Article:
Lineham, B, Altaie, A, Harwood, P et al. (3 more authors) (2022) A systematic review on the potential value of synovial fluid biomarkers to predict clinical outcomes in cartilage repair treatments. Osteoarthritis and Cartilage, 30 (8). pp. 1035-1049. ISSN 1063-4584

https://doi.org/10.1016/j.joca.2022.05.007

A systematic review on the potential value of synovial fluid biomarkers to predict clinical outcomes in cartilage repair and regeneration treatments

Beth Lineham[i], Ala Altaie[i], Paul Harwood[i,ii], Dennis McGonagle[i], Hemant Pandit[i,ii], Elena Jones[i]

i. Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, UK
ii. Trauma and Orthopaedics Department, Leeds Teaching Hospitals NHS Trust, UK

Attributed institute: Leeds Institute of Rheumatic and Musculoskeletal Medicine

Corresponding author: Beth Lineham mn13bl@leeds.ac.uk

Word count: 4754

Abstract word count: 247

Abstract

Objective

Multiple biochemical biomarkers have been previously investigated for the diagnosis, prognosis and response to treatment of articular cartilage damage, including osteoarthritis (OA). Synovial fluid (SF) biomarker measurement is a potential method to predict treatment response and effectiveness. However, the significance of different biomarkers and their correlation to clinical outcomes remains unclear. This systematic review evaluated current SF biomarkers used in investigation of cartilage degeneration or regeneration in the knee joint and correlated these biomarkers with clinical outcomes following cartilage repair or regeneration interventions.

Design

PubMed, Institute of Science Index, Scopus, Cochrane Central Register of Controlled Trials, and Embase databases were searched. Studies evaluating SF biomarkers and clinical outcomes following cartilage repair intervention were included. Two researchers independently performed data extraction and QUADAS-2 analysis. Biomarker inclusion, change following intervention and correlation with clinical outcome was compared.

Results

9 studies were included. Study heterogeneity precluded meta-analysis. There was significant variation in sampling and analysis. 33 biomarkers were evaluated in addition to microRNA and catabolic/anabolic ratios. Five studies reported on correlation of biomarkers with six biomarkers significantly correlated with clinical

outcomes following intervention. However, correlation was only demonstrated in isolated studies.

Conclusions

This review demonstrates significant difficulties in drawing conclusions regarding the importance of SF biomarkers based on the available literature. Improved standardisation for collection and analysis of SF samples is required. Future publications should also focus on clinical outcome scores and seek to correlate biomarkers with progression to further understand the significance of identified markers in a clinical context.

Registration number: PROSPERO CRD42022304298

Study protocol available on PROSPERO website

Keywords: Synovial fluid, biomarkers, cartilage repair, regeneration

Running headline: Biomarkers following cartilage repair

No conflicts of interest declared

Background

Damage to the articular cartilage of the knee is a common and challenging issue causing significant pain, functional deterioration and reduced quality of life. Cartilage tissue is avascular and therefore has limited intrinsic healing potential with no endogenous repair mechanism.[1] Cartilage defects have a variable natural history and can expand both in width and depth, often to involve subchondral bone.[2] Over time such lesions typically progress to symptomatic osteoarthritis (OA), with increasing loss of cartilage, synovial inflammation and changes to adjacent bone.[3]

Ultimately, many patients with advanced OA require knee replacement surgery for symptom relief. However, a variety of techniques are employed at an earlier stage, in an attempt to repair or regenerate damaged cartilage, with the aim of slowing disease progression. The type of intervention employed is determined by patient age and activity levels, as well as location, size and depth of the lesion.[4] Various nonsurgical treatment options are available. Visco-supplementation with intra-articular hyaluronic acid is proposed to slow progression by enhancing proteoglycan synthesis.[5,6] Platelet-rich plasma (PRP) has been shown to enhance chondrocyte proliferation in vitro and slow progression of OA in animal models. Clinical results are however inconsistent for both of these, with recent human studies unable to demonstrate any clinical advantage to their use. [7,8 9 10,11]

For small focal cartilage defects at the knee, marrow stimulating techniques including microfracture, abrasion chondroplasty and osteochondral drilling have been utilised. These are proposed to stimulate healing by introducing bone marrow cells into the lesion, including multipotent mesenchymal stem cells (MSCs), derived from the marrow and synovial fluid (SF), which may remodel fibrin clot into fibrocartilage.[12,13]

Other techniques include replacement of the cartilage defect with autologous cartilage from a lower weight bearing area (osteochondral autograft transfer)[14] or use of an autograft transplantation of chondrocytes to the defect under a patch (autologous chondrocyte implantation (ACI))[15] or embedded within a matrix (matrix-associated autologous chondrocyte implantation (MACI)).[16] Once lesions have progressed further to OA the outcomes from these techniques become less predictable.[17] Other surgical options that attempt to slow disease progression and relieve symptoms include high tibial osteotomy (HTO), and knee joint distraction (KJD). HTO attempts to correct mechanical alignment and offload the affected compartment.[18] Knee joint distraction is a newer technique, which temporarily offloads the joint using an external fixator, and is proposed to simulate cartilage healing.[19]

The effectiveness of different procedures used to treat cartilage damage at the knee can be assessed using clinical, radiological and biomarker outcome measures.[20] There are many different clinical scoring systems used for this purpose.[20] The Western Ontario and McMaster Universities Arthritis Index (WOMAC) is widely used in both hip and knee OA, and covers pain, stiffness and physical function.[21] The Knee injury and Osteoarthritis Outcome Score (KOOS) is a knee specific extension of the WOMAC tool, first published in 1998 and is also commonly used.[22,23] Pain visual analogue scales (VAS) are often used for many conditions to quantify pain levels and are not specific to the knee or OA.[24] The Larson knee score is an older score and less commonly used.[25] Various other scores are used with often subjective assessments of pain and function however scores with tested validity and reliability are preferred.[20]

Whilst clinical measures are well known and widely employed, biomarker outcomes are less well recognised in clinical practice, and none currently form part of the diagnostic criteria for OA. In healthy cartilage, the extracellular matrix is slowly turned over and therefore exists in a state of equilibrium between catabolism and anabolism.[26] In cartilage damage and OA, chondrocyte death leads to the release of damage-associated molecular patterns (DAMPs) and inflammatory cytokines with catabolic activation.[27,28] Following cartilage damage markers can remain elevated for years prior to the development of post traumatic OA.[29] Cartilage breakdown products, pro-inflammatory cytokines and proteolytic enzymes have been observed in serum, plasma, urine and synovial fluid (SF) of patients with knee OA.[30 31] These proteins are therefore recognised as potential biomarkers of cartilage breakdown and catabolism.[32] Measurement in the SF is preferable as it offers the potential for early diagnosis or evaluation of treatment response, as it is the initial location of relevant molecular alterations, markers found in SF may be undetectable in serum.[29,33,34] These biomarkers are also increasingly recognised as potentially important to the development of pharmacological agents to treat OA.[35] However, their role in the evaluation of cartilage repair or regeneration interventions and their correlation to clinical outcomes remains unclear. Understanding the relationship between biomarkers and clinical outcomes is integral in order to understand biomarker clinical relevance and importance as an outcome measure.

This systematic review was undertaken to determine current knowledge regarding the use of early biomarker measurement to indicate the effectiveness of articular cartilage regeneration treatments in the knee.

144

Methods

Search Terms and Search Strategy

This review was performed in accordance with published guidance[36] and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.[37] Electronic searches were performed using keywords including “knee”, “cartilage”, “regeneration”, “restoration”, “synovial fluid” of citation databases PubMed, Institute of Science Index, Scopus, Cochrane Central Register of Controlled Trials, and Embase. The full search strategy for the OVID search is included in the Supplemental material. A filter for human subjects was applied. A manual search was then performed utilising references and citations of previous systematic reviews and included trials. Two researchers independently performed searches and screened studies according to the inclusion and exclusion criteria.

Inclusion and Exclusion Criteria

All studies in human subjects undergoing articular cartilage regeneration interventions which had both SF biomarkers and clinical outcomes as an outcome measure of cartilage defects of the knee were included. There were no restrictions regarding patient age, sex or race or the date, or country of publication. Non-English language and animal studies were excluded.

Data Extraction

Two researchers independently reviewed the full text of all included papers and extracted relevant data. To prevent inclusion of duplicate data, papers from the same

research group were verified using year and place of recruitment with the largest data set chosen. Data extracted included patient age and sex, length of follow up, duration of symptoms, articular cartilage defect characteristics, intervention technique, lesion size, frequency of postoperative complications, all reported SF biomarker levels (including qualitative chemokine/cytokine profiles and quantitative levels), details of synovial fluid processing, and all reported pre- and postoperative clinical outcome scores (including Lysholm score, visual analogue scale-pain, Western Ontario and McMaster Universities Osteoarthritis Index and Knee Injury and Osteoarthritis Outcome Score). All treatments were included if they are currently licensed for use in the United Kingdom.

Appraisal of Bias

The quality of studies was evaluated in accordance with the QUADAS-2 tool. A review of the evidence in accordance with each item in the tool was prepared by two researchers with a discussion of specific issues and uncertainties. The level of evidence was determined independently by two researchers. Any discrepancies were discussed to reach a final decision.

Statistical analysis

All data extracted was entered into an electronic database (GraphPad Prism). The following were compared across different studies: (i) biomarkers included, (ii) biomarker change following intervention, (iii) biomarker change compared to control, (iv) biomarker correlation with clinical outcome. Meta-analysis was not undertaken due to clinical heterogeneity of participants, interventions and outcomes.

Results

Study characteristics

The PRISMA flowchart outlining study selection is shown in Figure 1. Initial searches identified 2715 studies, of which 1427 remained following removal of duplicates. All but 54 of these studies were excluded at the stage of abstract review, of these, a further 45 were excluded on reviewing the full manuscript. The reasons for exclusion at each stage are detailed in Figure 1.

Suggested position for Figure 1

Nine studies met the inclusion criteria and formed the study data, the characteristics of these are outlined in Table 1. These included studies examining four different cartilage regeneration or repair interventions, ACI (1 study), HA (5 studies), HTO (2 studies) and KJD (1 study). All reported results in adults only.

Suggested position for Table 1

The results of study quality and risk of bias as assessed by Quality Assessment of Diagnostic Accuracy Score 2 (QUADAS-2) questions are outlined in Figure 2. The study quality was variable, all studies were prospective with four out of the nine studies using a consecutive or randomised controlled design for selection of patients.

Four of the studies compared two different interventions or one intervention with a control group. Cole et al.[38] compared HA with PRP injections with a randomised

study design and identically timed sampling. Groups were matched across age, sex, Kellgren-Lawrence grade and laterality although there was a significant difference in BMI between the HA group (29.0 +/- 6.4 kg/m^2) and PRP group (27.4 +/- 3.9 kg/m^2). Creamer et al.[39] used a comparator group of saline placebo injection which was injected into the other knee of their patients. The knee to receive HA or placebo was randomised and sampling was taken at the same time points. Shimizu et al.[40] randomised patients to receive HA or CS intra-articular injection. Sampling was taken at equivalent time points. There was no significant difference in age, sex and BMI between groups. Ozcamdalli et al.[41] compared intra-articular N-acetyl cysteine and HA. Patients were randomly allocated to treatment and sampling was identical. Groups were matched on age, sex and BMI. Watt et al.[42] used comparator SF samples with ‘normal’ samples previously collected from patients undergoing amputation for treatment of lower limb tumour and osteoarthritis samples from research tissue bank samples. The comparator was used to establish 'normal’, ‘low’ and ‘high’ levels of biomarkers in SF samples only and other outcomes were not measured in these patients. Groups were not matched.

Suggested position for Figure 2

Synovial fluid processing and biomarker analysis methods

Methods of SF acquisition and analysis varied between studies (Table 2). Cole et al. utilised ultrasound for SF aspiration,[38] other studies did not mention use of imaging in sample acquisition. Schneider et al. injected 50mL of isotonic 0.9% saline into the joint prior to aspiration,[43] other studies did not mention the use of fluid injection in sample acquisition.

All studies used commercially available kits for biomarker analysis. Study methodology is outlined in Table 2.

Suggested position for Table 2

The studies included tested for a wide range of both catabolic and anabolic biomarkers, as shown in Figure 3. Thirty-three biomarkers were examined using traditional methods. The study utilising MiRNA-PCR profiled a panel of 84 miRNAs (Supplementary Table 1). There was a wide range of both catabolic and anabolic biomarkers tested with the majority catabolic. MMP-3, C-6S, IL-8 and TIMP-1 were measured most frequently. MMP-3 degrades extracellular matrix[44] and activates other pro-MMPs.[45] C-6S is an epitope of chondroitin sulphate, with raised levels a marker of cartilage destruction.[46] IL-8 is a chemoattractant cytokine and has been previously associated with WOMAC scores in knee OA.[47] TIMP-1 is an inhibitor of MMPs with increased levels seen in OA patients compared to controls[48] and may be predictive of progression of radiographic changes in hip OA.[49] The other biomarkers investigated are described further in the discussion.

Suggested position of Figure 3

A wide variety of different clinical outcome measures were reporting in the included studies as detailed in Table 3.

Suggested position of Table 3

Biomarker changes

Reported changes in biomarker concentrations and correlation with clinical outcome are summarised in Table 4. Of those biomarkers tested in a single study, for DPD,[43] NTX,[43] YKL-40,[43] FGF-2,[42] HA,[40] PICP,[43] proteoglycan,[43] MMP-1/TIMP-1,[43] MMP-3/TIMP-1[43] TGFβ1,[42] a significant increase in levels following intervention was demonstrated. For Activin A,[42] COMP,[50] MMP-2,[50] total oxidant concentration,[41] and VEGF,[50] a significant decrease in recorded concentrations following intervention was recorded. For CPII,[39] CTX-II,[41] IL-1ra,[38] IL-10,[50] LTBP2,[42] Tenascin-C,[51] C-3B3,[39] MMP-3[42] and total antioxidant concentration[41] no change following intervention was observed. Of eleven biomarkers tested in more than one study, C-4S,[51,40] C-6S,[51,41,40] keratan sulphate,[39,51] IL-6,[38,50,42] IL-8,[38,50] MMP-3,[41-43,50] MMP-9,[50,40] MMP-13,[50,40] and TIMP-1,[40,43,42] showed inconsistent results between studies, for IL-1B,[38,50] and TNF-a[38,50] no significant change following intervention was consistently reported. The study utilising MiRNA-PCR arrays recorded significant changes in hsa-miR-23a-3p and hsa-miR-30c-5p following intervention.

Suggested position of Table 4

Biomarker change over time

Most of the studies measured biomarker levels at one time point only following intervention. Those that measured at multiple time points often found significant variation in the levels over time. There was a significant difference in the absolute marker levels found between the studies. Figure 4 demonstrates the percentage change over time in MMP-3 levels in three of the four studies that investigated it

following intervention. Schneider et al. measured MMP3 normalised to total protein and therefore levels are not comparable. Significant variation is seen although decreased levels were eventually seen by Ozcamdalli et al. following HA injection, and Kumagai et al. following HTO with Watt et al. showing no significant change over three time points.

Suggested position of Figure 4

Correlation with clinical outcomes

Five studies specifically reported on correlation of biomarkers with clinical outcomes with three studies reporting a statistically significant correlation (Table 4).[51,52,42] For those studies with a comparator group, none showed a significant change in biomarker level in the tested intervention compared to the comparator group.

Discussion

There is an increasing interest in and understanding of the role of SF biomarkers in cartilage degeneration and regeneration. This review identified nine studies which evaluated both SF biomarkers and clinical outcomes following a cartilage regeneration or repair intervention. Studies were of variable quality regarding risk of bias, although all were prospective. Many studies did not have a comparator group, used convenience-based sampling and used a case control or case series design. Blinding of the SF biomarker analysis was not undertaken in any of the studies, although the effect this has on study quality is questionable, given that there is no current reference standard for any SF biomarker test. Two patient groups were identified, those with sports injuries and those with osteoarthritis. These are separate entities clinically and therefore introduce heterogeneity.

Methods of SF collection and analysis are important in the measurement of biomarker levels. Sample collection is not currently standardised in the available literature. There were major differences between the studies identified in this review, namely in lavage of the joint or dilution of SF and in digestion of hyaluronan which may affect biomarker levels. Different methods were used to obtain biomarker levels, although all tests used were commercially available, previously validated methods. Most studies employed traditionally used tests such as ELISA, one employed the use of miRNA PCR testing which is an emerging technique utilising mass spectrometry-based proteomics for experimental identification of potential targets.[30] There was significant variability in the presentation of biomarker levels and changes with actual levels often not detailed. These differences between collection, analysis and presentation of results makes direct comparison between studies difficult.

There was a wide range of biomarkers tested. Most of the biomarkers were tested in only one or two studies making direct comparison difficult. There is some controversy regarding the catabolic or anabolic nature of various biomarkers as cartilage is undergoing constant remodelling and therefore specific values of biomarkers may not give an overall impression of the state of cartilage condition. It has been proposed that the relationship between catabolic and anabolic markers may be more relevant than specific levels.[53] To elucidate the importance of results of this review, markers are categorised below according to the current state of understanding as to their relevance.

Aggrecan is one of the most abundant proteins in the cartilage matrix[46] and is an integral component, necessary for drawing water into the extracellular matrix. Raised levels are a marker of cartilage destruction and have been associated with OA severity.[54] Measurement of chondroitin sulfate indicates aggrecan degradation and is therefore a marker of cartilage destruction.[46] Three epitopes have been implicated in OA[55] and were evaluated in this review. C-3B3 showed no significant change following HA injection.[39] C-4S showed a decrease following HA injection in one study[51] with correlation with clinical outcomes at 6 weeks and no significant change following HA in another study.[40] C-6S was investigated in three studies with no significant change following HA injection in two,[40,41] and a significant decrease following HA injection in another.[51]

COMP is an extracellular matrix protein involved in the development of cartilage,[50] concentration of COMP has been found to reflect OA severity.[56,45] COMP was

evaluated in one study which demonstrated a decrease in measured concentrations following HTO.[50] Absolute levels of COMP were much higher in the study included in this review than seen in other previous studies.[57]

CPII appears to be activated in OA, increased levels have been demonstrated in SF in OA although not in serum.[58] CPII was investigated in one study in this review and showed no significant change in concentrations post HA.[39] CTX-II release is MMP-mediated and specifically reflects cartilage degeneration.[59] CTX-II was investigated in Ozcamdalli et al. in this review and showed no significant change following HA injection.[41] Keratan sulfate is a glycosaminoglycan and is a marker of cartilage catabolism.[60] Two studies in this review measured keratan sulfate following HA injection with one reporting no significant change in measured concentrations[39] and one a decrease.[51]

MCP-1, also referred to as CCL2, is a proinflammatory chemokine which recruits monocytes, T cells and dendritic cells to sites of inflammation.[61] A significant increase in levels was observed in one study included in this review after KJD.[42]

Proteoglycan is a non-collagenous protein of the extracellular matrix.[62] Degradation products have been correlated with increased osteoarthritic changes but the significance of absolute levels in SF are unclear. An increase was seen in one study in this review following ACI.[43]

Tenascin-C is a component of the extra-cellular matrix and SF levels are significantly correlated with radiographic knee OA.[63] This was investigated by one study in our

review and showed no significant change after HA injection however baseline values were correlated with clinical outcomes at 5 weeks.[51]

YKL-40 (also named human cartilage glycoprotein 39 or chitinase-3-like protein 1) is a glycoprotein induced by pro-inflammatory cytokines. Raised levels in SF have been previously demonstrated to correlate with disease severity in OA.[64] Levels increased initially then decreased back to baseline following HA intra-articular injection in one study in this review.[43]

With respect to bone markers, DPD stabilises collagen by crosslinking collagen peptides.[65] It is a marker of bone resorption.[66] DPD was investigated in only one study in this review, a significant but transient increase in SF levels was observed at six weeks post ACI, falling back to undetectable levels by 1 year post surgery.[43] NTX is a marker of bone resorption and is released during collagen degradation.[65] A significant initial increase in concentration was observed following ACI, returning to baseline by 1 year.[43] PICP is released during collagen I synthesis.[67] Type I collagen is involved in OA progression and is a potential marker for osteophyte progression.[68] In one study in this review a significant increase was observed initially following HA injection then a following decrease.[43]

Pro-inflammatory cytokines, including IL-1β, were measured by two studies in this review with both showing no significant change.[38,50] IL-1ra was measured in one paper[38] and showed no significant change. IL-1 has been extensively investigated and appears to affect susceptibility to OA and induce multiple inflammatory mediators.[69]

IL-6 is a marker of inflammation and has been shown to be correlated with radiographic changes,[70] knee pain[71] and lumbar pain in OA.[72] Levels were measured in three of the studies included in this review, with one reporting no significant change,[38] and one showing a significant decrease following intervention[50] and a further showing a significant increase at 6 weeks.[42] IL-8, another pro-inflammatory cytokine, has been previously correlated with WOMAC scores in knee OA.[47] Results reported by the studies included in this review were inconsistent, with no change in one[38] and a significant decrease in another.[50] IL-10, previously demonstrated to have higher levels in radiographically severe OA,[73] was found to have no significant change in measured concentrations following HTO by Kumagai et al.[50]

TNF-α is a pro-inflammatory cytokine that has been extensively researched with raised serum levels reported in osteoarthritis[70] and inhibition of TNF-α shown to be chondroprotective in animal studies.[74] No significant change following intervention was shown in either study included in this review.[38,50] VEGF release is also triggered by inflammation. This has been shown to be a mediator of endochondral ossification,[75] with levels associated with osteoarthritis severity.[56] In this review, one study demonstrated a decrease in measured SF concentrations following HTO.[50]

Matrix metalloproteinases (MMPs), including MMP-3, which degrades the extracellular matrix[44] and activates other pro-MMPs.[45] In this review, four studies investigated SF MMP-3 levels. Two studies recorded a decrease following intervention,[41,50] one an increase,[43] with no significant change in another.[42] MMP-9 degrades collagen as a type IV collagenase,[76] raised levels are seen in osteoarthritis.[77] Kumagai et al. found no significant change,[50] Shimizu et al.

demonstrated a significant decrease.[40] MMP-13 is increased in early stage OA and undetectable in normal tissues.[17] It was decreased in one study[50] and initially increased then decreased following HA injection.[43]

Recent literature has proposed a link between increased oxidative stress and development of osteoarthritis,[78] with decreased antioxidant capacity seen in end-stage OA.[79] Ozcamdalli et al. demonstrated a decrease in total oxidant concentration level and no significant change in total antioxidant concentration.[41]

With respect to Anabolic markers, Activin A is a member of the TGF-β superfamily and acts as an anabolic cytokine in articular cartilage.[80] Watt et al. reported significantly decreased levels at 3 and 6 weeks following KJD.[42] FGF-2 is a chondroprotective growth factor which suppresses interleukin-1 driven aggrecanase activity.[81] Levels were significantly increased at 6 weeks following KJD.[42]

HA is a non-protein glycosaminoglycan important in lubrication and viscoelasticity of SF.[82] The concentration and molecular weight of HA in SF in OA is significantly reduced and levels are associated with OA progression.[83] Measurement in one study in this review showed a significant increase following HA injection.[40]

LTBP2 is an extracellular glycoprotein cytokine which regulates the TGFβ growth factor pathway.[84,85] There was no significant change in the measured SF levels following intervention in the single study that investigated it.[42] TIMP-1 is an inhibitor of MMPs, and therefore considered a marker of anabolic activity, increased levels have been demonstrated in OA.[48] A significant increase was shown at 3 weeks

following KJD[42] and 6 weeks following ACI[43] with no significant increase seen following HA injection.[40] TGFβ-1 is a growth factor involved in cartilage maintenance.[86] Therefore increased levels may be expected in an anabolic response. This was investigated by Watt et al.[42] with a significant increase seen at 6 months post KJD.

It is increasingly recognised cartilage undergoes constant physiologic remodelling, similar to that in bone.[87] Therefore, levels of opposing markers may be important. MMP-1/TIMP-1 and MMP-3/TIMP-1 ratios, reflecting the relationship between the matrix metalloproteinase and its inhibitor, have been demonstrated to be significantly lower in OA patients than in control groups.[77] Schneider et al. demonstrated a significant initial increase in ratios of these proteins measured in the SF following HA injection, this same ratio was found to be decreased at six weeks.[43]

Thousands of miRNAs have been currently identified.[88] Understanding of their function is increasing but they appear to repress mRNA translation or cleavage[89] and are involved in regulation of cell differentiation and regulation.[90] Many miRNAs have been associated with OA.[91] In the study included in this review, Kwak et al. investigated miRNA expression in the SF, finding significant changes in two: hsa-miR-23a-3p and hsa-miR-30c-5p, with the latter correlated with clinical symptoms.[52] Hsa-miR-30c-5p is known to be associated with OA progression and has been named as a possible therapeutic target.[92,93] MiR-378a-5p, miR-140-3p, miR-27b-3p have been previously associated with osteoarthritis progression and were profiled in this study but showed no significant changes following intervention.[52]

There was significant variation in biomarker levels seen at different time points and following different interventions, as shown in Figure 4. This may demonstrate an inflammatory phase post operatively followed by a further phase of cartilage regeneration however it is difficult to draw conclusions without further study specifically examining this phenomenon.

Despite all included studies reporting SF biomarkers and clinical outcomes, only five studies directly correlated these. C-4S was investigated in two studies, both investigating HA injection, with a decrease in measured level and correlation with clinical response seen in one study[51] and no significant change and no correlation with clinical outcomes in the other.[40] C-6S was correlated in the same two studies, no correlation with clinical outcomes was seen in either study.[40,51] No other biomarkers were correlated with clinical outcomes by two or more studies using the same intervention. No biomarkers were found to correlate with clinical outcomes in more than one study. These results demonstrate the variability in findings throughout the literature and the difficulty in identifying a specific biomarker to assess the effectiveness of cartilage repair and regeneration interventions.

Conclusion and future research

This review demonstrates the difficulties with drawing conclusions regarding the importance of SF biomarkers in the assessment of OA based upon the current literature. Improved standardisation for the collection and analysis of SF samples is integral to improving this situation. It appears the relationship between biomarkers and progression over time may be more important than absolute levels at a single snapshot. Future research should also focus on standardising the use and presentation of clinical outcome scores and seeking to correlate biomarkers with clinical progression to further understand the significance of identified markers. Larger scale studies with consistent design will be required to develop reference standards that conform to international reporting guidelines.

Conflict of interest

Beth Lineham: There are no financial or personal interests that could have potentially and inappropriately influenced the integrity of the work in this manuscript to disclose by the author.

Ala Altaie: There are no financial or personal interests that could have potentially and inappropriately influenced the integrity of the work in this manuscript to disclose by the author.

Paul Harwood: There are no financial or personal interests that could have potentially and inappropriately influenced the integrity of the work in this manuscript to disclose by the author.

Dennis McGonagle: There are no financial or personal interests that could have potentially and inappropriately influenced the integrity of the work in this manuscript to disclose by the author.

Hemant Pandit: There are no financial or personal interests that could have potentially and inappropriately influenced the integrity of the work in this manuscript to disclose by the author.

Elena Jones: There are no financial or personal interests that could have potentially and inappropriately influenced the integrity of the work in this manuscript to disclose by the author.

References

1. Widuchowski W, Widuchowski J, Trzaska T. Articular cartilage defects: study of 25,124 knee arthroscopies. *Knee*. Jun 2007;14(3):177-82. doi:10.1016/j.knee.2007.02.001
2. Ding C, Cicuttini F, Jones G. Tibial subchondral bone size and knee cartilage defects: relevance to knee osteoarthritis. *Osteoarthritis Cartilage*. May 2007;15(5):479-86. doi:10.1016/j.joca.2007.01.003
3. Davies-Tuck ML, Wluka AE, Wang Y, et al. The natural history of cartilage defects in people with knee osteoarthritis. *Osteoarthritis Cartilage*. Mar 2008;16(3):337-42. doi:10.1016/j.joca.2007.07.005
4. Gelse K, Angele P, Behrens P, et al. [Debridement in Focal Cartilage Damage of the knee. Systematical review of the literature and recommendations of the working group "clinical tissue regeneration" of the German Society of Orthopaedics and Trauma (DGOU)]. *Z Orthop Unfall*. 08 2018;156(4):423-435. doi:10.1055/s-0044-101470
5. Altman RD, Manjoo A, Fierlinger A, Niazi F, Nicholls M. The mechanism of action for hyaluronic acid treatment in the osteoarthritic knee: a systematic review. *BMC Musculoskelet Disord*. Oct 26 2015;16:321. doi:10.1186/s12891-015-0775-z
6. Dougados M. Sodium hyaluronate therapy in osteoarthritis: arguments for a potential beneficial structural effect. *Semin Arthritis Rheum*. Oct 2000;30(2 Suppl 1):19-25. doi:10.1053/sarh.2000.0246
7. Altman R, Hackel J, Niazi F, Shaw P, Nicholls M. Efficacy and safety of repeated courses of hyaluronic acid injections for knee osteoarthritis: A systematic review. *Semin Arthritis Rheum*. 10 2018;48(2):168-175. doi:10.1016/j.semarthrit.2018.01.009
8. Jevsevar D, Donnelly P, Brown GA, Cummins DS. Viscosupplementation for Osteoarthritis of the Knee: A Systematic Review of the Evidence. *J Bone Joint Surg Am*. Dec 16 2015;97(24):2047-60. doi:10.2106/JBJS.N.00743
9. Drengk A, Zapf A, Stürmer EK, Stürmer KM, Frosch KH. Influence of platelet-rich plasma on chondrogenic differentiation and proliferation of chondrocytes and mesenchymal stem cells. *Cells Tissues Organs*. 2009;189(5):317-26. doi:10.1159/000151290
10. Saito M, Takahashi KA, Arai Y, et al. Intraarticular administration of platelet-rich plasma with biodegradable gelatin hydrogel microspheres prevents osteoarthritis progression in the rabbit knee. *Clin Exp Rheumatol*. 2009 Mar-Apr 2009;27(2):201-7.
11. Bennell KL, Paterson KL, Metcalf BR, et al. Effect of Intra-articular Platelet-Rich Plasma vs Placebo Injection on Pain and Medial Tibial Cartilage Volume in Patients With Knee Osteoarthritis: The RESTORE Randomized Clinical Trial. *JAMA*. 11 23 2021;326(20):2021-2030. doi:10.1001/jama.2021.19415
12. Baboolal TG, Khalil-Khan A, Theodorides AA, Wall O, Jones E, McGonagle D. A Novel Arthroscopic Technique for Intraoperative Mobilization of Synovial Mesenchymal Stem Cells. *Am J Sports Med*. 12 2018;46(14):3532-3540. doi:10.1177/0363546518803757
13. Magnussen RA, Dunn WR, Carey JL, Spindler KP. Treatment of focal articular cartilage defects in the knee: a systematic review. *Clin Orthop Relat Res*. Apr 2008;466(4):952-62. doi:10.1007/s11999-007-0097-z
14. Bobić V. Arthroscopic osteochondral autograft transplantation in anterior cruciate ligament reconstruction: a preliminary clinical study. *Knee Surg Sports Traumatol Arthrosc*. 1996;3(4):262-4. doi:10.1007/BF01466630

15. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med*. Oct 06 1994;331(14):889-95. doi:10.1056/NEJM199410063311401
16. Cherubino P, Grassi FA, Bulgheroni P, Ronga M. Autologous chondrocyte implantation using a bilayer collagen membrane: a preliminary report. *J Orthop Surg (Hong Kong)*. Jun 2003;11(1):10-5. doi:10.1177/230949900301100104
17. Meric G, Gracitelli GC, Görtz S, De Young AJ, Bugbee WD. Fresh osteochondral allograft transplantation for bipolar reciprocal osteochondral lesions of the knee. *Am J Sports Med*. Mar 2015;43(3):709-14. doi:10.1177/0363546514562549
18. Liu X, Chen Z, Gao Y, Zhang J, Jin Z. High Tibial Osteotomy: Review of Techniques and Biomechanics. *J Healthc Eng*. 2019;2019:8363128. doi:10.1155/2019/8363128
19. van der Woude JAD, Wiegant K, van Roermund PM, et al. Five-Year Follow-up of Knee Joint Distraction: Clinical Benefit and Cartilaginous Tissue Repair in an Open Uncontrolled Prospective Study. *Cartilage*. Jul 2017;8(3):263-271. doi:10.1177/1947603516665442
20. Harris K, Dawson J, Gibbons E, et al. Systematic review of measurement properties of patient-reported outcome measures used in patients undergoing hip and knee arthroplasty. *Patient Relat Outcome Meas*. 2016;7:101-8. doi:10.2147/PROM.S97774
21. Copsey B, Thompson JY, Vadher K, et al. Problems persist in reporting of methods and results for the WOMAC measure in hip and knee osteoarthritis trials. *Qual Life Res*. Feb 2019;28(2):335-343. doi:10.1007/s11136-018-1978-1
22. Roos EM, Toksvig-Larsen S. Knee injury and Osteoarthritis Outcome Score (KOOS) - validation and comparison to the WOMAC in total knee replacement. *Health Qual Life Outcomes*. May 25 2003;1:17. doi:10.1186/1477-7525-1-17
23. Rodriguez-Merchan EC. Knee instruments and rating scales designed to measure outcomes. *J Orthop Traumatol*. Mar 2012;13(1):1-6. doi:10.1007/s10195-011-0177-4
24. Downie WW, Leatham PA, Rhind VM, Wright V, Branco JA, Anderson JA. Studies with pain rating scales. *Ann Rheum Dis*. Aug 1978;37(4):378-81. doi:10.1136/ard.37.4.378
25. Larson R. Rating sheet for knee function. In: Smillie IS, ed. *Diseases of the Knee Joint*. Churchill-Livingstone; 1972:29-30.
26. Mueller MB, Tuan RS. Anabolic/Catabolic balance in pathogenesis of osteoarthritis: identifying molecular targets. *PM R*. Jun 2011;3(6 Suppl 1):S3-11. doi:10.1016/j.pmrj.2011.05.009
27. Statham P, Jones E, Jennings LM, Fermor HL. Reproducing the Biomechanical Environment of the Chondrocyte for Cartilage Tissue Engineering. *Tissue Eng Part B Rev*. Jun 01 2021;doi:10.1089/ten.TEB.2020.0373
28. Musumeci G, Castrogiovanni P, Trovato FM, et al. Biomarkers of Chondrocyte Apoptosis and Autophagy in Osteoarthritis. *Int J Mol Sci*. Aug 31 2015;16(9):20560-75. doi:10.3390/ijms160920560
29. Markus DH, Berlinberg EJ, Strauss EJ. Current State of Synovial Fluid Biomarkers in Sports Medicine. *JBJS Rev*. 08 16 2021;9(8)doi:10.2106/JBJS.RVW.21.00024
30. van Spil WE, Szilagyi IA. Osteoarthritis year in review 2019: biomarkers (biochemical markers). *Osteoarthritis Cartilage*. 03 2020;28(3):296-315. doi:10.1016/j.joca.2019.11.007
31. Domenis R, Zanutel R, Caponnetto F, et al. Characterization of the Proinflammatory Profile of Synovial Fluid-Derived Exosomes of Patients with Osteoarthritis. *Mediators Inflamm*. 2017;2017:4814987. doi:10.1155/2017/4814987

32. Lafeber FP, van Spil WE. Osteoarthritis year 2013 in review: biomarkers; reflecting before moving forward, one step at a time. *Osteoarthritis Cartilage*. Oct 2013;21(10):1452-64. doi:10.1016/j.joca.2013.08.012
33. Munjal A, Bapat S, Hubbard D, Hunter M, Kolhe R, Fulzele S. Advances in Molecular biomarker for early diagnosis of Osteoarthritis. *Biomol Concepts*. Aug 09 2019;10(1):111-119. doi:10.1515/bmc-2019-0014
34. Boffa A, Merli G, Andriolo L, Lattermann C, Salzmann GM, Filardo G. Synovial Fluid Biomarkers in Knee Osteoarthritis: A Systematic Review and Quantitative Evaluation Using BIPEDs Criteria. *Cartilage*. 12 2021;13(1_suppl):82S-103S. doi:10.1177/1947603520942941
35. Saberi Hosnijeh F, Bierma-Zeinstra SM, Bay-Jensen AC. Osteoarthritis year in review 2018: biomarkers (biochemical markers). *Osteoarthritis Cartilage*. 03 2019;27(3):412-423. doi:10.1016/j.joca.2018.12.002
36. Group CSaDTM. Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy Version 2.0. 2021/2022.
37. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *J Clin Epidemiol*. Oct 2009;62(10):e1-34. doi:10.1016/j.jclinepi.2009.06.006
38. Cole BJ, Karas V, Hussey K, Pilz K, Fortier LA. Hyaluronic Acid Versus Platelet-Rich Plasma: A Prospective, Double-Blind Randomized Controlled Trial Comparing Clinical Outcomes and Effects on Intra-articular Biology for the Treatment of Knee Osteoarthritis. *Am J Sports Med*. Feb 2017;45(2):339-346. doi:10.1177/0363546516665809
39. Creamer P, Sharif M, George E, et al. Intra-articular hyaluronic acid in osteoarthritis of the knee: an investigation into mechanisms of action. *Osteoarthritis Cartilage*. Jun 1994;2(2):133-40. doi:10.1016/s1063-4584(05)80063-9
40. Shimizu M, Higuchi H, Takagishi K, Shinozaki T, Kobayashi T. Clinical and biochemical characteristics after intra-articular injection for the treatment of osteoarthritis of the knee: prospective randomized study of sodium hyaluronate and corticosteroid. *J Orthop Sci*. Jan 2010;15(1):51-6. doi:10.1007/s00776-009-1421-0
41. Ozcamdalli M, Misir A, Kizkapan TB, et al. Comparison of Intra-articular Injection of Hyaluronic Acid and N-Acetyl Cysteine in the Treatment of Knee Osteoarthritis: A Pilot Study. *Cartilage*. Oct 2017;8(4):384-390. doi:10.1177/1947603516675915
42. Watt FE, Hamid B, Garriga C, et al. The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis. *Osteoarthritis Cartilage*. 03 2020;28(3):324-333. doi:10.1016/j.joca.2019.12.005
43. Schneider U, Schlegel U, Bauer S, Siebert CH. Molecular markers in the evaluation of autologous chondrocyte implantation. *Arthroscopy*. Apr 2003;19(4):397-403. doi:10.1053/jars.2003.50042
44. Roughley PJ, Nguyen Q, Mort JS, Hughes CE, Caterson B. Proteolytic degradation in human articular cartilage: its relationship to stromelysin. *Agents Actions Suppl*. 1993;39:149-59. doi:10.1007/978-3-0348-7442-7_17
45. Murphy G, Knäuper V, Atkinson S, et al. Matrix metalloproteinases in arthritic disease. *Arthritis Res*. 2002;4 Suppl 3:S39-49. doi:10.1186/ar572
46. Lotz M, Martel-Pelletier J, Christiansen C, et al. Value of biomarkers in osteoarthritis: current status and perspectives. *Ann Rheum Dis*. Nov 2013;72(11):1756-63. doi:10.1136/annrheumdis-2013-203726

47. Ruan G, Xu J, Wang K, et al. Associations between serum IL-8 and knee symptoms, joint structures, and cartilage or bone biomarkers in patients with knee osteoarthritis. *Clin Rheumatol*. Dec 2019;38(12):3609-3617. doi:10.1007/s10067-019-04718-8
48. Li X, Fu X, Gao Y, Li H, Wang W, Shen Y. Expression of tissue inhibitor of metalloproteinases-1 and B-cell lymphoma-2 in the synovial membrane in patients with knee osteoarthritis. *Exp Ther Med*. Jan 2018;15(1):885-889. doi:10.3892/etm.2017.5462
49. Chevalier X, Conrozier T, Gehrmann M, et al. Tissue inhibitor of metalloprotease-1 (TIMP-1) serum level may predict progression of hip osteoarthritis. *Osteoarthritis Cartilage*. May 2001;9(4):300-7. doi:10.1053/joca.2000.0389
50. Kumagai K, Fujimaki H, Yamada S, Nejima S, Matsubara J, Inaba Y. Changes of synovial fluid biomarker levels after opening wedge high tibial osteotomy in patients with knee osteoarthritis. *Osteoarthritis Cartilage*. 07 2021;29(7):1020-1028. doi:10.1016/j.joca.2021.03.013
51. Hasegawa M, Nakoshi Y, Tsujii M, et al. Changes in biochemical markers and prediction of effectiveness of intra-articular hyaluronan in patients with knee osteoarthritis. *Osteoarthritis Cartilage*. Apr 2008;16(4):526-9. doi:10.1016/j.joca.2007.09.014
52. Kwak YH, Kwak DK, Kim NY, Kim YJ, Lim JS, Yoo JH. Significant changes in synovial fluid microRNAs after high tibial osteotomy in medial compartmental knee osteoarthritis: Identification of potential prognostic biomarkers. *PLoS One*. 2020;15(1):e0227596. doi:10.1371/journal.pone.0227596
53. Kokebie R, Aggarwal R, Lidder S, et al. The role of synovial fluid markers of catabolism and anabolism in osteoarthritis, rheumatoid arthritis and asymptomatic organ donors. *Arthritis Res Ther*. Mar 24 2011;13(2):R50. doi:10.1186/ar3293
54. Dufield DR, Nemirovskiy OV, Jennings MG, Tortorella MD, Malfait AM, Mathews WR. An immunoaffinity liquid chromatography-tandem mass spectrometry assay for detection of endogenous aggrecan fragments in biological fluids: Use as a biomarker for aggrecanase activity and cartilage degradation. *Anal Biochem*. Nov 15 2010;406(2):113-23. doi:10.1016/j.ab.2010.06.044
55. Bautch JC, Clayton MK, Chu Q, Johnson KA. Synovial fluid chondroitin sulphate epitopes 3B3 and 7D4, and glycosaminoglycan in human knee osteoarthritis after exercise. *Ann Rheum Dis*. Nov 2000;59(11):887-91. doi:10.1136/ard.59.11.887
56. Haraden CA, Huebner JL, Hsueh MF, Li YJ, Kraus VB. Synovial fluid biomarkers associated with osteoarthritis severity reflect macrophage and neutrophil related inflammation. *Arthritis Res Ther*. 06 13 2019;21(1):146. doi:10.1186/s13075-019-1923-x
57. Arellano RD, Aguilar LS, Argüello R, Hernadez F, Gonzalez FF, Moran J. Cartilage Oligomeric Matrix Protein Levels in Synovial Fluid in Patients With Primary Knee Osteoarthritis And Healthy Controls: A Preliminary Comparative Analysis With Serum Cartilage Oligomeric Matrix Protein. *Arch Rheumatol*. Sep 2017;32(3):189-196. doi:10.5606/ArchRheumatol.2017.6220
58. Nelson F, Dahlberg L, Laverty S, et al. Evidence for altered synthesis of type II collagen in patients with osteoarthritis. *J Clin Invest*. Dec 15 1998;102(12):2115-25. doi:10.1172/JCI4853
59. Löfvall H, Katri A, Dąbrowska A, et al. GPDPLQ. *Sci Rep*. 02 28 2019;9(1):3050. doi:10.1038/s41598-019-39803-0
60. Thonar EJ, Schnitzer TJ, Kuettner KE. Quantification of keratan sulfate in blood as a marker of cartilage catabolism. *J Rheumatol*. May 1987;14 Spec No:23-4.

61. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res*. Jun 2009;29(6):313-26. doi:10.1089/jir.2008.0027
62. Hardingham T, Bayliss M. Proteoglycans of articular cartilage: changes in aging and in joint disease. *Semin Arthritis Rheum*. Dec 1990;20(3 Suppl 1):12-33. doi:10.1016/0049-0172(90)90044-g
63. Hasegawa M, Hirata H, Sudo A, et al. Tenascin-C concentration in synovial fluid correlates with radiographic progression of knee osteoarthritis. *J Rheumatol*. Oct 2004;31(10):2021-6.
64. Huang K, Wu LD. YKL-40: a potential biomarker for osteoarthritis. *J Int Med Res*. 2009 Jan-Feb 2009;37(1):18-24. doi:10.1177/147323000903700102
65. Seibel MJ. Clinical application of biochemical markers of bone turnover. *Arq Bras Endocrinol Metabol*. Aug 2006;50(4):603-20. doi:10.1590/s0004-27302006000400006
66. Kuo TR, Chen CH. Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark Res*. 2017;5:18. doi:10.1186/s40364-017-0097-4
67. Garvican ER, Vaughan-Thomas A, Innes JF, Clegg PD. Biomarkers of cartilage turnover. Part 1: Markers of collagen degradation and synthesis. *Vet J*. Jul 2010;185(1):36-42. doi:10.1016/j.tvjl.2010.04.011
68. Nguyen LT, Sharma AR, Chakraborty C, Saibaba B, Ahn ME, Lee SS. Review of Prospects of Biological Fluid Biomarkers in Osteoarthritis. *Int J Mol Sci*. Mar 12 2017;18(3)doi:10.3390/ijms18030601
69. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. *Arthritis Rheum*. Jun 2001;44(6):1237-47. doi:10.1002/1529-0131(200106)44:6<1237::AID-ART214>3.0.CO;2-F
70. Stannus O, Jones G, Cicuttini F, et al. Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis Cartilage*. Nov 2010;18(11):1441-7. doi:10.1016/j.joca.2010.08.016
71. Stannus OP, Jones G, Blizzard L, Cicuttini FM, Ding C. Associations between serum levels of inflammatory markers and change in knee pain over 5 years in older adults: a prospective cohort study. *Ann Rheum Dis*. Apr 2013;72(4):535-40. doi:10.1136/annrheumdis-2011-201047
72. Suyasa IK, Lestari AAW, Setiawan IGNY, Mahadewa TGB, Widyadharma IPE. Elevated High-Sensitivity C-Reactive Protein And Interleukin-6 Plasma As Risk Factors For Symptomatic Lumbar Osteoarthritis In Postmenopausal Women. *Open Access Maced J Med Sci*. Nov 25 2018;6(11):2107-2110. doi:10.3889/oamjms.2018.422
73. Barker T, Rogers VE, Henriksen VT, Trawick RH, Momberger NG, Lynn Rasmussen G. Circulating IL-10 is compromised in patients predisposed to developing and in patients with severe knee osteoarthritis. *Sci Rep*. 01 19 2021;11(1):1812. doi:10.1038/s41598-021-81382-6
74. Chisari E, Yaghmour KM, Khan WS. The effects of TNF-alpha inhibition on cartilage: a systematic review of preclinical studies. *Osteoarthritis Cartilage*. 05 2020;28(5):708-718. doi:10.1016/j.joca.2019.09.008
75. Duan X, Murata Y, Liu Y, Nicolae C, Olsen BR, Berendsen AD. Vegfa regulates perichondrial vascularity and osteoblast differentiation in bone development. *Development*. Jun 01 2015;142(11):1984-91. doi:10.1242/dev.117952

776 76. Okada Y, Gonoji Y, Naka K, et al. Matrix metalloproteinase 9 (92-kDa gelatinase/type
777 IV collagenase) from HT 1080 human fibrosarcoma cells. Purification and activation of the
778 precursor and enzymic properties. *J Biol Chem*. Oct 25 1992;267(30):21712-9.
779 77. Kanyama M, Kuboki T, Kojima S, et al. Matrix metalloproteinases and tissue
780 inhibitors of metalloproteinases in synovial fluids of patients with temporomandibular joint
781 osteoarthritis. *J Orofac Pain*. 2000;14(1):20-30.
782 78. Lepetsos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis.
783 *Biochim Biophys Acta*. 04 2016;1862(4):576-591. doi:10.1016/j.bbadis.2016.01.003
784 79. Tootsi K, Märtson A, Kals J, Paapstel K, Zilmer M. Metabolic factors and oxidative
785 stress in osteoarthritis: a case-control study. *Scand J Clin Lab Invest*. Nov 2017;77(7):520-
786 526. doi:10.1080/00365513.2017.1354255
787 80. Alexander S, Watt F, Sawaji Y, Hermansson M, Saklatvala J. Activin A is an
788 anticatabolic autocrine cytokine in articular cartilage whose production is controlled by
789 fibroblast growth factor 2 and NF-kappaB. *Arthritis Rheum*. Nov 2007;56(11):3715-25.
790 doi:10.1002/art.22953
791 81. Chia SL, Sawaji Y, Burleigh A, et al. Fibroblast growth factor 2 is an intrinsic
792 chondroprotective agent that suppresses ADAMTS-5 and delays cartilage degradation in
793 murine osteoarthritis. *Arthritis Rheum*. Jul 2009;60(7):2019-27. doi:10.1002/art.24654
794 82. Gupta RC, Lall R, Srivastava A, Sinha A. Hyaluronic Acid: Molecular Mechanisms and
795 Therapeutic Trajectory. *Front Vet Sci*. 2019;6:192. doi:10.3389/fvets.2019.00192
796 83. Filková M, Senolt L, Braun M, et al. Serum hyaluronic acid as a potential marker with
797 a predictive value for further radiographic progression of hand osteoarthritis. *Osteoarthritis*
798 *Cartilage*. Dec 2009;17(12):1615-9. doi:10.1016/j.joca.2009.06.002
799 84. Flaumenhaft R, Kojima S, Abe M, Rifkin DB. Activation of latent transforming growth
800 factor beta. *Adv Pharmacol*. 1993;24:51-76. doi:10.1016/s1054-3589(08)60933-3
801 85. Olofsson A, Ichijo H, Morén A, ten Dijke P, Miyazono K, Heldin CH. Efficient
802 association of an amino-terminally extended form of human latent transforming growth
803 factor-beta binding protein with the extracellular matrix. *J Biol Chem*. Dec 29
804 1995;270(52):31294-7. doi:10.1074/jbc.270.52.31294
805 86. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and
806 osteoarthritis. *Osteoarthritis Cartilage*. Jun 2007;15(6):597-604.
807 doi:10.1016/j.joca.2007.02.005
808 87. Li Y, Xu L. Advances in understanding cartilage remodeling. *F1000Res*. 2015;4(F1000
809 Faculty Rev):642. doi:10.12688/f1000research.6514.1
810 88. Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to
811 function. *Nucleic Acids Res*. 01 08 2019;47(D1):D155-D162. doi:10.1093/nar/gky1141
812 89. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. Jan 23
813 2004;116(2):281-97. doi:10.1016/s0092-8674(04)00045-5
814 90. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev*
815 *Genet*. Jul 2004;5(7):522-31. doi:10.1038/nrg1379
816 91. Nugent M. MicroRNAs: exploring new horizons in osteoarthritis. *Osteoarthritis*
817 *Cartilage*. Apr 2016;24(4):573-80. doi:10.1016/j.joca.2015.10.018
818 92. Song J, Kim D, Lee CH, Lee MS, Chun CH, Jin EJ. MicroRNA-488 regulates zinc
819 transporter SLC39A8/ZIP8 during pathogenesis of osteoarthritis. *J Biomed Sci*. May 20
820 2013;20:31. doi:10.1186/1423-0127-20-31
821 93. Kung LHW, Ravi V, Rowley L, et al. Cartilage MicroRNA Dysregulation During the
822 Onset and Progression of Mouse Osteoarthritis Is Independent of Aggrecanolysis and

823 Overlaps With Candidates From End-Stage Human Disease. *Arthritis Rheumatol*. 03
824 2018;70(3):383-395. doi:10.1002/art.40378
825