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REVIEW

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Biomarkers for intervertebral disc and associated back pain: From diagnosis to disease prognosis and personalized treatment

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Abstract

Biomarkers are commonly recognized as objective indicators of a medical state or clinical outcome and have been widely used as clinical and diagnostic tools and surrogate endpoints in many pathological conditions. In the context of intervertebral disc (IVD) and associated back pain, also known as degenerative disc disease (DDD), the use of biomarkers has been poorly explored. DDD is currently diagnosed using imaging techniques and subjective pain scales, limiting an objective association between DDD and pain levels, as well as an evaluation of disease progression. There is a need for objective and reliable measurements for DDD, pain and pathology progression. DDD predictors could also help clinicians in deciding on the optimal treatment for distinct patient groups. This review addresses the current candidate biomarkers in DDD, including imaging, genetic, metabolite and protein-based parameters, both at the tissue and systemic levels, that may become a major advance in the diagnosis and prognosis of the disease, as well as in the management of therapeutic approaches to DDD.

KEYWORDS
back pain, biomarkers, degenerative disc disease, diagnosis

1 | INTRODUCTION

Biomarkers are defined as a biological characteristic that can be objectively measured and evaluated as an indicator of normal versus pathological conditions, or a response to a therapeutic treatment. This might include altered pattern of gene expression, variation of a certain protein level in the body fluids or even alterations in the electrical activity, in the casa of the brain.[1] Biomarkers are currently explored in multiple diseases' contexts as targets/indicators of a pathological condition and as powerful tools in the diagnosis and in the assessment of treatment efficacy. Although most examples are related to cancer, biomarkers have also been explored in the context of other diseases, such as heart failure,[2] osteoarthritis (OA)[3] or more recently, mental disorders like depression.[4] Examples exploring biomarkers for disease diagnosis, include the CancerSEEK test, that allows the early stage detection of eight cancer types (ovarian, liver, stomach, pancreatic, esophageal, colorectal, lung, and breast), with an effectiveness of 98% in the case of ovarian cancers,[5] or the NIH OA Biomarkers Consortium that aims to identify biomarkers for drug development, preventive medicine, and medical diagnostics for OA,[3] such as urinary (u)C-terminal cross-linked telopeptide of type II collagen (uCTX-II), a molecule related to collagen degradation and inflammation.[6] Biomarkers are a crucial tool for disease diagnosis and can, if validated regarding specificity and sensitivity, be revolutionary in patient's management and disease treatment.

Disc degenerative disease (DDD), that is the group of pathologies related to intervertebral disc (IVD) degeneration, is recognized as the main contributor for low back pain (LBP) and radicular leg pain,[7] accounting for about 40% of the cases.[8] LBP is a highly prevalent condition, affecting more than 630 million people globally,[9] with indication to increase to more than three quarters of the population that might suffer from LBP at some point of their lives.[10] The identification and use of biomarkers of DDD and LBP are still poorly explored, owing to the heterogeneity of IVD associated pathologies but also due to the avascular nature of this organ, that might limit biomarkers detection systemically.

In the past years several studies have shed light on biomarkers for DDD and LBP, through the identification of numerous molecules that are distinctly expressed during the IVD degenerative process, or altered imaging patterns that can categorize disease stages or treatment efficacy. In this review we outline all the currently available and potential biomarkers in the context of DDD and LBP, aiming to provide a glance on the most recent advances in biomarkers identification. We discuss putative promising DDD biomarkers for the near future, while pinpointing the major challenges for their effective clinical translation.

2 | DDD: CAUSE, DIAGNOSIS, AND PROGNOSIS

The IVD is the major avascular organ in the human body, still, as the degenerative process progresses, tissue extracellular matrix undergoes multiple changes, whereby the degradation by matrix-metalloproteinases (MMPs) that weakens the IVD is most prominent. This weakening of the IVD matrix leads to altered biomechanical properties and triggers the appearance of fissures in the annulus fibrosus (AF), allowing the invasion and growth of nerve fibers and blood vessels into the otherwise aneural/avascular tissue.[11] This is believed to be a major cause of LBP, as the ingrowth of nerve fibers exposes them to inflammatory mediators such as TNF-α and IL-1β, inducing the expression of pain-related factors such as nitric oxide (NO), cyclooxygenase 2 (COX-2), and nerve growth factors (NGF).[12] The ingrowth of new vasculature provides a new and faster route for the release of mediators (cytokines, chemokines, growth factors) produced by the disc during the degenerative process. Owing to the alterations that occur both in the tissue matrix and in IVD cells during the pathological degenerative process, the pattern of molecules presented or released by the tissue are thought to be distinct from the those present in health/homeostasis. In fact, DDD has been associated to particular "patterns" of gene polymorphisms, considered as antecedent biomarkers of the disease[13,14] that impact the production of matrix components[15] and the catabolic/pro-inflammatory molecules production[16–18] as summarized in Table 1. Although genetic alterations are related to susceptibility or predisposition, they are not deterministic and therefore cannot be considered as true biomarkers for diagnosis and assessment of disease progression.

DDD diagnosis and the subsequent medical decision on the treatment is nowadays essentially based on IVD imaging by magnetic resonance imaging (MRI) and pain symptoms enumerated by the patient, using supporting grading scales to categorize pain levels. Changes in the IVD morphological aspect can be observed through MRI, that allows the visualization of water content through the intensity of the T2-weighted signal. MRI has been considered the best imaging technique to evaluate IVD degeneration,[48] but image interpretation is always subjected to a qualitative assessment, such as the Pfirrmann grading system, which presents a considerable degree of subjectivity.[49] This analysis only provides information on IVD hydration and structural changes like nucleus pulposus (NP) bulging, herniation, and sequestration. Though MRI analysis is well accepted as a diagnostic tool, the main pitfalls remain the lack of association between degeneration and pain levels, and the inability to estimate disease progression. On the other hand, pain levels are commonly assessed using the visual analogue scale (VAS). This scale, considered as the gold standard technique to categorize pain, can give a reliable measurement, that is valid and sensitive to change.[50] Nonetheless, pain is a personal and subjective experience that is influenced by several factors, including culture, personal learning and the psychological conditions of the patient at the moment.[51] Overall, the available tools to diagnose LBP and DDD have a shortage of predictors that could help clinicians in their decision for the optimal treatment.

3 | LOCAL BIOMARKERS

The degenerative IVD tissue itself provides relevant information that might help distinguish healthy from pathological conditions. These differences can be evidenced by imaging parameters, the abnormal expression of certain factors and consequently the synthesis and release of specific molecules to the blood or other body fluids. These parameters and molecules can represent good candidates for DDD and pain biomarkers, and their potential will be discussed in the following sections.

3.1 | Imaging biomarkers

In the past couple of years, a few studies have attempted to improve and surpass the common MRI limitations and provide new tools for DDD diagnosis (Table 2). More details on the technological developments in this field have been recently reviewed.[58,59] MR elastography-derived stiffness (MRE-DS), a noninvasive imaging technique allows a relative assessment of the shear stiffness by tracking propagating strain waves as they move through soft tissue (Table 2). The use of this technique was proposed by Walter et al. as an objective biomarker for DDD, by reflecting the alterations in tissue mechanical integrity, that may complement the common Pfirrmann classifications, thus improving DDD diagnosis.[52] Histogram analysis (HA) of MR images (analysis of regional variations of signal intensity [pixel values] across the IVD tissue) was also proposed as a sensitive

TABLE 1 Genes with polymorphisms known to be associated with DDD.

	Gene	Association	Patient cohort	Pathology	Reference
ECM	COL1A1	COLIA1 Sp1 polymorphism is a risk factor related to IVD degeneration in older people	966 men and women (>65 years old) of the Longitudinal Aging Study Amsterdam	DDD	19
		COLIA1 Sp1 polymorphism is associated with IVD degeneration in young male soldiers.	36 Greek army (24 patients [29 ± 7.6 years old], and 12 controls [25 ± 3.8 years old])	DDD	20
		COLIA1 Sp1 polymorphism is associated with disc signal intensity (degeneration).	Finnish population, 588 men 35–70 years old	DDD	21
	COL9A2	Single nucleotide polymorphism in COL9A is associated with disc degeneration	157 Finnish subjects (19–78 years old), 174 controls	DDD and LBP	22
	COL9A3	The presence of Trp3 allele increases the risk of developing dark nucleus pulposus	135 Finnish male patients (40–45 years old)	DDD	23
		Trp3 allele is associated with more severe degeneration based on Pfirrmann scores	75 Southern European, 25 controls (35–45 years old)	DDD	24
	COL11A1	Polymorphism in COL11A2 is associated with IVD degeneration and herniation	29 consecutive Finnish probands (14 male, 15 female; 42–75 years old)	LSS	25
		The carriers of the COL11A2 minor allele had an increased risk of disc bulges	135 Finnish male patients (40–45 years old)	DDD and herniation	23
	ACAN	Aggrecan polymorphisms are associated with multilevel and severe IVD degeneration at an early age	64 young women (20–29 years old), 32 cases, 32 controls	DDD	26
		Polymorphisms of the aggrecan gene are associated with IVD degeneration and herniation	150 Turkish young adults, 150 controls (20–30 years old)	DDD and herniation	27
		Aggrecan gene variable number of tandem repeats polymorphism was associated with IVD degeneration	132 Finnish middle-aged men (41–46 years old)	DDD	28
Catabolic molecules	IL-1A	IL1A −889 T allele represented a significant risk factor for the IVD degeneration	150 Finnish men (38–56 years old), 61 control subjects	DDD	29
		Single nucleotide polymorphisms of IL-1A is associated with IVD degeneration	96 Danish adolescents, 57 controls (mean age 13.1 years old)	DDD	30
	IL-6	IL-6 Single nucleotide polymorphism is involved in the etiology of IVD degeneration among young adults	538 young adults (mean age of 19 years old) belonging to the 1986 Northern Finland Birth Cohort	DDD	31
		Association of IL-6 genetic variations with discogenic pain	155 Finnish subjects (17–78 years old), 179 controls (20–69 years old)	DDD and LBP	32
	MMP-3	MMP-3 polymorphism is a possible risk factor for the acceleration of degenerative changes in the lumbar IVD in the elderly	54 young Japanese (18–28 years old) and 49 elderly (64–94 years old) patients	DDD	33
		Subjects who carry mutation alleles 5A of MMP-3 are more vulnerable to disc degeneration when exposed to whole-body vibration and/or bending/twisting under ergonomic loads	178 Chinese patients (mean age 48.5 years old), 284 controls (mean age 40.6 years old)	DDD	34
	MMP-2	Three-fold higher risk for lumbar disc disease in individuals with the MMP-2-1306CC genotype compared with individuals with at least 1 variant T allele	162 Chinese individuals (25.4 ± 3.5 years of age)	DDD	35
	MMP-9	1562C/T polymorphism of the MMP-9 gene is associated with a high risk of IVD degeneration	408 Chinese population	DDD	36

(Continues)

TABLE 1 (Continued)

	Gene	Association	Patient cohort	Pathology	Reference
GAG Metabolism	VDR	VDR polymorphisms are associated with IVD degeneration and reduced disc signal intensity on MRI	85 Pairs of Finnish twins (35–69 years old)	DDD	37
		Association of VDR gene polymorphisms with IVD degeneration and herniation	150 Turkish young adults, 150 controls (20–30 years old)	DDD and herniation	27
		A positive association between VDR FokI/T2C polymorphism and IVD degeneration was observed	121 Brazilian patients (mean age 46.0 years old [males], ~45.2 years old [females]), 131 controls (mean age 33.8 years old [males], ~33.9 years old [females])	LBP and DDD	38
		VDR polymorphisms were associated with multilevel and severe IVD degeneration and disc herniation	205 Japanese young adults (mean age 22 years old)	DDD and herniation	39
		Polymorphisms of the VDR gene were associated with disc degeneration and herniation	150 Turkish young adults, 150 controls (20–30 years old)	DDD and herniation	27
		Single-nucleotide polymorphisms occurring in the VDR gene were associated with the development of lumbar disc degeneration	75 Southern European men, 25 controls (35–45 years old)	DDD	24
		Subjects with mutation in the VDR gene are more vulnerable to lumbar disc degeneration when exposed to whole-body vibration and/or bending/twisting under ergonomic loads	178 Chinese patients (mean age 48.5 years old), 284 controls (mean age 40.6 years old)	LBP and DDD	34
		VDR polymorphisms are closely associated with the occurrence and severity of disc degeneration	84 Egyptian patients (mean age 44.2 years old) and 60 controls (mean age 43.3 years old)	DDD	40
	IGF-1R	The G allele (GG and GC) of the IGF-1R was overrepresented in the population showing more severe disc space narrowing on radiographs	434 postmenopausal Japanese women	Disc degeneration	41
	CILP	CILP plays a role in lumbar disc degeneration by regulating TGF-beta signaling pathway	467 Japanese men and women	DDD	42–44
Inflammatory Pathways	TIMP1	Polymorphism of TIMP1 is associated with the radiographic progression of lumbar disc degeneration	720 English women	DDD	45
	COX2	Polymorphism of COX2 is associated with the radiographic progression of lumbar disc degeneration	720 English women	DDD	45
		Single-nucleotide polymorphisms of COX2, rs5277 and rs5275 are significantly associated with IVD degeneration	308 Indian male patients with mild Total Disc Degenerative Score (TDDS; mean 29.6 years old), 387 Indian male patients with severe TDDS (mean 31.7 years old)	DDD	46
	THSD2	Polymorphism of THSD2 is associated with the radiographic progression of lumbar disc degeneration	720 English women	DDD	45
		Regulation of intervertebral disc ECM metabolism by the THBS2-MMP system plays an essential role in the etiology and pathogenesis of LDH	322 cases (35.7 ± 15.2 years old) and 332 controls (60.9 ± 14.1 years old; Japanese population)	DDD and herniation	47

Abbreviations: ACAN, Aggrecan; CILP, Cartilage Intermediate layer protein; COL11A1, Collagen XI alpha2; COL1A1, Collagen I alpha 1; COL9A1/A2/A3, Collagen IX alpha 1/Collagen IX alpha 2/Collagen IX alpha3; COX2, Cyclooxygenase-2; DDD, Disc degenerative disease; IGF-1R, Insulin-like growth factor 1 receptor; IL-1A/IL-1B, Interleukin 1 & 1ß; IL-6, Interleukin 6; LBP, low back pain; LSS, Lumbar spinal stenosis; MMP-2, Matrix metalloproteinase 2; MMP-3, Matrix metalloproteinase 3; MMP-9, Matrix metalloproteinase 9; THSD2, Thrombospondin; TIMP1, Tissue inhibitor of metalloproteinase 1; VDR, Vitamin D receptor.

TABLE 2 IVD imaging biomarkers.

Technique	Association	Patient cohort	Pathology	Reference
MRE-DS	Increase in MR elastography-derived stiffness measurements in the NP and AF with increasing Pfirrmann grade	47 subjects without LBP (age range 20–71 years old)	LBP	[52]
HA	This peak separation was shown to strongly correlate with Pfirrmann grade	49 subjects (age range 25–69 years old)	LBP	[53]
MRI and discography	T1ρ and T2 MRI and multilevel provocative discography. Opening Pressure (OP) recorded as the pressure when fluid first enters the IVD nucleus	17 LBP patients, age 30–53	LBP	[54]
MRI (modic changes, MC)	MC affecting whole anterior–posterior length and MC affecting 2/3 posterior length associated with prolonged severe LBP. Type I MC associate with pain more strongly than type II MC. Any MC were associated with disability	1142 subjects (age mean 53 years old)	LBP	[55]
MRI (UDS Score)	UDS noted in 39.8% of subjects, 61.4% at the lower lumbar spine. UDS subjects had greater severity and extent of IVD degeneration and Modic changes	108 subjects	LBP	[56]
MRI HIZ	Disc degeneration/displacement more prevalent in HIZ individuals. HIZ subjects had more frequently prolonged severe LBP and had higher Oswestry Disability Index score	1214 subjects, mean age 48.1 ± 6.3 years old	LBP and sciatica	[57]

imaging biomarker for IVD degeneration classification by Waldenberg et al. Healthy and well hydrated IVDs presented histograms with two separate peaks representing the NP and the AF, while in degenerated discs there was a decrease in peak separation.[53]

Already in 2011, Borthakur et al.[54] proposed to determine whether T1ρ MRI and discography opening pressure (OP) could be considered biomarkers of disc degeneration in LBP patients. This study showed that a significant and strong correlation exists between T1ρ values and OP measurements obtained by discography in LBP patients, with T1ρ being significantly lower in the painful versus non-painful discs. Later on, the same author proposed T1ρ MRI and disc height ratio (DHR) as potential biomarkers of degenerative disc disease, with painful discs presenting both low DHR and T1ρ values, while non-painful disks measured the highest DHR and extended to a higher range of T1ρ.[60] Nevertheless, discography has been almost abandoned due to the increased risk of initiation of IVD degenerative cascade.

The ratio of R1ρ dispersion and chemical exchange saturation transfer (CEST) (RROC) has also been proposed as a promising pH level dependent MRI technique with potential to be a noninvasive tool to detect painful IVDs.[61]

Samartzis's group has been investigating imaging biomarkers for a long time. For example, they were the first to definitively note Modic changes (MC) types with specific morphologies to be independently associated with prolonged severe LBP and back-related disability, in a large-scale study.[55] Furthermore, they propose ultra-short time-to-echo (UTE) Disc Sign (UDS) as a novel imaging biomarker, highly associated with degenerative spine changes, chronic LBP and disability.[56] More recently, the same group proposed lumbar high-intensity zones (HIZs) and specific patterns, such as homogenous multilevel HIZ, as potentially clinically-relevant imaging biomarkers, significantly associated with prolonged/severe LBP and sciatica.[57]

Recently, another imaging biomarker based on brain activity was proposed. A specific brain MRI signature that tracks induced tonic pain intensity was able to predict pain severity in LBP. Tonic and clinical pain showed similar network-level representations, particularly in somatomotor, frontoparietal and dorsal attention networks, distinct from experimental phasic pain.[62] Future brain neuroimaging holds promise for the discovery of biomarkers. Another example made use of morphological changes in cerebral cortical thickness (CT) and resting-state functional connectivity (rsFC) as brain biomarkers for LBP.[63]

All the proposed techniques represent relevant advances in DDD analysis that can complement the diagnostic techniques currently applied in clinical practice and be considered as DDD biomarkers. Still, these studies need further validation in bigger patient cohorts and more importantly, although these techniques contribute to patient stratification/classification, further studies are needed to correlate these parameters with pain to further distinguish symptomatic from asymptomatic discs.

3.2 | Metabolites as biomarkers

Alterations in the metabolic profile of LBP patients with DDD have been analyzed. The most significant contributions to this type of biomarkers have been reviewed elsewhere[64] and are here summarized in Table 3. High resolution magic angle spinning (HR MAS) 1H NMR spectroscopy is a non-destructive technique that has been applied to characterize the composition of several tissues. This noninvasive technique has been suggested by Radek et al. as a way to evaluate IVD

degeneration through the comparison of the metabolites of human degenerative discs at different levels of degeneration. The analysis revealed that the concentrations of creatine, glycine, hydroxyproline, alanine, leucine, valine, acetate, isoleucine, α,β-glucose and myo-inositol, and the N-acetyl peak of chondroitin sulfate were augmented in discs with severe degeneration (Pfirrmann grades of IV and V) when compared to moderate degeneration (Pfirrmann grade III) discs, suggesting that the ratio of these metabolites, as main constituents of proteoglycans and glycosaminoglycan breakdown, can be used as potential biomarkers of disease progression or more specifically, of increasing disc degeneration levels.[65]

Total plasma N-glycosylation pattern has been connected with CLBP. High-branched (tri-antennary and tetra-antennary) N-glycan structures were increased on patients' plasma glycoproteins, compared to healthy controls. Furthermore, Disialylated and trisialylated glycan structures were also increased in CLBP. Therefore, plasma glycomics can be used as potential biomarkers for this disorder.[66]

Metabolomic biomarkers for MC phenotypes of the lumbar spine via a combined metabolomic-genomic approach were also investigated by magnetic resonance spectroscopy. The results showed that decreased very-low-density lipoprotein (VLDL) mean diameter may lead to MC. This work opened the field of "spine-metabolomics."[67]

Advanced glycation end-products (AGEs) have been reported as a possible biomarker of aging and metabolic diseases, including LBP. Quantified AGE (qAGE) and VAS for leg numbness were positively correlated. qAGE showed potential as a biomarker for LBP, lower extremity pain, and numbness in patients under 50-years-old.[68]

3.3 | Tissue RNA based biomarkers

3.3.1 | Differentially expressed genes in IVD

DDD have also been associated with the expression of several genes related to inflammatory pathways. Most common mediators associated with the degenerative cascade are cytokines such as interleukins (IL) IL-1β, IL-6, IL-8, IL-10, IL-12, IL-17, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).[18,69] Some of these inflammatory mediators (RANTES/CCL5, CCR1/3/4/5, IL-1β, IL-6) were compared in the work of Kepler et al.,[70] in human discs of patients with discogenic pain, using as control healthy and "painless" discs from autopsies. RANTES (Regulated upon Activation, Normal T cell Expressed, and Secreted)/CCL5 (Chemokine [C-C motif] ligand 5) expression was reported as being significantly higher (3.60-fold) in painful discs versus painless discs, while correlating with increasing Pfirrmann grade and IL-1β expression,[70] suggesting RANTES/CCL5 as a potential biomarker of pain and disease progression.

With the development of new tools for high throughput screening of human patient samples, new potential biomarkers can be identified. A few studies focused on differently expressed genes (DEGs) in patients with IVD pathologies, to further elucidate the potential pathogenesis mechanisms of the disease and to unravel unobvious, and preferentially specific, disease markers. IGFBP3 has been identified to be strongly upregulated in degenerative human AF, with interferon pathway being found as the most significant canonical pathway induced in degenerative AF.[71] In another study, a DEGs analysis of 16 NP and AF samples of patients DDD using the gene expression dataset GSE70362, revealed 35 genes differently expressed in both NP and AF of degenerated discs, comparing to healthy control discs, including collagen type VI (COL6A2) an abundant protein of the IVD ECM, integrin binding sialoprotein (IBSP), that modulates ECM interactions, Ras-related protein (RAP1A), that regulates cell proliferation and adhesion and forkhead box F2 (FOXF2), an ECM-production transcription factor. The protein–protein interaction network modules revealed that interferon signaling has an important role in human IVD degeneration, through the negative regulation of the cell cycle by IFIT1, IFIT2, and IFIT3.[72] Additionally, six micro-RNAs (miRNAs) were found to target these common DEGs (miR-96, miR-182, miR-31, miR-526D, miR-188, and miR-19).[72] In a similar study using the gene expression dataset GSE17077 the comparison of senescent versus

TABLE 3 IVD metabolomic biomarkers.

Technique	Association	Patient cohort	Pathology	Reference
HR MAS	Metabolites ratio correlate with Pfirrmann grade	26 subjects (age range 18–72 years old)	DDD	65
Hydrophilic interaction ultra-performance liquid chromatography (HILIC-UPLC)	Increase in the relative amount of high-branched (tri-antennary and tetra-antennary) N-glycan structures on CLBP patients' plasma glycoproteins	1128 subjects	CLBP	66
Magnetic ressonance spectroscopy	MC metabolomic biomarkers include mean diameter of very-low-density lipoprotein (VLDL)/low-density lipoprotein (LDL) particles and cholesterol esters/phospholipids in large LDL	757 subjects	Lumbar Modic Changes	67
Skin autofluorescence	In LBP patients <50-years-old, qAGE was correlated with VAS, lower extremity pain, and numbness. qAGE was higher in LBP patients with diabetes and dialysis and osteoporosis	636 subjects		68

non-senescent AF cells obtained from patients with DDD revealed differences in genes involved in phosphorylation, regulation of apoptosis and regulation of programmed cell death, axon guidance, natural killer cell-mediated cytotoxicity, purine metabolism and the mitogen-activated protein kinase (MAPK) signaling pathway. MAPK-regulated AP1 pathway may contribute to senescence-associated disc degeneration and the increased expression of hub genes such as HSP90 and CXCL5 in senescent AF cells could be potential targets for future investigations into molecular biomarkers in DDD.[73]

The described studies provided evidence of multiple genes that are differently expressed in pathological IVDs (Table 4). Although these studies constitute great advances in the search of potential biomarkers, their power is limited due to the lack of standardization between the different conditions analyzed. Some have compared healthy versus pathological IVDs; while others compared differently expressed genes within the pathology, that is, different levels of degeneration or herniation. This discrepancy in the study design hinders the conclusions that can be drawn concerning unique biomarkers and highlights the need to standardize future studies.

3.3.2 | Differentially expressed noncoding RNAs in the IVD

Noncoding RNAs (ncRNAs) have been explored more recently as targets/candidates for IVD associated pathologies, namely, micro (mi) RNAs, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs; Table 4).[78–81] Only 1% of the genome is known to be involved in protein translation, leaving around 70%–90% to noncoding transcripts, known as ncRNAs. These ncRNAs have a key role in the regulation of biological events and depending on their size, are called miRNAs, small-interfering RNAs (siRNAs) or lncRNAs (>200 nucleotides). miRNAs are known to negatively regulate the stability and/or repress the translation of targeted mRNA and through this mechanism regulate essential functions such as cell proliferation, apoptosis, ECM metabolism, and so forth.[82,83]; whereas lncRNAs are able to activate or repress gene expression at multiple levels through diverse mechanisms, play a role in post-transcriptional events and contribute to splicing and mRNA translation and degradation.[84] CircRNAs are known to modulate gene expression levels primarily through sponging and interfering with miRNAs.[81] These ncRNA molecules can also be altered in pathological conditions such as DDD. Although the potential of noncoding transcripts to be used as systemic biomarkers is mostly limited to miRNAs, as other ncRNAs are more susceptible to degradation, several studies have addressed their patterns of expression in IVD degeneration.

3.3.3 | Differentially expressed LncRNAs in the IVD

The first study addressing the differentially expressed lncRNAs in patients with IVD pathologies compared lncRNAs expression in DDD versus spinal cord injury.[74] The microarray data analysis indicated that 116 lncRNAs were more highly expressed in degenerative discs and were mainly associated with cell migration and phosphorylation. The data identified Fas-associated protein factor-1 (FAF1), that potentiates the Fas-mediated apoptosis and its nearby enhancer-like lncRNA RP11-296A18.3, suggesting that this lncRNA might eventually promote disc cells apoptosis through the overexpression of FAF1, as demonstrated in degenerative discs. Other lncRNAs have also been described as being differentially expressed in patients with DDD, namely LINC00917, CTD-2246P4.1, CTC-523E23.5, RP4-639J15.1, RP11-363G2.4, AC005082.12, MIR132, and RP11-38F22.1.[75] Additionally, SPHK1, a gene known to be involved in endothelial cell migration and neovascularization, was shown to be upregulated, probably caused by the upregulation of lncRNAs LINC00917 or CTD-2246P4.1. Later, a similar study was conducted analyzing the whole IVD transcriptome by RNA sequencing, finding 1854 lncRNAs differentially expressed in degenerative conditions. Among these, NONHSAT031859 and NONHSAT006310 were the most significantly upregulated and downregulated in degenerative discs, respectively. KEGG pathway analysis for these target genes suggested that these lncRNAs were involved in diverse pathways, such as lysosome, focal adhesion, and MAPK signaling.[76]

The increasing number of available datasets facilitates the systematic analysis of RNA profiles from patients with DDD. For example, Qu et al. compared the gene and lncRNA expression of patients with degenerative lumbar NP with a non-degenerative control group.[85] They identified several potential genes and lncRNAs that were associated with disc degeneration. Among those, KCNQ1OT1 may be involved in DDD by regulating the expression of NCDN, while the lncRNAs OIP5-AS1 and UGDH-AS1 may be implicated in the molecular mechanisms of DDD by affecting the expression of FOXF1 and PKD1. These studies provide new insights on lncRNAs functional roles in IVD pathology and may detect novel potential candidates for diagnostic biomarkers. Nevertheless, validation studies with large and stratified study groups are still lacking.

3.3.4 | Differentially expressed miRNAs in IVD degeneration

In the IVD, miRNAs have been described to be involved in the degenerative process by mediating NP cells apoptosis (miR-155, miR-27a, and miR-494)[86–88] and ECM regulation through ADAMTSs and MMPs (miR377, miR-193a-3p).[89,90] Ohrt-Nissen et al. showed that 27 miRNAs were highly expressed in the AF and 10 in the NP from patients with DDD, and the most differently expressed miRNAs, mir-449a, mir-154 (in AF), mir-627, and mir-668 (in NP) were further validated by qPCR. The analysis of the top 15 signaling pathways most likely to be controlled by these miRNAs identified the transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) epidermal growth factor (EGF), and actin cytoskeletal pathway.[77] In another study, Zhao and colleagues characterized the miRNA expression in individuals with DDD versus spinal cord

TABLE 4 Potential RNA biomarkers of DDD and LBP identified in the IVD tissue.

	Molecule	Association	Patient cohort	Pathology	Reference
mRNA	RANTES/CCL5	Higher in painful discs and correlated with the increasing Pfirrmann grade and increasing IL-1β expression	18 patients (26–73 years old)	Painful versus non-painful discs	70
		MAPK-regulated AP1 pathway may contribute to senescence-associated disc degeneration through CXCL5	GSE17077 dataset	Senescent versus non-senescent AF from patients with DDD	73
	IGFBP3	Strongly upregulated in degenerative human AF	McGill Scoliosis and Spine Group 12 patients (21–82 years old)	DDD versus Health	71
	COL6A2 IBSP RAP1A FOXF2	Dysregulated in the NP and AF and associated with IVD degeneration progression by disrupting the extracellular matrix organization and focal adhesions pathway	GSE70362 dataset	DDD versus Health	72
Long noncoding RNAs	lncRNA RP11-296A18.3	Upregulated in DDD	10 patients (27–52 years old)	DDD versus Health	74
	LINC00917 CTC-523E23.5	Remarkably higher in DDD samples	10 Samples GSE56081 dataset	DDD versus Health	75
	NONHSAT031859	Upregulated in DDD	12 patients (29–63 years old)	DDD versus spinal cord injury	76
	NONHSAT006310	Downregulated in DDD			
Micro-RNAs	mir-449a mir-154	Upregulated in the AF of patients with DDD	14 patients (age is not reported)	AF versus NP of human discs with DDD/LBP	77
	mir-627 mir-668	Upregulated in the NP of patients with DDD			

injury. Their findings revealed 25 miRNAs upregulated and 26 downregulated in the DDD group, mostly related to the signaling pathways phosphoinositide 3-kinase (PI3K)-Akt, mitogen-activated protein kinase (MAPK), epidermal growth factor receptor (EGFR; ErbB), and Wnt.[91] Nonetheless, the validation of these dysregulated miRNAs was not performed and their role in DDD remains to be elucidated.

Although promising, these studies addressing differently expressed miRNAs in the human IVDs during the degenerative process present several limitations. Namely: (1) the lack of healthy controls; (2) the existence of a heterogeneous population with regards to pain levels and degenerative grades[77]; and (3) the small cohort of patients,[91] hindering any correlation of these miRNAs expression with the clinical symptoms.

4 | SYSTEMIC BIOMARKERS

4.1 | Protein biomarkers

In the context of DDD and associated back pain, there are few studies addressing the existence of systemic biomarkers for diagnosis and pain assessment (Table 5). One of the first studies was published in 1998 by Kuiper et al., that suggested keratan sulfate (KS) as a biomarker of IVD matrix degradation upon exposure to high loading. A correlation between KS serum level and degeneration grade was found in patients undergoing chemonucleolysis as a treatment for disc herniation, and a systemic measure of massive and rapid IVD degradation could be assessed through KS levels (3- to 10-times higher than the mean baseline).[102] Alterations in the immunoglobulins concentration in the serum (IgG, IgA, and IgM) have also been considered, but no differences were found when comparing healthy subjects with patients with DDD.[103] More recent data focused on key inflammatory biomarkers of LBP present in the blood and was reviewed in the work of Khan et al[104] It is consensual that IL-6 is a key player in the inflammatory environment of DDD and associated pain, although this cytokine shares this pivotal role with many other pathologies, including OA and rheumatoid arthritis,[105] limiting its potential as a diagnostic and prognostic marker alone. Individuals with disc degeneration and spinal stenosis also showed increased systemic levels of IL-2, IL-3, IL-8, HGF, IFN-α2, LIF, MCP-3, and TNF-ß when compared to patients with herniated discs.[94] Post-treatment, patients with disc pathologies (as degeneration and spinal stenosis) a decrease in IL-2Rα, IL-3, and SCGF-ß was noticed. Improvement of pain was shown to be related with a decrease of systemic levels of MCP-1 and MIG, SCGF-ß and other factors that participate in angiogenesis/fibrosis, such as the hepatocyte growth factor (HGF), nociception (stem cell factor [SCF], IFN-α2) and inflammation markers (IL-10, IL-6, IL-18, TRAIL9). These factors were suggested as predictors of pain response to treatment in patients with DDD and spinal stenosis.[94] Overall, these studies revealed the heterogeneity inherent to each lumbar back pain pathology and the consequences of these differences in the diagnosis and response to treatment. Grad and colleagues, identified CCL5/RANTES and CXCL6 in the blood plasma samples of individuals with DDD.[92] These cytokines are produced by the IVD during degeneration ex vivo, and were already identified in the IVD tissue, as previously mentioned.[70] The authors suggested that high levels of CCL5 and CXCL6 were associated with moderate/severe disc degeneration assessed by MRI. Although promising, this study requires further replication in other ethnic cohorts to validate the hypothesis. Although there is still few and heterogeneous data, CCL5/RANTES was the only molecule whose RNA expression in the degenerated IVD correlated with high protein levels in the serum of DDD patients. Additionally, CCL5/RANTES, along with the neuropeptide Y (NPY), have been previously correlated with LBP and with DDD progression in a study with 43 patients (age >60 years old).[93]

Potential biomarkers have been proposed through proteomic analysis of the human cerebrospinal fluid (CSF), comparing painful, and non-painful DDD.[96] From the CSF analysis, 27 proteins associated with DDD were differently expressed such as cystatin C, alpha-1-antichymotrypsin, gelsolin, chromogranin-A, neural cell adhesion molecule L1-like protein, and amyloid-like protein 1, that were increased in patients with DDD, while prostaglandin-H2 D-isomerase, serine/cysteine proteinase inhibitor clade G, superoxide dismutase, extracellular superoxide dismutase, calsyntenin-1, serum albumin, orosomucoid 1, and alpha-2-HS-glycoprotein were decreased, regardless of the patients' pain status. Most of the proteins were related to nerve injury (apoliprotein E, D, and A-IV, hemopexin, ProSAAS, β-2-microglobulin, prosaposin, and insulin-like growth factor II). Cystatin C was further shown to correlate with DDD severity and hemopexin demonstrated to correlate not only with disease severity, but also with pain intensity and physical disability of the patients.[96] This study suggests that LBP is more likely to be related to nerve injury and that the inflammatory mechanisms might not be central to pain genesis. Although this study presents a panel of potential candidate markers of IVD degeneration and associated pain, the use of CSF sampling can hinder the utilization of this marker in daily clinics.

Recently, Brayda-Bruno et al. analyzed the levels of vitamin D, known to have a role in collagen type I and type II turnover, and other osteo-cartilaginous markers (cross-linked C-telopeptides of type I [CTx-I] and type II [CTx-II] collagen) in the plasma of patients suffering from DDD. This study demonstrated that CTx-II was increased in patients with DDD, while both vitamin D and CTx-I levels were unable to differentiate DDD patients from healthy controls.[95] All these data portray the diverse panoply of molecules that can be altered systemically in the context of DDD, with and without associated pain, despite the avascular nature of IVD. It is important to refer that although these markers are of easy access (blood sampling), none of the studies mentions the impact of circadian variations on the systemic levels of these cytokines/proteins, which may vary depending on the time of blood collection from the patient.[106] This reinforces the need for standardized procedures not only in the selection of the included pathologies but also in the sampling procedures, for the analysis of potential blood biomarkers in future studies.

Boisson et al. compared serum biomarkers of inflammation, redox status and cartilage degradation in CLBP patients with and without Modic1 changes, but did not find significant differences between

TABLE 5 Potential protein and RNA biomarkers of DDD and LBP identified systemically.

Source	Molecule	Association	Patient cohort	Pathology	Reference
Blood	RANTES/CCL5	High levels were correlated with moderate/severe disc degeneration	80 patients (Average age 49.25 years old [control group]/49.5 years old DDD group)	DDD versus Health	92
		Basal levels were altered with pain and in response to activity	43 patients Age ≥ 60 years old	LBP	93
	IL-6	Increased in DDD and spinal stenosis in comparison to DDD/herniation	16 patients 55 ± 15 years old	DDD versus DDD/DH versus SS before and post ESI treatment	94
	IFN-γ				
	TNF-α				
	IL-2				
	IL-3				
	IL-8				
	IFN-α2				
	LIF				
	MCP-3				
	TNF-ß				
	HGF	Increased in DDD and spinal stenosis in comparison to DDD/herniation; HGF seems to mediate pain relief in herniated discs.			
	CXCL6	High levels were correlated with moderate/severe disc degeneration	80 patients (Average age 49.25 years old [control group]/49.5 years old DDD group)	DDD versus health	92
	NPY	Increased with pain and in response to activity	43 patients Age ≥ 60 years old	LBP	93
	CTx-II	CTx-II increased in patients with DDD	158 patients (average age 40.4 years old [control group]/41.1 years old DDD group)	DDD versus Health	95
CSF	Cystatin C	Correlates with IVD degeneration severity	54 patients 30–51 years old	Painful and non-painful intervertebral DDD	96
	Hemopexin	Correlates with IVD degeneration severity, pain intensity and physical disability			
	Neurofilament protein	Increased levels in the CSF of patients with DH	22 patients (22–64 years old)	DDD/DH versus Health	97
	S-100 protein				
lncRNA	MAGI2-AS3	Decreased in plasma of IDD patients	66 patients (58.6 ± 4.8 years old)	DDD versus Healthy	98
	LINC00324	Decreased after treatment of DDD patients with occupational, physical, or epidural steroid therapy	60 patients (53.1 ± 6.8 years old)	DDD versus Control	99
miRNA	miR-766-3p	Upregulated in blood	10 patients (53.1 ± 24.2 years old)	DDD and manifest lumbar disc herniation (LDH) versus control group	100
	miR-6749-3p				
	miR4632-5p	Downregulated in blood			
	miR-155	Decreased in patients' blood serum	69 patients	Patients with single level decompression and fusion surgery for DDD versus controls	101

inflammation (IL-1β, IL-6, IL-8, and TNF-α), redox status (total thiols, advanced oxidation protein products, and carbonyl groups) or cartilage degradation (Coll2-1 and Coll2-1NO2) markers between patients' groups.[107] Klyne et al. investigated systemic inflammation associated with acute back pain, pointing C-reactive protein (CRP), and IL-6 as important contributors to inflammation in the early post-onset phase of LBP.[108] The same authors also investigated these markers in LBP patients who did or did not recover by 6 months. Their results pointed to a high inflammation (CRP/IL-6) response associated with good recovery, while specific elevation of TNF, along with depressive symptoms, was associated with bad recovery.[109] Also from the same group, an investigation of systemic inflammatory markers from LBP patients with sleep, depression and fear was performed. Their results point towards an early role of CRP (and perhaps IL-6) in control of inflammation and recovery, and a pathological role of persistent TNF overexpression, perpetuated by depressive-like behaviors.[110]

Systemic inflammatory mediators from patients with vertebral endplate bone marrow lesions visualized on MRI as Modic changes (MCs) were analyzed. The results suggest MIF was strongly expressed in LBP patients with MC.[111] In a similar study, Karppinen et al. screened diverse panels of biomarkers in serum of CLBP patients with MCs compared to a matched pain-free control group.[112] Interestingly, several biomarkers were suppressed, whereas IL-1sRII and HGF were elevated among the MC patient group. Moreover, MC type or size had no influence on biomarker expression. In a subsequent study the investigators quantified a set of biomarkers in the serum of CLBP patients with MC who were treated with zoledronic acid and were followed-up for 1 year.[113] As expected, treatment with zoledronic acid downregulated bone turnover markers. Interestingly the drug also increased the chemokine IP-10 compared to placebo treatment. These findings improve our knowledge of the effects of specific treatments and the biomarkers that signal biochemical processes.

The role of excessive adipose tissue in aggravating the inflammatory processes and in the development of LBP was also disclosed. Adipsin, CS-846 and GDF-15 aspire to be LBP biomarkers specific for women with obesity.[114]

4.2 | Systemic lncRNAs

There are a few reports on the analysis of lncRNAs in peripheral blood samples. LncRNA MAGI2-AS3 is known to upregulate Fas ligand (FasL), a factor that has been implicated in the development of DDD. A total of 66 patients with DDD who were diagnosed and treated the first time, and a respective control group were assessed regarding the expression of lncRNA MAGI2-AS3 in peripheral plasma.[98] Interestingly, plasma lncRNA MAGI2-AS3 levels were significantly lower in the DDD group compared to the control group. ROC curve analysis revealed an AUC of 0.9, indicating a good predictive value of lncRNA MAGI2-AS3 to effectively distinguish patients with DDD. Furthermore, plasma concentration of lncRNA MAGI2-AS3 increased after treatment compared to pre-treatment levels, suggesting the use of this marker to monitor treatment success. In vitro, overexpression of lncRNA MAGI2-AS3 suppressed the gene and protein expression of FasL in human NP cells, confirming the role of this ncRNA in IVD cells. In a similar study, the expression of lncRNA LINC00324, which is known to upregulate FasL, was analyzed in plasma of 60 patients with newly diagnosed DDD and a corresponding control group.[99] Significantly enhanced levels of lncRNA LINC00324 were detected in plasma samples of the DDD group compared to the control group. There was also a significant positive relation between the FasL and lncRNA LINC00324 concentrations in the patient group. Interestingly, the expression of circulating lncRNA LINC00324 was decreased after treatment of DDD patients with occupational, physical, or epidural steroid therapy, suggesting this ncRNA may be a marker of treatment outcome. In addition, overexpression of lncRNA LINC00324 resulted in increased FasL expression in NP cells from DDD patients but not from healthy NP cells. To conclude, lncRNAs may serve as tissue and systemic biomarkers of DDD, although the specificity of such markers will need to be confirmed with larger patient populations.

4.3 | Systemic miRNA

Given the remarkable stability of miRNAs in serum or plasma samples, their profiles or specific expression levels have increasingly been analyzed in blood circulation. Cui et al. used RNA sequencing analysis to compare circulating miRNA profiles of patients with DDD with a control group of patients without DDD.[100] Seventeen upregulated and 56 downregulated miRNAs were identified between the study groups. Gene mapping analysis revealed that upregulation of miR-766-3p, miR-6749-3p, and downregulation of miR-4632-5p could target multiple genes related to DDD. The study suggests combinations of biomarkers may be most valuable for predictive diagnosis of DDD.

Another study investigated the expression of miR-29a in plasma and IVD tissue of patients with lumbar spinal stenosis (LSS).[115] In addition, plasma from healthy individuals and tissue from postmortem resections were used as controls. The plasma level of miR-29a was significantly lower in the LSS group compared to the control group. Decreased miR-29a levels were associated with elevated plasma concentrations of MMP9 and ADAMTS5. The expression levels of miR-29a, MMP9, and ADAMTS5 were positively correlated with the expression of these markers in the IVD tissue of LSS patients, suggesting release of these markers in the circulation. ROC analysis was performed to evaluate the diagnostic value of plasma miR-29a to distinguish patients with LSS. The resulting AUC of 0.97 indicated a high diagnostic accuracy of this miRNA for predicting LSS. Nevertheless, miR-29 has been implicated with several other pathological processes and diseases and may only be used in combination with other diagnostic methods.

Divi et al. screened various miRNAs in blood serum of patients undergoing single level decompression and fusion surgery for DDD.[101] In comparison to a healthy control group, 11 downregulated and 2 upregulated miRNAs were identified. ROC curve analysis indicated that only the AUC for decreased miR-155 was significant for predicting DDD, although its value of 0.720 can be considered as

moderate. Again, miR-155 is known to be involved in cell apoptosis in many different tissues and is not specific for IVD, necessitating combination of biomarkers for accurate diagnosis.

These studies indicate that the level of correlation between local and systemic biomarkers depend on the specific marker and may be related to the release of the RNA molecules in the circulation as well as the stability of the RNA. Additional studies will be required with larger cohorts and precisely defined study and control groups to assess the validity of the identified biomarkers for diagnosis of DDD subtypes.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

It is worldwide recognized that finding a biomarker or a set of them that could guide clinicians in their assessments, stratify patients accordingly, and facilitate a more precise and personalized treatment, avoiding a great number of unnecessary surgeries, would represent a revolutionary advance in the spine field.

Although a number of potential biomarkers (Figure 1) of DDD and back pain have been suggested in the studies herein described, some limitations and questions remain to be addressed, and need to be emphasized, such as: (a) variability between the control groups (e.g., painful vs. non-painful IVDs; degenerated vs. non-degenerated) among different studies, that hinders interpretation of the data; (b) lack of correlation between the markers identified in the IVD tissue versus systemic analysis; and (c) biomarker specificity to the DDD, disease stages, and/or response to treatment. In particular, it has been difficult to define a potential systemic biomarker of DDD as a diagnostic tool, since it been difficult to differentiate it from osteoarthritis.[104] In particular, IVD degeneration and facet joint osteoarthritis have common features as destruction of cartilage and other joint tissues, subchondral bone changes, osteophyte formation and reduced joint space. These changes are accompanied of an overlap of molecules related with ECM degeneration, inflammation, oxidative stress, apoptosis, senescence and reduced autophagy, as recently reviewed.[116] In order to define new biomarkers or validate the targets identified so far, future studies should consider standardizing the methodology and results obtained and compared those obtained with osteoarthritis. In addition, new guidelines on imaging analysis combined with systemic biomarkers to improve the classification of degenerated IVDs, would benefit the research in this field.

In this review we focus on clinical biomarkers. Nevertheless, we cannot exclude the contribution of animal models to uncover and validate biomarkers associated with LBP and DDD. Traditionally animal models in the field were considered not so reliable due to their stricking differences compared with human spine, namely its content in notochordal cells and lack of biped position. Moreover, LBP symptoms in human patients often do not reflect the observed degree of IVD pathology, and the variability observed in the patients due to the diversity of causes (injury, infection, disease), comorbidities, genetic,

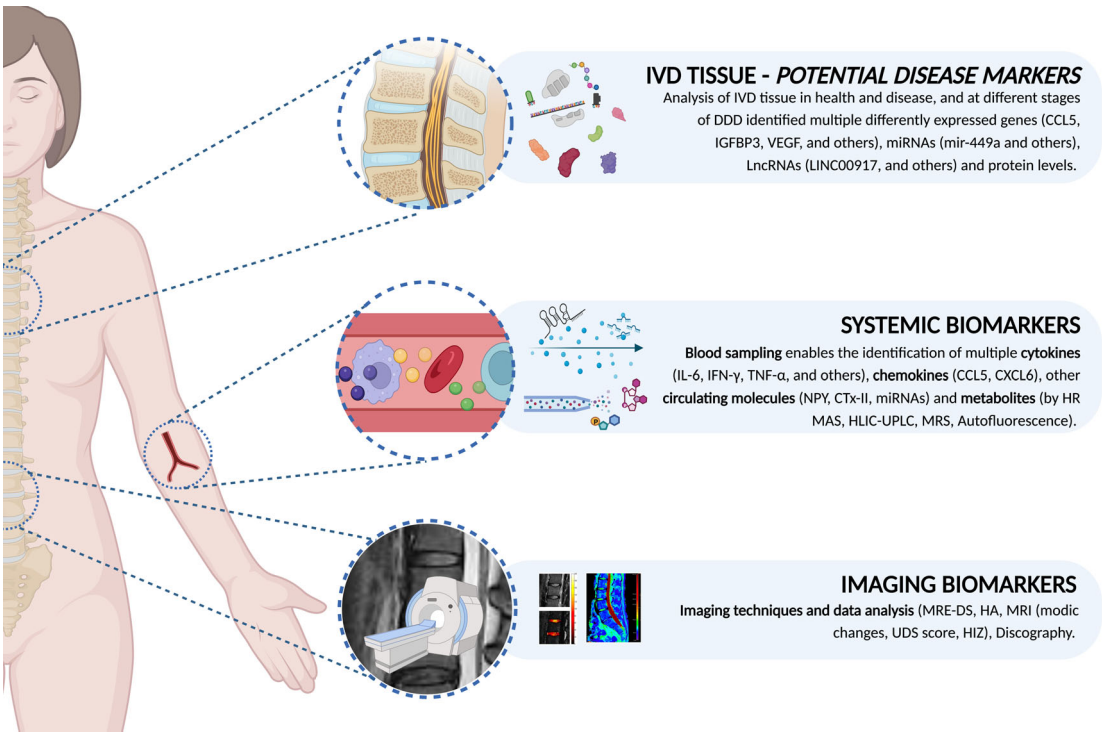

FIGURE 1 Candidate biomarkers of IVD degeneration and associated pain identified through imaging techniques, gene expression, protein analysis, in the IVD tissue or systemically. *Source*: This figure was created with Biorender.com under the Biorender agreement number: MN259AP2SA, and partly adapted from: Mallio et al.[59]

and psychosocial factors that may have an impact on pain perception, has bringing challenges to their replication in animals.[117]

Nevertheless, with the development of verticality-inducing models and the closer mimicking of pain-associated mechanisms, that may include large animals (the most closed to human IVD and LBP) or animals with endogenous pain, novel methods to quantify pain-related behavior (e.g., facial grimace, open-field test or rotarod test) and imaging analysis of central nervous system (e.g., brain MRI), would be a very important area to identify disease biomarkers.[117] Moreover, it is expected that the advances in artificial intelligence to analyze complex data sets using these models would also contribute to use animal models for biomarkers discovery/validation.[118]

Overall, the investment in the identification/validation of new biomarkers would provide: (1) new means of diagnosing IVD degeneration and associated pain by a quantitative approach, as alternative to the current methods (MRI and pain assessment scales); (2) the identification of patients with DDD that might be more prone to develop disc herniation; and (3) help to highlight novel mechanisms to explain the heterogeneity of pain symptoms associated with DDD. Pursuing the identification of sensitive and specific biomarkers will then open new avenues in patient care worldwide and will contribute to spread the field of personalized medicine.

AUTHOR CONTRIBUTIONS

Catarina Leite Pereira and Raquel M. Gonçalves conceived the idea. Catarina Leite Pereira performed the first version of the revision. Sibylle Grad and Raquel M. Gonçalves reviewed the manuscript and updated the revision.

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