissues could be considered as candidates for biological markers in OA, as these molecules have a role in metabolic processes in the joints. Recent data indicates that some markers could be valuable to diagnose, predict OA progression and assess therapeutic response; however, the interpretation of results should be careful because tissue specificity, clearance rates and circadian variations are still under investigation in most of biomarkers. Although biomarkers could be considered valuable tools, they still have some limitations in clinical practice and it is necessary to develop and validate specific and sensitive biomarkers.

**Keywords:** Osteoarthritis, Biomarkers.

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**Received:** April 11, 2015

**Accepted:** May 05, 2015

**Published:** May 09, 2015

**Citation:** Rubén Daniel Arellano Pérez Vertti, et al., (2015) Biochemical Markers in Osteoarthritis. *Int J Bone Rheumatol Res*, 2(2), 11-17.

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#### Introduction

Osteoarthritis (OA) is the most frequent degenerative joint disease and one of the leading causes of morbidity and economic burden on health resources. It is a slow progressive disease which alters all tissues of the affected joint with a long asymptomatic period [1].

OA includes progressive degradation of cartilage, menisci, ligaments, synovial inflammation and changes to the subchondral bone. Current diagnosis of osteoarthritis is mainly based on radiographic criteria (eg, joint space width, osteophyte formation, subchondral sclerosis) and clinical symptoms (eg, pain, rigidity and loss of function) [2].

Although plain radiography is considered to be the gold standard, not only to support diagnosis but to estimate the extent of the disease, its poor sensitivity does not allow early detection of joint degradation and the monitoring of potential treatments; additionally, radiography may not show early biochemical changes within joint, as these may occur many years before symptoms become apparent. Unfortunately, when the patient seeks for medical attention, the disease has already progressed in such a way that the treatment options are limited to surgical alternatives (total joint replacement).

Magnetic resonance imaging (MRI) is a more sensitive imaging method but it is still less used than X-rays due to cost and limited availability.

For that reason, as the prevalence of OA is increasing and early detection of OA has become a clinical challenge, biochemical

markers for OA have been proposed to be useful tools not only for early diagnosis and prognostic purpose but for drug development, monitoring intervention and for the development of personalized evidence-based action plans [3].

A biomarker has been defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention” [4].

Biochemical markers can inform about the molecular events underlying the structural joint changes that characterize knee OA. In addition, they can suggest which metabolic processes may be involved in the development of knee pain [5, 6].

There have been several attempts to improve the accuracy on early diagnosis of OA. In some studies, high sensitive C - reactive protein (hsCRP) has been considered as an early biomarker in OA supporting a pathophysiological role of inflammation in the disease process. However, the association of hsCRP with some other entities (eg. obesity) has limited its use as a tool for early diagnosis of OA [7, 8].

In this review, we describe and summarize the current knowledge of biochemical markers related to components of synovial joints (collagen, proteoglycans, and synovium and subchondral bone metabolism).

### Biped Classification

The Osteoarthritis Biomarkers Network has developed the BIPED scheme to provide a common communication on this field. Burden of disease, investigative, prognostic, efficacy of intervention and diagnostic are the components of this classification scheme [9].

A sixth category has been added recently under the acronym BIPED: “safety”, (BIPEDS) considering that safety is an important issue for more invasive investigations (eg, exposure to drugs, radiation or contrast agents) [3].

Table 1 depicts BIPED classification.

### Biochemical Markers in OA

The study of biochemical markers in OA has recently received attention as alterations involved in the disease include the three main components of the sinovial joint: cartilage, synovium and subchondralbone [10].

#### Articular Cartilage Metabolism Biomarkers

Articular cartilage is mainly composed of water, collagen (most abundantly type II), proteoglycans (aggrecan), glycoproteins and chondrocytes. A balance between catabolic and anabolic processes normally maintains integrity of tissue; such balance allows keeping the mechanical and physiological properties of cartilage.

Articular cartilage is the target tissue in OA and its gradual degradation causes loss of joint function. It’s described that collagen type II disruption is an early event during the cartilage degradation, preceding proteoglycans loss [11].

#### Biomarkers reflecting collagen type II synthesis activity

During collagen type II synthesis and secretion process, N and C propeptides are released from the procollagen molecules. For that reason, it is rational to measure biomarkers that reflect the balance between synthesis and degradation of type II collagen.

There are two alternative forms of Type II procollagen. The difference between these two molecules is determined by the presence (IIA) or absence (IIB) of a 69 aminoacids sequence in the N-propeptide. Several studies using assays to measure procollagen type II C-terminal propeptide (PIICP) and the IIA form of type II collagen N-propeptide (PIIANP) in synovial fluid and serum, respectively, have detected changes in levels of these two markers in patients with OA [12-16].

**Table 1. Cartilage, bone and synovium biomarkers mainly studied according to BIPED classification.**

TISSUE	MARKER	FLUID	BURDEN	INVESTIGATION	PROGNOSTIC	EFFICACY	DIAGNOSTIC
CARTILAGE							
Type II collagen	PIIANP*	Serum			*		
	PIICP*	Serum, SF			*		*
	CTX-II**	Urine, SF	*		*	*	*
	Coll 2-1**	Urine, serum			*		
	Coll 2-1 NO2**	Urine, serum			*		
	C2C**	Urine, serum			*	*	
	C1,2C	Urine, serum			*		*
Aggrecan	Epitope 846*	SF				*	
Nonaggrecan and noncollagenous-proteins	COMP**	Serum, SF	*		*		*
Synovium	Glc-Gal-PYD	Urine				*	
	YKL-40	Serum, SF	*			*	
	Hyaluronicacid (HA)	Serum	*		*		

\*Markers of synthesis; \*\*Markers of degradation; SF sinovial fluid

**Biomarkers reflecting collagen Type II degradation**

C-telopeptide of type II collagen (CTX-II) is a biomarker extensively studied which measures concentrations in urine. CTX-II is the result of collagen type II degradation by collagenases at 3/4 length of the triple helix. This degradation process results in two fragments: 1/4 length and 3/4 length. CTX-II assays recognize on the 1/4 length a sequence of 6 aminoacids in the C telopeptide of type II collagen. Elevated levels of UCTX-II have been reported in OA, but also in Rheumatoid Arthritis (RA), non- mineralized cartilage lesions, bone marrow lesions and subcondral bone in animal models. Thus, it has been suggested that CTX-II is not a specific marker of articular cartilage degradation, but could also reflect the remodeling of calcified cartilage [17-21].

Two assays detect the 3/4 fragments by its C-terminal neo-end have been described: The Col 2-3/4 assay (short; C1,C2) recognizing both Type I and typeII collagen because of sequence homology, and the Col 2-3/4 assay (long mono; C2C) recognizing specifically Type II collagen [22].

Protein nitration appears as a key phenomenon in OA. At this respect, two immunoassays detected elevated serum levels of a peptide of 9 amino acids (Coll 2-1) or its nitrated form (Coll 2-1 NO<sub>2</sub>) inpatients with OA and RA and the ratio Coll2-1 NO<sub>2</sub>/Coll 2- 1 was significantly higher in RA than in OA subjects [23].

There are some other biomarkers related to collagen metabolism such as type II collagen α chains collagenase neoepitope (α-CTX-II)and Glc-Gal-PYD, collagen type II-specific neoepitope (C2M), C-terminal telopeptide of collagen type I (CTX-I, α-CTX-I), N-terminal telopeptide of collagen type I (NTX-I), and amino terminal propeptide of collagen type I (PINP) [24-27].

Table 2 depicts some biomarkers related to articular cartilage degradation and synthesis.

**Biomarkers related to proteoglicans metabolism**

From 10 to 20% of wet weight of cartilage matrixare proteoglycans. These molecules play two fundamental roles in this tissue: the compressive function and maintaining the fluid and electrolyte balance in the articular cartilage.

There are two major classes of proteoglycans found in articular cartilage: aggrecans, consisting in large aggregating proteoglycan monomers and small proteoglycans including decorin, biglycan and fibromodulin [28].

**Aggrecan**

Aggrecan is the major proteoglycan in the articular cartilage. This molecule plays an important role in maintaining the proper functioning of articular cartilage as it provides a hydrated gel structure (via its interaction with hyaluronan and link protein) that endows the cartilage with load-bearing properties [29].

The level of aggrecan synthesis is analyzed with antibodies recognizing the epitope 846 located on the chondroitin sulfatechains, which increases significantly in OA cartilage [30].

The catabolism of aggrecan is a proteolytic process mediated by aggrecanase activity. Aggrecanases cleave the core protein of aggrecan at several sites generating neoepitopes. These neoepitopes can be detected by several assays.

Aggrecanases are members of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) gene family and have been designated ADAMTS-4 and ADAMTS-5, respectively [31].

Through different preclinical models of arthritis, it's been shown that ADAMTS-5 is protease responsible for driving cartilage loss [32-34].

The presence of aggrecan neoepitopes has shown to be associated with the presence of osteoarthritis. In urine, ARGS (Aggrecan Antibody, N-terminal neoepitope) assay showed a significant elevation in patients with OA [35, 36].

Recently Germaschewski and colleagues developed a novel assay to evaluate ARGS neoepitope concentrations in OA patients. In this study, ARGS neoepitope appears not only as promising prognostic/stratification marker to facilitate patient selection but as an early pharmacodynamic marker for OA therapeutic trials [37].

The core protein of aggrecan has many chondroitin sulfate and keratan sulfate (KS) chains. Several assays used to assess KS levels in serum or sinovial fluid suggest that KS could be a potential marker of cartilage destruction [38].

**Other non-collagenous proteins**

**COMP:** COMP (Cartilage Oligomeric Matrix Protein) is a non-collagenous protein present in cartilage. It is a 524-kDa homopentameric, extracellular matrix glycoprotein member of the thrombospondin family of calcium binding proteins [39].

**Table 2. Cartilage collagen Type II degradation and synthesis biomarkers**

BIOMARKER AND MOLECULE TARGET	CARTILAGE METABOLISM	BIOLOGICAL FLUID
CTX-II Type II collagen	Degradation	Urine
PIICP C-propeptide of type II collagen	Synhtesis	Synovial fluid, serum
PIIANP N-terminus propeptide of type II procollagen, splice variant A	Synhtesis	Synovial fluid, serum
C1, C2 Type II collagen fragment	Degradation	Synovial fluid, serum
C2C Type II collagen fragment	Degradation	Synovial fluid, serum

Although function of COMP remains unclear, it may have a role in endochondral ossification, interacting with collagen fibrils via each C-terminal globule, for extracellular matrix stabilization, influencing fibril formation for collagens type I and II accelerating fibrillogenesis and binding to aggrecan, mediating the organization of cartilage matrix for its load bearing function [40, 41].

It is important to highlight that COMP levels increase with age, with the number of affected joints, are higher in men than in women and differ among ethnicities [42].

In different studies, high level of COMP was detected in all fluids, but was ten times higher in synovial fluid than in serum indicating preferential release from the affected joints [43].

COMP has shown to be a diagnostic and prognostic biomarker of arthritis, to correlate with the disease severity and have the potential to be used for monitoring articular cartilage destruction and response to different therapeutic modalities [42, 44, 45].

Deamidation is a nonenzymatic mechanism of amino acid damage and aging occurring in numerous proteins. An assay developed against a desaminated form of COMP (D-COMP) has shown that serum levels were correlated with hip OA suggesting that D-COMP could be the first OA specific biomarker in a specific joint [46].

Finally, Henrotin et al., have proposed a specific immunoassay through proteomic approach, showing Fibulin-3 (Fibulin 3-1 and 3-2) as a potential diagnostic biomarker of OA in urine samples [47].

### Synovial metabolism biomarkers

**Hyaluronic acid (HA):** Hyaluronic acid (HA) is a constituent of cartilage and the synovium. HA is widely distributed throughout many body tissues, and its presence in the serum can be caused by conditions other than arthritis (e.g. liver disease). Although its increased levels have been described in OA, this relationship has been demonstrated more specifically in rheumatoid arthritis [48, 49].

**Urinary Glucosyl-Galactosyl-Pyridinoline:** Crosslinking molecules such as pyridinoline (PYD), which involves the C- and N-telopeptides link collagen molecules together. Glucosyl galactosyl - pyridinoline (Glc-Gal-PYD), a glycosylated analog of free PYD, is present in human synovial tissue but also reflects degradation of synovial tissue. It is absent from bone, cartilage, and other soft tissues. Urinary excretion is elevated in OA patients; for that reason is considered a significant predictor of pain and physical function [50, 51].

In other study by Jordan et al, Glc-Gal-Pyd, was associated with OA and disease severity at the tibiofemoral and patellofemoral joints in men [52].

**Human Cartilage Protein (YKL-40):** Chitinase-3-like protein 1 or human cartilage glycoprotein 39 is a protein known as YKL-40; belongs to family 18 of the mammalian glycosyl hydrolases and weights 40 kDa [53].

Several different cell types in the joint tissue, including macrophages, articular chondrocytes, synoviocytes and other tissues

like brain, kidney and placenta secrete YKL-40. A subpopulation of macrophages expresses YKL-40 mRNA; this is important as these cells participate in inflammatory and extracellular matrix (ECM) remodelling processes in different tissues [54].

Although YKL-40 lacks enzymatic activity and specific receptor is unknown, may be involved in inflammatory processes in arthritis, asthma, COPD, liver fibrosis, and cancer. YKL-40 binds to important components in cartilage extracellular matrix, which is, proteoglycans and collagens, and influence their production and assembly [55, 56].

*In vitro*, the expression of YKL-40 is increased in redifferentiation of dedifferentiated chondrocytes and observed with *in vitro* chondrogenesis, indicating that YKL-40 is a differentiation marker in chondrocytes [57].

Synoviocytes can secrete YKL-40 during various inflammatory reactions; additionally YKL-40 is produced by osteoblasts, and the primary osteocytes present in osteophytes [58, 59].

Although the molecular processes governing the induction and inhibition of YKL-40 are poorly understood, some studies have reported that IL-1 $\beta$  and TGF- $\beta$  decreased the secretion of YKL-40 associated with a reduction in YKL-40 mRNA levels [60, 61].

In neonatal rat chondrocytes the inflammatory cytokines TNF- $\alpha$  and IL-1 potently induced increased levels of YKL-40 mRNA. In a recent study, YKL-40 level were increased by inflammatory cytokines IL-6 and IL-17, correlating the levels of intra-articular YKL-40 MMP-1 and MMP-3, suggesting that YKL-40 is a factor associated with inflammatory and catabolic processes in OA joints [62, 63].

Also, YKL-40 is produced *in vivo* in older and osteoarthritic cartilage and explant cultures with normal tissue produce low levels of this protein.

According to several studies, changes in the biochemical or biomechanical environment, removal of chondrocytes from their native ECM environment and injury to the cartilage matrix stimulate YKL-40 production. In OA, chondrocytes alter their gene expression patterns in response to changes in their surrounding matrix, the mechanical properties of the cartilage, and various growth factors, cytokines and inflammatory mediators, resulting in continued YKL-40 expression [61, 62, 64].

In synovial membranes from OA patients, YKL-40-positive cells were found and the number of YKL-40-positive cells correlated with the severity of the synovitis [65].

Despite several studies have demonstrated that YKL-40 levels to be higher in OA patients compared to healthy controls with a significant correlation between synovial fluid and serum, the role of YKL-40 in OA has remained unclear. Is it a molecule related to pathogenesis of disease or is it only a biomarker reflecting severity of inflammatory process? [66-68].

### Subchondral bone metabolism biomarkers

The relationship between cartilage and subchondral bone in OA has been matter of controversy. In clinical setting, although unconvincing results have been obtained by using existing bone

markers to assess OA [69], some studies have demonstrated that bone turnover is increased and highly associated to OA and bone marrow lesions detected by magnetic resonance imaging and associated to progression of OA [70, 71].

The increased interest in subchondral bone and OA is due to altered metabolism during OA initial phase and progression of disease. During early phase of disease, the trabecular thickness is reduced due to bone resorption with increased production of cathepsin K and MMPs [72, 73].

As the disease progresses, subchondral bone becomes sclerotic with a thickening of the subchondral plate and higher levels of IGF-1 and TGF- $\beta$ 1. Phenotypic changes in osteoblasts from subchondral bone explains bone sclerosis as an increase in material density and not mineral density with abnormal collagen type I fibers [72, 74].

Subchondral bone Type I collagen degradation can be assessed by measurement of pyridinoline cross-links in urine and excretion is significantly elevated in patients with OA [75].

In addition, NTX-I and CTX-I assays to detect epitopes located in the N-terminal and C-terminal cross-linked telopeptides, respectively, of type I collagen can be used to measure high levels in patients with early and progressive OA [76].

In a recent study by Huebner et al, they evaluate joint tissue remodelling using  $\alpha$ -C-telopeptide of type I collagen ( $\alpha$ -CTX) and urinary C-telopeptide of type II collagen (CTX-II). These markers were associated to severity and progression of OA and localized knee bone turnover. They concluded that  $\alpha$ -CTX is indicative of dynamic bone turnover and subsequent radiographic progression of disease, suggesting a role as a sensitive and prognostic marker for the subchondral bone remodeling associated to OA and progression of disease [77].

## Future Directions

The focus of this review has been on biochemical markers of OA related to components of synovial joints. At present, it remains unclear if biochemical markers demonstrate optimal validity to evaluate OA patients. OA affects one or a group of joints. Therefore, the optimal marker of disease must provide information of the disease in one joint.

Unfortunately, serum and urine biomarkers exhibit large inter-individual variations, thus requiring highly sensitive techniques. Synovial fluid markers may be more informative than systemic markers because they relate to structural damage within a joint. Therefore, synovial fluid markers are promising as diagnostic, prognostic tools and to measure efficacy of intervention.

Early diagnosis of OA is necessary to improve patient outcomes after treatment. Thus, a challenge for the future is the need to develop sensitive biomarkers as surrogates to identify the patient with early symptoms and prediction of clinical response.

Although is not in the scope of this review, including imaging studies such as MRI and genetic tools, such as genome wide association studies, combined with chemical markers may provide relevant information to early detection, evaluate risk of disease progression and improve disease prognosis for personalized evi-

dence-based action plans.

Further understanding of the molecular and cellular basis of OA is fundamental to guide and validate the development of specific markers, which provide new information on the pathogenesis of OA and might lead to the identification of new markers with potential clinical utility for specific strategies to diagnosis and measure efficacy of interventions.

## Conclusions

In this brief review, we summarize recent progress in chemical markers investigation. We describe a comprehensive approach, which is necessary to understand the complexity and heterogeneity of the disease.

Osteoarthritis is the most common joint disease, with cartilage loss leading to joint destruction and severe functional impairment. The goal of OA research is to search for new diagnostic and therapeutic strategies. This may help to early diagnose, prevent, reduce or stop the progression of the disease.

In many studies, molecular biomarkers of bone, cartilage, and synovium have been associated with OA, mainly in cross sectional studies. Undoubtedly, there is a need to develop alternative methods with a better sensitivity than plain radiography and less expensive than an MRI.

Several markers, (such as COMP, U-CTX II, C2C, coll2-1, coll2-1NO<sub>2</sub>, CP II, PIINP, COMP, 846 epitope, HA, Fib3-1 and Fib 3-2), have been largely studied and proposed to identify patients with OA, at high risk for rapid progression and for monitoring drug efficacy. However, there is still controversy whether these biomarkers can be used routinely in clinical practice.

Recent encouraging results have been provided by many preclinical and clinical studies on diagnostic, prognostic and efficacy of intervention, as shown in a recent meta-analysis by Valdes et al, [78]. In that study, it was analysed the largest sample with regards to biochemical markers of cartilage degradation (uCTX-II, COMP, C2M) showing that biomarkers can be valuable prognostic tools for progression of knee OA. However, it is important to keep in mind some limitations when biomarkers are analyzed, regarding preanalytical parameters such as diurnal variation, physical activity or diet, clearance rates, and some covariates (age, gender, BMI, concomitant diseases, drugs) that may affect the concentration of a biomarker and hinder its use as a valuable tool for evaluation of OA.

Another important issue is that we must to increase our knowledge about metabolism of biomarkers in different fluids. Usually, biomarkers generated within a joint are released into the synovial fluid; the clearance rates and levels in blood or urine depend on synovial vascularity. Additionally, many of these molecules arise throughout the body, one or a group of joints, and not only from the knee or from the hip and elevated levels do not necessarily reflect disease in one or more peripheral joints. Therefore as noted, by Felson, (2014), the optimal marker of disease may be one that provides information on the disease in a joint [79].

Finally, although biomarkers could be valuable to diagnose, predict OA progression and assess therapeutic response, they have still limitations in clinical practice and it is necessary to develop

and validate more specific and sensitive biomarkers.

## Acknowledgement

Authors acknowledge Juanita Oropeza for her support in typing this manuscript.

## References

- [1]. Kraus VB, M. Nevitt, L J. Sandell (2010) Summary of the OA biomarkers workshop 2009--biochemical biomarkers: biology, validation, and clinical studies. *Osteoarthritis Cartilage* 18(6): 742-5.
- [2]. Bijlsma JW, F. Berenbaum, FP. Lafeber (2011) Osteoarthritis: an update with relevance for clinical practice. *Lancet* 377(9783): 2115-26.
- [3]. Kraus VB, Burnett B, Coindreau J, Cottrell S, Eyre D, et al. (2011) Application of biomarkers in the development of drugs intended for the treatment of osteoarthritis. *Osteoarthritis Cartilage* 19(5): 515-42.
- [4]. (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 69(3): 89-95.
- [5]. Ishijima M, Watari T, Naito K, Kaneko H, Futami I, et al. (2011) Relationships between biomarkers of cartilage, bone, synovial metabolism and knee pain provide insights into the origins of pain in early knee osteoarthritis. *Arthritis Res Ther* 13(1).
- [6]. Inoue R, Ishibashi Y, Tsuda E, Yamamoto Y, Matsuzaka M, et al. (2011) Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. *Osteoarthritis Cartilage* 19(1): 51-7.
- [7]. Saxne T, Lindell M, Månsson B, Petersson IF, Heinegård D (2003) Inflammation is a feature of the disease process in early knee joint osteoarthritis: *Rheumatology (Oxford)* 42(7): 903-4.
- [8]. Sowers M, Jannausch M, Stein E, Jamadar D, Hochberg M, et al. (2002) C-reactive protein as a biomarker of emergent osteoarthritis. *Osteoarthritis Cartilage* 10(8): 595-601.
- [9]. Bauer DC, Hunter DJ, Abramson SB, Attur M, Corr M, Felser D, et al. (2006) Classification of osteoarthritis biomarkers: a proposed approach. *Osteoarthritis Cartilage* 14(8): 723-7.
- [10]. Samuels J, S. Krasnokutsky, S.B. Abramson (2008) Osteoarthritis: a tale of three tissues. *Bull NYU Hosp Jt Dis* 66(3): 244-50.
- [11]. Huebner JL, Williams JM, Deberg M, Henrotin Y, Kraus VB (2010) Collagen fibril disruption occurs early in primary guinea pig knee osteoarthritis. *Osteoarthritis Cartilage* 18(3): 397-405.
- [12]. Shinmei M, Ito K, Matsuyama S, Yoshihara Y, Matsuzawa K (1993) Joint fluid carboxy-terminal type II procollagen peptide as a marker of cartilage collagen biosynthesis. *Osteoarthritis and Cartilage* 1(2): 121-128.
- [13]. Nelson F, Dahlberg L, Laverty S, Reiner A, Pidoux I, et al. (1998) Evidence for altered synthesis of type II collagen in patients with osteoarthritis. *J Clin Invest* 102(12): 2115-25.
- [14]. Rousseau JC, Zhu Y, Miossec P, Vignon E, Sandell L J, et al. (2004) Serum levels of type IIA procollagen amino terminal propeptide (PIIANP) are decreased in patients with knee osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage* 12(6): 440-7.
- [15]. Garnero P, Ayrat X, Rousseau JC, Christgau S, Sandell L J, et al. (2002) Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. *Arthritis Rheum* 46(10): 2613-24.
- [16]. Sharif M, Kirwan J, Charni N, Sandell LJ, Whittles C, et al. (2007) A 5-yr longitudinal study of type IIA collagen synthesis and total type II collagen degradation in patients with knee osteoarthritis--association with disease progression. *Rheumatology* 46(6): 938-43.
- [17]. Bay-Jensen AC, Tabassi NC, Sondergaard LV, Andersen TL, Dagnaes-Hansen F, et al. (2009) The response to oestrogen deprivation of the cartilage collagen degradation marker, CTX-II, is unique compared with other markers of collagen turnover. *Arthritis Res Ther* 11(1).
- [18]. Oestergaard S, Sondergaard BC, Hoegh-Andersen P, Henriksen K, Qvist P, et al. (2006) Effects of ovariectomy and estrogen therapy on type II collagen degradation and structural integrity of articular cartilage in rats: implications of the time of initiation. *Arthritis Rheum* 54(8): 2441-51.
- [19]. Meulenbelt I, Kloppenburgh M, Kroon HM, Houwing-Duistermaat JJ, Garnero P, et al. (2006) Urinary CTX-II levels are associated with radiographic subtypes of osteoarthritis in hip, knee, hand, and facet joints in subject with familial osteoarthritis at multiple sites: the GARP study. *Ann Rheum Dis* 65(3): 360-5.
- [20]. Garnero P, Pterfey C, Zaim S, Schoenharth M (2005) Bone marrow abnormalities on magnetic resonance imaging are associated with type II collagen degradation in knee osteoarthritis: a three-month longitudinal study. *Arthritis Rheum* 52(9): 2822-9.
- [21]. Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, et al. (2001) Collagen type II C-telopeptide fragments as an index of cartilage degradation. *Bone* 29(3): 209-15.
- [22]. Elsaid K.A, C.O Chichester (2006) Review: Collagen markers in early arthritic diseases. *Clin Chim Acta* 365(1-2): 68-77.
- [23]. Abramson S.B (2008) Osteoarthritis and nitric oxide. *Osteoarthritis Cartilage* 16(2): 60008-4.
- [24]. Huebner J.L, Jensen A.B, Leeming D. J, Coleman R. E, McDaniel G, et al. (2010) 048 URINARYMARKERS, ALPHA CTX AND CTXII, ARE INDICATIVE OF OA SEVERITY AND BONE TURNOVER. *Osteoarthritis and Cartilage* 18: S29-S30.
- [25]. Bay-Jensen AC, Liu Q, Byrjalsen I, Li Y, Wang J, Pedersen C, Leeming DJ, et al. (2011) Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIM--increased serum CIIM in subjects with severe radiographic osteoarthritis. *Clin Biochem* 44(5-6): 423-9.
- [26]. Deberg M, Labasse A, Christgau S, Cloos P, Bang Henriksen D, et al. (2005) New serum biochemical markers (Coll 2-1 and Coll 2-1 NO<sub>2</sub>) for studying oxidative-related type II collagen network degradation in patients with osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage* 13(3): 258-65.
- [27]. Deberg MA, Labasse AH, Collette J, Seidel L, Reginster JY, et al. (2005) One-year increase of Coll 2-1, a new marker of type II collagen degradation, in urine is highly predictive of radiological OA progression. *Osteoarthritis Cartilage* 13(12): 1059-65.
- [28]. Bhosale A.M, J.B. Richardson (2008) Articular cartilage: structure, injuries and review of management. *Br Med Bull* 87: 77-95.
- [29]. Kiani C, Liwen C. H. E. N, WU Y. J, Albert J. Y, Burton B.Y (2002) Structure and function of aggrecan. *Cell Res* 12(1): 19-32.
- [30]. Poole AR, Ionescu M, Swan A, Dieppe PA (1994) Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan. Implications for pathogenesis. *J Clin Invest* 94(1): 25-33.
- [31]. Tang, B.L (2001) ADAMTS: a novel family of extracellular matrix proteases. *Int J Biochem Cell Biol* 33(1): 33-44.
- [32]. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, et al. (2005) Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature* 434(7033): 644-8.
- [33]. Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, et al. (2005) ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature* 434(7033): 648-652.
- [34]. Little CB, Meeker CT, Golub SB, Lawlor KE, Farmer PJ, et al. (2007) Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair. *J Clin Invest* 117(6): 1627-36.
- [35]. Dufield DR, Nemirovskiy OV, Jennings MG, Tortorella MD, Malfait AM, et al. (2010) An immunoaffinity liquid chromatography-tandem mass spectrometry assay for detection of endogenous aggrecan fragments in biological fluids: Use as a biomarker for aggrecanase activity and cartilage degradation. *Anal Biochem* 406(2): 113-23.
- [36]. Larsson S, Englund M, Struglics A, Lohmander LS (2010) Association between synovial fluid levels of aggrecan ARGS fragments and radiographic progression in knee osteoarthritis. *Arthritis Res Ther* 12(6): 31.
- [37]. Germaschewski FM, Matheny CJ, Larkin J, Liu F, Thomas LR, et al. (2014) Quantitation OF ARGS aggrecan fragments in synovial fluid, serum and urine from osteoarthritis patients. *Osteoarthritis Cartilage* 22(5): 690-7.
- [38]. Wakitani S, Okabe T, Kawaguchi A, Nawata M, Hashimoto Y (2010) Highly sensitive ELISA for determining serum keratan sulphate levels in the diagnosis of OA. *Rheumatology* 49(1): 57-62.
- [39]. Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, et al. (1992) Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. *J Biol Chem* 267(9): 6132-6.
- [40]. Halász K, Kassner A, Mörgelin M, Heinegård D (2007) COMP acts as a catalyst in collagen fibrillogenesis. *J Biol Chem* 282(43): 31166-73.
- [41]. Chen FH, Herndon ME, Patel N, Hecht JT, Tuan RS, et al. (2007) Interaction of cartilage oligomeric matrix protein/thrombospondin 5 with aggrecan. *J Biol Chem* 282(34): 24591-8.
- [42]. Clark AG, Jordan JM, Vilim V, Renner JB, Dragomir AD, et al. (1999) Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the Johnston County Osteoarthritis Project. *Arthritis Rheum* 42(11): 2356-64.
- [43]. Saxne T, D. Heinegard (1992) Cartilage oligomeric protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. *Br J Rheumatol* 31(9): 583-91.
- [44]. Verma P, d K. Dalal (2013) Serum cartilage oligomeric matrix protein (COMP) in knee osteoarthritis: a novel diagnostic and prognostic biomarker. *J Orthop Res* 31(7): 999-1006.
- [45]. El-Arman MM, El-Fayoumi G, El-Shal E, El-Boghdady I, El-Ghaweet A (2010) Aggrecan and cartilage oligomeric matrix protein in serum and synovial fluid of patients with knee osteoarthritis. *Hss J* 6(2): 171-6.

- [46]. Catterall JB, Hsueh MF, Stabler TV, McCudden CR, Bolognesi M, et al. (2012) Protein modification by deamidation indicates variations in joint extracellular matrix turnover. *Journal of Biological Chemistry* 287(7): 4640-4651.
- [47]. Henrotin Y, Gharbi M, Mazzucchelli G, Dubuc JE, De Pauw E, et al. (2012) Fibulin 3 peptides Fib3-1 and Fib3-2 are potential biomarkers of osteoarthritis. *Arthritis & Rheumatism* 64(7): 2260-2267.
- [48]. Elliott AL, Kraus VB, Luta G, Stabler T, Renner JB, et al. (2005) Serum hyaluronan levels and radiographic knee and hip osteoarthritis in African Americans and Caucasians in the Johnston County Osteoarthritis Project. *Arthritis Rheum* 52(1): 105-111.
- [49]. Kraus V.B (2006) Do biochemical markers have a role in osteoarthritis diagnosis and treatment? *Best Pract Res Clin Rheumatol* 20(1): 69-80.
- [50]. Garnero P, Gineyts E, Christgau S, Finck B, Delmas PD (2002) Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. *Arthritis Rheum* 46(1): 21-30.
- [51]. Gineyts E, P. Garnero, P. Delmas (2001) Urinary excretion of glucosyl-galactosyl pyridinoline: a specific biochemical marker of synovium degradation. *Rheumatology* 40(3): 315-323.
- [52]. Jordan KM, Syddall HE, Garnero P, Gineyts E, Dennison EM, et al. (2006) Urinary CTX-II and glucosyl-galactosyl-pyridinoline are associated with the presence and severity of radiographic knee osteoarthritis in men. *Annals of the rheumatic diseases* 65(7): 871-877.
- [53]. Huang K, L.D Wu (2009) YKL-40: a potential biomarker for osteoarthritis. *J Int Med Res* 37(1): p. 18-24.
- [54]. Volck B, Johansen JS, Stoltenberg M, Garbarsch C, Price PA, et al. (2001) Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. *Osteoarthritis Cartilage* 9(3): 203-14.
- [55]. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, et al. (2011) Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annu Rev Physiol* 73: 479-501.
- [56]. Shao R, Hamel K, Petersen L, Cao QJ, Arenas RB, et al. (2009) YKL-40, a secreted glycoprotein, promotes tumor angiogenesis. *Oncogene* 28(50): 4456-68.
- [57]. Imabayashi H, Mori T, Gojo S, Kiyono T, Sugiyama T, et al. (2003) Redifferentiation of dedifferentiated chondrocytes and chondrogenesis of human bone marrow stromal cells via chondrosphere formation with expression profiling by large-scale cDNA analysis. *Exp Cell Res* 288(1): 35-50.
- [58]. Johansen JS (2006) Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. *Dan Med Bull* 53(2): 172-209.
- [59]. Connor JR, Dodds RA, Emery JG, Kirkpatrick RB, Rosenberg M, et al. (2000) Human cartilage glycoprotein 39 (HC gp-39) mRNA expression in adult and fetal chondrocytes, osteoblasts and osteocytes by in-situ hybridization. *Osteoarthritis Cartilage* 8(2): 87-95.
- [60]. De Ceuninck F, Pastoureaux P, Bouet F, Bonnet J, Vanhoutte PM (1998) Purification of guinea pig YKL40 and modulation of its secretion by cultured articular chondrocytes. *J Cell Biochem* 69(4): 414-24.
- [61]. Johansen JS, Olee T, Price PA, Hashimoto S, Ochs RL, et al. (2001) Regulation of YKL-40 production by human articular chondrocytes. *Arthritis Rheum* 44(4): 826-37.
- [62]. Recklies AD, Ling H, White C, Bernier SM (2005) Inflammatory cytokines induce production of CHI3L1 by articular chondrocytes. *Journal of Biological Chemistry* 280(50): 41213-41221.
- [63]. Väänänen T, Koskinen A, Paukkeri EL, Hämäläinen M, Moilanen T, et al. (2014) YKL-40 as a novel factor associated with inflammation and catabolic mechanisms in osteoarthritic joints. *Mediators Inflamm* 215140(10): 15.
- [64]. Volck B, Ostergaard K, Johansen JS, Garbarsch C, Price PA (1999) The distribution of YKL-40 in osteoarthritic and normal human articular cartilage. *Scandinavian journal of rheumatology* 28(3): 171-179.
- [65]. Kawasaki M, Hasegawa Y, Kondo S, Iwata H (2001) Concentration and localization of YKL-40 in hip joint diseases. *The Journal of rheumatology* 28(2): 341-345.
- [66]. Volck B, Johansen JS, Stoltenberg M, Garbarsch C, Price PA, et al. (2001) Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. *Osteoarthritis and Cartilage* 9(3): 203-214.
- [67]. Johansen JS, Hvolris J, Hansen M, Backer V, Lorenzen I, et al. (1996) Serum YKL-40 levels in healthy children and adults. Comparison with serum and synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. *Br J Rheumatol* 35(6): 553-9.
- [68]. Huang K, L Wu (2009) YKL-40: a potential biomarker for osteoarthritis. *Journal of International Medical Research* 37(1): 18-24.
- [69]. Hunter DJ, TD Spector (2003) The role of bone metabolism in osteoarthritis. *Current rheumatology reports* 5(1): 15-19.
- [70]. Bailey AJ, Mansell JP, Sims TJ, Banse X (2004) Biochemical and mechanical properties of subchondral bone in osteoarthritis. *Biorheology* 41(3): 349-358.
- [71]. Garnero P, Peterfy C, Zaim S, Schoenharth M (2005) Bone marrow abnormalities on magnetic resonance imaging are associated with type II collagen degradation in knee osteoarthritis: a three-month longitudinal study. *Arthritis & Rheumatism* 52(9): 2822-2829.
- [72]. Kwan Tat S, Lajeunesse D, Pelletier JP, Martel-Pelletier J (2010) Targeting subchondral bone for treating osteoarthritis: what is the evidence? *Best Practice & Research Clinical Rheumatology* 24(1): 51-70.
- [73]. Hayami T, Pickarski M, Zhuo Y, Wesolowski GA, Rodan GA, et al. (2006) Characterization of articular cartilage and subchondral bone changes in the rat anterior cruciate ligament transection and meniscectomized models of osteoarthritis. *Bone* 38(2): 234-243.
- [74]. Bailey AJ, TJ Sims, L. Knott (2002) Phenotypic expression of osteoblast collagen in osteoarthritic bone: production of type I homotrimer. *The international journal of biochemistry & cell biology* 34(2): 176-182.
- [75]. Stewart A, Black A, Robins S P, Reid DM (1999) Bone density and bone turnover in patients with osteoarthritis and osteoporosis. *The Journal of rheumatology* 26(3): 622-626.
- [76]. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD (2002) Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. *Arthritis & Rheumatism* 46(12): 3178-3184.
- [77]. Huebner JL, Bay-Jensen AC, Huffman KM, He Y, Leeming DJ, et al. (2014) Alpha C-Telopeptide of Type I Collagen Is Associated With Subchondral Bone Turnover and Predicts Progression of Joint Space Narrowing and Osteophytes in Osteoarthritis. *Arthritis & Rheumatology* 66(9): 2440-2449.
- [78]. Valdes AM, Meulenbelt I, Chassaing E, Arden NK, Bierma-Zeinstra S, et al. (2014) Large scale meta-analysis of urinary C-terminal telopeptide, serum cartilage oligomeric protein and matrix metalloprotease degraded type II collagen and their role in prevalence, incidence and progression of osteoarthritis. *Osteoarthritis Cartilage* 22(5): 683-9.
- [79]. Felson DT (2014) The current and future status of biomarkers in osteoarthritis: *J Rheumatol* 41(5):834-6.