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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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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 asympto-

matic 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, ra-

diation 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 $\frac{3}{4}$ length of the triple helix. This degradation process results in two fragments: $\frac{1}{4}$ length and $\frac{3}{4}$ length. CTX-II assays recognize on the $\frac{1}{4}$ 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 $\frac{3}{4}$ 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 NO₂) inpatients with OA and RA and the ratio Coll2-1 NO₂/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 proteoglycans metabolism

From 10 to 20% of wet weight of cartilage matrix are 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 sulfate chains, 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 synovial 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	Synthesis	Synovial fluid, serum
PIIANP N-terminus propeptide of type II procollagen, splice variant A	Synthesis	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 mac-

rophages, 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 β and TGF- β 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- α 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 un-

convincing 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- β 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 α -C-telopeptide of type I collagen (α -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 α -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 evidence-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, coll2-1NO₂, 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.

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