

# Biomarkers for Early Diagnosis of Osteoarthritis

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Osteoarthritis (**OA**) is the most common form of degenerative joint disease characterized by progressive degeneration of articular cartilage, osteophyte formation and joint space narrowing. OA may commonly develop in knees, hips, hands, facet joints and feet. OA is a significant public health problem that costs society, at least, \$200 billion per year and the incidence of OA is estimated over 100 million people in the world per year. The diagnosis of OA is based on clinical and radiological imaging. However, onset of the OA is before radiological diagnosis. So that radiological methods are not sensitive enough in the early stages of OA. Early diagnosis of OA may allow preventive treatments without the destruction of joints. Therefore, there is a need for diagnostic methods. The term “biomarker” refers to a measurable indicator for biological processes including osteogenesis and inflammation. OA is believed to be caused by osteogenesis, mechanical stress on the joint and inflammatory process. So there are lots of candidate biomarkers, for mechanisms of OA and there are some novel biomarkers which may be used to predict OA. These biomarkers

have two groups including non-inflammatory (Type II collagen, NTX-I, CTX-I, CTX-II, aggrecans, Fib3-1, COMP, FSTL1, hyaluronic acid, BGP/OC, MGP, urotensin-II, OPN, ALP, BMPs, P1CP, P1NP, RANKL, MMPs, TRAcP, YKL-40) and inflammatory (IL-1 $\beta$ , IL-4, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, IL-18, TNF- $\alpha$  and CRP) biomarkers. Inflammatory biomarkers have also two groups which are pro-inflammatory and anti-inflammatory. Thus, OA is a multifactorial disease but so far its mechanism is not clear yet. The mentioned and unmentioned or unknown biomarkers are important for OA and this chapter highlights the recent advances in the use of biomarkers of OA and osteogenesis that could have facilitated the screening, diagnosis and management of OA.

## INTRODUCTION

OA is the most common form of degenerative joint diseases [1,2]. OA is the cause of functional limitations and characterized by progressive degeneration of articular cartilage, osteophyte formation, joint space narrowing, sclerosis on subchondral bone and inflammation of tissue, proliferation of synovial and cellular differentiation [2-5]. Although OA is the most common form of degenerative joint diseases, its pathogenesis is unclear [6]. Its major risk factors such as, genetic predisposition, aging and obesity, are associated with the pathogenesis of OA [7-9]. Since 2012 OA has become a significant public health problem that costs society, at least, \$200 billion per year and the incidence of OA is estimated over 100 million people in the world per year. [10]. Moreover, OA causes the loss of labor productivity among the mid-aged and the elderly people [11]. The diagnosis of OA is based on clinical and radiological imaging. However, the onset of OA is before radiological diagnosis. So that radiological methods are not sensitive enough in the early stages of OA. Early diagnosis of OA may allow preventive treatments without the destruction of joints, therefore there is an (urgent) need for diagnostic methods [12]. The term biomarker refers to a measurable indicator for biological processes including osteogenesis and inflammation. OA is believed to be caused by osteogenesis, mechanical stress on the joint and inflammatory process. Therefore, many biomarkers, due to related mentioned mechanisms, are candidate for OA. There are some novel biomarkers which may be used to predict OA [13].

OA biomarkers indicating the changes in serum, urine or synovial fluid levels, can be detected earlier than radiological changes and they indicate growth or degradation of tissue. There are some biomarkers available for the diagnosis of early stage of OA. Regarding OA, a lot of novel biomarkers should have some characteristics in order to be used for prognostic and diagnostic purposes at early stage [14]. Nowadays lots of research have focused on two groups of biomarkers including non-inflammatory (biomarkers in metabolism of collagen and in non-collagen proteins) and inflammatory biomarkers [2-9]. Inflammatory biomarkers include two groups which are pro-inflammatory and anti-inflammatory [15-18].

### I. Non-inflammatory biomarkers;

1. Biomarkers in metabolism of collagen
2. Biomarkers in metabolism of non-collagen

2.1. Aggrecan biomarkers

2.2. Non-aggrecan biomarkers

## II. Inflammatory biomarkers

1. Pro-inflammatory

2. Anti-inflammatory

OA is a multifactorial disease, but so far its mechanism is not clear yet. Inflammatory and non-inflammatory biomarkers are important for OA (Figure 1). This chapter highlights the recent advances in the use of biomarkers of OA and osteogenesis that could have facilitated the screening, diagnosis and management of OA.

## NON-INFLAMMATORY BIOMARKERS

For OA, there are two groups in non-inflammatory markers including biomarkers in the metabolism of collagen and non-collagen.

### Biomarkers in Metabolism of Collagen

Collagen is the most abundant insoluble fibrous protein in the Extracellular Matrix (**ECM**) of bone and cartilage [19]. Collagen plays an important role in the metabolism of bone and cartilage. The collagen network works metabolically active, meaning, the balance between the synthesis and degradation defines the turnover rate of collagens. This process is estimated to be 80 to 120 days and all biomolecules which work in this process are candidate biomarkers for OA [20]. Associated with OA pathogenesis, it has been shown a clear evidence of increased bone and cartilage mass along with increases in denaturation, cleavage and loss of type II collagen and proteoglycan. Previous studies have shown product of type II collagen denaturation [21-23].

Type II collagen is synthesized as a procollagen at articular cartilage. This procollagen is cleaved by the procollagen N- and C- proteinases and form Procollagen Type II C-Propeptide (**PIICP**) and Procollagen Type II N-Propeptide (**PIINP**) [24]. Half-life of PIICP is relatively short (14-16 hours) and it is a putative marker for synthesis of type II collagen. Furthermore, synthesis and degradation of type II collagen are increased in osteoarthritic cartilage in early stage of OA [25]. There is also positive correlation between the level of PIICP and OA in early stage [21]. The advantages of PIICP and PIINP as a biomarker are their determined levels in serum, synovial fluid, and tissue as well as their altered concentrations in early stage of OA.

Cross linked N-Telopeptide of Type I Collagen (**NTX-I**) and cross linked C-Telopeptide of Type I Collagen (**CTX-I**), which are metabolites of type I collagen, are associated with progression of OA [26]. CTX-I and NTX-I are used as a biomarker for resorption and formation of bone respectively. Procollagen type I N-propeptide (**PINP**) is also a biomarker for resorption of bone.  $\alpha$ -CTX-I, a form of CTX-I, is an indicator for new bone formation [27]. C-Telopeptide of Type II Collagen (CTX-II) is a product of type II collagen denaturation and is an important biomarker for damaged

cartilage. Joint erosion correlated with increased CTX-II [28]. A (recent) study emphasizes the association between CTX-II concentration and radiographic progression of OA [29]. Moreover, there is a correlation between CTX-II levels and osteophyte formation. As a result it is proposed that CTX-II can be a sensitive biomarker to assess OA severity [27].

## Biomarkers in Metabolism of Non-Collagen

Biomarkers in metabolism of non-collagen comprise two groups including biomarkers in metabolism of aggrecan and non-aggrecan.

### Aggrecans

These biomarkers are aggrecans including chondroitin sulfate and keratan sulfate which are the basic proteoglycans of cartilage. Aggrecan is the major proteoglycan in the joint cartilage and is important for the proper functioning of articular cartilage. Moreover, it plays an important role in chondroskeletal morphogenesis during development and in mediating chondrocyte-chondrocyte and chondrocyte-matrix interactions, and its ability to bind Hyaluronic Acid (**HA**) [30]. Keratan sulfate level indicates the turnover of aggrecan and is measured in body fluids [31]. Both keratan sulfate and chondroitin sulfate are sulfated glycosaminoglycans. Larsson et al. report that aggrecan fragments in synovial fluids are associated with pain, articular function and reduced articular distance in OA patients [27]. A study by Wakitani confirms that keratan sulfate level is significantly higher in the knee trauma patients than those in control group. In this study, serum keratan sulfate level of OA patients with Kellgren and Lawrence grades 0 and I is higher than the OA patients with Kellgren and Lawrence grades II, III, IV. Moreover, the serum concentration of keratan sulfate has been correlated with the damage of the articular cartilage and significantly has been increased even at an early stage after the injury [32]. Piscoya et al. report that keratan sulfate level and chondroitin sulfate release increased in a bimodal of increasing stress in porcine with articular stress [33]. In order to identify biochemical markers of OA in the guinea pig, Huebner et al have characterized keratan sulfate. Synovial fluid concentration of keratan sulfate increased in OA patients and was correlated with the severity of histological damage [34].

### Non-aggrecan biomarkers

These biomarkers consist of via formation and degradation of ECM components except collagen. One of the members of ECM components is called Cartilage Oligomeric Matrix Protein (**COMP**) also known as thrombospondin-5 in cartilage. COMP is synthesized by chondrocytes, osteoblasts, fibroblasts of tendon and synovial cells. The best known resorption marker is COMP and increased COMP level is correlated with the late stage of OA. Collagen degradation, also known as Col2-3/4C (C2C), is another biomarker for resorption of cartilage. It is reported that the serum levels of COMP and C2C are increased in patients with knee OA [27].

Fibulin-3 peptides (Fib3-1 and Fib3-2) are the members of ECM protein family. They are synthesized as glycoproteins that incorporate into a fibrillar ECM [35]. A previous study has

suggested that Fib3-1 and Fib3-2 are potential biomarkers of OA. This study reported that Fib3-1 and Fib3-2 are expressed in cartilage and serum of OA patients. The levels of Fib3-1 and Fib3-2 are significantly elevated in the superficial layer of fibrillated cartilage in OA patients [36]. Fibulin-3 peptides are associated with the tissue inhibitor of matrix metalloproteinase 3 (TIMP3). The overexpression of fibulin-3 suppresses the differentiation of chondrocytes, formation of cartilage nodules, production of proteoglycan, and matrix gene expression [37]. A recent study has shown that the levels of Fib3-1 and Fib3-2 significantly increased in OA patients compared to control group [38].

Follistatin-like protein 1 (**FSTL1**) is a novel OA biomarker in serum. FSTL1 is secreted as glycoprotein and can bind to heparin. This protein can modulate the action of Bone Morphogenetic Proteins (**BMPs**) on cell proliferation and differentiation [35]. A previous study showed that FSTL1 is overexpressed in synovium of rheumatoid arthritis patients which inhibits synovial cell growth. FSTL1 mRNA and protein levels increased in synovial tissues of OA patients. It is expressed in the cytoplasm of synovial and endothelial cells but is weakly expressed in chondrocytes. However, FSTL1 expression in serum and synovial cells is strongly elevated in OA patients [39]. In vivo and in vitro studies have shown that FSTL1 inhibits both MMPs and cytokines thus protects the joint from articular damage [39]. Serum FSTL1 concentrations are also correlated with age and OA progression. Therefore, FSTL1, which reflects the severity of joint damage, monitors OA progression and is an effective pharmacotherapeutic agent against OA, is a useful serum biomarker in OA [40].

Hyaluronic Acid (**HA**), also known as hyaluronon or hyaluronate, is a marker for early stage of OA [41]. HA has some physiological and biological functions and plays a role in ECM construction of cartilage and bone tissues. It can be measured in serum, skin, urine and synovial tissue [42]. The serum of OA patients contains a lower concentration of HA than healthy ones. HA prevents degeneration of cartilage and promotes regeneration of cartilage [43]. The serological HA level is correlated with the degree of synovial proliferation and the length of osteophytes in OA patients [41]. Intra-articular HA injection is a useful method for the treatment of OA. It has been shown that HA treatment improves the joint function and reduces pain [44]. The level of HA can be a biomarker for prediction of OA [27].

YKL-40 (Chitinase-3-Like-1 [**CHI3L1**], human cartilage glycoprotein) is a potential biomarker for diagnosis of OA progression. The level of YKL-40 indicates regeneration of cartilage but the exact functions of YKL-40 are still unknown. However, it is known that YKL-40 stimulates growth of fibroblast cells, activates the AKT and phosphoinositide-3 kinase signaling pathway [45]. YKL-40 is expressed in chondrocytes, synovial cells and macrophages of articular cartilage [46]. In a study, it is reported that the serum and synovium fluids of late stage OA patients contain higher concentration of YKL-40 than healthy ones. However, YKL-40 level is not useful during early stage of OA. The YKL-40 level in synovium and cartilage is correlated with the degree of OA [27,46].

Matrix metalloproteinases (MMPs; also known as matrixin) are also potential biomarkers for OA. MMPs have biological roles including degradation and turnover of most of the ECM components such as fibronectin, laminin, proteoglycan and collagen. MMPs which are catalytic enzymes (proteinases) break peptide bounds of target proteins in ECM. MMPs are expressed in synovial cells and chondrocytes, and comprise a family of over 20 proteins. According to structural and functional characteristics of MMPs, their family members are classified into six groups; collagenases (MMP-1, 8, 13, and 18), gelatinases (MMP-2 and 9), stromelysins (MMP-3, 10, 11), elastases (MMP-7 and 12), membrane type MMPs (MT-MMPs, MMP-14, 15, 16 and 17) and a group of unnamed MMPs [47]. A recent study showed that there is a correlation between MMP-1, MMP-3 and cartilage loss in 161 OA patients [48].

OA has been shown to be associated with basal calcium deposition in articular cartilage, synovial fluid and synovial membrane [49]. Bone Gamma-Carboxyglutamic Acid Protein (**BGP**), also known as Osteocalcin (**OC**), and Matrix Gamma-Carboxyglutamic Acid Protein (**MGP**) are vitamin K-dependent proteins. Both of them have been isolated from bone. BGP/OC, characterized by the presence of Gla residues, is the amplest non-collagenous protein in bone [50,51]. The level of BGP/OC in serum is closely linked to bone metabolism, thus its serum concentration serves as an indicator for OA [50]. Previous studies show that local reductions of BGP/OC levels in human osteons are associated with reduced cortical remodeling [52]. Gla-Rich Protein (**GRP**) is also a vitamin K-dependent protein and molecular mechanism of its function is still unknown. However, GRP has been suggested to act as a negative regulator of osteoblastic differentiation, a modulator of calcium availability and an inhibitor of soft tissue calcification [53]. Rafael et al. have demonstrated the carboxylated-GRP accumulation in control group whereas un-carboxylated-GRP was the predominant form in OA-affected tissues, co-localizing at sites of ectopic calcification. On the other hand, MGP is widely accepted as one of the strongest physiological inhibitors of soft tissue calcification [54]. According to unpublished data of our group, in human synovial cells with OA, MGP concentration is significantly higher than control group. Furthermore, OA leads to synovial fibrosis, and Urotensin II (**U-II**) may cause pathologic fibrosis in cardiovascular system, lung and liver. Gogebakan et al. report that the level of U-II is elevated in synovial fluid of OA patients [55]. However, further studies are needed to recognize U-II and MGP as candidate biomarkers.

The regulation of calcium availability and subsequent deposition in the ECM is also reported to be determinant for disease progression, which prompted us to investigate the relation between calcium mineral deposition and GRP expression and accumulation. Furthermore, GRP, also named Ucpa (upper zone of growth plate and cartilage matrix associated protein), was reported as a specific cartilage associated protein and suggested to be a negative regulator of osteogenic differentiation in mice [16].

Alkaline Phosphatase (**ALP**) is a hydrolase enzyme and removes phosphate groups from molecules such as nucleotides, proteins, etc. Bone and calcifying cartilage tissues express ALP which is observed on the cell surface and within matrix vesicles. ALP is a glycoprotein and

regulates other genes (e.g. BGP/OC) in the developmental program [56]. Therefore, ALP is the most frequently used biomarker in detection of osteoblastic bone formation. The ALP concentrations in liver and bone have been applied in routine diagnosis of OA [57]. Moreover, the level of ALP indicates that there can be active bone formation occurring as ALP is a byproduct of osteoblast activity [58]. Corrado et al. showed in OA cells that BGP/OC and ALP production have enhanced cell proliferation. In the same study, mentioned biomarkers have reduced cell proliferation in osteoporotic cells [59].

Osteopontin (OPN; also known as Bone Sialoprotein [**BSP-1**]) is an extracellular structural glycoprotein. OPN is expressed in bone cells (pre-osteoblasts, osteoblasts, osteoclasts, osteocytes, odontoblasts, and hypertrophic chondrocytes), macrophages, endothelial cells, smooth muscle cells and epithelial cells [60]. OPN plays role in diverse biological processes including bone morphogenesis, bio-mineralization, leukocyte recruitment, cell survival, and inflammation [61]. The role of OPN has been demonstrated as a biomarker that both as a synovial lining layer at the site of cartilage invasion and as mediating the attachment of synovial fibroblasts to cartilage at the sites of invasion. Furthermore, it contributes to matrix degradation in rheumatoid arthritis [62]. In cartilage and synovial fluid, OPN expression is associated with destruction and swelling of the joint, and loss of proteoglycan substance in articular cartilage of mice [63]. Cheng et al. report that high level of OPN is associated with OA progression [64]. A study by Xu et al. represents that phosphorylation of OPN induces activation of MMP-13 expression at gene and protein levels in the cartilage, and it has been further correlated with the cartilage degeneration [65].

## **INFLAMMATORY BIOMARKERS**

Inflammation has a crucial role in the development and progression of OA. Inflammatory cytokines is the most important biomarkers participating in the pathogenesis of OA. They are responsible for loss of metabolic homeostasis of tissues forming joints because of promoting catabolic and destructive processes. As to OA, inflammatory markers are divided into two groups; pro-inflammatory and anti-inflammatory biomarkers [15-18].

### **Pro-inflammatory Biomarkers**

There are many pro-inflammatory cytokine studies with respect to using as diagnostic in the literature. Interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, IL-15, IL-17, IL-18, tumor necrosis factor alpha (TNF- $\alpha$ ), CRP, and adipokines are pro-inflammatory biomarkers [66,67].

IL-1 $\beta$ , which is a member of the IL-1 family consisting of 11 members, plays a role in the pathogenesis of OA [68]. IL-1 $\beta$  induces catabolic activity independently or dependently with other mediators, and suppresses type II collagen and aggrecan [15,16,69]. IL-1 $\beta$  is expressed by chondrocytes, osteoblasts, synovial membrane cells and mononuclear cells in joint [70-75]. IL-1 $\beta$  induces also the production of several cytokines and chemokines such as IL-6 and IL-8 [76,77]. IL-1 $\beta$  levels highly increased in synovial fluids, synovial membranes, cartilage and subchondral bone

layers of OA patients [71,72,74,78]. Furthermore, Ning et al. show that the expression of IL-1 $\beta$  is correlated with severity of OA. In the same study they propose that IL-1 $\beta$  is a potential biomarker indicating the severity (or grade) of OA disease [79]. Another study affirms that IL-1 $\beta$  is a useful biomarker assessing the effect of treatment with HA in OA patients [80].

IL-6 is another pro-inflammatory biomarker for OA pathogenesis. Previous studies show that IL-6 inhibits the production of type II collagen in animal models of OA [81]. IL-6 is induced by IL-1 $\beta$  in chondrocytes during inflammation process. IL-6 is also a regulator of inflammatory and immunological processes. High level of IL-6 is shown to be present in synovial fluids of rheumatoid arthritis and OA patients [76,82].

Another well-known pro-inflammatory cytokine is IL-15 used as a biomarker in OA diagnosis. Recent studies show that IL-15 indicates disease level and is a progressive marker for OA [83,84]. Teunis et al. show that the serum level of IL-15 is higher in OA patients compared to healthy controls [85]. Another previous study demonstrates that MMP-7 and IL-15 can play crucial roles in the pathogenesis of OA [86].

Another member of pro-inflammatory markers is IL-18. A recent study demonstrates that the IL-18 level significantly increased in OA patients compared to control groups. Another study shows that the more severity of disease increases, the more IL-18 level increases. The same study proposes that IL-18 can be used as a biomarker for OA and in assessing of disease severity [87]. In a previous study, IL-18 level significantly increased in synovial fluid and articular cartilage of OA patients, which is positively correlated with radiographic severity. According to this result, Wang et al. support the role of IL-18 in the pathophysiology of OA. They also demonstrate that IL-18 stimulates the COX-2 and TNF- $\alpha$  expressions in primary synovial cells, while increasing Prostaglandin-E2 (**PGE<sub>2</sub>**) and TNF- $\alpha$  levels in the supernatant of the culture medium in primary synovial cells and inducing high PGE<sub>2</sub> level production in second-generation synovial cells [87].

TNF- $\alpha$  that is an inflammatory marker plays a role in pathophysiologic processes of OA. The TNF- $\alpha$  secretion is correlated synergistically with the level of IL-1 $\beta$  [70-77,88]. A major risk factor in injuries is mechanical stress which results in the subsequent release of cartilage matrix degradation products during starting and progression of OA. This situation triggers the same signaling pathways as those induced by pro-inflammatory biomarkers including TNF- $\alpha$  and IL-1 $\beta$ . It means that the mentioned cytokines trigger inflammation and catabolic processes in joint tissues such as cartilage and synovial fluids, and so activate inner cell pathways [89,90]. For example, TNF- $\alpha$  suppresses the syntheses of type II collagen and proteoglycan. Hence, MMP-1, MMP-3, MMP-13, a kind of disintegrin, and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4) are synthesized in induced chondrocytes [91,92]. It is shown that there is a positive correlation between the expression of TNF receptor and pain, joint stiffness, and disease severity in serum of OA patients [93]. As a result, it is proposed that TNF- $\alpha$  is a good biomarker for OA.

C-Reactive Protein (**CRP**) is a pentameric protein in blood plasma and its level rises in response to inflammation. Increased CRP levels following IL-6 secretion induces pro-inflammatory cytokines [94]. Jin et al. report that the CRP level is increased in OA patients compared to healthy control group [95]. Moreover, a previous study shows that there is an association between CRP and the loss of physical function in OA patients [96].

Adipokines are expressed from adipocytes and related with pathogenesis of OA. Adipokines including leptin, adiponectin, visfatin and resistin play role in cartilage and bone homeostasis [97]. They have inflammatory effect and are associated with OA [27]. It is shown that adiponectin and leptin are correlated with extra articular symptoms [98]. Popa et al. demonstrate the existence of an inverse correlation between severity of inflammation and plasma leptin levels in active rheumatoid arthritis [99]. A study by Berner proved that adiponectin is also expressed by osteoblasts and has a role in bone homeostasis [100]. The increased level of synovial adiponectin has been confirmed by Senolt et al. in rheumatoid arthritis [101].

## Anti-Inflammatory Biomarkers

In OA, anti-inflammatory cytokines are responsible for anabolic or catabolic mechanism of chondrocytes. There are many anti-inflammatory cytokines including IL-4, IL-7, IL-8, IL-10 and IL-13 which play role in OA pathophysiology [13].

IL-4 has a crucial role in shaping the nature of immune responses. Moreover, it suppresses bone resorption in vitro and in vivo [102,103]. This activity is indicated through ability of IL-4 to inhibit the expression of IL-1, TNF and RANKL in adjacent cells which modulate osteoclast proliferation and life span [103]. Assirelli et al. have also shown the differences in the spectrum of biological effects promoted by IL-4 in human OA cartilage. Their outcomes indicate that IL-4 has the ability to inhibit the IL-1 $\beta$  -suppressed release of MMP-13 and “chemokine (C-C motif) lig and 5 regulated on activation normal T cell expressed and secreted” (CCL5/RANTES). Both MMP-13 and CCL5/RANTES are synthesized in OA chondrocytes which indicates IL-4 as a vital anabolic cytokine during OA cartilage pathogenesis [104]. IL-4 and IL-13 are closely related cytokines which are known to inhibit osteoclastogenesis by aiming osteoblasts to produce an inhibitor against OPG. Yamada et al. report that the inhibitory effect of IL-4 is stronger than that of IL-13 throughout osteoclast differentiation in cell culture system [105].

IL-7 is also a cytokine and induces bone loss through increased osteoclastogenesis. It regulates bone resorption both peri-articular and systemically. High level of IL-7 is associated with rheumatoid arthritis [106]. Toralto et al. propose that elevated levels of IL-7 lead to bone loss by two mechanisms. First one is that, IL-7 causes the T cell proliferation of the key osteoclastogenic cytokines RANKL and TNF. And the second one is that IL-7 leads to the expansion of the OC precursor pool by inducing the osteoclast proliferation. The increased concentrations of circulating osteoclastogenic cytokines cause the increased bone loss associated with inflammation [107].

IL-8, also known as CXCL8, is a neutrophil selective chemo-attractant cytokine. IL-8 is expressed in macrophages, neutrophils and endothelial cells. A study by Bendre shows that IL-8 may be a potential activator of osteoclast mediated bone resorption [108]. Previous studies reveal that IL-8 has multiple functions like chemotactic activity [109].

IL-10 is an anti-inflammatory cytokine that inhibits immunoproliferative and inflammatory processes. IL-10 is produced by B cells, mast cells, eosinophils, macrophages and dendritic cells. IL-10 can also down-regulate the synthesis of pro-inflammatory cytokines and chemokines as well as the synthesis of nitric oxide, gelatinases and collagenases [110]. The low level of IL-10 causes the inhibition of the pro-inflammatory cytokines and collagenases. Previous studies have confirmed that lack of IL-10 causes femur bone loss and alveolar bone loss in various animal models [111,112]. Recent studies show that IL-10 has potent inhibitory effects on osteoclastogenesis [113]. The molecular mechanism is that IL-10 up-regulates OPG expression but down-regulates RANKL [114].

## CONCLUSION

In addition to all OA biomarkers described above, there are epigenetic biomarkers which have also emerged as a key mechanism in the development of OA. Moreover, Vascular Endothelial Growth Factor (**VEGF**) can be used for determining severity of OA [115].

In conclusion, biomarkers are important for early diagnosis of OA which is a multifactorial disease. Radiological methods are not sensitive enough in the early stages of OA. Early diagnosis may allow preventive treatments without (before?) the joint destruction. As a final remark, there is an urgent need for novel diagnostic methods to improve prognosis of OA patients.

## References

1. Pereira D, Ramos E, Branco J. Osteoarthritis. *Acta Med Port.* 2015; 28: 99-106.
2. Bitton R. The economic burden of osteoarthritis. *Am J Manag Care.* 2009; 15: S230-235.
3. Verma P, Dalal K. Serum Cartilage Oligomeric Matrix Protein (COMP) in knee osteoarthritis: a novel diagnostic and prognostic biomarker. *J Orthop Res.* 2013; 31: 999-1006.
4. Kumm, J, Tamm A, Lintrop M. Diagnostic and prognostic value of bone biomarkers in progressive knee osteoarthritis: a 6-year follow-up study in middle-aged subjects. *Osteoarthritis Cartilage,* 2013; 21: 815-822.
5. Bay-Jensen AC, Liu Q, Byrjalsen I, Li Y, Wang J, Pedersen C, et al. Enzyme-linked Immunosorbent Assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIM--increased serum CIIM in subjects with severe radiographic osteoarthritis. *Clin Biochem.* 2011; 44: 423-429.
6. Pelletier JP, Raynauld JP, Caron J, Mineau F, Abram F, et al. Decrease in serum level of matrix metalloproteinase is predictive of the disease-modifying effect of osteoarthritis drugs assessed by quantitative MRI in patients with knee osteoarthritis. *Annals of the Rheumatic Diseases.* 2010; 69: 2095-2101.
7. Swearingen CA, Carpenter JW, Siegel R, Brittain IJ, Dotzlaw J, et al. Development of a novel clinical biomarker assay to detect and quantify aggrecanase-generated aggrecanase fragments in human synovial fluid, serum and urine. *Osteoarthritis Cartilage.* 2010; 18: 1150-1158.
8. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol.* 2011; 7: 33-42.
9. Hart PH, Ahern MJ, Smith MD, Finlay-Jones JJ, et al. Comparison of the suppressive effects of interleukin-10 and interleukin-4 on synovial fluid macrophages and blood monocytes from patients with inflammatory arthritis. *Immunology.* 1995; 84: 536-542.

10. Ma VY, Chan L, Carruthers KJ. Incidence, prevalence, costs, and impact on disability of common conditions requiring rehabilitation in the United States: stroke, spinal cord injury, traumatic brain injury, multiple sclerosis, osteoarthritis, rheumatoid arthritis, limb loss, and back pain. *Arch Phys Med Rehabil.* 2014; 95: 986-995.
11. Shi D, Dai J, Xu Z, Chen D, Jiang Q. Update on basic and clinical aspects of osteoarthritis. *Ann Transl Med.* 2015; 3: 142.
12. Braun HJ, Gold GE. Diagnosis of osteoarthritis: imaging. *Bone.* 2012; 51: 278-288.
13. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis.* 2013; 5: 77-94.
14. Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyère O. Value of biomarkers in osteoarthritis: current status and perspectives. *Ann Rheum Dis.* 2013; 72: 1756-1763.
15. Stove J, Huch K, Günther KP, Scharf HP. Interleukin-1beta induces different gene expression of stromelysin, aggrecanase and tumor-necrosis-factor-stimulated gene 6 in human osteoarthritic chondrocytes *in vitro*. *Pathobiology.* 2000. 68: 144-149.
16. Chadjichristos C, Ghayor C, Kypriotou M, Martin G, Renard E, et al. Sp1 and Sp3 transcription factors mediate interleukin-1 beta down-regulation of human type II collagen gene expression in articular chondrocytes. *J Biol Chem.* 2003; 278: 39762-39772.
17. E X, Cao Y, Meng H, Qi Y, Du G. Dendritic cells of synovium in experimental model of osteoarthritis of rabbits. *Cell Physiol Biochem.* 2012; 30: 23-32.
18. Hammacher A, Ward LD, Weinstock J, Treutlein H, Yasukawa K, Simpson RJ. Structure-function analysis of human IL-6: identification of two distinct regions those are important for receptor binding. *Protein Sci.* 1994; 3: 2280-2293.
19. Halper J, M Kjaer. Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins. *Adv Exp Med Biol.* 2014; 802: 31-47.
20. Laurent GJ. Dynamic state of collagen: pathways of collagen degradation *in vivo* and their possible role in regulation of collagen mass. *Am J Physiol.* 1987; 252: C1-9.
21. Elsaid KA, Chichester CO. Review: Collagen markers in early arthritic diseases. *Clin Chim Acta.* 2006; 365: 68-77.
22. Kraus VB. Biomarkers in osteoarthritis. *Curr Opin Rheumatol.* 2005; 17: 641-646.
23. Charni-Ben Tabassi N, Garnero P. Monitoring cartilage turnover. *Curr Rheumatol Rep.* 2007; 9: 16-24.
24. Peltonen L, Halila R, Ryhänen L. Enzymes converting procollagens to collagens. *J Cell Biochem.* 1985; 28: 15-21.
25. Nelson F, Dahlberg L, Laverty S, Reiner A, Pidoux I. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. *J Clin Invest.* 1998; 102: 2115-2125.
26. Bettica P, Cline G, Hart DJ, Meyer J, Spector TD. Evidence for increased bone resorption in patients with progressive knee osteoarthritis: longitudinal results from the Chingford study. *Arthritis Rheum.* 2002; 46: 3178-3184.
27. Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyère O. Value of biomarkers in osteoarthritis: current status and perspectives. *Ann Rheum Dis.* 2013; 72: 1756-1763.
28. Sowers MF, Karvonen-Gutierrez CA, Yosef M, Jannausch M, Jiang Y, et al. Longitudinal changes of serum COMP and urinary CTX-II predict X-ray defined knee osteoarthritis severity and stiffness in women. *Osteoarthritis Cartilage.* 2009; 17: 1609-1614.
29. Kumahashi N, Swärd P, Larsson S, Lohmander LS, Frobell R. Type II collagen C2C epitope in human synovial fluid and serum after knee injury--associations with molecular and structural markers of injury. *Osteoarthritis Cartilage.* 2015; 23: 1506-1512.
30. Kiani C, Chen L, Wu YJ, Yee AJ, Yang BB. Structure and function of aggrecan. *Cell Res.* 2002; 12: 19-32.
31. Fraser A, Fearon U, Billingham RC, Ionescu M, Reece R, et al. Turnover of type II collagen and aggrecan in cartilage matrix at the onset of inflammatory arthritis in humans: relationship to mediators of systemic and local inflammation. *Arthritis Rheum.* 2003; 48: 3085-3095.
32. Wakitani S, Nawata M, Kawaguchi A, Okabe T, Takaoka K, et al. Serum keratan sulfate is a promising marker of early articular cartilage breakdown. *Rheumatology (Oxford).* 2007; 46: 1652-1656.
33. Piscocya JL, Fermor B, Kraus VB, Stabler TV, Guilak F, et al. The influence of mechanical compression on the induction of osteoarthritis-related biomarkers in articular cartilage explants. *Osteoarthritis Cartilage.* 2005; 13: 1092-1099.
34. Huebner JL, Kraus VB. Assessment of the utility of biomarkers of osteoarthritis in the guinea pig. *Osteoarthritis Cartilage.* 2006; 14: 923-930.
35. Mobasheri A. Osteoarthritis year 2012 in review: biomarkers. *Osteoarthritis Cartilage.* 2012; 20: 1451-1464.
36. Henrotin Y, Gharbi M, Mazzucchelli G, Dubuc JE, De Pauw E. Fibulin 3 peptides Fib3-1 and Fib3-2 are potential biomarkers of osteoarthritis. *Arthritis Rheum.* 2012; 64: 2260-2267.

37. Wakabayashi T, Matsumine A, Nakazora S, Hasegawa M, Iino T. Fibulin-3 negatively regulates chondrocyte differentiation. *Biochem Biophys Res Commun.* 2010; 391: 1116-1121.
38. Ishijima M, Kaneko H, Kaneko K. The evolving role of biomarkers for osteoarthritis. *Ther Adv Musculoskelet Dis.* 2014; 6: 144-153.
39. Wang Y, Li D, Xu N, Tao W, Zhu R. Follistatin-like protein 1: a serum biochemical marker reflecting the severity of joint damage in patients with osteoarthritis. *Arthritis Res Ther.* 2011; 13: R193.
40. Murakami K, Tanaka M, Usui T, Kawabata D, Shiomi A. Follistatin-related protein/follistatin-like 1 evokes an innate immune response via CD14 and toll-like receptor 4. *FEBS Lett.* 2012; 586: 319-324.
41. Singh S, Kumar D, Sharma NR. Role of hyaluronic Acid in early diagnosis of knee osteoarthritis. *J Clin Diagn Res.* 2014; 8: LC04-07.
42. Cao JJ, Singleton PA, Majumdar S, Boudignon B, Burghardt A. Hyaluronan increases RANKL expression in bone marrow stromal cells through CD44. *J Bone Miner Res.* 2005; 20: 30-40.
43. Moreland LW. Intra-articular hyaluronan (hyaluronic acid) and hyalans for the treatment of osteoarthritis: mechanisms of action. *Arthritis Res Ther.* 2003; 5: 54-67.
44. Migliore A, Procopio S. Effectiveness and utility of hyaluronic acid in osteoarthritis. *Clin Cases Miner Bone Metab.* 2015; 12: 31-33.
45. Recklies AD, C White, H Ling. The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase beta-mediated signalling pathways. *Biochemical Journal.* 2002; 365: 119-126.
46. Huang K, Wu LD. YKL-40: a potential biomarker for osteoarthritis. *J Int Med Res.* 2009; 37: 18-24.
47. Ishiguro N, Kojima T, Poole AR. Mechanism of cartilage destruction in osteoarthritis. *Nagoya J Med Sci.* 2002; 65: 73-84.
48. Pelletier JP, Raynaud JP, Caron J, Mineau F, Abram F, Dorais M, et al. Decrease in serum level of matrix metalloproteinases is predictive of the disease-modifying effect of osteoarthritis drugs assessed by quantitative MRI in patients with knee osteoarthritis. *Ann Rheum Dis.* 2010. 69: 2095-2101.
49. Liu YZ, AP Jackson, SD Cosgrove. Contribution of calcium-containing crystals to cartilage degradation and synovial inflammation in osteoarthritis. *Osteoarthritis Cartilage.* 2009; 17: 1333-1340.
50. Hoang QQ, Sicheri F, Howard AJ, Yang DS. Bone recognition mechanism of porcine osteocalcin from crystal structure. *Nature.* 2003; 425: 977-980.
51. Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiol Rev.* 1989; 69: 990-1047.
52. Ingram RT, Park YK, Clarke BL, Fitzpatrick LA. Age- and gender-related changes in the distribution of osteocalcin in the extracellular matrix of normal male and female bone. Possible involvement of osteocalcin in bone remodeling. *J Clin Invest.* 1994; 93: 989-997.
53. Viegas CS, Rafael MS, Enriquez JL, Teixeira A, Vitorino R, et al. Gla-rich protein acts as a calcification inhibitor in the human cardiovascular system. *Arterioscler Thromb Vasc Biol.* 2015; 35: 399-408.
54. Cranenburg EC, LJ Schurgers, C Vermeer. Vitamin K: the coagulation vitamin that became omnipotent. *Thromb Haemost.* 2007; 98: 120-125.
55. Gögebakan B, Uruc V, Ozden R, Duman IG, Yagiz AE. Urotensin II (U-II), a novel cyclic peptide, possibly associated with the pathophysiology of osteoarthritis. *Peptides.* 2014; 54: 159-161.
56. Golub EE. Role of matrix vesicles in biomineralization. *Biochem Biophys Acta.* 2009; 1790: 1592-1598.
57. Epstein E, Kiechle FL, Artiss JD, Zak B. The clinical use of alkaline phosphatase enzymes. *Clin Lab Med.* 1986; 6: 491-505.
58. Rodan GA, R SB. *Advances in bone and mineral research annual II*, edn. P. WA1984, Amsterdam: Excerpta Medica.
59. Corrado A, Neve A, Macchiarola A, Gaudio A, Marucci A. RANKL/OPG ratio and DKK-1 expression in primary osteoblastic cultures from osteoarthritic and osteoporotic subjects. *J Rheumatol.* 2013; 40: 684-694.
60. Fisher LW, NS Fedarko. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. *Connect Tissue Res.* 2003; 44: 33-40.
61. Lund SA, Giachelli CM, Scatena M. The role of osteopontin in inflammatory processes. *J Cell Commun Signal.* 2009; 3: 311-322.
62. Petrow PK, Hummel KM, Schedel J, Franz JK, Klein CL, et al. Expression of osteopontin messenger RNA and protein in rheumatoid arthritis: effects of osteopontin on the release of collagenase 1 from articular chondrocytes and synovial fibroblasts. *Arthritis Rheum.* 2000; 43: 1597-605.

63. Yumoto K, Ishijima M, Rittling SR, Tsuji K, Tsuchiya Y, et al. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. *Proc Natl Acad Sci U S A*. 2002; 99: 4556-4561.
64. Cheng C, Gao S, Lei G. Association of osteopontin with osteoarthritis. *Rheumatol Int*. 2014; 34: 1627-1631.
65. Xu M, Zhang L, Zhao L, Gao S, Han R. Phosphorylation of osteopontin in osteoarthritis degenerative cartilage and its effect on matrix metalloproteinase 13. *Rheumatol Int*. 2013; 33: 1313-1319.
66. Schlaak JF, Schwarting A, Knolle P, Meyer zum Büschenfelde KH, Mayet W. Effects of Th1 and Th2 cytokines on cytokine production and ICAM-1 expression on synovial fibroblasts. *Ann Rheum Dis*. 1995; 54: 560-565.
67. Wojdasiewicz P, Poniatowski A, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm*. 2014; 2014: 561459.
68. A Dinarello C. Overview of the interleukin-1 family of ligands and receptors. *Semin Immunol*. 2013; vol. 25: 389-393.
69. Shakibaei M, Schulze-Tanzil G, John T, Mobasheri A. Curcumin protects human chondrocytes from IL-1 $\beta$ -induced inhibition of collagen type II and  $\beta$ 1-integrin expression and activation of caspase-3: an immunomorphological study. *Ann Anat*. 2005; 187: 487-497.
70. de Lange-Brokaar BJ, Ioan-Facsinay A, van Osch GJ, Zuurmond AM, Schoones J. Synovial inflammation, immune cells and their cytokines in osteoarthritis: a review. *Osteoarthritis Cartilage*. 2012; 20: 1484-1499.
71. Melchiorri C, Meliconi R, Frizziero L, Silvestri T, Pulsatelli L. Enhanced and coordinated *in vivo* expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. *Arthritis Rheum*. 1998; 41: 2165-2174.
72. Massicotte F, Lajeunesse D, Benderdour M, Pelletier JP, Hilal G, et al. Can altered production of interleukin-1 $\beta$ , interleukin-6, transforming growth factor- $\beta$  and prostaglandin E (2) by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthritis Cartilage*. 2002; 10: 491-500.
73. Ribel-Madsen S, Else Marie Bartels, Anders Stockmarr, Arne Borgwardt, Claus Cornett, et al. A synoviocyte model for osteoarthritis and rheumatoid arthritis: response to Ibuprofen, betamethasone, and ginger extract—a cross-sectional *in vitro* study. *Arthritis*. 2012; 505842.
74. Farahat MN, Yanni G, Poston R, Panayi GS. Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis*. 1993; 52: 870-875.
75. Szekanecz Z, AE Koch, Cell-cell interactions in synovitis. Endothelial cells and immune cell migration. *Arthritis Res*. 2000; 2: 368-373.
76. Guerne PA, DA Carson, M Lotz. IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones *in vitro*. *J Immunol*. 1990; 144: 499-505.
77. Lotz M, Terkeltaub R, Villiger PM. Cartilage and joint inflammation. Regulation of IL-8 expression by human articular chondrocytes. *J Immunol*. 1992; 148: 466-473.
78. Sohn DH, Sokolove J, Sharpe O, Erhart JC, Chandra PE, et al. Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. *Arthritis Res Ther*. 2012; 14: R7.
79. Ning L, Ishijima M, Kaneko H, Kurihara H, Arikawa-Hirasawa E, et al. Correlations between both the expression levels of inflammatory mediators and growth factor in medial perimeniscal synovial tissue and the severity of medial knee osteoarthritis. *Int Orthop*. 2011; 35: 831-838.
80. Vincent HK, Percival SS, Conrad BP, Seay AN, Montero C. Hyaluronic Acid (HA) Viscosupplementation on Synovial Fluid Inflammation in Knee Osteoarthritis: A Pilot Study. *Open Orthop J*. 2013; 7: 378-384.
81. Poree B, Kyriotou M, Chadjichristos C, Beauchef G, Renard E, Legendre F, et al. Interleukin-6 (IL-6) and/or soluble IL-6 receptor down-regulation of human type II collagen gene expression in articular chondrocytes requires a decrease of Sp1. Sp3 ratio and of the binding activity of both factors to the COL2A1 promoter. *J Biol Chem*. 2008; 283: 4850-4865.
82. Mabey T, Honsawek S. Cytokines as biochemical markers for knee osteoarthritis. *World J Orthop*. 2015; 6: 95-105.
83. Ling SM, Patel DD, Garner P, Zhan M, Vaduganathan M, et al. Serum protein signatures detect early radiographic osteoarthritis. *Osteoarthritis Cartilage*. 2009; 17: 43-48.
84. Sun JM, Liang-Zhi Sun, Jun Liu, Bao-hui Su, Lin Shi, et al. Serum interleukin-15 levels are associated with severity of pain in patients with knee osteoarthritis. *Dis Markers*. 2013; 35: 203-206.
85. Teunis T, Beekhuizen M, Kon M, Creemers LB, Schuurman AH. Inflammatory mediators in posttraumatic radiocarpal osteoarthritis. *J Hand Surg Am*. 2013; 38: 1735-1740.
86. Tao Y, Qiu X, Xu C, Sun B, Shi C. Expression and correlation of matrix metalloproteinase-7 and interleukin-15 in human osteoarthritis. *Int J Clin Exp Pathol*. 2015; 8: 9112-9118.

87. Wang Y, Xu D, Long L, Deng X, Tao R, Huang G. Correlation between plasma, synovial fluid and articular cartilage Interleukin-18 with radiographic severity in 33 patients with osteoarthritis of the knee. *Clin Exp Med.* 2014; 14: 297-304.
88. Aggarwal BB, SC Gupta, B Sung. *Br J Pharmacol.* 2013; 169: 1672-1692.
89. Roman-Blas JA, Jimenez SA. NF-kappaB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage.* 2006; 14: 839-848.
90. Marcu KB, Otero M, Olivotto E, Borzi RM, Goldring MB. NF-kappaB signaling: multiple angles to target OA. *Curr Drug Targets.* 2010; 11: 599-613.
91. Saklatvala J. Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature.* 1986; 322: 547-549.
92. Seguin CA, Bernier SM. TNFalpha suppresses link protein and type II collagen expression in chondrocytes: Role of MEK1/2 and NF-kappaB signaling pathways. *J Cell Physiol.* 2003; 197: 356-369.
93. Penninx BW, Abbas H, Ambrosius W, Nicklas BJ, Davis C. Inflammatory markers and physical function among older adults with knee osteoarthritis. *J Rheumatol.* 2004; 31: 2027-2031.
94. Bay-Jensen AC, Reker D, Kjelgaard-Petersen CF, Mobasher A, Karsdal MA. Osteoarthritis year in review 2015: soluble biomarkers and the BIPED criteria. *Osteoarthritis Cartilage.* 2016; 24: 9-20.
95. Jin X, Beguerie JR, Zhang W, Blizzard L, Otahal P. Circulating C reactive protein in osteoarthritis: a systematic review and meta-analysis. *Ann Rheum Dis.* 2015; 74: 703-710.
96. Smith JW, Martins TB, Gopez E, Johnson T, Hill HR. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther Adv Musculoskelet Dis.* 2012; 4: 315-325.
97. Poonpet T, Honsawek S. Adipokines: Biomarkers for osteoarthritis? *World J Orthop.* 2014; 5: 319-327.
98. Krysiak R, Handzlik-Orlik G, Okopien B. The role of adipokines in connective tissue diseases. *Eur J Nutr.* 2012; 51: 513-528.
99. Popa C, Netea MG, Radstake TR, van Riel PL, Barrera P. Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. *Ann Rheum Dis.* 2005; 64: 1195-1198.
100. Berner HS, Lyngstadaas SP, Spahr A, Monjo M, Thommesen L. Adiponectin and its receptors are expressed in bone-forming cells. *Bone.* 2004; 35: 842-849.
101. Senolt L, K Pavelka D, Housab M, Haluzik. Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. *Cytokine.* 2006; 35: 247-252.
102. Miossec P, Chomarat P, Dechanet J, Moreau JF, Roux JP, Delmas P, et al. Interleukin-4 inhibits bone resorption through an effect on osteoclasts and proinflammatory cytokines in an ex vivo model of bone resorption in rheumatoid arthritis. *Arthritis Rheum.* 1994; 37: 1715-1722.
103. Lacey DL, Erdmann JM, Teitelbaum SL, Tan HL, Ohara J. Interleukin 4, interferon-gamma, and prostaglandin E impact the osteoclastic cell-forming potential of murine bone marrow macrophages. *Endocrinology.* 1995; 136: 2367-2376.
104. Assirelli E, Lia Pulsatelli, Paolo Dolzani, Daniela Platano, Eleonora Olivotto, Giuseppe Filardo, et al. Human osteoarthritic cartilage shows reduced in vivo expression of IL-4, a chondroprotective cytokine that differentially modulates IL-1beta-stimulated production of chemokines and matrix-degrading enzymes in vitro. *PLoS One.* 2014; 9: e96925.
105. Yamada, A, Takami M, Kawawa T, Yasuhara R, Zhao B, et al, Interleukin-4 inhibition of osteoclast differentiation is stronger than that of interleukin-13 and they are equivalent for induction of osteoprotegerin production from osteoblasts. *Immunology.* 2007; 120: 573-579.
106. Miyaura C, Onoe Y, Inada M, Maki K, Ikuta K. Increased B-lymphopoiesis by interleukin 7 induces bone loss in mice with intact ovarian function: similarity to estrogen deficiency. *Proc Natl Acad Sci U S A.* 1997; 94: 9360-9365.
107. Toraldo G, Roggia C, Qian WP, Pacifici R, Weitzmann MN. IL-7 induces bone loss in vivo by induction of receptor activator of nuclear factor kappa B ligand and tumor necrosis factor alpha from T cells. *Proc Natl Acad Sci U S A.* 2003; 100: 125-130.
108. Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ. Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. *Bone.* 2003; 33: 28-37.
109. Kim SJ, Uehara H, Karashima T, Mccarty M, Shih N. Expression of interleukin-8 correlates with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in nude mice. *Neoplasia.* 2001; 3: 33-42.
110. Zhang Q, Chen B, Yan F, Guo J2, Zhu X. Interleukin-10 inhibits bone resorption: a potential therapeutic strategy in periodontitis and other bone loss diseases. *Biomed Res Int.* 2014; 2014: 284836.

111. Dresner-Pollak R, Gelb N, Rachmilewitz D, Karmeli F, Weinreb M, et al. Interleukin 10-deficient mice develop osteopenia, decreased bone formation, and mechanical fragility of long bones. *Gastroenterology*. 2004; 127: 792-801.
112. Al-Rasheed A, Scheerens H, Rennick DM, Fletcher HM, Tatakis DN. Accelerated alveolar bone loss in mice lacking interleukin-10. *J Dent Res*. 2003; 82: 632-635.
113. Xu LX, Kukita T, Kukita A, Otsuka T, Niho Y, Iijima T. Interleukin-10 selectively inhibits osteoclastogenesis by inhibiting differentiation of osteoclast progenitors into preosteoclast-like cells in rat bone marrow culture system. *Journal of Cellular Physiology*. 1995; 165: 624-629.
114. Liu D, S Yao, GE Wise. Effect of interleukin-10 on gene expression of osteoclastogenic regulatory molecules in the rat dental follicle. *Eur J Oral Sci*. 2006; 114: 42-49.
115. Saetan N, Honsawek S, Tanavalee A, Yuktanandana P, Meknavin S, et al. Relationship of plasma and synovial fluid vascular endothelial growth factor with radiographic severity in primary knee osteoarthritis. *Int Orthop*. 2014; 38: 1099-1094.