

Osteoarthritis Year in Review 2024: Molecular Biomarkers of Osteoarthritis

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Osteoarthritis Year in Review 2024: Molecular Biomarkers of Osteoarthritis

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ABSTRACT

Objective: To provide a comprehensive and insightful summary of studies on molecular biomarkers at the gene, protein, and metabolite levels across different sample types and joints affected by osteoarthritis (OA).

Methods: A literature search using the PubMed database for publications on OA biomarkers published between April 1, 2023 and April 30, 2024 was performed. Publications were then screened, examined at length, and summarized in a narrative review.

Results: Out of the 364 papers initially identified, 44 publications met inclusion criteria, were relevant to OA, and were further examined for data extraction and discussion. These studies included 1 genomic analysis, 22 on protein markers, 6 on metabolite markers, 9 on inflammatory mediators, and 6 integrating multiple molecular levels.

Conclusions: Significant advancements have been made in identifying molecular biomarkers for OA, encompassing various joints, sample types, and molecular levels. Despite this progress, gaps remain, particularly in the need for validation, larger sample sizes, the integration of more clinical data, and consideration of covariates. For early detection and improved treatment of OA, continued efforts in biomarker identification are needed. This effort should seek to identify effective biomarkers that advance early detection, support prevention, evaluate interventions, and improve patient outcomes.

INTRODUCTION

Osteoarthritis (OA), a chronic joint disease characterized by the breakdown of cartilage and other tissues, is the most prevalent form of arthritis and the leading cause of disability worldwide. In 2020, OA affected 595 million people globally, about 7.6% of the population¹. Economically, OA's direct costs range from 1-2.5% of the Gross National Product in the US, UK, Canada, and Australia^{2, 3}. OA commonly affects the hips, knees, hands, and feet, leading to pain, stiffness, reduced mobility, fatigue, and diminished work performance^{4, 5}. Despite these significant physical and economic burdens, there are no clinical biomarkers for early OA detection or to monitor disease progression.

A biomarker is defined as “a characteristic that is measured as an indicator of normal biological processes, pathological processes or responses to an exposure or intervention^{6, 7}.” This broad definition includes molecular, histological, radiographic, and physiological characteristics. This review focuses on molecular biomarkers, exploring their potential complex roles as diagnostic, prognostic, monitoring, and predictive markers for OA. This review will examine molecular biomarkers at the gene, protein, and metabolite levels across sample types and joints affected by OA. Effective OA biomarkers are urgently needed to understand OA's heterogeneity, enable early detection, improve disease management, and enhance patient outcomes and quality of life.

SYSTEMATIC REVIEW: METHODS AND RESULTS

A PubMed search for “Osteoarthritis Biomarkers” identified 364 English-language studies published in English between April 1, 2023 and April 30, 2024. Duplicate and non-OA papers were excluded (n=272). Studies were selected based on inclusion criteria requiring original research on molecular biomarkers (gene, protein, metabolite)

for OA in any joint in human and animal models. Exclusion criteria included imaging studies, reviews, editorials, and inappropriate methodology resulting in the removal of 48 additional publications. Inappropriate methodology consisted of (1) absence of consideration of relevant covariates (e.g. age, sex, etc) (2) extensive use of previously published publicly available data and (3) insufficiently clear description of methods.. Various sample types and joints were considered, with most studies (82.6%) focusing on the knee. In total, 46 studies are discussed in this review including 1 genomic analysis, 22 studies on protein markers, 6 on metabolite markers, 9 on inflammatory mediators, and 6 that integrated multiple molecular levels. Studies were categorized into two groups based on the number of biomarkers examined (<5 or >5), with most exploring more than five (Figure 1, Table 1).

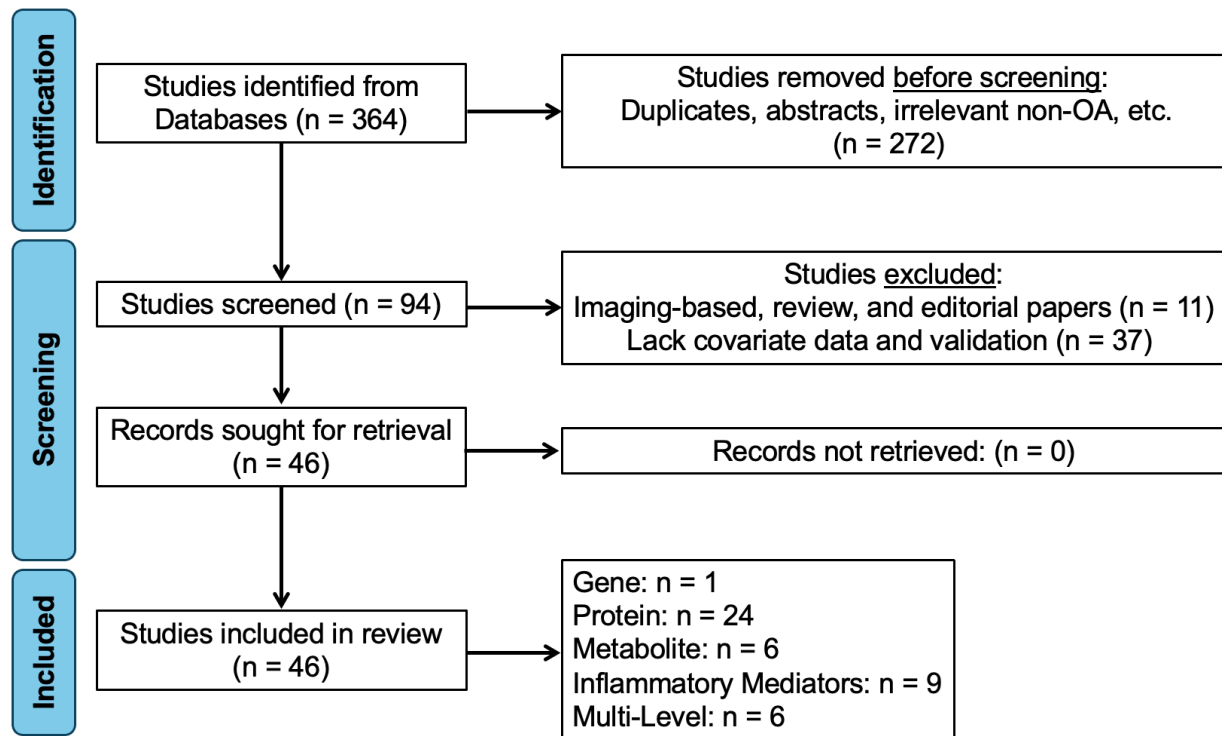


Figure 1. Flow diagram summarizing systematic review methods.

DISCUSSION

Gene-level

The search for genomic biomarkers of OA has been a research priority for decades as identification of genomic biomarkers could allow population-scale screening and early detection, minimizing the burden of OA. Many studies examining gene expression were excluded because they did not appropriately consider relevant covariates such as age and sex. Readers should be careful because of the proliferation of studies of dubious quality in this issue⁸. Potentially important developments for genetic biomarkers of OA include the demonstration of the feasibility of loop-mediated isothermal amplification (LAMP) for point-of-care detection of specific genes and downstream mRNA expression in synovial fluid (SF)⁹. There remains much progress needed to develop clinically useful genetic biomarkers of OA.

Protein-level

Proteins—large complex molecules essential for physiology—are key indicators for diagnosing and monitoring diseases such as OA. Joint components like cartilage, synovium, and bone are often investigated to provide direct insights into OA pathophysiology. SF, pivotal for joint lubrication and nourishment, directly reflects the biochemical and cellular changes during disease and health. SF studies can uncover critical biomarkers and molecular pathways involved in OA, thereby enhancing diagnostic accuracy, monitoring disease progression, and developing targeted treatments that address the underlying pathology of the condition.

96 Rocchetti et al. analyzed the SF proteomes from patients with severe OA
97 undergoing total knee replacement (TKR) and controls (those undergoing knee
98 arthroscopy for acute meniscus injuries). Dysregulated SF levels of α -Enolase (ENO1)
99 and Fibrinogen β -Chain (FBG) correlated with Knee-injury-and-Osteoarthritis-Outcome-
100 Scores (KOOS) and visual analog scale (VAS) scores among the OA group, suggesting
101 diagnostic and prognostic potential¹⁰. Rai et al. studied SF proteome changes post-
102 anterior cruciate ligament (ACL) injury over time (initial visit, time of surgery). Analysis
103 revealed 130 differentially expressed proteins between the two aspirations, including
104 inflammatory (CRIP1, S100A11, PLS3, POSTN, and VIM) and
105 chondroprotective/homeostatic proteins (CHI3L2, TNFAIP6/TSG6, DEFA1, SPP1,
106 CILP)¹¹. Ryden et al. analyzed proteolytic activity in SF from OA patients undergoing TKR
107 and controls (SF from diseased donors with no history of joint disease), revealing 429
108 differentially expressed peptides, including increased cleaved APOA1, suggesting
109 increased enzymatic degradation by metalloproteinases and chymase¹².

110 Paz-Gonzalez et al., using proteomics to compare healthy and diseased menisci
111 from mildly degenerated and end-stage OA joints, identified 42 candidate protein
112 biomarkers with lower CYTL1 levels potentially marking early joint degeneration¹³.
113 Another study harvested knee subchondral bone to explore differentially expressed
114 proteins (DEPs) associated with OA. SerpinA5, a complement and coagulation protein,
115 was significantly increased in severe OA subchondral bone compared to controls. These
116 findings were also validated in a rat model¹⁴. Thus, it is possible that SerpinA5 may play
117 a role in subchondral bone OA pathogenesis, similar to their role in the OA pathogenesis
118 of cartilage and synovial tissues¹⁵, and could be a potential drug target for OA treatment.

OA, now recognized as a systemic disease, affects not only joint components but SF and the circulatory system¹⁶, making analysis of serum and plasma, which contain numerous biomarkers, critical for advancing our understanding of OA as they can unveil insights into disease mechanisms, progression, and treatment response.

Three studies by Kraus et al. demonstrate the diagnostic capabilities of serum protein profiles using mass spectrometry-based approaches¹⁷. The first analyzed 107 peptides between knee osteoarthritis (kOA) patients and healthy controls and identified an 8-peptide panel (ACTG, HEMO, C1R, HRG, CNDP1, CRAC1, MASP1, HABP2) that distinguished kOA patients from controls with high accuracy (ROC-AUC=0.833, PR-AUC=0.929) and could distinguish between symptomatic and non-symptomatic cases equally. ACTG, a type of actin, was the strongest single indicator of OA within the panel. A follow-up validation study examined if serum proteomics could predict kOA progression¹⁸. Serum from participants with symptomatic kOA (n=104), of which 46 were progressors, underwent multiple reaction monitoring mass spectrometry analysis. The 11-peptide panel (C1R, CD14, CRAC1, HRG, IC1, PLF4, RET4, SHBG, VTDB, ZPI) when paired with demographic data, served as a moderate predictor of kOA progression. However, it was not significantly better at predicting progression than the 11-peptide panel alone. Their third study sought to evaluate the serum protein panel and its predictive capabilities of kOA in a separate cohort of 200 females without evidence of radiographic OA (KL grade <1). Results indicate that a 6-protein panel was able to predict incident RkOA development up to 8 years later (AUC=0.77) and was superior to other predictive methods such as age, BMI, and joint pain. COMP, CRAC1, and ZPI—which have also been noted as potential biomarkers in other studies—were considered essential peptides

and those alone were able to significantly discriminate incident RkOA¹⁹. Similarly, Paz-Gonzalez et al. developed a prognostic panel for predicting kOA in non-radiographically confirmed subjects (n=282 training and n=100 validation) and to identify individuals with high risk of disease development. Models built with clinical/demographic variables and protein markers (APOA1, ZAG2, A2AP) had an AUC=0.831 for predicting kOA incidence during the 96-month follow-up period²⁰. Collectively, proteomic panels could aid in early OA diagnosis and treatment.

By investigating pre- and post-operative serum levels of inflammatory protein biomarkers in total knee arthroplasty patients, Giordano et al. identified 12 differentially expressed inflammatory protein markers between participants and controls. Of these, 4 proteins (IL-6, HGF, CSF-1, and CCL20) were upregulated and 8 (CASPASE-8, AXIN1, SCF, EN-RAGE, STAMPB, 4E-BP1, SIRT2, and TWEAK) were downregulated in kOA patients. Additionally, they were able to distinguish 2 subgroups of kOA patients using six differentially expressed proteins (CD244, STAMPB, CASP-8, CD40, PD-L1, TNFRSF9). Both groups had significantly different 12-month post-operative KOOS and VAS, suggesting that differential expression of these proteins may be applicable as prognostic biomarkers following total knee arthroscopy²¹.

Armitano-Lago et al. studied how changing load during gait affects serum levels of cartilage oligomeric matrix protein (COMP), a biomarker of cartilage turnover, in patients who had undergone ACL reconstruction (ACLR). While overall differences in serum COMP (sCOMP) among the cohort were not found, post hoc analysis revealed a significant time x condition interaction in a subgroup of patients (sCOMP Increasers). Additionally, sCOMP levels were lower during high loading when compared to low loading,

and sCOMP levels during habitual loading were higher than during symmetrical loading (3.5 hours post-loading). Overall, this study suggests that changes in sCOMP dynamics following load manipulation during gait could optimize gait in ACLR patients but further optimization is warranted.²².

A 6-month weight loss study in older patients with and without OA found that weight loss increased serum levels of adiponectin, an anti-inflammatory hormone, while COMP levels decreased suggesting weight loss could protect against OA, and be targeted therapeutically²³. Chen et al. investigated the use of bone turnover markers for OA diagnosis in middle-aged subjects. Beta-C-Terminal telopeptide (β -CTX), and parathyroid hormone (PTH), and 25-hydroxy-cholecalciferol (25(OH)D) were significantly associated with OA in middle age. β -CTX and PTH levels increased and decreased with OA grade, respectively²⁴, indicating their diagnostic and prognostic potential.

In a human plasma study, Styrkarsdottir et al. examined cartilage acidic protein 1 (CRTAC1) role in OA risk and progression to confirm their previous findings that CRTAC1 is significantly associated with disease²⁵. Three proteins CRTAC1, ACAN, and NCAN were associated with prevalent KOA, hip OA, and disease risk. CRTAC1 was highly associated with prevalent KOA and ACAN was highly associated with hip OA and KOA. Plasma levels of CRTAC1, but not ACAN and NCAN, were able to predict progression to joint replacement for both knee and hip OA²⁶. Thus, these three proteins may be useful biomarkers to improve OA diagnosis, risk assessment, and predict joint replacement.

Zou et al. used mendelian randomization to analyze nearly 5,000 plasma proteins, highlighting 12 proteins (CRYZ, MAPK3, LGALS3, GZMK, MAX, CFHR3, OMG, DNAJB12, ITIH1, ULK3, USP8, CSK) as potential drug targets for KOA and hip OA, with

MAPK3 and GZMK identified as core targets for kOA and ITIH1 for hip OA. Gene expression of MAPK3 and MAX reduced the risk of kOA, while levels of LGALS3, GZMK, OMG, and DNAJB12 increased the risk of kOA²⁷. These proteins shed light on new avenues for OA treatment.

Multi-tissue studies are essential for understanding OA's complex interactions across joint and systemic tissues, potentially revealing interconnected pathological mechanisms and potential therapeutic targets. An example of this was showcased in a study by Kumavat et al. where expression of Fibrinogen Alpha (FGA) in human plasma and SF samples was examined, revealing that FGA is higher in OA plasma and SF samples compared to healthy controls, suggesting its role in OA pathogenesis²⁸. A cross-sectional study of human serum and SF examined relationships between ER stress-related glucose-related protein 78 (GRP78) and CCAAT/enhancer-binding protein homologous protein (CHOP) concentrations with disease severity of primary kOA (pkOA). While serum levels of the two proteins did not differ significantly between pkOA patients and healthy controls, SF concentrations of GRP78 and CHOP across Kellgren-Lawrence (KL) grades differed. Moreover, SF levels, but not serum levels, of the two proteins were positively correlated with KL grade, IL-6, and matrix-metalloproteinase (MMP)-3 levels²⁹.

Meehan et al. studied how clinical improvement in pain and function after injection of Intra-Articular-Hyaluronic Acid is associated with changes in SF volume as well as serum and SF biomarkers in patients with symptomatic kOA. Results indicate that, although clinical improvements were observed at 3-, 6-, and 12-months following injection, no significant changes from baseline in SF volume, serum biomarkers, or SF biomarkers were detected, except for SF MMP-10 which decreased by 16% at 3 months.

A lack of controls, such as a placebo treatment, is a major limitation of this study, indicating the need for further research into MMP-10 and other SF biomarker levels as a result of treatment with hyaluronic acid³⁰. Liu et al. correlated calcitonin gene-related peptide (CGRP) levels in serum and SF with knee osteophyte formation, finding significant associations in both fluids with osteophyte grade. These data suggest that CGRP may play a role in osteophyte formation in those with KOA and could indicate OA severity and risk of progression³¹.

Kalogera et al. compared serum and SF levels of biomarkers related to extracellular matrix (ECM) remodeling in KOA patients. Biomarkers included collagen types I (C1M), II (C2M), III (C3M), and X (C10C)), and aggrecan (ARGS). Serum C1M levels negatively correlated with subchondral bone density. C2M negatively correlated with KL grade and positively correlated with minimum joint space width (minJSW). SF levels of C10C were negatively correlated with minJSW and positively correlated with total osteophyte area and KL grade. Data suggests that these ECM biomarkers were linked to structural outcomes and might be related to different processes related to disease³².

In an immobilization study of human serum and urine, Liphardt et al. studied the effects of bedrest on various serum and urine biomarkers of collagen type II degradation in healthy individuals over time— with and without exercise and nutritional supplementation. After 21 days of bedrest, serum CPII, C2C, and C1,2C were increased, with C2C levels remaining elevated 1-6 days after resuming activity. Urine levels of CTX-II were also increased during bedrest, indicating that immobilization for 21 days causes degradation of type II collagen, with effects extending days after resuming activity³³.

The last multi-fluid study meeting inclusion criteria was conducted by Goode et al. where baseline biomarkers associated with longitudinal changes in the worsening of disc space narrowing and low back pain were examined in human serum and urine. Baseline biomarkers like Lumican (proteoglycan) were associated with worsening disc space narrowing, while brain-derived neurotrophic factor (neuropeptide) was associated with worsening of vertebral osteophytes. Baseline levels of hyaluronic acid were associated with worsening lower back pain³⁴. These baseline biomarkers may prove as useful prognostic biomarkers and therapeutic targets for back pain management and spine OA.

Taking a different approach, Adepu et al. used horse saliva to quantify salivary biglycan-neo-epitope-BGN(262), a potential biomarker of equine OA subchondral bone sclerosis, using the rationale that saliva reflects disease and contains many of the same molecules present in SF and serum. Results show that levels of the candidate marker are higher in osteoarthritic horses than control horses and may be useful for assessing OA severity³⁵.

Metabolite-level

Metabolites are small molecule intermediates of metabolism (amino acids, lipids, peptides, nucleic acids, vitamins, etc.) Previous studies have indicated that OA pathological changes are linked to imbalanced metabolism. Thus, identification of metabolic biomarkers is paramount. Like proteins, metabolites have been examined in SF and in circulatory fluids.

Using an inflammatory OA rat model, Berenguer et al. identified changes in serum metabolites, including organic acids (lactacystin, taurine), benzenoids (styrene oxide, tyramine), and lipids (prenols, sphingolipids), within the first 28 days³⁶. Similarly, Wang et

al. found 61 differentially regulated metabolites in serum between healthy and kOA patients, with metabolites like carnitine, hypoxanthine, xanthine, palmitoleic acid, and N-alpha-acetyl-L-asparagine correlated with kOA severity. Pathway enrichment analyses linked these metabolites to arachidonic acid, linoleic acid, caffeine, and purine metabolism³⁷. Collectively, the wide array of metabolites in human and animal models warrants further investigation into these metabolites as OA biomarkers.

Beyond the knee, Cambon-Binder et al. found that serum concentration of carboxymethyllysine (CML) and homocitruline—products of two non-enzymatic post-translational modifications of proteins and are related to age—were lower in erosive OA patients compared to non-erosive hand OA patients, while cartilage CML levels were higher in OA cartilage versus non-OA cartilage, suggesting CML could be a prognostic biomarker for hand OA³⁸.

Two studies by Turnbull et al. identified associations between serum lipid mediators and KOA. In patients with knee injuries, baseline omega-3 polyunsaturated fatty acids (PUFAs) levels (eicosapentaenoic acid, docosahexaenoic acid, 17- and 14-hydroxydocosahexaenoic acid, 18-hydroxyeicosapentaenoic acid) were higher compared to later timepoints (3 months, 2 years) and to healthy controls. Other lipid mediators like 8,9- and 14,15-dihydroxyeicosatrienoic acid (DHET) were associated with severe knee pain and symptoms at two years³⁹. These findings were reinforced in their second study examining these same associations in serum of kOA patients, where arachidonic acid, DHET, as well as 8,9-epoxyeicosatrienoic acid (EET) and 12-hydroxyeicosatetraenoic acid (HETE) were linked to pain scores and KL grade. Over three years, concentrations of 8,9-EET and 5-HETE had the strongest longitudinal association with pain scores and

could distinguish patients with pain from those without⁴⁰. These findings suggest that serum levels of certain PUFAs and those related to arachidonic acid metabolism may have prognostic ability to predict knee pain.

Lastly, Mehta et al. used metabolic phenotyping to identify serum metabolites associated with pain and inflammatory cytokines (IL-10, IL-13, IL-1B, IL-2, IL-8, TNFa) in symptomatic OA. Key pain-associated metabolites included acyl ornithine, carnosine, cortisol, cortisone, cystine, DOPA, glycolithocholic acid sulphate, phenylethylamine, and succinic acid. Cystine and glycolithocholic acid sulphate were positively correlated with all measured inflammatory cytokines, indicating that targeting these pathways, metabolites, and cytokines could improve OA pain management.⁴¹

Inflammatory Mediators

Individuals with OA often experience acute and chronic inflammation affecting multiple tissues within the joint⁴². While inflammation aids tissue repair and homeostasis, it also contributes to OA development. Consequently, many studies have focused on inflammatory mediators as potential biomarkers including IL-6, IL-7, IL-1B, TNFa, leptin, adiponectin, MMPs, and systemic inflammation indices.

In a study by McAlindon et al. inflammatory biomarkers in serum associated with the development of erosive hand OA were examined, revealing strong associations with elevated IL-7, suggesting it may mediate inflammation and disease development⁴³. Three papers by Amirkhizi, Andersson, and Ilia et al. examined similar inflammatory cytokines in serum and SF⁴⁴⁻⁴⁶. Amirkhizi et al. discovered an inverse correlation between dietary total antioxidant capacity (TAC) and serum IL-6, TNFa, MMP-1, and NF-kB indicating that higher TAC scores were linked to lower disease severity and inflammatory cytokine

levels⁴⁴. Andersson et al. tested the potential of circulating galectin, IL-1B, IL-6, and TNF α as biomarkers for predicting KOA and/or hand OA at baseline and at a 2-year follow-up. Galectin levels at baseline were predictive of hand OA development but not KOA, and were more effective than IL-6, IL-1B, and TNF α in predicting radiographic hand OA⁴⁵. Similarly, Ilia et al. analyzed adiponectin, IL-10, TNF α , and IL-6 in SF of KOA patients, reporting that adiponectin levels correlated with IL-6 and that higher IL-6 levels were linked to increased IL-10. TNF α was positively correlated with BMI, WOMAC scores, and activity of daily living subscales but no cytokines were significantly correlated with KL grade⁴⁶.

Leptin and IL-1B, biomarkers associated with metabolic syndrome (MetS) and OA, were investigated by Mai et al. in a Vietnamese patient population. They found high plasma leptin levels in OA and MetS patients correlated with BMI, waist-to-hip ratio, visual analog scale scores, and HbA1c and A1C levels. Conversely, IL-1B levels were higher in OA patients and correlated with insulin levels, and ROC analysis revealed that leptin and IL-1B could distinguish individuals with and without OA and those with and without MetS, suggesting that these biomarkers play key roles in the pathogenesis of MetS-related OA⁴⁷.

Two studies by Singh et al. examined the potential for MMP-3 to detect early KOA and to differentiate between OA grades^{48, 49}. Serum MMP-3 levels were higher in OA individuals compared to healthy controls, varying significantly between KL grades⁴⁹. They also compared serum levels of MMP-3 and CTX-II, finding that both increased significantly with OA grade. CTX-II was better at discriminating between healthy and early KOA, while MMP-3 was better at discriminating between early and mild KOA⁴⁸. Combined,

both studies suggest that serum MMP-3 estimation could be a useful tool for diagnosis of OA and discriminating between OA grades.

Systemic inflammation indices and their association with OA were studied. Tarabeih et al. found that the systemic immune-inflammation index (SII) - a newly developed index used to predict the risk of chronic inflammation disorders - emerged as a reliable prognostic biomarker for OA development⁵⁰ in the first reported study of SII and OA. Zhou et al. identified systemic inflammation response index (SIRI) as an emerging biomarker for OA⁵¹. Both SIRI and SII, novel composite indices integrating three independent white blood cell subsets and platelets, represent inflammatory and immune status⁵². These indices—SIRI and SII—stand out as minimally-invasive biomarkers that characterize inflammatory and immune status as well as OA, warranting further investigation.

Multi-Level Integration

Several studies have integrated various experimental measures to develop comprehensive OA biomarkers, reflecting the disease's complex impact on multiple joint tissues. A significant focus has been extracellular vesicles (EVs) and surface markers in KOA. Clarke et al. combined proteomics and phospholipidomics to identify biomarkers associated with SF EVs from healthy, mildly, and severely OA-affected horse joints. Several proteins and phospholipids emerged as potential composite biomarkers for OA that either decreased (phosphatidylinositol 32:1, phosphatidylcholine 34:4, phosphatidylinositol 38:6, CD163, annexin A8) or increased (sphingomyelin 36:0;2, sphingomyelin 41:3,2, phosphatidylcholine O-32:3, CD109, moesin) with OA severity⁵³. Similarly, Zhang et al. characterized EV surface markers, cells, tissues, and effectors

associated with KOA pathogenesis. Proteomic analysis of EVs from KOA patients revealed 692 peptides associated with OA severity. Key surface markers including BGN, NRP1, CD109, and CSPG4 and coagulation peptides – including FGA – were highly expressed in chondrocytes from damaged cartilage and synovial immune cells, suggesting that osteoarthritic SF EVs could serve as diagnostic biomarkers⁵⁴.

Matejova et al. also focused on EVs and surface markers as potential biomarkers, analyzing plasma from healthy, early-, and end-stage OA individuals. Stage-specific EV surface markers included CD326, CD45, and CD31, which were higher in early-stage OA compared to healthy. In contrast, CD63 and CD31 expression was higher in end-stage OA. Furthermore, CD56, ROR1, and CD326 expression was significantly higher in early-stage OA compared to end-stage OA⁵⁵. Surface marker expression and overlapping markers (CD163, CD109) require further investigation to determine their potential as OA biomarkers.

At the microRNA level, Zhou et al. examined plasma microRNA-320c (miR-320c)—previously implicated to be involved in OA development—as a novel predictor and biomarker of the severity of KOA. MiR-320c expression was significantly higher in KOA individuals compared to healthy (AUC=0.942), demonstrated high diagnostic accuracy for KOA (AUC=0.985), and correlated with WOMAC score, IL-1B, and IL-6. MiR-320c also regulated cyclic AMP (cAMP) responsive element binding protein 5 (CREB5) expression, influencing OA pathogenesis via the cAMP pathway, indicating its potential as a novel biomarker of OA severity⁵⁶.

Nielson et al. used a data-driven approach to develop a model identifying OA biomarkers by integrating multi-omic, clinical, lifestyle, and biomarker data from 19,120

OA patients. They identified predictive biomarkers from various levels: genetic (TGFB1, GDF5, PTCH1, FAm53A), protein (CRTAC1, COL91, ACTA2, EDA2R, TACSTD2), and metabolite (acetate, valine, 3-hydroxybutyrate, citrate)⁵⁷. This approach, along with abundant patient data, reveals OA pathogenesis and predictive biomarkers, paving the way for personalized medicine in OA diagnosis, prevention, and treatment.

Lastly, Almalki et al. examined the influence of genetics and environmental factors on OA progression in obese females, focusing on vitamin D deficiency and GDF5. Vitamin D, GDF5, and calcium were lower while uric acid, lipid profiles, c-reactive protein, and erythrocyte sedimentation rate were higher in kOA females compared to healthy. Genotyping replicated an association between the rs143383 variant of GDF5 and kOA, where kOA patients had a higher TT (homozygous) and TC (heterozygous) genotype prevalence compared to healthy controls⁵⁸. Further investigation is required to better understand the association of these biomarkers—vitamin D levels and GDF5—and their role in the pathogenesis and progression of OA. Taken together, these studies highlight the complexity of OA and the potential for multi-level integrated approaches to develop various biomarkers for OA.

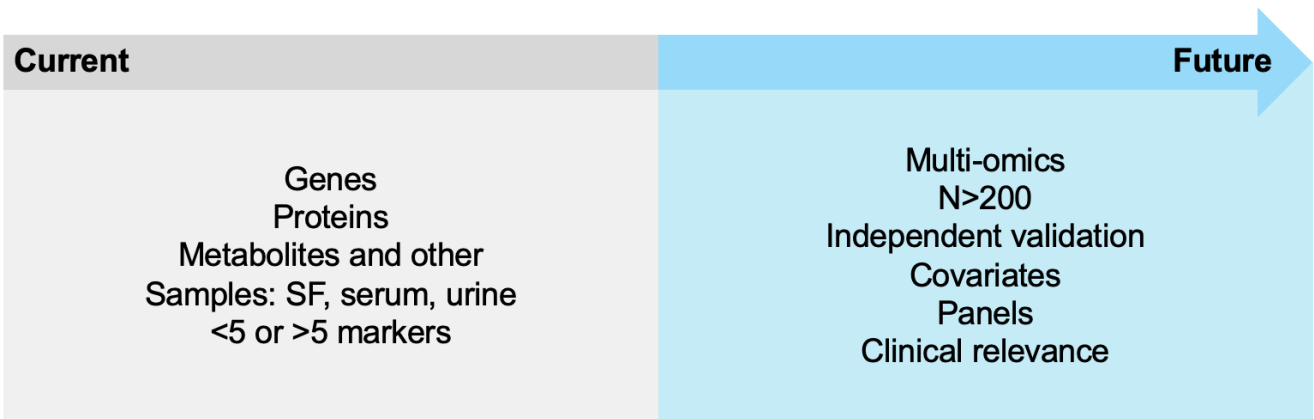


Figure 2 Progress and future directions for biomarkers in osteoarthritis.

CONCLUDING REMARKS

Significant progress has been made in identifying molecular biomarkers for diagnosing, predicting, and monitoring OA across various joints and molecular levels. However, gaps remain in our approach to studying these markers (Figure 2). Firstly, validation in independent datasets is crucial, especially given the small sample sizes ($n < 200$) in most studies. Larger cohorts, integration of clinical data, and the consideration of covariates like sex and age is necessary to account for the complex context of OA when developing biomarkers. Multi-omic studies show promise but must be coupled with rigorous validation measures. Lastly, while many potential diagnostic biomarkers have been identified, the priority should be in identifying patients with painful OA, as asymptomatic patients likely do not require treatment. We must advance promising candidate biomarkers beyond the discovery stage to improve early detection, disease monitoring, prevention, and intervention, ultimately enhancing patient outcomes and quality of life.

Contributions

All three authors equally contributed to the article including database searching, screening, analysis, interpretation, and writing and revising the manuscript.

Conflict of Interest/Disclosures

Authors have no conflicts of interest to disclose. Dr. June owns stock in Beartooth Biotech and OpenBioWorks, which were not involved in this study.

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415

416 **Abbreviations**

417 ACL - anterior cruciate ligament; ACLR - ACL reconstruction; cAMP - cyclic AMP; CGRP
418 - calcitonin gene-related peptide; CML - carboxymethyllysine; COMP - cartilage
419 oligomatrix protein; CREB5 - cAMP responsive element binding protein 5; CRTAC1 -
420 cartilage acidic protein 1; DEGs - differentially expressed genes; DEPs - differentially
421 expressed proteins; DHET - dihydroxyeicosatrienoic acid; ECM - extracellular matrix;
422 EET - epoxyeicosatrienoic acid; ENO1 - α -Enolase; EVs - extracellular vesicles; FBG -
423 Fibrinogen β -Chain; FGA - Fibrinogen Alpha; GRP78 - glucose-related protein 78; HETE
424 - hydroxyeicosatetraenoic acid; IA-HA - IA-Hyaluronic acid; KL - Kellgren-Lawrence;
425 KOOS - Knee-injury-and-Osteoarthritis-Outcome-Scores; LAMP - loop-mediated
426 isothermal amplification; Met - metabolic syndrome; miR-320c - microRNA-320c;
427 minJSW - minimum joint space width; MMPs - matrix-metalloproteinases; OA -
428 osteoarthritis; OASF - osteoarthritis synovial fluid; pKOA - primary kOA; PTH -
429 parathyroid hormone; PUFAs - polyunsaturated fatty acids; SF - synovial fluid; SII -
430 systemic immune-inflammation index; SIRI - systemic inflammation response index;
431 sCOMP - serum cartilage oligomatrix protein; TAC - total antioxidant capacity; TKR -
432 total knee replacement; VAS - visual analog scale.

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