Chapter

Value of Biomarkers in Osteoarthritis

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Abstract

Biochemical markers in osteoarthritis are molecules that occur during the physiological cycle of the bone and cartilage matrix, and they can be detected in body fluids. The most important goal of marker metrology in osteoarthritis is that cartilage damage can be recognized at the early stage when it has not yet been detected radiologically. In addition to early recognition, follow-up of disease activity, determination of disease severity, prediction of prognosis, and evaluation of response to treatment are other purposes of marker measurement. Type II collagen is the most important structural element of joint cartilage and is relatively specific to hyaline cartilage. The main event in osteoarthritis pathophysiology is the damage of the Type II collagen network. For this reason, researches aimed at detecting osteoarthritis-specific and specific biochemical markers have focused on Type II collagen. CTX-II is currently the most investigated and promising biomarker in relation to osteoarthritis clinic.

Keywords: biomarkers, CTX-II, fibulin-3, osteoarthritis, Type II collagen

1. Introduction

Osteoarthritis (OA) is a multifactorial, dynamic disease process characterized by erosion in the joint cartilage, bone hypertrophy at the joint edges, subchondral sclerosis, synovial membrane, and biochemical and morphological changes in the joint capsule. It is more common in the elderly [1].

The diagnosis of OA is classically performed by radiological imaging methods that support clinical findings. However, primary OA developed without any trauma, especially those without any traumas, started many years before the radiological findings became evident and the pathology is often not revealed early. The progression of the disease is often slow and is spread over the years. Radiological findings in OA can provide indirect information about the cartilage tissue. For this reason, radiological methods are not sensitive especially in the early stage [2, 3]. Early diagnosis allows the joint to treat OA conservatively without interruption.

Imaging methods in OA provide information about the accumulated image that already existed in the past, rather than the current assessment of how far the disease has progressed. Therefore, there is a need for alternative methods that can detect joint changes in a quantitative, reliable, and sensitive manner. Biochemical markers can serve this purpose. For this reason, recent research on OA has focused on the development of disease-specific biochemical markers, with an increasing number of publications in this area in recent decades [4]. Biochemical markers, which stand

out among laboratory methods, will be discussed in this section in the context of recent developments.

A good biochemical marker, which is specific to the disease, reflects the disease activity at that time, is susceptible to posttreatment changes, predicts the outcome of the disease, and has knowledge of its metabolism and biological properties [5]. Clinical use of the markers should be based on a number of criteria such as clearance rates, circadian differences, diet, physical activity, and drug use [6].

Biochemical markers in OA are molecules that occur during the physiological cycle of the connective tissue matrix and can be detected in body fluids. One of the most important purposes of biochemical markers measurement in OA is the recognition of cartilage damage at the early stages when it has not yet been detected radiologically. In addition to early recognition, follow-up of disease activity, determination of the severity of the disease, prediction of prognosis, and evaluation of response to treatment are other purposes of marker measurement [7].

Until now, no notable findings of a laboratory for primary OA have been reported. Routine laboratory tests, including the highly sensitive CRP, cannot provide definitive information regarding disease activity in OA. Although the quantitative values of CRP may increase in synovitis in case of inflammation, the results are usually normal. Similarly, the serum levels of antinuclear antibodies, rheumatoid factor, and complement components are normal. These laboratory findings are important in terms of differential diagnosis from other diseases with arthritis and metabolic disorders [7].

In OA, the synovial fluid is noninflammatory, pale yellow, and brittle. It is mononuclear cell-weighted, with a small number of leucocytes. The viscosity of the liquid is normal. Excess synovial fluid may suggest that the course of the disease is going worse [7].

New diagnostic methods are being developed for early diagnosis since OA has positive responses to early-stage treatment interventions. Biochemical markers showing bone and cartilage recurrence in recent years have been shown to be useful in identifying patients at risk for high joint deterioration [8]. They have also been reported to be compatible with magnetic resonance (MR) imaging [9, 10]. There is evidence that these markers can distinguish not only the cartilage surface change in knee OA but also the specific forms of damage to the bone and the surrounding soft tissue. For this reason, different combinations of markers may play an important role in the prognosis of the disease.

Despite the lack of specific laboratory methods in diagnosis, many biomarkers have been recently developed for the diagnosis and follow-up of OA. The joint is a complex structure of bone, cartilage, and synovial tissue; for this reason, it is useful to use the markers of these three constructs when determining the degree of the degeneration of the insert. The extracellular matrix of bone, cartilage, and synovial tissues forms mainly collagens. These are Type I (bone and synovium), Type II (cartilage), and Type III (synovium) collagens. Collagen is found together with aggrecan and other glycoproteins. The speed of construction and destruction of the cartilaginous structure is slow, so the cartilaginous structure has a long half-life. Extracellular matrix, mainly composed of collagen in normal conditions, balances between construction and continuous renewal. However, when the speed of construction cannot capture the speed of destruction, the cartilaginous structure gradually loses its integrity [11].

In general, cartilage, proenzymes, active proteinases, proteinase inhibitors, matrix fragments that are released by proteinases, and antibodies developed by the organism against cartilage components are among the markers of cartilage cycling in pathologies involving joint cartilage. Among these markers, the proteoglycans most frequently researched are the smaller fragments of the structure

that eventually result in proteolytic destruction. Knowledge of the biochemistry and immunological properties of the proteoglycans has allowed the measurement of proteoglycan components and degradation products with more sensitive methods [12].

Nowadays, these methods can be used to measure proteoglycan degradation products in the serum and synovial fluid inflammation and degenerative joint diseases. Some studies have found that there is a correlation between proteoglycan levels in the synovial fluid and severity of the disease [12].

Several biochemical markers such as Type II collagen, proteoglycans, hyaluronan, cartilage oligomeric matrix protein (COMP), and matrix metalloproteinases (MMPs) have been investigated in relation to OA and radiological progression, and frequently conflicting results have been obtained [13].

In some studies with COMP, it was concluded that OA progression was positively related, while in other studies, it was shown that it was affected by factors such as age, ethnicity, and BMI (body mass index), which had a weak relationship with the narrowing of the joint space and disease progression. In addition, it is reported that cartilage is not specific, and it is also found in structures such as the synovium and meniscus [14].

Glucosyl-galactosyl-pyridinoline (Gly-Gal-Pyd), Type I, and Type III are cross-links of the collagenous roof. It is found in the synovial tissue and has been identified in in vitro studies that have occurred during the cartilage destruction process. In a study conducted, urinary Gly-Gal-Pyd levels were found to correlate strongly with pain and disability scores and radiological disease stage in patients with knee OA [15].

Type II collagen is the most important structural element of the joint cartilage and is relatively specific to hyaline cartilage. The main event in OA pathophysiology is the damage in the Type II collagen network. Therefore, investigations aimed at detecting OA-specific and specific biochemical markers have focused on Type II collagen [15].

Experimental arthritis models are exploring cartilage metabolism in a variety of ways. In one study, an increase in synovial fluid proteoglycan fragments in the experimental OA model showed concordance with the severity of arthritis [12].

The use of biomarkers in OA has some significant purposes. One of these is the predetermination of patients with rapid cartilage destruction in order to prevent joint destruction in the future because the period of the radiographic degeneration of the joints and the diagnosis of OA are usually detected in the advanced stages of cartilage damage from the molecular point of view. In addition to early recognition, monitoring of disease activity and determination of disease severity, prediction of prognosis, and cartilage degradation should be tracked in order to monitor the efficacy of new drugs developed as cartilage protectants [16].

Some criteria must be considered for a biomarker measurement to be valid in OA. First of all, it is necessary to know what kind of pathology the specimen measured reflects since there are different types of markers for tissue damage, tissue repair, anabolic or catabolic processes, or pathologies at the cell or tissue level. It is also important that the measured indicator is indeed the marker to be measured. For this reason, the method should be well investigated and the most appropriate method should be selected according to the conditions. Furthermore, the biomarker measurement results should be compatible with the clinical and radiological findings of the disease and with the pain-function score. It should also reveal the smallest change in the severity of the disease [16].

In order to understand the clinical benefit of biomarkers, it is necessary to initially standardize the measurement method used. Sample receipt time, purchase and storage conditions, and each biomarker circadian rhythm should be known.

Some may be affected by factors such as physical activity, age, and gender. For example, C-terminal cross-linked Type II collagen (CTX-II) and serum COMP (sCOMP) from cartilage markers show very little circadian variation [11].

At the later stages of OA, cartilage damage to the tissue occurs at a high rate, making it difficult to interpret when the marker is detected at a very low concentration. Serum can change the levels of biomarkers with foods such as hyaluronic acid. At the first hour after feeding, hyaluronic acid levels reach the highest point. For this reason, the serum levels of biomarkers in OA should be checked on an empty stomach. Metabolism of biomarkers, kidney excretion, and drugs can also be affected. The level of urine CTXII is affected by ibuprofen [11].

The levels of certain biomarkers such as COMP, chondroitin sulfate, and urine CTX-II may vary with age and sex, as well as joint pathology. In addition, a patient's ethnicity and BMI may affect the baseline measurement values of biomarkers. There are different classifications for biomarkers to be used in OA. These may be direct and indirect markers, cartilage, bone and synovial tissue, or markers of synthesis and destruction. It is more accurate to classify OA in comparison to the tissues from which they originate if the bone and the synovial tissue as well as cartilage are thought to have contributed to the development and the course of OA [16].

2. Metabolic processes of osteoarthritis during which biomarkers emerge

In osteoarthritis, significant changes that cause an inflammatory cascade which in turn triggers the chronic overproduction of factors at the metabolic level occur. These factors may aggravate osteoarthritis. Biomarkers are metabolic processes of all kinds which develop during the inflammatory process in osteoarthritis.

Hyaline cartilage structure is principally composed of water, collagen, and proteoglycans, which include sparsely distributed chondrocytes. Chondrocytes provide a balance between the anabolic and catabolic activities that protect the aggrecan structure [17]. Deprivation of the cartilaginous matrix results in an imbalance between the cartilage synthesis (anabolic) and resorption (catabolic) processes in the joint. Mechanical strain causes upregulation of cytokines like interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) with a rapid transcription through a shock sensor system in chondrocytes and tendons [18]. The mechanical strain resulting from normal activity or therapeutic exercise in fact inhibits this upregulation and helps in the remodeling of the cartilage through collagen synthesis. The upregulation of cytokines causes induction of MMPs which enzymatically disrupts the cartilage structure [19]. In addition, mechanical strains cause microcellular damage which leads to the release of extracellular membrane particles and intracellular microtubule elements into the joint [20].

These mechanical strains also produce other metabolic changes such as the release of arachidonic acid from the phospholipids in the damaged intra-articular cell membranes after phospholipase A2 (PLA2) action [21]. The continuous strain together with the metabolic processes causes inflammation in the joint tissues. The main cytokines that cause degradation in the synovia are the IL-1, IL-6, IL-17, and TNF-α [22]. Other elements and side products which further increase the cartilage degradation and play a role in osteoarthritis include insulin-like growth factor 1 (IGF-1), transforming growth factor beta1 (TGF-β1), and chondrodegradative enzymes [23].

In cases of acute or chronic joint damages, arachidonic acid is the primary fatty acid produced by the metabolic conversion of the cell membrane phospholipids through PLA_2. Through other enzymatic activities, arachidonic acid transforms into

various inflammatory mediators such as cytokines and eicosanoids, which lead to a progression of the disease.

During the metabolic conversion of arachidonic acid into inflammatory metabolites, the two most important enzymatic pathways are the 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) [24]. These parallel pathways produce the leukotrienes, prostaglandins, thromboxanes, and prostacyclins, which play a significant role in the onset and progression of the inflammatory response. The conversion of arachidonic acid through COX-2 leads to production of prostaglandins, which are physiologically important mediators in tissue repair and prostacyclins [25].

The metabolic transformation of arachidonic acid through 5-LOX, another inducible enzyme, leads to the production of leukotrienes. Leukotriene B4, specifically, is a chemoattractant and a fatty acid metabolite that causes damage in the cells and tissues [26]. Leukotrienes initiate the production of new reactive oxygen species. The upregulation of the inflammatory cascade of cytokines causes permanent disruption of the cell membrane, thus leading to formation of more arachidonic acid [27].

Matrix metalloproteinases produced from chondrocytes are zinc-containing proteinases which degrade the cartilage. In particular, the expression of MMP-1 and MMP-13 is induced by IL-1β, which in turn causes the degradation of Type II collagen [28].

Chondrocytes also produce the reactive oxygen species. The production of reactive oxygen species causes damage on the components of the cartilage matrix and induces apoptosis [29]. Another form of transformation is the nonenzymatic lipid peroxidation of arachidonic acid. When arachidonic acid is exposed to reactive oxygen species, the molecule is oxidized to three main products: F2-isoprostanes, 4-hydroxynonenal, and malondialdehyde [30]. All three molecules directly destroy the hyaline cartilage. Chondrocytes also produce the reactive oxygen species like xanthine-hypoxanthine system, hydroxyl radicals, peroxide, and hydroxyproline [31].

Each of the biochemical products produced along the sequence of inflammatory cascade in osteoarthritis mentioned briefly above is investigated as a biochemical marker in the early diagnosis, during treatment and later during follow-up of the disease.

3. Biochemical markers of bone origin

The bone matrix consists mainly of Type I collagen molecules linked by pyridinoline (PyD) and deoxypyridinoline (D-PyD) cross-links. The degradation of Type I collagen can be assessed by pyridinoline cross-links in the urine. NTX-I and CTX-I, the epitopes of N-terminal and C-terminal cross-link telopeptides, are the most studied bone resorption markers. Bone formation and degradation markers shown in **Table 1** can be affected by regional subchondral bone structure defects [32, 33]. Serum and urine concentrations may vary due to age, menopause, osteoporosis, and other bone diseases. Bone markers in osteoarthritis give incompatible results due to several factors that can affect the results.

Bone	Production	Demolition
Type I collagen	N- and C-propeptides (PICP and PINP)	Pyridinoline (PYD), deoxypyridinoline C-terminal and N-terminal telopeptides (CTX-I, NTX-I)
Noncollagen protein	Osteocalcin Bone alkaline phosphatase	Sialoprotein (BSP) Tartrate-resistant acid phosphatase (TRAP)

Table 1.
Biochemical markers of bone origin used in OA.

Urine C-terminal and Type I collagen telopeptide levels (CTX-I) were higher in cases with a rapid onset of osteoarthritis than slow-onset events [34]. Bone sialoprotein (BSP) is a product of active osteoblasts located in the junctions of mineralized cartilage and subchondral bone tissue. Elevated levels of serum BSP reflect the bone matrix cycle [35].

There is evidence that combined measurement of COMP and BSP may be a prognostic marker to determine the development of OA in chronic knee pain cases [36]. Osteocalcin, an important component of bone noncollagen matrix, is released during mineralization. This measurement gives information about bone formation. It is important to demonstrate subchondral bone metabolism [16].

During cartilage damage, changes in the bone metabolism occur and the molecules of the bone increase in body fluids. In general, elevated serum BSP reflects the bone matrix cycle [33].

Serum and urine levels of bone markers may vary due to menopause, osteoporosis, and other bone diseases. It is an important question in terms of OA performance [37].

Bone markers have shown more pronounced circadian rhythm changes and more concentrated on cartilage and synovial tissue markers in recent years due to inconsistent results.

3.1 Biochemical markers of cartilage production

The main event in the pathophysiology of OA is the destruction of the Type II collagen nerve which is formed by COL-2 α1 fibrils. For this reason, OA studies have focused on Type II collagen. Type II collagen is only 1% of all collagen in the body, and the normal cycle is slow. Type II collagen, predominantly found in the joint cartilage, is synthesized procollagen in chondrocytes (**Table 2**). Subsequently, the extracellular fluid is released, where the procollagen carboxy-terminal and amino-terminal propeptides (PIICP and PIINP, respectively) are separated from the parent construct, and mature collagen synthesis is completed. These are important indicators of collagen synthesis in the articular cartilage. And the levels of cartilage tissue, serum, and synovial fluid can be measured [11].

PIICP and PIINP may be the most common Type II collagen [12] in the cartilage (**Table 2**). COL-2 molecules are synthesized as propeptides from the carboxy-terminal and amino-terminal regions of the extracellular domain before forming fibrils. These peptides are cysteine-rich PIINAP, a prokaryotic Type II C-terminal propeptide (PIICP), a procollagen Type II N-propeptide (PIINP), and a second form of PIINP. They are indicators of chondrocyte synthase activity. It has been detected that serum PIINAP levels are inversely correlated with the loss of cartilage induced by MR or radiography in patients with OA [38].

Cartilage	Production	Demolition
Type II collagen	N- and C-propeptides (PIICP, PIIANP, PIIBNP)	PYD, CTX-II Type II collagen fragments
Aggrecan	Chondroitin sulfate epitopes	Keratan sulfate epitopes
Aggrecan and noncollagenous proteins	Glycoprotein-39 (YKL-40) Cartilage-derived retinoic acid-sensitive protein	COMP SLRPs

Table 2.
Biochemical markers of cartilage origin used in OA.

The development of OA in the synovial fluid of individuals with knee injuries has reached maximum levels of propeptide levels in the preradiological period [39].

In a study by Garnero, serum PIIANP levels in OA patients showed a decrease [14]. The increase in the urine CTX-II levels with serum PIIANP levels may indicate that joint destruction develops more rapidly. PIICP levels give hope to early detection of OA.

Nine amino acid peptides (COL2-1) and their nitrated form (COL2-1 NO2) of Type II collagen are localized peptides in the collagen network of the triple helix structure and show oxidative degradation of this helix structure. In a 3-year follow-up study in patients with knee OA, it was observed that initial increases in urine levels were associated with high disability assessed by the Western Ontario and the McMaster University Osteoarthritis Index (WOMAC) [40]. These results suggest that the urinary levels of COL2-1 and COL2-1 NO2 may reflect the clinical severity of OA. However, a significant association of COL2-1 NO2 with CRP and an increased synovial inflammation requires caution in the differentiation of other arthritic patients [5].

Another name for YKL-40, a cartilaginous marker, is glycoprotein-39. In advanced stage OA, serum and synovial fluid are present in high amounts. Elevated serum levels were detected in hip OA. YKL-40 levels may also increase depending on other pathologies, especially inflammation. For this reason, inflammation can also be considered as a marker [41].

3.2 Biochemical markers of cartilage destruction

The most well-known marker in the demolition reagents is COMP. Increasing levels are thought to indicate that OA is advancing. Since COMP is synthesized not only by cartilage but also by synovial cells, tendon fibroblasts, and osteoblasts, the increase may be due to cartilage destruction or synovial inflammation. In the knee OA, the COMP level is synovitis grade compatible, but it is shown that OA is not compatible with the grade. The absence of COMP specificity may limit the use of OA-RA (rheumatoid arthritis) to assess changes in joint damage [42].

4. Type II collagen destruction products

There is a consensus that Type II collagen degradation products can be used as markers in the diagnosis and follow-up of OA and RA [43]. C2C and C1-2C are new epitopes formed after destruction of Type II collagen speckle collagenases. For this reason, it can give opinion on the destruction of cartilage. The levels of C1-2C were found higher in OA cartilage than in normal cartilaginous tissue [11].

CTX-II is also a Type II collagen degradation product and an important indicator of cartilage damage. Urine CTX-II levels were elevated in RA and OA, and high levels were found to be compatible with joint erosion [43]. In patients with knee OA, urine CTX-II measurement has shown that it may be useful in determining the prognosis of joint damage, and they have been found useful as a determinant for rapid degeneration of joint cartilage [44].

In another study, it was determined that the urine CTX-I and CTX-II and sCOMP levels can determine patients with focal cartilage lesions in the early stages of knee OA [45]. At the beginning of OA, new epitopes emerged from the triple helix of collagen collapsed by collagenases. The C-terminal telopeptide, one of these epitopes, is now the most searched for association with OA clinic and is most promising as a specific marker for OA [44]. In many studies, OA was found to be particularly high in urine levels compared to controls and that it can be used as a

diagnostic marker [46]. Another publication has shown that high CTX-II levels are associated with radiological progression of the knee and hip in OA and that they increase eight times the risk of progression in these individuals [44].

Bettica et al. found a relationship between urinary CTX-I and knee OA development in terms of cartilage derivation markers [34]. Urine CTX-II has been reported as a good marker of knee and hip OA progression [47, 48].

5. Oligosaccharides

Chondroitin sulfate and keratan sulfate are oligosaccharides that bind to the aggrecan protein and are the first molecules in which cartilage formation and degradation are evaluated. The affinities of these oligosaccharides depend on the length and sulfation of the molecule and thus may vary from person to person. It is present at high concentration in the circulation during prolonged disease and significant loss of cartilage. Although cartilage is at the highest concentration in the tissue, chondroitin sulfate and keratan sulfate epitopes can be found in the cartilage as well as in the extracellular matrix of the molecules outside the acceptor. For these reasons, their use as a marker in clinical evaluation and treatment follow-up is very limited.

Biglycan, decorin, fibromodulin, and lumican are small leucine-rich proteoglycans (SLRPs). The destruction of these small proteoglycans, along with the large molecule of cartilage-like aggrecan, suggests that it is active OA [11].

6. Biochemical markers of synovial tissue construction

6.1 Hyaluronan

It has been shown that radiological progression is faster in OA patients with high serum hyaluronan (sHA) levels [49]. It is not useful as a marker in everyday practice due to its distinctive circadian rhythm [37].

6.2 Highly responsive CRP

Osteoarthritis becomes defective in the chondrocyte metabolism and therefore there is an increased interest in acute phase proteins in OA, despite a common systemic manifestation of RA in nature. It has been reported that high-sensitivity CRP (hs-CRP) levels may be a prognostic feature of rapid progressive hip and knee OA (**Table 3**). In a study conducted to investigate the association between hs-CRP

Synovial tissue	Production	Demolition
Type III collagen	Type II N-propeptide (PIINP)	PYD, CTX-I, NTX-I Glucosyl-galactosyl-pyridinoline (Gly-Gal-Pyd)
Noncollagenous proteins	Hyaluronan, YKL-40, COMP	
Proteases and inhibitors	Tissue matrix proteinases (TIMP 1, 2)	Matrix metalloproteinases (MMP 1, 2, 3, 9)
Systemic infection	Highly sensitive CRP	

Table 3.
Biochemical markers originating from the synovial tissue used in OA.

and the OA severity and size in patients with advanced hip and knee OA, the severity of pain in the advanced OA patient group, although not the extent of OA, was associated with hs-CRP [50]. In a study designed to determine whether the levels of IL-6, TNF-α, and CRP in the normal population could be an adjunct marker in radiographic knee OA, the prevalence and incidence of radiological knee OA and the circulating levels of IL-6 were found closely related [51].

7. Biochemical markers of synovial tissue demolition

7.1 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases have been measured mainly in studies related to RA. The metalloproteinase enzyme group may cause collapse of the extracellular matrix elements by acting as collagen and Type II collagen [10]. The tissue inhibitors of metalloproteinases (TIMPs), which are natural inhibitors of metalloproteinases, are released from both chondrocytes and synovial cells. The synovial fluid and serum MMP-1 and MMP-3 levels have been shown to be elevated in patients with hip or knee OA. It has been reported that MMP-1 and MMP-3 levels can be detected not only in RA and OA but also in other adult states such as systemic lupus erythematosus [52]. MMP-3 has been reported radiographically to predict narrowing of the joint space [53].

7.2 Glucoside-galactose-pyridinoline

In the extracellular matrix, collagen Type II fibrils are placed in triple alpha helix. They are present at very low levels in the cartilage and other tissues found abundantly in the human synovium, thus showing an increase in urine Gly-Gal-Pyd levels in knee OA [54].

8. Other biochemical markers

Metabolic changes associated with obesity are possible causal agents for OA. Leptin is released primarily from adipocytes but is also released from chondrocytes and production increases in the cartilaginous form of OA. Leptin levels in synovial fluid are a possible metabolic factor in the pathogenesis of OA.

The role of markers such as leptin and IL-6 in obese-associated hip OA is unclear. In a study, it was determined that metabolic and ambulatory mechanisms may play a role in the etiology of hip OA and that the relationship between bone composition and the narrowing hip joint space was mediated by leptin, particularly in women [53].

Proteomic studies, which reveal the protein content of the cell tissue and biological fluids, distinguish related proteins and show functional changes in proteins have become more prominent in recent years [55]. In 2011, studies describing new proteomes in the urine, serum, and chondrocyte vesicles of OA patients were published [56]. In one of these studies, two fragments of fibulin-3 (Fib 3-1 and Fib 3-2) were shown to increase in the urine of OA patients.

Fibulin-3 is a proteome closely related to the TIMP, which plays an important role in the pathogenesis of OA. While it is suggested that they are biomarkers with high sensitivity and specificity, more work is needed to confirm them [57].

Deamidated COMP (D-COMP) hip joint was associated with OA radiological severity, but the same relationship was not detected with knee OA. It was suggested that D-COMP may be a biomarker specific to the hip joint [58].

It is thought that soluble leptin receptor (sOB-R) may be a marker of cartilage damage because of the significant relationship between the basal sOB-R level and low osteocalcin and PIIANP levels [59].

Since sOB-R is an adipokine receptor, it may be a promising marker for clarifying the relationship between obesity and pathogenesis of OA, especially in load-bearing joints [60].

9. Clinical evaluation of the osteoarthritis biomarkers present

Osteoarthritis Research Society International (OARSI) has published a series of recommendations for the use of soluble biomarkers in clinical trials. Publications supported by OARSI summarize the basic steps for a biomarker to be used as a drug development tool and various situations that OA biomarkers can be used [61, 62]. The Foundation for the National Institutes of Health/Osteoarthritis Initiative (FNIH/OAI) has published the results of an analysis on soluble biomarkers in a study that investigated the use of biomarkers as a drug development tool [61, 62]. The FNIH/OAI researchers found that time-dependent concentrations of urine C-terminal telopeptide of Type II collagen (uCTX-II), sHA, and serum N-terminal telopeptide of Type I collagen (sNTX-I) over a 24-month period were associated with subject cases that had both progressive pain and radiographic progression of knee OA over a 4-year period. Baseline levels of uCTX-II and sNTX-I predicted pain progression and radiographic progression. Plans are underway to qualify these biomarkers using samples and data from already-completed DMOAD (disease-modifying osteoarthritis drugs) trials.

Over the past years, several biomarkers have been tested in samples taken from patients with OA of various degrees. However, the number of newly found biomarkers was limited; most of them were already discovered molecules including MMPs, interleukins, adipokines and joint-related serum biomarkers, MMP-mediated degradation of C-reactive protein (CRPM), MMP-mediated degradation of Type III collagen (C3M), cartilage oligomeric matrix protein (COMP), HA (hyaluronic acid), N-terminal propeptide of collagen IIA (PIIANP), COL2-3/4 C-terminal cleavage product of Types I and II collagen, uCTX-II, MMP-3, and urine-nitrated Type II collagen degradation fragments (uCOL2-1 NO2).

The first analytical data came from the OAI. Eighteen biomarkers believed to be associated with OA were tested in the 129 blood or urine samples collected from OA patients [61]. The results showed that three commercially available biomarkers were related to age: sHA, P2ANP, and C1,2C. Similarly, uCTX-II, MMP-3, uCOL2-1 NO2, and sHA showed gender-related differences [61]. In a study, the concentration levels of sCOMP, sCTX-II, sMMP-3, sPIIINP, and sHA were identified in 79 patients who had cartilage damage and underwent knee arthroscopy or total knee replacement [63]. PIIANP, serum CTX-II, HA, and COMP levels were measured; however, only the concentration levels of HA and COMP were found significantly higher in OA patients with cartilage damage in the early term. These results suggest that the concentration levels of sCOMP and HA may be used in predicting the early-term cartilage lesions in the knee.

In a study on CRP [64], 58 cases of knee OA and 33 controls were examined for CRP and MMP-derived collagen types C1M, C2M, and C3M. The knee OA cases had elevated levels of C1M, C2M, and CRP and significantly lower level of C3M in comparison to controls.

Over the past few years, a limited number of studies have attempted to validate the existing OA biomarkers in the context of a clinical DMOAD study. Karsdal et al. [65] studied uCTX-I, uCTX-II, and serum osteocalcin. After 24-months, the biomarkers declined in all patients who had a positive Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain response to calcitonin. However, in another calcitonin study [65], the pain and biomarker responses after 24 months were not significant and the radiographic responses between the two studies were also different. This made it difficult to confirm these biomarkers for pain or radiographic response. The authors of the clinical trial of calcitonin have concluded that a precisely successful DMOAD study will be necessary to confirm the predictive and surrogate biomarkers for OA drug development.

Researchers have come up with studies that examine large OA cohorts dealing with the predictive ability of established OA biomarkers [66, 67]. In one of these studies, the sHA was associated with the joint space width (JSW) in the Iwaki Health Promotion Project over a period of 5 years [66].

In a Chingford cohort study [67] with a 20-year data history of radiographic knee OA progression in a group of middle-aged women with a Kellgren and Lawrence (KL) score of 0 at baseline, the high sCOMP levels were significantly related to painful radiographic OA development in the knee. The increase in the risk of radiographic knee OA was found in relation to sCOMP during the 5-year follow-up of 493 cases. In another report [68], 5 years of data from the Rotterdam study cohort were used to determine the relationship between the incidence of OA and KL score progression and biomarkers. As reported by Van Spil et al. [68], uCTX-II and sCOMP were found to be significantly associated with the incidence and progression of OA. The researchers investigating the 5-year data from the Cohort Hip and Cohort Knee (CHECK) study found that some biomarkers measured at the baseline were related to the incidence and progression of OA in the knee. Interestingly, uCTX-II and sCOMP were the most consistent biomarkers associated with the presence, incidence, and progression of knee OA.

UCTX-II and sCOMP had a positive effect on the presence and progression of OA in the knee. Both biomarkers showed negative correlation with knee OA. The authors suggested that the low cartilage and subchondral bone turnover in the earliest stages of knee OA may explain this second finding [67].

Over the past years, the mechanisms and benefits of inflammatory biomarkers in the pathogenesis and progression of OA have also been studied. The data from the Rotterdam study showed that CRP was independently associated with the incidence and progression of OA, similar to uCTX-II and sCOMP, and CRPM showed positive correlation to the progression of OA [69].

In a meta-analysis of the knee, hip, and hand OA studies from 1992 to 2012 [70], no correlation between the pain symptoms of OA and hs-CRP levels was found. However, radiographic findings showed strong correlation with hs-CRP levels. As shown in another study, inflammatory macrophages in the joints of knee OA patients might be a potential source of inflammation that triggers CRP production [71].

Soluble markers of the synovial fluid (SF) and inflammatory macrophages (CD14 and CD163) were shown to be associated with abundance of active macrophages in the knee joint as measured by EC20 SPECT imaging. These soluble markers were associated with narrowing of the joint space, osteophytes, and severity of the knee pain [72].

The best known inflammation markers have been confirmed in a study by Attur et al. [73]. Previously, proinflammatory mediators such as IL-1β, TNF-α, and COX-2 in peripheral blood leukocytes have been shown to identify the patients under risk for knee OA [74]. In a cohort of symptomatic knee OA patients prospectively evaluated for 24 months, an increase in the peripheral blood transcripts regarding

the basal levels of IL-1β, TNF-α, and COX-2 was shown to predict the narrowing of the joint space [73]. In another study assessing the samples taken from symptomatic knee OA patients under prospective evaluation for 24 months, the authors concluded that the levels of plasma interleukin-1 receptor antagonist (IL-1Ra) were positively correlated with the severity and progression of knee OA [75].

In addition to the above findings, reduced serum and uncarboxylated matrix Gla protein (ucMGP) levels were detected in OA patients. The mean serum ucMGP levels in knee OA patients were significantly lower than the healthy controls and showed negative correlation with radiographic severity [76].

Mabey et al. reported that the IL-4 and IL-6 levels in OA patients were significantly higher than the controls and showed positive correlation with radiographic severity [77].

In a study conducted on 138 OA patients [78], adipsin (complement factor D), leptin, adiponectin, resistin, and serpin E1 levels in the serum and cartilage volume with MRI were measured at the baseline and after 24 months. The elevated levels of adipsin and leptin were correlated to the increased cartilage volume in the global knee and medial femur. Adiponectin levels showed negative correlation with the cartilage volume in the medial compartment and femur. No correlation between resistin and serpin E1 and cartilage volume was detected.

10. Conclusion

In conclusion, biochemical markers, especially Type II collagen production, demolition, and synovial tissue markers, are important contributors in the early diagnosis, treatment, and follow-up of OA. Numerous biochemical markers that can potentially predict the progression of OA are still under research, but the progress is slow. For a molecule to qualify as a marker, it must be biologically and methodologically sensitive and specific. COMP, antigenic keratan sulfate, hyaluronan, YKL-40, Type III collagen N-propeptide, and urine Gly-Gal-Pyd are the most promising biochemical markers. The only predictor of cartilage loss determined by MR in the knee OA is sCOMP [79].

Early identification of OA with possible identification of new biochemical biomarkers with proteomic studies in the future seems possible. More comprehensive randomized and controlled studies of biomarkers will provide useful information in early diagnosis, prognosis, and response to treatment in OA.

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