Review

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Unveiling Prognostic and Diagnostic Biomarkers in Knee and Hip Osteoarthritis: A Targeted Review

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Osteoarthritis is a multifactorial condition marked by the gradual deterioration of joint cartilage, synovial inflammation, alterations in the subchondral bone and changes in the surrounding soft tissues. Clinical assessments and patient-reported outcome measures can identify pathological tissue alterations in osteoarthritis, in conjunction with radiographic evaluation of osteophytes, bone sclerosis, and joint space reduction. Although available treatments can help manage symptoms, early identification of prognostic factors for osteoarthritis progression is crucial for personalizing interventions and improving long-term outcomes. Therefore, it is essential to identify the key factors that can influence the disease's progression, including biological, mechanical, and clinical aspects. This review synthesizes current findings on the prognostic and diagnostic value of various biomarkers (systemic, intrinsic) and prognostic factors (biochemical, genetic, epigenetic) in knee and hip osteoarthritis. We also discuss the role of machine learning tools in identifying new biomarkers associated with osteoarthritis development and progression, paving the way for translation to clinical studies. In addition, we discuss recent studies aimed at identifying potential biomarkers and molecules that could serve as therapeutic strategies for osteoarthritis treatment.

Keywords: osteoarthritis; synovial biomarkers; inflammation; cartilage; micro-RNA; bioinformatics

Introduction

Osteoarthritis (OA) refers to a particular category of pathological processes impacting the joint in degenerative joint disease, characterized by the chronic, gradual deterioration of articular cartilage [1]. OA is exceedingly common, with an estimated frequency reaching tens of millions in the United States, with an increasing incidence associated with advanced age and elevated body mass index (BMI) [2]. In 2020, almost 595 million individuals globally were affected by OA, constituting 7.6% of the total population. In Western Europe, OA impacts more than 57 million individuals, with a prevalence of 10% in males and 13% in women aged 60 and older [3]. The pooled prevalence of OA in South Asia is estimated at 16.4%, whereas in East Asia and the Pacific, it is roughly 15.7% [4]. In the last 30 years, OA prevalence increased by 132.2% and is expected to reach 60–100% by 2050. The high prevalence is linked to a north-south gradient and to socio-economic status. After age 70, OA ranks as the seventh leading cause of disability, mainly affecting the knee. Rising prevalence is partly linked to high BMI, contributing ~20% of the increase [3] and high socio-demographic index countries like Australia [5,6] and China [7,8]. By 2030, incidence may decrease in women but rise in men. Early-onset OA (<55 years) now represents over half of new cases and 26.1% of total years lived with disability and causes global economic costs exceeding US$106.87 billion, with indirect productivity losses reaching almost 60% [9].

Clinical assessments and patient-reported outcome measures can identify pathological tissue alterations in OA in conjunction with radiographic evaluation of osteophytes, bone sclerosis, and joint space reduction. Nonetheless, these approaches are constrained in monitoring the initial molecular alterations occurring in joint tissues [10]. Thus, there is an immediate need for economical and precise approaches to detect molecular alterations in joints that occur prior to the identification of imaging-detected diseases.

Utilizing these compounds as biomarkers might facilitate earlier detection and more efficacious OA treatment [11].

The National Institutes of Health characterizes biomarkers as molecules that signify normal or pathological processes, or responses to therapeutic interventions [12]. They play a crucial role in the development of disease-modifying drugs by providing rapid and pertinent insights into the effects of treatments on biological pathways [13]. The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis, and Musculoskeletal Diseases (ESCEO) has advocated for the establishment of international standards to validate OA biomarkers, emphasizing the importance of sensitivity, specificity, and reproducibility [2,13]. This initiative necessitates cooperation among researchers, the pharmaceutical sector, and regulatory agencies. In 2012, the Foundation for the National Institutes of Health (FNIH) OA Biomarkers Consortium initiated the identification of knee OA biomarkers that possess prognostic significance [2]. Several were validated in Phase 1 and subsequently progressed to Phase 2 within the PROGRESS OA trial [14].

In this context, this review synthesizes current findings on the prognostic and diagnostic value of various biomarkers (systemic, intrinsic) and prognostic factors (biochemical, genetic, epigenetic) in knee and hip OA. We aimed to identify the most relevant studies in the field to provide an overview of prognostic and diagnostic factors and their integration into medical practice. We also discuss the role of machine learning tools in identifying new biomarkers associated with OA development and progression, paving the way for translation to clinical studies. Also, we discuss recent studies focusing on the identification of potential biomarkers and molecules that may offer new therapeutic strategies for OA treatment.

Materials and Methods

This review integrates findings from relevant peer-reviewed articles retrieved from PubMed/MEDLINE (https://pubmed.ncbi.nlm.nih.gov) and EMBASE (https://www.embase.com), focusing on studies published between January 2001 and February 2025. The search strategy involved the use of keywords and medical subject heading (MeSH) terms, such as “prognostic factors”, “osteoarthritis”, “gene expression”, “biomarkers”, “inflammatory cytokines”, “extracellular matrix proteins”, and “regulatory RNA molecules”. Various Boolean operators (“OR” and “AND”) were employed to develop the search strategies. The search was conducted from 05 January to 20 February 2025, and all English-published articles available online until the day of data collection were considered. The search terms were refined based on preliminary searches, and duplicate studies were removed.

Prognostic Factors of Knee and Hip OA

In recent years, multiple studies have been conducted to evaluate the prognostic factors of knee and hip OA, based on a standardized set of criteria [15–21]. Next, we will summarize and discuss the prognostic factors investigated within promising studies, including systemic and intrinsic. We divided prognostic factors as systemic or intrinsic based on whether their influence on knee OA progression stems from metabolic, biochemical, or demographic factors versus joint-related characteristics (e.g., structural, biomechanical, and functional). To discriminate between prognostic factors and biomarkers, we will present the insights each category provides below. Firstly, it is important to note that while some biomarkers act like prognostic indicators, not all prognostic factors fit this category. Prognostic factors comprise clinical, demographic, or structural characteristics and could predict disease outcomes, such as OA progression or symptom severity, regardless of treatment. Meanwhile, biomarkers refer to measurable biological indicators indicating the likely response to a specific therapy [22]. Using a biomarker with primarily prognostic value as a predictive tool may lead to unjustified treatment constraints and high costs. In addition, treating a predictive marker as prognostic could result in neglecting opportunities for targeted therapy. Though most biomarkers contain a mix of prognostic and predictive properties, their dominant utility must be appropriately identified [2].

Systemic Prognostic Factors

Systemic factors, including metabolic (body mass index—BMI, obesity, comorbidity count) [21], biochemical and inflammatory (serum and synovial hyaluronic acid—HA, tumor necrosis factor-α—TNF-α, magnetic resonance image (MRI)-detected infrapatellar synovitis, joint effusion) [16,23], demographic (age, sex, ethnicity, education level, vitality, smoking) [17,18,21], and psychosocial and clinical symptoms-related factors (knee pain intensity, baseline OA severity, pain catastrophizing, neuropathic pain symptoms, preoperative pain, other pain sites, central sensitization-associated symptoms, social support, Western Ontario and McMaster Universities Osteoarthritis Index—WOMAC score, and depression) [20], have renewed interest based on their role in the progression of knee OA. This section synthesizes the current evidence on these factors, highlighting consistent findings and gaps in prognostic reliability.

Several studies revealed the positive association between increased BMI and progression of knee OA [15,18,24–27]. A Mendelian Randomization strategy applied to data from the Open Genome-Wide Association Studies Project and FinnGen databases exposed that BMI significantly increases OA risk, emphasizing the importance of weight reduction, joint protection, and physical activity as preventive measures [28]. A study including data from a

healthcare dataset has demonstrated the correlation between BMI and increasing costs in patients with an OA diagnosis. In the same vein, the examination of 10,366 primary total hip arthroplasty patients demonstrated that increasing BMI is associated with increased index and 90-day costs for total hip arthroplasty [29]. A cross-sectional study including 952 knee OA outpatients found that BMI could seriously affect the health-related quality of life, emphasizing the importance of weight examination [30]. Using data from the National Health and Nutrition Examination Survey, Ren and collaborators [31] investigated the relationship between conicity index (C-index) and relative fat mass (RFM) and OA. The evaluation of these anthropometric measures of obesity in 34,707 subjects revealed a strong relation between C-index, RFM, and OA, which may be better indicators of obesity in assessing OA risk [31]. A cross-sectional analysis linked to the Deficit of Inhibition as a marker of Neuroplasticity (DEFINE) Study in Rehabilitation, including 113 patients with knee OA, demonstrated that a higher BMI impairs the restorative effects of endogenous pain control. Consequently, BMI could seriously diminish the positive associations between conditioned pain modulation and clinical outcomes, including depression, quality of life, and pain [32]. Similarly, a retrospective analysis of 1714 patients enrolled in the Osteoarthritis Healthy Weight for Life program found a significant dose-dependent effect between weight loss and reduced symptoms of hip OA [33].

The study revealed that serum hyaluronic acid and tumor necrosis factor-α are associated with knee OA progression [16]. A meta-analysis including 591 knee OA cases found that TNF-α G-308A polymorphisms are a susceptible genotype for OA in the Asian population [34]. Other analyses support the link between an increase in the concentration of hyaluronic acid in synovial fluid and the progression of knee OA [21,23]. In addition, factors including MRI-detected infrapatellar synovitis and joint effusion could place individuals with knee OA at greater risk of a poor functional outcome [15,35].

Critical analysis of the findings from eight prospective cohort studies, including patients with musculoskeletal knee pain, revealed that bilateral knee pain and a lower educational level were associated with persistent knee pain [17]. In addition, moderate evidence suggested that education level and vitality could lead to OA progression [15,35]. However, limited or conflicting evidence was observed regarding sex, former knee injury, quadriceps strength, smoking, running, and regular sports performance, and association with knee OA progression [16]. Another study conducted by Collins *et al*. [24] on a group of 1753 participants with symptomatic knee OA found that female sex was associated with trajectories of persistently severe pain, whereas male sex was associated with trajectories of less intense pain in symptomatic knee OA. Consistent with these results, a prospective cohort study determining prognostic patient factors for knee and hip arthroplasty revealed that the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) total sum score in females is associated with knee arthroplasty. However, when it comes to hip arthroplasty, an increased risk is associated with males having a higher WOMAC total sum score [18].

In addition to disease development, other systemic and psychological factors have been demonstrated to affect surgical outcomes in knee or hip OA. An umbrella analysis comprising 18 systematic reviews (nine pertaining to knee surgery, four concerning hip replacement, and seven addressing both hip and knee replacement) identified 20 predictive markers with high or moderate confidence. Factors such as pain catastrophizing, neuropathic pain symptoms, preoperative discomfort, additional pain locations, symptoms associated with central sensitization, social support, and depression were correlated with postoperative chronic pain. Thus, subsequent research may leverage these findings to examine the influence of these prognostic markers on treatment decisions [20].

To conclude, several studies, including large-scale genetic and epidemiological analyses, consistently confirm that BMI and obesity are strongly linked with the risk, progression, cost burden, and reduced quality of life in knee OA. Furthermore, data from OpenGWAS and FinnGen further reinforce the causal relationship, highlighting the relevance of anthropometric measures of obesity and weight management as key targets for OA prevention and care. However, certain studies reported inconsistent data for BMI and age and found no strong associations for sex, pain severity, or previous injury [21,36]. Strong evidence supports the role of biochemical factors such as hyaluronic acid, TNF-α, and MRI based on results like synovitis and joint effusion in predicting knee OA progression. Psychosocial and functional factors, including bilateral knee pain, WOMAC scores, and depression, also influence long-term outcomes. However, demographic and lifestyle-related predictors (e.g., sex, physical activity, prior injury) exhibited conflicting results in OA prediction. The consensus is that the current understanding, accuracy, and reliability of the prognostic factors and consultation data for knee pain are limited [17].

Intrinsic Prognostic Factors

Intrinsic factors comprise a set of structural, biomechanical, and functional characteristics of the joint, including cartilage degradation, bone marrow lesions, malalignment, joint space narrowing, and muscle strength [19]. In this section, we present the current evidence of the association between intrinsic prognostic factors and the development and progression of knee OA.

A study conducted by Bruyere *et al*. [37] showed a significant association between joint space narrowing of 0.5–0.8 mm over a period of 3 years and the progression of knee OA, leading to the need for surgical intervention. This

positive association between joint space narrowing, pain, joint stiffness, and knee OA progression is also supported by other findings [38–40].

A higher baseline range of motion of the knee is associated with a slower progression of knee OA [25,41]. In contrast, knee locking in flexion or painful knee flexion has been positively associated with a faster progression of knee OA, leading to the need for surgical intervention [27]. Steultjens *et al.* [42] showed that reduced muscle strength of the quadriceps was associated with the progression of knee OA. Recent studies regarding the association between changes in knee alignment and the development and progression of knee OA showed that the presence of varus alignment was significantly associated with increased development of medial knee OA [43–45].

Macri and colleagues [19] examined the correlation between baseline patellofemoral or tibiofemoral OA and the incidence of knee arthroplasty after a decade, as per the multicenter Cohort Hip and Cohort Knee (CHECK) study. The decade-long study indicated that the relative risk of getting arthroplasty was elevated when OA affected the patellofemoral joint [19]. While patellofemoral OA may serve as a clinically valuable prognostic marker in early knee OA, further research is required to elucidate the causative mechanisms underlying this association.

These findings support the notion that the risk of developing knee OA is higher in patients with valgus deformity of the knee. Thus, any malalignment of the knee beyond the normal range is significantly associated with the development and progression of gonarthrosis, especially in overweight or obese patients.

The presence of bone marrow lesions (BMLs) in the knee joint has been associated with significant progression of knee OA, with differences depending on the location of the BMLs. The presence of BMLs in the medial compartment, as well as in both compartments, was significantly associated with OA progression, compared to the presence of BMLs in the lateral compartment, where a less significant association was observed [46].

Studies using MRI detection demonstrated that the loss of articular cartilage was significantly associated with the OA progression to the point of requiring total knee arthroplasty [47–49]. A positive correlation was found between OA progression and the presence of hypertrophic synovium detected by MRI, particularly in the infrapatellar, intercondylar, and posterior edge of the knee [37,50].

Biomarkers in OA

Biochemical Biomarkers

For a systematic evaluation of OA-specific biomarkers, the Osteoarthritis Biomarkers Network, funded by the National Institutes of Health, classified biomarkers into five categories according to the Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic (BIPED) classification: Burden of disease, Investigative, Prognosis, Efficacy of intervention, and Diagnostic [51]. As mentioned above, the role of biomarkers is to identify patients with OA in its early stages so that the administered treatment can demonstrate its effectiveness [52–54]. Therefore, in this section, we aim to present insights from several studies [51,55–65] investigating various biomarkers present in urine or serum that may be specific for OA and can help initiate treatment in the early stages of the disease (Fig. 1).

Numerous studies indicate that the link between Col2-1 and its nitrated variant, Col2-1 NO2, with heightened disability in OA patients, suggests that urinary concentrations of Col2-1 and Col2-1 NO2 may serve as possible indicators of OA development [66]. A prospective study by Dore and collaborators [67] found that N-telopeptide of type I collagen (*NTX-I*), a biomarker derived from the N-telopeptide portion of collagen type I, was the only biomarker significantly increased in early OA, suggesting a complex relationship between cartilage degeneration and bone turnover. In addition, a recent meta-analysis exploring the diagnostic value of several OA biochemical markers found that NTX-I was the only biomarker differently expressed in patients with early OA versus controls. However, due to a low number of studies regarding any given biomarker, the diagnostic value of these biomarkers could not be determined, requiring extensive prospective studies [68].

In addition to these biomarkers, cartilage oligomeric matrix protein (*COMP*) and type II collagen cleavage product (*C2C*) have shown a correlation with the progression of knee OA in symptomatic patients. A study by Udomsinprasert and collaborators [55] found high *COMP* protein levels in 270 knee OA patients. They also found a correlation between serum *COMP* and radiological severity, measures of body composition, physical performance, knee pain, and disability. All these findings provide strong evidence regarding *COMP*'s potential role as a prognostic marker for knee OA [55]. Berry *et al.* [69] demonstrated in their study that the presence of *COMP* and procollagen type IIA N-terminal propeptide (PIIANP) was positively associated with reduced progression of knee OA, and the presence of C2C in serum was not significantly associated with OA progression. An analysis conducted by Catterall *et al.* [56] on 450 participants demonstrated a significant and specific association between native *COMP* and the severity of knee OA, whereas the deamidated form of *COMP* was found to be more specific for hip OA.

Several studies have demonstrated the association between C-terminal cross-linked telopeptide of type II collagen (CTX-II) levels and the imaging progression of knee OA [58,70,71]. Reijman *et al.* [57] analyzed the relationship between elevated urinary CTX-II levels and the radiographic progression and prevalence of knee OA, concluding that patients with CTX-II levels in the highest quartile had a sixfold higher risk of radiographic progression of knee

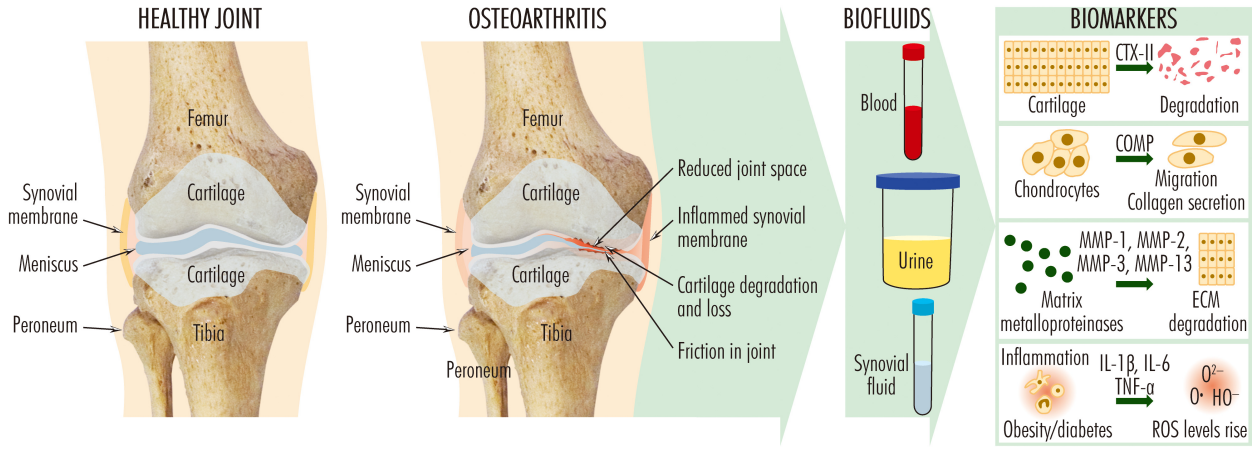

Fig. 1. Schematic representation of key biomarkers in body fluids of osteoarthritis (OA) patients. Left panel: joint degeneration in OA, including synovial membrane inflammation, cartilage degradation, and joint space narrowing, compared to a healthy joint. Central panel: primary biofluids—blood, urine, and synovial fluid—are usually chosen for biomarker detection. Right panel: key OA biomarkers: C-terminal cross-linked telopeptides of type II collagen (CTX-II) indicates cartilage degradation; Cartilage oligomeric matrix protein (COMP) is involved in chondrocyte activity and collagen secretion; Matrix metalloproteinases (MMPs) (e.g., MMP-1, -2, -3, -13) contribute to extracellular matrix degradation; Pro-inflammatory cytokines (interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α)) mediate inflammation and oxidative stress. These biomarkers collectively predict the biochemical changes underlying OA progression (created with Adobe Illustrator, version CS6, and Adobe Photoshop, version CS3, Adobe Systems Incorporated, San Jose, CA, USA).

OA. Similarly, Cheng *et al*. [58], in a study including 2856 participants, demonstrated a strong association between elevated CTX-II levels and the progression of knee OA. MRI can identify patients with knee OA even in its early stages, but when combined with elevated CTX-II levels, the identification of patients with rapid OA progression is 10 times more likely [59]. A study by Garnero *et al*. [51] evaluated biomarker levels related to articular cartilage and synovium between 67 patients with knee OA and 67 patients without knee OA. This cross-sectional study revealed that urinary CTX-II, urinary Glc-Gal-Pyd, and serum N-propeptide II of type II collagen (PIINP) levels were markedly elevated in individuals with knee OA. This indicates that these molecules may function as particular biomarkers for knee OA, as they correlate with joint surface area [51]. In 1255 knee OA patients, Bihlet and collaborators [70] confirmed that baseline u-CTX-II was associated with an elevated risk of radiographic progression in terms of joint space narrowing and predicted total joint replacement within the 2 years.

It has also been demonstrated that increased levels of matrix metalloproteinase (MMP)-1 and MMP-3 correlate with cartilage volume loss, as assessed by MRI over a two-year period [60]. Although inflammatory biomarkers such as C-reactive protein (CRP), TNF-alpha, interleukin-6 (IL-6), IL-5, and IL-10 have low specificity for OA, as they are present in many inflammatory conditions, their elevated levels are associated with cartilage volume loss [60,61,72]. A predominant involvement of one or both compartments of the knee joint has been observed depending on the increase in different inflammatory biomarkers. Specifically, increased levels of IL-6 and CRP were associated with greater involvement of the medial compartment, whereas higher levels of MMP-1 and MMP-3 were mainly associated with cartilage damage in the lateral compartment [60]. An increased expression of MMP-13 has been associated with the rapid progression of OA and with joint space narrowing in OA patients [73]. Stannus *et al*. [61] demonstrated that simultaneously elevated levels of IL-6 and TNF-alpha are associated with specific damage to the medial compartment. However, only changes in IL-6 levels were linked to bicompartmental knee joint involvement in OA [61]. During the Childhood Determinants of Adult Health (CDAH)-1 study, blood serum samples were tested using non-isotopic Enzyme-Linked Immunosorbent Assay (ELISA) and demonstrated the negative association of COMP, MMP-3, and hyaluronic acid biomarkers with knee cartilage thickness and volume in OA patients [74].

Genetic Biomarkers

Several studies investigated the role of genetic factors in knee OA and provided valuable evidence for the development and progression mechanisms. This section reviews several genetic biomarkers identified through genome-wide association studies and bioinformatic analyses, underscoring their applicability for diagnosis, prognosis, and treatment development.

Regarding the genetic variation of the Mothers Against Decapentaplegic Homolog 3 (*SMAD3*) gene, Valdes *et al*. [75] demonstrated in their study an association between this gene and knee and hip OA. The growth differentiation factor 5 (*GDF5*) *rs143383* single nucleotide polymorphism (SNP) has been associated with knee OA, partic-

ularly in the Caucasian population [76]. A study conducted by Attur *et al*. [77] with over 1000 patients showed a strong association between the interleukin 1 receptor antagonist (*IL1RN*) *TTG* haplotype and radiologically severe forms of knee OA. Hulin-Curtis *et al*. [78] demonstrated that the G allele of the C-C motif chemokine ligand 2 (*CCL2*) *rs2857657* variant predominantly influences knee OA in women.

Badshah and colleagues [79] conducted a genetic analysis utilizing amplification refractory mutation system (ARMS) PCR to investigate the correlation between IL-6 (IL-6-174G/C), transforming growth factor-β1 (TGF-beta1-29C/T), and calmodulin 1 gene-16C/T (*CALM1-16C/T*) polymorphisms and OA in a cohort of 295 patients. A favorable correlation was identified between the *GG* genotype of IL-6-174G/C and the *TT* genotype of *CALM1-16C/T* polymorphisms with OA, indicating its potential as a susceptibility marker [79].

Data analysis from genome-wide association studies found target genes associated with OA that have received treatment approval or are in clinical trials, including transforming growth factor beta 1 (*TGFB1*), *GDF5*, fibroblast growth factor 18 (*FGF18*), cathepsin K (*CTSK*), *IL11*, dipeptidase 1 (*DPEP1*), direct IAP-binding protein with low PI (*DIABLO*), corticotropin-releasing hormone receptor 1 (CRHR1), microtubule-associated protein tau (MAPT), and tumor necrosis factor (ligand) superfamily member 15 (*TNFSF15*) [80]. These genes encode proteins associated with several biological activities related to OA, including extracellular signaling (*GDF5* and *TGFB1*) and transcription factors critical for osteoblastic differentiation and skeletogenesis (runt-related transcription factor 2, *RUNX2*) [81]. A recent Russian study using OA samples from 1500 women employed several genotyping techniques, such as restriction fragment length polymorphism and TaqMan technology, to identify genetic markers associated with knee OA development. The examination of polymorphic variants in candidate genes (aggrecan, *ACAN*; a disintegrin and metalloproteinase with thrombospondin motifs 5, *ADAMTS5*; carbohydrate sulfotransferase 11, *CHST11*; SRY-box transcription factor 9, *SOX9*; collagen type I alpha 1 chain, *COL1A1*) and genes identified through genome-wide analysis (astrotactin 2, *ASTN2*; aldehyde dehydrogenase 1 family member A2, *ALDH1A2*; double von Willebrand factor A domains, *DVWA*; *CHST11*; G protein nucleolar 3, *GNL3*; nuclear receptor coactivator 3, *NCOA3*; filamin A interacting protein/sentrin-specific protease 1, *FILIP/SENP1*; MCF.2 cell line derived transforming sequence-like, *MCF2L*; glycosyltransferase 8 domain containing, *GLT8D*; disruptor of telomeric silencing like-1, *DOT1L*) indicated that the variable number of tandem repeats (VNTR) locus of *ACAN* and the *rs7639618* locus of *DVWA* are associated with an increased risk of knee OA [82].

Activating Protein-1 (*AP-1*) is primarily composed of proteins such as Jun proto-oncogene, AP-1 transcription factor subunit (*JUN*), JunD proto-oncogene, AP-1 transcription factor subunit (*JUND*), *Fos* proto-oncogene, *AP-1* transcription factor subunit (*FOS*), and *FOS* like 2, *AP-1* transcription factor subunit (*FOSL2*). It plays a crucial role in the inflammatory process by regulating the expression of the pro-inflammatory cytokine IL-1, which, in a cascade, leads to an increase in MMP-13 levels, responsible for the degradation of articular cartilage in knee OA. However, all these processes originate from the stimulation and activation of JUN/FOS heterodimers [83–85].

Other genetic biomarkers associated with knee OA include four genes: *MYC* proto-oncogene, BHLH transcription factor (*MYC*), *JUN*, dual specificity phosphatase 1 (*DUSP1*), and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (*NFKBIA*). These genes are involved in multiple signaling pathways of pro-inflammatory cytokines, such as TNF and IL-17, as well as in the hormonal response to steroids, glucocorticoids, and corticosteroids, as stated by Zhang *et al*. [86]. Therefore, these four genes may have a positive role in the development of potential therapies for knee OA [86].

Recently, several studies based on bioinformatic approaches aimed to explore the potential prognostic and diagnostic value of genetic biomarkers for OA, providing new research directions for personalized therapy. Wang and colleagues [87] utilized the Gene Expression Omnibus (GEO) library to uncover gene signatures for OA through the screening and functional annotation of the most prevalent differentially expressed genes (DEGs) in synovial membrane and blood samples. Following the identification of 379 DEGs, subsequent screenings revealed 22 DEGs of diagnostic significance for OA. Only nine DEGs, namely toll like receptor 7 (*TLR7*), receptor transporter protein 4 (*RTP4*), cysteine rich protein 1 (*CRIP1*), zinc finger protein 688 (*ZNF688*), DNA topoisomerase I (*TOP1*), eukaryotic translation initiation factor 1A, Y-linked (*EIF1AY*), member RAS oncogene family (*RAB2A*), zinc finger protein 281 (*ZNF281*), and ubiquitin interaction motif containing 1 (*UIMC1*), may serve as potential diagnostic biomarkers for OA [87]. Hu and collaborators [88] used the same GEO database to select gene expression profiles (*GSE55235*, *GSE55457*, *GSE77298*, and *GSE82107*), aiming to identify the key biomarkers and immune infiltration in OA by machine learning approaches. Notably, functional and genomic enrichment analysis and biological investigations were performed to discover and validate the important genes in cartilage cells. They found 105 DEGs from which transfer RNA selenocysteine/phosphoserine (*TCA1*), *TLR7*, *MMP-9*, C-X-C motif chemokine ligand 10 (*CXCL10*), C-X-C motif chemokine ligand 13 (*CXCL13*), major histocompatibility complex, class II, DR alpha (*HLA-DRA*), and adiponectin, C1Q and collagen domain containing (*ADIPOQSPP1*) could be used as OA biomarkers [88]. From

159 papers involving multi-omics data sets, Li and collaborators [89] classified 2405 OA-related marker genes and 5459 cell-type gene markers identified in single-cell RNA-seq studies. They integrated all these markers into an online database named OAomics, including 6765 OA marker genes and omics features, paving the way for future biological investigation of these biomarkers [89]. Another study performed a comprehensive bioinformatics analysis to identify and investigate OA-associated hub genes. The authors identified DEGs and performed functional enrichment analysis starting from two sample sets (*GSE46750*, *GSE98918*) retrieved from the GEO database, comprising OA synovial membrane and normal samples harvested from patients. They identified eight hub genes, including integrin subunit beta 2 (*ITGB2*), membrane spanning 4-domains A6A (*MS4A6A*), *HLA-DRA*, complement C1q B chain (*C1QB*), complement C1q C chain (*C1QC*), CD74 molecule (*CD74*), colony stimulating factor 1 receptor (*CSF1R*), and major histocompatibility complex, class II, DP alpha 1 (*HLA-DPA1*), and verified the gene expression levels in an OA rat model using RT-qPCR, observing the up-regulation among all of them. The results demonstrated that these hub genes could be used as OA biomarkers, discriminating between OA patients and controls [90]. Wang *et al*. [91] utilized the limma program to analyze several DEGs between normal healthy and OA cartilage tissue samples associated with diverse biological processes, including cytokine activity, receptor interactions, extracellular matrix binding, and immune receptor activation. The seven most predictive OA genes were identified, including anthrax toxin receptor 1 (*ANTXR1*), potassium voltage-gated channel modifier subfamily S member 3 (*KCNS3*), sarcoglycan delta (*SGCD*), and *Lin-7* homolog A, crumbs cell polarity complex component (*LIN7A*), which exhibited correlations with immune cell infiltration analysis results, offering significant insights for the early diagnosis and treatment of OA [91].

Notably, multiple studies published this year used various bioinformatic tools and machine learning algorithms (e.g., Limma package; weighted gene co-expression network analysis, WGCNA; protein-protein interaction, PPI; random forest; support vector machine; generalized linear model; extreme gradient boosting; least absolute shrinkage and selection operator; logistic regression and support vector machine-recursive feature elimination) aiming to identify genetic biomarkers associated with OA development (Fig. 2).

These studies found several OA biomarkers with promising prognostic and diagnostic value, including T-cell surface glycoprotein (*CD4*), *CSF1R*, and transmembrane immune signaling adaptor (*TYROBP*), upregulated in OA samples [92], age-related biomarkers (CCAAT enhancer binding protein beta, *CEBPB*; phosphatase and tensin homolog, *PTEN*; actin related protein 2/3 complex subunit 1B, *ARPC1B*; phosphoinositide-3-kinase regulatory subunit 1, *PIK3R1*; cell division cycle 42, *CDC42*) [93], integrin subunit beta 5 (*ITGB5*), elevated in OA cartilage and synovial samples [94], B-cell CLL/lymphoma 3 (*BCL3*) [95], ubiquitination-related genes (WD repeat domain 74, *WDR74*; tumor necrosis factor receptor superfamily member 12A, *TNFRSF12A*) [96], and *SEH1L* and *BIRC2* [97].

Epigenetic Biomarkers

Although epigenetic studies are not yet as extensive as genetic studies, there are still a number of studies that report an association between different types of microRNAs and OA. DNA methylation patterns and histone modifications represent other epigenetic modifications associated with OA. This section reviews some of the most relevant miRNAs, methylation patterns, and histone modifications in OA.

For example, Iliopoulos *et al*. [98] identified 16 such types of microRNAs, while Díaz-Prado *et al*. [99] identified 7 types of microRNAs. All of these offer potential alternative strategies for diagnosis and treatment [98,99].

Elevated levels of miR-497 were observed in the blood of women with hip OA [100], while elevated concentrations of plasmatic miR-146a and miR-365 were recorded in patients with knee OA [101]. Simultaneously, miRNA let-7e has been documented to be markedly diminished in knee OA, influencing the apoptosis and autophagy of articular chondrocytes [102]. OA progression was forecasted by many miRNAs, including plasma miR-140-3p, miR-1307-5p, miR-181a-3p, miR-221-5p, miR-4326, miR-443, and miR-99a-5p, derived from hip and knee OA patients in the Research Arthritis and Articular Cartilage and Genetics Osteoarthritis and Progression study cohorts [103]. Plasmatic miR-320 family predicted radiographic knee OA progress over 4 years [104], while miR-146a-5p, miR-145-5p, and miR-130b-3p predicted postoperative pain relief [105].

Other miRNA studies found miR-584-5p, miR-183-5p, and miR-4435 in serum [106], while miR-206 was identified in peripheral blood mononuclear cells [107]. Recently, a research group evaluated the role of 768 circulating miRNAs in 30 sera retrieved from 10 patients with erosive hand OA, 10 patients with non-erosive OA, and controls without hand OA. Subsequently, after the screening phase, they validated the identified miRNAs in larger samples. Firstly, the authors identified nine micro-RNAs (miR-373-3p, miR-558, miR-607, miR-653-5p, miR-137 and miR-448, miR-142-3p, miR-144-3p and miR-34a-5p). However, only miR-196-5p was significantly down-regulated between erosive and non-erosive hand OA in the screening and validation phases, suggesting that it could play a role in OA [108].

Recently, another study aimed to identify sensitive, noninvasive early OA biomarkers in donkeys with chemical-induced OA. The analysis of serum and synovial samples collected at different intervals revealed that miR-146b and miR-27b were upregulated as OA progressed,

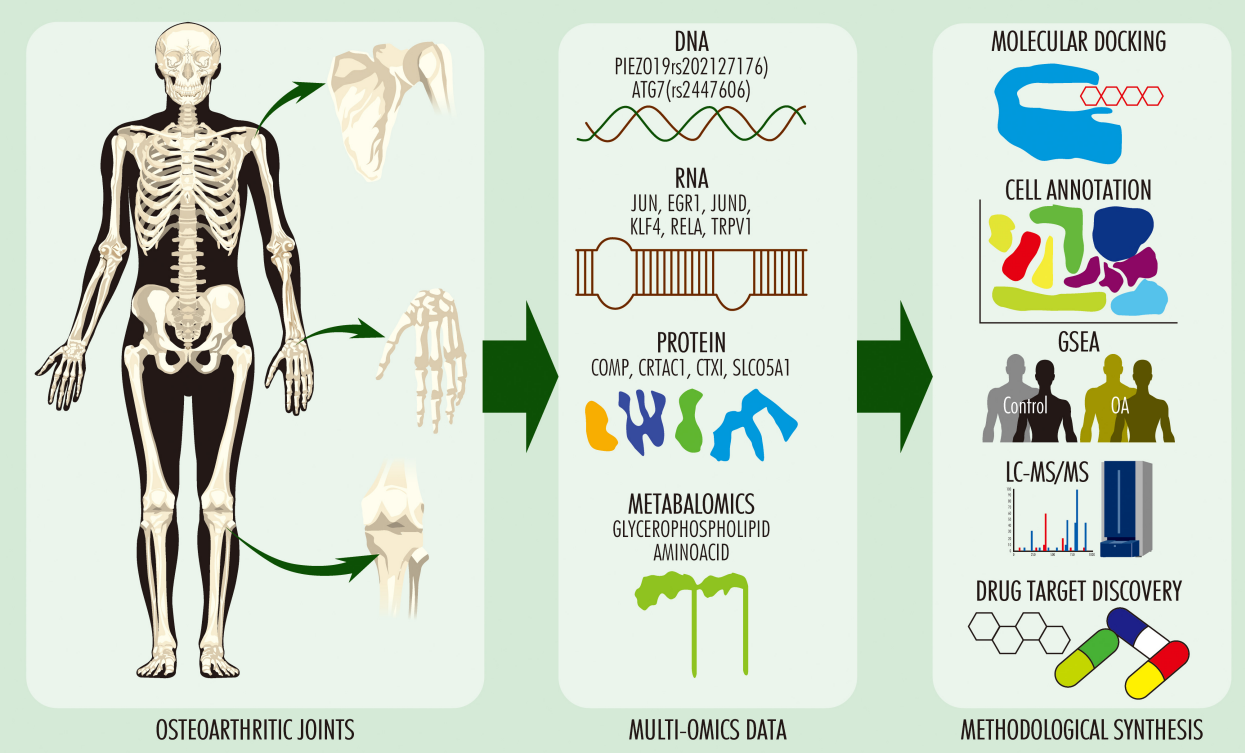

Fig. 2. The application of bioinformatic tools for methodological identification and validation of OA biomarkers. Left panel: anatomical regions commonly impacted by OA, including the knee, hip, spine, and hand. Central panel: multi-omics data used in OA investigations, including genomics (e.g., piezo type mechanosensitive ion channel component 1, *PIEZO1*; autophagy-related 7, *ATG7*), transcriptomics (e.g., Jun proto-oncogene, AP-1 transcription factor subunit (*JUN*); early growth response 1, *EGR1*; transient receptor potential cation channel subfamily V member 1, *TRPV1*), proteomics (e.g., COMP; cartilage acidic protein 1, *CRTAC1*; *CTXI*), and metabolomics (e.g., glycerophospholipid and amino acid pathways). Right panel: commonly used methods for analyzing and synthesizing multi-omics datasets, including cell type annotation via single-cell sequencing, molecular docking, gene set enrichment analysis (GSEA), mass spectrometry (LC-MS/MS), and drug target discovery (created with Adobe Illustrator, version CS6, and Adobe Photoshop, version CS3, Adobe Systems Incorporated, San Jose, CA, USA).

suggesting that they could be candidates for noninvasive biomarkers for the early diagnosis of OA [109]. Studies of articular cartilage and subchondral bone found long noncoding RNA genes, including ILF3 divergent transcript (*ILF3-DT*) (lncRNA), metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*, lncRNA), and *SPRY4* Antisense RNA 1 (*SPRY4-AS1*, lncRNA), as potential OA risk genes [110,111].

DNA methylation patterns and histone modifications represent other epigenetic modifications associated with OA. DNA methylation involves adding methyl groups to the DNA molecule, catalyzed by a family of DNA methyltransferases (DNMTs), mainly DNMT1, DNMT3A, and DNMT3B [112]. These DNMTs transfer a methyl group from S-adenyl methionine to the fifth carbon of a cytosine residue to form 5 mC. DNA demethylation is catalyzed by translocation enzymes 1, 2, and 3 (*TET1, TET2*, *TET3*), which form 5-hydroxymethylcytosine (5-hmC) [113]. Methylation processes play an important role in OA pathogenesis. The overexpression of the fat mass and obesity-associated demethylase gene (*FTO*) positively affected knee OA by directly modulating miR-515-5p [114]. DNA methylation profiling was performed to establish an association between CpG methylation and human chondrocyte age. Chondrocytes' exposure to signal transducer and activator of transcription 3 (*STAT3*) agonist decreased DNA methylation, while conditional deletion of Stat3 in cartilage cells increased DNMT3B expression in articular chondrocytes in the knee joint *in vivo*, leading to a more prominent OA progression in a post-traumatic OA mouse model [115]. Various studies found that several DNMT polymorphisms are linked to knee OA [113,116,117]. While the CT haplotype of DNMT1 polymorphisms was associated with a lower risk of OA, the CC genotype of *rs2424913* of DNMT3b was associated with an increased risk (Miranda-Duarte *et al.* [117], 2020). DMNT1 demethylation could elevate the expression of pro-inflammatory or matrix carbolic proteins, including prostaglandin-endoperoxide synthase 2 (*PTGS2*), *MMP-9*, and *MMP-13* [113]. In addition, DNMT3b overexpression could decrease the onset of OA caused by trauma or chemicals [113,116].

Histone alterations, such as methylation, phosphorylation, acetylation, and ubiquitination at lysine residues within histone cores, represent key epigenetic alterations that profoundly influence gene expression by regulating the accessibility of transcription factors to DNA regions [118]. While histone post-translational modifications pertinent to OA have not been thoroughly characterized, there are instances suggesting their potential role as epigenetic regulators in the disease. Among histone methyltransferases, the enhancer of zeste homolog 2 (EZH2) has recently gained significance in the field of OA. Growing data indicate that EZH2 is a pivotal mediator of cartilage degradation, as it is significantly increased in OA. Another histone methyltransferase (HMT) with significant promise is the disruptor of telomeric silencing like-1 (DOT1L), which serves a crucial function in safeguarding cartilage from OA [119]. Histone acetylation occurs on lysine residues situated within the N-terminal tails of histones, modifying their positive charge. A recent study investigated the correlation of H3K27me3 lysine demethylase 6A (*KDM6A*) demethylase with knee OA and revealed that its elevated expression UTX aggravated the signs of OA, including articular cartilage damage, osteophyte formation, synovitis, and subchondral bone loss in mice [120]. Despite the inadequate characterization of histone acetyltransferases (HATs) in OA, certain instances may serve as prospective therapeutic targets. P300/CBP-associated factor (PCAF) is a HAT that, in conjunction with H3K9ac, is increased in osteoarthritic cartilage and in chondrocytes triggered by TNF-α. PCAF may serve as a crucial regulator of the inflammatory response in cartilage owing to its involvement in the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway [121].

Although numerous miRNAs have been associated with various OA phenotypes across joint sites, future studies are needed to validate the diagnostic value, facing challenges regarding sample size and site choice, lack of reproducibility, and clinical trial assessment.

Therapeutic Role of Biomarkers for OA Treatment

The therapeutic implications of biomarkers are represented by their ability to represent not only an indicator of disease progression but also to become potential therapeutic targets. In this section, we discuss recent studies aimed at identifying potential biomarkers and molecules that could serve as therapeutic strategies for OA treatment.

Due to their modulation of a myriad of OA-relevant signaling pathways, various inflammatory mediators, including IL-1β, TNF-α, and IL-6, have become attractive biomarkers for OA diagnostic and therapeutic intervention efficacy tracking purposes [1]. Inflammatory cytokines such as IL-1β and TNF-α, which play a role in the mechanism of articular cartilage degradation, have been shown in preclinical studies to reduce inflammation and slow disease progression in patients with OA when used with specific inhibitors. The use of non-steroidal anti-inflammatory drugs decreases the level of C-reactive protein, which is correlated with the severity of OA. Preclinical studies have demonstrated that intra-articular administration of IL1 receptor antagonists (IL1Ra) is effective and safe in horses, dogs, mice and rabbits with OA [122–124]. The use of FGF18 as a potential anabolic agent has shown promising results in a randomized clinical trial. The use of Sprifermin has shown an increase in articular cartilage thickness and an improvement in WOMAC score [125]. Disease-modifying osteoarthritis drugs (DMOADs) are still under investigation in terms of their efficacy and safety. However, to date, no DMOAD has been approved for human use [126]. There are several new therapies still in the investigational phase that have received approval for use in humans, like TPX-100, AKL4/APPA (Oral NFkB and nuclear factor erythroid 2–related factor 2 modulator (Nrf2)), Lorecivivint (Wnt pathway inhibitor), KA34/Kartogenin, UBX0101 (p53/ mouse double minute 2 homolog (MDM2) inhibitor), GLPG1972 and M6495 (a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) inhibitor), MIV-711 (Cathepsin K inhibitors), Invossa-K (Transforming Growth Factor-β) [127].

Recently, a study using the GEO database for bioinformatics analysis combined with Mendelian randomization identified two core genes, ADP ribosylation factor like GTPase 4C (*ARL4C*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Their expression levels were found to decrease in OA pathology and exhibited a protective effect, suggesting that these genes may serve as therapeutic targets, offering promise for personalized treatment of OA [128]. A systematic review highlighting the advances in OA treatment outcomes exposed the potential role of MMP-13 in OA treatment, being an attractive target for inhibitor development. This conclusion is based on its role in digesting type II collagen and the proteoglycan molecule aggrecan, giving it a dual role in matrix destruction [129]. Tuerlings and collaborators [130] performed RNA sequencing of subchondral bone in OA and conducted unsupervised hierarchical clustering and differential expression analyses. They found that IL11 and chondroadherin like (*CHADL*) genes were among the most consistently differentially expressed OA pathophysiology-related genes, classifying them as strong potential therapeutic targets for OA [130].

Studies across DNMT3B roles demonstrated its elevated expression in healthy human cartilage and decreased expression in OA human chondrocytes and OA mouse models [131,132]. Even though Dnmt3b knockdown leads to early onset and progression of OA, functionalized Dnmt3b could exert a chondroprotective effect [131]. In addition, several downstream targets of Dnmt3b, including 4-aminobutyrate aminotransferase, could be a suitable therapeutic target since its inhibition *in vivo* at-

tenuates murine OA progression [132]. Recently, two related studies demonstrated that the knockdown of specific demethylases could represent a suitable therapeutic strategy for OA treatment [133,134]. Firstly, Assi and collaborators [134] found that inhibiting the Jumonji-C demethylase family reduced H3K79me. In addition, the authors injected daminozide in mice, a KDM2/7 subfamily inhibitor, leading to blocked KDM2/7 histone demethylase activity, resulting in high H3K79me and glycosaminoglycan levels. Upon KDM7A/B demethylase knockdown by daminozide, the results showed that these demethylases could protect from daminozide on H3K79me and thus OA. Therefore, blockage of KDM7A/B histone demethylases could be a promising strategy for OA treatment [134]. Similarly, the inhibition of histone demethylase lysine demethylase 6B (*KDM6B*) could impair force-induced injury to chondrocytes by promoting H3K27me3 expression. These results were validated in a mouse model of injury-induced OA, where Jmjd3 inhibition significantly mitigated OA, classifying this strategy as an epigenetic approach to treating OA [133].

Discussion

Regarding prognostic factors, this review of the literature identified factors associated with both the clinical and imaging progression of knee, hip, or hand OA. However, for a more accurate assessment of gonarthrosis progression, many more studies are still needed. Prognostic factors for evaluating the symptomatic progression of gonarthrosis have been analyzed in only a few specialized studies.

Several studies revealed the relationship between increased BMI and hyaluronic acid levels and knee OA progression [23–27], while findings on age remain inconsistent. Factors like varus alignment, synovitis, and baseline OA severity displayed good prognostic value [15,21]. Conflicting or limited evidence can be depicted from studies investigating predictors, including sex, pain, and activity level. Higher BMI and WOMAC scores predict knee arthroplasty in women, while older men are at greater risk for hip arthroplasty [18,24]. Overall, prognostic evidence remains limited and inconclusive.

A strong relationship was found between intrinsic factors, including joint space narrowing [37,38,40], malalignment [43,44], cartilage loss, bone marrow lesions, muscle weakness [19], and knee OA development and progression. In time, joint space narrowing could lead to increased pain, stiffness, and a high risk of surgical intervention. Knee malalignment and quadriceps weakness contribute to OA progression, while patellofemoral OA increases the long-term risk of arthroplasty [20].

Even though various biochemical biomarkers such as CTX-II [58,59,70,71], COMP [55], Col2-1 [66], NTX-I [67,68], and PIINP [51] have been evaluated for the early detection and progression monitoring of knee OA, their specificity and predictive value vary across studies. Although inflammatory biomarkers like CRP and IL-6 are linked to cartilage loss [60,61,72–74], their specificity is poor due to their elevation in other inflammatory conditions. The Burden of Disease, Investigative, Prognostic, Efficacy of Intervention and Diagnostic (BIPED) classification provides a helpful framework [51], but inconsistent findings and a lack of standardization across studies still limit the clinical utility of many biomarkers.

Multiple studies revealed the associations between OA and various polymorphisms in genes such as *GDF5* [76], *SMAD*3 [75], *IL1RN* [77], IL-6 and *CALM1* [79], *CCL2* [78], *TGFB1* and *FGF18* [80], and *RUNX2* [81] in OA. Recent bioinformatic and machine learning-driven analyses identified several DEGs and hub genes with diagnostic and prognostic potential, such as *CSF1R*, *TLR7*, *MMP9*, *HLA-DRA*, *CD4* [92], *ITGB5* [94], and *BCL3* [95], paving the way for future OA personalized therapies.

Promising findings highlight the miRNAs as potential biomarkers for OA diagnosis and treatment [103,105,108,111], contributing towards the future early-stage diagnosis of this disease. However, factors like low or different sample sizes could lead to a lack of reproducibility in microRNAs across studies.

The key to identifying patients with knee OA is the combination of multiple prognostic factors. Once patients with such prognostic factors are identified, they should be carefully monitored, as they could serve as a starting point for testing new disease-modifying antirheumatic drugs (DMARDs). In the study conducted by Lapane *et al*. [135] regarding the administration of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with radiologically confirmed knee OA, it was found that long-term NSAID use was associated with a moderate effect on structural progression, joint stiffness, and other symptoms of knee OA.

Our endeavor to better understand disease mechanisms and medications has helped us realize that variations in disease expression and response to therapy are caused by a variety of underlying pathological mechanisms, as well as other individual patient characteristics that influence treatment efficacy and tolerability. Personalized care is the best option for OA due to its diverse characteristics, the large number of affected patients, and the demand for new, effective, and safe treatments. The ultimate goal of the meeting among ESCEO working group members was to determine the value of biomarkers from multiple sources to describe different phenotypes of OA patients. Clinical decision-making could be based on the characterization of OA patient phenotypes. The attempt to select patients for whom a disease-modifying OA drug (DMOAD) intervention could prevent the development of OA would help create medications tailored to each patient.

This review possesses multiple limitations that warrant acknowledgment. Despite including recent and perti-

nent studies, the variability in study designs and diagnostic criteria may influence data comparability. The review predominantly concentrated on biomarkers validated in knee and hip OA, perhaps restricting their applicability to other joints. Moreover, publication bias and insufficient long-term follow-up in certain studies may have affected the reported correlations.

Conclusions

Pain levels, functional limitations, and the presence of additional chronic conditions, such as frailty, can be utilized to create patient profiles. Furthermore, accumulating evidence endorses the use of MRI for diagnostic purposes in clinical settings, enabling physicians to discern patients who are more likely to derive benefit from treatment. Further research is necessary to validate these preliminary findings and to determine the association between the improvements and robust clinical outcomes. The advancement of high-resolution imaging techniques, including MRI, and their growing availability are facilitating the identification of correlations between genetic alterations and the intricate intra-articular changes associated with knee OA. Numerous systemic and intrinsic prognostic factors, such as BMI, joint space narrowing, and malalignment, are associated with the progression of knee OA; however, evidence for additional factors is inconsistent. Emerging biomarkers and targeted therapies, including cytokine inhibitors, growth factors, and epigenetic modulators, show promising results in improving the diagnosis and treatment of knee and hip OA. However, future research on biomarkers for the diagnosis and prediction of development in OA must address several limitations, including insufficient specificity, inadequate standardization for clinical application, and issues of reliability.

Availability of Data and Materials

Not applicable.

Author Contributions

Conceptualization, FCC, SAI, and AC; Methodology and data interpretation, SAI, AC, BS, IAE, COV, MC, SS, and FCC; Writing—original draft preparation, SAI, AC, COV, and BS; Writing—review and editing, SAI, AC, BS, IAE, COV, MC, SS, and FCC; Funding acquisition, COV; Figure design, MC. All authors contributed significantly to the interpretation of data and to editorial changes of important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of it, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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