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Advances in Molecular biomarker for early diagnosis of Osteoarthritis

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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.

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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 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 (eg. 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-486-5-, 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 miRNA 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 cycloxygease-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 ILl-1B, IL-6, ILl-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 progressers 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, ColX, 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]. ColX 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 (11-17 also found in synovia)	Il-21, Il-17A, IFN-γ	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	ColX	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	Il-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 a. (2019)
Synovial protein	TNF-α	Signaling cell death	Significantly elevated prior to disease progression	Larrson 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-11 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.

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