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INTEGRATIVE ANALYSIS OF MICROBIAL, GENOMIC, AND BIOCHEMICAL BIOMARKERS FOR EARLY DETECTION AND FORENSIC CHARACTERIZATION OF CHRONIC INFLAMMATORY JOINT AND SPINE DISORDERS

Bhriganka Bharadwaj[1], Dr. Dhanya Sudheesh[2], Dr. Sukanta Bandyopadhyay[3], Dr Bright Olickal Philip[4], T.selvamohan[5], Dr. Mukta Sharma[6], Dr. Sangita Boro[7]

[1]Assistant Professor, Department of Microbiology, Specialization in Fermented Beverages, Fermented food, Medical Microbiology, Medicinal Plants, Assam down town University, Guwahati, Assam
Pin – 781026, India, E-mail id: bhriganka@adtu.in, Orcid id: 0000-0002-9402-1536
[2]Reader, Department of Biochemistry, Specialization in Medical Biochemistry, KUHS
E-mail id:sudhidhanya09@gmail.com Orcid Id: 0009-0002-9990-0617
[3]Professor, Department of Biochemistry, Rama Medical College Hospital & Research Centre, Kanpur, India.
E-mail id: sukantoaxum@gmail.com, Orcid Id: 0009-0002-3664-0475
[4]Associate Professor, Department of Chemistry, K J Somaiya College of Science and Commerce, Vidyavihar, Mumbai, Pincode-400077, Maharashtra, India, E-mail Id- bright@somaiya.edu , Orcid Id- 0009-0005-8021-3448
[5]Assistant Professor, Department of Zoology, Specialization in Enzymology, Rani Anna government college
E-mail Id - tsmohan71@gmail.com, Orcid Id - 0009-0000-1605-137X
[6]Professor and Director Research, Department of Microbiology, Specialization in Medical Microbiology and Food Microbiology, School of Sciences, IIMT University, Meerut, Pincode- 250001, E-mail Id- muktavats@yahoo.com , Orcid Id- 0000-0001-6784-3932
[7] Assistant Professor, Department of Microbiology, Specialization in Fermented Beverage, Assam down town University Pin code: 781026, Guwahati, India, Orcid Id- 0000-0003-0772-8451, E-mail Id : sangita.boro@adtu.in

ABSTRACT

The aetiologies of chronic inflammatory joint and spine disorders are complex, involving interaction between immune dysregulation, imbalance in microbiomes, genetic susceptibility, epigenetic regulation, biochemical disruption and tissue degeneration. This comprehensive review synthesizes current evidence on microbial, genomic, and biochemical biomarkers for early detection, disease stratification, and forensic characterization of these disorders. Microbial biomarkers, including gut dysbiosis, bacterial DNA, and bacterial–fungal networks, provide insight into host–microbiome interactions and their contribution to systemic inflammation. Genomic and epigenetic biomarkers clarify inherited risk, immune regulation, cellular heterogeneity, and disease susceptibility. Biochemical biomarkers, including cytokines, chemokines, proteomic signatures, metabolites, and synovial fluid markers, reflect active inflammation, tissue injury, and therapeutic response. The review further highlights the value of multi-omics integration, artificial intelligence, network biology, imaging biomarkers, and clinical data systems in developing more precise diagnostic and prognostic models. Forensic applications are also considered, particularly the use of integrated molecular signatures for biological profiling, disease reconstruction, and pathological characterization. Although a wide range of clinical applications have been proposed, their implementation is limited by the need for validation of these biomarkers, heterogeneity of data, ethical issues, and the requirement for standardizing analytical workflows. Precise diagnostics and forensic interpretation in chronic inflammatory joint and spine diseases may be feasible by integrated biomarker strategies.

KEYWORDS: Microbial biomarkers, Genomic biomarkers, Biochemical biomarkers, multi-omics integration, Chronic inflammatory spine disorders

1. INTRODUCTION

Osteoarthritis and rheumatoid arthritis (RA) are among the most important chronic inflammatory disorders of the joints and spine that cause long-term disability, physical impairment and health burden globally. These conditions include a wide range of musculoskeletal conditions in which inflammation will persist, the tissue will begin to break down, pain will be chronic, and the function of the involved tissues will be impaired to a degree significantly impacting the patient's quality of life. The impact of musculoskeletal pain from these disorders is not only physical but also psychological, social and economic, with a need for better diagnostic and therapeutic approaches [1]. Joint diseases have always been known as a complex disease, with both structural, inflammatory and degenerative changes in the joints that may, in some stages of the disease, prove irreparable if diagnosis and intervention have been delayed [2]. Chronic inflammatory conditions of the joints and spine are complex interactions between immune-mediated inflammatory mechanisms, tissue remodeling, genetic susceptibility and environmental factors. Many inflammatory and degenerative processes occur within the spine and often simultaneously, leading to progressive anatomical change and problems in the assessment of the disease. This is why inflammatory and degenerative diseases are often difficult to differentiate, especially in the early stages of disease development [3]. Likewise, inflammatory arthritis of the axial skeleton has significant clinical heterogeneity, and

differences in onset, progression, and severity of the disease can delay diagnosis and therapeutic interventions in early stages [4].

The biological changes that precede clinical signs and symptoms of musculoskeletal conditions are of increasing importance in the context of recent advances in musculoskeletal diagnostics. The clinical examination, radiology, and laboratory tests are used to diagnose the disease and are standard, but may be insufficiently sensitive to detect early disease. New technologies in the diagnosis are promising to increase the accuracy of diagnosis and aid in earlier detection of disease, with advanced imaging modalities, molecular biomarkers and comprehensive clinical assessment frameworks demonstrating significant potential [5]. This has caused growing interest in the use of biomarkers, which can give objective and reproducible measurements of disease activity and disease course. Biomarkers play a key role in precision medicine as "signatures" that contain information that is measurable regarding molecular, physiological and pathological processes. These uses are not limited to diagnosis, but are also used for disease stratification, prognostic evaluation, disease monitoring, and personalized treatment planning. The use of biomarkers in precision healthcare has also been growing with the development of biosensor technologies and molecular profiling technologies, which enable the further detailed characterization of disease-specific biological signatures [6]. As a parallel development, computational methods have also been used to drive biomarker discovery, taking advantage of the growing number of biological data and uncovering complex molecular patterns not analyzable by traditional methods [7]. These innovations are very similar to the overall vision of precision medicine: to make the best healthcare decisions by integrating individual biological variability and disease-specific molecular information [8].

Although there have been significant advances in the research of biomarkers, most of the current studies have focused on the individual assessment of microbial, genomic, and biochemical biomarkers. This specialized approach to research hampers knowledge of the complex biological processes that contribute to the initiation, progression and clinical heterogeneity of disease. In addition, the intersection of these categories of biomarkers for forensic characterization has received little attention, although there is increasing evidence that multi-dimensional molecular signatures may help to provide insight into disease history, biological profiling, and retrospective assessment. In recent years, the integration of multi-omics has been shown to be useful for producing comprehensive, very informative molecular signatures, useful for both clinical and forensic applications [9].

Hence, the purpose of this review is to critically synthesize the existing knowledge on microbial, genomic and biochemical biomarkers associated with chronic inflammatory joint and spine disorders. Special focus is dedicated to their integrative use for early disease detection, precision diagnostic development and forensic characterization, as well as exploring how multi-omics approaches can be used to improve future disease stratification, biomarker validation and translation.

2. REVIEW METHODOLOGY

This is a comprehensive review of published literature, which has resulted in this extensive review of microbial, genomic, epigenetic, biochemical and multi-omics biomarkers of chronic inflammatory joint and spine conditions. Relevance to disease pathobiology, biomarker discovery, early diagnosis, clinical translation and forensic characterization were criteria used to guide the selection of studies. The articles were prioritized based on peer-reviewed articles, recent reviews, genomic and microbiome studies, proteomic and metabolomic studies, and on multi-omics integration or on forensic biomarker studies. The literature collected was compiled and critically summarized, which helped to identify the essential biological mechanisms, classes of biomarkers, translation issues and future research directions. Evidence was organised by theme for a comprehensive review framework linking mechanisms of disease to the clinical and forensic potential of biomarkers.

3. Pathobiology of Chronic Inflammatory Joint and Spine Disorders: Foundations for Biomarker Discovery

Chronic inflammatory joint and spinal disorders occur due to a complicated balance between the elements of immune dysregulation, genetics, microbes and tissue degeneration. The disorders are defined by chronic inflammatory reactions that result in loss of tissue homeostasis and trigger molecular pathways that result in cartilage destruction, bone erosion and structural remodeling. In RA and other inflammatory forms of arthritis, activated immune cells invade synovial tissues and secrete pro-inflammatory factors that perpetuate the chronic inflammation and drive joint damage [10]. Many joint conditions exhibit the same immunopathological patterns: An inappropriate immune reaction that causes chronic damage to the tissues and leads to functional impairment [11]. Diseases commence and develop with the help of immune-mediated inflammation. The dysregulated interactions between innate and adaptive immune cell components lead to increased production of cytokines, chemokines and inflammatory signaling molecules that lead to local and systemic inflammatory responses. Constant stimulation of these pathways breaks down the normal immune tolerance, leading to increased tissue-destructive pathways and ongoing disease activity and structural damage [12]. These inflammatory networks are an important biological basis for the discovery of molecular markers that could reflect the onset, severity and progression of disease.

There is increasing evidence that host–microbiome interactions play a role in the pathogenesis of chronic inflammatory musculoskeletal disorders. Changes in gut microbiota composition can affect the systemic immune response in a variety of ways, including through microbial metabolites, immune signaling pathways, and gut barriers. According to the new idea of the “gut–spine axis,” gut dysbiosis can lead to the development of spinal degeneration through triggering of chronic

inflammatory conditions and metabolic disorders that impact the tissues within the vertebral and intervertebral discs [13]. Likewise, the gut–joint axis has been reported to be important in regulating the development of other diseases, such as osteoarthritis, rheumatoid arthritis and spondyloarthropathies, suggesting the importance of microbial signatures as potential markers for detecting and stratifying disease [14]. Genetic predisposition has a further influence on genetic susceptibility and the course of disease. Inherited genetic variations have been linked to susceptibility to spinal deformities, intervertebral disc degeneration and inflammatory musculoskeletal diseases, suggesting that biological heterogeneity and disease risk in these diseases are related to genetic susceptibility [15]. There is evidence that genetic susceptibility is involved in the pathogenesis of ankylosing spondylitis, because there is an abnormality of inflammatory responses and pathological bone formation [16]. The results confirm the emerging trend of using genomic markers to identify individuals who are at risk and to predict disease courses.

Cytological alterations linked to ageing have also been linked to the onset of chronic musculoskeletal diseases. Due to the ageing process, the joints and spine start to accumulate cells and produce chemicals which cause inflammation, leading to further tissue breakdown and healing capacity loss. This SASP drives the progression of the disease and can also cause structural damage by promoting a pro-inflammatory environment [17]. At the same time, proteomic studies have identified significant changes in protein levels in extracellular matrix remodeling, inflammatory and metabolic proteins in degenerative spinal tissues, offering valuable insights into molecular signature proteins that are specific to disease [18]. One of the other mechanisms that is relevant is oxidative stress, which has been shown to be a connection between inflammation and tissue damage. Reactive oxygen species (ROS) cause abnormal function in the cellular structure, degradation of the extracellular matrix, damage to mitochondria and release of inflammatory signals from intervertebral disc tissues when levels are high. These molecular changes accelerate the disease process and produce measurable biochemical changes that could be used as potential biomarkers for therapeutic studies and disease monitoring [19].
Combining these pieces of evidence suggests that chronic inflammatory joint and spine diseases are the result of a complex interplay among immunity, microbiology, genetics, senescence, proteomics and oxidative stress. To discover reliable biomarkers that truly capture disease pathogenesis and can facilitate early diagnosis, it is crucial to understand these mechanisms. Combining molecular data from these domains of pathogenicity provides a solid basis for creating multi-omics-based biomarker strategies that will enhance disease characterization and precision diagnostics. Table 1 highlights the key pathobiological mechanisms that underpin the biological basis for the discovery of biomarkers in chronic inflammatory joint and spine diseases.

Table 1. Pathobiological Mechanisms Underlying Biomarker Discovery in Chronic Inflammatory Joint and Spine Disorders

Pathobiological domain	Main biological process	Relevant disorder context	Biomarker implication
Immune dysregulation	Cytokine release, immune-cell activation, chronic inflammation	Rheumatoid arthritis, axial spondyloarthritis	Indicates inflammatory activity and disease progression
Host–microbiome interaction	Gut–joint and gut–spine immune signaling	Spondyloarthritis, intervertebral disc degeneration	Supports microbial biomarker discovery
Genetic susceptibility	HLA and non-HLA genetic variation	Ankylosing spondylitis, spinal degeneration	Enables risk prediction and disease stratification
Cellular senescence	Senescence-associated inflammatory signaling	Osteoarthritis, degenerative spinal disorders	Reflects age-related tissue damage
Oxidative stress	Reactive oxygen species, mitochondrial dysfunction	Intervertebral disc degeneration	Indicates biochemical tissue injury

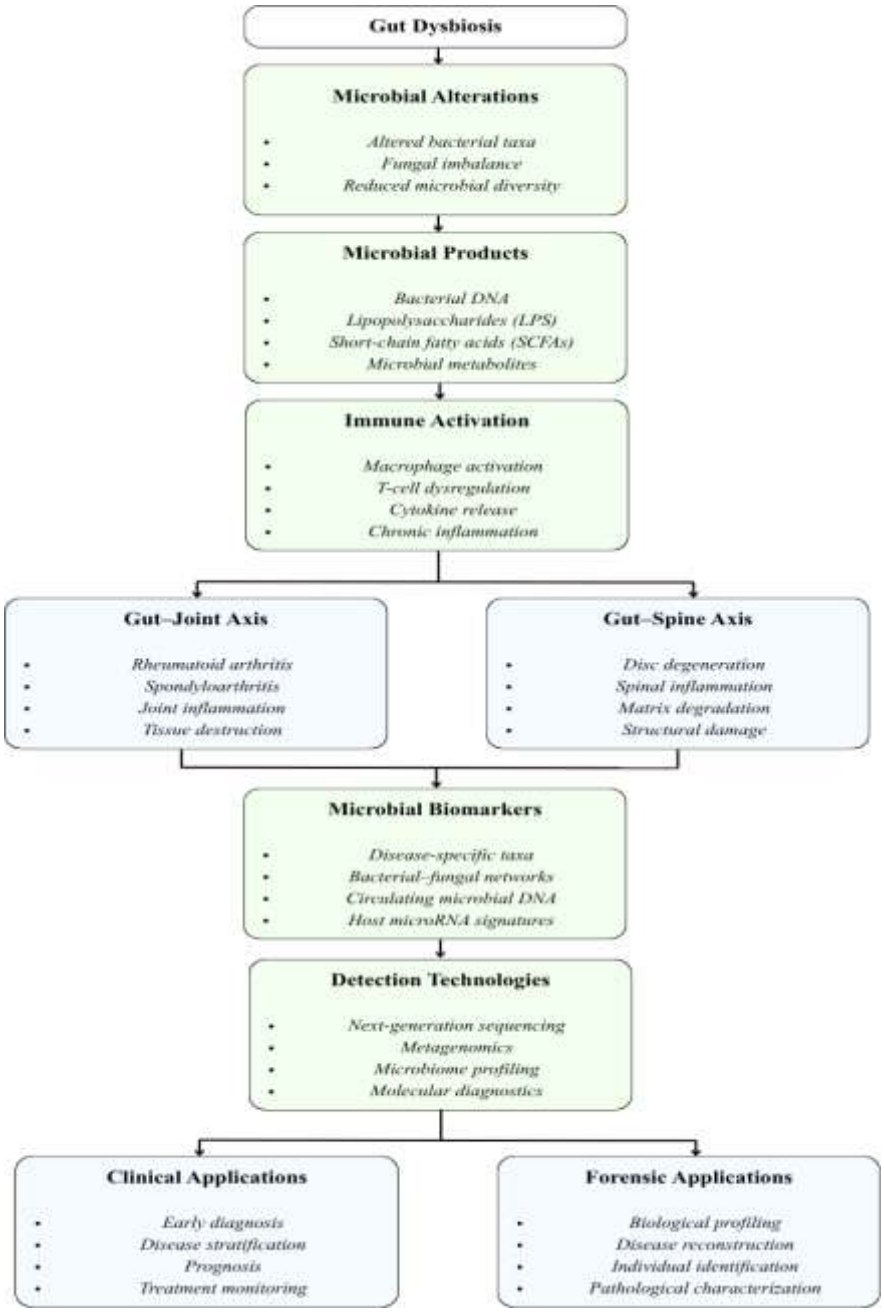
4. Microbial Biomarkers: The Microbiome as a Diagnostic and Forensic Signature

The human microbiome has been identified as an important modulator of musculoskeletal health and disease, as well as a regulator of immune homeostasis, inflammatory responses and tissue metabolism. There are growing indications that changes in the microbial community may play a role in the initiation and progression of chronic inflammatory joint and spine diseases via immune modulation, metabolic signaling pathways, and microbial translocation. This has led the focus to shift to microbial biomarkers as a promising option for early disease detection and personalized disease risk assessment. Targeting the gut microbiome as a therapeutic and diagnostic axis has attracted a great deal of interest, based on its close relationship to the systemic inflammatory processes involving skeletal tissues [20]. Inflammatory diseases such as spondyloarthritis have a special link with the gut microbiome and the immune system. Chronic inflammation and pathological tissue remodeling are associated with microbial metabolites and antigens from the gut, affecting innate and adaptive immune responses. Dysbiosis may lead to a dysregulation of the immune system, triggering inflammatory pathways not only in the GI tract but also in joints and in spinal structures, thus providing diagnostic signatures of microbes [21].
Recent studies have extended the concepts of the microbiome paradigm from the gut–joint axis to the microbiome–intervertebral disc axis. Microbial communities or bacterial products can affect the disc homeostasis by inflammatory signaling, extracellular matrix degradation, and modulation of local immune response. These results indicate that

microbial changes might be used as a diagnostic tool for spinal degeneration and chronic inflammatory spinal diseases in the future, offering a new approach to the non-invasive monitoring of the disease process. [22] This is supported by the fact that gut-derived bacteria DNA has been found in circulation and is linked to autoimmune responses, and may play a role in the pathogenesis of spondyloarthropathies in pro-inflammatory macrophages. Moreover, microbial signatures are relevant to host immune responses due to the presence of microbial genetic material in host tissues [23]. AS has emerged as one of the most well-studied models of "microbiome-associated musculoskeletal disease". Unique microbial signatures have been found in patients that may be associated with disease susceptibility and severity, implying distinct microbial taxa and functional pathways associated with disease. These changes in microbiome may be a source for diagnostic biomarkers that can be used to differentiate disease states and predict clinical outcome [24]. In addition to the abundance of bacteria, bacterial–fungal interkingdom networks have been found to be disrupted in ankylosing spondylitis, suggesting that microbial ecosystem dynamics can be a more holistic representation of dysbiosis associated with disease than just individual taxa [25].

With the advent of sophisticated molecular technologies, the discovery of microbial biomarkers is gaining momentum, which enables the detection of nucleic acids produced by the pathogen as well as host responses. Furthermore, the identification of circulating microRNA profiles in patients with infectious and inflammatory conditions of the spine that are disease-specific provides a glimpse into the potential for infection-related molecular signatures in differential diagnosis and clinical decision making [26]. In the same way, next-generation sequencing has been a tremendous improvement in pathogen detection in spinal infection, providing more pathogen characterization and greater sensitivity than traditional microbiological techniques. As a result of these advances, microbial biomarkers can be used in various complex conditions of the spine, where conventional diagnostic methods are not enough. [27] Microbial biomarkers have the potential to be used in forensic science as well as for clinical diagnosis. Each person's microbiome is unique, evolves, and is influenced by health status, exposures to the environment and diseases experienced. The development of new properties has led to an increasing interest in forensic identification based on microbiome, in which microbial signatures can assist with biological profiling (along with traditional forensic evidence) [28]. The alterations in microbial structures observed in disease states in chronic inflammation conditions support the hypothesis that microbiome patterns may be used in forensic characterization of disease states. This finding is echoed by other chronic inflammatory diseases, which have shown that microbial communities unique to disease can be used to inform about the disease and potentially be used for diagnostic and investigative purposes [29].

The microbiome is a dynamic relationship of host biology, immunity and environment, which can be a valuable source of biomarkers in chronic inflammatory joint and spine disorders. The new sequencing technologies and advances in microbial profiling keep uncovering signatures of microbes associated with disease, which can help to better identify early diagnosis, patient stratification and forensic characterization. The use of microbial biomarkers in combination with genomic and biochemical data could help to further improve the development of comprehensive multi-omics diagnostic tools. The mechanistic connection between gut dysbiosis, immune dysregulation, generation of microbial biomarkers and their clinical/forensic applications in chronic inflammatory joint/axis disorders is shown in Figure 1.



5. Genomic Biomarkers: Genetic and Epigenetic Determinants of Disease Susceptibility

Genomic biomarkers offer a mechanism for the understanding of chronic inflammatory joint and spine conditions and why some people are affected by similar environmental or biomechanical exposures, while others are not. As part of a role of inherited susceptibility, genome-wide association studies (GWAS) have identified genetic loci associated with low back pain and to the presence of lumbar spinal disorders, including variants associated with lumbar spinal stenosis [30]. This evidence was subsequently greatly extended by large biobank-based genomic analyses which uncovered novel genetic markers associated with spinal pathologies and revealed the power of population scale datasets to unravel disease relevant genomic signatures [31]. The major histocompatibility complex is still one of the most significant regions of the genome affecting the risk for inflammatory spine disease. Traditionally, ankylosing spondylitis has been linked to HLA-B27, but studies of ethnic groups from East Asia suggested that the susceptibility to the disease is related to a more complex MHC architecture, which also involves non-HLA-B27 components [32]. In the context of axial spondyloarthritis, a more general genetic context is becoming relevant, as it is the non-HLA-B27 variants that could explain variations in disease presentation, immune activation and diagnostic uncertainty in genetically diverse populations [33]. In addition to inherited variation in DNA sequence, there are also epigenetic and regulatory genomic mechanisms that contribute to disease susceptibility. In chronic musculoskeletal disorders, LNCs play a role in gene regulation, inflammatory signaling, cell differentiation and tissue remodeling, among other things, and thus become important candidate biomarkers [34]. The inflammatory gene expression of immune cells can be modulated by epigenetic changes at the DNA level and at the chromatin level, which can lead to chronic immune dysregulation in RA [35]. The same pathway reveals disease-relevant epigenetic changes affecting immune pathways, cytokine regulation and pathological bone remodeling in AS [36]. These results indicate that epigenetic markers could be used in future to better understand the basis of why some individuals

with a genetic predisposition to develop clinically active disease do not, and others do. The ability to perform single-cell RNA sequencing has also bolstered the discovery of genomic biomarkers by enabling the cell-specific analysis of skeletal tissues, immune populations and stromal cell states, which would otherwise be masked by bulk sequencing, and has uncovered disease-associated cell heterogeneity [37]. While genomic and epigenetic biomarkers play a key role in susceptibility assessment, measurable downstream molecular products provide an added value for genomic and epigenetic biomarkers. Biomarkers in the serum and urine provide non-invasive markers of disease activity, prognosis and systemic biological change, and clinical relevance of genomic regulation to a clinically accessible molecular signature [38]. All these links between genetic susceptibility, epigenetic modification, transcriptomic changes and protein expression provide a hierarchical model of biomarkers for early diagnosis and disease stratification. Epigenomic and transcriptomic markers provide insights into how immune and skeletal cells get remodeled in disease, while genomic biomarkers help to understand inherited risk. They are most useful when used in conjunction with one another rather than singly; particularly for disorders which have overlapping inflammatory and degenerative aspects. The joint and spine disease classification could be enhanced by a combined genomic–epigenomic approach for early detection, risk prediction and precise classification. Genetic susceptibility, epigenetic regulation, molecular expression and clinically actionable genomic biomarkers are vertically organized, with genetic susceptibility influencing epigenetic regulation, which, in turn, affects molecular expression, which, in turn, affects clinically actionable genomic biomarkers, as shown in Figure 2.

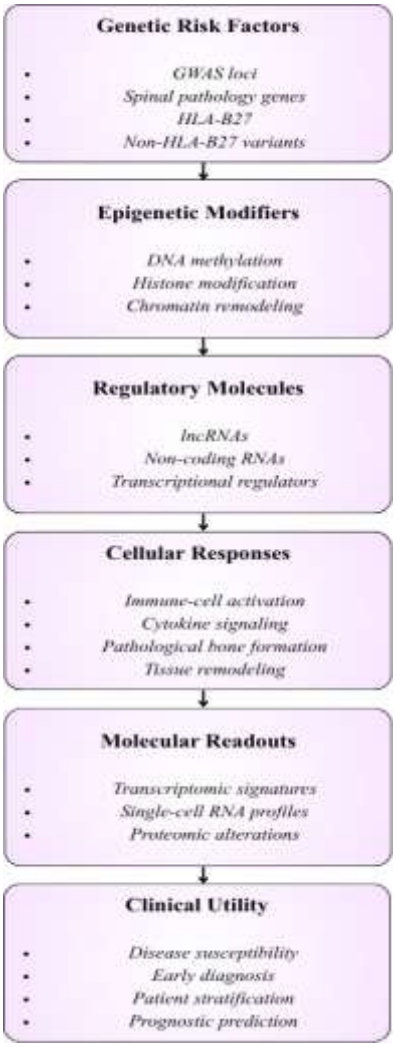


Figure 2. Multi-Layer Genomic Biomarker Framework for Chronic Inflammatory Joint and Spine Disorders

6. Biochemical Biomarkers: Molecular Indicators of Inflammation and Tissue Damage

Biochemical biomarkers are dynamic molecular markers which reflect the current inflammatory activity, tissue damage, metabolic disturbance and therapeutic response in chronic inflammatory joint and spine diseases. These biomarkers are dynamic, as opposed to a set of static genetic determinants, and thus give information that is useful for disease diagnosis, monitoring, prognosis and evaluation of treatment. They have found their place as part of the biomarker discovery and precision medicine strategies for musculoskeletal diseases, since they can almost directly reflect the changes in the biological tissues that are affected by the disease [39]. Biochemical markers, such as inflammatory cytokines and chemokines, are some of the most thoroughly studied markers in musculoskeletal disorders. The imbalance of cytokines plays an important role in the pathogenesis of intervertebral disc degeneration through the mechanisms of extracellular matrix degradation, apoptosis, angiogenesis and chronic inflammation. These molecular mediators are seen to have a role in the progression of disease and are also measurable markers of disease activity and therapeutic response [40]. Additional

inflammatory signaling is enhanced by the involvement of chemokines in recruiting and activating immune cells. Macrophage-associated chemokines have been shown to have diagnostic value in early axial spondyloarthritis and could be used as a biomarker to detect inflammatory activity prior to irreversible structural damage.

The range of biochemical biomarkers that can be detected has been greatly increased with the advent of proteomic technologies. Using quantitative serum proteomics, several proteins related to immune regulation, complement activation, inflammatory signaling and tissue remodeling have been identified as being associated with AS. These protein signatures provide information about disease mechanisms, and also have the potential to be used as non-invasive diagnostic and prognostic markers [41]. Biochemical biomarker clinical value is further strengthened by their proven roles in the diagnosis of axial spondyloarthritis, its assessment, predicting disease progression, and evaluation of therapeutic efficacy, and therefore their use as part of precision diagnostic tools [42]. A wide range of molecular changes are apparent in tissue degradation in joints and spine that can be identified by biochemical profiling. Several pathogenetic pathways, including the involvement of inflammatory mediators, matrix-degrading enzymes, cellular stress responses and extracellular matrix disruption, have been demonstrated to be shared between intervertebral disc degeneration and osteoarthritis. The overlapping mechanisms suggest that common biochemical markers could be used in a variety of inflammatory and degenerative musculoskeletal diseases [43].

Metabolomics has become a promising method for the discovery of biochemical signatures unique to disease states. Metabolomic analyses offer valuable information about the cellular metabolism and biochemical pathways associated with disease through characterizing alterations in small molecule metabolites. Multi-layer molecular profiling of disease through integrated metabolomic–proteomic approaches has shown the value of multi-layer molecular disease characterization, with key compounds in the serum identified that are associated with spinal tissue pathology [44]. In the same way, careful metabolomic studies have revealed unique amino acid metabolic patterns in AS, which could have diagnostic and prognostic value for inflammatory musculoskeletal diseases [45]. The use of biofluid-derived biomarkers offers a much more practical pathway to clinical implementation, as they can be obtained via minimally invasive sampling procedures in many cases. Although there are a number of other types of biological material available for the assessment of joint disease, synovial fluid is the most informative because it mirrors the local inflammatory activity, cellular interactions and tissue degradation processes in the affected joints. Comprehensive synovial fluid analyses have been demonstrated to have significant diagnostic and prognostic value, and have been included in the biomarker assessment and monitoring of the disease [46].

Biochemical biomarkers are molecular markers that reflect the inflammatory response, tissue damage and metabolic changes during disease pathogenesis. Their clinical uses are not limited to diagnosis, but also prognosis, therapeutic monitoring and disease stratification. Biochemical signatures can be added to microbial and genomic signatures to create a more comprehensive multi-omics model for early detection and characterization of chronic inflammatory Joint and Spine Disorders. The principal classes of biomarkers discussed in this review are summarized and their sample sources and diagnostic relevance are highlighted in Table 2.

Table 2. Major Biomarker Classes for Early Detection and Disease Characterization

Biomarker class	Representative markers	Main sample source	Diagnostic relevance
Microbial biomarkers	Gut dysbiosis, bacterial DNA, bacterial–fungal networks	Stool, blood, tissue, synovial fluid	Early immune activation and disease-associated dysbiosis
Genomic biomarkers	HLA variants, non-HLA loci, lncRNAs	Blood, saliva, tissue	Susceptibility, risk profiling, disease stratification
Epigenetic biomarkers	DNA methylation, immune-cell epigenetic changes	Blood, immune cells, tissue	Functional regulation of inflammatory pathways
Proteomic biomarkers	Serum proteins, complement-related proteins, tissue-remodeling proteins	Serum, urine, synovial fluid	Disease activity and prognostic assessment
Metabolomic biomarkers	Amino acid profiles, inflammatory metabolites	Serum, plasma, urine	Metabolic disturbance and disease monitoring

7. Integrative Multi-Omics Approaches for Early Detection and Forensic Characterization

Chronic inflammatory arthritis of the joints and spine is a complex disease requiring analytical methods that can account for interactions between several biological levels. Single classes of biomarkers may only offer limited information about disease mechanisms; however, multi-omics integration brings together the information from the genome, microbiome, biochemistry, and clinical measurements to provide a more complete picture of disease biology. This holistic perspective enables identification of molecular pathways related to each other and the identification of biomarker sets associated with clinical heterogeneity, disease onset and progression [47].

Systems biology and network-based analytical methods have now become a cornerstone to understanding the biological systems with large-scale biological data sets. These approaches focus on the interactions among genes, proteins, metabolites and microbial communities instead of individual molecules to network functional groups related to the disease process. Network-based bioinformatics can be useful to identify important regulatory hubs and molecular interactions,

which can be utilized as robust diagnostic and/or prognostic biomarkers to improve the accuracy of the disease characterization and therapeutic targeting [48].

There has been a growing need for advanced computing techniques to extract clinically relevant information from the complex biological information that is created with the increasing number of multi-omics data sets. Artificial intelligence (AI) and machine learning algorithms have been found to be effective in linking the various omics layers and uncovering unexplained patterns linked to disease susceptibility, disease development, and treatment response. These computational models enable the discovery of biomarkers, which can identify multidimensional relations that are not easily identified with traditional statistical methods, and thus help develop strategies for precision medicine [49].

The predictive modelling capabilities of musculoskeletal diseases have also been enhanced by deep learning methodologies that can analyze thousands of images, tens of thousands of molecular and clinical data to inform the predictive model. In the field of musculoskeletal research, deep-learning applications range from detecting lesions to evaluating disease progression and predicting future changes in the disease. These methods, if combined with omics-derived biomarkers, can lead to better early detection of disease and individualized risk profile, which can result in timely clinical intervention [50]. Integrated biomarker data to generate clinically useful prediction tools is still a challenge. Common challenges with spinal disorder prognostic models include data heterogeneity, population variability, model overfitting, and restricted external model validation. To overcome these challenges, it is essential to have strict methodological frameworks and validated assessments of biomarkers following standard protocols in order to be reproducible and clinically applicable in different patient populations [51].

In recent years, there has been significant progress in biomedical informatics that has demonstrated the ability to merge multi-modal predictive modeling with imaging biomarkers and electronic health record (EHR) related decision support systems. These holistic platforms have the potential to integrate molecular, imaging, and clinical data to create comprehensive predictive models, which can improve diagnostic precision and enable personalized disease management strategies. These developments are especially important for the assessment of chronic inflammatory joint and spine diseases, where the complexity of the disease may require multidimensional assessment approaches [52]. Multi-omics data integration also has great potential for forensic characterization. All-encompassing biological signatures from genomic, microbial, proteomic and metabolomic data sources could offer insights into disease history, biological state, and individual-specific pathological signatures. The integrated molecular fingerprints may complement the traditional forensic approach and may help in a more precise reconstruction of biological processes involved in disease and better pathological attribution in medico-legal settings.

Multi-omics integration allows for mapping separate observations of disease-associated biomarkers into the network of biological interactions. Systems biology plus artificial intelligence, plus predictive analytics, plus clinical informatics, can all be used to enhance early diagnosis, patient stratification, forensic characterization. Such integrative frameworks will become more crucial for future biomarker research to provide clinically actionable and biologically meaningful insights. Table 3 provides an overview of the potential use of integrated multi-omics biomarker models for early detection, disease stratification, clinical prediction and forensic characterization.

Table 3. Clinical and Forensic Utility of Integrated Multi-Omics Biomarker Models

Application area	Integrated data type	Analytical approach	Expected utility
Early detection	Microbial, genomic, biochemical data	Multi-omics profiling	Identifies preclinical disease signatures
Disease stratification	Genomic, proteomic, metabolomic data	Network-based analysis	Classifies disease subtypes and severity
Prognostic modeling	Biomarkers, imaging, clinical data	Machine learning and AI	Predicts progression and treatment response
Forensic characterization	Microbiome, molecular, clinical signatures	Molecular fingerprinting	Supports disease history reconstruction
Clinical translation	Biomarkers, EHR, imaging data	Decision-support systems	Improves personalized diagnosis and monitoring

8. Clinical Translation, Challenges, and Future Perspectives

Rigorous validation and the ability to reproduce results in clinical use and incorporate the microbial, genomic, and biochemical biomarkers into precision medicine frameworks are key to the successful clinical implementation of these biomarkers. There are many candidate biomarkers that have shown diagnostic and prognostic value, but only a few have reached the stage of being used in routine clinical practice. There is a need for standardized pipelines to discovery, good validation across independent populations, and full evaluation of clinical utility in order to make the translation effective. Systematic validation processes play a key role in the translation of these approaches to precision medicine, and in the use of microbial predictors, advanced analytics, and machine learning to enhance disease stratification and therapeutic decision making [53]. One of the key challenges in the implementation of biomarkers is the ability to integrate different and heterogeneous datasets obtained from multiple omics platforms. There is a considerable variation in the collection,

analytical methods, data processing and population parameters that can have a strong impact on biomarker reproducibility and comparability. The integration of the data from multi-omics studies, such as genome, transcriptome, proteome, metabolome and microbiome, is often challenging. To overcome these challenges, there is a need for standardized computational workflows, more interoperability between datasets and the evolution of scalable analytical methods to process increasingly complex biological information [54].

As the application of microbial and genomic data in the clinical and forensic context has increased, there are also important ethico-legal and regulatory concerns. Biomarker studies frequently include information that is sensitive to the biological nature and can include health status, ancestry, susceptibility to disease and even personal identity. As a result, the concerns of data privacy, informed consent, biosafety, biological information ownership, and regulation will have to be taken seriously to ensure the implementation of this initiative on a large scale. In the context of microbiome studies, biological signatures have medical and forensics implications and thus it is critical to have proper ethical governance mechanisms [55]. Future efforts to discover and validate biomarkers are likely to be greatly improved by emerging technologies. Single-cell and spatial omics platforms have revolutionized the capacity to comprehensively characterize cellular heterogeneity, tissue organization and microenvironmental interactions at a level of unprecedented resolution. They allow to determine cell-specific molecular signatures that are not detectable by classic bulk analyses, and offer a greater understanding of disease mechanisms in chronic inflammatory joint and spine diseases. The development of highly precise and clinically actionable biomarker panels is expected to continue to be rapid thanks to the continued evolution of commercial single-cell and spatial omics technologies [56].

The integration of the validated biomarkers with AI, Digital Health Platforms, imaging systems and real-world clinical data will drive future advancements. Such convergence can aid in the creation of dynamic diagnostic models that better predict a disease's onset and progression and therapeutic response than is currently possible. Moreover, increased integration of clinical, molecular science, bioinformations and forensic scientists will be crucial to bringing discoveries of biomarkers to fruition and making them useful tools for personalized healthcare and evidence-based decision making. The final phase of clinical translation is vital in deciding the impact that biomarker discoveries can have in the area of patient care and forensics. Success will depend on overcoming issues with data validation, integration and regulation. The development of multi-omics technologies and precision medicine frameworks offers a promising approach to the development of more accurate, personalized and clinically relevant biomarker-based diagnostics.

9. CONCLUSION

Chronic inflammatory joint and spine diseases are a result of interrelated immune, microbial, genomic, epigenetic, biochemical and degenerative processes. This review points out that none of the individual categories of biomarkers is enough to reflect the biological complexity of these diseases. Microbial signatures indicate host–microbiome interactions and could be useful to diagnose early events and to use for forensic profiling, and genomic and epigenetic signatures would explain susceptibility, immune regulation and disease heterogeneity. Biochemical markers offer dynamic information on inflammation, tissue damage, metabolic disruption and therapeutic response. The most powerful diagnostic and translational potential will be provided by integrating these layers of biomarkers into multi-omics frameworks, which include systems biology, artificial intelligence, imaging biomarkers and clinical data. These types of integration can enhance the early diagnosis, patient stratification, disease-associated biological pattern modelling, and forensic reconstruction of disease-related biological patterns. But for clinical application, there is a need for standardized sampling, biomarker panels validated, reproducible analytical pipelines, ethical governance, as well as external validation in different populations. Integrated biomarker strategies could be a viable alternative to precision diagnostics and forensic characterization of chronic inflammatory joint and spine diseases, but need to be well validated and clinically actionable.

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