Advances in Molecular biomarker for early diagnosis of Osteoarthritis

Abstract: Osteoarthritis (OA) is a chronic degenerative joint disease. The pathogenesis is poorly understood. What is known is that OA is characterized by imbalance in anabolic and catabolic gene expression in articular chondrocytes. This results in bone on bone articulations resulting in impaired mobility and joint pain. Although the cause of OA is unknown, comorbidities include: aging, obesity, and mechanical stress. Currently the only diagnostic modalities are radiology and physical examination, and early detection is rare. Biomarkers are quantifiable substances, and their presence can be suggestive of a certain phenomenon or disease. Biomarkers are popular for early diagnosis for pathological conditions in the fields of oncology, cardiology, and endocrinology. This review has systematically reviewed the literature about biomarkers in the field of OA, specifically protein, miRNA, and metabolic biomarkers found in the blood, urine, and synovial fluid.

Keywords: Osteoarthritis; Biomarker; Diagnosis.

Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease that affects the articular cartilage of joints. 52.5 million Americans have been diagnosed with OA, and the economic burden of OA is more than $185 million a year [1]. OA is characterized by an inability of chondrocytes (cartilage cells) to produce viable matrix, which is intended to replace matrix that has been lost. This results in loss of protective articular cartilage resulting in bone on bone articulations, causing pain and immobility. OA has been classically categorized as a disease of aging and a symptom of joint use; however, current OA research indicates that OA is an inflammatory disease associated with biochemical and molecular changes [2-4]. Comorbidities associated with OA include aging, obesity, diabetes, and mechanical stress on joints; however periodic weight bearing exercise is protective against OA [5, 6]. The exact pathophysiology of OA progression is unknown; however, it is likely due to several heterogeneous factors.

Currently OA is diagnosed with radiographic and physical examinations. The loss of cartilage is best visualized via radiography. The predominant scale to evaluate OA severity is the Kellgren-Lawrence (KL) scale which rates OA severity between 0-4 based on radiological visualization of joint width [7, 8]. The physical exam can be used to determine how much pain is present, and to what degree mobility has been compromised [9]. These methods are reactive not predictive. The goal of OA biomarker research is to develop tests that are predictive rather than reactive. According to the World Health Organization a biomarker is, “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease,” [10]. Biomarkers can be found in biological fluids, including urine, serum, feces, lymph, and synovial fluid. The primary bio-fluids that have been used to identify biomarkers in pathophysiology of OA are urine, synovial fluid, and blood. Blood is easily withdrawn from the body, and it mediates many of the immune and immunologic pathways of the body. Urine is easily accessible. Synovial fluid is more difficult to assess; however, it is the first fluid initially altered in the pathogenesis of OA. In the past 5 years the scientific community has attempted to identify various OA biomarkers. This review aims to provide a comprehensive summary of these findings.
Serum biomarkers

Serum is the liquid portion of blood that does not clot, because clotting factors have been removed [11]. Immune reactions occur primarily in the blood, and therefore serum serves as an important bio-fluid. Serum biomarker research has been primarily focused on cytokines, Interleukins (IL), proteins and miRNA molecules.

Interleukins (IL) are proteins that regulate the cellular immune response. One important role interleukins play is promoting the proliferation and differentiation of B-cells [12]. IL mediated dysfunction in B-cells is responsible for the pathogenesis of various systemic diseases. It has been hypothesized that dysfunctions in B cells may leads to initiation and progression of OA [13]. To investigate this, several labs have analyzed concentrations of various IL proteins in serum. Shan et al. (2017) found elevated serum IL-21 levels in OA patients. They performed a study involving 53 subjects, 40 of whom were recently diagnosed with OA and 13 healthy controls. They found significantly elevated levels of IL-21, IL-17A and IFN-γ levels in the group of patients with OA; however, they found no correlation between IL-4 OA [14], Silvestri et al. (2006) conducted a study on 243 subjects: seventy (70) had nodal hand OA, 71 had erosive hand OA, 64 were about to undergo total joint replacement, 34 with hip OA, 30 with knee OA, and 38 healthy controls. They found significantly elevated levels of IL-4R in all the groups with OA [15]. This contrasts with the results of Shan et. al. as it indicates that serum IL-4 may be involved in the pathogenesis of OA. This highlights that interleukins may serve as OA biomarkers but these findings need to be validated with additional research.

Fibulin-3 is an extracellular glycoprotein found in Connective Tissue (CT) including blood and cartilage. Fibulin-3 inhibits Tissue differentiation of chondrocytes and suppresses angiogenesis [16, 17]. Runhar et al. (2016) elucidate a relationship between Fibulin-3 levels and OA pathogenesis. They recruited 241 women between the ages of 50 and 60 with a BMI >27, that did not have knee OA. They measured baseline serum concentration of 3 fibulin-3 peptides: Fib3-1, Fib3-2, Fib3-3, and measured the concentration of the three peptides 30 months later. They found baseline Fibulin-3 peptide concentration significantly higher in patients that developed OA than those that did not. This indicates that baseline Fibulin-3 concentration could serve as a prognostic biomarker for OA pathogenesis [18]. There are many Fibulin-3 peptides, and future research could be conducted on the impact of various subtypes of Fibulin-3 and OA.

NADPH Oxidase generates reactive oxidative species (ROS), which put oxidative stress on cells. High levels of ROS and oxidative stress induce apoptosis [19]. Age related NADPH oxidase (arNOX) is a surface oxidase that increases with age [20]. High ROS levels may induce apoptosis in chondrocytes thus arNOX may be implicated in the pathogenesis of OA. Kim et. al. (2015) tested this hypothesis by conducting a cross sectional study with 40 subjects, 20 control subjects, and 20 patients with OA. They found that patients with severe OA had significantly elevated levels of arNOX [21]. This study illustrates a relationship between OA and arNOX activity; however, it did not determine whether high arNOX levels cause OA. Further research can determine whether arNOX expression and or hyperactivity cause OA.

Collagen is a key structural component in cartilage. Balance between different collagen types maintains cartilage health. Collagen Type X (ColX) is up-regulated when chondrocytes undergo hypertrophy. It maintains ECM metabolism, and is often found at the epiphysis of long bones during endochondral ossification [22]. Elevated ColX levels could reflect changes in chondrocyte metabolism and may be implicated in the development of OA. He et al. (2014) performed a competitive ELISA C-Col10 to measure concentration of ColX within sera. They found ColX concentration significantly elevated in patients with a KL score >2 when compared to the concentration found in the sera of healthy control subjects (p=0.04) [23]. This data supports that elevated ColX concentration can be indicative of OA pathogenesis.

Cartilage Oligomeric Matrix Protein (COMP) is present in the matrix of cartilage cells. The exact function of COMP is unknown; however, it likely plays a role in cartilage stabilization. Elevated COMP levels have been observed with reactive and rheumatoid arthritis [24]. Verma et al. (2013) tested the efficacy of COMP as an OA biomarker using a case control study analyzing the serum of 150 subjects. One Hundred (100) subjects had knee OA, and 50 subjects were healthy controls. They found average serum COMP levels to be 1117.21 ng/ml in OA patients and 338.62 ng/ml in the control group. These results were statistically significant; furthermore, they found COMP levels negatively correlated with disease duration (R=-0.88) and positively correlation with age and pain score [25]. This provides evidences of COMP as a prognostic biomarker for the pre-emptive diagnosis of OA. Serum Biomarker research is also being conducted on: cartilage breakdown products, bone markers, and chemokines. Much of the other protein research is in early phases. OA biomarker research has predominantly focused on serum proteins; however, miRNA markers are also important.
Serum MiRNA

MiRNA molecules are non-coding RNA molecules which regulate gene expression by targeting the 3’ UTR of mRNA and induce mRNA degradation or inhibit translation. MiRNA expression patterns change with age and various degenerative musculoskeletal diseases. [26]. Wan et al. (2018) collected plasma samples from 74 patients and 79 healthy controls and analyzed expression of miRNA-136. They found plasma levels of miRNA-136 significantly decreased in patients with OA when compared to healthy controls. They also found miRNA-136 levels inversely proportional to disease severity and IL-17. [27]. They additionally performed a dual luciferase assay and a western blot to illustrate that miRNA-136 target IL-17 for degradation. It has been previously reported that IL-17 may mediate cartilage breakdown in OA [28]. This is significant because it illustrates a possible mechanism of OA pathogenesis. Zheng et al (2018) devised an innovative method by correlating type III collagen CTX (CTX-III) and miRNA-98 expression in serum samples of OA patients. They performed this assay on 20 individuals with OA and 20 healthy controls. They reported both CTX-III (p=0.0013) and miR-98 (p=0.0065) expression significantly higher in OA patients than in healthy controls [29].

Li et al. (2017) performed a microarray analysis on arthritic mouse ankle samples to identify novel mRNA and miRNAs that might play a role in OA [28]. They discovered several mRNAs (e.g. Adam8, Arg1, Ccl2) and miRNAs (e.g. miR-150, miR-7, and miR200b) differentially regulated in mice OA samples [30]. Ntoumou et al. (2017), collected serum samples from 24 human subjects: 12 samples from healthy controls and 12 samples from individuals with OA. They performed a miRNA array and found 279 miRNAs differentially expressed in osteoarthritic conditions. Further, they validated their preliminary findings and showed 3 signature miRNAs: 140-3p, 33b-3p, and 671-3p down-regulated in OA. These miRNA are known to be involved in several molecular pathways including: Wnt, ErbB, TGF-beta, etc. [31]. These miRNA may serve as biomarkers for OA; however, there is need to test on large number of sample size. Kong et al. (2017) performed a microarray analysis on the plasma of 100 knee OA patients and the plasma of 100 healthy controls. They discovered 41 miRNAs up-regulated and 29 miRNAs down-regulated. They further validated several differentially regulated miRNAs and found miR-486-5p, 19b-3p, 122-5p to be independent risk factors for knee OA. They also found that a combination analysis involving these miRNA molecules had the greatest diagnostic value [32].

Dong et al. (2018) performed a microRNA expression profile on plasma samples derived from 218 patients prior to treatment of Celecoxib and after 6 weeks of treatment with Celecoxib. Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) that selectively inhibits the cyclooxygenase-2 (COX-2) pathway. It reduces OA associated joint pain [33]. They found 10 key miRNA signatures dysregulated with treatment: miR-675-5p, miR-126-5p, miR-155-5p, miR-320a, miR-210, miR-3197, miR-17-3p, miR-146a-5p, miR-4796, and miR-92a-3p [34]. MiR-155-5p has been shown to up-regulate pro-inflammatory cytokines including IL1-1B, IL-6, IL-8, and TNF-α [35]. MiR-146a is involved in inducing apoptosis in response to mechanical injury. These results are important because these miRNA molecules may not only serve as markers of OA, they may also illustrate potential therapeutic targets for OA. Although the serum is an important source of biomarkers, the urine also represents many metabolic changes occurring in the body, and is thus an important source of biomarkers.

Urine biomarkers

Urine serves as an important bio-fluid because large volumes can be acquired with noninvasive techniques. Urine contains plasma filtration waste products, and the urine reflects the current physiologic state of the body [36].

Ions such as Calcium$^{2+}$ and Zinc$^{2+}$ play a role in tissue preservation. In addition, both of the ions mediate bone formation [37, 38]. High levels of Zinc$^{2+}$ inhibit chondrocyte differentiation, and Calcium$^{2+}$ is a key signaling molecule. Both Calcium$^{2+}$ and Zinc$^{2+}$ are cofactors of MMP proteins [39]. Calcium$^{2+}$ and Zinc$^{2+}$ levels could serve as markers of MMP activity, thus they may serve as biomarkers for OA progression. This hypothesis was tested by Xin, et al. (2017). They performed a case control study involving 102 subjects: 82 with knee OA and 20 healthy controls. The experimental group was further subdivided based on the KL criterion. They found significant elevated level of CTX-II in OA patients compared to the healthy controls; however, there was no statistically significant difference between group 1 (early OA) and health controls [40]. This indicates that elevated levels could be witnessed only in the late pathogenesis of OA and could not serve as a prognostic biomarker for early OA.

MMP proteins cleave Collagen type 2 (CTX-II). Therefore, an elevated concentration of CTX-II fragments should be expected when MMP activity is up-regulated [41]. This would suggest that CTX-II fragments could serve a similar role to that of Zinc$^{2+}$ and Calcium$^{2+}$ in predicting MMP activity and mirroring OA pathogenesis. This hypothesis
was tested by Poole et al. (2016) who conducted a population-based cohort study involving 253 subjects with knee pain; the subjects were placed into 3 groups. Group 1 had no cartilage pathology. Group 2 had pre-radiologic cartilage pathology. Group 3 had radiologic cartilage pathology. The subjects were then analyzed an average of 3.3 years later. The study found statistically significant differences in the CTX-II fragment concentrations at baseline; they found baseline CTX-II concentrations higher in progressors than in non-progressers (p=0.003) [42]. This may mean that CTX-II fragment concentration may serve as a predictive biomarker for the development of OA.

**Synovial Fluid**

The synovial fluid is the most applicable bio-fluid to investigate progression of OA because of its direct and intimate relationship with various tissues of knee joint. Change in knee joint/tissue environment will directly affect synovia gene expression [43] and synovial fluid composition. Research is currently focused on protein, metabolic and miRNA biomarkers within the synovia.

**Synovial Protein**

As was previously discussed IL-17 shows potential as a serum marker of OA. IL-17 is an inflammatory cytokine responsible for mediating the body’s immune system [44, 45]. Yiu et al. (2015) conducted a study with 332 subjects including 226 OA patients and 106 controls. The OA patients were further divided into groups based on the KL grading criteria. They analyzed IL-17 levels in synovial fluid using ELISA. They found significantly higher synovia concentrations of IL-17 in OA patients (P<0.01) [46].

Interleukin-6 (IL-6) is a cytokine with several functions, it may be responsible for the differentiation of osteoblasts and or osteoclasts [47, 48]. Osteoclasts are responsible for the resorption of bone matrices [49, 50]. A possible hypothesis for OA pathogenesis could include an IL-6-osteoclast interaction. This hypothesis was tested by Doss et al. (2007). They collected synovial fluid from 49 end stage OA patients who had recently undergone joint replacement surgery. They measured levels of IL-6 with ELISA, and found that out among the 49 patients tested, 8 (16%) had elevated IL-6 levels in the synovial fluid [50, 51]. Animal studies also suggested that inflammatory markers such as IL-6, IL-1 and TNF-α can serve as OA biomarkers. Recently, Castrogiovanni et al. (2019) investigated role of exercise on rat OA model. The analysis involved 32 rats, one group of rats served as the experimental group in which OA was induced and the rats performed moderate physical activity. They found that anterior cruciate ligament transection (ACLT)-rats with OA have elevated level of inflammatory markers (IL-1, IL-6, and TNF-α) and moderate physical activity reduced expression of these OA markers [52].

OA is characterized by synovial fluid hypoxia. Hypoxia-inducible factor (HIF) is a transcription factor that promotes chondrocyte survival in times of hypoxia; therefore, HIF concentration may be associated with OA [53, 54]. Chu et al. (2014) tested this hypothesis by performing a cross sectional study involving 278 patients with knee OA and 203 healthy controls. They further subdivided the OA patients based upon the KL grading system and determined HIF-1α levels using ELISA. They found the average HIF-1α concentration in synovial fluid to be 542.98 pg/mL for patients with OA, and 113.45 pg/mL in the control group. They also found that as the grades of OA changed the concentration of HIF-1α levels significantly increased (p<0.001). They found the average synovial concentration HIF-1α concentration to be 508.98 pg/mL in grade 2 OA, 530.36 pg/mL in grade 3 OA, and 588.71 pg/mL in grade 4 OA [55]. They performed the same assay on serum samples and they validated the results. The correlation found between the concentration of HIF-1α and the severity of OA in the patient indicates that HIF may play a role in the pathogenesis of OA, and may serve as a biomarker.

TNF-α is a cellular signaling protein that communicates cellular stress to nearby cells, and is responsible for signaling cell death [56, 57]. It is commonly elevated after injuries, specifically knee injuries and meniscus tears [58]. Larsson et al. (2015) conducted a cross sectional study on 132 subjects to determine the relationship between TNF-α and OA progression; the subjects had undergone a meniscectomy an average of 18 years ago. They measured TNF-α concentrations by immunoassays. Subjects with higher first examination concentrations of TNF-α were more likely to have OA progression. They also found that higher second examination concentrations of TNF-α were associated with additional loss of joint space [59]. Similarly, to Doss et al., Larsson et al. also found elevated levels of IL-6 associated with OA progression. This indicated that TNF-α may serve as a biomarker for OA pathogenesis.

Lubricin is a glycoprotein secreted by synovial fibroblast and found in synovial membranes and fluid; it protects chondrocytes by reducing joint friction. It is
downregulated after joint injuries [60, 61]. Several studies have demonstrated the role of Lubricin in the pathogenesis of OA. Musemueci et. al (2014) performed a histologic analysis of 40 patients with OA and 9 control subjects. They found that the synovial fluid of patients with OA had significantly decreased lubricin [62]. Musemueci et. al group also performed another study (2019) in which they induced OA in rats and found that physical activity and the Mediterranean diet increased lubricin expression [63]. These findings all suggest the role of lubricin in the pathogenesis of OA.

Bradykinin is a vasodilator and mediates inflammation. Vasodilation enlarges blood vessels and decreases blood pressure [64, 65]. Since bradykinin is involved with increasing tissue perfusion, it may be associated with OA progression. Belluci et al. (2013) tested this hypothesis by obtaining synovial fluid from 30 patients with knee OA. They measured levels of bradykinin by performing an ELISA, and found bradykinin levels positively associated with cartilage degradation [66]. Synovial protein markers are vital in understanding OA; however, miRNA markers are becoming more important.

**miRNA markers**

Murata et al. (2009) performed an analysis on miR-16, miR-132, miR146, miR-155 and miR-223 in the synovial fluid and the plasma of patients with OA, RA, and in healthy controls. They identified miRNA-132 as a signature miRNA marker with diagnostic value. They concluded that plasma miR-132 levels could be used to distinguish individuals with OA/RA from healthy controls [67]. A similar study was performed by Li et al. (2016) and identified OA specific miRNAs in synovial fluid. They found miRNA: 23a-3p, 24-3p, 27a-3p, 27b-3p, 29c-3p, 34a-5p, and 186-5p to be differentially regulated in early and late phase OA [68].

As was stated earlier the prevalence of OA is higher in women than in men, and this risk increases after menopause. This is a poorly understood phenomenon. Kohle et al. (2017) performed a study on the miRNA cargo of exosomes isolated from the synovial fluid. They found differential expression of miRNA signatures in men and women that suffered from OA. They found miRNA: 181d-3p, 3904-3p, 155-3p, 4532, 185-5p, 7107-5p, 6865-3p, 4459, and 7107-5p dysregulated in female OA patients [4]. These findings are significant because little research has been done to isolate gender specific biomarkers for OA, despite the increased prevalence of OA in females. These findings are also significant because they illustrated a direct relationship between female sex hormones and OA. MiRNA research is an important aspect of OA biomarker research. Much of the research is in early stages. These include markers that have been demonstrated in vitro or on non-human derived cells. These markers include miRNA-29a, 145-5p, and 122 [69-71].

**Conclusion**

In the age of personalized and genomic medicine biomarkers are going to continue to gain importance. OA biomarkers will become especially important due to the high prevalence and cost of OA. OA biomarker research has already helped to deepen our understanding of the pathologic changes that occur in OA (Table 1). The ideal biomarker would be one that could be collected non-invasively, be predictive of the outcome of the disease, and provide potential therapeutic targets. The current markers all have advantages and disadvantages, as do each of the fluids studied. Blood and urine are easily accessible; however, changes in the synovial fluid can be detected earlier.

Serum is fairly easily accessible, because it can be extracted from the body with minimally invasive techniques. Serum is also the site of much of the body’s metabolism, and therefore many of the changes that occur with OA may be represented by serum biomarkers. There is data to support the use of various serum interleukins as biomarkers for OA. IL-21 and IL-17a are secreted by T-cells and mediate immune responses. They are up-regulated in individuals with OA, and seem to show promise as OA biomarkers. IFN-γ is another serum cytokine that is elevated in OA; however, IFN-γ is responsible for up-regulating MHC class I in times of viral infections [72]. Thus, it may be elevated during times of aseptic viral arthritis, and may not be specifically elevated for OA. Other serum proteins studied include Fibulin-3, COMP, CoIX, and arNOX. Fibulin-3 is informative because the baseline elevations provide prognostic value. COMP was another protein marker with prognostic value, and it is also important how COMP levels negatively correlate with disease progression. The most significant problem associated with the use of COMP as a marker for OA is the elevation of COMP in the serum of individuals with RA; however, RA is associated with other markers including rheumatoid factor, and anti-citrulline antibodies [73]. CoIX is a potential biomarker for OA; however, significant elevations were not evidenced early in the pathogenesis of OA, they were evidenced when patients had a KL score >2.
**Table 1:** Molecular biomarkers and their role in pathophysiological function of osteoarthritis.

| Sample type   | Molecule name | Function                                                                 | Study outcome                                                                 | Reference                  |
|---------------|---------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------|----------------------------|
| Serum protein | IL-21, IL-17A, IFN-γ (11-17 also found in synovia) | Serum cytokines, B-cell proliferation and differentiation | Significantly elevated levels found in OA patients | Shan et al./ Yiu et al. |
| Serum protein | IL-4/ IL-4R   | Serum cytokine                                                             | Conflicting                                                                   | Shan et al./ Silvestri et al. |
| Serum protein | Fibulin-3 fragments | Inhibition of chondrocyte differentiation and suppresses angiogenesis | Baseline levels indicative of OA progression                                  | Runhar et al. |
| Serum protein | arNOX         | ROS generation                                                             | Elevated in cases of severe OA                                                | Kim et al.                 |
| Serum protein | CoIX          | ECM maintenance during times of chondrocyte hypertrophy                   | Elevated when KL score >2                                                    | He et al.                  |
| Serum protein | COMP          | Likely involved in cartilage stabilization                                 | Elevated in cases of OA, negatively correlated with disease duration         | Verma et al. |
| Serum miRNA   | miRNA-136     | Multifactorial                                                             | Levels inversely proportional to IL-17                                       | Wan et al.                 |
| Serum miRNA   | miRNA-98      | Related to immune system                                                   | Levels correlated with CTX-III and OA                                         | Zheng et al. |
| Serum miRNA   | miRNA 140-3p, 33b-3p, 671-3p, etc. | Involved in many metabolic pathways including: Wnt, ErbB, | Dysregulated in OA                                                          | Ntoumou et al./ Kong et al. |
| Serum miRNA   | miR-675-5p, miR-126-5p, miR-155-5p, etc. | Many including apoptosis, and cytokine expression | Dysregulated in OA with Celecoxib treatment                                  | Dong et al. |
| Urine ions    | Ca$^{2+}$ and Zn$^{2+}$ | Tissue preservation, bone formation, signaling | Elevated when KL score ≥2                                                    | Xin et al.                 |
| Urine metabolite | CTX-II      | Associated with MMP activity                                              | Baseline elevations higher in OA progressors than in non-progressors         | Poole et al. |
| Synovial protein | IL-6        | Osteoblast/ osteoclast differentiation                                       | Mixed                                                                        | Doss et al. |
| Synovial protein | HIF         | Chondrocyte survival during hypoxia                                         | Significantly elevated in OA                                                 | Chu et al. |
| Synovial protein | II-1B, TNF, and MMP | Markers of OA                                                              | Decreased expression after exercise in rats                                  | Castrogiovanni et. al |
| Synovial protein | lubricin     | Protects chondrocytes                                                      | Decreased expression in OA                                                   | Musumecia et al., Szychlinska et al. (2016), Szychlinska et al. (2019) |
| Synovial protein | TNF-α        | Signaling cell death                                                       | Significantly elevated prior to disease progression                          | Larsson et al. |
| Synovial Peptide | Bradykinin | Vasodilation                                                               | Associated with cartilage degradation                                        | Belluci et al. |
| Synovial miRNA | miRNA 132, 16, 146, etc. | Multifactorial                                                             | Differentially regulated in OA                                               | Murata et al./ Li et al. |
| Synovial miRNA | miRNA 181d-3p, 3904-3p, 155-3p, etc. | Multifactorial, possibly involved in estrogen signaling                    | Dysregulated in females with OA                                             | Kohle et al. |
Urine markers are the most easily accessible. Calcium and Zinc are cofactors for MMP proteins however, elevated urine concentrations are non-specific. Urinary levels of CTX-I1 are better than Calcium and Zinc levels because they are more specifically elevated in OA, and elevated CX-II fragment levels were found at baseline in individuals that went on to develop OA. Synovial fluid is the most representative of the state of joints in the body; however, it is the most difficult to assess, because of the invasive techniques involved with its collection. Several serum proteins have been studied, and the most relevant include HIF, TNF-α, and Bradykinin. HIF and TNF-α levels appear to serve a prognostic role, while Bradykinin may not.

MiRNAs based diagnosis are one the most dynamic areas in OA biomarker research. Several studies identified OA specific miRNAs in serum and synovial fluid but there is a need to validate on a large scale sample size to get such type of diagnostic test from bench to clinic. For early and accurate diagnosis of an OA, we conclude that there is need to identify panels of biomarkers in various bio-fluids (serum, urine and synovial fluid) to predict early stage OA in precise manner.

Conflict of interest: Authors state no conflict of interest

Acknowledgements: We would like to thank The Department of Orthopaedic Surgery for their support.

References

1. CDC. Arthritis In America. 2017.
2. Huang G, Hua S, Yang T, Ma J, Yu W, Chen X. Platelet-rich plasma shows beneficial effects for patients with knee osteoarthritis by suppressing inflammatory factors. Journal of Experimental and Therapeutic Medicine. 2018;15(3):6.
3. Litwic A, Edwards MH, Dennis EM, Cooper C. Epidemiology and Burden of Osteoarthritis. British Medical Bulletin, 2013;105:185-199.
4. Kolhe R, Hunter M, Liu S, Jadeja R, Pandkar C, Mondal A. Gender-specific differential expression of exosomal miRNA in synovial fluid of patients with osteoarthritis. Nature Scientific Reports. 2017;7:14.
5. Musumeci G, Castrogiovanni P, Trovato FM, Imbesi R, Giunta S, Szychlinska MA, et al. Physical activity ameliorates cartilage degeneration in a rat model of aging: a study on lubricin expression. Scandinavian Journal of Medicine and Science In Sports. 2015;25(3):e222-30.
6. Musumeci G, Loreto C, Imbesi R, Trovato FM, Di Giunta A, Lombardo C, et al. Advantages of exercise in rehabilitation, treatment and prevention of altered morphological features in knee osteoarthritis. A narrative review. Histology And Histopathology. 2014;29(6):707-19.
7. McAlindon TE, Kannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthritis Cartilage. 2016;22(5):363-388.
8. Kellgren JH, Lawrence JS. Radiological Assessment of Osteo-Arthrosis. Annals of the Rheumatic Diseases. 1957;16(4): 494-502.
9. Iversen M, Lyn Price L, von Heideken J, Harvey W, Wang Ch. Physical examination findings and their relationship with performance-based function in adults with knee osteoarthritis. BioMed Central Musculoskeletal Disorders. 2016;17(273):12.
10. Strimbu K, Tavel J. What are Biomarkers. Current Opinion HIV AIDS. 2010;5(6):463-466.
11. Yu Z, Kastenmüller G, He Y, Belcvedi P, Müller G, Prehn C, et al. Differences between Human Plasma and Serum Metabolite Profiles. PloS One. 2010;6(7):e21230.
12. Akdis M, Burgler S, Cramer R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-g: Receptors, functions, and roles in diseases. The Journal of Allergy and Clinical Immunology. 2011;127(3):701-721.
13. Zharkova O, Celhar T, Cravens PD, Satterthwaite AB, Fairhurst AM, Davis LS. Pathways leading to an immunological disease: systemic lupus erythematous. Rheumatology. 2017;56(1):i55-i66.
14. Shan Y, Qi C, Liu Y, Gao H, Zhao D, Jiang Y. Increased frequency of peripheral blood follicular helper T cells and elevated serum IL-21 levels in patients with knee osteoarthritis. Molecular Medicine Reports. 2006;15(3): 1095-1102.
15. Silvestri T, Pulsatelli L, Dolzani P, Facchini A, Meliconi R. Elevated Serum levels of Soluble Interleukin-4 receptor. Osteoarthritis and Cartilage. 2006;14(7):717-9.
16. Nandhu MS, Kwiatkowska A, Bhaskaran V, Hayes J, Hu B, Viapiano MS. Tumor-derived fibulin-3 activates pro-invasive NF-kappa B signaling in glioblastoma cells and their microenvironment. Oncogene. 2017;36(34):4875-4886.
17. Ehlermann J, Weber S, Pfisterer P, Schorle H. Cloning, expression and characterization of the murine Efemp1, a gene mutated in Dovey-Honeycomb retinal dystrophy. Gene Expression Patterns. 2003;3: 441-7.
18. Runhaar J, Sanchez C, Taralla S, Henrotin Y, Bierma-Zeinstra SM. Fibulin-3 fragments are prognostic biomarkers of osteoarthritis incidence in overweight and obese women. Osteoarthritis and Cartilage. 2016;24(4):672-8.
19. Van Heerebeek L, Meischi C, Stocker W, Meijer CJLM, Niessen HWM, Roos D. NADPH oxidative(s): new source(s) of reactive oxygen species in the vascular system?”. Journal of Clinical Pathology. 2002;55(7): 561-568.
20. Meadows C, Morré OJ, Morré DM, Draelos ZD, Kern D. Age-related NADH oxidase (arNOX)-catalyzed oxidative damage to skin proteins. Archives of Dermatological Research. 2014;306(7):645-52.
21. Kim MJ, Kim HJ, Hong YH, Lee CK, Kim YW, Shon OJ, et al. Age-related NADPH Oxidase (arNOX) Activity Correlated with Cartilage Degradation and Bony Changes in Age-related Osteoarthritis. Journal of Korean Medical Science. 2015;30(9):1246-52.
22. Shen G. The Role of Type X Collagen in Facilitating and Regulating Endochondral Ossification of Articular Cartilage. Orthodontics and Craniofacial Research. 2005;8(1):11-17.
23. He Y, Siebuhr AS, Brandt-Hansen NU, Wang J, Su D, Zheng Q, et al. Type X collagen levels are elevated in serum from human osteoarthritis patients and associated with biomarkers of cartilage degradation and inflammation. BMC Musculoskeletal Disorders. 2014;15(309):10.

24. Tseng S, Hari Reddi A, Di Cesare P. Cartilage Oligomeric Matrix Protein: A biomarker of Arthritis. Biomarker Insights. 2009;4:33-44.

25. Verma P, Dalal K. Serum cartilage oligomeric matrix protein (COMP) in knee osteoarthritis: a novel diagnostic and prognostic biomarker. Journal of Orthopaedic Research. 2013;31(7):999-1006.

26. Macfarlane LA, Murphy PR. MicroRNA: Biogenesis, Function and Role in Cancer. Current Genomics. 2010;11(7):537-67.

27. Wan L, Zhao Q, Niu G, Xiang T, Ding C, Wang S. Plasma miR-136 can be used to screen patients with knee osteoarthritis from healthy controls by targeting IL-17. Experimental and Therapeutic Medicine. 2018;16(4):3419–3424.

28. Honorati MC, Bovara M, Cattini L, Piccini A, Faccini A. Contribution of interleukin 17 to human cartilage degradation and synovial inflammation in osteoarthritis. Osteoarthritis and Cartilage. 2002;10:799-807.

29. Zheng WD1, Zhou FL, Lin N, Liu J. Investigation for the role of CTX-III and microRNA-98 in diagnosis and treatment of osteoarthritis. European Review for Medical and Pharmacological Sciences. 2018;22:5424-5428.

30. Li H, Bai B, Wang J, Xu Z, Yan S, Liu G. Identification of key mRNAs and microRNAs in the pathogenesis and progression of osteoarthritis using microarray analysis. Molecular Medicine Reports. 2017;16:5565-5666.

31. Ntoumou E, Tzetis M, Braoudaki M, Lambrou G, Poulou M, Malizos K, et al. Anastasopoulou and A. Tsezou. Serum microRNA array analysis identifies miR-140-3p, miR-33b-3p and miR-671-3p as potential osteoarthritis biomarkers involved in metabolic processes. Clinical Epigenetics. 2017;12(9):15.

32. Kong R, Gao J, Si Y, Zhao D. Combination of circulating miR-19b-3p, miR-122-5p and miR-486-5p expression correlates with risk and disease severity of knee osteoarthritis. American Journal of Translational Research. 2017;9(6):2852-2864.

33. Xu C, Gu K, Yasen Y, Hou Y. Efficacy and Safety of Celecoxib Therapy in Osteoarthritis: A Meta-Analysis of Randomized Controlled Trials. Medicine. 2016;95(20):e3585.

34. Dong Z, Jiang H, Jian X, Zhang W. Change of miRNA expression profiles in patients with knee osteoarthritis before and after celecoxib treatment. Journal of Clinical Laboratory Analysis. 2019;33(3):e22648.

35. Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL, Millar NL, et al. Gilchrist DS. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(27):5.

36. Harpole M, Davis J, Espina V. Current State of the Art for Enhancing Urine Biomarker Discovery Volume 13. Expert Review Proteomics. 2016;13(6):609-26.

37. Yamaguchi M. Role of Zinc in Bone Formation and Bone Resorption. The Journal of Trace Elements in Experimental Medicine. 1998;11(2):119-135.

38. Boonrungsiiman S, Gentleman E, Carzaniga R, Evans ND, McComb DW, Porter AE, et al. The role of intracellular calcium phosphate in osteoblast-mediated bone apatite formation. PNAS. 2012;109(35):14170-5.

39. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjäderhane L, et al. The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. Dental Materials. 2010;26(11):1059-67.

40. Xin L, Wu Z, Qu Q, Wang R, Tang J, Chen L. Comparative study of CTX-II, Zn2+, and Ca2+ from the urine for knee osteoarthritis patients and healthy individuals. Medicine. 2017;96(32):e7593.

41. Zhen ET, Brittain LJ, Laska DA, Mitchell PG, Sumer EU, Karsdal MA, et al. Characterization of Metalloprotease Cleavage Products of Human Articular Cartilage. Arthritis and Rheumatism. 2008;58(8):2420-31.

42. Poole AR, Ha N, Bourdon S, Sayre EC, Guermazi A, Cibere J. Ability of a Urine Assay of Type II Collagen Cleavage by Collagenases to Detect Early Onset and Progression of Articular Cartilage Degeneration: Results from a Population-based Cohort Study. The Journal of Rheumatology. 2016;43(10):1864-1870.

43. Catterall JB, Stabler TV, Flannery CR, Kraus VB. Changes in Serum and Synovial Fluid Biomarkers after Acute Injury. Arthritis Research and Therapy. 2010;12:R229.

44. Jin W, Dong C. IL-17 Cytokines in Immunity and Inflammation. Emerging Microbes and Infections. 2013;2(60):e60.

45. Zhu S, Qian Y. IL-17/IL-17 receptor system in autoimmune disease: mechanisms and therapeutic potential. Clinical Science. 2012;122(11):487-511.

46. Yingsong L, Peng H, Meng Z, Wei M. Correlation of IL-17 Level in Synovia and Severity of Knee Osteoarthritis. International Medical Journal of Experimental and Clinical Research. 2015;21:1732-1736.

47. Tseng W, Lu J, Bishop G, Watson A, Sage A, Demer L, et al. Demer, and Y. Tintut. Regulation of interleukin-6 expression in osteoblasts by oxidized phospholipids. Journal of Lipid Research. 2010;51(10):1010-1016.

48. Yoshitake F, Itoh S, Narita H, Ishihara K, Ebisu S. Interleukin-6 Directly Inhibits Osteoclast Differentiation by Suppressing Receptor Activator of NF-κB Signaling Pathways. Journal of Biological Chemistry. 2008;283(5):11535-40.

49. Teitelbaum S. Bone Resorption by Osteoclasts. Science. 2000;289(5484):1504-8.

50. Miyamoto T, Suda T. Differentiation and Function of Osteoclasts. The Keio Journal of Medicine. 2003;52(1):1-7.

51. Doss F, Menard J, Haushchild M, Kreutzer HJ, Mittlmeier T, Müller-Steinhardt M, et al. Elevated IL-6 levels in the synovial fluid of osteoarthritis patients stem from plasma cells. Scandinavian Journal of Rheumatology. 2007;36(2):136-9.

52. Castrogiovanni P, Di Rosa M, Ravalli S, Castorina A, Guglielmino C, Imbesi R, et al. Moderate Physical Activity as a Prevention Method for Knee Osteoarthritis and the Role of Synoviocytes as Biological Key. International Journal of Molecular Science. 2019;20(3):e511.

53. Lafont JE. Lack of Oxygen in Articular Cartilage: Consequences For Chondrocyte Biology. International Journal of Experimental Pathology. 2010;91(2):99-106.

54. Hubbi ME, Semenza GL. Regulation of Cell Proliferation by Hypoxia-Inducible Factors. American Physiological Society. 2015;309(12):C775-82.
with the Radiographic Severity of Knee Osteoarthritis. Genetic Molecular Research. 2014;14(4):10529-36.

56. Xue M, Qiique C, Zhang Q, Zhao H, Su L, Sun P, et al. Effects of Tumor Necrosis Factor α (TNF-α) and Interleukina 10 (IL-10) on Intercellular Cell Adhesion Molecule-1 (ICAM-1) and Cluster of Differentiation 31 (CD31) in Human Coronary Artery Endothelial Cells. Medical Science Monitor. 2018;24:4433-4439.

57. Balkwill F. TNF-α in Promotion and Progression of Cancer. Cancer and Metastasis Reviews. 2006;25(3):409-16.

58. Liu C, Tang J. Expression levels of tumor necrosis factor-α and the corresponding receptors are correlated with trauma severity. Oncology Letters. 2014;8(6):2747-2751.

59. Larsson S, Englund M, Struglits C, Lohmander LS. Interleukin-6 and Tumor Necrosis Factor Alpha in Synovial Fluid Are Associated with Progression of Radiographic Knee Osteoarthritis Subjects with Previous Meniscectomy. Osteoarthritis and Cartilage. 2015;21(11):1906-14.

60. Elsaid KA, Fleming BC, Oksendahl HL, Machan JT, Fadale PD, Hulstyn ML, et al. Decreased Lubricin Concentrations and Markers of Joint Inflammation in Synovial Fluids from Patients with Anterior Cruciate Ligament Injury. Arthritis and Rheumatism. 2009;58(6):1707-15.

61. Szychlinska MA, Leonardi R, Al-Qahtani M, Mobasher A, Musumeci G. Altered joint tribology in osteoarthritis: Reduced lubricin synthesis due to the inflammatory process. New horizons for therapeutic approaches. Analis of Physical and Rehabilitation Medicine. 2016;59(3):149-156.

62. Musumeci G, Trovato FM, Loreto C, Leonardi R, Szychlinska MA, Castorina S, et al. Lubricin expression in human osteoarthritic knee meniscus and synovial fluid: A morphological, immunohistochemical and biochemical study. Acta Histochemica. 2014;116(5):965-72.

63. Szychlinska MA, Castrogiovanni P, Trovato FM, Nsir H, Zarrouk M, Lo Furno D, et al. Physical activity and Mediterranean diet based on olive tree phenolic compounds from two different geographical areas have protective effects on early osteoarthritis, muscle atrophy and hepatic steatosis. European Journal of Nutrition. 2019;58(2):565-581.

64. Golas Ch, Charalabopoulos A, Stagakis D, Charalabopoulos K, Batistatou A. The Kinin System - Bradykinin: Biological Effects and Clinical Implications. Multiple Role of the Kinin system - Bradykinin. Hippokratia. 2007;11(4):124-8.

65. Howl J, Payne SJ. Bradykinin receptors as a therapeutic target. Expert Opinion on Therapeutic Targets. 2003;7(2):277-85.

66. Bellucci F, Meini S, Cucchi P, Catalani C, Nizzardo A, Riva A, et al. Synovial fluid levels of bradykinin correlate with biochemical markers for cartilage degradation and inflammation in knee osteoarthritis. Osteoarthritis and Cartilage. 2013;21(11):6.

67. Koichi Murata HY, Shimei Tanida, Masahiro Ishikawa, Kohei Nishitani, Hiromu Ito and Takashi Nakamura. Plasma and synovial fluid microRNAs as potential biomarkers of rheumatoid arthritis and osteoarthritis. Arthritis Research and Therapy. 2009;12(86):1774-80.

68. Li YH, Tavallaee G, Tokar T, Nakamura A, Sundrarajan K, Weston A, et al. Identification of synovial fluid microRNA signature in knee osteoarthritis: differentiating early- and late-stage knee osteoarthritis. Osteoarthritis and Cartilage. 2016;24(9):1577-86.