INTERVERTEBRAL DISC DEGENERATION:

SYMPTOMATIC AND ASYMPTOMATIC DISTINGUISHERS

A Dissertation

Presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirement for the Degree

Doctor of Philosophy

By

JACOB S. KRAMER

Dr. Aaron Stoker, Dissertation Supervisor

JULY 2023

The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled:

INTERVERTEBRAL DISC DEGENERATION: SYMPTOMATIC AND ASYMPTOMATIC DISTINGUISHERS

presented by Jacob S. Kramer,

a candidate for the degree of Doctor of Philosophy in Pathobiology

and hereby certify that, in their opinion, it is worthy of acceptance.

_____

Professor Aaron Stoker

_____

Professor James Cook

_____

Professor Emily Leary

_____

Professor Theodore Choma

_____

Professor Kei Kuroki

ACKNOWLEDGEMENTS

This work was inspired and guided by the committee, which was composed of an exceptional group of multidisciplinary mentors – Dr. Aaron Stoker, Dr. James Cook, Dr. Emily Leary, Dr. Theodore Choma, and Dr. Kei Kuroki. I have been immensely fortunate to be supported by them during this work.

I especially thank the clinical coordinators at the Missouri Orthopaedic Institute and the Musculoskeletal Transplant Network for their invaluable roles in tissue collection for these projects.

Additionally, thank you to Dr. Rui Zhang and Dr. Bret Ulery for your patience and guidance in the early days.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS ii

TABLE OF CONTENTS iii

LIST OF TABLES vi

LIST OF GRAPHS ix

ABSTRACT xii

CHAPTER 1: LITERATURE REVIEW OF SPINAL STRUCTURE AND PATHOLOGY 1

INTRODUCTION 2

CLINICAL PROFILES OF *S*-IVDD 10

GAPS IN UNDERSTANDING IN S-IVDD DEVELOPMENT 36

CONCLUSION 38

REFERENCES 40

CHAPTER 2: RELATIONSHIPS AMOUNG PATIENT DEMOGRAPHIC CHARACTERISTICS AND THE RADIOGRAPHIC, GROSS, AND HISTOLOGICAL ASSESSMENT OF IVDD IN SYMPTOMATIC AND ASYMPTOMATIC IVDS 64

INTRODUCTION 65

METHODS 70

RESULTS 73

DISCUSSION 75

LIMITATIONS 78

CONCLUSIONS 80

FIGURES 81

REFERENCES 90

CHAPTER 3: RELATIONSHIPS BETWEEN PATIENT CHARACTERISTICS TO SIGNALING PEPTIDES AND MATRIX AFFECTING ENZYMES WITHIN AND BETWEEN ASYMPTOMATIC AND SYMPTOMATIC POPULATIONS 94

INTRODUCTION 95

METHODS 102

RESULTS 105

DISCUSSION 109

LIMITATIONS 112

CONCLUSIONS 114

FIGURES 115

FULL DISSERTATION (D) FIGURES 118

REFERENCES 131

CHAPTER 4: RELATIONSHIPS BETWEEN MACROSCOPIC INTERVERTEBRAL DISC DEGENERATION TO SIGNALING PEPTIDES AND MATRIX AFFECTING ENZYMES WITHIN AND BETWEEN PATIENTS WITH ASYMPTOMATIC AND SYMPTOMATIC INTERVERTEBRAL DISC DEGENERATION 139

INTRODUCTION 140

METHODS 145

RESULTS 148

DISCUSSION 152

LIMITATIONS 157

CONCLUSIONS 160

FIGURES 162

REFERENCES 170

CHAPTER 5: RELATIONSHIPS BETWEEN HISTOLOGICAL IVDD TO SIGNALING PEPTIDES AND MATRIX AFFECTING ENZYMES WITHIN AND BETWEEN ASYMPTOMATIC AND SYMPTOMATIC POPULATIONS 174

INTRODUCTION 175

METHODS 182

RESULTS 185

DISCUSSION 192

LIMITATIONS 195

CONCLUSIONS 198

FIGURES 199

REFERENCES 219

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS 225

VITA 231

LIST OF TABLES

Chapter 1 (Literature Review)

1. Table of Biomarkers and studies relevant to IVDD

Chapter 2

1. Demographic and IVD information for *S*-IVDs or *A*-IVDs
2. *S*-IVD Histology Total and Category Score Distributions
3. *A*-IVD Histology Total and Category Score Distributions
4. *S* versus *A* Model Results
5. *S* or *A* Model Results
6. Summary of Significant Differences Within and Between *S*-IVDs and *A*-IVDs

Chapter 3

1. Demographic and IVD information for *S*-IVDs or *A*-IVDs
2. *S* vs *A* Model Results
3. Ex vivo production of Inflammatory Proteins in *S*-IVDs versus *A*-IVDs
4. Ex vivo production of MMPs in *S*-IVDs versus *A*-IVDs
5. Ex vivo production of TIMPs and Growth Factors in *S*-IVDs versus *A*-IVDs
6. Significant *S* or *A* Model Results
7. Summary of significant effects on AF by Patient Characteristics
8. Summary of significant effects on NP by Patient Characteristics

Chapter 4

1. Demographic and IVD information for *S*-IVDs or *A*-IVDs

2. *S* vs *A* Model Results
3. Summary of Significant *S*-IVDs versus *A*-IVDs ANOVA Results
4. *S* or *A* T/P-Grade ANOVA Results
5. *S* or *A* Tukey HSD Post-hoc Results
6. Summary of significant differences in inflammatory cytokine release
7. Summary of significant differences in MMP release
8. Summary of significant differences in TIMP and growth factor release

Chapter 5

1. Demographic and IVD Information for *S*-IVDs or *A*-IVDs
2. *S*-IVD Histology Total and Category Score Distributions
3. *A*-IVD Histology Total and Category Score Distributions
4. Ex vivo inflammatory cytokine/chemokine release by *S*-IVDs versus *A*-IVDs within TOTAL HDS
5. Ex vivo MMP release by *S*-IVDs versus *A*-IVDs within TOTAL HDS
6. Ex vivo TIMP release by *S*-IVDs versus *A*-IVDs within TOTAL HDS
7. Ex vivo protein release by *S*-IVDs versus *A*-IVDs within CELL-M
8. Ex vivo protein release by *S*-IVDs versus *A*-IVDs within DEATH
9. Ex vivo protein release by *S*-IVDs versus *A*-IVDS within MUCOUS
10. Ex vivo protein release by *S*-IVDs versus *A*-IVDs within TEAR
11. Ex vivo protein release by *S*-IVDs versus *A*-IVDs within GRAN
12. Ex vivo inflammatory cytokine/chemokine release by *S*-IVDs or *A*-IVDs between TOTAL HDS
13. Ex vivo protein release by *S*-IVDs or *A*-IVDs between CELL-M scores

14. Ex vivo protein release by *S*-IVDs or *A*-IVDs between DEATH scores

15. Ex vivo protein release by *S*-IVDs or *A*-IVDs between MUCOUS scores

16. Ex vivo protein release by *S*-IVDs or *A*-IVDs between TEAR scores

17. Ex vivo protein release by *S*-IVDs or *A*-IVDs between GRAN scores

LIST OF GRAPHS

Chapter 2

1. *S*-IVDs vs *A*-IVDs by Histological or Macroscopic IVDD Severity
2. Relationships to Patient Characteristics in *S*-IVDD
3. Relationships to Patient Characteristics in *A*-IVDD

Chapter 3

1. Inflammatory cytokine/chemokine release by *S*-AF versus *A*-AF tissues
2. Inflammatory cytokine/chemokine release by *S*-NP versus *A*-NP tissues
3. MMP Release by *S*-AF versus *A*-AF tissues
4. MMP release by *S*-NP versus *A*-NP tissues
5. Growth Factor and TIMP Release by *S*-AF versus *A*-AF tissues
6. Growth Factor and TIMP Release by *S*-NP versus *A*-NP tissues

Differences within S-IVDD or A-IVDD Cohorts by Patient Characteristics

7. Age in *S*-AF
8. Age in *A*-AF
9. Age in *S*-NP
10. Age in *A*-NP
11. Sex in *A*-AF
12. Sex in *A*-NP
13. Age:Sex in *S*-AF
14. Age:Sex in *A*-AF
15. Age:Sex in *S*-NP

16. Age:Sex in *A*-NP
17. Obesity:Age in *A*-AF
18. Obesity:Age in *A*-NP
19. Obesity:Sex in *A*-AF
20. Obesity:Sex in *A*-NP
21. Age:Sex:Obesity in *A*-AF
22. Age:Sex:Obesity in *A*-NP (1)
23. Age:Sex:Obesity in *A*-NP (2)

Chapter 4

1. Ex vivo inflammatory cytokine/chemokine release by *S*-IVDs versus *A*-IVDs
2. Ex vivo MMP release by *S*-IVDs versus *A*-IVDs
3. Ex vivo TIMP and Growth Factor release by *S*-IVDs versus *A*-IVDs
4. Significant ex vivo Biomarkers in *S*-AF Tissues
5. Significant ex vivo Biomarkers in *A*-AF Tissues
6. Significant ex vivo Biomarkers in *S*-NP Tissues
7. Significant ex vivo Biomarkers in *A*-NP Tissues

Chapter 5

1. Ex vivo release of inflammatory cytokine/chemokine by *S*-AF versus *A*-AF tissues within TOTAL HDS
2. Ex vivo release of inflammatory cytokines/chemokines by *S*-NP versus *A*-NP tissues within TOTAL HDS
3. Ex vivo release of MMPs by *S*-AF versus *A*-AF tissues within TOTAL HDS

4. Ex vivo release of MMPs by *S*-NP versus *A*-NP tissues within TOTAL HDS
5. Ex vivo release of TIMPs and Growth Factors by *S*-AF versus *A*-AF tissues within TOTAL HDS
6. Ex vivo release of TIMPs and Growth Factors by *S*-NP versus *A*-NP tissues within TOTAL HDS
7. Ex vivo protein release by *S*-AF tissues between TOTAL HDS
8. Ex vivo protein release by *A*-AF tissues between TOTAL HDS
9. Ex vivo protein release by *S*-NP tissues between TOTAL HDS
10. Ex vivo protein release by *A*-NP tissues between TOTAL HDS

DETERMININATION OF FACTORS RELATED TO THE SYMPTOMATIC DEGENERATION OF INTERVERTEBRAL DISCS

Jacob S. Kramer

Aaron M. Stoker, Dissertation Supervisor

ABSTRACT

Introduction: It is currently not possible to distinguish symptomatic (*S*) from asymptomatic (*A*) intervertebral disc degeneration (IVDD) based on patient factors or the degree of degeneration. Additionally, it is not known if age, sex, and BMI contribute to the gross/radiographic or histological progression of IVDD. These studies were performed to determine patient, gross/radiographic, histological, and protein differentiators of *S*-IVDD or *A*-IVDD.

Methods: IVD tissues were recovered from 202 *S* clinical patients and 36 *A* tissue donors with IRB Approval (#2010692), informed patient consent, or a legal permit under the Uniform Anatomical Gift Act as appropriate. Age, sex, BMI, Pfirrmann grade, Thompson grade, and histological degeneration scores (HDS) were determined. Each isolated tissue was cultured for 6 days in supplemented DMEM which was collected every 3 days, and tissue culture media were assessed for their content of inflammatory cytokines/chemokines, degradative enzymes (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors. Gross/radiographic grades, histological scores, and ex vivo biomarker data were compared within and between *S*-IVD and *A*-IVDs using multivariable generalized linear models or ANOVAs while adjusting for age, sex, and BMI as covariates with significance set at $p<0.05$.

Results: Overall, *S*-IVDs produced significantly increased levels of inflammatory cytokines/chemokines, specific degradative enzymes, and lower levels of TIMPs compared to *A*-IVDs regardless of IVDD severity. Obesity significantly affected the progression of IVDD in *S* and *A* populations.

Discussion: Obesity and inflammatory cytokines may represent key factors in distinguishing *S*-IVDD versus *A*-IVDD towards earlier identification and symptom mitigation in clinical patients.

CHAPTER 1: LITERATURE REVIEW OF SPINAL STRUCTURE AND PATHOLOGY

Jacob S. Kramer[1,2], Aaron M. Stoker[1,2]

[1]Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO

[2]Department of Orthopaedic Surgery, University of Missouri, Columbia, MO

INTRODUCTION

The spine (spinal column, vertebral column) is an S-shaped combination of bone and soft tissues that physically supports the skeleton and protects the spinal cord while allowing for freedom of movement.[1] The primary structures of the spinal column are the vertebrae and intervertebral discs (IVDs).[2] The vertebrae are bones that form the structural units of the spinal column, protect spinal nerves, and serve as attachment points for ligaments and tendons.[1,3] IVDs are soft tissue transitional structures that flexibly connect superior and inferior vertebrae.[1] The structure of the vertebrae and IVDs contribute to the spine's ability to resist and distribute complex biomechanical forces.[2] The flexible articulation between vertebral bodies is further stabilized by facet joints, which are capsular, articulated joints formed by an adjoining superior/inferior vertebrae pair.[2] Facet joints allow flexibility while limiting translational motion and resultant shear forces in the spine.[4] Rigidity is crucial to protect nerve structures, and flexibility in the torso is equally vital to allow diverse motion patterns for daily tasks.[1,3] Thus, the articulation of vertebral bodies involves a complex interplay between maintaining the S-shaped curve of the spine, allowing passage and projection of the central nervous system and its vascular structures, and allowing freedom of movement in the trunk and neck.[1,3,4]

The human spine is composed of 7 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 4 coccygeal vertebrae that each form either a ventrally concave (kyphotic) or convex (lordotic) curve.[5,6] The kyphotic thoracic spine connects the lordotic cervical and lumbar regions to each other, while the kyphotic sacrococcygeal spine connects the axial skeleton to the hip.[1,5] The alternating curves of the spinal regions results in the development of a non-linear column, which is highly stable and resistant to buckling.[7] The resulting column

acts as an incredibly rigid tension spring, with the interface between the lumbar and sacral spine reportedly able to withstand more than 10kN of compressive force.[8,9] The five spinal regions work in concert to withstand the complex biomechanical forces experienced by the spine during normal activities of daily living.[10]

Structural components of normal vertebrae and intervertebral discs (IVDs)

Each vertebra contains a ventral, large, cancellous cylindrical body (centrum, vertebral body).[11] The vertebral body acts as the main point of articulation between vertebrae, and is the where the IVD connects to the vertebrae.[11] The trabecular bone of the vertebral body features robust vascularization that penetrates the bony endplate and outer layers of the otherwise avascular IVD.[12] The avascular evolution of the IVD has been attributed to the proximity of the IVDs to the central nervous system, which is well established to be sensitive to systemic circulating molecules and immune cells.[13,14] Dorsal to the vertebral body, each vertebrae contains a cortical vertebral arch, which includes two pedicles originating from the vertebral body.[15] The pedicles of the vertebral arch circle around the spinal canal, joining dorsally by two flat laminae to form the spinous processes.[11] The spinous processes emanate inferiorly and posteriorly from the vertebral arch, and serve as anchor points for tendon and ligament connections.[16] Laterally, one transverse process projects from each side of the vertebral arch.[16] Directly superiorly and inferiorly, two articular processes project to adjoin adjacent vertebrae.[2]

Together, the IVD, vertebral bodies, laminae, pedicles, and articular processes form the vertebral foramen, through which the spinal cord and dura mater pass.[2,3,17] The vertebral foramen is the primary protecting structure of the spinal cord, nerve roots, and associated vascular networks.[2,15,17] While the general structure and function of the vertebrae and IVDs

are similar throughout the spine, there is regional variation in the structure of these tissues related to the balance of flexibility and stability required for each section of the spine.[1,3,10,18]

The cervical spine is divided into the craniocervical junction and the subaxial spine, wherein the former allows freedom of movement of the head and the latter provides structural support.[15] The cervical spine experiences the widest range of motion of the three spinal regions (80-90° flexion, 70° extension, 20-45° lateral flexion, 90° rotation).[10] Cervical IVDs are smaller than the thoracic and lumbar IVDs, but are the tallest IVDs relative to the size of adjacent vertebra.[10] The increased relative height of the cervical IVD contributes to the flexibility and range of motion observed in the cervical spine that are required for normal movement of the head.[3,4,19,20] The seventh cervical vertebrae is unique and contains one large spinous process (vertebra prominens) that serves as a transitionary structure to the thoracic spine (mid-back).[17]

The thoracic spine rigidly links the cervical and lumbar spine to each other and provides stability to the trunk via articulation with the ribcage via costal facets, narrow IVDs relative to cervical and lumbar IVDs, and facet joint orientation.[17] Relative to the cervical spine, the facet joints of thoracic vertebrae are also tighter, which limits flexion and extension in the thoracic spine.[17,21,22] The thoracic spine is therefore limited to 50° rotation and 20-45° of flexion, extension, and lateral bending.[17,21,23]

The lumbar spine supports the upper body and features robust process attachment to the larger back muscles (erector spinae, latissimus dorsi).[2] The low back experiences the greatest biomechanical demand compared to cervical and thoracic regions, which is reflected by larger vertebral bodies and IVDs.[1] The lumbar spine is capable of up to 49° of

flexion/extension, 10° of lateral bending, and 7° of rotation.[24] Physiologic loads in the low back are transferred to the legs via the sacrum and sacroiliac joint.[1,25]

The ability of the spine to transmit forces applied during motion is largely owed to the structure and extracellular matrix (ECM) composition of the two main tissue types that comprise IVDs: the annulus fibrosus (AF) and the nucleus pulposus (NP).[26,27] The AF forms the firmer outer rings of the IVD that surrounds the highly hydrophilic softer gel-like NP tissue near the center of the IVD.[28–30] These two tissues are contained between a pair of endplates, which are the most direct nutrition routes for IVD tissues.[28] Each endplate contains a cartilaginous and osseus region, wherein calcification occurs distally relative to the center of the IVD.[16] The macroscopic stability of IVDs relies on the production of crucial materials including collagens and proteoglycans by AF and NP cells, which respectively provide structure and ensure hydration of each tissue in the IVD.[18]

Annulus fibrosus (AF)

The AF contains a concentric series of firm collagenous sheets (lamellae) that lay 30° to the transverse plane of the vertebral body to which they are attached.[31] Each lamellae is composed of primarily collagen I fiber bundles that can range from 150-450 um in thickness depending on spinal level, with directionality rotating 60° between alternating lamellae.[16] The annulus fibrosus contains (20%) proteoglycan content by weight, primarily including aggrecan and versican.[32,33] The AF is rooted in the bony endplate, passing through a chondroid region.[32] The resulting network of interwoven fibrocartilage forms an incredibly rigid cage which exerts inward pressure during axial compression.[32,34] Crucially, the NP tensions the AF during compression, which distributes force equally throughout the volume of the IVD.[3,35]

Nucleus pulposus (NP)

The NP is a highly hydrated structure that is contained within the vertebral end plates and the AF.[29] The ECM of the NP is primarily composed of water (70-90%), small collagen II fibers (20%), and proteoglycans (up to 50%).[35] Aggrecan is the primary proteoglycan found in the ECM of the NP, which is responsible for the hydrophilic properties of the tissue.[29,35] The NP releases higher ratios of collagen II and collagen VI compared to the AF, which are thought to contribute to the gelatinous consistency and properties of the NP.[29,36]

Optimal IVD loading preserves the biomechanical integrity of the spinal column and the IVD itself, as the avascularity of the inner AF and NP necessitate compression mediated nutrient/waste exchange through the endplate.[37] Compression drives water and waste out of the IVD, resulting in higher osmolarity within the IVD.[38] Upon decompression, water and nutrients are drawn back into the IVD by the osmotic gradient, supplying cells deeper in the IVD.[39] The outer AF is supplied directly by blood vessels, which represents the secondary route for nutrient/waste exchange in the IVD.[26,34]

Burden and impact of back pain and symptomatic IVD degeneration (S-IVDD)

Globally, more than 500 million people experience low back pain (LBP) at any given time.[40] Acute back pain is also common, affecting 80% of people at least once in their lifetime.[41] Episodes of nonspecific LBP are widespread, and resolve without direct intervention in 90% of cases.[41] However, non-resolving chronic back pain is a significant clinical issue, and the total population prevalence of chronic LBP in some nations has been reported to exceed 30%.[42,43] The symptoms of chronic LBP are often disabling, and it was estimated that LBP accounted for 65 million years lived with disability (YLD) for patients in 2017.[44] Because the symptoms of chronic LBP can substantially reduce quality of life in patients of all ages, there is considerable need to determine mechanisms that contribute to the development and progression of chronic LBP clinical patients.

While root causes in the development of LBP may vary widely between individuals, disruptions in the IVD and nearby nerve structures are widely considered to be a common factor.[43,45] However, while IVDD is commonly observed in patients with chronic LBP, IVDD occurs as a part of normal age related changes, and many patients with significant IVDD are asymptomatic for LBP.[46,47] [48] Thus, while IVDD may play a role in the development of LBP, it is unclear why some patients develop *S*-IVDD and patients with similar levels of IVDD remain asymptomatic (*A*-IVDD).[46] Therefore, it is widely accepted that the development of *S*-IVDD is a multifactorial process that is likely affected by patient characteristics.[42]

The prevalence of chronic LBP increases with age, is more common in females than males, and is more common in obese than non-obese individuals.[40,49–51] However, it is currently not possible to predict the development and progression of LBP within the

clinical patient population based on patient demographic fators.[42–45] The significant barriers to characterizing the root causes of LBP, and prognosticating the development and progression of LBP in patients, is the similarity in the radiographic and gross presentation of patients with *S*-IVDD and *A*-IVDD, and the considerable patient-to-patient variability in *S*-IVDD development and progression observed in clinical pateints.[45,52–54] Therefore, there is a significant clinical need to determine factors that cause the development of *S*-IVDD in patients to improve the lives of patients with LBP. Because of the considerable patient to patient variability in the development of *S*-IVDD, the causes of *S*-IVDD development and progression are likely due to a complex combination of biological and biomechanical factors that affect biochemical signals released by IVDs and surrounding tissues that contribute to the patient-to-patient variability in the timing, severity, and presentation of LBP.

Therefore, studies are needed to identify patient and tissue characteristics that differentiate patients who develop *S*-IVDD versus *A*-IVDD. Determining if patient factors like age, sex and BMI relate to measures of the physical development of IVDD differently in patients with *S*-IVDD and *A*-IVDD may help to determine enate differences in these patient populations. Further, determining the differences biochemical responses of the tissues of the IVD recovered from patients with *S*-IVDD and *A*-IVDD, and relating those responses to measures of IVDD severity and patient demographics may further clarify clinically important factors that differentiate between patients with *S*-IVDD and *A*-IVDD. The data from these study may allow for the development of novel diagnostic methods to identify patients at risk for developing chronic LBP, allowing for earlier interventions which may improve outcomes and the quality of life for patients. Further, these studies may

identify novel targets for treatment that could be more effective a preventing or slowing down the progression of *S*-IVDD.[55,56]

CLINICAL PROFILES OF *S*-IVDD

It has been reported that 90-95% of low back pain reported in primary care settings have nonspecific causes, regardless of the duration of pain.[57,58] Most cases of IVD-related LBP are caused by herniated IVDs, age related disc degeneration (spondylosis), and/or instability (spondylolisthesis) that result in narrowing of nerve passageways (stenosis) within the IVD.[59,60]

Herniations – Age, Sex, Obesity, Occupation

IVD bulges and herniations typically present as the NP pushing against, and eventually puncturing, the AF.[60,61] This usually occurs in the thinner, posterior aspect of the IVD, frequently compressing nearby nerves.[60,61] However, IVD bulges and herniations are often found incidentally, and more than 85% of patients presenting with symptoms of acute IVD herniation will resolve within 2-3 months without treatment.[61,62] Therefore, six weeks of persistent symptoms from a symptomatic herniation (*S*-IVD herniation) are usually required before diagnostic imaging is performed on a pateint.[62] *S*-IVD herniations are most common in lumbar IVDs and occur at an approximate annual rate of 0.5-2% in human adults, though they are most common in patients between 30-50 years and occur up to twice as frequently in males.[61] Previous patient cohort studies (Spine Patient Outcomes Research Trial (SPORT) and National Surgical Quality Improvement Program (NSQIP) have indicated that *S*-IVD herniation occurs most frequently between L4-S1,with a mean age of 45 years, are 60% males, and have an average BMI of 28.[63 64]

Historically, herniations have been attributed in some way to environmental factors, namely through activities and/or occupations that expose an individual to repetitive loaded flexion, long-term low frequency vibrations, or a sudden, high energy torsion.[65] At least

one case control study found that occupational exertion and higher levels of loaded flexion were significantly related to *S*-IVD herniations with spondylosis, but was not significantly related to *S*-IVD herniation cases without evident spondylosis, indicating age as a potential mitigator for occupation related *S*-IVD herniations.[65] However, there is no literature consensus regarding the impact of any occupation or occupation type on the development of *S*-IVD herniation.[65,66]

Stenosis -Age, Sex, Obesity

The changes observed in lumbar spondylosis and spondylolisthesis cause lumbar stenosis through a loss in stability and alignment of the vertebrae.[16,67] Spondylosis specifically refers to age-related posterior vertebral body osteophyte formation, facet joint hypertrophy, synovial facet cysts, and increased size of the ligamentum flavum.[67,68] Further, spondylosis changes contribute to degenerative spondylolisthesis, wherein alignment between vertebral bodies is lost, resulting in abnormal biomechanical loads in the IVD.[53,69] The gradual degeneration of IVDs forces the thicker, anterior portion of the spinal column to experience increasingly maladaptive biomechanical loads.[67] The subsequent narrowing of nerve passageways including the central canal, lateral foramen, or lateral recess is called lumbar stenosis; however, there is currently no standardized definition of lumbar spinal stenosis or universal diagnostic criteria for the condition.[67] Studies have estimated lumbar spinal stenosis to range in prevalence from 2-13%.[67,70] Patients with symptomatic lumbar stenosis (*S*-stenosis) are a mean age of 68 years, and the rate of *S*-stenosis has been reported to increase with age (4% of people aged 40-49, 12% of people aged 60+).[71,72] However, asymptomatic lumbar stenosis (*A*-stenosis) is a common incidental finding that also increase with increasing age.[48]

It has been reported that *S*-stenosis is more common in women than men, though at least one study has reported that *S*-stenosis was more common in women only if spondylolisthesis was present.[49,67,70,73] Obesity has also long been a factor suspected of affecting the development and/or severity of *S*-stenosis.[74] A nationwide study in Sweden analyzing nearly 350,000 patients found incident rate ratios (IRR) of 2.18 and 1.68 in obese and overweight patients compared to normal patients, and further found that obese patients were in some cases twice as likely to develop *S*-stenosis.[49] However, analyses of the commonly cited SPORT population did not identify a significant relationship between patient obesity and the effectiveness of treatment of stenosis.[75] The variability in the findings of these studies relating patient sex or BMI to the development and treatment effectiveness *S*-stenosis indicates that there may be an interaction between patient age and BMI and the development and treatment of *S*-stenosis in clinical patients.

Genetic Factors in S-IVD Herniation and S-Stenosis

Previous twin and family studies have indicated that patients with a family history of *S*-IVD herniation and/or *S*-stenosis were significantly more likely to develop these pathologies in their lifetime.[76–82] These findings have led to numerous investigations into genetic risk factors associated with *S*-IVD herniation and *S*-stenosis. [76,82,83 81] While the genetic risk factors for lumbar IVD herniation and stenosis are likely complex, many studies have focused on the proteins of the ECM of the IVD.[76,81–83] Since collagen I, collagen II, aggrecan, and versican are the primary structural components of the IVD ECM, these genes have been a focus of many of these studies. [30,76,81–83] However, while mutations of the collagen I and collagen II genes have been found to cause pathogenic connective

tissue changes, the specific contribution of these mutations to *S*-IVD herniation or *S*-stenosis have not been clarified in human clinical pateints.[76 67,76,82]

Overall, clinically meaningful literature examining the effects of specific gene polymorphisms on *S*-IVD herniations or *S*-stenosis is sparse.[76,77,82,84] The bulk of these publications are two or more decades old, which indicates the difficulty in isolating genetic factors for *S*-IVDD. Despite the development and use of broad genetic databases, the range in variability of collagen and aggrecan gene structure is such that many polymorphisms may cause similar effects or none at all.[76,77,82] Even the most recent studies can only conclude what has been long suspected and affirmed by twin studies: that variants in collagen and aggrecan gene structure *likely* affect the development of *S*-IVDD pathologies.[82] However, these conclusions have yet to result in the development of clinical therapies for *S*-IVDD.

Evaluation and Diagnosis

Herniations

An IVD herniation results when the AF weakens, allowing the nucleus pulposus to bulge from within or even exit the boundary of the AF.[60] When this occurs, compression of the thecal sac, spinal cord, and/or cauda equina can occur.[85,86] IVD herniations may occur acutely, though they do not always result in either acute or chronic symptoms.[62] Patients suffering from *S*-IVD herniation often report a burning or stinging pain that radiates and intensifies with activity or sitting.[60,62,87] Herniations can result from injury and as a secondary effect of IVDD, which may not be radiographically evident.[46,88] In the lumbar spine, there are hallmark radicular signs of nerve lesions commonly resulting from *S*-IVD herniations at each lumbar level.[60,62]

Lesions in the L1 nerve typically feature inguinal pain and/or numbness/tingling (paresthesia) without hip flexor involvement and without stretch reflex disruption.[61] Lesions in mid-lumbar nerves (L2-L4) frequently present with back pain that radiates to the anterior/medial thigh and lower leg, which may be accompanied with paresthesia.[60] A defining feature of herniations in the L2-4 nerves is weakness in hip flexion, hip adduction, knee extension, and the patellar reflex.[61,62] Herniations in the lowest lumbar IVDs (L4-S1) commonly present with back pain and sensory loss radiating into the buttock, thigh, lower leg, and foot.[61] Herniations in the L4-S1 region may also affect the S1 nerve, which triggers similar symptoms to higher lumbar herniations with the addition of incontinence and sexual dysfunction in some cases.[61]

Stenosis

The gradual wear of IVDs, instability and misalignment (degenerative spondylolisthesis), facet joint arthrosis, and hypertrophy of the ligamentum flavum each contribute to the manifestation of stenosis.[71] Lumbar stenosis is categorized by the location and cause of the thecal sac/nerve root compression which can occur in the central canal, lateral recess, lateral foramen, or outside the foramen (extraforaminal).[67] Central canal stenosis is often a consequence of anterior hypertrophy of the ligamentum flavum and posterior IVD bulging.[67] Stenosis in the lateral recess is usually a result of facet joint arthropathy, wherein the superior articular facet forms osteophytes.[67] Foraminal stenosis is primarily associated with a loss of IVD height, foraminal protrusion, or uncinate shaped osteophytes.[67] Stenosis can also occur when IVDs herniate far laterally, which results in extraforaminal nerve root compression.[67]

Patients suffering from *S*-stenosis often experience asymmetric pain in the legs during extended ambulation/standing and lumbar extension, with numbness and tingling that usually affects the entire limb.[67] Additionally, neurogenic claudication (or pseudo claudication) is a common feature of lumbar stenosis, wherein muscles of the legs fatigue quickly with activity due to restricted nerve supply, which simulates blood restriction to the muscle; approximately 50% of patients with *S*-stenosis experience muscle weakness.[67]

Diagnostic Imaging

Low back pain has diverse causes including muscle spasms, IVD protrusions, discitis, osteomyelitis, or malignancies.[89] The variety and frequency of back pain causes and complaints requires diagnostic imaging methods to deduce these causes. Methods including x-ray, computed tomography (CT), magnetic resonance imaging (MRI), and provocative discography have been used in differential diagnoses for back pain.[89] Among these, x-ray, MRI and CT imaging are the most commonly utilized clinical means to evaluate patients with *S*-IVDD symptoms.[70,71]

Lumbar radiographs are often used as an initial method to evaluate a patient with LBP because of the relatively low cost and ease of access.[90] This method captures details of the bony structure and anatomy of the spine, but does not directly image soft tissues like the IVDs.[90] Dynamic and/or weight bearing radiographs are often utilized to make a diagnoses (such as listhesis and/or hypermobility) that may only become evident with activity.[90] Further, x-rays can also be used to identify uncinate osteophytes, IVD space narrowing, and flexion/extension angle changes often indicative of chronic IVDD.[90] However, since IVD injuries/defects are difficult to assess using x-rays; this method is most suitable for evaluating alignment of the vertebrae during earlier stages of IVDD.[90]

Computed tomography (CT) scans are less commonly used than MRI or x-rays in routine diagnoses of LBP.[90] CT scans can be used to confirm findings seen on initial x-ray assessment, but alone are limited in their diagnostic power.[90] In cases where specific defects (pars defects, spondylolisthesis, osteophyte formation, endplate sclerosis, vacuum disc sign) are suspected, CT can be a useful mode of lumbar spine imaging.[90] CT scanning may be additionally used to visualize the spinal canal and available clearance for its associated nerves.[90]

Magnetic resonance imaging (MRI) represents the gold-standard diagnostic imaging method for tissue defects associated with *S*-IVDD.[90] T2 weighted MRI imaging is capable of visualizing the entire IVD and nearby nerve structures, as well as the hydration of the IVD.[90] Physicians quantify radiographic *S*-IVDD using the T2 weighted MRI based Pfirrmann grading system, which assigns a score of 1-5 to an IVD.[91] Increasing Pfirrmann grades indicate reduced IVD homogeneity/reduction in T2 signal intensity, narrowed IVD space, and a loss of distinction between the AF and NP.[91] A study published by Yu et al. assessed radiographs from 108 *S*-IVDD cases and observed Pfirrmann grade 3, 4, and 5 changes in approximately 13%, 75%, and 12% of *S*-IVDs, respectively.[92]

Discography is a method that utilizes the injection of a contrast dye into the IVD space that is then visualized using x-ray imaging.[90] Provocative discography can be used to evaluate patients that have radicular pain or claudication but do not have radiographic changes observed using other methodologies.[90] This method is intended to reproduce and visualize the physical source of a patient's symptoms.[93] This is achieved by injecting a suspected and adjacent IVDs with a volume of contrast dye and subsequently x-ray imaging the spine.[90] The physician records the subjective pain response of the patient to dye

injection in each IVD, the volume of dye injected in each IVD, the morphology of each injected IVD in the x-ray, and lack of pain response in adjacent control injected IVDs.[90] These measures are then used to identify the IVD responsible for the patients symptoms.[90] Provocative discography is the sole imaging modality capable of differentiating *S*-IVDs from *A*-IVDs within a patient.[90] However, because non-contrasted x-ray, CT, and MRI imaging are less invasive, they are more commonly used for diagnostic visualization of lumbar IVDs.[58,94]

Nonsurgical Treatments

Because many *S*-IVD herniations resolve without the need for surgical intervention, nonsurgical management is the first treatment option for most patients.[61,62,95] For patients with *S*-stenosis, non-surgical treatment strategies are indicated for patients with mild or moderate radicular signs or claudication.[71,96] The most common nonsurgical treatment options for herniations and stenosis include physical therapy, medications, and injections.[97]

Herniation

Physical therapy (physiotherapy) is a commonly recommended option for the nonsurgical management of *S*-IVD herniation due to its non-invasive nature and potential improvements in muscular strength and coordination that may contribute to the alleviation of herniation symptoms.[95,98,99] Typically, a physical therapy treatment regimen is initiated at least three weeks after symptom development.[61] Physical therapy regimes aimed at treating *S*-IVD herniations typically include a combination of aerobic exercises (walking and cycling), directional preference strategies (yoga and stretching), coordination drills, and any combination of other exercises intended to improve motor control or muscular strength.[100]

The conceptual foundation justifying physiotherapy involves the idea that appropriate muscular recruitment and strength in the torso translates to spinal stability, which ideally reduces undesirable biomechanical loads on the IVDs and nociceptive tissues.[1,99,101] Motor control exercises are commonly employed with the intent of gaining or regaining the ability to appropriately recruit abdominal, paraspinal, gluteal, and pelvic floor muscles to improve movement patterns and reduce pain.[99]

In a meta-analysis conducted in 2021 of eleven randomized, controlled trials, the authors concluded that physiotherapy can be reliably associated with significant decreases in pain and disability in *S*-IVD herniation.[99] However, five of the eleven analyzed studies showed no statistically significant differences between *S*-IVD herniation patients who received physiotherapy compared to those who did not.[99] However, because there is not a consensus on the optimal physical therapy protocol used to treat patients with LDH, there is considerable study to study variability in the physical therapy protocol used to treat patients with LDH, which makes it difficult compare data and outcomes between studies. While the data have not yet established a consensus on the correct protocol or timing to treat LDH patients using physical therapy, they do consistently provide statistical support for the use of physical therapy as a prerequisite or in combination with surgical intervention for LDH with case-by-case discretion by physicians.[53,95,98,99,102–105]

The conservative treatment of LDH to address patient symptoms typically involves a combination of physical therapy and medication use by the patient. The most commonly utilized medications in nonsurgical management of *S*-IVD herniations include nonsteroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase (Cox) inhibitors, narcotics, and muscle relaxants.[95,98] However, there is a lack of high quality evidence supporting a statistically significant improvement in metrics of LDH patient outcomes based on the implementation of any of these medications.[106]

Injections are another non-surgical treatment option for patient with LDH. These typically include an analgesic and/or corticosteroid with the intent of reducing the presence or activity of pro-inflammatory cytokines and/or degradative enzymes, thereby reducing inflammation related pain.[107] High quality evidence indicating a statistically supported

benefit in the implementation of injections for the management of *S*-IVD herniation is rare.[106] A prominent challenge in delineating the efficacy of epidural injections in comparison to surgical interventions (discectomy) is the high rate of injections received by patients who eventually have surgery.[102,107] This crossover is difficult to avoid, making it burdensome to distinguish outcomes related to injection vs surgery related outcomes.

Stenosis

While the progressive degeneration of spinal tissues observed in lumbar stenosis is difficult to mitigate, the role of posture, muscular strength, and activity is thought to be related to tissue pathology and symptom development.[3,108] The goal of physical therapy for conservative management of *S*-stenosis is to re-establish appropriate muscular strength and coordination in the trunk and legs such that appropriate stabilization may prevent additional unwanted biomechanical strain on herniated or stenosed tissues.[3,109] While large, systematic reviews are not yet robust enough to offer clinical guidelines, stenosis is subjectively considered to respond favorably to conservative management and particularly physical therapy.[67,70,71,96,110] Physical therapies employed to relieve stenosis symptoms include isometric and stretching exercises, postural modification, muscle strengthening, endurance training, and ambulation/stabilization exercises.[109] The diverse methods of physical therapy and their nonstandard prescription for LSS has made it difficult to discern the effectiveness of any single method or type.[109]

Patients with lumbar spinal stenosis are often prescribed analgesic, anti-inflammatory, steroidal, and/or muscle relaxing drugs upon initial patient complaint.[70,110] Due to the potential long-term side effects of NSAIDs and acetaminophen, muscle relaxants including gabapentin and pregabalin have become favorable for long term

management of neuropathic pain.[110,111] Despite their prevalent use, the long-term effects of these oral medications on LSS symptoms have not been conclusively established.[71,112,113]

Injections represent an intermediately invasive option for patients with *S*-stenosis. The rationale for their use is founded upon the widespread consensus that inflammatory molecule production is increased in stenosed tissues and affected nerves and vascular networks, wherein a strong, local agent could mitigate these factors.[67,71,96] While several combinations of injection components (usually a corticosteroid and an analgesic) have been used, none have shown quantitative, clinical efficacy in mitigating long term LSS symptoms.[107,114]

Surgical Treatments

Herniations

In patients who are not responsive to conservative management for lumbar IVD herniations/protrusions, lumbar discectomy is the standard-of-care for symptom resolution.[115,116] Each type of discectomy aims to eliminate the immediate source of nerve disruption by removing IVD material that is compressing nerve fibers.[115] Patients undergoing discectomy generally experience symptom remission after surgery.[107,115,117] Procedures performed with (microdiscectomy) or without (open discectomy) magnification represent the two categories of discectomies.[115] However, microdiscectomies (MD) and microendoscopic discectomies (MED) are the standards-of-care for lumbar disc herniations due to reduced epidural fibrosus, segmental instability, and postoperative pain compared to open discectomies.[118] In a randomized clinical trial study analyzing outcomes of MD vs MED procedures, significantly less pain was only observed in the very short term (<3 months) in MED patients.[119]

Most *S*-IVD herniations resolve conservatively, though it is not uncommon for symptoms to reemerge from subsequent IVDD and chronic nerve root compression.[62,120] It has been reported that 85-90% of patients with *S*-IVD herniations see symptom resolution within 3 months without notable intervention, though the likelihood of resolution decreases after symptoms persist for 6 weeks.[62] If patients require surgery, intuitive factors such as preoperative pain level, symptom duration, age, mental health, and preoperative activity level are the most consistent predictors of post-surgical success.[61,62,121]

Stenosis

Surgery is indicated in cases of *S*-stenosis that does not respond to conservative treatment within three months.[122] While stenosis is related to IVDD, the source of nerve compression in stenosis is a narrowing of nerve passageways, rather than the IVD itself causing nerve compression as in herniation cases.[62,67] Therefore, decompression treatments for *S*-stenosis commonly include removal of bone from the lamina of the vertebral arch (laminectomy, laminotomy) or the vertebral foramen (foraminotomy) in order to increase the available space for compressed nerve structures.[110,113] However, because *S*-stenosis patients typically present with a combination of spondylosis, spondylolisthesis, facet joint arthrosis, and IVDD, the further removal of bone introduces notable instability in most cases.[110,122] Therefore, surgical correction for *S*-stenosis commonly includes laminectomy/laminotomy or foraminotomy in combination with an appropriate fusion procedure.[71]

The core purpose of a fusion procedure is to correct instability in vertebral segments in order to restore optimal biomechanical loading patterns to the greatest possible extent.[67,123] A fusion is performed by first removing the degenerative IVD and preparing

the endplate for implantation of a cage, spacer, or bone graft.[124] Success of the fusion hinges on the growth of trabecular bone of the former IVD space and integration of the bone with the implant.[124] The most common lumbar interbody fusion approaches include posterior, transverse, oblique, and anteriorly initiated procedures.[124] Each of these approaches is indicated based on patient pathology wherein specific anatomic features might be avoided or more easily accessed by the surgeon.[124] Investigators have not conclusively established high levels of evidence to support improved long-term outcome differences between surgically and non-surgically treated stenosis patients.[122] For patients failing three months of conservative treatment, decompression and fusion is related to significantly improved short and mid-term outcomes.[122,123]

Histological Features of IVD Tissues

Histological features of IVD tissues broadly include assessments of cellularity, lesions, and extracellular matrix structure.[125,126] The tissue types comprising the IVD unit include the bony (osseus) and cartilaginous (hyaline) endplates, the highly collagenous annulus fibrosus, and the hydrophilic nucleus pulposus.[18,30,127] Specific cell types and tissues develop within an IVD unit that are uniquely adapted for cyclic loading in a highly osmolar, hypoxic environment.[128–130] The tissues comprising the IVD each serve distinct roles and subsequently feature cells with distinct lineages and/or matrix properties.[29,32,33,36,131,132]

Bony and cartilaginous endplates

The endplates of the IVD each contain a bony (BEP) and hyaline cartilage (CEP) region that integrate with the AF and NP of the IVD.[133] The hyaline cartilage layer is arranged continuously with the lamellae of the inner portions of the annulus fibrosus.[134] The outermost fibers of the AF are anchored in the cancellous vertebral bone.[134] Blood vessels and nerves from the superior and inferior vertebral bodies extend through the endplates, however their capillary nets penetrate only the outer third of the AF.[135] Nutrients from the blood must diffuse through the endplate and through the outer layers of the IVD to supply glucose, while compression and relaxation act to dispel waste and drive water into the IVD, respectively.[26] With age and/or pathology, both the bony and cartilaginous endplates undergo substantial tissue changes.[126]

Bony Endplate Histopathological Changes

In the bony endplate of the IVD, normally hematopoietic marrow can become fatty and/or develop zones of fibrovascular tissue.[126] Less degenerative BEPs feature these bone

changes primarily at sites of CEP damage, while more severe degeneration results in fatty or fibrovascular material to extend variably into the vertebral body.[126,133] Additionally, neovascularization through the subchondral bone and endplate may be observed.[126] The degeneration of the bony endplate is implicated in impaired biomechanical loading as well as impaired nutrition/waste exchange in the IVD.[126,136,137]

Healthy IVDs feature a clear distinction between the bony and cartilaginous endplates, and this demarcation is gradually lost with age and/or degeneration.[126,137] In the most severe cases, large defects in the endplate allow substantial IVD material to enter the vertebral body.[126] Osteophyte formation is also observed as a degenerative change in the bony endplate, wherein bone forms in an uncinate shape around the AF.[126,137]

The osseus endplate of healthy IVDs contain thin plates of subchondral bone with distinct trabeculae.[126] With age and degeneration, this bone can thicken (sclerosis), impairing the biomechanical properties and nutrient transport of the IVD.[126,133,138] Further, it is not uncommon for the hydrostatic pressure of the NP to wear away the superior and inferior endplates (Schmorl's nodes), in which case the formation of fibrocartilage within the vertebral bone defect is common, and increases in severity with the size of the defect.[126]

Cartilaginous Endplate Histopathological Changes

Healthy IVDs with nondegenerative CEP tissues feature no nuclei condensation and single chondrocytes within a single lacuna. As the IVD ages and degeneration increases, there is an increase in the proliferation of the chondrocytes in the CEP.[126] At earlier stages of degeneration clone pairs of chondrocytes within a lacunae along with mild condensation of nuclei, reduced cell size (apoptosis), cellular debris (remaining from necrosis), or large, irregular cells (senescence) can be observed.[126] In more severe

degeneration of the CEP, increasingly large clone clusters can be observed with increasing evidence of apoptosis, necrosis, senescence, and neovascularization.[126]

Lesions in the CEP can be observed as irregularities (or loss of thickness) in the hyaline cartilage ranging from minor superficial zone defects to partial/complete separation of the CEP from the BEP (avulsion).[126] Varying thickness fissures and/or erosion of the CEP also progress with degenerative severity.[126] With degeneration, the CEP thins and can become calcified or experience the ingrowth of fibrocartilage that increases in abundance with increasing degenerative severity.[126]

Annulus Fibrosus Histopathological Changes

Normal AF cells are fibroblast like and have an elongated shape in the outer lamellae and gradually become more chondrocyte like (rounded) in the inner AF.[126] With degeneration, the morphology of AF cells becomes increasingly irregular, with progressive loss of the demarcation between inner and outer AF cells or loss of cellularity in more severe cases.[126] Further, apoptotic/necrotic cells and vascular ingrowth becomes more prevalent in degenerative AF tissues. In the most severely degenerated tissues, senescent cells and/or vessel invasion of the inner AF may be observed.[126]

Nondegenerative AF tissues have clearly defined lamella and a distinct region of transition from the bony endplate.[126] Additionally, AF tissues are clearly distinct from the NP and endplates with a convex IAF. During the development of IVDD, fissures of increasing size between and then across lamella can be observed, and the demarcation between lamellae of the AF and tissues of the IVD is gradually lost. Further, convexity of the IAF is lost as IVDD progresses.[126] In severely degenerative tissues, the fiber structure of the AF is lost and avulsion of the AF from the enthesis can occur.[126]

Nucleus Pulposus Histopathological Changes

Healthy NP tissues include single cells in each lacuna without substantial apoptosis/necrosis or senescence. As degeneration progresses, an increasing proportion of the NP cells cluster together with increasing prevalence of cell death and senescence.[126] In the most degenerative NP tissues, cells become highly proliferative, resulting in marked hypercellularity.[126] Similar to the AF, fissures and tears can form in the NP with degeneration. These defects increase in size with increasing degenerative severity.[126] Nondegenerative NP tissues exhibit consistent eosin staining without ordered collagen fibers and with clear distinction from the AF and endplates.[126] As degeneration progresses, reduced eosin staining and fiber formation are observed in the NP.[126] Eosin staining is weak throughout severely degenerative NP tissues and accompanied by substantial fibrosis and/or mucoid degeneration.[126]

Histopathological Scoring for IVDD

A detailed histologic IVDD grading scheme was published by Boos et al. in 2002, which assigned a total histologic degeneration score (HDS) of 0-22 based on presence and/or severity of 11 features of histological IVDD in sagittal *A*-IVD sections or *S*-IVD explants.[139] In that study, Boos et al. observed a significant correlation between total HDS, age, and *A*-IVD T-grade.[139] While their study included surgical samples (n=23 patients), the authors concluded that histological grading of complete IVD sections was more accurate due to potential loss of granular changes and difficulty assessing cell death accurately in *S*-IVD tissues.[139]

Drs. Christoph Weiler, Norbert Boos, and Andreas Nerlich streamlined their histological IVDD grading scheme in 2011 to include 4 histological features that showed

the greatest agreement in their original study: AF/NP cell morphology (0-6), mucous degeneration in the AF (0-3), tear and cleft formation (0-3), and granular changes in the NP (0-3), resulting in total HDS scores ranging from 0-15.[140] This system is sometimes further modified to include cell death scoring (0-4), which raises the highest possible total HDS to 19.[139,141] Increased scores indicate AF/NP cell proliferation and aggregation (cell morphology), loss of AF fiber demarcation and excessive proteoglycan staining (mucous degeneration), ruptures in the AF/NP matrix (tear and cleft formation), collections of eosinophilic staining proteins in the NP matrix (granular changes), and the presence of apoptotic bodies and/or ruptured cells (cell death).[140] In their 2011 study, Weiler et al. examined *S*-IVD specimens from 854 patients and reported significantly higher scores in NP (total HDS ranging from ~10-12) compared to AF (total HDS ranging from ~7-9) tissues and similar scores across ages in both AF and NP tissues.[140]

A review of histological IVDD grading schemes published in 2021 by Le Maitre et al. reported that the streamlined Weiler, Boos, and Nerlich grading scheme has not been used to analyze sagittal lumbar *A*-IVD sections.[126] While a reduced number of criteria were included in the scheme published by Weiler et al. in 2011, there was no statistical relationship between age and histological *S*-IVDD, potentially indicating that age-related changes in histological IVDD are more common in *A*-IVDs than *S*-IVDs.[140]

Cellular and Molecular Behaviors of IVDs

The maintenance of IVD cells requires a complex and sensitive molecular balance between inflammation, anabolism, and catabolism.[134,138,142–146] These regulatory functions are carried out by signaling cytokines and chemokines, matrix degrading enzymes, and growth factors.[134,147,148] A broad summary of the role of each of these proteins in the development of *S*-IVDD or *A*-IVDD is provided in Table 1.

Inflammatory IVD Signaling

IVD cells exhibit distinct molecular signals in response to normal and pathological stress, damage, and degeneration.[147] These signals initially take the form of small peptides called cytokines that carry out diverse and tissue specific functions.[33,149] Cytokines can attract and stimulate immune cells to produce more cytokines, chemokines, or other molecular agents intended to maintain homeostasis.[14] An imbalance in the production of these inflammatory factors has been found to induce variable cellular effects including autophagy, apoptosis, and senescence in AF and NP cells.[147,150] It is well recognized that an aberrant inflammatory cascade contributes to the development and progression of IVDD.[147] The most commonly studied secreted inflammatory cytokines and chemokines related to the development and progression of *S*-IVDD and/or *A*-IVDD are tissue necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-17, interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1 (CCL2), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES) (CCL5), and MCP-3 (CCL7).[147]

Inflammatory cytokines are produced and released by IVD cells as signaling agents for homeostasis.[147,151] Chemokines respond to inflammatory cytokines to promote or

inhibit the movement and activity of immune cells.[147,151] An imbalance in inflammatory mediators has been found to induce variable cellular effects including immune cell infiltration, autophagy, apoptosis, and senescence in AF and NP cells.[147,150] Excessive inflammation contributes to the development of *S*-IVDD, though inflammatory mediators also regulate homeostasis in *A*-IVDs.[152] Previous studies and reviews have reported significantly increased expression and production of numerous inflammatory cytokines/chemokines with increasing IVDD, though the direct comparison of *S*-IVDs versus *A*-IVDs is somewhat rare.[147,152–156]

A review published by K. Wuertz and L. Haglund in 2013 included twenty studies analyzing expression, production, or in situ localization of inflammatory cytokines in *S*-IVDs and/or *A*-IVDs.[152] In their review, two studies statistically compared inflammatory cytokine presence between *S*- versus *A*-AF or NP tissues: Shamji et al. in 2010 reported significantly increased immunohistochemical (IHC) staining of IL-17 in *S*-IVD versus *A*-IVD tissues; Weiler et al. in 2005 reported significant, positive correlations between TNF-α IHC staining, histological IVDD, and age in *S*-IVD and *A*-IVD tissues.[155,157] *S*-IVDs have also been shown to express increasing levels of chemokines MCP-1, MCP-3, MIP-1α, MIP-1β, RANTES, and IL-8 with increasing degeneration.[149,158,159] Low levels of TNF-α, TNFR1, and TNFR2 expression have been observed in non-degenerated animal IVDs, indicating the involvement of TNF as a regulator of pathological processes in the IVD.[160] Further, when comparing TNF staining across histological IVDD severity scores, it was found that histologically normal tissues exhibited significantly decreased TNF expression compared to histologically degenerative IVD tissues from a population of *S*-IVDs and *A*-IVDs.[161] Histologically, across age, *A*-IVD tissues have been found to express higher levels

of TNF in older individuals, indicating that the inflammatory activities mediated by TNF may change in the IVD over time.[162] At least one immunohistochemistry (IHC) study found significantly increased immunopositivity for IL-1α/β with increasing histological degeneration in *S*-IVDs and *A*-IVDs.[163] Therefore, current literature supports a role for inflammatory cytokines and chemokines in the development of *S*-IVDD.[147,151,152]

Degradative Enzymes and their inhibitors

Degradation of damaged extracellular matrix components is among the most important biological functions of a cell and/or tissue. The communication of the need for matrix degradation is carried out by cytokines and chemokines in concert with other complex biological mechanisms.[147,164] Specialized enzymes each with unique substrate kinetics are responsible for cleaving damaged extracellular matrix components (i.e. collagen, aggrecan) as a normal part of tissue maintenance.[165] Matrix degrading enzymes include the metzincin matrix metalloproteinases (MMPs) and the disintegrin/metalloproteinases with thrombospondin motifs (aggrecanases, ADAMTS).[165,166] Most members of these enzyme subfamilies can bind multiple ligands due to their variable collagenase, gelatinase, stromelysin, and aggrecanase activities.[165,167] The most studied matrix degrading enzymes in the IVD include MMP-1, 2, 3, 13, 14, and ADAMTS-4/5.[147,165] Each of these enzymes is produced in an inactive (latent, pro) form that must be disrupted in order to become active.[168] Briefly, pro-MMPs are sustained by the electrostatic attraction between a highly conserved cysteine thiol group and a histidine linked zinc ion within the proteinase.[168] For the pro-MMP to become active, this association must be disrupted, freeing the catalytic domain of the proteinase to associate with a ligand.[168] Additionally, MMPs are regulated by the production of tissue inhibitors

of matrix metalloproteinases (TIMPs), which prevent protease activity through irreversible binding.[164,165] A cycle of inflammation and imbalances in MMP/TIMP levels in the IVD are thought to contribute to excessive degradation and fibrotic tissue formation, though the roles of specific MMPs in the development of *S*-IVDD have not been precisely established.[164,169–171]

A review published by Wang et al. in 2015 reported that six studies examining MMP expression, production, or in situ localization/activity in human IVD tissues, two studies included tissues from *S*-IVDs and *A*-IVDs, and no study included in the review delineated AF and NP tissues.[172] The two studies comparing *S*-IVD versus *A*-IVD tissues observed significant increases in MMP-8 and MMP-10 mRNA expression in *S*-IVDs.[169,173] Within *S*-IVDs, Weiler et al in 2002 reported significant, positive correlations between IHC staining for MMPs-1, -2, -3, and total histological degeneration scores (HDS).[174] Le Maitre et al. in 2004 additionally reported no observable MMP-3 or MMP-13 IHC staining in histologically non-degenerative IVDs, though *S*-IVDs and *A*-IVDs were not differentiated.[148] Roberts et al in 2000 reported significantly increased MMP-3 and MMP-7 IHC staining with increasing *S*-IVDD, and further that *S*-IVDs and *A*-IVDs demonstrated consistent and similar TIMP-2 IHC staining, though TIMP-1 staining was present in *S*-IVD samples but not in *A*-IVDs.[167] When assessing *S*-IVD mRNA expression of TIMPs, Bachmeier et al. in 2009 found significantly increased expression of TIMP-1 and TIMP-2 in *S*-IVD tissues with higher total HDS.[169] However, Deng et al. in 2015 found no significant difference in TIMP-1 in *S*-IVD versus *A*-IVD tissues.[175] Other studies have found increasing levels of ADAMTS-4 with increasing degenerative severity while ADAMTS-5 did not exhibit this pattern.[176,177] Taken together, the current literature

characterizing the role of MMP/TIMP balance in the development of *S*-IVDD or *A*-IVDD is incomplete and sometimes conflicting.[167,169,172,175] Regardless, the balance between catabolism and anabolism is considered particularly important in the development of *S*-IVDD.[161,178,179]

Growth Factors

While the degradation of ECM components is crucial, promoting appropriate cell proliferation and subsequent ECM deposition is vital in the cycle of tissue maintenance.[134,180] Growth factors are able to activate immune cells, promote or inhibit cell proliferation and matrix synthesis, and stimulate the production of inflammatory cytokines, chemokines, and MMPs.[180] However, the imbalanced inflammation and catabolism of a degenerating IVD contributes to the altered production of anabolic growth factors.[146,181] This catabolic/anabolic imbalance is evident in the degenerating IVD by simultaneous matrix destruction, fibrotic tissue formation, and blood vessel/nerve ingrowth, which are each governed in part by growth factor production.[135,182] The clinical scope and specific effects of neovascularization and innervation on *S*-IVDD have not been fully characterized, but have been associated with the degenerative radiographic, gross, and histological IVDD changes that have been previously discussed.[183] Thus, the modulation of growth factors is often studied as a potential therapy for IVDD with the intent of inhibiting excessive matrix degradation while also preventing pathological vascularization and innervation.[184,185]

Commonly studied growth factors in the IVD include platelet derived (PDGF), vascular (VEGF), transforming growth factor β (TGF-β), insulin-like growth factor (IGF), bone morphogenic proteins (BMP), and fibroblast growth factor (FGF).[186] These growth

factors are each expressed and produced in both *S*-IVDs and *A*-IVDs, indicating to many investigators that anabolic signaling by IVD cells may be modulated to a non-pathological range.[181,186] Many animal studies have found that treatment of IVD cells (especially NP) with growth factors can increase the production of desirable ECM components including collagen II and aggrecan.[186] However, these studies commonly report cell proliferation, which must be carefully balanced in the IVD.[187] The application of growth factors as a treatment for *S*-IVDD has been hindered by the potentially unintended anabolic effects that growth factors may induce despite reducing degradation.[186]

Paglia et al. in 2016 reported significantly decreased cell death and matrix degradation following PDGF intradiscal injection in a preclinical rabbit model of IVDD.[188] In a bovine IVDD model testing the effects of FGF2, Li et al. in 2008 reported suppression of excessive proteoglycan production and dose dependent modulation of MMP-13 expression and production.[189] VEGF has been investigated due to its role in the proliferation of blood vessels, which has may be a significant factor affecting IVD nutrient access.[190] While *S*-IVD tissues have been shown to produce VEGF, the presence of blood vessels in VEGF positive *S*-IVD tissues is not consistently observed.[191,192] To the authors' knowledge, no published studies have directly compared growth factor expression and/or production between *S*-IVD versus *A*-IVD tissues.[181,193,194]

Broad Overview of IVDD Biomarker Literature

Biomarker Category	Biomarker	Other names	Producing Cells/Tissues	Biological Roles	Literature Relation to Disc Degeneration
Pro-Inflammatory	**GRO-α**	CXCL1, neutrophil-activating protein 3 (NAP-3), KC.[195(p1)]	Macrophages, neutrophils, epithelial cells.[196(p)]	Neutrophil chemoattractant, growth.[196,197]	Expression of CXCL1 upregulated in degenerative IVDs.[198] Cytokine stimulation upregulates CXCL1 expression in nucleus pulposus cells.[151]
	MCP-1	CCL2, small inducible cytokine A2, GDCF-2, SMC-CF.[199]	Monocytes, macrophages, dendritic cells.[199]	Basophil and monocyte chemoattractant, modulator of immune cell activity.[200]	Mast cell/IVD interactions mediated in part by CCL2.[178] Cytokine stimulation modulates annulus cell production of CCL2.[201]
	MCP-3	CCL7, FIC, MARC, NC28, SCYA6.[202]	Monocytes, macrophages.[202]	Macrophage chemoattractant, leukocyte activation, *in-vivo* substrate of MMP-2.[203]	Greater production of MCP-3 in systemic fluids of stenosis compared to herniation patients.[204]
	IL-1β	Leukocytic pyrogen, leukocyte endogenous mediator, mononuclear cell factor, ILF2.[205]	Activated macrophages, lymphocytes, neutrophils, fibroblasts, endothelial cells.[205,206(p1)]	Cell proliferation, differentiation, apoptosis, inflammation.[207]	IL-1β produced by degenerate IVDs, stimulation with IL-1β affects catabolic anabolic balance in IVD.[151,206,208(p1)]
	IL-6	CDF, HGF, HSF, BSF2.[209(p6)]	T-cells, B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells.[210]	B-cell stimulation, hepatocyte stimulation, antiviral activity, platelet activation.[210,211]	Elevated IL-6 expression associated with disc degeneration[212], serum IL-6 levels may differentiate between IVD pathology[213]
	IL-8	NAF, GCP1, LECT, NAP1, LYNAP.[214(p8)]	Mononuclear macrophages, neutrophils, eosinophils.[197] T lymphocytes, epithelial cells, fibroblasts.[214(p8)]	Neutrophil chemotaxis, angiogenesis.[214,215]	IL-8 increased in CSF of painful disc degeneration patients[216], mRNA analysis of degenerative disc tissue showed association between radicular pain and IL-8 expression.[149]
	MIP-1α	CCL3, SCYA3, G0S19-1, LD78ALPHA.[217]	Monocytes, T lymphocytes, B lymphocytes, neutrophils, dendritic cells, NK T cells.[218,219]	Leukocyte chemotaxis, inhibition of hematopoietic stem cell proliferation.[218,220]	Human disc cell expression of MIP-1α increases with IL-1β stimulation, NP degeneration grade positively correlated to MIP-1α expression[219], elevated MIP-1α in serum of degenerative disc patients[221]
	MIP-1β	CCL4, ACT2, G-26, HC21, LAG1, SCYA2.[222]	CD8+ T-cells, Monocytes, NK T-cells.[222]	Macrophage chemotaxis, T-cell chemoattraction.[223,224]	MIP-1β and resistin expression elevated in degenerative NP tissues[225], TGF-β inhibits MIP-1β in *in-vivo* rat model.[226]
	RANTES	CCL5, SISd, eoCP, SCYA5, TCP228.[227]	CD4+ T Cells, Monocytes.[228]	Chemotaxis for T-cells, eosinophils and basophils.[229]	RANTES expression significantly increased in painful versus painless IVDs[158], human disc cells stimulated with inflammatory cytokines produce RANTES.[230]
Degradative	**MMP-1**	CLG, CLGN.[231]	Activated macrophages, endothelial cells, granulocytes, lymphocytes.[232,233]	Cleavage of Collagen Types I, II, and III.[234]	MMP-1 expression found to be upregulated in degenerative disc tissue[164], MMP-1 expression is correlated with age in lumbar IVDD.[170]
	MMP-2	CLG4, MONA, TBE-1.[235]	Most cell types, endothelial, epithelial, macrophages.[236]	Type IV collagenase, gelatinase A.[237]	Increased MMP-2 content found in nucleus pulposus of degenerative discs[238], compression and/or puncture of rodent caudal discs increased MMP-2 activity.[239]
	MMP-3	SL-1, STMY, STR1, CHDS6.[240]	Most cell types, macrophages, cardiomyocytes, fibroblasts.[240]	Degradation of proteoglycan, fibronectin, laminin, casein, nonhelical collagen.[241]	MMP-3 expression positively correlated to degenerative score and herniation grade[242], MMP-3 expression up-regulated in samples with histological disc degeneration.[169]
	MMP-7	MPSL1, PUMP-1.[243]	Epithelial cells, pancreatic cells, salivary gland, hepatocytes, macrophages.[244]	Degradation of casein, gelatins type I, II and IVD, fibronectin, proteoglycan.[245]	Immunohistochemistry of MMP-7 showed increased positivity with increased degeneration[246], MMP-7 is highly expressed in degenerated inner nucleus pulposus and annulus fibrosus.[164]
	MMP-8	HNC, CLG1, PMNL-CL.[247]	Neutrophils, macrophages, epithelial/endothelial cells, fibroblasts.[248]	Degradation of type I, II, and III collagens.[247]	Treatment of cartilage endplate with MMP-8 increases nutrient uptake[131], MMP-8 expression within histologically degenerative discs is upregulated.[169]
	MMP-9	GELB, CLG4B, MANDP2.[249]	Neutrophils, macrophages, fibroblasts, endothelial/epithelial cells.[250]	Degradation of collagen types IV and V, gelatinase B activity.[249]	MMP-9 expression correlated to histological degeneration and herniation grade in young patients[251], MMP-9 expression significantly elevated in disc extrusion cases.[252(p1)]
Anti-Degradative	**TIMP-1**	EPA, EPO, HCI, CLGI.[253]	Most cell types, fibroblasts, endothelial/epithelial cells.[253(p1)]	Inhibition of MMPs, weak inhibition of membrane type MMPs, anti-apoptotic and cell proliferative effects.[254,255]	Immunohistochemistry of lumbar herniation tissues showed greater number of TIMP-1 positive cells with worsening herniation and degeneration grade[256], TIMP-1 expression increased in degenerative discs in review of literature.[164]
	TIMP-2	DDC8, CSC-21K.[257]	Most cell types, endothelial/epithelial cells, fibroblasts.[257]	Inhibition of MMPs, inhibition of endothelial cell proliferation.[257,258]	Human degenerate discs showed more immunopositive staining for TIMP-2 compared to control tissues[148], TIMP-2 immunopositivity correlates to MMP-14 and MMP-2 in degenerate IVDs.[238]
	TIMP-3	SFD, K222, HSMRK222.[259]	Most cell types, endothelial/epithelial cells, fibroblasts.[259]	Inhibition of MMPs 1, 2, 3, 7, 9, 13,14, and 15, inhibition of aggrecanase.[260,261]	TIMP-3 expression negatively correlated to degree of IVD degeneration[261], neovascularization was inhibited by TIMP-3 overexpression *in-vivo*.[182(p3)]
	TIMP-4	TIMP-4	Lymphocytes, monocytes, mast cells.[262]	Inhibition of MMPs, proliferative and anti-apoptotic functions.[263]	Degenerative nucleus pulposus cell mays produce less TIMP-4 than any other tissue inhibitor of metalloproteinases.[179]

GAPS IN UNDERSTANDING IN S-IVDD DEVELOPMENT

The potential relationships between patient demographic factors (age, sex, BMI) and development and progression of IVDD as measured grossly, radiographically, and histologically are still poorly understood.[40,47,65,76] Histologically, Weiler et al in 2011 observed increased *S*-IVD total HDS in men compared to women, and a significant correlation between *S*-IVD total HDS and BMI.[140] Radiographically, Takatalo et al. in 2013 observed a significant association between waist circumference and radiographic lumbar IVDD in males.[264] A review of the associations between obesity and spinal diseases published by Sheng et al. in 2017 also observed significantly higher risk for radiographic *S*-IVDD in overweight and obese individuals.[74] However, these findings have not been sufficient to develop diagnostic/prognostic methods for *S*-IVDD because the relationship of these patients factors to *A*-IVDD were not considered.[126,133,139,140] An improved understanding of the fundamental differences in *S*-IVDD versus *A*-IVDD tissues and individuals is therefore needed in order to determine which histological and/or radiographic/gross IVDD features and patient characteristics may be associated with *S*-IVDD versus *A*-IVDD development.

Fundamental differences between *S*-IVDD versus *A*-IVDD patients could be reflected in the protein content and/or production of *S*-IVDs or *A*-IVDs. Numerous published reports have investigated changes in IVD tissue metabolic responses related to IVDD using flow cytometry, gene expression, and/or IHC techniques to analyze changes in biomarkers.[155,157,161,163,265–268] While cellular contents, mRNA expression, and protein localization are important data needed to characterize the IVD microenvironment, the assessment of proteins released *ex vivo* can reflect a standardized stress response by *S*-

IVDs and *A*-IVDs.[269–273] Previous human IVD tissue and cell culture studies have primarily aimed to characterize *S*-IVD matrix metabolism and develop models for evaluation of potential *S*-IVDD therapies.[272–274] However, few studies have characterized differences in the *ex vivo* protein production by *S*-IVDs and *A*-IVDs.[152,175]

The relationship between IVD biomarker production and *S*-IVDD development may also be affected by patient characteristics including age, sex, and BMI.[155,159,167,275] Several previous studies have reported that radiographic and histologic *S*-IVDD and *A*-IVDD progress with age and obesity, and additionally that obesity may increase the risk of *S*-IVDD development.[74,140,264] Obesity is also associated with increased inflammatory cytokine production, which may be related to *S*-IVDD onset/severity.[74,275–277] Some studies have reported earlier *A*-IVDD in males, though females may be more susceptible to *S*-IVDD development.[47,48,73,278] While some extensive proteomic characterization studies have been published using IVD tissues, studies associating *S*-IVD and/or *A*-IVD biomarker content/production to patient characteristics are rare.[279–283] Additionally, interactions between patient characteristics may affect *S*-IVDD and *A*-IVDD progression in specific subgroups (i.e., older, obese males), and few, if any published studies assess these effects in their analyses.

In addition to patient factors, as changes to the tissues and structure of the IVD progress during IVDD, the production of specific biological factors by the tissues of the IVD change. However, the changes in tissue metabolic response related to gross changes in tissue structure may be different in patients with *A*-IVDD and *S*-IVDD. Therefore, relating the *ex vivo* production of biological factors by *S*-IVDs and *A*-IVDs to the level of IVDD based on changes to the tissue observed grossly and radiographically, may provide

insight into the biological factors that contribute to IVDD. Further, comparing the differences in the ex vivo production of these biological factors between *S*-IVDs and *A*-IVDs based on these same measures of IVDD tissue change may identify clinically important changes in the metabolic responses of the IVD tissue that contribute to the development and progression of *S*-IVDD.[152]

Despite the widespread use of the IVDD histological scoring system published by Boos et al., the relationship between histological IVDD and *S*-IVDD development has not yet been clarified in any more meaningful way than the relationship between *S*-IVDD and radiographic IVDD progression.[133,137] Further, most published studies compare IVDs based on total HDS without differentiating between patients with *S*-IVDD and *A*-IVDD.[133,246] Therefore, the relationship between symptoms development and changes in histological tissue architecture of the IVD during IVDD require further study. Additionally, the *ex vivo* production of inflammation related and degradative enzyme related proteins by tissue recovered from *S*-IVDs and *A*-IVDs based on changes in histological measures of IVDD may provide insight into biological factors that differentiate patients who develop *S*-IVDD and those who maintain *A*-IVDD.

CONCLUSION

To address these gaps in the current understanding of the development and progression of *S*-IVDD the studies outlined in the following chapters were performed to identify potential distinguishers of *S*-IVDD and *A*-IVDD. The studies aim to identify significant relationships between patient characteristics (age sex, BMI), *ex vivo* IVD tissue biomarker production, and IVDD severity as reflected by radiographic, gross, and/or histological assessments within and between *S*-IVD and *A*-IVD. Understanding

the potential roles of each of these factors in the development of *S*-IVDD may allow for the identification of risk factors S-IVDD development, as well as, diagnostic, prognostic and/or therapeutic targets to improve outcomes for patients with *S*-IVDD.

REFERENCES

1. Ombregt L. Applied anatomy of the lumbar spine. In: *A System of Orthopaedic Medicine*. Elsevier; 2013:415-436.e4. doi:10.1016/B978-0-7020-3145-8.00031-4

2. Waxenbaum JA, Reddy V, Williams C, Futterman B. Anatomy, Back, Lumbar Vertebrae. In: *StatPearls*. StatPearls Publishing; 2020. Accessed May 7, 2020. http://www.ncbi.nlm.nih.gov/books/NBK459278/

3. Banton RA. Biomechanics of The Spine. 2012;7(2):9.

4. Jaumard NV, Welch WC, Winkelstein BA. Spinal Facet Joint Biomechanics and Mechanotransduction in Normal, Injury and Degenerative Conditions. *J Biomech Eng*. 2011;133(7):71010-NaN. doi:10.1115/1.4004493

5. Giglio CA, Volpon JB. Development and evaluation of thoracic kyphosis and lumbar lordosis during growth. *J Child Orthop*. 2007;1(3):187-193. doi:10.1007/s11832-007-0033-5

6. Kalamchi L, Valle C. Embryology, Vertebral Column Development. In: *StatPearls*. StatPearls Publishing; 2022. Accessed May 24, 2022. http://www.ncbi.nlm.nih.gov/books/NBK549917/

7. Meakin JR, Hukins DWL, Aspden RM. Euler buckling as a model for the curvature and flexion of the human lumbar spine. *Proc R Soc Lond B Biol Sci*. 1996;263(1375):1383-1387. doi:10.1098/rspb.1996.0202

8. Jäger M, Luttmann A. Biomechanical analysis and assessment of lumbar stress during load lifting using a dynamic 19-segment human model. *Ergonomics*. 1989;32(1):93-112. doi:10.1080/00140138908966070

9. Hartmann H, Wirth K, Mickel C, Keiner M, Sander A, Yaghobi D. Stress for Vertebral Bodies and Intervertebral Discs with Respect to Squatting Depth. *J Funct Morphol Kinesiol*. 2016;1:254-268. doi:10.3390/jfmk1020254

10. Swartz EE, Floyd RT, Cendoma M. Cervical Spine Functional Anatomy and the Biomechanics of Injury Due to Compressive Loading. *J Athl Train*. 2005;40(3):155-161.

11. DeSai C, Reddy V, Agarwal A. Anatomy, Back, Vertebral Column. In: *StatPearls*. StatPearls Publishing; 2020. Accessed May 7, 2020. http://www.ncbi.nlm.nih.gov/books/NBK525969/

12. Gofur EM, Singh P. Anatomy, Back, Vertebral Canal Blood Supply. In: *StatPearls*. StatPearls Publishing; 2020. Accessed May 7, 2020. http://www.ncbi.nlm.nih.gov/books/NBK541083/

13. Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. CNS immune privilege: hiding in plain sight. *Immunol Rev*. 2006;213:48-65. doi:10.1111/j.1600-065X.2006.00441.x

14. Sun Z, Zhang M, Zhao XH, et al. Immune cascades in human intervertebral disc: the pros and cons. *Int J Clin Exp Pathol*. 2013;6(6):1009-1014.

15. Kaiser JT, Reddy V, Lugo-Pico JG. Anatomy, Head and Neck, Cervical Vertebrae. In: *StatPearls*. StatPearls Publishing; 2022. Accessed April 4, 2022. http://www.ncbi.nlm.nih.gov/books/NBK539734/

16. Ombregt L. Applied anatomy of the lumbar spine. In: *A System of Orthopaedic Medicine*. Elsevier; 2013:415-436.e4. doi:10.1016/B978-0-7020-3145-8.00031-4

17. Waxenbaum JA, Reddy V, Futterman B. Anatomy, Back, Thoracic Vertebrae. In: *StatPearls*. StatPearls Publishing; 2022. Accessed April 4, 2022. http://www.ncbi.nlm.nih.gov/books/NBK459153/

18. Fearing BV, Hernandez PA, Setton LA, Chahine NO. Mechanotransduction and cell biomechanics of the intervertebral disc. *JOR Spine*. 2018;1(3). doi:10.1002/jsp2.1026

19. Spine biomechanical testing methodologies: The controversy of consensus vs scientific evidence - PMC. Accessed April 4, 2022. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7984003/

20. An HS, Masuda K, Inoue N. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthop Sci*. 2006;11(5):541-552. doi:10.1007/s00776-006-1055-4

21. Singh R, Srivastva SK, Prasath CSV, Rohilla RK, Siwach R, Magu NK. Morphometric Measurements of Cadaveric Thoracic Spine in Indian Population and Its Clinical Applications. *Asian Spine J*. 2011;5(1):20-34. doi:10.4184/asj.2011.5.1.20

22. Weiler C, Schietzsch M, Kirchner T, Nerlich AG, Boos N, Wuertz K. Age-related changes in human cervical, thoracal and lumbar intervertebral disc exhibit a strong intra-individual correlation. *Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc*. 2012;21 Suppl 6:S810-818. doi:10.1007/s00586-011-1922-3

23. Hwang D, Lee JH, Moon S, Park SW, Woo J, Kim C. The reliability of the nonradiologic measures of thoracic spine rotation in healthy adults. *Phys Ther Rehabil Sci*. 2017;6(2):65-70. doi:10.14474/ptrs.2017.6.2.65

24. Bible JE, Biswas D, Miller CP, Whang PG, Grauer JN. Normal functional range of motion of the lumbar spine during 15 activities of daily living. *J Spinal Disord Tech*. 2010;23(2):106-112. doi:10.1097/BSD.0b013e3181981823

25. Sattar MH, Guthrie ST. Anatomy, Back, Sacral Vertebrae. In: *StatPearls*. StatPearls Publishing; 2022. Accessed May 24, 2022. http://www.ncbi.nlm.nih.gov/books/NBK551653/

26. Fluid-flow dependent response of intervertebral discs under cyclic loading_ On the role of specimen preparation and preconditioning | Elsevier Enhanced Reader. doi:10.1016/j.jbiomech.2015.10.029

27. Chan SCW, Ferguson SJ, Gantenbein-Ritter B. The effects of dynamic loading on the intervertebral disc. *Eur Spine J*. 2011;20(11):1796-1812. doi:10.1007/s00586-011-1827-1

28. Raj PP. Intervertebral disc: anatomy-physiology-pathophysiology-treatment. *Pain Pract Off J World Inst Pain*. 2008;8(1):18-44. doi:10.1111/j.1533-2500.2007.00171.x

29. Nedresky D, Reddy V, Singh G. Anatomy, Back, Nucleus Pulposus. In: *StatPearls*. StatPearls Publishing; 2022. Accessed April 11, 2022. http://www.ncbi.nlm.nih.gov/books/NBK535373/

30. Waxenbaum JA, Reddy V, Futterman B. Anatomy, Back, Intervertebral Discs. In: *StatPearls*. StatPearls Publishing; 2022. Accessed April 11, 2022. http://www.ncbi.nlm.nih.gov/books/NBK470583/

31. Gofur EM, Singh P. Anatomy, Back, Vertebral Canal Blood Supply. In: *StatPearls*. StatPearls Publishing; 2020. Accessed May 7, 2020. http://www.ncbi.nlm.nih.gov/books/NBK541083/

32. Nosikova YS, Santerre JP, Grynpas M, Gibson G, Kandel RA. Characterization of the annulus fibrosus–vertebral body interface: identification of new structural features. *J Anat*. 2012;221(6):577-589. doi:10.1111/j.1469-7580.2012.01537.x

33. Torre OM, Mroz V, Bartelstein MK, Huang AH, Iatridis JC. Annulus fibrosus cell phenotypes in homeostasis and injury: implications for regenerative strategies. *Ann N Y Acad Sci*. 2019;1442(1):61-78. doi:10.1111/nyas.13964

34. Jackson AR, Yuan TY, Huang CY, Brown MD, Gu WY. NUTRIENT TRANSPORT IN HUMAN ANNULUS FIBROSUS IS AFFECTED BY COMPRESSIVE STRAIN AND ANISOTROPY. *Ann Biomed Eng*. 2012;40(12):2551-2558. doi:10.1007/s10439-012-0606-4

35. Newell N, Little JP, Christou A, Adams MA, Adam CJ, Masouros SD. Biomechanics of the human intervertebral disc: A review of testing techniques and results. *J Mech Behav Biomed Mater*. 2017;69:420-434. doi:10.1016/j.jmbbm.2017.01.037

36. The viscoelastic behavior of the non-degenerate human lumbar nucleus pulposus in shear - ScienceDirect. Accessed January 10, 2020. https://www.sciencedirect.com/science/article/abs/pii/S0021929097000699

37. Walter BA, Torre OM, Laudier D, Naidich TP, Hecht AC, Iatridis JC. Form and function of the intervertebral disc in health and disease: a morphological and stain comparison study. *J Anat*. 2015;227(6):707-716. doi:10.1111/joa.12258

38. Ferguson SJ, Ito K, Nolte LP. Fluid flow and convective transport of solutes within the intervertebral disc. *J Biomech*. 2004;37(2):213-221. doi:10.1016/s0021-9290(03)00250-1

39. De Geer CM. Intervertebral Disk Nutrients and Transport Mechanisms in Relation to Disk Degeneration: A Narrative Literature Review. *J Chiropr Med*. 2018;17(2):97-105. doi:10.1016/j.jcm.2017.11.006

40. Ganesan S, Acharya AS, Chauhan R, Acharya S. Prevalence and Risk Factors for Low Back Pain in 1,355 Young Adults: A Cross-Sectional Study. *Asian Spine J*. 2017;11(4):610-617. doi:10.4184/asj.2017.11.4.610

41. Chiodo AE, Bhat SN, Van Harrison R, et al. *Low Back Pain*. Michigan Medicine University of Michigan; 2020. Accessed August 29, 2022. http://www.ncbi.nlm.nih.gov/books/NBK572334/

42. Meucci RD, Fassa AG, Faria NMX. Prevalence of chronic low back pain: systematic review. *Rev Saúde Pública*. 2015;49:1. doi:10.1590/S0034-8910.2015049005874

43. Fujii K, Yamazaki M, Kang JD, et al. Discogenic Back Pain: Literature Review of Definition, Diagnosis, and Treatment. *JBMR Plus*. 2019;3(5). doi:10.1002/jbm4.10180

44. Wu A, March L, Zheng X, et al. Global low back pain prevalence and years lived with disability from 1990 to 2017: estimates from the Global Burden of Disease Study 2017. *Ann Transl Med*. 2020;8(6):299. doi:10.21037/atm.2020.02.175

45. Geurts JW, Willems PC, Kallewaard JW, van Kleef M, Dirksen C. The Impact of Chronic Discogenic Low Back Pain: Costs and Patients' Burden. *Pain Res Manag*. 2018;2018. doi:10.1155/2018/4696180

46. Brinjikji W, Luetmer PH, Comstock B, et al. Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations. *AJNR Am J Neuroradiol*. 2015;36(4):811-816. doi:10.3174/ajnr.A4173

47. Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. *Spine*. 2002;27(2):125-134. doi:10.1097/00007632-200201150-00002

48. Brinjikji W, Luetmer PH, Comstock B, et al. Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations. *AJNR Am J Neuroradiol*. 2015;36(4):811-816. doi:10.3174/ajnr.A4173

49. Knutsson B, Sandén B, Sjödén G, Järvholm B, Michaëlsson K. Body Mass Index and Risk for Clinical Lumbar Spinal Stenosis: A Cohort Study. *Spine*. 2015;40(18):1451-1456. doi:10.1097/BRS.0000000000001038

50. Kalichman L, Guermazi A, Li L, Hunter DJ. Association between age, sex, BMI and CT-evaluated spinal degeneration features. *J Back Musculoskelet Rehabil*. 2009;22(4):189-195. doi:10.3233/BMR-2009-0232

51. Oh CH, Yoon SH. Whole Spine Disc Degeneration Survey according to the Ages and Sex Using Pfirrmann Disc Degeneration Grades. *Korean J Spine*. 2017;14(4):148-154. doi:10.14245/kjs.2017.14.4.148

52. Buser Z, Ortega B, D'Oro A, et al. Spine Degenerative Conditions and Their Treatments: National Trends in the United States of America. *Glob Spine J*. 2018;8(1):57-67. doi:10.1177/2192568217696688

53. Bydon M, Alvi MA, Goyal A. Degenerative Lumbar Spondylolisthesis: Definition, Natural History, Conservative Management, and Surgical Treatment. *Neurosurg Clin N Am*. 2019;30(3):299-304. doi:10.1016/j.nec.2019.02.003

54. Choi YS. Pathophysiology of Degenerative Disc Disease. *Asian Spine J*. 2009;3(1):39-44. doi:10.4184/asj.2009.3.1.39

55. Iwabuchi M, Murakami K, Ara F, Otani K, Kikuchi SI. The Predictive Factors for the Resorption of a Lumbar Disc Herniation on Plain Mri. *Fukushima J Med Sci*. 2010;56(2):91-97. doi:10.5387/fms.56.91

56. Vadapalli R, Mulukutla R, Vadapalli AS, Vedula RR. Quantitative Predictive Imaging Biomarkers of Lumbar Intervertebral Disc Degeneration. *Asian Spine J*. 2019;13(4):527-534. doi:10.31616/asj.2018.0166

57. Kalichman L, Hunter DJ. Diagnosis and conservative management of degenerative lumbar spondylolisthesis. *Eur Spine J*. 2008;17(3):327-335. doi:10.1007/s00586-007-0543-3

58. Zhang Y gang, Guo T mao, Guo X, Wu S xun. Clinical diagnosis for discogenic low back pain. *Int J Biol Sci*. 2009;5(7):647-658.

59. Dydyk AM, Khan MZ, Singh P. Radicular Back Pain. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK546593/

60. Windt DA van der, Simons E, Riphagen II, et al. Physical examination for lumbar radiculopathy due to disc herniation in patients with low-back pain. *Cochrane Database Syst Rev*. 2010;(2). doi:10.1002/14651858.CD007431.pub2

61. Dulebohn SC, Ngnitewe Massa R, Mesfin FB. Disc Herniation. In: *StatPearls*. StatPearls Publishing; 2019. Accessed December 6, 2019. http://www.ncbi.nlm.nih.gov/books/NBK441822/

62. Al Qaraghli MI, De Jesus O. Lumbar Disc Herniation. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK560878/

63. Surgical vs Nonoperative Treatment for Lumbar Disk Herniation: The Spine Patient Outcomes Research Trial (SPORT): A Randomized Trial | Orthopedics | JAMA | JAMA Network. Accessed August 31, 2022. https://jamanetwork.com/journals/jama/article-abstract/204281

64. Golinvaux NS, Bohl DD, Basques BA, Yacob A, Grauer JN. Comparison of the lumbar disc herniation patients randomized in SPORT to 6,846 discectomy patients from NSQIP: demographics, perioperative variables, and complications correlate well. *Spine J Off J North Am Spine Soc*. 2015;15(4):685-691. doi:10.1016/j.spinee.2014.12.008

65. Seidler A, Bolm-Audorff U, Siol T, et al. Occupational risk factors for symptomatic lumbar disc herniation; a case-control study. *Occup Environ Med*. 2003;60(11):821-830. doi:10.1136/oem.60.11.821

66. Zielinska N, Podgórski M, Haładaj R, Polguj M, Olewnik Ł. Risk Factors of Intervertebral Disc Pathology—A Point of View Formerly and Today—A Review. *J Clin Med*. 2021;10(3):409. doi:10.3390/jcm10030409

67. Wu L, Cruz R. Lumbar Spinal Stenosis. In: *StatPearls*. StatPearls Publishing; 2022. Accessed September 19, 2022. http://www.ncbi.nlm.nih.gov/books/NBK531493/

68. Middleton K, Fish DE. Lumbar spondylosis: clinical presentation and treatment approaches. *Curr Rev Musculoskelet Med*. 2009;2(2):94-104. doi:10.1007/s12178-009-9051-x

69. Hasegewa K, Kitahara K, Hara T, Takano K, Shimoda H. Biomechanical evaluation of segmental instability in degenerative lumbar spondylolisthesis. *Eur Spine J*. 2009;18(4):465-470. doi:10.1007/s00586-008-0842-3

70. Kalichman L, Cole R, Kim DH, et al. Spinal stenosis prevalence and association with symptoms: The Framingham Study. *Spine J Off J North Am Spine Soc*. 2009;9(7):545-550. doi:10.1016/j.spinee.2009.03.005

71. Bagley C, MacAllister M, Dosselman L, Moreno J, Aoun SG, El Ahmadieh TY. Current concepts and recent advances in understanding and managing lumbar spine stenosis. *F1000Research*. 2019;8:F1000 Faculty Rev-137. doi:10.12688/f1000research.16082.1

72. Försth P, Ólafsson G, Carlsson T, et al. A Randomized, Controlled Trial of Fusion Surgery for Lumbar Spinal Stenosis. *N Engl J Med*. 2016;374(15):1413-1423. doi:10.1056/NEJMoa1513721

73. Ishimoto Y, Yoshimura N, Muraki S, et al. Prevalence of symptomatic lumbar spinal stenosis and its association with physical performance in a population-based cohort in Japan: the Wakayama Spine Study. *Osteoarthritis Cartilage*. 2012;20(10):1103-1108. doi:10.1016/j.joca.2012.06.018

74. Sheng B, Feng C, Zhang D, Spitler H, Shi L. Associations between Obesity and Spinal Diseases: A Medical Expenditure Panel Study Analysis. *Int J Environ Res Public Health*. 2017;14(2):183. doi:10.3390/ijerph14020183

75. Rihn JA, Radcliff K, Hilibrand AS, et al. Does obesity affect outcomes of treatment for lumbar stenosis and degenerative spondylolisthesis? Analysis of the Spine Patient Outcomes Research Trial (SPORT). *Spine*. 2012;37(23):1933-1946. doi:10.1097/BRS.0b013e31825e21b2

76. Ala-Kokko L. Genetic risk factors for lumbar disc disease. *Ann Med*. 2002;34(1):42-47. doi:10.1080/078538902317338634

77. Heikkilä JK, Koskenvuo M, Heliövaara M, et al. Genetic and environmental factors in sciatica. Evidence from a nationwide panel of 9365 adult twin pairs. *Ann Med*. 1989;21(5):393-398. doi:10.3109/07853898909149227

78. Varlotta GP, Brown MD, Kelsey JL, Golden AL. Familial predisposition for herniation of a lumbar disc in patients who are less than twenty-one years old. *J Bone Joint Surg Am*. 1991;73(1):124-128.

79. Familial predisposition and clustering for juvenile lumbar disc herniation - PubMed. Accessed October 25, 2022. https://pubmed.ncbi.nlm.nih.gov/1462208/

80. Scapinelli R. Lumbar disc herniation in eight siblings with a positive family history for disc disease. *Acta Orthop Belg*. 1993;59(4):371-376.

81. Kuivaniemi H, Tromp G, Prockop DJ. Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. *Hum Mutat*. 1997;9(4):300-315. doi:10.1002/(SICI)1098-1004(1997)9:4<300::AID-HUMU2>3.0.CO;2-9

82. Battié MC, Ortega-Alonso A, Niemelainen R, et al. Lumbar spinal stenosis is a highly genetic condition partly mediated by disc degeneration. *Arthritis Rheumatol Hoboken NJ*. 2014;66(12):3505-3510. doi:10.1002/art.38823

83. Noponen-Hietala N, Kyllönen E, Männikkö M, et al. Sequence variations in the collagen IX and XI genes are associated with degenerative lumbar spinal stenosis. *Ann Rheum Dis*. 2003;62(12):1208-1214. doi:10.1136/ard.2003.008334

84. Paassilta P, Lohiniva J, Göring HH, et al. Identification of a novel common genetic risk factor for lumbar disk disease. *JAMA*. 2001;285(14):1843-1849. doi:10.1001/jama.285.14.1843

85. Lin TY, Wang YC, Chang CW, Wong CB, Cheng YH, Fu TS. Surgical Outcomes for Upper Lumbar Disc Herniation: Decompression Alone versus Fusion Surgery. *J Clin Med*. 2019;8(9). doi:10.3390/jcm8091435

86. Sugiura T, Okuda S, Matsumoto T, et al. Surgical Outcomes and Limitations of Decompression Surgery for Degenerative Spondylolisthesis. *Glob Spine J*. 2018;8(7):733-738. doi:10.1177/2192568218770793

87. Cunha C, Silva AJ, Pereira P, Vaz R, Gonçalves RM, Barbosa MA. The inflammatory response in the regression of lumbar disc herniation. *Arthritis Res Ther*. 2018;20. doi:10.1186/s13075-018-1743-4

88. Goode AP, Marshall SW, Kraus VB, et al. Association between serum and urine biomarkers and lumbar spine individual radiographic features: the Johnston County Osteoarthritis Project. *Osteoarthritis Cartilage*. 2012;20(11):1286-1293. doi:10.1016/j.joca.2012.08.003

89. Lumbar Spine Imaging - StatPearls - NCBI Bookshelf. Accessed August 30, 2022. https://www.ncbi.nlm.nih.gov/books/NBK553181/

90. Hasz MW. Diagnostic Testing for Degenerative Disc Disease. *Adv Orthop*. 2012;2012:413913. doi:10.1155/2012/413913

91. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine*. 2001;26(17):1873-1878. doi:10.1097/00007632-200109010-00011

92. Yu LP, Qian WW, Yin GY, Ren YX, Hu ZY. MRI Assessment of Lumbar Intervertebral Disc Degeneration with Lumbar Degenerative Disease Using the Pfirrmann Grading Systems. *PLoS ONE*. 2012;7(12):e48074. doi:10.1371/journal.pone.0048074

93. Stretanski MF, Vu L. Fluoroscopy Discography Assessment, Protocols, and Interpretation. In: *StatPearls*. StatPearls Publishing; 2022. Accessed October 25, 2022. http://www.ncbi.nlm.nih.gov/books/NBK572119/

94. Tamagawa S, Sakai D, Nojiri H, Sato M, Ishijima M, Watanabe M. Imaging Evaluation of Intervertebral Disc Degeneration and Painful Discs—Advances and Challenges in Quantitative MRI. *Diagnostics*. 2022;12(3):707. doi:10.3390/diagnostics12030707

95. Kim CH, Choi Y, Chung CK, et al. Nonsurgical treatment outcomes for surgical candidates with lumbar disc herniation: a comprehensive cohort study. *Sci Rep*. 2021;11:3931. doi:10.1038/s41598-021-83471-y

96. Theodoridis T, Krämer J, Kleinert H. [Conservative treatment of lumbar spinal stenosis--a review]. *Z Orthopadie Unfallchirurgie*. 2008;146(1):75-79. doi:10.1055/s-2007-989394

97. Thackeray A, Fritz JM, Lurie JD, Zhao W, Weinstein JN. Non-surgical Treatment Choices by Individuals with Lumbar Intervertebral Disc Herniation in the United States: Associations with Long-Term Outcomes. *Am J Phys Med Rehabil*. 2017;96(8):557-564. doi:10.1097/PHM.0000000000000685

98. Atlas SJ, Keller RB, Wu YA, Deyo RA, Singer DE. Long-Term Outcomes of Surgical and Nonsurgical Management of Sciatica Secondary to a Lumbar Disc Herniation: 10 Year Results from the Maine Lumbar Spine Study. *Spine*. 2005;30(8):927-935. doi:10.1097/01.brs.0000158954.68522.2a

99. Singh V, Malik M, Kaur J, Kulandaivelan S, Punia S. A systematic review and meta-analysis on the efficacy of physiotherapy intervention in management of lumbar prolapsed intervertebral disc. *Int J Health Sci*. 2021;15(2):49-57.

100. Pourahmadi MR, Taghipour M, Ebrahimi Takamjani I, Sanjari MA, Mohseni-Bandpei MA, Keshtkar AA. Motor control exercise for symptomatic lumbar disc herniation: protocol for a systematic review and meta-analysis. *BMJ Open*. 2016;6(9):e012426. doi:10.1136/bmjopen-2016-012426

101. Wagner H, Liebetrau A, Schinowski D, Wulf T, de Lussanet MH. Spinal lordosis optimizes the requirements for a stable erect posture. *Theor Biol Med Model*. 2012;9:13. doi:10.1186/1742-4682-9-13

102. Gugliotta M, da Costa BR, Dabis E, et al. Surgical versus conservative treatment for lumbar disc herniation: a prospective cohort study. *BMJ Open*. 2016;6(12):e012938. doi:10.1136/bmjopen-2016-012938

103. Peul WC, Hout WB van den, Brand R, Thomeer RTWM, Koes BW, for the Leiden-The Hague Spine Intervention Prognostic Study Group. Prolonged conservative care versus early surgery in patients with sciatica caused by lumbar disc herniation: two year results of a randomised controlled trial. *BMJ*. 2008;336(7657):1355-1358. doi:10.1136/bmj.a143

104. Vroomen PCAJ, de Krom MCTFM, Slofstra PD, Knottnerus JA. Conservative Treatment of Sciatica: A Systematic Review. *Clin Spine Surg*. 2000;13(6):463-469.

105. Suri P, Hunter DJ, Jouve C, et al. Nonsurgical Treatment of Lumbar Disk Herniation: Are Outcomes Different in Older Adults? *J Am Geriatr Soc*. 2011;59(3):423-429. doi:10.1111/j.1532-5415.2011.03316.x

106. Jordan JL, Konstantinou K, O'Dowd J. Herniated lumbar disc. *BMJ Clin Evid*. 2011;2011:1118.

107. Buttermann GR. Treatment of Lumbar Disc Herniation: Epidural Steroid Injection Compared with Discectomy: A Prospective, Randomized Study. *JBJS*. 2004;86(4):670-679.

108. Mann SJ, Lam JC, Singh P. McKenzie Back Exercises. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 31, 2022. http://www.ncbi.nlm.nih.gov/books/NBK539720/

109. Macedo LG, Hum A, Kuleba L, et al. Physical Therapy Interventions for Degenerative Lumbar Spinal Stenosis: A Systematic Review. *Phys Ther*. 2013;93(12):1646-1660. doi:10.2522/ptj.20120379

110. Inoue G, Miyagi M, Takaso M. Surgical and nonsurgical treatments for lumbar spinal stenosis. *Eur J Orthop Surg Traumatol*. 2016;26(7):695-704. doi:10.1007/s00590-016-1818-3

111. Wiffen PJ, Derry S, Bell RF, et al. Gabapentin for chronic neuropathic pain in adults. *Cochrane Database Syst Rev*. 2017;2017(6):CD007938. doi:10.1002/14651858.CD007938.pub4

112. Diwan S, Sayed D, Deer TR, Salomons A, Liang K. An Algorithmic Approach to Treating Lumbar Spinal Stenosis: An Evidenced-Based Approach. *Pain Med Off J Am Acad Pain Med*. 2019;20(Suppl 2):S23-S31. doi:10.1093/pm/pnz133

113. Zaina F, Tomkins-Lane C, Carragee E, Negrini S. Surgical versus non-surgical treatment for lumbar spinal stenosis. *Cochrane Database Syst Rev*. 2016;(1). doi:10.1002/14651858.CD010264.pub2

114. Manchikanti L, Knezevic NN, Boswell MV, Kaye AD, Hirsch JA. Epidural Injections for Lumbar Radiculopathy and Spinal Stenosis: A Comparative Systematic Review and Meta-Analysis. *Pain Physician*. 2016;19(3):E365-410.

115. Butler AJ, Donnally III CJ. Discectomy. In: *StatPearls*. StatPearls Publishing; 2022. Accessed September 7, 2022. http://www.ncbi.nlm.nih.gov/books/NBK544281/

116. Aljoghaiman M, Martyniuk A, Farrokhyar F, Cenic A, Kachur E. Survey of lumbar discectomy practices: 10 years in the making. *J Spine Surg*. 2020;6(3):572-580. doi:10.21037/jss-20-519

117. Blamoutier A. Surgical discectomy for lumbar disc herniation: surgical techniques. *Orthop Traumatol Surg Res OTSR*. 2013;99(1 Suppl):S187-196. doi:10.1016/j.otsr.2012.11.005

118. Calikoglu C, Cakir M. Open Discectomy vs. Microdiscectomy: Results from 519 Patients Operated for Lumbar Disc Herniation. *Eurasian J Med*. 2018;50(3):178-181. doi:10.5152/eurasianjmed.2018.18016

119. MEYER G, DA ROCHA ID, CRISTANTE AF, et al. Percutaneous Endoscopic Lumbar Discectomy Versus Microdiscectomy for the Treatment of Lumbar Disc Herniation: Pain, Disability, and Complication Rate—A Randomized Clinical Trial. *Int J Spine Surg*. 2020;14(1):72-78. doi:10.14444/7010

120. Traeger A, Buchbinder R, Harris I, Maher C. Diagnosis and management of low-back pain in primary care. *CMAJ Can Med Assoc J*. 2017;189(45):E1386-E1395. doi:10.1503/cmaj.170527

121. Goupille P, Mulleman D, Valat J. Radiculopathy associated with disc herniation. *Ann Rheum Dis*. 2006;65(2):141-143. doi:10.1136/ard.2005.039669

122. Lee BH, Moon SH, Suk KS, Kim HS, Yang JH, Lee HM. Lumbar Spinal Stenosis: Pathophysiology and Treatment Principle: A Narrative Review. *Asian Spine J*. 2020;14(5):682-693. doi:10.31616/asj.2020.0472

123. Omidi-Kashani F, Hasankhani EG, Ashjazadeh A. Lumbar Spinal Stenosis: Who Should Be Fused? An Updated Review. *Asian Spine J*. 2014;8(4):521-530. doi:10.4184/asj.2014.8.4.521

124. Mobbs RJ, Phan K, Malham G, Seex K, Rao PJ. Lumbar interbody fusion: techniques, indications and comparison of interbody fusion options including PLIF, TLIF, MI-TLIF, OLIF/ATP, LLIF and ALIF. *J Spine Surg*. 2015;1(1):2-18. doi:10.3978/j.issn.2414-469X.2015.10.05

125. Walter BA, Torre OM, Laudier D, Naidich TP, Hecht AC, Iatridis JC. Form and function of the intervertebral disc in health and disease: a morphological and stain comparison study. *J Anat*. 2015;227(6):707-716. doi:10.1111/joa.12258

126. Le Maitre CL, Dahia CL, Giers M, et al. Development of a standardized histopathology scoring system for human intervertebral disc degeneration: an Orthopaedic Research Society Spine Section Initiative. *JOR Spine*. 2021;4(2):e1167. doi:10.1002/jsp2.1167

127. Inoue N, Espinoza Orías AA. Biomechanics of Intervertebral Disc Degeneration. *Orthop Clin North Am*. 2011;42(4):487-499. doi:10.1016/j.ocl.2011.07.001

128. Spillekom S, Smolders LA, Grinwis GCM, et al. Increased Osmolarity and Cell Clustering Preserve Canine Notochordal Cell Phenotype in Culture. *Tissue Eng Part C Methods*. 2013;20(8):652-662. doi:10.1089/ten.tec.2013.0479

129. Erwin WM, Heras FL, Islam D, Fehlings MG, Inman RD. The regenerative capacity of the notochordal cell: tissue constructs generated in vitro under hypoxic conditions: Laboratory investigation. *J Neurosurg Spine*. 2009;10(6):513-521. doi:10.3171/2009.2.SPINE08578

130. Effects of Glucose Deprivation on ATP and Proteoglycan Production of Intervertebral Disc Cells under Hypoxia | Scientific Reports. Accessed November 16, 2020. https://www.nature.com/articles/s41598-020-65691-w

131. Dolor A, Sampson SL, Lazar AA, Lotz JC, Szoka FC, Fields AJ. Matrix modification for enhancing the transport properties of the human cartilage endplate to improve disc nutrition. *PLoS ONE*. 2019;14(4). doi:10.1371/journal.pone.0215218

132. de Bree K, de Bakker BS, Oostra RJ. The development of the human notochord. *PLoS ONE*. 2018;13(10):e0205752. doi:10.1371/journal.pone.0205752

133. Histology and Pathology of the Human Intervertebral Disc : JBJS. Accessed September 21, 2022. https://journals.lww.com/jbjsjournal/Abstract/2006/04002/Histology_and_Pathology_of_the_Human.3.aspx

134. Taylor JR. Growth of human intervertebral discs and vertebral bodies. *J Anat*. 1975;120(Pt 1):49-68.

135. Fournier DE, Kiser PK, Shoemaker JK, Battié MC, Séguin CA. Vascularization of the human intervertebral disc: A scoping review. *JOR Spine*. 2020;3(4):e1123. doi:10.1002/jsp2.1123

136. Wade KR, Robertson PA, Broom ND. A fresh look at the nucleus-endplate region: new evidence for significant structural integration. *Eur Spine J*. 2011;20(8):1225-1232. doi:10.1007/s00586-011-1704-y

137. Roberts S, Evans H, Trivedi J, Menage J. Histology and Pathology of the Human Intervertebral Disc. *JBJS*. 2006;88(suppl_2):10-14. doi:10.2106/JBJS.F.00019

138. Grunhagen T, Wilde G, Soukane DM, Shirazi-Adl SA, Urban JPG. Nutrient Supply and Intervertebral Disc Metabolism. *JBJS*. 2006;88(suppl_2):30. doi:10.2106/JBJS.E.01290

139. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine*. 2002;27(23):2631-2644. doi:10.1097/00007632-200212010-00002

140. Weiler C, Lopez-Ramos M, Mayer HM, et al. Histological analysis of surgical lumbar intervertebral disc tissue provides evidence for an association between disc degeneration and increased body mass index. *BMC Res Notes*. 2011;4:497. doi:10.1186/1756-0500-4-497

141. Lee NN, Kramer JS, Stoker AM, et al. Canine models of spine disorders. *JOR Spine*. 2020;3(4):e1109. doi:10.1002/jsp2.1109

142. Fernando HN, Czamanski J, Yuan TY, Gu WY, Abdi S, Huang CYC. Mechanical Loading Affects the Energy Metabolism of Intervertebral Disc Cells. *J Orthop Res Off Publ Orthop Res Soc*. 2011;29(11):1634-1641. doi:10.1002/jor.21430

143. Iatridis JC, MacLean JJ, Roughley PJ, Alini M. Effects of Mechanical Loading on Intervertebral Disc Metabolism In Vivo. *J Bone Joint Surg Am*. 2006;88(0 2):41-46. doi:10.2106/JBJS.E.01407

144. Molinos M, Almeida CR, Caldeira J, Cunha C, Gonçalves RM, Barbosa MA. Inflammation in intervertebral disc degeneration and regeneration. *J R Soc Interface*. 2015;12(104). doi:10.1098/rsif.2014.1191

145. Lu P, Takai K, Weaver VM, Werb Z. Extracellular Matrix Degradation and Remodeling in Development and Disease. *Cold Spring Harb Perspect Biol*. 2011;3(12). doi:10.1101/cshperspect.a005058

146. Ali R, Maitre CL, Richardson S, Hoyland PJ, Freemont A. Connective tissue growth factor expression in human intervertebral disc: implications for angiogenesis in intervertebral disc degeneration. *Biotech Histochem*. 2008;83(5):239-245. doi:10.1080/10520290802539186

147. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol*. 2014;10(1):44-56. doi:10.1038/nrrheum.2013.160

148. Maitre CLL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J Pathol*. 2004;204(1):47-54. doi:10.1002/path.1608

149. Ahn SH, Cho YW, Ahn MW, Jang SH, Sohn YK, Kim HS. mRNA Expression of Cytokines and Chemokines in Herniated Lumbar Intervertebral Discs. *Spine*. 2002;27(9):911-917.

150. ZHANG F, ZHAO X, SHEN H, ZHANG C. Molecular mechanisms of cell death in intervertebral disc degeneration (Review). *Int J Mol Med*. 2016;37(6):1439-1448. doi:10.3892/ijmm.2016.2573

151. Phillips KLE, Cullen K, Chiverton N, et al. Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master regulator of catabolic processes. *Osteoarthritis Cartilage*. 2015;23(7):1165-1177. doi:10.1016/j.joca.2015.02.017

152. Wuertz K, Haglund L. Inflammatory Mediators in Intervertebral Disk Degeneration and Discogenic Pain. *Glob Spine J*. 2013;3(3):175-184. doi:10.1055/s-0033-1347299

153. Jacobsen HE, Khan AN, Levine ME, Filippi CG, Chahine NO. Severity of Intervertebral Disc Herniation Regulates Cytokine and Chemokine Levels In Patients

with Chronic Radicular Back Pain. *Osteoarthritis Cartilage*. 2020;28(10):1341-1350. doi:10.1016/j.joca.2020.06.009

154. De Geer CM. Cytokine Involvement in Biological Inflammation Related to Degenerative Disorders of the Intervertebral Disk: A Narrative Review. *J Chiropr Med*. 2018;17(1):54-62. doi:10.1016/j.jcm.2017.09.003

155. Shamji MF, Setton LA, Jarvis W, et al. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum*. 2010;62(7):1974-1982. doi:10.1002/art.27444

156. Mf S, La S, W J, et al. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum*. 2010;62(7). doi:10.1002/art.27444

157. Weiler C, Nerlich AG, Bachmeier BE, Boos N. Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. *Spine*. 2005;30(1):44-53; discussion 54. doi:10.1097/01.brs.0000149186.63457.20

158. Kepler CK, Markova DZ, Dibra F, et al. Expression and Relationship of Proinflammatory Chemokine RANTES/CCL5 and Cytokine IL-1β in Painful Human Intervertebral Discs. *Spine*. 2013;38(11):873. doi:10.1097/BRS.0b013e318285ae08

159. Kawaguchi S, Yamashita T, Katahira G ichiro, Yokozawa H, Torigoe T, Sato N. Chemokine profile of herniated intervertebral discs infiltrated with monocytes and macrophages. *Spine*. 2002;27(14):1511-1516. doi:10.1097/00007632-200207150-00006

160. Anderson DG, Izzo MW, Hall DJ, et al. Comparative gene expression profiling of normal and degenerative discs: analysis of a rabbit annular laceration model. *Spine*. 2002;27(12):1291-1296. doi:10.1097/00007632-200206150-00009

161. Le Maitre CL, Hoyland JA, Freemont AJ. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1β and TNFα expression profile. *Arthritis Res Ther*. 2007;9(4):R77. doi:10.1186/ar2275

162. Tumor necrosis factor-α: a key contributor to intervertebral disc degeneration. *Acta Biochim Biophys Sin*. Published online January 1, 2017:1-13. doi:10.1093/abbs/gmw112

163. Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human Intervertebral disc degeneration. *Arthritis Res Ther*. 2005;7(4):R732-R745. doi:10.1186/ar1732

164. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA, Kang JD. Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and

degeneration. *Spine J Off J North Am Spine Soc*. 2013;13(3):331-341. doi:10.1016/j.spinee.2012.02.027

165. Visse R, Nagase H. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Circ Res*. 2003;92(8):827-839. doi:10.1161/01.RES.0000070112.80711.3D

166. Kelwick R, Desanlis I, Wheeler GN, Edwards DR. The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. *Genome Biol*. 2015;16(1):113. doi:10.1186/s13059-015-0676-3

167. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. Matrix Metalloproteinases And Aggrecanase: Their Role in Disorders of the Human Intervertebral Disc. *Spine*. 2000;25(23):3005.

168. Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem*. 1997;378(3-4):151-160.

169. Bachmeier BE, Nerlich A, Mittermaier N, et al. Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. *Eur Spine J*. 2009;18(11):1573-1586. doi:10.1007/s00586-009-1031-8

170. Basaran R, Senol M, Ozkanli S, Efendioglu M, Kaner T. Correlation of matrix metalloproteinase (MMP)-1, -2, -3, and -9 expressions with demographic and radiological features in primary lumbar intervertebral disc disease. *J Clin Neurosci*. 2017;41:46-49. doi:10.1016/j.jocn.2017.03.001

171. Vergroesen PPA, Kingma I, Emanuel KS, et al. Mechanics and biology in intervertebral disc degeneration: a vicious circle. *Osteoarthritis Cartilage*. 2015;23(7):1057-1070. doi:10.1016/j.joca.2015.03.028

172. Wang WJ, Yu XH, Wang C, et al. MMPs and ADAMTSs in intervertebral disc degeneration. *Clin Chim Acta*. 2015;448:238-246. doi:10.1016/j.cca.2015.06.023

173. Richardson SM, Doyle P, Minogue BM, Gnanalingham K, Hoyland JA. Increased expression of matrix metalloproteinase-10, nerve growth factor and substance P in the painful degenerate intervertebral disc. *Arthritis Res Ther*. 2009;11(4):R126. doi:10.1186/ar2793

174. Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 SSE Award Competition in Basic Science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. *Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc*. 2002;11(4):308-320. doi:10.1007/s00586-002-0472-0

175. Deng B, Ren JZ, Meng XQ, et al. Expression profiles of MMP-1 and TIMP-1 in lumbar intervertebral disc degeneration. *Genet Mol Res*. 2015;14(4):19080-19086. doi:10.4238/2015.December.29.16

176. Patel KP, Sandy JD, Akeda K, et al. Aggrecanases and aggrecanase-generated fragments in the human intervertebral disc at early and advanced stages of disc degeneration. *Spine*. 2007;32(23):2596-2603. doi:10.1097/BRS.0b013e318158cb85

177. Effect of small interference RNA (siRNA) for ADAMTS5on intervertebral disc degeneration in the rabbit anular needle-puncture model | Arthritis Research & Therapy | Full Text. Accessed October 25, 2022. https://arthritis-research.biomedcentral.com/articles/10.1186/ar2851

178. Wiet MG, Piscioneri A, Khan SN, Ballinger MN, Hoyland JA, Purmessur D. Mast Cell-Intervertebral disc cell interactions regulate inflammation, catabolism and angiogenesis in Discogenic Back Pain. *Sci Rep*. 2017;7(1):12492. doi:10.1038/s41598-017-12666-z

179. Mern DS, Beierfuß A, Fontana J, Thomé C, Hegewald AA. Imbalanced Protein Expression Patterns of Anabolic, Catabolic, Anti-Catabolic and Inflammatory Cytokines in Degenerative Cervical Disc Cells: New Indications for Gene Therapeutic Treatments of Cervical Disc Diseases. Kletsas D, ed. *PLoS ONE*. 2014;9(5):e96870. doi:10.1371/journal.pone.0096870

180. Stone WL, Leavitt L, Varacallo M. Physiology, Growth Factor. In: *StatPearls*. StatPearls Publishing; 2023. Accessed April 27, 2023. http://www.ncbi.nlm.nih.gov/books/NBK442024/

181. Tolonen J, Grönblad M, Vanharanta H, et al. Growth factor expression in degenerated intervertebral disc tissue. *Eur Spine J*. 2006;15(5):588-596. doi:10.1007/s00586-005-0930-6

182. Li Y, Zhang T, Tian W, et al. Loss of TIMP3 expression induces inflammation, matrix degradation, and vascular ingrowth in nucleus pulposus: A new mechanism of intervertebral disc degeneration. *FASEB J*. 2020;34(4):5483-5498. doi:10.1096/fj.201902364RR

183. Lama P, Le Maitre CL, Harding IJ, Dolan P, Adams MA. Nerves and blood vessels in degenerated intervertebral discs are confined to physically disrupted tissue. *J Anat*. 2018;233(1):86-97. doi:10.1111/joa.12817

184. Pratsinis H, Kletsas D. Growth factors in intervertebral disc homeostasis. *Connect Tissue Res*. 2008;49(3):273-276. doi:10.1080/03008200802147951

185. Masuda K, An HS. Prevention of disc degeneration with growth factors. *Eur Spine J*. 2006;15(Suppl 3):422-432. doi:10.1007/s00586-006-0149-1

186. Masuda K, An HS. Prevention of disc degeneration with growth factors. *Eur Spine J*. 2006;15(Suppl 3):422-432. doi:10.1007/s00586-006-0149-1

187. Wang SZ, Rui YF, Lu J, Wang C. Cell and molecular biology of intervertebral disc degeneration: current understanding and implications for potential therapeutic strategies. *Cell Prolif*. 2014;47(5):381-390. doi:10.1111/cpr.12121

188. Paglia DN, Singh H, Karukonda T, Drissi H, Moss IL. PDGF-BB Delays Degeneration of the Intervertebral Discs in a Rabbit Preclinical Model. *Spine*. 2016;41(8):E449. doi:10.1097/BRS.0000000000001336

189. Li X, An HS, Ellman M, et al. Action of fibroblast growth factor-2 on the intervertebral disc. *Arthritis Res Ther*. 2008;10(2):R48. doi:10.1186/ar2407

190. Fournier DE, Kiser PK, Shoemaker JK, Battié MC, Séguin CA. Vascularization of the human intervertebral disc: A scoping review. *JOR Spine*. 2020;3(4):e1123. doi:10.1002/jsp2.1123

191. David G, Ciurea A, Iencean S, Mohan A. Angiogenesis in the degeneration of the lumbar intervertebral disc. *J Med Life*. 2010;3(2):154-161.

192. Capossela S, Bertolo A, Gunasekera K, Pötzel T, Baur M, Stoyanov JV. VEGF vascularization pathway in human intervertebral disc does not change during the disc degeneration process. *BMC Res Notes*. 2018;11. doi:10.1186/s13104-018-3441-3

193. Zhang W, Gong Y, Zheng X, et al. Platelet-Derived Growth Factor-BB Inhibits Intervertebral Disc Degeneration via Suppressing Pyroptosis and Activating the MAPK Signaling Pathway. *Front Pharmacol*. 2022;12:799130. doi:10.3389/fphar.2021.799130

194. Masuda K, An HS. Growth factors and the intervertebral disc. *Spine J Off J North Am Spine Soc*. 2004;4(6 Suppl):330S-340S. doi:10.1016/j.spinee.2004.07.028

195. CXCL1 C-X-C motif chemokine ligand 1 [Homo sapiens (human)] - Gene - NCBI. Accessed May 28, 2020. https://www.ncbi.nlm.nih.gov/gene/2919

196. Ahuja SK, Murphy PM. The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. *J Biol Chem*. 1996;271(34):20545-20550. doi:10.1074/jbc.271.34.20545

197. Geiserls T, DewaldS B, EhrengruberSII MU, Clark-Lewis I, BaggioliniS M. The Interleukin-8-relatedChemotactic Cytokines GROa, GROB, and GROT Activate Human Neutrophil and Basophil Leukocytes. :6.

198. Phillips KLE, Chiverton N, Michael AL, et al. The cytokine and chemokine expression profile of nucleus pulposus cells: implications for degeneration and regeneration of the intervertebral disc. *Arthritis Res Ther*. 2013;15(6):R213. doi:10.1186/ar4408

199. CCL2 C-C motif chemokine ligand 2 [Homo sapiens (human)] - Gene - NCBI. Accessed May 28, 2020. https://www.ncbi.nlm.nih.gov/gene/6347

200. Gschwandtner M, Derler R, Midwood KS. More Than Just Attractive: How CCL2 Influences Myeloid Cell Behavior Beyond Chemotaxis. *Front Immunol*. 2019;10. doi:10.3389/fimmu.2019.02759

201. Proinflammatory cytokines modulate the chemokine CCL2 (MCP-1) in human annulus cells in vitro: CCL2 expression and production. - Abstract - Europe PMC. Accessed May 28, 2020. https://europepmc.org/article/med/25527176

202. CCL7 C-C motif chemokine ligand 7 [Homo sapiens (human)] - Gene - NCBI. Accessed May 28, 2020. https://www.ncbi.nlm.nih.gov/gene/6354

203. Menten P, Wuyts A, Van Damme J. Monocyte chemotactic protein-3. *Eur Cytokine Netw*. 2001;12(4):554-560.

204. Weber KT, Satoh S, Alipui DO, et al. Exploratory study for identifying systemic biomarkers that correlate with pain response in patients with intervertebral disc disorders. *Immunol Res*. 2015;63:170-180. doi:10.1007/s12026-015-8709-2

205. IL1B interleukin 1 beta [Homo sapiens (human)] - Gene - NCBI. Accessed June 1, 2020. https://www.ncbi.nlm.nih.gov/gene/3553

206. The role of interleukin-1 in the pathogenesis of human Intervertebral disc degeneration. Accessed June 1, 2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1175026/

207. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281(1):8-27. doi:10.1111/imr.12621

208. Yang W, Yu XH, Wang C, et al. Interleukin-1β in intervertebral disk degeneration. *Clin Chim Acta*. 2015;450:262-272. doi:10.1016/j.cca.2015.08.029

209. IL6 interleukin 6 [Homo sapiens (human)] - Gene - NCBI. Accessed June 1, 2020. https://www.ncbi.nlm.nih.gov/gene/3569

210. Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther*. 2006;8(Suppl 2):S3. doi:10.1186/ar1917

211. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harb Perspect Biol*. 2014;6(10). doi:10.1101/cshperspect.a016295

212. DENG X, ZHAO F, KANG B, ZHANG X. Elevated interleukin-6 expression levels are associated with intervertebral disc degeneration. *Exp Ther Med*. 2016;11(4):1425-1432. doi:10.3892/etm.2016.3079

213. Weber KT, Alipui DO, Sison CP, et al. Serum levels of the proinflammatory cytokine interleukin-6 vary based on diagnoses in individuals with lumbar intervertebral disc diseases. *Arthritis Res Ther*. 2016;18(1):3. doi:10.1186/s13075-015-0887-8

214. Russo RC, Garcia CC, Teixeira MM, Amaral FA. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. *Expert Rev Clin Immunol*. 2014;10(5):593-619. doi:10.1586/1744666X.2014.894886

215. Baggiolini M, Clark-Lewis I. Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett*. 1992;307(1):97-101. doi:10.1016/0014-5793(92)80909-Z

216. Krock E, Millecamps M, Anderson KM, et al. Interleukin-8 as a therapeutic target for chronic low back pain: Upregulation in human cerebrospinal fluid and pre-clinical validation with chronic reparixin in the SPARC-null mouse model. *EBioMedicine*. 2019;43:487-500. doi:10.1016/j.ebiom.2019.04.032

217. CCL3 C-C motif chemokine ligand 3 [Homo sapiens (human)] - Gene - NCBI. Accessed June 10, 2020. https://www.ncbi.nlm.nih.gov/gene/6348

218. Bhavsar I, Miller CS, Al-Sabbagh M. Macrophage Inflammatory Protein-1 Alpha (MIP-1 alpha)/CCL3: As a Biomarker. In: Preedy VR, Patel VB, eds. *General Methods in Biomarker Research and Their Applications*. Biomarkers in Disease: Methods, Discoveries and Applications. Springer Netherlands; 2015:223-249. doi:10.1007/978-94-007-7696-8_27

219. Menten P, Saccani A, Dillen C, et al. Role of the autocrine chemokines MIP-1α and MIP-1β in the metastatic behavior of murine T cell lymphoma. *J Leukoc Biol*. 2002;72(4):780-789. doi:10.1189/jlb.72.4.780

220. Driscoll KE. Macrophage inflammatory proteins: biology and role in pulmonary inflammation. *Exp Lung Res*. 1994;20(6):473-490. doi:10.3109/01902149409031733

221. Zhang YL, Li B, Zhou ZH. A cross-sectional study: serum CCL3/MIP-1α levels may reflect lumbar intervertebral disk degeneration in Han Chinese people. *J Pain Res*. 2018;11:497-503. doi:10.2147/JPR.S152349

222. CCL4 C-C motif chemokine ligand 4 [Homo sapiens (human)] - Gene - NCBI. Accessed June 11, 2020. https://www.ncbi.nlm.nih.gov/gene/6351

223. Zack Howard OM, Turpin JA, Goldman R, Modi WS. Functional redundancy of the human CCL4 and CCL4L1 chemokine genes. *Biochem Biophys Res Commun*. 2004;320(3):927-931. doi:10.1016/j.bbrc.2004.06.039

224. Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG. B cells and professional APCs recruit regulatory T cells via CCL4. *Nat Immunol*. 2001;2(12):1126-1132. doi:10.1038/ni735

225. Li Z, Wang X, Pan H, et al. Resistin promotes CCL4 expression through toll-like receptor-4 and activation of the p38-MAPK and NF-κB signaling pathways: implications for intervertebral disc degeneration. *Osteoarthritis Cartilage*. 2017;25(2):341-350. doi:10.1016/j.joca.2016.10.002

226. Zhang J, Li Z, Chen F, et al. TGF-β1 suppresses CCL3/4 expression through the ERK signaling pathway and inhibits intervertebral disc degeneration and inflammation-related pain in a rat model. *Exp Mol Med*. 2017;49(9):e379. doi:10.1038/emm.2017.136

227. CCL5 C-C motif chemokine ligand 5 [Homo sapiens (human)] - Gene - NCBI. Accessed June 11, 2020. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=6352

228. Miyagishi R, Kikuchi S, Takayama C, Inoue Y, Tashiro K. Identification of cell types producing RANTES, MIP-1 alpha and MIP-1 beta in rat experimental autoimmune encephalomyelitis by in situ hybridization. *J Neuroimmunol*. 1997;77(1):17-26. doi:10.1016/s0165-5728(97)00040-4

229. Crawford A, Angelosanto JM, Nadwodny KL, Blackburn SD, Wherry EJ. A role for the chemokine RANTES in regulating CD8 T cell responses during chronic viral infection. *PLoS Pathog*. 2011;7(7):e1002098. doi:10.1371/journal.ppat.1002098

230. Production and expression of RANTES (CCL5) by human disc cells and modulation by IL-1-β and TNF-α in 3D culture | Elsevier Enhanced Reader. doi:10.1016/j.yexmp.2014.01.002

231. MMP1 matrix metallopeptidase 1 [Homo sapiens (human)] - Gene - NCBI. Accessed June 11, 2020. https://www.ncbi.nlm.nih.gov/gene/4312

232. Edsparr K, Basse PH, Goldfarb RH, Albertsson P. Matrix Metalloproteinases in Cytotoxic Lymphocytes Impact on Tumour Infiltration and Immunomodulation. *Cancer Microenviron*. 2010;4(3):351-360. doi:10.1007/s12307-010-0057-0

233. Goetzl EJ, Banda MJ, Leppert D. Matrix metalloproteinases in immunity. :5.

234. Iyer S, Visse R, Nagase H, Acharya KR. Crystal Structure of an Active Form of Human MMP-1. *J Mol Biol*. 2006;362(1):78-88. doi:10.1016/j.jmb.2006.06.079

235. MMP2 matrix metallopeptidase 2 [Homo sapiens (human)] - Gene - NCBI. Accessed June 11, 2020. https://www.ncbi.nlm.nih.gov/gene/4313

236. Reference GH. MMP2 gene. Genetics Home Reference. Accessed June 14, 2020. https://ghr.nlm.nih.gov/gene/MMP2

237. Devarajan P, Johnston JJ, Ginsberg SS, Wart HEV, Berliner N. Structure and expression of neutrophil gelatinase cDNA. Identity with type IV collagenase from HT1080 cells. *J Biol Chem*. 1992;267(35):25228-25232.

238. Rutges JPHJ, Kummer JA, Oner FC, et al. Increased MMP-2 activity during intervertebral disc degeneration is correlated to MMP-14 levels. *J Pathol*. 2008;214(4):523-530. doi:10.1002/path.2317

239. Rastogi A, Kim H, Twomey JD, Hsieh AH. MMP-2 mediates local degradation and remodeling of collagen by annulus fibrosus cells of the intervertebral disc. *Arthritis Res Ther*. 2013;15(2):R57. doi:10.1186/ar4224

240. MMP3 matrix metallopeptidase 3 [Homo sapiens (human)] - Gene - NCBI. Accessed June 14, 2020. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=4314

241. Bord S, Horner A, Hembry RM, Compston JE. Stromelysin-1 (MMP-3) and Stromelysin-2 (MMP-10) Expression in Developing Human Bone: Potential Roles in Skeletal Development. *Bone*. 1998;23(1):7-12. doi:10.1016/S8756-3282(98)00064-7

242. Zigouris A, Batistatou A, Alexiou GA, et al. Correlation of matrix metalloproteinases–1 and –3 with patient age and grade of lumbar disc herniation: Clinical article. *J Neurosurg Spine*. 2011;14(2):268-272. doi:10.3171/2010.9.SPINE09935

243. MMP7 matrix metallopeptidase 7 [Homo sapiens (human)] - Gene - NCBI. Accessed June 14, 2020. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=4316

244. Yokoyama Y, Grünebach F, Schmidt SM, et al. Matrilysin (MMP-7) Is a Novel Broadly Expressed Tumor Antigen Recognized by Antigen-Specific T Cells. *Clin Cancer Res*. 2008;14(17):5503-5511. doi:10.1158/1078-0432.CCR-07-4041

245. Ra HJ, Parks WC. Control of Matrix Metalloproteinase Catalytic Activity. *Matrix Biol J Int Soc Matrix Biol*. 2007;26(8):587-596. doi:10.1016/j.matbio.2007.07.001

246. Le Maitre C, Freemont A, Hoyland J. Human disc degeneration is associated with increased MMP 7 expression. *Biotech Histochem*. 2006;81(4-6):125-131. doi:10.1080/10520290601005298

247. MMP8 matrix metallopeptidase 8 [Homo sapiens (human)] - Gene - NCBI. Accessed June 14, 2020. https://www.ncbi.nlm.nih.gov/gene/4317

248. Thirkettle S, Decock J, Arnold H, Pennington CJ, Jaworski DM, Edwards DR. Matrix Metalloproteinase 8 (Collagenase 2) Induces the Expression of Interleukins 6 and 8 in Breast Cancer Cells. *J Biol Chem*. 2013;288(23):16282-16294. doi:10.1074/jbc.M113.464230

249. MMP9 matrix metallopeptidase 9 [Homo sapiens (human)] - Gene - NCBI. Accessed June 14, 2020. https://www.ncbi.nlm.nih.gov/gene/4318

250. Yabluchanskiy A, Ma Y, Iyer RP, Hall ME, Lindsey ML. Matrix Metalloproteinase-9: Many Shades of Function in Cardiovascular Disease. *Physiology*. 2013;28(6):391-403. doi:10.1152/physiol.00029.2013

251. Zigouris A, Alexiou GA, Batistatou A, Voulgaris S, Kyritsis AP. The role of matrix metalloproteinase 9 in intervertebral disc degeneration. *J Clin Neurosci*. 2011;18(10):1424-1425. doi:10.1016/j.jocn.2011.01.036

252. Li PB, Tang WJ, Wang K, Zou K, Che B. Expressions of IL-1α and MMP-9 in degenerated lumbar disc tissues and their clinical significance. *Eur Rev Med Pharmacol Sci*. 2017;21(18):4007-4013.

253. TIMP1 TIMP metallopeptidase inhibitor 1 [Homo sapiens (human)] - Gene - NCBI. Accessed June 14, 2020. https://www.ncbi.nlm.nih.gov/gene/7076

254. Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): An ancient family with structural and functional diversity. *Biochim Biophys Acta*. 2010;1803(1):55-71. doi:10.1016/j.bbamcr.2010.01.003

255. Ries C. Cytokine functions of TIMP-1. *Cell Mol Life Sci*. 2014;71(4):659-672. doi:10.1007/s00018-013-1457-3

256. Nishida T. Kinetics of Tissue and Serum Matrix Metalloproteinase-3 and Tissue Inhibitor of Metalloproteinases-1 in Intervertebral Disc Degeneration and Disc Herniation. *Kurume Med J*. 1999;46(1):39-50. doi:10.2739/kurumemedj.46.39

257. TIMP2 TIMP metallopeptidase inhibitor 2 [Homo sapiens (human)] - Gene - NCBI. Accessed June 15, 2020. https://www.ncbi.nlm.nih.gov/gene/7077

258. Bourboulia D, Stetler-Stevenson WG. Matrix MetalloProteinases (MMPs) andTissue Inhibitors of MetalloProteinases (TIMPs): positive and negative regulators intumor cell adhesion. *Semin Cancer Biol*. 2010;20(3):161-168. doi:10.1016/j.semcancer.2010.05.002

259. TIMP3 TIMP metallopeptidase inhibitor 3 [Homo sapiens (human)] - Gene - NCBI. Accessed June 15, 2020. https://www.ncbi.nlm.nih.gov/gene/7078

260. Kishnani N. Identification and characterization of human tissue inhibitor of metalloproteinase-3 and detection of three additional metalloproteinase inhibitor activities in extracellular matrix. *Matrix Biol*. 1995;14(6):479-488. doi:10.1016/0945-053X(95)90005-5

261. The imbalance between TIMP3 and matrix-degrading enzymes plays an important role in intervertebral disc degeneration | Elsevier Enhanced Reader. doi:10.1016/j.bbrc.2015.12.020

262. Koskivirta I, Rahkonen O, Mäyränpää M, et al. Tissue inhibitor of metalloproteinases 4 (TIMP4) is involved in inflammatory processes of human

cardiovascular pathology. *Histochem Cell Biol*. 2006;126(3):335-342. doi:10.1007/s00418-006-0163-8

263. Melendez-Zajgla J, Pozo LD, Ceballos G, Maldonado V. Tissue Inhibitor of Metalloproteinases-4. The road less traveled. *Mol Cancer*. 2008;7(1):85. doi:10.1186/1476-4598-7-85

264. Takatalo J, Karppinen J, Niinimäki J, et al. Does Lumbar Disc Degeneration on Magnetic Resonance Imaging Associate With Low Back Symptom Severity in Young Finnish Adults? *Spine*. 2011;36(25):2180. doi:10.1097/BRS.0b013e3182077122

265. Akyol S, Eraslan BS, Etyemez H, Tanriverdi T, Hanci M. Catabolic cytokine expressions in patients with degenerative disc disease. *Turk Neurosurg*. 2010;20(4):492-499. doi:10.5137/1019-5149.JTN.3394-10.1

266. Grönblad M, Virri J, Tolonen J, et al. A Controlled Immunohistochemical Study of Inflammatory Cells in Disc Herniation Tissue. *Spine*. 1994;19(24):2744.

267. Dongfeng R, Hou S, Wu W, et al. The expression of tumor necrosis factor-α and CD68 in high-intensity zone of lumbar intervertebral disc on magnetic resonance image in the patients with low back pain. *Spine*. 2011;36(6):E429-433. doi:10.1097/BRS.0b013e3181dfce9e

268. Analysis of tissue distribution of TNF-alpha, TNF-alpha-receptors, and the activating TNF-alpha-converting enzyme suggests activation of the TNF-alpha system in the aging intervertebral disc - PubMed. Accessed March 27, 2023. https://pubmed.ncbi.nlm.nih.gov/17405915/

269. Yoon ST, Patel NM. Molecular therapy of the intervertebral disc. *Eur Spine J*. 2006;15(Suppl 3):379-388. doi:10.1007/s00586-006-0155-3

270. Ponnappan RK, Markova DZ, Antonio PJ, et al. An organ culture system to model early degenerative changes of the intervertebral disc. *Arthritis Res Ther*. 2011;13(5):R171. doi:10.1186/ar3494

271. Salzer E, Mouser VHM, Tryfonidou MA, Ito K. A bovine nucleus pulposus explant culture model. *J Orthop Res*. n/a(n/a). doi:10.1002/jor.25226

272. Du J, Pfannkuche J, Lang G, et al. Proinflammatory intervertebral disc cell and organ culture models induced by tumor necrosis factor alpha. *JOR Spine*. 2020;3(3):e1104. doi:10.1002/jsp2.1104

273. Stern S, Lindenhayn K, Perka C. Human intervertebral disc cell culture for disc disorders. *Clin Orthop*. 2004;(419):238-244. doi:10.1097/00003086-200402000-00039

274. Le Maitre CL, Hoyland JA, Freemont AJ. Studies of Human Intervertebral Disc Cell Function in a Constrained In Vitro Tissue Culture System. *Spine*. 2004;29(11):1187.

275. Ruiz-Fernández C, Francisco V, Pino J, et al. Molecular Relationships among Obesity, Inflammation and Intervertebral Disc Degeneration: Are Adipokines the Common Link? *Int J Mol Sci*. 2019;20(8):2030. doi:10.3390/ijms20082030

276. Shiri R, Karppinen J, Leino-Arjas P, Solovieva S, Viikari-Juntura E. The Association Between Obesity and Low Back Pain: A Meta-Analysis. *Am J Epidemiol*. 2010;171(2):135-154. doi:10.1093/aje/kwp356

277. Shin JH, Park S, Cho H, Kim JH, Choi H. Adipokine human Resistin promotes obesity-associated inflammatory intervertebral disc degeneration via pro-inflammatory cytokine cascade activation. *Sci Rep*. 2022;12:8936. doi:10.1038/s41598-022-12793-2

278. Donnally III CJ, Hanna A, Varacallo M. Lumbar Degenerative Disk Disease. In: *StatPearls*. StatPearls Publishing; 2020. Accessed April 23, 2020. http://www.ncbi.nlm.nih.gov/books/NBK448134/

279. Zhang Y, Yao L, Robinson KM, Dillingham TR. Biomarkers in the Degenerative Human Intervertebral Disc Tissue and Blood. *Am J Phys Med Rehabil*. 2022;101(10):983-987. doi:10.1097/PHM.0000000000001943

280. Gruber HE, Hanley EN. Do We Need Biomarkers for Disc Degeneration? *Biomark Insights*. 2007;1:131-133.

281. Hider SL, Konstantinou K, Hay EM, Glossop J, Mattey DL. Inflammatory biomarkers do not distinguish between patients with sciatica and referred leg pain within a primary care population: results from a nested study within the ATLAS cohort. *BMC Musculoskelet Disord*. 2019;20(1):202. doi:10.1186/s12891-019-2604-2

282. Khan AN, Jacobsen HE, Khan J, et al. Inflammatory biomarkers of low back pain and disc degeneration: a review. *Ann N Y Acad Sci*. 2017;1410(1):68-84. doi:10.1111/nyas.13551

283. Rajasekaran S, Tangavel C, Soundararajan DCR, et al. Proteomic Signatures of Healthy Intervertebral Discs From Organ Donors: A Comparison With Previous Studies on Discs From Scoliosis, Animals, and Trauma. *Neurospine*. 2020;17(2):426-442. doi:10.14245/ns.2040056.028

CHAPTER 2: RELATIONSHIPS AMOUNG PATIENT DEMOGRAPHIC CHARACTERISTICS AND THE RADIOGRAPHIC, GROSS, AND HISTOLOGICAL ASSESSMENT OF IVDD IN SYMPTOMATIC AND ASYMPTOMATIC IVDS

Jacob S. Kramer[1,2], Aaron M. Stoker[1,2], Theodore J. Choma[2], Emily V. Leary[1,2], Jinpu Li[1,2], Don K. Moore[2], James L. Cook[1,2]

[1]Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO

[2]Department of Orthopaedic Surgery, University of Missouri, Columbia, MO

INTRODUCTION

Back pain is among the leading causes of disability worldwide.[1,2] However, the sources of back pain are diverse and sometimes obscure.[3] While the majority of cases resolve without intervention, there is currently not a clinically accepted method to prognosticate the development and progression of back pain onset or severity.[4–7] Lumbar intervertebral disc degeneration (IVDD) has long been considered a significant contributor to the development of spinal pathologies and back pain.[5,8–11] IVDD involves the wearing of annulus fibrosus (AF) and nucleus pulposus (NP) tissues, which can reduce alignment and the space available for nerves in the spine.[8,12] Lumbar IVDD can be symptomatic (*S*) or asymptomatic (*A*), and is involved in tissue changes including protrusions/herniations, stenosis, spondylolisthesis, and degenerative disc disease.[3] At least two, large (n>500 individuals) published studies have reported significant, positive correlations between radiographic *S*-IVDD severity and back pain severity.[13,14] Therefore, patients with persistent (>6 weeks) back pain are commonly assessed for physical and radiographic signs of *S*-IVDD.[3]

Physicians evaluate radiographic *S*-IVDD using the T2 weighted magnetic resonance imaging (MRI) based Pfirrmann grading system, which assigns a score of 1-5 to an IVD.[9] Increasing Pfirrmann grades indicate reduced IVD homogeneity/reduction in T2 signal intensity, narrowed IVD space, and a loss of distinction between the AF and NP.[9] A study published by Yu et al. assessed radiographs from 108 *S*-IVDD cases and observed Pfirrmann grades 3, 4, and 5 in approximately 13%, 75%, and 12% of *S*-IVDs, respectively.[15]

However, radiographic *A*-IVDD is a common incidental finding in patients.[4,16] A study published by Belavy et al. in 2020 analyzed MRI images from young (25-35 years) athletes without back pain and observed primarily Pfirrmann grade 2 changes (>75%), but Pfirrmann grades 3 and 4 were observed in ~10% and ~7% of *A*-IVDs, respectively.[16] In a review published by Rahyussalim et al. in 2020, Pfirrmann grade 3 *A*-IVDs were more common (26-100% based on increasing age) than Pfirrmann grade 4 or 5 *A*-IVDs (35-72% based on increasing age).[17] Reporting *A*-IVDD prevalence across life, a review published by Brinjikji et al. in 2015 found that nearly 40% of individuals aged 20-30 years exhibited radiographic *A*-IVDD, which consistently increased in prevalence (~1.5%/year) until age 60, when average yearly increases slowed considerably (~0.5%/year).[4] These studies clearly indicate that radiographic *S*-IVDD features are also present in age-related *A*-IVDD, and while symptom *severity* appears to be related to *S*-IVDD severity, it is known that radiographic IVDD alone is not a predictor of *S*-IVDD *development*.[3]

While not a clinically relevant method to evaluate IVDD, gross changes in IVD structure related to IVDD can also be assessed visually using the Thompson grading system, which assigns a score of 1-5 based on gross assessment of the IVD in the mid-sagittal plane.[18] Increasing Thompson grades indicate increased fibrous tissue and cleft formation in the NP, decreased demarcation of AF fibers, endplate cartilage loss and sclerosis, and vertebral body osteophyte formation.[18] In 1990, Thompson et al. published this proposed IVD grading scheme and analysis of 136 *A*-IVDs, and observed approximately 15%, 30%, 27%, 20%, and 10% prevalence for Thompson grades 1-5, respectively.[18] Pękala et al. in 2022 published a well powered (n=100 donor spine segments) study assessing the agreement between Thompson and Pfirrmann grading

systems, and found that the majority of disagreement was present at the lowest scores, where Thompson Grade 1 *A*-IVDs were most often classified as Pfirrmann Grade 2.[19] Therefore, comparisons within or between Thompson/Pfirrmann grades ≤2 should be interpreted cautiously.

Unfortunately, the Thompson/Pfirmann grading systems (T/P-grades) are not sensitive to smaller scale tissue changes that occur during IVDD.[9,18] Therefore, standardized histological grading systems have been developed with the goal of characterizing IVDD changes that are inhomogeneous throughout the disc.[20] A detailed histologic IVDD grading system was published by Boos et al. in 2002, which assigned a total histologic degeneration score (HDS) of 0-22 based on presence and/or severity of 11 features of histological IVDD in sagittal *A*-IVD sections or *S*-IVD explants.[20] In that study, Boos et al. observed a significant correlation between total HDS, age, and *A*-IVD T-grade.[20] While their study included surgical samples (n=23 patients), the authors concluded that histological grading of complete IVD sections was more accurate due to potential loss of granular changes and difficulty assessing cell death accurately in *S*-IVD tissues.[20]

The original histological scoring system published by Boos et al. has been modified by Drs. Christoph Weiler, Norbert Boos, and Andreas Nerlich includes 4 histological features: AF/NP cell morphology (0-6), mucous degeneration in the AF (0-3), tear and cleft formation (0-3), and granular changes in the NP (0-3), resulting in total HDS scores ranging from 0-15.[21] This system is sometimes further modified to include cell death scoring (0-4), which raises the highest possible total HDS to 19.[20,22] Increased scores indicate AF/NP cell proliferation and aggregation (cell morphology), loss of AF fiber demarcation and excessive proteoglycan staining (mucous degeneration), ruptures in the AF/NP matrix (tear

and cleft formation), collections of eosinophilic staining proteins in the NP matrix (granular changes), and the presence of apoptotic bodies and/or ruptured cells (cell death).[21] Assessing these tissue level changes may help differentiate patients with similar P/T-grades, but present with different clinical symptoms or have different responses to treatment and/or clinical outcomes after treatment.

While these methods can provide insight into the level of IVDD occurring in a patient, the diagnostic and prognostic utility of these methods for symptom development, symptom progression, and response to treatment in clinical patients has not been established. This is because development of *S*-IVDD is likely due to a complex set of factors including patient specific demographic factors such as age, sex, obesity, lifestyle, and genetic factors.[24–27] A review of the associations between obesity and spinal diseases published by Sheng et al. in 2017 observed significantly higher risk for radiographic *S*-IVDD in overweight and obese individuals.[28] However, these findings have not been sufficient to develop diagnostic/prognostic systems for *S*-IVDD, indicating a need to understand how patient demographic characteristics interact in relation to IVDD in both symptomatic and asymptomatic patient populations.

Taken together, current literature indicates that it is difficult to clinically stage or predict the development of *S*-IVDD versus *A*-IVDD based on patient characteristics and IVD morphology, and further that few studies directly compare *S*-IVDs to *A*-IVDs histologically based on patient characteristics and their interactions.[12,20,21,23] An improved understanding of the fundamental differences in *S*-IVDD versus *A*-IVDD tissues and individuals is therefore needed in order to determine which histological and/or

radiographic/gross IVDD features and patient characteristics may be associated with *S*-IVDD versus *A*-IVDD development.

Therefore, this study was designed to determine if histologic or gross/radiographic IVDD severity are significantly different between *S*-IVDs and *A*-IVDs. Further, this study aimed to identify relationships between patient age, sex, and/or obesity and IVDD development in *S*-IVDs and *A*-IVDs, and to determine if there were significant differences in these relationships between *S*-IVDs and *A*-IVDs. It was hypothesized that total HDS and T/P-grades would be significantly higher in *S*-IVDs compared to *A*-IVDs. It was further hypothesized that age, sex, and/or obesity would be significantly related to T/P-grades and total HDS within *S*-IVDD and *A*-IVDD cohorts, and that these relationships would be significantly different between *S*-IVDD and *A*-IVDD cohorts.

METHODS

S-IVD and A-IVD Populations: With IRB approval (IRB#2010692), and informed patient consent, IVD tissues were recovered from clinical patients (*S*-IVDD cohort, n=202, mean age 55.3y, 118F, 89 obese) undergoing surgery to treat symptomatic degenerative IVD disorders such as herniation, stenosis, spondylolisthesis, and degenerative disc disease. *S*-IVD tissues were not recovered from tumor or infection related cases.

With consent as recorded in a legal permit under the Uniform Anatomical Gift Act[29], IVDs were also recovered from qualified tissue donors (*A*-IVDD cohort, n=25, mean age 53.4y, 12F, 10 obese) by the Midwest Transplant Network. Upon recovery at the Midwest Transplant Network facility, donor medical history was assessed during a Donor Risk Assessment Interview (DRAI). *A*-IVDD tissues testing positive for pathogens or diseases including HIV/AIDS, hepatitis, HTLV-I/II, Chagas' disease, hepatitis, malaria, cancers, Ebola virus, and Zika virus were excluded from this study. Additionally, *A*-IVDD tissues with a history of discogenic back pain or spinal surgery were not recovered for this study. Patient/donor age was restricted to 17-95 years.

S-IVD Tissue Processing and Culture: *S*-IVD tissues recovered from clinical procedures (n=210 identified levels) were suspended in sterile phosphate buffered saline (PBS) within one hour of surgery completion. A 6mm diameter explant from the tissues recovered from each *S*-IVD were created for *ex vivo* tissue culture. *S*-IVD explants were cultured for 6 days in supplemented (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium) DMEM.

A-IVD Tissue Processing: Spine segments were recovered from the deceased tissue donors and transported to the lab within 24 hours of death. *A*-IVDs were recovered from all available lumbar levels (n=73) of each received lumbar spinal column (n=25).

Gross/radiographic IVDD Assessment: *S*-IVDs with identified levels (n=199) were each assigned a P-grade[9] (1-5) by one observer blinded to patient history using preoperative MRI data. *A*-IVDs (n=60) were bisected in the mid-sagittal plane at the time of receipt and assigned a T-grade[18] (1-5) by one observer blinded to patient history.

Histological Assessment: A portion of each cultured *S*-IVD specimen and sagittal/transverse sections in the median plane of each *A*-IVD were formalin fixed, decalcified in 10% EDTA, paraffin-embedded, and tissue sections were stained using H&E, Toluidine blue, and Picrosirius red. For *S*-IVD tissues, histological classification of AF or NP tissue was performed, and tissues found to contain a mixture of AF/NP and/or bone were excluded from analysis in this study.

A modified IVD scoring system based on the system published by Boos et al.[30] was used to evaluate each tissue by one blinded pathologist. *S*-IVD and *A*-IVD tissues were assessed for total HDS (TOTAL HDS, 0-19) by summing the scores for cell morphology (CELL-M, 0-6), mucous degeneration in the AF (MUCOUS, 0-3), cell death (DEATH, 0-4), tear and cleft formation (TEAR, 0-3), and granular changes in the NP (GRAN, 0-3). TOTAL HDS ranging from 0-6 were classified as mild, 7-13 as moderate, and 14-19 as severe histological IVDD.

Statistical analysis: For simplicity, "patient characteristics" will refer to *S* patient *and/or A* donor demographics. In order to account for patient characteristics (i.e., age, sex, and BMI) as factors, BMI was dichotomized as obese/not obese ($\leq$30/>30 kg/m^2). In the *S*-IVDD

cohort, 89 (44%) patients were classified as not obese and 113 (56%) as obese. In the *A*-IVDD cohort, 13 (52%) individuals were classified as not obese (≤30 kg/m^2) and 10 (40%) as obese (>30 kg/m^2). Additionally, T/P-grades "2" were aggregated with T/P-grades "3" due to low frequencies of the former. Multivariable linear models (LMs) were created for T/P-grades and histology scores, and *S*-IVDs were compared to *A*-IVDs with adjustment for patient characteristics and their interactions. Then, LMs were created for T/P-grades and histology scores within each cohort, and patient characteristics were compared. Two-sided significance was set at α=0.05. Predicted LM values were plotted for each comparison within and between *S*-IVDD and *A*-IVDD cohorts.

RESULTS

S-IVD and A-IVD Characteristics: Of 202 *S*-IVDD patients, 170 (84%) had single level procedures and 32 (16%) had multi-level procedures. Tissues from n=11 multilevel patients were pooled in the operating room, resulting in 199 *S*-IVDs with identified levels collected from L1-L5. The Pfirrmann grades of *S*-IVDs ranged from 3-5 (mean ± SD = 3.95±0.74). Of 199 *S*-IVDs, 64 (30%) received a P-grade of 3, 97 (46%) a P-grade of 4, and 38 (18%) a P-grade of 5. From the 25 *A* spine segments received, 73 *A*-IVDs were recovered from L1-L5 (Table 1). The range of Thompson grades in *A*-IVDs included grades 2-5 (mean ± SD = 3.46±0.86). Of the 60 *A*-IVDs assigned a T-grade, 7 (12%) were assigned T-grade 2, 27 (45%) grade 3, 19 (32%) grade 4, and 7 (12%) grade 5. *S*-IVDs and *A*-IVDs demonstrated clear evidence of histological IVDD in all assessed categories. *S*-IVD TOTAL HDS were most frequently moderate (7-13), though scores for each category were more evenly distributed than in *A*-IVDs (Table 2). *A*-IVD TOTAL HDS were also most frequently moderate (7-13); within each category, higher (2-3) MUCOUS, TEAR, and DEATH scores were particularly common (Table 3).

Differences between S-IVDD and A-IVDD Scores: Radiographically/grossly, T/P-grades for *S*-IVDs were significantly ($p<0.001$) higher than those observed in *A*-IVDs (Figure 1). There was not significant difference in TOTAL HDS observed between *S*-IVDs and *A*-IVDs, though DEATH and TEAR scores were significantly ($p\leq0.005$) higher in *S*-IVD and *A*-IVD tissues, respectively (Table 4).

Relationship between Patient Characteristics and level of IVDD in S-IVD or A-IVD cohorts:

The data from this study indicated significant relationships between patient characteristics

and the radiographic and histological scores of both the S and A patient cohorts. For the *S*-IVDD cohort, P-grades were significantly (p=0.01) increased in younger, obese patients, though considerable overlap in models was observed for this comparison (Figure 2, Table 5). For the *A*-IVDD cohort, T-grades significantly (p=0.0001) increased with age in males and females (Figure 3A, Table 5).

Histologically, TOTAL HDS were significantly (p=0.02) increased in older, obese males in the *A*-IVDD cohort (Figure 3B, Table 5). However, a significant relationship between patient demographics and TOTAL HDS was not observed in the *S*-IVD cohort. (Table 5) Further, significant relationships were observed between the patient demographic characteristics and the scores for each histologic scoring category. (Table 5) For the *A*-IVDD cohort, TEAR scores were significantly (p=0.03) increased in older, obese individuals; CELL-M and DEATH scores were significantly increased ($p\leq0.02$) in older, obese males; and GRAN scores significantly (p=0.014) decreased with increasing age. For the *S*-IVDD cohort, MUCOUS scores were significantly (p=0.03) increased in older females

DISCUSSION

The data from this study indicated potentially important differences in IVDD progression between *S*-IVDD and *A*-IVDD patient cohorts. The degree of radiographic IVDD in the *S*-IVDD cohort was significantly greater than the degree of gross IVDD in the *A*-IVDD, indicating that patients with *S*-IVDD have higher levels of IVDD based on radiographic/gross assessment of tissue structure. The data in this study also indicated that age is a significant factor IVDD progression. In the *A*-IVDD cohort the T-grade of the IVD increased as patient age increased, supporting the belief that age is an import contributor to *A*-IVDD progression. In the *S*-IVDD cohort, the P-grade if the IVD increased with patient grade in the non-obese patient population, but not in the obese *S*-IVDD patient population. This finding supports the belief that obesity is a factor in the development of *S*-IVDD in younger patients. Taken together, these data indicate that *S*-IVDs and *A*-IVDs undergo similar age-related radiographic/gross changes, but obesity may contribute to development of *S*-IVDD in younger patients.

Several published studies have reported significant associations between obesity, back pain, and radiographic IVDD.[28,31–34] Among these is a well powered, (n=23,048 cases from the 2014 Medical Expenditure Panel Study) retrospective analysis published by Sheng et al. in 2017, which reported that age and obesity were both significantly related to back pain and *S*-IVDD occurrence.[28] In a subsample of males, a study published by Chou et al in 2016 reported significantly increased BMI in participants with severe compared to no/mild back pain.[2] Therefore, it is possible that obesity may be related to earlier/more severe symptom onset in *S* patients in the present study.

In contrast to the T/P-Grade data, a significant difference between the *S*-IVDD and *A*-IVDD cohorts was not observed for the level of histological IVDD based on Total HDS. However, increased DEATH and decreased TEAR scores were observed in *S*-IVDs compared to *A*-IVDs. This finding indicates that increased cell death in the IVD may contribute to the development of *S*-IVDD and is a distinguishing factor between patients who develop *S*-IVDD and those who have *A*-IVDD. It is possible that the increase in tear formation observed in the *A*-IVDD cohort is due to differences in how the tissues from the *S*-IVDD and *A*-IVDD cohorts were processed for histological analysis. Because a sagittal section of the IVD was assessed in the *A*-IVDD cohort and only an explant of tissue was assessed in the *S*-IVDD cohorts, it is possible that tear formation in the IVD can be more accurately assessed in the sagittal *A*-IVD sections than *S*-IVD tissue explants assessed in this study.

Interestingly, the data from this study indicated more significant relationships between patient demographics and histological measures of IVDD in the *A*-IVDD cohort than the *S*-IVDD cohort. In agreement with the T-grade data, increasing age was associated with increased TOTAL HDS, TEAR, CELL-M, and DEATH scores in the *A*-IVDD cohort. Further, obesity and male sex were consistently associated with age as a significant factor with increases in these histological scores. This finding may indicate that these patient factors contribute to the histological changes that occur during normal *A*-IVDD development and progression. Since similar associations between patient demographic factors and histological scores of IVDD were not observed in the *S*-IVDD cohort, it is possible that other factors are driving the progression of these measures of histological IVDD in patients with *S*-IVDD.

In contrast to the other histological scores assessed in the study, scores for granular changes to the NP decreased with patient age in the *A*-IVDD cohort. This finding indicates a potential role for changes to the NP and the development of *S*-IVDD. It is possible that as patients with higher granular changes to the NP age, they are more likely to develop *S*-IVDD. However, there was not a significant difference between the *A*-IVDD cohort and the *S*-IVDD cohort for this histological score, indicating similar levels of granular changes to the NP in these two patient populations. Therefore, further study is required to determine if granular changes to the NP are related to the development of *S*-IVDD.

For the *S*-IVDD cohort, the only histological score with a significant relationship to patient demographics was the MUCOUS score. The data from this study indicated MUCOUS scores increased significantly in older female patients with *S*-IVDD. While further research is required to determine the clinical significance for this association, overall, the data from this study indicates that there is not a significant relationship between patient demographic factors and histological indicators of IVDD in the *S*-IVDD cohort. Therefore, in contrast to the *A*-IVDD cohort, these data indicate that other factors besides patient demographic factors may be contributing to the histological progression of IVDD for patients with *S*-IVDD. Because the development of *S*-IVDD is complex and multifactorial, there are numerous possible co-factors that contribute to histological IVDD progression in the *S*-IVDD cohort, including injury, patient occupation, and biological factors produced by the IVD tissues. Further study is required to determine how these, and other potential factors, contribute to the histological progression IVDD in the *S*-IVDD patient population.

LIMITATIONS

The primary limitations of this study were the differences in type and timing of histological and gross/radiographic assessments for *S*-IVD and *A*-IVD tissues. However, these differences are due to the innate differences in tissue recovery between the two cohorts. Because the entire IVD was recovered from tissue donors in the *A*-IVDD cohort, it was possible to assess the entire IVD histologically for this study. However, removal of the entire IVD *en bloc* is not standard of practice for the surgical treatment of patients with *S*-IVDD. Therefore, IVDs in the *S*-IVDD cohort would not be assessed as whole sagittal sections like IVDs in *A*-IVDD cohort. Further, the tissue samples recovered from surgical patients were pooled tissue fragments from IVD, and it was not possible to differentiate between AF and NP tissues prior to culture. Therefore, histological assessment of the cultured IVD explant was performed to ensure accurate identification of the tissue that was cultured. Since AF and NP tissues were easily distinguishable prior to culture in the *A*-IVDD cohort, histological assessment was not required to determine tissue type of the cultured explant. Therefore, it is possible that more significant associations were observed between in the *A*-IVDD cohort and patient demographics in this study because the entire IVD was assessed histological, and only one tissue explant was assessed in the *S*-IVDD cohort.

Regarding gross/radiographic IVD assessments, it was not financially feasible to acquire MRI images for donor segments, therefore, this study relied on the comparison of T-grading system to assess gross IVDD in the *A*-IVDD. While it is possible that the T-grading and P-grading of IVDD performed in this study were not assessing similar level of IVDD, a previous study reported a Cohen's kappa of .61 ($p<.001$) for the two systems

across grades, and 92% of disagreements were by a difference of 1 grade (usually T grade 1 IVDs were scored as P grade 2).[19] Therefore, since the T-grades and P-grades of the IVDs in this study were mostly ≥3, it is likely that the two measures were assessing similar levels of IVDD in the two patient populations.

Finally, the tissues from the *S*-IVDD cohort were cultured for six days prior to processing for histological analysis. Therefore, it is possible that the histological scores of the cultured tissues in the *S*-IVDD cohort were increased due to factors related to the culturing process. However, previous studies using animal tissues have indicated that significant changes in tissue histological scores were not observed in whole IVDs and tissue explants after >6 days of *ex vivo* tissue culture.[35,36] Therefore, it is unlikely that the 6 day culture period used in this study had a significant effect on the histological scores of the tissues in the *S*-IVDD cohort.

CONCLUSIONS

This study aimed to determine gross/radiographic and/or histologic distinguishers of *S*-IVDD and *A*-IVDD, and to determine if specific patient demographic measures (age, sex, BMI) had a significant association with level of IVDD in each cohort. The data from this study indicated that a range of IVDD levels could be observed in both the *S*-IVDD and *A*-IVDD cohorts, supporting previous research indicating the IVDD can occur with or without the presentation of symptoms. The data also indicated that IVDs in the *S*-IVDD cohort had significantly higher gross measures of IVDD than IVDs in the *A*-IVDD cohort, when accounting for patient age, sex, and BMI. This is not surprising since the patients in the *S*-IVDD cohort were surgical patients being treated for back pain associated with *S*-IVDD. However, there was not a significant difference between the *S*-IVDD and *A*-IVDD cohorts for the total HDS of the IVD when accounting for patient age, sex, and BMI, indicating that similar changes to the IVD occurs at the microscopic level in the *S*-IVDD and *A*-IVDD cohorts. Interestingly, the *S*-IVDD cohort had significantly higher DEATH scores than the *A*-IVDD cohort, indicating that increased cell death in the IVD tissue may contribute to symptom development in patients.

The data from this study also identified more significant relationships between patient demographic factors and measures of IVDD in the *A*-IVDD cohort compared to the *S*-IVDD cohort. This finding may indicate that increased IVDD in the *A*-IVDD cohort are more related to patient demographic factors, but other factors are contributing to the increases in IVDD observed in *S*-IVDD cohort. Therefore, future studies are needed to identify factors that contribute to IVDD progression in the patients with *S*-IVDD.

FIGURES

Patient Characteristics		
	S-IVDs	*A*-IVDs
Sex		
Male	84	13
Female	118	12
Total	202	25
Mean age ± SD	55.3 ± 15.4	53.4 ± 14.7
<30 kg/m^2	89	13
>30 kg/m^2	113	10
Sample Characteristics		
Identified Levels		
L1	4	14
L2	14	15
L3	36	20
L4	76	16
L5	77	8
Total	210	73

Table 1: Demographic and IVD information for *S*-IVDs or *A*-IVDs. BMI information was not available for n=2 *A*-IVD patients.

S-IVD Histology Total and Category Score Distributions (% of tissues)

SCORE	TOTAL	CELL-M	DEATH	MUCOUS	TEAR	GRAN
0	0 (0)	7 (2.5)	1 (.36)	1 (.6)	8 (2.9)	45 (42)
1	0 (0)	7 (2.5)	29 (10.4)	18 (10.5)	74 (26.6)	31 (29)
2	0 (0)	39 (14.0)	109 (39.2)	54 (31.4)	153 (55.0)	25 (24)
3	0 (0)	133 (47.8)	110 (39.6)	99 (57.6)	43 (15.5)	5 (4.7)
4	3 (1.1)	42 (15.1)	29 (10.4)			
5	5 (1.8)	20 (7.2)				
6	13 (4.7)	30 (10.8)				
7	28 (10.1)					
8	41 (14.8)					
9	45 (16.2)					
10	45 (16.2)					
11	38 (13.7)					
12	27 (9.7)					
13	17 (6.1)					
14	8 (2.9)					
15	6 (2.2)					
16	1 (.36)					
17	1 (.36)					
18	0 (0)					
19	0 (0)					

Table 2: Frequency distribution of TOTAL HDS, CELL-M, DEATH, MUCOUS, TEAR, and GRAN scores in *S*-IVDs

A-IVD Histology Total and Category Score Distributions (% of tissues)

SCORE	TOTAL	CELL-M	DEATH	MUCOUS	TEAR	GRAN
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	24 (32.9)
1	0 (0)	0 (0)	8 (5.5)	5 (6.9)	14 (9.7)	22 (30.1)
2	0 (0)	2 (1.4)	85 (58.2)	27 (37.5)	80 (55.2)	13 (17.8)
3	0 (0)	79 (54.5)	50 (34.5)	40 (55.6)	51 (35.2	14 (19.2)
4	0 (0)	46 (31.7)	2 (1.4)			
5	0 (0)	16 (11.0)				
6	4 (2.8)	2 (1.4)				
7	12 (8.3)					
8	19 (13.1)					
9	28 (19.3)					
10	22 (15.2)					
11	26 (17.9)					
12	14 (9.7)					
13	17 (11.7)					
14	3 (2.1)					
15	0					
16	0					
17	0					
18	0					
19	0					

Table 3: Frequency distribution of TOTAL HDS, CELL-M, DEATH, MUCOUS, TEAR, and GRAN scores in *A*-IVDs.

***S* vs *A* Model Results**

IVDD Measures	*Coefficient Estimate (β)*	*P-value*
T/P Grade	**-0.36**	**0.001***
TOTAL HDS	0.41	0.087
CELL-M	0.24	0.058
DEATH	**-0.24**	**0.005***
MUCOUS	0.07	0.490
TEAR	**0.45**	**0.001***
GRAN	0.31	0.058

Table 4: Significant ($p<0.05$) differences in gross/radiographic or histologic IVDD in *S*-IVDs vs *A*-IVDs while accounting for age, sex, and obesity status. A negative β coefficient indicates significantly increased scores in *S*-IVDs.

***S* or *A* Model Results**

	***S*-IVDD Cohort Characteristics**		***A*-IVDD Cohort Characteristics**	
IVDD Measures	***Coefficient Estimate (β)***	***P-value***	***Coefficient Estimate (β)***	***P-value***
T/P Grade	**Age:Obesity (-0.02)**	**0.029***	**Age (0.03)**	**0.001***
TOTAL HDS			**Age:Sex:Obesity (0.36)**	**0.020***
CELL-M			**Age:Sex:Obesity (0.12)**	**0.015***
DEATH			**Age:Sex:Obesity (0.13)***	**0.002***
TEAR			**Age:Obesity (0.03)**	**0.027***
MUCOUS	**Age:Sex (-0.02)**	**0.011***		
GRAN			**Age (-0.04)**	**0.002***

Table 5: Significant (* = $p<0.05$) effects of age, sex, obesity, and their interactions on gross/radiographic or histological IVDD in *S*-IVDs or *A*-IVDs.

S-IVDs VS *A*-IVDS BY HISTOLOGICAL OR MACROSCOPIC IVDD SEVERITY

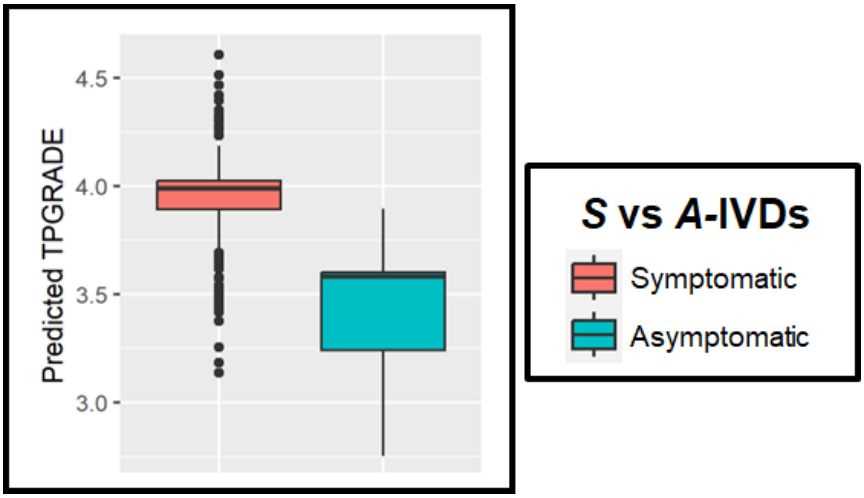


Figure 1: Significant ($p<0.05$) difference in radiographic/gross IVDD between *S*-IVDs vs. *A*-IVDs with cofactor adjustment for patient/donor age, sex, and obesity status.

RELATIONSHIPS TO PATIENT CHARACTERISTICS IN *S*-IVDD

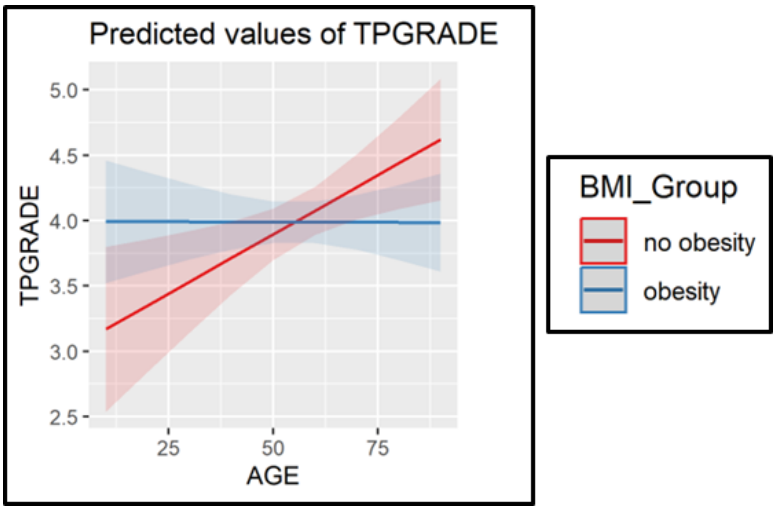


***Figure 2**:* Significant ($p<0.05$) relationship between gross/radiographic IVDD and obesity with increasing age in *S*-IVDs.

RELATIONSHIPS TO PATIENT CHARACTERISTICS IN *A*-IVDD

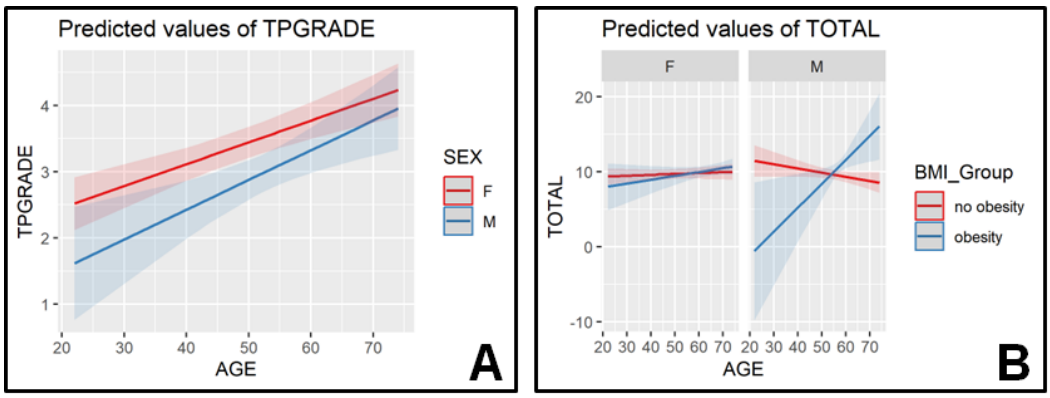


Figure 3: Significant ($p<0.05$) relationship between gross/radiographic IVDD and obesity with increasing age in male and female *A*-IVDs

SUMMARY OF SIGNIFICANT DIFFERENCES WITHIN AND BETWEEN *S*-IVDs AND *A*-IVDs

	IVDD	
COHORT	T/PGRADE	TOTAL
A vs S	S>A	
S	↑OB<55	
A	↑	↑M>55, OB

Table 6: Summary of significant ($p<0.05$) differences within and between *S*-IVDD and *A*-IVDD cohorts. "↑/↓" indicates a significant increase/decrease with age or across factor levels, "F" = female, "M" = male, "OB" = obesity, ">55" = more than 55 years old, "OB" = within obese individuals.

REFERENCES

1. Murray CJL, Vos T, Lozano R, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet*. 2012;380(9859):2197-2223. doi:10.1016/S0140-6736(12)61689-4

2. Chou L, Brady SRE, Urquhart DM, et al. The Association Between Obesity and Low Back Pain and Disability Is Affected by Mood Disorders. *Medicine (Baltimore)*. 2016;95(15):e3367. doi:10.1097/MD.0000000000003367

3. Berry JA, Elia C, Saini HS, Miulli DE. A Review of Lumbar Radiculopathy, Diagnosis, and Treatment. *Cureus*. 11(10). doi:10.7759/cureus.5934

4. Brinjikji W, Luetmer PH, Comstock B, et al. Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations. *AJNR Am J Neuroradiol*. 2015;36(4):811-816. doi:10.3174/ajnr.A4173

5. Derby R, Kim BJ, Lee SH, Chen Y, Seo KS, Aprill C. Comparison of discographic findings in asymptomatic subject discs and the negative discs of chronic LBP patients: Can discography distinguish asymptomatic discs among morphologically abnormal discs? *Spine J*. 2005;5(4):389-394. doi:10.1016/j.spinee.2005.01.007

6. Kalichman L, Cole R, Kim DH, et al. Spinal stenosis prevalence and association with symptoms: The Framingham Study. *Spine J Off J North Am Spine Soc*. 2009;9(7):545-550. doi:10.1016/j.spinee.2009.03.005

7. Ishimoto Y, Yoshimura N, Muraki S, et al. Prevalence of symptomatic lumbar spinal stenosis and its association with physical performance in a population-based cohort in Japan: the Wakayama Spine Study. *Osteoarthritis Cartilage*. 2012;20(10):1103-1108. doi:10.1016/j.joca.2012.06.018

8. An HS, Masuda K, Inoue N. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthop Sci*. 2006;11(5):541-552. doi:10.1007/s00776-006-1055-4

9. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine*. 2001;26(17):1873-1878. doi:10.1097/00007632-200109010-00011

10. Antoniou J, Steffen T, Nelson F, et al. The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration. doi:10.1172/JCI118884

11. Inoue N, Espinoza Orías AA. Biomechanics of Intervertebral Disc Degeneration. *Orthop Clin North Am*. 2011;42(4):487-499. doi:10.1016/j.ocl.2011.07.001

12. Histology and Pathology of the Human Intervertebral Disc : JBJS. Accessed September 21, 2022. https://journals.lww.com/jbjsjournal/Abstract/2006/04002/Histology_and_Pathology_of_the_Human.3.aspx

13. Takatalo J, Karppinen J, Niinimäki J, et al. Does Lumbar Disc Degeneration on Magnetic Resonance Imaging Associate With Low Back Symptom Severity in Young Finnish Adults? *Spine*. 2011;36(25):2180. doi:10.1097/BRS.0b013e3182077122

14. Cheung KMC, Karppinen J, Chan D, et al. Prevalence and Pattern of Lumbar Magnetic Resonance Imaging Changes in a Population Study of One Thousand Forty-Three Individuals. *Spine*. 2009;34(9):934. doi:10.1097/BRS.0b013e3181a01b3f

15. Yu LP, Qian WW, Yin GY, Ren YX, Hu ZY. MRI Assessment of Lumbar Intervertebral Disc Degeneration with Lumbar Degenerative Disease Using the Pfirrmann Grading Systems. *PLoS ONE*. 2012;7(12):e48074. doi:10.1371/journal.pone.0048074

16. Belavy DL, Brisby H, Douglas B, et al. Characterization of Intervertebral Disc Changes in Asymptomatic Individuals with Distinct Physical Activity Histories Using Three Different Quantitative MRI Techniques. *J Clin Med*. 2020;9(6):1841. doi:10.3390/jcm9061841

17. Rahyussalim AJ, Zufar MLL, Kurniawati T. Significance of the Association between Disc Degeneration Changes on Imaging and Low Back Pain: A Review Article. *Asian Spine J*. 2020;14(2):245-257. doi:10.31616/asj.2019.0046

18. Thompson JP, Pearce RH, Schechter MT, Adams ME, Tsang IK, Bishop PB. Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. *Spine*. 1990;15(5):411-415. doi:10.1097/00007632-199005000-00012

19. Pękala P, Taterra D, Krupa K, et al. Correlation of morphological and radiological characteristics of degenerative disc disease in lumbar spine: a cadaveric study. *Folia Morphol*. 2022;81(2):503-509. doi:10.5603/FM.a2021.0040

20. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine*. 2002;27(23):2631-2644. doi:10.1097/00007632-200212010-00002

21. Weiler C, Lopez-Ramos M, Mayer HM, et al. Histological analysis of surgical lumbar intervertebral disc tissue provides evidence for an association between disc degeneration and increased body mass index. *BMC Res Notes*. 2011;4:497. doi:10.1186/1756-0500-4-497

22. Lee NN, Kramer JS, Stoker AM, et al. Canine models of spine disorders. *JOR Spine*. 2020;3(4):e1109. doi:10.1002/jsp2.1109

23. Le Maitre CL, Dahia CL, Giers M, et al. Development of a standardized histopathology scoring system for human intervertebral disc degeneration: an Orthopaedic Research Society Spine Section Initiative. *JOR Spine*. 2021;4(2):e1167. doi:10.1002/jsp2.1167

24. Ganesan S, Acharya AS, Chauhan R, Acharya S. Prevalence and Risk Factors for Low Back Pain in 1,355 Young Adults: A Cross-Sectional Study. *Asian Spine J*. 2017;11(4):610-617. doi:10.4184/asj.2017.11.4.610

25. Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. *Spine*. 2002;27(2):125-134. doi:10.1097/00007632-200201150-00002

26. Ala-Kokko L. Genetic risk factors for lumbar disc disease. *Ann Med*. 2002;34(1):42-47. doi:10.1080/078538902317338634

27. Seidler A, Bolm-Audorff U, Siol T, et al. Occupational risk factors for symptomatic lumbar disc herniation; a case-control study. *Occup Environ Med*. 2003;60(11):821-830. doi:10.1136/oem.60.11.821

28. Sheng B, Feng C, Zhang D, Spitler H, Shi L. Associations between Obesity and Spinal Diseases: A Medical Expenditure Panel Study Analysis. *Int J Environ Res Public Health*. 2017;14(2):183. doi:10.3390/ijerph14020183

29. Verheijde JL, Rady MY, McGregor JL. The United States Revised Uniform Anatomical Gift Act (2006): New challenges to balancing patient rights and physician responsibilities. *Philos Ethics Humanit Med PEHM*. 2007;2:19. doi:10.1186/1747-5341-2-19

30. Classification of Age-Related Changes in Lumbar Intervertebr... : Spine. Accessed August 24, 2022. https://journals.lww.com/spinejournal/Fulltext/2002/12010/Classification_of_Age_Related_Changes_in_Lumbar.2.aspx

31. Andersen RE, Crespo CJ, Bartlett SJ, Bathon JM, Fontaine KR. Relationship between body weight gain and significant knee, hip, and back pain in older Americans. *Obes Res*. 2003;11(10):1159-1162. doi:10.1038/oby.2003.159

32. Liuke M, Solovieva S, Lamminen A, et al. Disc degeneration of the lumbar spine in relation to overweight. *Int J Obes 2005*. 2005;29(8):903-908. doi:10.1038/sj.ijo.0802974

33. Xu X, Li X, Wu W. Association Between Overweight or Obesity and Lumbar Disk Diseases: A Meta-Analysis. *J Spinal Disord Tech*. 2015;28(10):370-376. doi:10.1097/BSD.0000000000000235

34. Koyanagi A, Stickley A, Garin N, et al. The association between obesity and back pain in nine countries: a cross-sectional study. *BMC Public Health*. 2015;15(1):123. doi:10.1186/s12889-015-1362-9

35. Pelle DW, Peacock JD, Schmidt CL, et al. Genetic and Functional Studies of the Intervertebral Disc: A Novel Murine Intervertebral Disc Model. *PLoS ONE*. 2014;9(12):e112454. doi:10.1371/journal.pone.0112454

36. Le Maitre CL, Fotheringham AP, Freemont AJ, Hoyland JA. Development of an in vitro model to test the efficacy of novel therapies for IVD degeneration. *J Tissue Eng Regen Med*. 2009;3(6):461-469. doi:10.1002/term.180

37. McDonnell EE, Buckley CT. Investigating the physiological relevance of ex vivo disc organ culture nutrient microenvironments using in silico modeling and experimental validation. *JOR Spine*. 2021;4(2):e1141. doi:10.1002/jsp2.1141

CHAPTER 3: RELATIONSHIPS BETWEEN PATIENT CHARACTERISTICS TO SIGNALING PEPTIDES AND MATRIX AFFECTING ENZYMES WITHIN AND BETWEEN ASYMPTOMATIC AND SYMPTOMATIC POPULATIONS

Jacob S. Kramer[1,2], Aaron M. Stoker[1,2], Theodore J. Choma[2], Emily V. Leary[1,2], Jinpu Li[1,2], Don K. Moore[2], James L. Cook[1,2]

[1]Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO

[2]Department of Orthopaedic Surgery, University of Missouri, Columbia, MO

INTRODUCTION

Back pain and the need for surgery have been linked to lumbar intervertebral disc degeneration (IVDD).[1] IVDD involves the wearing of annulus fibrosus (AF) and nucleus pulposus (NP) tissues, which reduces the available space and alignment in the spine.[2] Hallmark features often accompany IVDD, including herniation of the NP, stenosis, and spondylolisthesis.[2] The development of any of these changes may elicit painful symptoms (*S*-IVDD) that are often debilitating.[3–5] However, these changes are also found in patients with asymptomatic IVDD (*A*-IVDD).[6] Because similar levels of IVDD can occur in *S*-IVDD and *A*-IVDD patients, the factors that contribute to the development of *S*-IVDD are poorly understood. However, the aberrant production of inflammatory cytokines/chemokines, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors by the AF and NP tissues of the IVD are thought to potentially contribute to the development of *S*-IVDD.[7–10] The production of the proteins by the cells have to the potential to be used as protein biomarkers to indicate changes to the IVD related to symptom development in patients.[11]

Inflammatory cytokines are produced and released by IVD cells as signaling agents for homeostasis.[7,12] Chemokines respond to inflammatory cytokines to promote or inhibit the movement and activity of immune cells.[7,12] The most commonly studied secreted inflammatory cytokines and chemokines related to the development and progression of IVDD are tissue necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-17, interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1 (CCL2), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES, CCL5), and MCP-3

(CCL7).[7] An imbalance in inflammatory mediators has been found to induce variable cellular effects including immune cell infiltration, autophagy, apoptosis, and senescence in AF and NP cells.[7,13] These changes in the IVD caused by excessive IVD tissue inflammation are thought to contribute to the development of *S*-IVDD.[14] Previous studies and reviews have reported significantly increased expression and production of numerous inflammatory cytokines and chemokines with increasing IVDD, supporting a role for inflammatory cytokines and chemokines in the development of *S*-IVDD .[7,14–18]

While there are numerous studies relating changes in inflammatory biomarker production by IVD tissues to level of IVDD, there few studies that compare the changes in these biomarkers between IVDs with *S*-IVDD and *A*-IVDD. A review published by K. Wuertz and L. Haglund in 2013 included 20 studies analyzing expression, production, or *in situ* localization of inflammatory cytokines tissues recovered from *S*-IVDs and/or *A*-IVDs.[14] In their review, only 2 studies statistically compared inflammatory cytokine presence between *S*-IVD and *A*-IVD tissues. These two studies used IHC staining of *S*-IVD and *A*-IVD tissues to determine that S-IVDs had higher level of IL-17 than *A*-IVD tissues, and that there was a positive correlation between tissue TNF-α staining and histological IVDD level and patient age in *S*-IVD and *A*-IVD tissues.[17,19] Therefore, there is currently only limited information on the differences in tissue inflammatory biomarker production during IVDD progression in patients with *S*-IVDD and *A*-IVDD.

Inflammatory mediators can also stimulate the production of proteases including MMPs and a disintegrins and metalloproteinases with thrombospondin motifs (ADAMTS).[7,20] These proteases can be subdivided into collagenases (MMP-1, 8, 13), gelatinases (MMP-2, 9), stromelysins (MMP-3, 10), matrilysin (MMP-7) and aggrecanases

(ADAMTS-1, 4, 5, 9).[7,21] MMPs and ADAMTS degrade extracellular matrix (ECM) proteins (i.e., collagen, proteoglycan) as a normal part of tissue maintenance.[8,9,14] Because these degradative enzymes degrade tissue ECM, their activity is highly regulated through multiple mechanisms. Most of these enzymes are produced in an inactive (latent) form that must be modified in order to become active.[22] Additionally, MMPs are regulated by proteins produced by the tissue called tissue inhibitors of matrix metalloproteinases (TIMPs), which block protease activity by binding to the MMP.[21,23] A cycle of inflammation and imbalances in MMP/TIMP levels in the IVD are thought to contribute to excessive degradation and fibrotic tissue formation, though the roles of specific degradative enzymes in the development of *S*-IVDD, or the differences between patients with *S*-IVDD and *A*-IVDD, have not been fully established.[20,23–25]

A review published by Wang et al. reported that of six studies examining differences in MMP levels in human IVD tissues related to IVDD, only two studies included tissues from *S*-IVDs and *A*-IVDs, and no study included in the review delineated differences based on AF or NP tissue types.[26] The two studies comparing *S*-IVD versus *A*-IVD tissues observed significant increases in MMP-8 and MMP-10 mRNA expression in *S*-IVDs.[20,27] Within *S*-IVDs, Weiler et al. reported significant, positive correlations between IHC staining for MMP-1, MMP-2, MMP-3, and total histological degeneration scores (total HDS).[28] Le Maitre et al. reported no observable MMP-3 or MMP-13 IHC staining in histologically normal IVDs, though the study did not differentiate between patients with *S-IVDD* and *A*-IVDD.[8] Roberts et al. reported significantly increased MMP-3 and MMP-7 IHC staining with increasing *S*-IVDD and that TIMP-1 staining was present in *S*-IVD samples but not in *A*-IVDs, but that TIMP-2 IHC staining was similar for *S-IVDs* and *A*-

IVDs.[29] When assessing *S*-IVD mRNA expression of TIMPs, Bachmeier et al. found significantly increased expression of TIMP-1 and TIMP-2 in *S*-IVD tissues with higher total HDS.[20] However, Deng et al. in 2015 found no significant difference in TIMP-1 gene expression in *S*-IVD versus *A*-IVD tissues.[30] Taken together, the current literature characterizing the role of MMP/TIMP balance in the development of *S*-IVDD or *A*-IVDD is incomplete and conflicting.[20,26,29,30] However, because significant changes in IVD tissue structure and composition occurs during IVDD, a loss of balance between catabolism and anabolism is considered particularly important in the development of *S*-IVDD.[31–33]

The controlled growth of cells/tissues is an important component of tissue maintenance.[34] Growth factors are able to activate immune cells, promote or inhibit cell proliferation and matrix synthesis, and stimulate the production of inflammatory cytokines, chemokines, and MMPs.[34] The expression and production of growth factors including insulin growth factor (IGF-1), fibroblastic growth factor (FGF), bone morphogenic protein (BMP), growth differentiation factor (GDF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF) have been observed in *S-IVDs* and/or *A*-IVDs, though their association to *S*-IVDD development and progression is not known.[10,35–38] VEGF has been investigated due to its role in the proliferation of blood vessels, which may be a significant factor affecting IVD nutrient access.[39] While *S*-IVD tissues have been shown to produce VEGF, the presence of blood vessels in VEGF positive *S*-IVD tissues is not consistently observed.[40,41] To the authors' knowledge, no published studies have directly compared growth factor expression and/or production between *S*-IVD versus *A*-IVD tissues.[38,42,43]

While the role of growth factors in IVDD are poorly understood, studies that focus on the potential of growth factors as therapeutic agents for the treatment of *S*-IVDD may provide insight into potential roles for growth factors during the development and progression of IVDD clinically.[35,36,42] Paglia et al. reported significantly decreased cell death and matrix degradation following PDGF intradiscal injection in a preclinical rabbit model of degenerative disc disease (DDD).[44] In a bovine IVDD model testing the effects of FGF-2, Li et al. reported suppression of excessive proteoglycan production and dose dependent modulation of MMP-13 expression and production.[45] Therefore, growth factor production by the IVD tissue during IVDD may be associated with the tissues attempt to decrease tissue ECM degradation.

Previous studies assessing changes in IVD tissue metabolic responses during IVDD have primarily focused on flow cytometry, gene expression, and IHC techniques to analyze cell types and protein biomarker production.[17,19,32,46–50] While these measures do provide important data needed to characterize the IVD microenvironment, the assessment of proteins released during *ex vivo* culture has to the potential to indicate what the IVD tissues are actively producing.[51–55] However, most previous human IVD *ex vivo* tissue and cell culture studies have primarily aimed to characterize *S*-IVD matrix metabolism and develop models to use as a tool to screen potential treatments for *S*-IVDD, and few studies have characterized differences in ex vivo protein production by *S*-IVDs versus *A*-IVDs.[14,17,33]

The relationship between IVD biomarker production and *S*-IVDD development may also be affected by patient characteristics including age, sex, and BMI.[17,29,56,57] Several previous studies have reported that radiographic and histologic *S*-IVDD and *A*-IVDD progress with age and obesity, and additionally that obesity may increase the risk of *S*-

IVDD development.[58–60] Obesity is also associated with increased systemic inflammatory cytokine concentrations, which may further affect inflammatory responses of IVD tissues during *S*-IVDD.[57,60–62] Additionally, some studies have reported earlier *A*-IVDD in males, though females may be more susceptible to *S*-IVDD development, which indicates there may be sex specific factors that contribute to IVDD that could affect the biological response of the IVD tissues.[6,63–65] While some extensive proteomic characterization studies have been published regarding IVD tissues, studies associating *S*-IVD and/or *A*-IVD biomarker content/production to patient characteristics are rare.[11,66–69] Additionally, interactions between patient characteristics may have a differential effect on *S*-IVDD and *A*-IVDD progression.

Therefore, this study aimed to identify significant differences in the *ex vivo* production of inflammatory cytokines/chemokines, MMPs, TIMPs, and growth factors by AF and NP tissues recovered from patients with *S*-IVDD and *A*-IVDD, when accounting for patient age, sex, and BMI. Further, this study aimed to identify significant relationships between the *ex vivo* production of these proteins by the IVD tissues and patient age, sex, and BMI within the *S*-IVDD and *A*-IVDD cohorts. An increased understanding of the individual and biochemical differences of *S*-IVDD and/or *A*-IVDD patients and tissues is needed to develop patient and stage specific therapies for the mitigation or prevention of *S*-IVDD. Therefore, it was hypothesized that *S*-AF and *S*-NP tissues would release significantly ($p<0.05$) higher concentrations of inflammatory cytokines/chemokines, MMPs, and significantly decreased concentrations of TIMPs and growth factors, compared to *A*-AF and *A*-NP tissues during *ex vivo* culture, while adjusting for patient age, sex, BMI, and their interactions. Additionally, it was hypothesized that patient age, sex, BMI or their

interactions would be associated with significantly ($p<0.05$) different levels of biomarker production within *S*-IVDD and *A*-IVDD cohorts.

METHODS

S-IVDD Population: IRB approval (IRB#2010692), and informed patient consent, spine tissues were recovered from patients (*S*, n=202, mean age 55.2y, 118F, 113 obese) undergoing surgery to treat *S*-IVDD disorders such as herniation, stenosis, spondylolisthesis, and DDD. *S*-IVD tissues were not recovered from tumor or infection related cases. Patient/donor age was restricted to 17-95 years.

A-IVDD Population: With consent as recorded in a legal permit under the Uniform Anatomical Gift Act[70], IVD tissues from qualified tissue donors (*A*, n=36, mean age 52.3y, 18F, 19 obese) were recovered by the Midwest Transplant Network. Medical history was thoroughly evaluated during a Donor Risk Assessment Interview (DRAI). *A*-IVD patient tissues testing positive for pathogens or diseases including HIV/AIDS, hepatitis, HTLV-I/II, Chagas' disease, hepatitis, malaria, cancers, Ebola virus, and Zika virus were excluded from this study. Additionally, IVD tissues were not recovered from patients with a history of discogenic back pain or spinal surgery were not recovered. Patient age was restricted to 17-95 years.

S-IVD Tissue Collection and tissue culture: *S*-IVD issues recovered from clinical procedures (n=210 specimens) were suspended in sterile phosphate buffered saline (PBS) within one hour of surgery completion. A 6 mm diameter explant from *S*-IVD tissues and from *A*-AF and -NP tissues were created for ex vivo tissue cultures. Tissue explants were cultured for 3 days in supplemented DMEM (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium). Media were collected after culture and stored at -20°C.

A-IVD Tissue Collection: Spine segments were recovered from the deceased and transported to the lab within 24 hours of death. IVDs were recovered from all available lumbar levels (n=103) of each received lumbar spinal column (n=36). A 6 mm diameter explant from AF and NP were created for *ex vivo* tissue cultures. Tissue explants were cultured separately for 3 days in supplemented DMEM (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium). Media were collected after culture and stored at -20°C.

Histological Assessment of S-IVD Tissues: Following tissue culture, a portion of each *S*-IVD specimen was formalin fixed, decalcified in 10% EDTA, paraffin-embedded, and stained using H&E, Toluidine blue, and Picrosirius red. Histological classification of *S*-AF or *S*-NP tissue was performed. *S*-IVD specimens that were a mixture of tissue types (AF,NP, cartilage endplate, and/or bone) were excluded from analysis in this study.

Media biomarker analysis: Using commercially available Luminex multiplex magnetic bead assays, *S* and *A*-IVD tissue culture media were tested for inflammatory cytokines/chemokines: GRO-α, MCP-1, MCP-3, TNF-α, IL-1RA, IL-6, IL-8, MIP-1α, MIP-1β, RANTES (Millipore); matrix metalloproteinases: MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13 (R&D Systems); tissue inhibitors of matrix proteinases: TIMP-1, TIMP-2, TIMP-3, TIMP-4 (Millipore); and growth factors: PDGF-AA, PDGF-AB/BB, VEGF, and FGF-2 (Millipore).

Statistical analysis: In this report, ":" will be used to refer to an interaction between patient characteristics (e.g., "Age:Obesity" indicates the interaction between age and obesity). BMI was dichotomized as obese/not obese ($\leq$30/>30 kg/m^2). For simplicity, "patient characteristics" will refer to *S*-IVDD or *A*-IVDD patient demographics (age, sex, obesity).

Media biomarker concentrations were standardized to the wet weight of each explant and natural log transformed. Multivariable linear models (LMs) were then created for each biomarker, and *S*-IVDs were compared to *A*-IVDs with adjustment for patient characteristics and their interactions. Then, LMs were created within *S*-IVDD or *A*-IVDD cohorts, and patient characteristics associated with significantly different biomarker production(s) were determined. Two-sided significance was set at $\alpha=.05$. Significant differences are reported as β coefficients followed by the associated p-value. Interaction plots of predicted biomarker values were used to interpret LM results.

RESULTS

Donor/Patient Characteristics:

Of 202 *S*-IVDD patients, 170 (84%) had single level procedures and 32 (16%) had multi-level procedures. Tissues from n=11 multi-level *S*-IVDD patients were pooled in the operating room, and with exclusion of non-AF or -NP tissues, 199 S-IVDs with identified levels of L1-L5 were analyzed. From n=36 *A*-IVDD patients, n=103 IVDs were recovered from L1-L5 (Table 1). Mean age ± SD in the *S*-IVDD cohort was 55.2 ± 15.4 years. Mean age ± SD in the *A*-IVDD cohort was 52.3 ± 14.5 years. In the *S*-IVDD cohort, n=86 patients were classified as not obese (<30 kg/m^2) and n=113 as obese (>30 kg/m^2). In the *A*-IVDD cohort, n=19 were obese and n=15 individuals were not obese.

Symptomatic versus Asymptomatic IVDs: Ex vivo Protein Production in the AF or NP

All significant differences in ex vivo protein production in *S* IVDs versus *A*- IVDs are listed in Table 2.

Inflammatory Cytokines/Chemokines

The release of GRO-α, MCP-3, IL-1Ra, IL-8, MCP-1, MIP-1α, MIP-1β, RANTES, and TNF-α were significantly ($p \leq 0.043$) higher by *S*-AF and *S*-NP tissues compared to *A*-AF and *A*-NP tissues, respectively (Table 3). The release of IL-6 by *S*-NP tissues was the significantly ($p < 0.001$) higher than *A*-NP tissues during *ex vivo* culture (Figures D1-D2).

Degradative Enzymes (MMPs)

S-AF and *S*-NP tissues released significantly ($p \leq 0.001$) higher levels of MMP-1, MMP-2, and MMP-13, and significantly ($p \leq 0.008$) lower levels of MMP-3 and MMP-8

compared to *A*-AF and *A*-NP tissues, respectively (Table 4). The release of MMP-7 was significantly (p=0.030) higher by *S*-NP tissues compared to *A*-NP tissues (Figure D3-D4).

Anti-Degradative Proteins (TIMPs, growth factors)

The release of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 were significantly ($p \leq 0.002$) higher by the *A*-AF and *A*-NP compared to the S-AF and S-NP tissues, respectively (Table 5). The release of PDGF-AA by the *S*-AF and *S*-NP were significantly ($p \leq 0.001$) higher than the *A*-AF and *A*-NP. The release of VEGF by the *S*-NP was significantly ($p \leq 0.001$) higher than the *A*-NP tissues (Figures D5-D6).

Patient Characteristics and Ex Vivo Protein Production

All significant differences observed within each tissue group (*S*-AF, *S*-NP, *A*-AF, *A*-NP) are reported in Table 6. Summaries of the directionality of each observed difference in each tissue type are reported in Tables 7-8.

Age, Sex, or Obesity alone

Increasing age was associated with a significant ($p \leq 0.037$) decrease in the release of the degradative enzymes MMP-1, MMP-7, and MMP-13 by *S*-AF tissues during *ex vivo* culture. Increasing age was also associated with a significant (p=0.002) decrease in the release of FGF-2 by *A*-AF during *ex vivo* culture (Figures D7-D8). Increasing age was associated with a significant ($p \leq 0.05$) increase in the release of TIMP-4, PDGF-AA, and VEGF by *S*-NP tissues during ex vivo culture. Increasing age was associated with a significant ($p \leq 0.05$) increase in the release of PDGF-AA, MMP-2, MMP-9, and MMP-13 by *A*-NP tissues during *ex vivo* culture (Figures D9-D10). The release of MMP-8 was significantly (p=0.047) higher by female *A*-AF tissues compared to male *A*-AF tissues

during *ex vivo* culture (Figure D11). The release of TIMP-3 was significantly (p=0.025) higher by female *A*-NP tissues compared to male *A*-NP tissues during *ex vivo* culture (Figure D12). No significant differences in *ex vivo* protein release were observed in relation to obesity alone as a factor.

Age:Sex, Age:Obesity, or Sex:Obesity interactions

When the interaction between patient age and sex were assessed, several significant relationships to protein release during ex vivo culture by the AF and NP tissues were observed. For *S*-AF tissues, the release of MCP-1 by was significantly (p=0.036) increased in older females. For *A*-AF tissues, the release of MMP-3 by was significantly (p=0.001) increased in younger females (Figures D13). For *S*-NP tissues, the release of RANTES was significantly (p=0.021) increased in older females, and the release of MMP-2 was significantly (p=0.014) decreased in younger females, compared to males (Figure D14). For *A*-NP tissues, the release of IL-1Ra, MMP-3, MMP-8, TIMP-1, and TIMP-2 by younger females was significantly ($p \leq 0.047$) higher compared to males, though there was considerable overlap in the confidence intervals for predicted values of MMP-8, TIMP-1, and TIMP-2. (Figure D15).

When the interaction between patient age and obesity were assessed, several significant relationships to protein release during ex vivo culture by the *A*-AF and *A*-NP tissues, but not for the *S*-AF and *S*-NP tissues. For *A*-AF tissues, obesity was associated with a significantly ($p \leq 0.025$) increased release of MCP-1 and MCP-3, and a significantly ($p \leq 0.020$) decreased release of PDGF-AA, MMP-1, TIMP-1, and TIMP-4, by younger patients compared to older patients. Additionally, no obesity was associated with a significantly ($p \leq 0.030$) increased release of TIMP-2 and TIMP-3 *A*-AF tissues from

younger patients compared to older patients (Figure D16). For *A*-NP tissues, obesity was associated with a significantly ($p=0.001$) increased release of FGF-2, and significantly ($p\leq0.048$) decreased levels of TIMP-1, TIMP-2, TIMP-3, TIMP-4 in younger patients compared to older patients (Figure D17).

When the interaction between patient sex and obesity were assessed, a few significant relationships to protein release during *ex vivo* culture by the *A*-AF and *A*-NP tissues were observed, but not for the *S*-AF and *S*-NP tissues. For *A*-NP tissues, obesity was associated with a significantly ($p\leq0.038$) increased release of MMP-8 and MMP-9 by male patients compared to female patients. For *A*-AF tissues, obesity was associated with a significantly ($p\leq0.045$) increased release of MMP-9 by male patients compared to female patients (Figures D18-D19).

Age:Sex:Obesity interactions

When the interaction between patient age, sex, and obesity were assessed, several significant relationships to protein release during ex vivo culture by the *A*-AF and *A*-NP tissues, but not for the *S*-AF and *S*-NP tissues. For *A*-AF tissues, obesity in females was associated with a significantly ($p=0.021$) increased release of TNF-α release by younger patient compared to older patients (Figure D20). For *A*-NP tissues, obesity in females was also associated with a significantly ($p\leq0.004$) increased release of MCP-1 and RANTES by younger patients compared to older patients, but the confidence intervals for predicted RANTES values overlapped considerably (Figure D21). Additionally, obesity in males was associated with a significantly ($p\leq0.047$) increased release of MMP-1 and MMP-7 by *A*-NP from younger patients compared to older patients (Figure D22).

DISCUSSION

The data from this study indicates that the release of inflammation related proteins by *S*-IVD tissues during *ex vivo* culture is significantly higher than *A*-IVD tissues, which indicates that increased inflammatory signaling by IVD tissues may be an important factor in the development of *S*-IVDD. Further, the data from this study indicates potentially important differences in the production of MMPs and TIMPs by the AF and NP tissues recovered from patients with *S*-IVDD and *A*-IVDD. Tissues from *S*-IVDs released significantly higher levels of MMP-1, MMP-7, and MMP-13, and significantly lower levels of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 compared to tissues from *A*-IVDs. This observation indicates that during *S*-IVDD, there may be a significant shift toward increased degradative enzyme activity that does not occur in patients with *A*-IVDD. Interestingly, growth factor release was increased in *S*-IVDs, potentially reflecting an attempted response by the AF and NP tissues to counter the effects of excessive inflammation and degradation that occurs during *S*-IVDD.[58,71–73] In agreement with the analysis of the gross and histological data, there were more significant associations identified between patient demographics (age, sex, BMI) and the release of the proteins assessed in this study by *A*-IVD tissues compared to *S*-IVD tissues. This observation further supports the concept that patient demographic factors are a significant contributor to *A*-IVDD development and progression, while other factors may be significant contributors to *S*-IVDD development, or development of *S*-IVDD disrupts the influence of patient demographic factors on IVDD.

The release of the majority of the tested inflammation related proteins were significantly increased by both AF and NP tissues from *S*-IVDD patients compared to tissues from *A-IVDD* patients. However, the release of IL-6 during culture was only

significantly higher by *S*-NP tissues compared to *A*-NP tissues. This finding may indicate an important difference in the response of AF and NP tissues to the development of *S*-IVDD. The NP is often the first IVD tissue to exhibit degenerative changes with age or pathology, and significantly higher levels of IL-6 have been detected in herniated *S*-IVD tissues compared to scoliosis patient IVDs.[7,74,75] Further, a meta-analysis by Deng et al. found a significant, positive correlation between serum IL-6 concentrations and early *S*-IVDD changes (i.e., bulging, protrusion).[76] Therefore, the NP may be the source of the IL-6 being detected in these patients with earlier stages of *S*-IVDD. However, the level and duration of *S*-IVDD in the patients were not considered in this analysis, so further study is required to determine if IL-6 production by the NP is increased at earlier stages of IVDD.

The significantly higher release of MMP-3 and MMP-8, and significantly lower release of MMP-1, MMP-7, and MMP-13, by *A*-AF and *A*-NP tissues compared to *S*-AF and *S*-NP may represent a distinct degradative phenotype in tissues from patients with *A*-IVDD compared to patients with *S*-IVDD. While the role and reason for the shift in MMP production cannot be elucidated by this study, the data indicates a shift towards higher production of MMP-1 and MMP-13, which are consistently associated with increased production and matrix degradation by orthopaedic tissues during disease.[20,26,77] When considered in the context of the significantly lower release of TIMP-1, TIMP-2, TIMP-3, and TIMP-4 released by *S*-IVD tissues compared to *A*-IVD tissues, the data from this study indicates a significant shift towards higher degradative enzyme activity and matrix degradation occurs in patients with *S*-IVDD compared to patients with *A*-IVDD.

As indicated earlier, there were more significant associations identified between patient demographics (age, sex, BMI) and the release of the proteins assessed in this study

by *A*-IVD tissues compared to *S*-IVD tissues. Increasing age was associated with significantly decreased MMP release by *S*-AF tissues and significantly increased MMP release by *A*-NP tissues, potentially indicating different degradative timing in *S*-IVD and *A*-IVD tissues. Additionally, younger age and obesity were associated with significant increases in inflammatory cytokine/chemokine release by *A*-AF and *A*-NP tissues, and significant decreases in age-related *A*-IVD TIMP release, which could indicate that obesity may contribute to the convergence of *A*-IVDD and *S*-IVDD metabolic profiles in younger patients. Further, there were more significant associations identified between patient demographics (age, sex, BMI) and the release of the proteins assessed in this study by *S*-NP and *A*-NP compared to *S*-AF and *A*-AF tissues, potentially reflecting the greater range of possible NP tissue changes that occur with age and degeneration.[72,78–80]

LIMITATIONS

As with any study, there are numerous limitations to consider when interpreting the data from this study. First, there was a notable disparity in the number of *A*-IVDD versus *S*-IVDD patients, and multiple samples were analyzed from each *A*-IVDD tissue donor. However, due to the inherent difficulty in obtaining tissues from patients with *A*-IVDD, it would be difficult to recover tissues from a similar number of patients with *A*-IVDD as were obtained from patients with *S*-IVDD who were undergoing surgery to treat their disease. Further, assessing samples from multiple levels of the same patient, which may have significantly different grades of IVDD, may better reflect the variability in IVD pathobiology observed in clinical patients with *A*-IVDD.

Additionally, there was a disparity in the distribution of the levels of the lumbar spine analyzed in the *A*-IVDD and *S*-IVDD patients. For the majority of the patients in the *A*-IVDD cohort, IVD tissues were recovered from lumbar (L1-L5) levels. However, the clinical distribution of *S*-IVDD levels was concentrated in L4-L5 and L5-S1, potentially presenting unique biochemical changes that reflect the increased biomechanical load experienced in the lower lumbar levels.[81,82] While analysis could have been focused on just these two levels of the lumbar spine, the significant decrease in sample size could have resulted in a significant decrease in study power. On going studies aimed at increasing the number of *A*-IVDD patients could allow for analysis of the data that includes IVD level as a factor or focused on specific levels of the lumbar spine. The data from this study will be used to help guide the analysis of the samples in these future studies.

Additionally, tissues were not stratified by grade of IVDD, nor were the *S*-IVDD patients grouped by surgery type in this study, which could be a significant indicators of

level of *S*-IVDD.[83–85] Since the level of IVDD degradation could have a significant impact on the production of the protein biomarkers assessed in this study, future studies should include measures of IVDD to ensure samples with similar levels of IVDD are used when comparing the release of these proteins by IVD tissues during *ex vivo* culture. However, the data from this study does provide a foundation of inherent differences between IVD tissues from patients with *A*-IVDD and *S*-IVDD that can be used to guide the development of future studies that include this sample information as a factor in the analysis.

Finally, the timeframe for recovering tissues from *A*-IVDD patients and *S*-IVDD patients and processing and culturing the tissues in the lab were different. Tissues recovered from *S*-IVDD surgical patients recovered from the patient and processed for culture in the laboratory within an hour of surgery, while tissues recovered from the *A*-IVDD patients were recovered from the deceased tissue donor and processed in the laboratory within 24 hours. It is possible that the delay in the *A*-IVDD cohort could have significantly affected the viability the IVD and the release of proteins from the tissues during culture. However, the protocol for tissue recovery used for the *A*-IVDD patients is the same as the protocol used to recover osteochondral allograft tissues used clinically, and a significant reduction in tissue viability is not observed in this timeframe in osteochondral allograft tissues.

CONCLUSIONS

The data from this study demonstrated numerous significant differences in *ex vivo* protein production between *S*-IVD and *A*-IVD tissues. The significantly higher release of TIMPs, the significant disparity in the release of specific MMPs, and the significantly lower release of inflammatory cytokines and chemokines by tissues from patients *A*-IVDD compared to *S*-IVDD tissues may be indicative of key pathways that drive the development of *S*-IVDD in patients. Additionally, the differences in the number of significant relationships between patient demographic data (age, sex, and BMI) and release of proteins by the *S*-IVD and *A*-IVD tissues, further supports the concept that patient factors may be more important in the progression and metabolic response of IVDD in patients with *A*-IVDD than patients with *S*-IVDD. While patient demographics likely still contribute to the development of *S*-IVDD, other factors appear to have a significant influence on the release of the protein biomarkers assessed in this study. Identifying what these additional factors may help improve diagnostic, prognostic, and treatment methodologies for patient with *S*-IVDD, or are at risk for developing *S*-IVDD.

FIGURES

Patient Characteristics		
	S-IVDs	*A*-IVDs
Sex		
Male	84	18
Female	118	18
Total	202	36
Mean Age	55.2 ± 15.4	52.3 ± 14.5
<30 kg/m^2	89	19
>30 kg/m^2	113	15
Sample Characteristics		
Identified Levels		
L1	4	16
L2	14	17
L3	36	27
L4	76	24
L5	77	19
Total	210	103

***Table 4*:** Demographics and IVD levels for *S*-IVDs and *A*-IVDs. BMI information was not available for n=2 *A*-IVDD patients.

***S* vs *A* Model Results**

Biomarker	*AF*		*NP*	
	Coefficient Estimate (β)	***P-value***	***Coefficient Estimate (β)***	***P-value***
GRO-α	**-0.53**	**0.006***	**-0.86**	**0.001***
IL-1RA	**-0.12**	**0.001***	**-0.10**	**0.001***
IL-6	-0.22	0.212	**-0.77**	**0.001***
IL-8	**-0.57**	**0.010***	**-1.10**	**0.001***
MCP-1	**-0.75**	**0.001***	**-0.55**	**0.001***
MCP-3	**-0.10**	**0.010***	**-0.07**	**0.043***
MIP-1α	**-0.35**	**0.001***	**-0.50**	**0.001***
MIP-1β	**-0.21**	**0.003***	**-0.26**	**0.001***
RANTES	**-1.10**	**0.001***	**-0.95**	**0.001***
TNF-α	**-0.15**	**0.001***	**-0.19**	**0.002***
MMP-1	**-0.75**	**0.003***	**-1.30**	**0.001***
MMP-2	**-2.50**	**0.001***	**-1.80**	**0.001***
MMP-3	**0.90**	**0.001***	**0.70**	**0.008***
MMP-7	0.25	0.130	**-0.37**	**0.030***
MMP-8	**1.80**	**0.001***	**2.00**	**0.001***
MMP-9	**-0.52**	**0.005***	-0.02	0.040
MMP-13	**-2.50**	**0.001***	**-1.90**	**0.001***
TIMP-1	**0.55**	**0.002***	**1.00**	**0.001***
TIMP-2	**1.30**	**0.001***	**2.10**	**0.001***
TIMP-3	**0.84**	**0.001***	**1.20**	**0.001***
TIMP-4	**1.20**	**0.001***	**1.50**	**0.001***
PDGF-AA	**-0.14**	**0.001***	**-0.18**	**0.001***
VEGF	-0.18	0.104	**-0.50**	**0.001***

Table 5: Results of multivariate, generalized LMs comparing *S*-IVD versus *A*-IVD ex vivo release of inflammatory, degradative, and anti-degradative molecules by AF or NP tissues with adjustment for patient age, sex, and obesity status. β coefficients and their associated p-values are listed. A negative β coefficient indicates significantly higher production by *S*-IVDs.

Significant *S* or *A* Model Results

S-AF		
Biomarkers	***Coefficient Estimate (β)***	***P-value***
MCP-1	Age:Sex (-0.040)	0.036*
MMP-1	Age -(.050)	0.021*
MMP-7	Age (-0.030)	0.037*
MMP-13	Age (-0.030)	0.012*

A-AF		
Biomarkers	***Coefficient Estimate (β)***	***P-value***
MCP-1	Age:Obesity (-0.040)	0.004*
MCP-3	Age:Obesity (-0.003)	0.025*
TNF-α	Age:Sex:Obesity (0.009)	0.021*
MMP-1	Age:Obesity (0.100)	0.011*
MMP-3	Age:Sex (0.050)	0.001*
MMP-8	Sex (-2.00)	0.047*
MMP-9	Sex:Obesity (4.30)	0.013*
TIMP-1	Age:Obesity (0.045)	0.020*
TIMP-2	Age:Obesity (.050)	0.030*
TIMP-3	Age:Sex (.045)	0.010*
	Age:Obesity (.042)	0.048*
TIMP-4	Age:Obesity (.066)	0.001*
PDGF-AA	Age:Obesity (0.003)	0.045*
FGF2	Age (-0.011)	0.002*

S-NP		
Biomarkers	***Coefficient Estimate (β)***	***P-value***
RANTES	Age:Sex (-0.043)	0.007*
MMP-2	Age:Sex (-0.099)	0.014*
	Age:Obesity (-0.084)	0.015*
TIMP-4	Age (0.025)	0.025*
PDGF-AA	Age (0.014)	0.002*
VEGF	Age (0.034)	0.020*

A-NP		
Biomarkers	***Coefficient Estimate (β)***	***P-value***
IL-1RA	Age:Sex (-0.001)	0.001*
MCP-1	Age:Sex:Obesity (-0.050)	0.004*
RANTES	Age:Sex:Obesity (-0.010)	0.002*
MMP-1	Age:Sex:Obesity (-0.088)	0.041*
MMP-2	Age (0.074)	0.001*
MMP-3	Age:Sex (0.042)	0.037*
MMP-7	Age:Sex:Obesity (-0.059)	0.047*
MMP-8	Age:Sex (0.037)	0.047*
	Sex:Obesity (4.32)	0.038*
MMP-9	Sex:Obesity (4.61)	0.022*
	Age (0.035)	0.001*
MMP-13	Age (0.058)	0.006*
TIMP-1	Age:Sex (0.038)	0.027*
	Age:Obesity (0.062)	0.004*
TIMP-2	Age:Sex (0.041)	0.03*
	Age:Obesity (0.058)	0.012*
TIMP-3	Sex (-2.40)	0.025*
	Age:Obesity (0.050)	0.048*
TIMP-4	Age:Obesity (0.061)	0.017*
PDGF-AA	Age (0.002)	0.005*
FGF2	Age:Obesity (-0.014)	0.001*

Table 6: Results of multivariate, generalized LMs of patient characteristics within *S*-AF/NP or *A*-AF/NP IVD tissue groups. Each cell indicates characteristics associated with significantly ($p<0.05$) different ex vivo biomarker release followed by their associated β coefficient and p-value.

FULL DISSERTATION (D) FIGURES

Inflammatory Cytokine/chemokine release by S-AF versus A-AF tissues

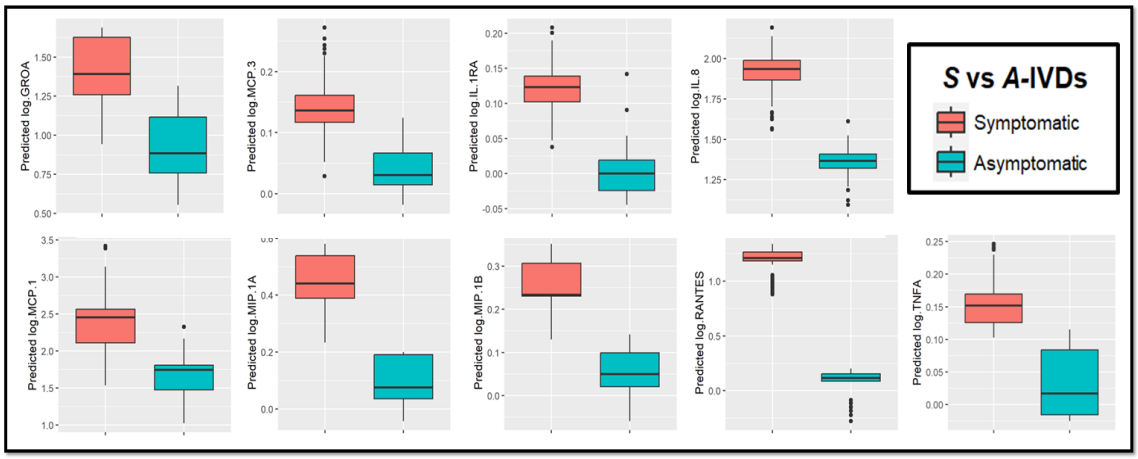


***Figure D1*:** Significant ($p<0.05$) differences in the ex vivo release of inflammatory cytokines and chemokines by *S*-AF vs. *A*-AF tissues.

Inflammatory Cytokine/chemokine release by S-NP versus A-NP tissues

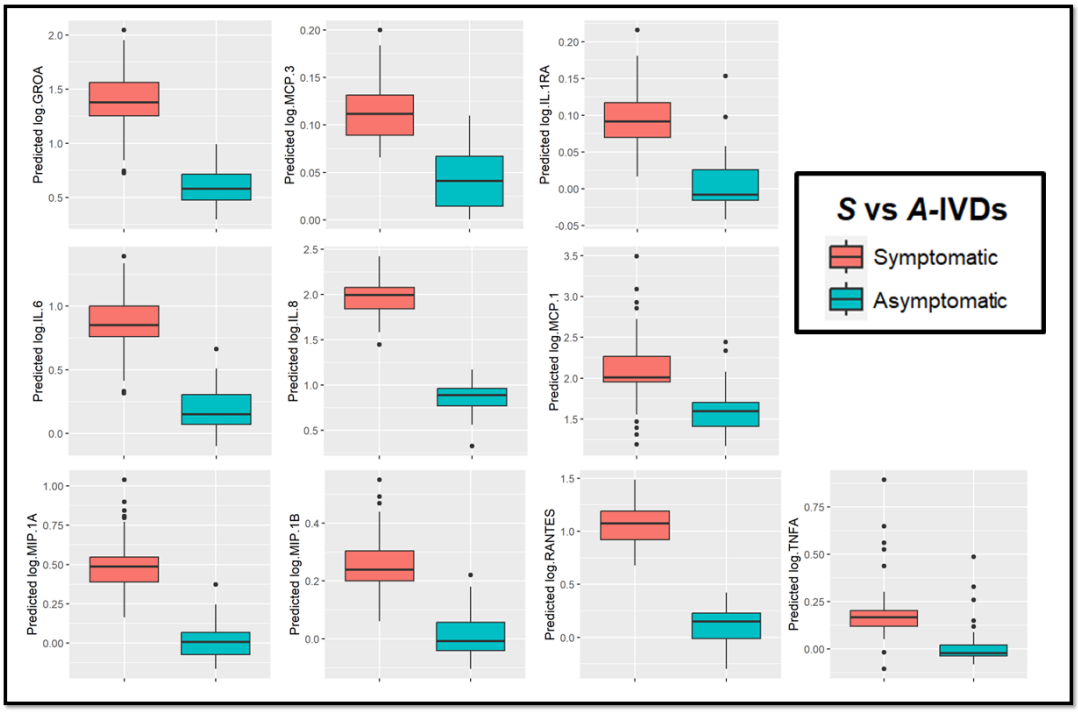


***Figure D2*:** Significant ($p<0.05$) differences in the ex vivo release of inflammatory cytokines and chemokines by *S*-NP vs. *A*-NP tissues.

MMP Release by S-AF versus A-AF Tissues

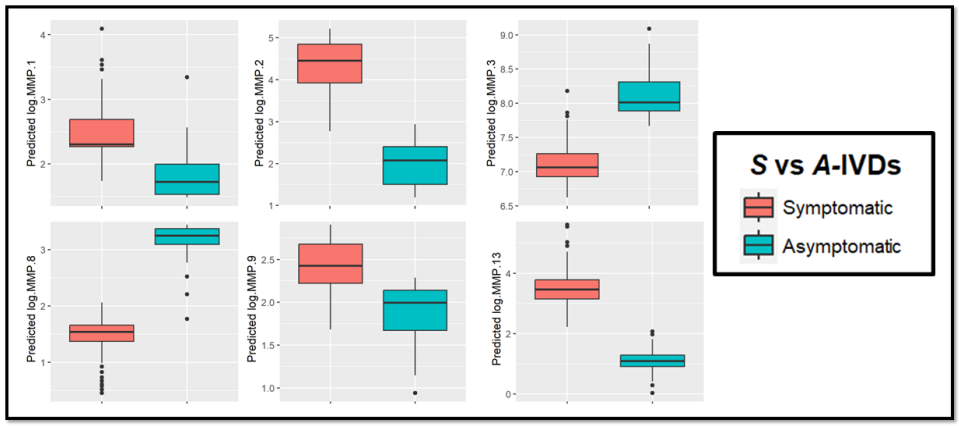


Figure D3: Significant ($p<0.05$) differences in the ex vivo release of MMPs by *S*-AF vs. *A*-AF tissues.

MMP Release by S-NP versus A-NP Tissues

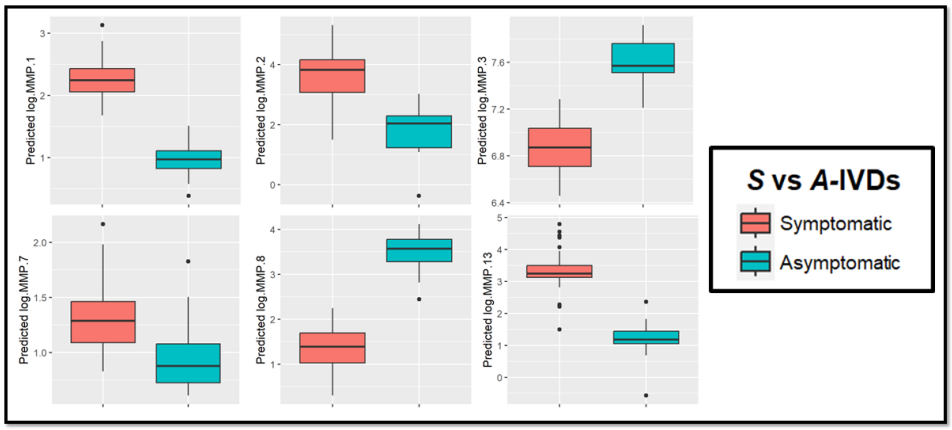


Figure D4: Significant ($p<0.05$) differences in the ex vivo release of MMPs by *S*-NP vs. *A*-NP tissues.

Growth Factor and TIMP Release by S-AF versus A-AF Tissues

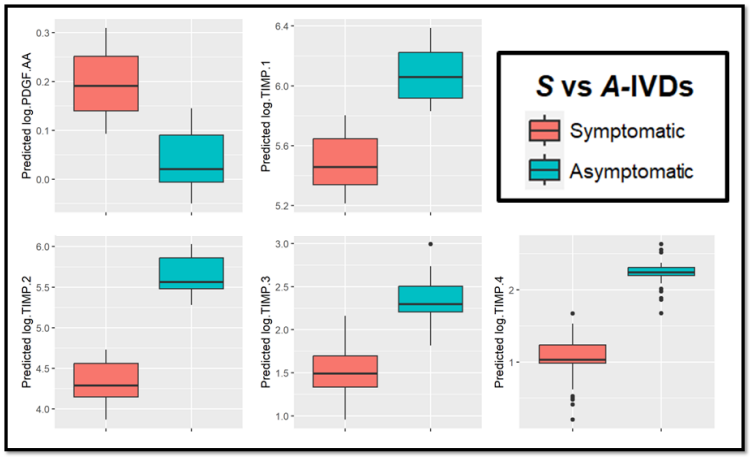


Figure D5: Significant ($p<0.05$) differences in the ex vivo release of growth factors and TIMPs by *S*-AF vs. *A*-AF tissues.

Growth Factor and TIMP Release by S-NP versus A-NP Tissues

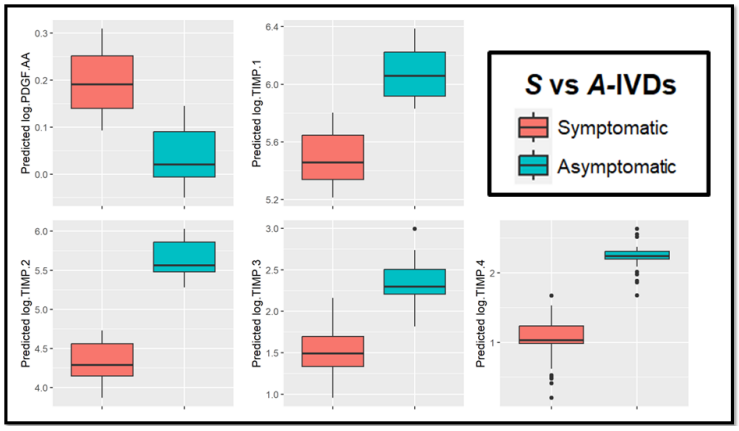


Figure D6: Significant ($p<0.05$) differences in the ex vivo release of growth factors and TIMPs by *S*-NP vs. *A*-NP tissues.

DIFFERENCES WITHIN *S*-IVDD OR *A*-IVDD COHORTS BY PATIENT CHARACTERISTICS

Age – S-AF

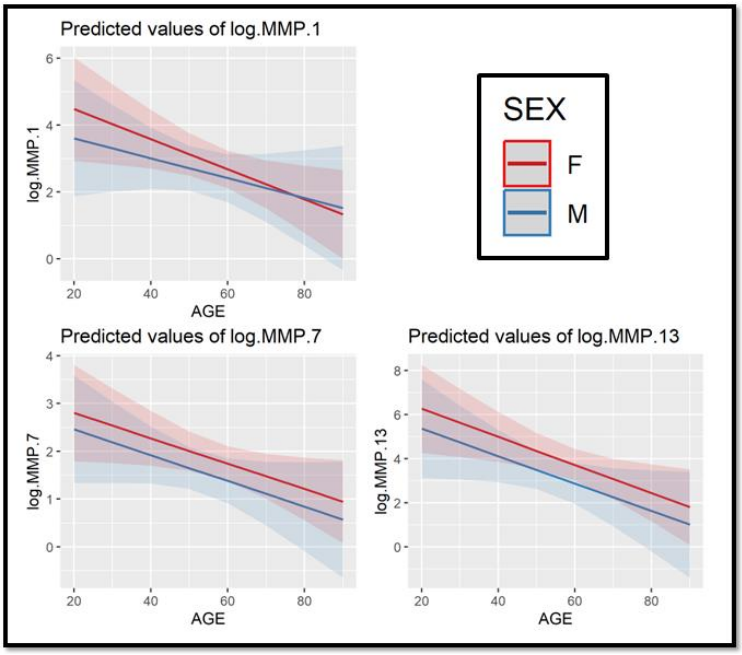


Figure D7: MMPs 1, 7, and 13 release significantly ($p<0.05$) affected by *S*-IVDD cohort age in *S*-AF tissues.

Age – A-AF

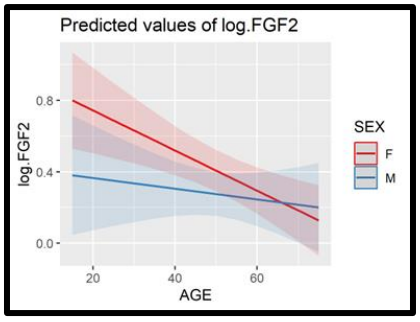


Figure D8: FGF-2 release significantly ($p<0.05$) affected by *A*-IVDD cohort age in *A*-AF tissues.

Age – S-NP

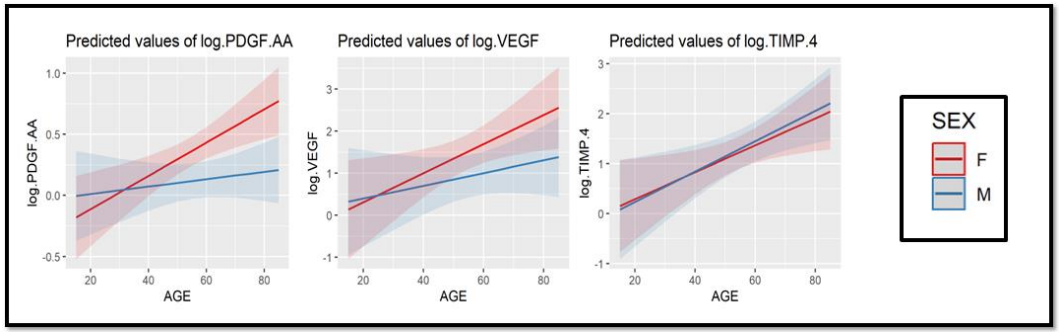


Figure D9: PDGF-AA, VEGF, and TIMP-4 release significantly ($p<0.05$) affected by *S*-IVDD cohort age in *S*-NP tissues.

Age – A-NP

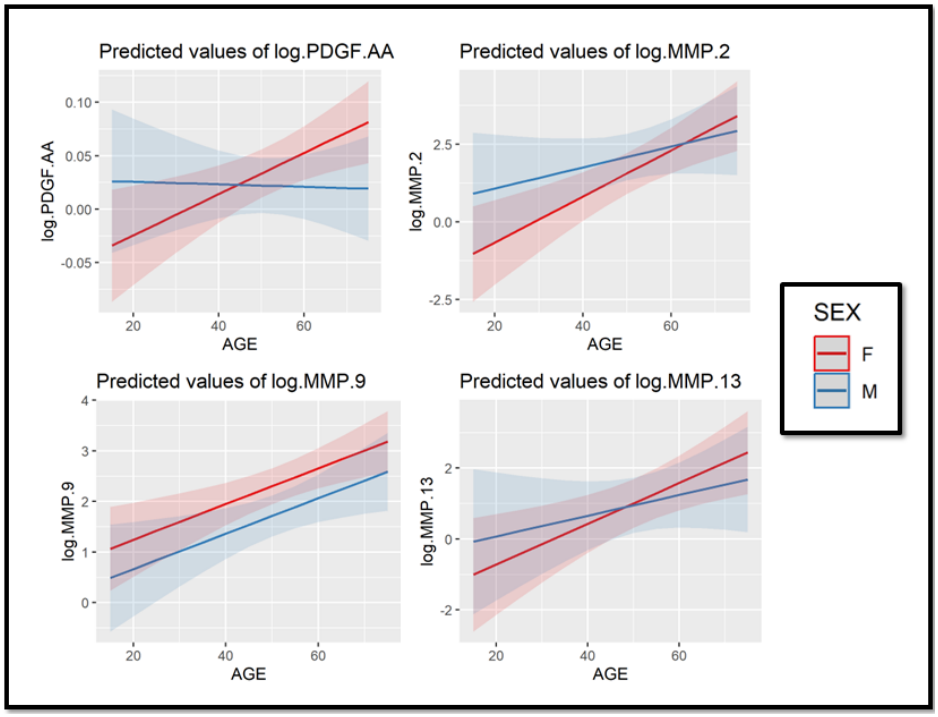


Figure D10: PDGF-AA and MMPs 2, 9, and 13 release significantly ($p<0.05$) affected by *A*-IVDD cohort age in *A*-NP tissues.

Sex – A-AF

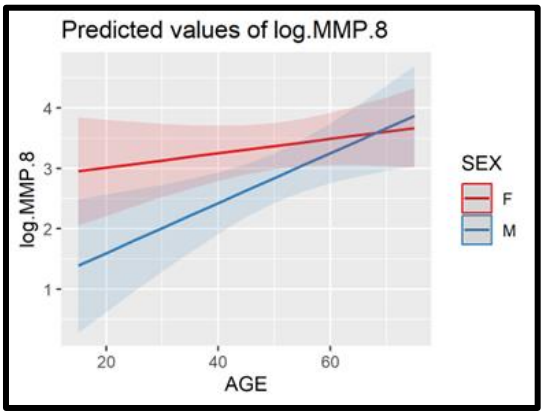


Figure D11: Significant ($p<0.05$) associations between asymptomatic donor sex and the production of FGF-2, and MMP-8 in asymptomatic donor annulus fibrosus tissues (AAF).

Sex – A-NP

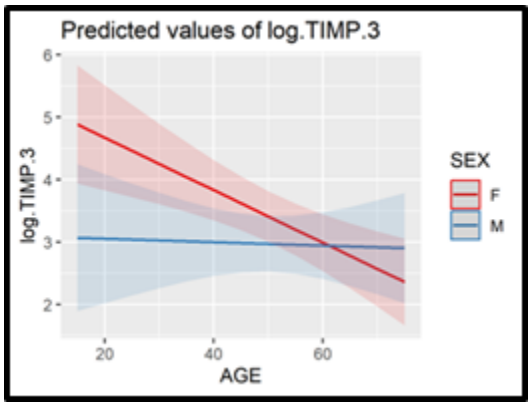
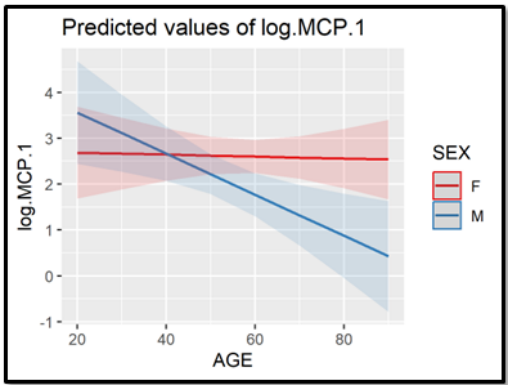


Figure D12: TIMP-3 production significantly ($p<0.05$) affected by donor sex in *A*-NP tissues.

Age:Sex

S-AF



A-AF

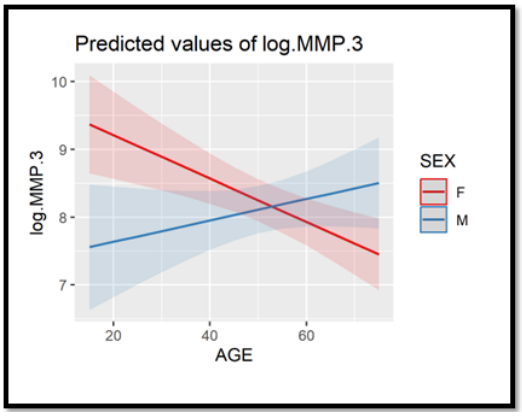


Figure D13: MCP-1 production significantly ($p<0.05$) affected by the interaction of age:sex in *S*-AF tissues. MMP-3 production significantly ($p<0.05$) affected by the interaction of age:sex in A-AF tissues.

Age:Sex – S-NP

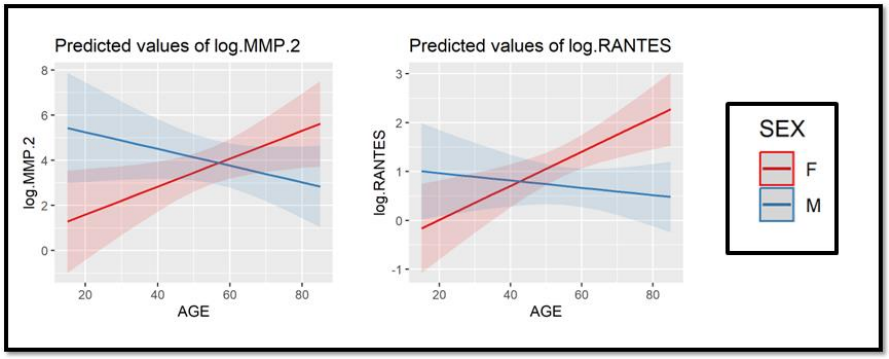


Figure D14: MMP-2 and RANTES production significantly ($p<0.05$) affected by the interaction of age:sex in *S*-NP tissues.

Age:Sex – A-NP

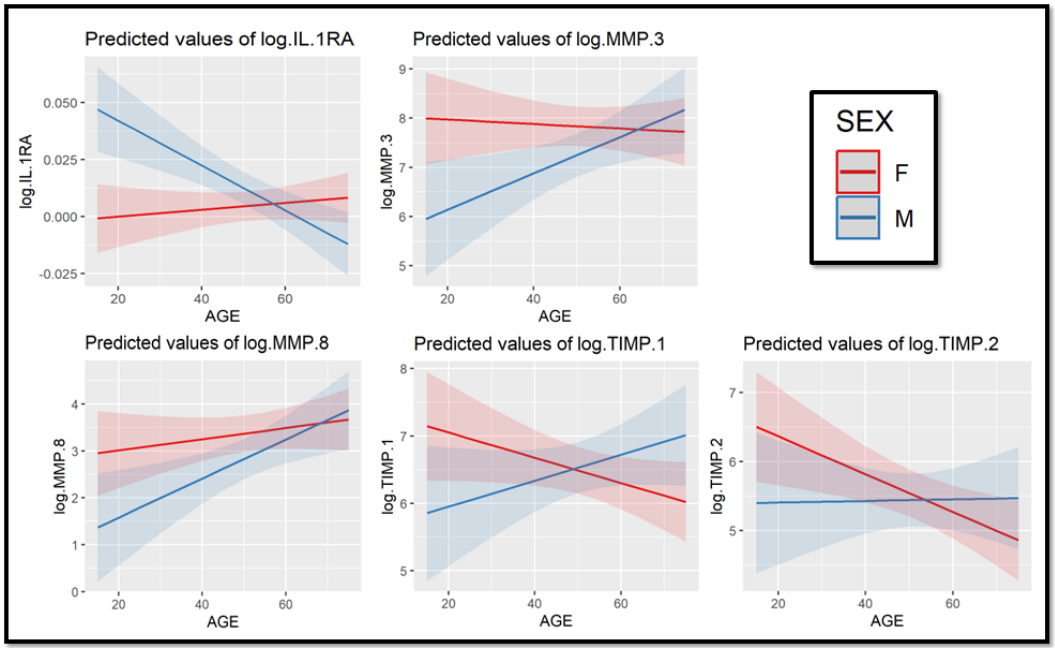


Figure D15: Il-1Ra, MMP-3, MMP-8, TIMP-1, and TIMP-2 release significantly ($p<0.05$) affected by the interaction of age:sex in *A*-NP tissues.

Obesity:Age – A-AF

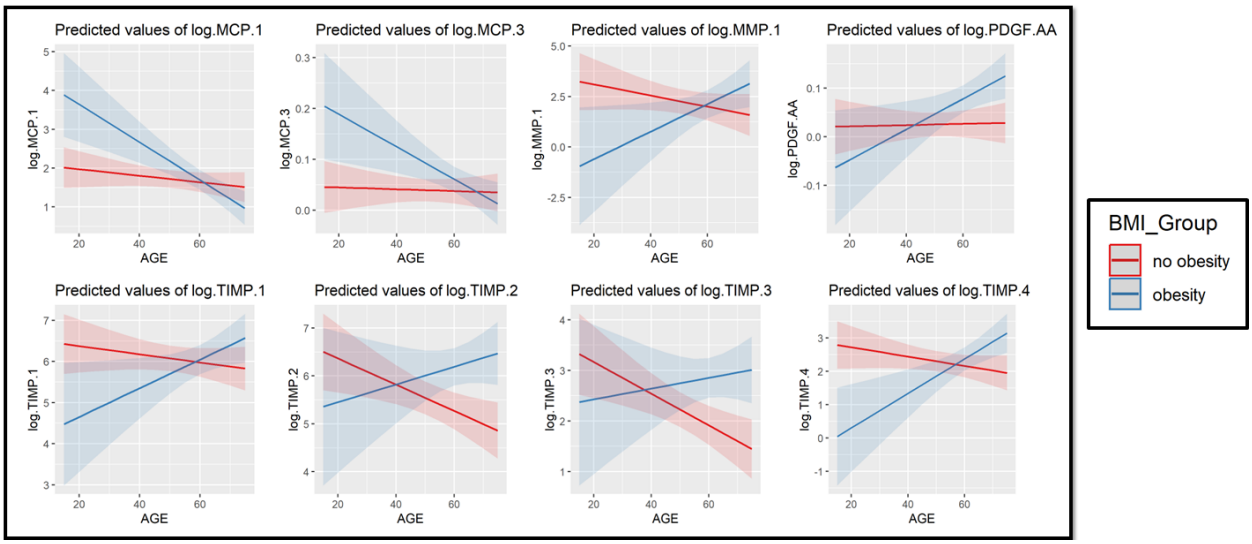


Figure D16: Inflammatory cytokines/chemokines, matrix regulating proteins, and growth factor production significantly ($p<0.05$) affected by the interaction of age:obesity in *A*-AF tissues.

Obesity:Age – A-NP

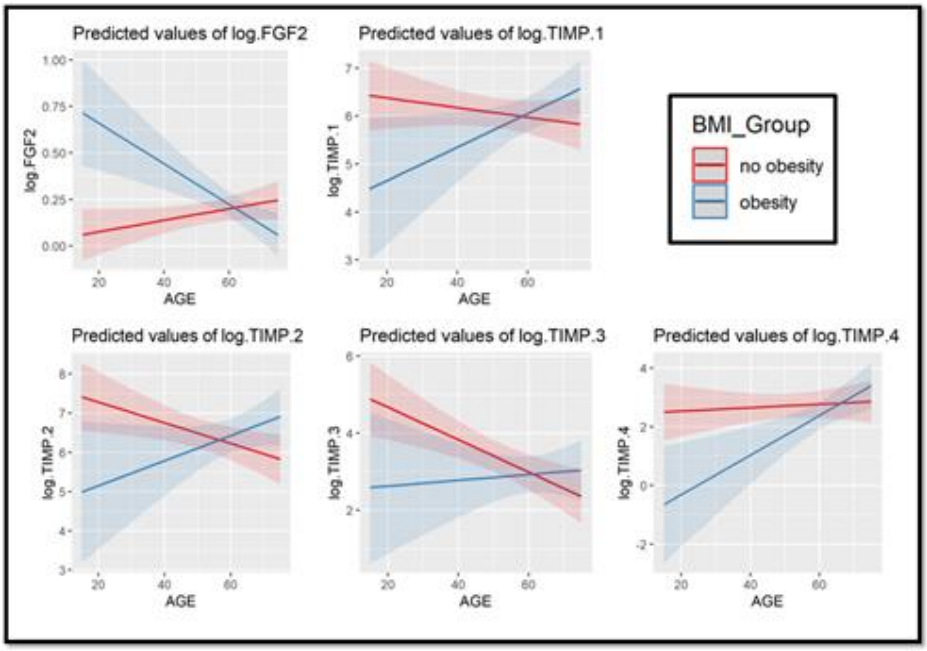


Figure D17: FGF-2 and TIMPs 1, 2, 3, and 4 release significantly ($p<0.05$) affected by the interaction of obesity:age in *A*-NP tissues.

Obesity:Sex – A-AF

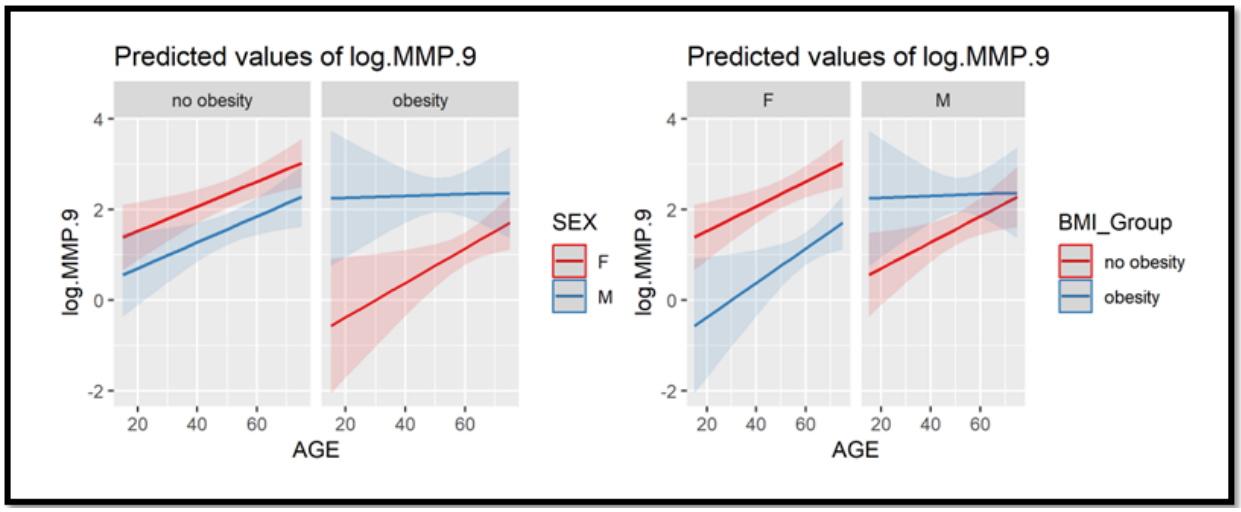


Figure D18: MMP-9 production significantly ($p<0.05$) affected by the interaction of obesity:sex in *A*-AF tissues.

Obesity:Sex – A-NP

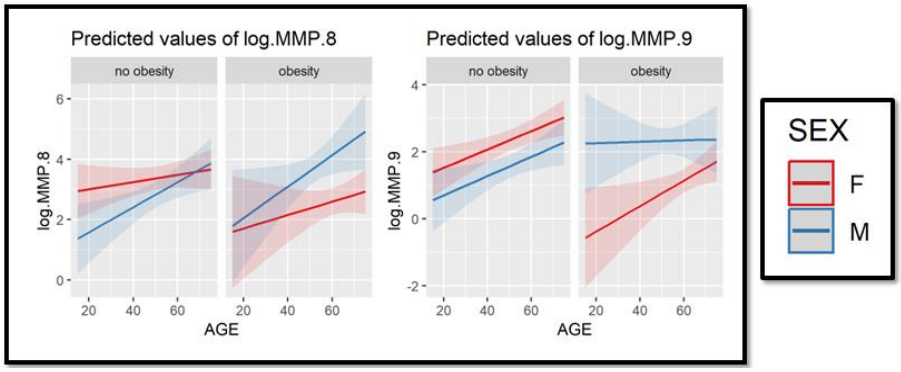


Figure D19: MMPs 8 and 9 release significantly ($p<0.05$) affected by the interaction of obesity:sex in *A*-NP tissues.

Age:Sex:Obesity – A-AF

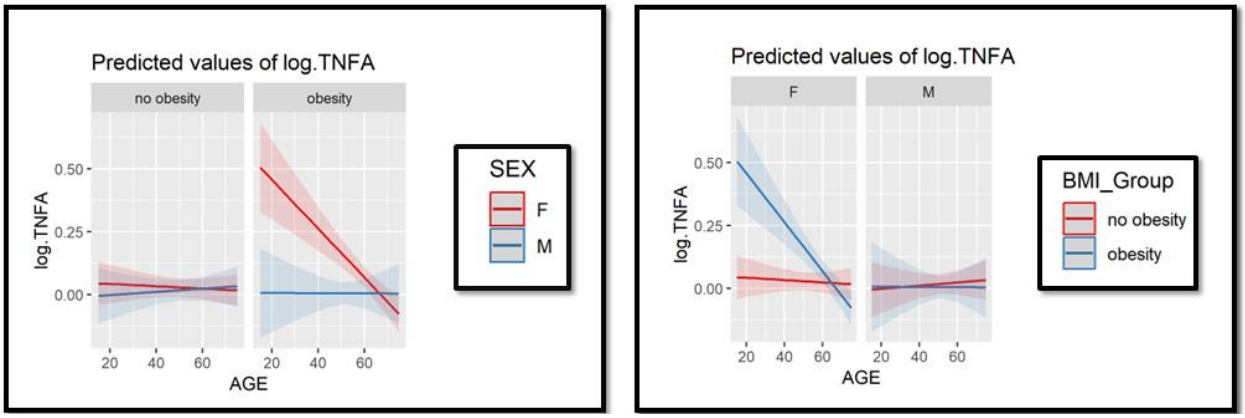


Figure D20: TNF-α production significantly ($p<0.05$) affected by the interactions of age:sex:obesity in *A*-AF tissues.

Age:Sex:Obesity – A-NP (1)

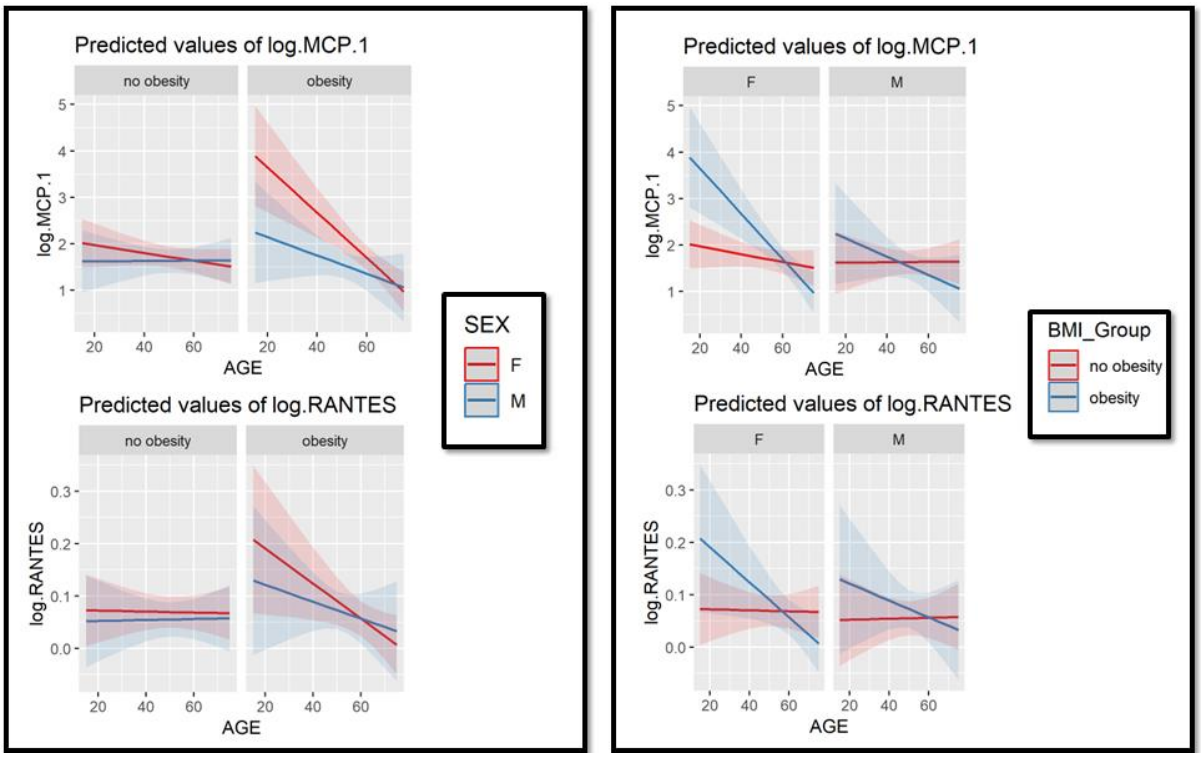


Figure D21: MCP-1 and RANTES production significantly ($p<0.05$) affected by the interactions of age:sex:obesity in *A*-NP tissues.

Age:Sex:Obesity – A-NP (2)

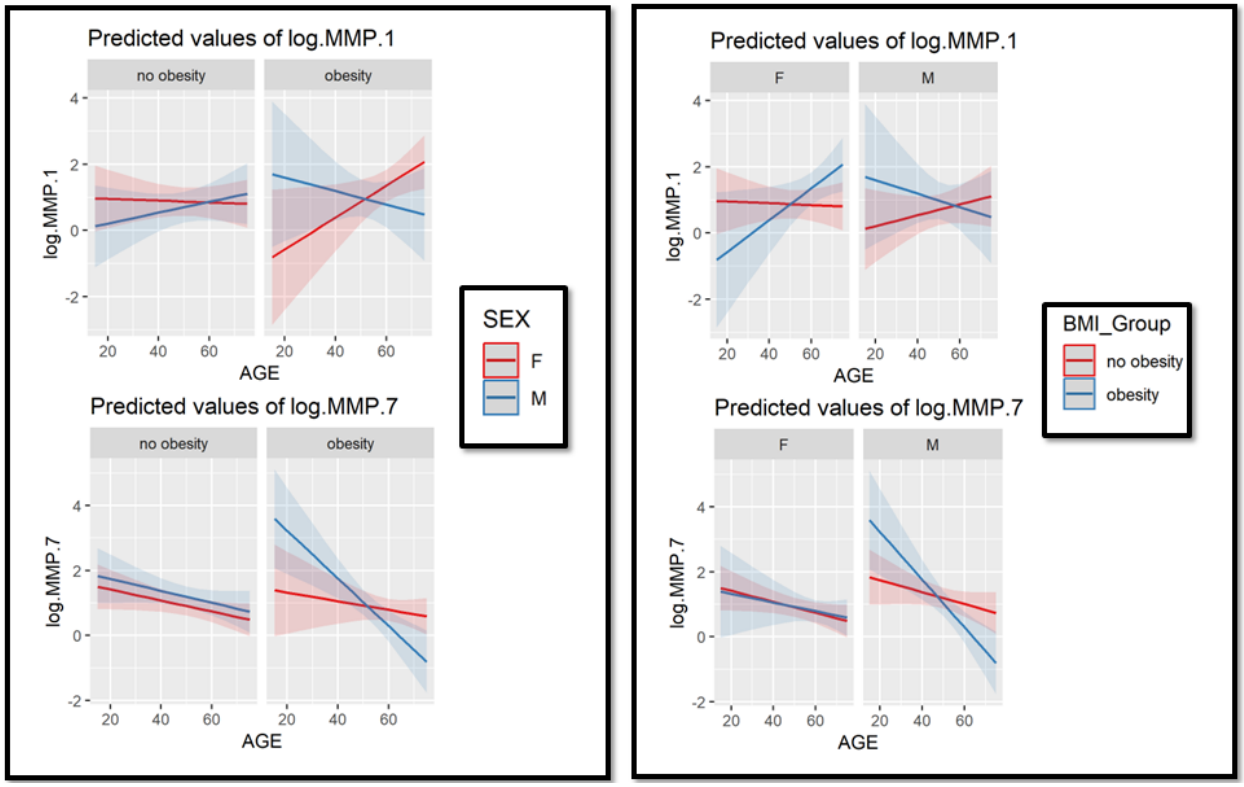


***Figure D22*:** MMP-1 and MMP-7 significantly ($p<0.05$) affected by the interactions between age:sex:obesity in *A*-NP tissues.

REFERENCES

1. Fujii K, Yamazaki M, Kang JD, et al. Discogenic Back Pain: Literature Review of Definition, Diagnosis, and Treatment. *JBMR Plus*. 2019;3(5). doi:10.1002/jbm4.10180

2. Berry JA, Elia C, Saini HS, Miulli DE. A Review of Lumbar Radiculopathy, Diagnosis, and Treatment. *Cureus*. 11(10). doi:10.7759/cureus.5934

3. Kalichman L, Cole R, Kim DH, et al. Spinal stenosis prevalence and association with symptoms: the Framingham Study. *Spine J Off J North Am Spine Soc*. 2009;9(7):545-550. doi:10.1016/j.spinee.2009.03.005

4. Al Qaraghli MI, De Jesus O. Lumbar Disc Herniation. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK560878/

5. Bydon M, Alvi MA, Goyal A. Degenerative Lumbar Spondylolisthesis: Definition, Natural History, Conservative Management, and Surgical Treatment. *Neurosurg Clin N Am*. 2019;30(3):299-304. doi:10.1016/j.nec.2019.02.003

6. Brinjikji W, Luetmer PH, Comstock B, et al. Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations. *AJNR Am J Neuroradiol*. 2015;36(4):811-816. doi:10.3174/ajnr.A4173

7. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol*. 2014;10(1):44-56. doi:10.1038/nrrheum.2013.160

8. Maitre CLL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J Pathol*. 2004;204(1):47-54. doi:10.1002/path.1608

9. Li Y, Zhang T, Tian W, et al. Loss of TIMP3 expression induces inflammation, matrix degradation, and vascular ingrowth in nucleus pulposus: A new mechanism of intervertebral disc degeneration. *FASEB J*. 2020;34(4):5483-5498. doi:10.1096/fj.201902364RR

10. Pratsinis H, Kletsas D. Growth factors in intervertebral disc homeostasis. *Connect Tissue Res*. 2008;49(3):273-276. doi:10.1080/03008200802147951

11. Zhang Y, Yao L, Robinson KM, Dillingham TR. Biomarkers in the Degenerative Human Intervertebral Disc Tissue and Blood. *Am J Phys Med Rehabil*. 2022;101(10):983-987. doi:10.1097/PHM.0000000000001943

12. Phillips KLE, Cullen K, Chiverton N, et al. Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master

regulator of catabolic processes. *Osteoarthritis Cartilage*. 2015;23(7):1165-1177. doi:10.1016/j.joca.2015.02.017

13. ZHANG F, ZHAO X, SHEN H, ZHANG C. Molecular mechanisms of cell death in intervertebral disc degeneration (Review). *Int J Mol Med*. 2016;37(6):1439-1448. doi:10.3892/ijmm.2016.2573

14. Wuertz K, Haglund L. Inflammatory Mediators in Intervertebral Disk Degeneration and Discogenic Pain. *Glob Spine J*. 2013;3(3):175-184. doi:10.1055/s-0033-1347299

15. Jacobsen HE, Khan AN, Levine ME, Filippi CG, Chahine NO. Severity of Intervertebral Disc Herniation Regulates Cytokine and Chemokine Levels In Patients with Chronic Radicular Back Pain. *Osteoarthritis Cartilage*. 2020;28(10):1341-1350. doi:10.1016/j.joca.2020.06.009

16. De Geer CM. Cytokine Involvement in Biological Inflammation Related to Degenerative Disorders of the Intervertebral Disk: A Narrative Review. *J Chiropr Med*. 2018;17(1):54-62. doi:10.1016/j.jcm.2017.09.003

17. Shamji MF, Setton LA, Jarvis W, et al. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum*. 2010;62(7):1974-1982. doi:10.1002/art.27444

18. Mf S, La S, W J, et al. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum*. 2010;62(7). doi:10.1002/art.27444

19. Weiler C, Nerlich AG, Bachmeier BE, Boos N. Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. *Spine*. 2005;30(1):44-53; discussion 54. doi:10.1097/01.brs.0000149186.63457.20

20. Bachmeier BE, Nerlich A, Mittermaier N, et al. Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. *Eur Spine J*. 2009;18(11):1573-1586. doi:10.1007/s00586-009-1031-8

21. Visse R, Nagase H. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Circ Res*. 2003;92(8):827-839. doi:10.1161/01.RES.0000070112.80711.3D

22. Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem*. 1997;378(3-4):151-160.

23. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA, Kang JD. Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration. *Spine J Off J North Am Spine Soc*. 2013;13(3):331-341. doi:10.1016/j.spinee.2012.02.027

24. Basaran R, Senol M, Ozkanli S, Efendioglu M, Kaner T. Correlation of matrix metalloproteinase (MMP)-1, -2, -3, and -9 expressions with demographic and radiological features in primary lumbar intervertebral disc disease. *J Clin Neurosci*. 2017;41:46-49. doi:10.1016/j.jocn.2017.03.001

25. Vergroesen PPA, Kingma I, Emanuel KS, et al. Mechanics and biology in intervertebral disc degeneration: a vicious circle. *Osteoarthritis Cartilage*. 2015;23(7):1057-1070. doi:10.1016/j.joca.2015.03.028

26. Wang WJ, Yu XH, Wang C, et al. MMPs and ADAMTSs in intervertebral disc degeneration. *Clin Chim Acta*. 2015;448:238-246. doi:10.1016/j.cca.2015.06.023

27. Richardson SM, Doyle P, Minogue BM, Gnanalingham K, Hoyland JA. Increased expression of matrix metalloproteinase-10, nerve growth factor and substance P in the painful degenerate intervertebral disc. *Arthritis Res Ther*. 2009;11(4):R126. doi:10.1186/ar2793

28. Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 SSE Award Competition in Basic Science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. *Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc*. 2002;11(4):308-320. doi:10.1007/s00586-002-0472-0

29. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. Matrix Metalloproteinases And Aggrecanase: Their Role in Disorders of the Human Intervertebral Disc. *Spine*. 2000;25(23):3005.

30. Deng B, Ren JZ, Meng XQ, et al. Expression profiles of MMP-1 and TIMP-1 in lumbar intervertebral disc degeneration. *Genet Mol Res*. 2015;14(4):19080-19086. doi:10.4238/2015.December.29.16

31. Wiet MG, Piscioneri A, Khan SN, Ballinger MN, Hoyland JA, Purmessur D. Mast Cell-Intervertebral disc cell interactions regulate inflammation, catabolism and angiogenesis in Discogenic Back Pain. *Sci Rep*. 2017;7(1):12492. doi:10.1038/s41598-017-12666-z

32. Le Maitre CL, Hoyland JA, Freemont AJ. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1β and TNFα expression profile. *Arthritis Res Ther*. 2007;9(4):R77. doi:10.1186/ar2275

33. Mern DS, Beierfuß A, Fontana J, Thomé C, Hegewald AA. Imbalanced Protein Expression Patterns of Anabolic, Catabolic, Anti-Catabolic and Inflammatory Cytokines in Degenerative Cervical Disc Cells: New Indications for Gene Therapeutic Treatments of Cervical Disc Diseases. Kletsas D, ed. *PLoS ONE*. 2014;9(5):e96870. doi:10.1371/journal.pone.0096870

34. Stone WL, Leavitt L, Varacallo M. Physiology, Growth Factor. In: *StatPearls*. StatPearls Publishing; 2023. Accessed April 27, 2023. http://www.ncbi.nlm.nih.gov/books/NBK442024/

35. Masuda K, An HS. Prevention of disc degeneration with growth factors. *Eur Spine J*. 2006;15(Suppl 3):422-432. doi:10.1007/s00586-006-0149-1

36. Kennon JC, Awad ME, Chutkan N, DeVine J, Fulzele S. Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration. *Biomol Concepts*. 2018;9(1):43-52. doi:10.1515/bmc-2018-0003

37. Ali R, Maitre CL, Richardson S, Hoyland PJ, Freemont A. Connective tissue growth factor expression in human intervertebral disc: implications for angiogenesis in intervertebral disc degeneration. *Biotech Histochem*. 2008;83(5):239-245. doi:10.1080/10520290802539186

38. Zhang W, Gong Y, Zheng X, et al. Platelet-Derived Growth Factor-BB Inhibits Intervertebral Disc Degeneration via Suppressing Pyroptosis and Activating the MAPK Signaling Pathway. *Front Pharmacol*. 2022;12:799130. doi:10.3389/fphar.2021.799130

39. Fournier DE, Kiser PK, Shoemaker JK, Battié MC, Séguin CA. Vascularization of the human intervertebral disc: A scoping review. *JOR Spine*. 2020;3(4):e1123. doi:10.1002/jsp2.1123

40. David G, Ciurea A, Iencean S, Mohan A. Angiogenesis in the degeneration of the lumbar intervertebral disc. *J Med Life*. 2010;3(2):154-161.

41. Capossela S, Bertolo A, Gunasekera K, Pötzel T, Baur M, Stoyanov JV. VEGF vascularization pathway in human intervertebral disc does not change during the disc degeneration process. *BMC Res Notes*. 2018;11. doi:10.1186/s13104-018-3441-3

42. Masuda K, An HS. Growth factors and the intervertebral disc. *Spine J Off J North Am Spine Soc*. 2004;4(6 Suppl):330S-340S. doi:10.1016/j.spinee.2004.07.028

43. Tolonen J, Grönblad M, Vanharanta H, et al. Growth factor expression in degenerated intervertebral disc tissue. *Eur Spine J*. 2006;15(5):588-596. doi:10.1007/s00586-005-0930-6

44. Paglia DN, Singh H, Karukonda T, Drissi H, Moss IL. PDGF-BB Delays Degeneration of the Intervertebral Discs in a Rabbit Preclinical Model. *Spine*. 2016;41(8):E449. doi:10.1097/BRS.0000000000001336

45. Li X, An HS, Ellman M, et al. Action of fibroblast growth factor-2 on the intervertebral disc. *Arthritis Res Ther*. 2008;10(2):R48. doi:10.1186/ar2407

46. Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human Intervertebral disc degeneration. *Arthritis Res Ther*. 2005;7(4):R732-R745. doi:10.1186/ar1732

47. Akyol S, Eraslan BS, Etyemez H, Tanriverdi T, Hanci M. Catabolic cytokine expressions in patients with degenerative disc disease. *Turk Neurosurg*. 2010;20(4):492-499. doi:10.5137/1019-5149.JTN.3394-10.1

48. Grönblad M, Virri J, Tolonen J, et al. A Controlled Immunohistochemical Study of Inflammatory Cells in Disc Herniation Tissue. *Spine*. 1994;19(24):2744.

49. Dongfeng R, Hou S, Wu W, et al. The expression of tumor necrosis factor-α and CD68 in high-intensity zone of lumbar intervertebral disc on magnetic resonance image in the patients with low back pain. *Spine*. 2011;36(6):E429-433. doi:10.1097/BRS.0b013e3181dfce9e

50. Analysis of tissue distribution of TNF-alpha, TNF-alpha-receptors, and the activating TNF-alpha-converting enzyme suggests activation of the TNF-alpha system in the aging intervertebral disc - PubMed. Accessed March 27, 2023. https://pubmed.ncbi.nlm.nih.gov/17405915/

51. Yoon ST, Patel NM. Molecular therapy of the intervertebral disc. *Eur Spine J*. 2006;15(Suppl 3):379-388. doi:10.1007/s00586-006-0155-3

52. Ponnappan RK, Markova DZ, Antonio PJ, et al. An organ culture system to model early degenerative changes of the intervertebral disc. *Arthritis Res Ther*. 2011;13(5):R171. doi:10.1186/ar3494

53. Salzer E, Mouser VHM, Tryfonidou MA, Ito K. A bovine nucleus pulposus explant culture model. *J Orthop Res*. n/a(n/a). doi:10.1002/jor.25226

54. Du J, Pfannkuche J, Lang G, et al. Proinflammatory intervertebral disc cell and organ culture models induced by tumor necrosis factor alpha. *JOR Spine*. 2020;3(3):e1104. doi:10.1002/jsp2.1104

55. Stern S, Lindenhayn K, Perka C. Human intervertebral disc cell culture for disc disorders. *Clin Orthop*. 2004;(419):238-244. doi:10.1097/00003086-200402000-00039

56. Kawaguchi S, Yamashita T, Katahira G ichiro, Yokozawa H, Torigoe T, Sato N. Chemokine profile of herniated intervertebral discs infiltrated with monocytes and macrophages. *Spine*. 2002;27(14):1511-1516. doi:10.1097/00007632-200207150-00006

57. Ruiz-Fernández C, Francisco V, Pino J, et al. Molecular Relationships among Obesity, Inflammation and Intervertebral Disc Degeneration: Are Adipokines the Common Link? *Int J Mol Sci*. 2019;20(8):2030. doi:10.3390/ijms20082030

58. Weiler C, Lopez-Ramos M, Mayer HM, et al. Histological analysis of surgical lumbar intervertebral disc tissue provides evidence for an association between disc degeneration and increased body mass index. *BMC Res Notes*. 2011;4:497. doi:10.1186/1756-0500-4-497

59. Takatalo J, Karppinen J, Niinimäki J, et al. Does Lumbar Disc Degeneration on Magnetic Resonance Imaging Associate With Low Back Symptom Severity in Young Finnish Adults? *Spine*. 2011;36(25):2180. doi:10.1097/BRS.0b013e3182077122

60. Sheng B, Feng C, Zhang D, Spitler H, Shi L. Associations between Obesity and Spinal Diseases: A Medical Expenditure Panel Study Analysis. *Int J Environ Res Public Health*. 2017;14(2):183. doi:10.3390/ijerph14020183

61. Shiri R, Karppinen J, Leino-Arjas P, Solovieva S, Viikari-Juntura E. The Association Between Obesity and Low Back Pain: A Meta-Analysis. *Am J Epidemiol*. 2010;171(2):135-154. doi:10.1093/aje/kwp356

62. Shin JH, Park S, Cho H, Kim JH, Choi H. Adipokine human Resistin promotes obesity-associated inflammatory intervertebral disc degeneration via pro-inflammatory cytokine cascade activation. *Sci Rep*. 2022;12:8936. doi:10.1038/s41598-022-12793-2

63. Donnally III CJ, Hanna A, Varacallo M. Lumbar Degenerative Disk Disease. In: *StatPearls*. StatPearls Publishing; 2020. Accessed April 23, 2020. http://www.ncbi.nlm.nih.gov/books/NBK448134/

64. Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. *Spine*. 2002;27(2):125-134. doi:10.1097/00007632-200201150-00002

65. Ishimoto Y, Yoshimura N, Muraki S, et al. Prevalence of symptomatic lumbar spinal stenosis and its association with physical performance in a population-based cohort in Japan: the Wakayama Spine Study. *Osteoarthritis Cartilage*. 2012;20(10):1103-1108. doi:10.1016/j.joca.2012.06.018

66. Gruber HE, Hanley EN. Do We Need Biomarkers for Disc Degeneration? *Biomark Insights*. 2007;1:131-133.

67. Hider SL, Konstantinou K, Hay EM, Glossop J, Mattey DL. Inflammatory biomarkers do not distinguish between patients with sciatica and referred leg pain within a primary care population: results from a nested study within the ATLAS cohort. *BMC Musculoskelet Disord*. 2019;20(1):202. doi:10.1186/s12891-019-2604-2

68. Khan AN, Jacobsen HE, Khan J, et al. Inflammatory biomarkers of low back pain and disc degeneration: a review. *Ann N Y Acad Sci*. 2017;1410(1):68-84. doi:10.1111/nyas.13551

69. Rajasekaran S, Tangavel C, Soundararajan DCR, et al. Proteomic Signatures of Healthy Intervertebral Discs From Organ Donors: A Comparison With Previous Studies on Discs From Scoliosis, Animals, and Trauma. *Neurospine*. 2020;17(2):426-442. doi:10.14245/ns.2040056.028

70. Verheijde JL, Rady MY, McGregor JL. The United States Revised Uniform Anatomical Gift Act (2006): New challenges to balancing patient rights and physician responsibilities. *Philos Ethics Humanit Med PEHM*. 2007;2:19. doi:10.1186/1747-5341-2-19

71. Roberts S, Evans H, Trivedi J, Menage J. Histology and Pathology of the Human Intervertebral Disc. *JBJS*. 2006;88(suppl_2):10-14. doi:10.2106/JBJS.F.00019

72. Le Maitre CL, Dahia CL, Giers M, et al. Development of a standardized histopathology scoring system for human intervertebral disc degeneration: an Orthopaedic Research Society Spine Section Initiative. *JOR Spine*. 2021;4(2):e1167. doi:10.1002/jsp2.1167

73. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine*. 2002;27(23):2631-2644. doi:10.1097/00007632-200212010-00002

74. Kos N, Gradisnik L, Velnar T. A Brief Review of the Degenerative Intervertebral Disc Disease. *Med Arch*. 2019;73(6):421-424. doi:10.5455/medarh.2019.73.421-424

75. Andrade P, Hoogland G, Garcia MA, Steinbusch HW, Daemen MA, Visser-Vandewalle V. Elevated IL-1β and IL-6 levels in lumbar herniated discs in patients with sciatic pain. *Eur Spine J*. 2013;22(4):714-720. doi:10.1007/s00586-012-2502-x

76. DENG X, ZHAO F, KANG B, ZHANG X. Elevated interleukin-6 expression levels are associated with intervertebral disc degeneration. *Exp Ther Med*. 2016;11(4):1425-1432. doi:10.3892/etm.2016.3079

77. Li H, Wang D, Yuan Y, Min J. New insights on the MMP-13 regulatory network in the pathogenesis of early osteoarthritis. *Arthritis Res Ther*. 2017;19(1):248. doi:10.1186/s13075-017-1454-2

78. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine*. 2001;26(17):1873-1878. doi:10.1097/00007632-200109010-00011

79. Thompson JP, Pearce RH, Schechter MT, Adams ME, Tsang IK, Bishop PB. Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. *Spine*. 1990;15(5):411-415. doi:10.1097/00007632-199005000-00012

80. Jahnke MR, McDevitt CA. Proteoglycans of the human intervertebral disc. Electrophoretic heterogeneity of the aggregating proteoglycans of the nucleus pulposus. *Biochem J*. 1988;251(2):347-356.

81. An HS, Masuda K, Inoue N. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthop Sci*. 2006;11(5):541-552. doi:10.1007/s00776-006-1055-4

82. Fearing BV, Hernandez PA, Setton LA, Chahine NO. Mechanotransduction and cell biomechanics of the intervertebral disc. *JOR Spine*. 2018;1(3). doi:10.1002/jsp2.1026

83. Wu H, Yu WD, Jiang R, Gao ZL. Treatment of multilevel degenerative lumbar spinal stenosis with spondylolisthesis using a combination of microendoscopic discectomy and minimally invasive transforaminal lumbar interbody fusion. *Exp Ther Med*. 2013;5(2):567-571. doi:10.3892/etm.2012.812

84. Adilay U, Guclu B. Comparison of Single-Level and Multilevel Decompressive Laminectomy for Multilevel Lumbar Spinal Stenosis. *World Neurosurg*. 2018;111:e235-e240. doi:10.1016/j.wneu.2017.12.033

85. Cho HJ, Ko YS, Won YI, et al. The Efficacy of Lumbar Hybrid Fusion for the Prevention of Adjacent Segment Disease: Fact or Artifact? A Meta-analysis. *Clin Spine Surg*. 2021;34(7):260-268. doi:10.1097/BSD.0000000000001097

CHAPTER 4: RELATIONSHIPS BETWEEN MACROSCOPIC INTERVERTEBRAL DISC DEGENERATION TO SIGNALING PEPTIDES AND MATRIX AFFECTING ENZYMES WITHIN AND BETWEEN PATIENTS WITH ASYMPTOMATIC AND SYMPTOMATIC INTERVERTEBRAL DISC DEGENERATION

Jacob S. Kramer[1,2], Aaron M. Stoker[1,2], Theodore J. Choma[2], Emily V. Leary[1,2], Jinpu Li[1,2], Don K. Moore[2], James L. Cook[1,2]

[1]Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO

[2]Department of Orthopaedic Surgery, University of Missouri, Columbia, MO

INTRODUCTION

Back pain is the most common source of lost labor and reduced activity worldwide.[1] Back pain and the need for surgery have been further linked to lumbar intervertebral disc degeneration (IVDD).[2] IVDD involves the wearing of annulus fibrosus (AF) and nucleus pulposus (NP) tissues, which reduces the available space and alignment in the spine.[3] IVDD is also frequently related to herniation of the NP, stenosis, and spondylolisthesis.[3] These changes are often associated with the development of painful symptoms (*S*-IVDD) that may be debilitating.[4–6]

Physicians evaluate radiographic *S*-IVDD using the T2 weighted magnetic resonance imaging (MRI) based Pfirrmann grading system, which assigns an *S*-IVD a score of 1-5.[7] Increasing Pfirrmann grades indicate reduced IVD homogeneity/reduction in T2 signal intensity, narrowed IVD space, and a loss of distinction between the AF and NP.[7] A study published by Yu et al. in 2012 assessed radiographs from 108 *S*-IVDD cases and observed Pfirrmann grade 3, 4, and 5 changes in approximately 13%, 75%, and 12% of *S*-IVDs, respectively.[8] However, these same changes in IVD tissue structure in asymptomatic patients (*A*-IVDD) is a common incidental finding that increases with patient age.[9,10] Previous studies have determined that a range of Pfirrmann grades in asymptomatic patients, with Pfirrmann grade 3 (26-100% based on increasing age), 4 or 5 (35-72% based on increasing age) commonly observed in these pateints.[11] Unfortunately, it is not possible to determine based on imaging if a patient has *S*-IVDD or will develop *S*-IVDD in the future.

While not a clinically relevant method to evaluate IVDD, gross changes in IVD structure related to IVDD can also be assessed visually using the Thompson grading

system, which assigns a score of 1-5 based on gross assessment of the IVD in the mid-sagittal plane. [12] Increasing Thompson grades indicate increased fibrous tissue and cleft formation in the NP, decreased demarcation of AF fibers, endplate cartilage loss and sclerosis, and vertebral body osteophyte formation.[12] In 1990, Thompson et al. published this proposed IVD grading scheme and analysis of 136 *A*-IVDs, and observed approximately 15%, 30%, 27%, 20%, and 10% prevalence for Thompson grades 1-5, respectively.[12] These studies clearly indicate that radiographic and gross *S*-IVDD features are also present in age-related *A*-IVDD.

However, researchers often cannot perform both Pfirrmann grading and Thompson grading on the IVD samples used in a study, which could make it difficult to compare data from samples that were grade using different IVDD grading system. A previous study addressed this concern by grading 100 donor spine segments using both the Thompson and Pfirrmann grading scales.[19] In this study, there was significant agreement between the Thompson and Pfirrmann grading scales, and the majority of disagreement in scores between the two scales were present at the lowest scores, where Thompson Grade 1 *A*-IVDs were most often classified as Pfirrmann Grade 2. Therefore, comparisons between IVDs graded using the Thompson grading scale and the Pfirrmann grading scale appear to represent similar levels of gross tissue changes to the IVD if the grade of the IVD is ≥3 for each system.

Since radiographic evidence of IVDD is not diagnostic or predictive of *S*-IVDD development and progression, other factors must be contributing to the development of *S*-IVDD in patients.[3] Many biological factors are also thought to contribute to the transition from *A*-IVDD to *S*-IVDD, though their specific roles and timings are not known.[13]

Therefore, comparing biological factors released by IVD tissues recovered from patients with similar levels of *S*-IVDD and *A*-IVDD may provide critical clinically relevant information on the factors that contribute to the development of *S*-IVDD.[14]

Previous study have indicated significant changes in the production of inflammatory cytokines/chemokines, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors by AF and NP tissues recovered from patients with *S*-IVDD.[15–18] Inflammatory cytokines are produced and released by IVD cells to regulate normal tissue functions.[15,20] Chemokines respond to inflammatory cytokines to promote or inhibit the movement and activity of immune cells.[15,20] In *S*-IVDD, the regulation of inflammatory cytokines/chemokines may be disrupted, leading to an aberrant increase in localized tissue inflammation resulting in excessive cell proliferation, apoptosis, and/or necrosis.[15,20] The most commonly studied secreted inflammatory cytokines/chemokines related to the development and progression of IVDD are tissue necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-17, interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1 (CCL2), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES, CCL5), and MCP-3 (CCL7).[15]

Inflammatory cytokines and chemokines can also stimulate the production of proteases including MMPs and a disintegrins and metalloproteinases with thrombospondin motifs (ADAMTS).[15,21] MMPs and ADAMTS degrade extracellular matrix (ECM) proteins (i.e., collagen, proteoglycan) as a normal part of tissue maintenance.[14,16,17] These proteases can be subdivided into collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10),

matrilysin (MMP-7) and aggrecanases (ADAMTS1, ADAMTS4, ADAMTS5, ADAMTS9).[15,22] Additionally, these degradative enzymes are regulated by the production of TIMPs, which inhibit the activity of these enzymes by directly binding to the protein and prevent matrix degradation.[22,23] The progressive loss of IVD tissue structure and composition during IVDD indicates that a shift toward increased ECM catabolism during IVDD.[21,23–25] This tissue degradation can lead to a cycle of inflammation and degradation in the IVD that are thought to contribute to progressive tissue degeneration and fibrotic tissue formation. However, the role of specific MMPs and TIMPs and during progression of IVDD, and the differences in the production of these proteins by patients with *S*-IVDD and *A*-IVDD, are not well understood and require further study.

The controlled growth of cells/tissues is an important component of tissue maintenance.[26] Growth factors are able to activate immune cells, promote or inhibit cell proliferation and ECM synthesis, and stimulate the production of inflammatory cytokines, chemokines, and MMPs.[26] The expression and production of growth factors including insulin growth factor (IGF-1), fibroblastic growth factor (FGF), bone morphogenic protein (BMP), growth differentiation factor (GDF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF) have been observed in IVDD, though their associations to *S*-IVDD development are not known.[18,27–30]

Therefore, this study was designed to characterize the ex vivo production of inflammatory cytokines/chemokines, MMPs, TIMPs, and growth factors by IVD tissues recovered from patients with *S*-IVDD and *A*-IVDD. The level of gross IVDD was determined for each IVD using the Pfirrmann grading system (*S*-IVDD samples) or the

Thompson grading system (*A*-IVDD samples), and differences in protein release within and between the *S*-IVDD and *A*-IVDD cohorts were determined based on level of gross IVDD accounting for patient age, sex, and BMI. It was hypothesized that within *S*-IVDD and *A*-IVDD cohorts, that AF and NP tissues would release significantly ($p<0.05$) higher level of inflammatory cytokines/chemokines and MMPs, and would release significantly lower levels of TIMPS and growth factors, as the T/P-grade of IVDD increased, while adjusting for patient age, sex, and obesity. Further, that AF and NP tissues recovered from patients with *S*-IVDD would release significantly ($p<0.05$) higher levels of inflammatory cytokines/chemokines and MMPs, and would release significantly lower levels of TIMPS and growth factors, at each T/P-grade of IVDD, while adjusting for patient age, sex, and obesity. Determining how the production of these proteins by the tissues of the IVD may be differentially affected by progression of IVDD in patients with *S*-IVDD and *A*-IVDD has the potential to provide foundational information for the development of patient and stage specific therapies for the mitigation or prevention of *S*-IVDD.

METHODS

S-IVDD Population: IRB approval (IRB#2010692), and informed patient consent, spine tissues were recovered from patients (*S*-IVDD patients, n=184, mean age 55.8y, 105F, 102 obese) undergoing surgery to treat *S*-IVDD disorders such as herniation, stenosis, spondylolisthesis, and DDD. *S*-IVD tissues were not recovered from tumor or infection related cases. Patient/donor age was restricted to 17-95 years.

A-IVDD Population: With consent as recorded in a legal permit under the Uniform Anatomical Gift Act[31], IVD tissues from qualified tissue donors (*A*-IVDD patients, n=20, mean age 51.8y, 10F, 9 obese) were recovered by the Midwest Transplant Network. Medical history was evaluated during a Donor Risk Assessment Interview (DRAI). *A*-IVDD patient tissues testing positive for pathogens or diseases including HIV/AIDS, hepatitis, HTLV-I/II, Chagas' disease, hepatitis, malaria, cancers, Ebola virus, and Zika virus were excluded from this study. Additionally, IVD tissues were not recovered from patients with a history of discogenic back pain or spinal surgery. Patient age was restricted to 17-95 years of age.

S-IVD Tissue Collection and culture: *S*-IVD tissues normally discarded during surgery (n=203) were recovered and suspended in sterile phosphate buffered saline (PBS). Within one hour of surgery completion tissues were processed in the lab for culture. A 6 mm diameter explant from *S*-IVD tissues and from *A*-AF and -NP tissues were created for ex vivo tissue cultures. Tissue explants were cultured for 3 days in supplemented DMEM (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium). Media were collected after

culture and stored at -20°C. After culture, each tissue explant was weighed to determine the wet weight of the tissue. Further, the cultured tissue explants were processed for histological analysis as described below.

A-IVD Tissue Collection: Spine segments were recovered from deceased tissue donors and transferred to the lab for within 24 hours to be assessed for Thompson grade as described below and processed for tissue explant culture. IVDs were recovered from all available lumbar levels (n=67) of each received lumbar spinal column (n=20). A 6 mm diameter explant from AF and NP were created for *ex vivo* tissue cultures. Tissue explants were cultured separately for 3 days in supplemented DMEM (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium). Media were collected after culture and stored at -20°C. After culture, each tissue explant was weighed to determine the wet weight of the tissue.

Pfirrmann and Thompson grading of IVDD: *S*-IVDs with identified levels were each assigned a P-grade (1-5) by one observer blinded to patient history using preoperative MRI images as previously described.[7] *A*-IVDs were bisected in the mid-sagittal plane using a diamond wet saw at the time of receipt and each level was assigned a T-grade(1-5) by one observer blinded to patient history as previously described.[12]

Histological Assessment of S-IVD Tissue type: Following tissue culture, a portion of each *S*-IVD specimen was formalin fixed, decalcified in 10% EDTA, paraffin-embedded, and stained using H&E, Toluidine blue, and Picrosirius red. Histological classification of *S*-AF or *S*-NP tissue was performed. *S*-IVD specimens that were a mixture of tissue types (AF, NP, cartilage endplate, and/or bone) were excluded from analysis in this study.

Media biomarker analysis: Using commercially available Luminex multiplex magnetic bead assays, *S*-IVD and *A*-IVD tissue culture media were tested for inflammatory cytokines/chemokines: GRO-α, MCP-1, MCP-3, TNF-α, IL-1RA, IL-6, IL-8, MIP-1α, MIP-1β, RANTES (Millipore); matrix metalloproteinases: MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13 (R&D Systems); tissue inhibitors of matrix proteinases: TIMP-1, TIMP-2, TIMP-3, TIMP-4 (Millipore); and growth factors: PDGF-AA, PDGF-AB/BB, VEGF, and FGF-2 (Millipore).

Statistical analysis: In order to account for patient characteristics (i.e., age, sex, and BMI) as factors, BMI was dichotomized as obese/not obese (<30/>30 kg/m^2). T/P-grades of "2" were combined with T/P-grades of "3" due to low frequency of the former. For simplicity, "patient characteristics" will refer to *S*-IVDD and/or *A*-IVDD patient demographics (age, sex, obesity).

Media biomarker concentrations were standardized to the wet weight of each explant and natural log transformed. Analyses of variance (ANOVAs) were then created for each biomarker within each T/P-grade, and *S*-IVDs were compared to *A*-IVDs with adjustment for patient characteristics and their interactions. Then, ANOVAs were created within *S*-IVDD or *A*-IVDD cohorts, and biomarker concentrations between T/P-grades were compared. Two-sided significance was set at $\alpha=0.05$. Interaction plots of predicted biomarker values were used to interpret ANOVA results.

RESULTS

Donor/Patient Characteristics:

From 184 *S*-IVDD patients, tissues from 203 IVDs were collected from L1-L5. The distribution of P-grades in the *S*-IVDD cohort were 64 P-grade 2/3 (31%), 99 (49%) P-grade 4, and n=40 (20%) P-grade 5. From 20 *A*-IVDD patients, 67 lumbar IVDs were recovered from L1-L5 (Table 1). The distribution of T-grades in the *A*-IVDD cohort were 39 (58%) T-grade 2/3, 19 T-grade 4 (28%), and 9 (13%) T-grade 5. In the *S*-IVDD cohort, 82 patients were classified as not obese and 102 as obese. In the *A*-IVDD cohort, 9 individuals were classified as not obese (<30 kg/m^2) and 9 as obese (>30 kg/m^2), and BMI information was not available for 2 patient in the *A*-IVDD cohort.

Ex vivo biomarker release by S-AF or S-NP tissues compared to A-AF or A-NP tissues within T/P-Grades

All significant differences in biomarker release by *S*-IVDD versus *A*-IVDD tissues at each T/P-Grade are listed in Table 2.

Inflammatory Cytokines/Chemokines

Within T/P-grades, AF and NP tissues from the *S-IVDD* cohort released significantly ($p \leq 0.037$) higher levels of GRO-α (within T/P-grade 5), IL-1Ra (T/P-grades 2/3), MCP-1 (T/P-grade 5) and RANTES (All T/P-grades) compared to AF and NP tissues from the *A*-IVDD cohort (Figures 1A and 1B). Additionally, NP tissues from the *S-IVDD* cohort released significantly ($p \leq 0.039$) higher levels of IL-1Ra (within T/P-grade 5), IL-6 and IL-8 (T/P-grades 2/3 and 5), MIP-1α (All T/P-grades), MIP-1β (T/P-grades 2/3, 5), and TNF-α (T/P-grade 5) compared to NP tissues from the *A*-IVDD cohort.

MMPs

Within T/P-grades, AF and NP tissues from the *S-IVDD* cohort released significantly ($p \leq 0.019$) higher levels of MMP-2 and MMP-13 (within T/P-grades 2/3 and 4) compared to AF and NP tissues from the *A*-IVDD cohort. Further, AF tissues from the *S-IVDD* cohort released significantly ($p \leq 0.037$) higher levels of MMP-13 (within T/P-grade 5) compared to AF tissues from the *A*-IVDD cohort. NP tissues from the *S-IVDD* cohort released significantly ($p \leq 0.037$) higher levels of MMP-1 (within T/P-grade 5) compared to NP tissues from the *A*-IVDD cohort. (Figures 2A and 2B). The release of MMP-3 (within T/P-grades 2/3) and MMP-8 (within all T/P-grades) by AF and NP tissues from the *A*-IVDD cohort were significantly ($p \leq 0.023$) higher than levels released by AF and NP tissues from the *S-IVDD* cohort. Further, AF tissues from the *A*-IVDD cohort released significantly ($p = 0.010$) higher levels of MMP-3 (within T/P-grade 4) increased levels of MMP-3 compared to AF tissues from the *S*-IVDD cohort.

TIMPs and Growth Factors

Within T/P-grades, AF and NP tissues from the *A*-IVDD cohort released significantly ($p \leq 0.050$) higher levels of TIMP-2, TIMP-3, and TIMP-4 (within all T/P-grades) and TIMP-1 (within T/P-grades 2/3) compared to AF and NP tissues from the *S-IVDD* cohort (Figures 3A and 3B). NP tissues from the *S-IVDD* cohort released significantly ($p \leq 0.04$) higher level of PDGF-AA and VEGF (within T/P-grades 2/3 and 4) compared to NP tissues from the *A*-IVDD cohort. AF tissues from the *S-IVDD* cohort released significantly ($p \leq 0.022$) higher level of PDGF-AA (within T/P-grades 2/3) compared to AF tissues from the *A-IVDD* cohort.

Ex vivo biomarker release by S-AF, S-NP, A-AF, or A-NP tissues between T/P-Grades

All significant differences in *S*-IVDD or *A*-IVDD cohorts are listed in Table 4. Tukey post-hoc analyses for ANOVAs within each cohort are reported in Table 5. Plots for significant differences within *S*-AF, *A*-AF, *S*-NP, *A*-NP tissue groups are shown in Figure 4, 5, 6, and 7, respectively.

Inflammatory Cytokines/Chemokines

For *S*-AF tissues, the release of GRO-α and IL-6 by tissues from *S*-IVDs with P-grade 5 were significantly ($p \leq 0.020$) higher than *S*-AF tissues from *S*-IVDs with P-grade 2/3 (Table 6, Figure 4). For *S*-NP tissues, the release of RANTES and TNF-α by tissues from *S*-IVDs with P-grade 5 were significantly ($p \leq 0.04$) higher than *S*-NP tissues from *S*-IVDs with P-grade 2/3 and 4, and the release of MCP-1 by tissues from *S*-IVDs with P-grade 5 were significantly ($p \leq 0.047$) higher than *S*-NP tissues from *S*-IVDs with P-grade 2/3. For *A*-AF and *A*-NP tissues, the release of MCP-1 by tissues from *A*-IVDs with T-grade 2/3 was significantly ($p \leq 0.020$) higher than tissues from *A*-IVDs with T-grade 4 and 5.

MMPs

For *S*-AF tissues, the release of MMP-8 by tissues from *S*-IVDs with P-grade 5, and MMP-3 and MMP-9 by tissues from *S*-IVDs with P-grade 4 or 5, were significantly ($p \leq 0.035$) higher than *S*-AF tissues from *S*-IVDs with P-grade 2/3. For *S*-NP tissues, the release of MMP-2 and MMP-8 by tissues from *S*-IVDs with P-grade 4 or 5 were significantly ($p \leq 0.04$) higher than *S*-NP tissues from *S*-IVDs with P-grade 2/3 and 4. For *A*-AF and *A*-NP tissues, the release of MMP-2 and MMP-8 by tissues from *A*-IVDs with

T-grades 4 or 5 were significantly ($p \leq 0.040$) higher than tissues from *A*-IVDs with T-grade 2/3. For *A*-AF tissues, the release of MMP-13 by *A*-AF tissues from *A*-IVDs with T-grades 4 or 5 was significantly ($p \leq 0.012$) higher than *A*-AF tissues from *A*-IVDs with T-grade 2/3.

TIMPs and Growth Factors

For *S*-AF tissues, the release of FGF-2 by tissues from *S*-IVDs with P-grades 4 or 5 was significantly ($p \leq 0.03$) higher than *S*-AF tissues from *S*-IVDs with P-grade 2/3. For *S*-NP tissues, the release of PDGF-AA by tissues from *S*-IVDs with P-grade 5 was significantly ($p = 0.037$) higher than *S*-NP tissues from *S*-IVDs with P-grade 2/3. For *A*-AF tissues, the release of FGF-2 by tissues from *A*-IVDs with T-grades 4 or 5 was significantly ($p \leq 0.030$) higher than *A*-AF tissues from *A*-IVDs with T-grade 2/3. For *A*-NP tissues, the release of TIMP-4, PDGF-AA, and VEGF by tissues from *A*-IVDs with T-grades 4 or 5 was significantly ($p \leq 0.032$) higher than *A*-NP tissues from *A*-IVDs with T-grade 2/3.

DISCUSSION

The data from this study indicates that the level of degradation of the IVD as assessed by the Pfirrmann and Thompson grading systems does have a significant effect on the release of inflammatory and degradative enzymes related biomarkers by IVD tissues recovered from patients with *S*-IVDD and *A*-IVDD during *ex vivo* culture. While the analysis of data in chapter 3 indicated that tissues from patients with *S*-IVDD released significantly higher level so all inflammation related biomarkers and specific MMPs and growth factors assessed in this study and significantly lower levels of all TIMPs compared to tissues from patients with *A*-IVDD, the data from this study indicate that these differences are significantly affected by the level of IVDD in the patient. Therefore, these data indicate tissue specific differences in the *ex vivo* release of these biomarkers between patients with *S*-IVDD and *A*-IVDD, which may be indicative of clinically important differences in IVD tissue metabolism related to the development and progression of *S*-IVDD and *A*-IVDD in patients.

In general, the biomarkers that were significantly different between tissues from patients with *S*-IVDD and *A*-IVDD were similar for AF and NP tissues. The significant differences identified between *S*-IVDD and *A*-IVDD patients for the release of GRO-α, IL-1RA, MCP-1, RANTES, MMP-2, MMP-8, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, and PDGF-AA were similar for both AF and NP tissues. However, notable differences in NP tissues biomarker release during ex vivo culture were observed between *S*-IVDD and *A*-IVDD patients that were not observed in AF tissues. The *S*-NP tissues released significantly higher levels of IL-6, IL-8, MIP-1α, MIP-1β, MMP-1, and VEGF during *ex vivo* culture compared to *A*-NP tissues. This indicates that an increase in inflammatory

signaling in the NP may be one of the factors that differentiates patients who develop *S*-IVDD from those that develop *A*-IVDD. Future studies aimed at determining the factors that stimulate an increase in the production of these biomarkers by the NP during IVDD may provide insight into targets for treatment to prevent the development of *S*-IVDD.

In agreement with the data from Chapter 3 and previous published studies, the analysis in this study indicates that an increase in tissue inflammation may be a key factor in the development of *S*-IVDD in patients.[21,32–34] The data from this study further clarifies differences in tissue inflammation related to progression of IVDD, and potentially in determining if a patient will develop *S*-IVDD or *A*-IVDD. The inflammatory biomarker that was consistently released at higher concentrations by tissues from patients with *S*-IVDD was RANTES. The release of RANTES by *S*-IVDD was significantly higher in both AF and NP tissues, and at all T/P-grades assessed. Therefore, RANTES appears to be an important factor in the development of *S*-IVDD in patients. Since the data from this study cannot determine the role of RANTES in *S*-IVDD, and if it is a causative or responsive factor for the development of *S*-IVDD, future studies will aim to understand the role of RANTES in the development and progression of *S*-IVDD.

In addition to RANTES, the significant difference in the release of other inflammation related biomarkers followed similar patterns in the AF and NP. The release of GRO-α and MCP-1 by the tissues from patients with *S*-IVDD were significantly higher than tissues from patient with *A*-IVDD when the T/P-grade of the IVD was higher. Additionally, while the release of MCP-1 by the *S*-IVDD cohort increased significantly as T/P-grade increased, the release of MCP-1 by the *A*-IVDD cohort decreased significantly as T/P-grade increased. This indicates that factors related to the progression of *S*-IVDD in

patients are activating pathways related to MCP-1 and GRO-α production by the tissues. Additionally, the significantly higher release of IL-6, IL-8, IL-1RA, MIP-1α, and MIP-1β by *S*-NP tissues with T/P-grades 2/3 and 5 compared to *A*-NP tissues, indicates that increased inflammatory signaling localized to the NP may be another important factor in differentiating patient who will develop *S*-IVDD from those who will develop *A*-IVDD.

In agreement with previous studies, the data from this study indicated that development and progression of IVDD is associated with a significant increase in MMP production by IVD tissues.[24,35–39] However, the data from this study indicates potentially clinically important differences in the production and regulation of degradative enzymes by IVDD tissues based on the development of symptoms in the patient. The upregulation of specific degradative enzymes by the AF and NP of *S*-IVDs (MMP-1, MMP-2, and MMP-13) and *A*-IVDs (MMP-3, and MMP-8) within specific T/P-grades may be indicative of the role of specific degradative enzymes during the development and progression of IVDD in each tissue. The release of MMP-8 by tissues from the *A*-IVDD cohort was significantly higher than tissues from the *A*-IVDD cohort at all T/P-grades assessed in this study, indicating that MMP-8 may be a degradative enzyme that is normally produced by the tissues of the IVD, and the factors that associated with the development of *S*-IVDD may decrease the production by the tissues. For IVDs with lower T/P-Grades, the release of MMP-2 was higher from the *S*-IVDD cohort, and the release of MMP-3 was higher from the *A*-IVDD cohort may be indicative of another potential difference between patients with *S*-IVDD and *S*-IVDD at the earlier stages of IVDD development. The data appears to indicate that, similar to MMP-8, the normal production of MMP-3 by the *S*-IVD tissues is decreased by factors associated with the earlier stages of IVDD in symptomatic patients.

Conversely, it is possible that the same factors that are decreasing MMP-3 production by the *S*-IVD tissues are also stimulating an increase in the production of MMP-2 by these tissues. Understanding the factors that are regulating the production of these two MMPs during the early stages of IVDD may provide insight into factors that contribute to the development of *S*-IVDD in patients.

Interestingly, while the release of MMP-13 by the *S*-AF tissues was significantly higher than the release from *A*-AF tissues at all T/P-Grades, the significantly higher release of MMP-13 by the *S*-NP tissues followed a similar pattern as MMP-2, indicating the production of these two degradative enzymes be regulated through similar mechanisms by the NP during IVDD development and progression, and may be indicative of important factors that contribute to the development of *S*-IVDD in patients. Additionally, the AF and NP from patients *A*-IVDD significantly increased the production of MMP-2 and MMP-13 as the T/P-Grade of the IVD increased. This observation further supports that concept that MMP-2 and MMP-13 may have a key role in the degradation of the tissues that occurs during IVDD, as increased tissue degradation was associated with a significant increase in the release of these MMPs in *A*-IVDD. However, it would appear that there is a threshold of production for MMP-2 and MMP-13 that is required during the earlier stages of IVDD development for patients to develop *S*-IVDD.

Another consistent finding from the data of this study, is the significantly higher release of TIMP-2, TIMP-3, and TIMP-4 by tissues from the *A*-IVDD cohort compared to tissues from the *S*-IVDD cohort across all T/P-Grades. This finding indicates that there is a shift in the regulation of degradative enzyme activity towards increased catabolism during the development of *S*-IVDD in patients. The decrease in the regulation of degrative

enzyme regulation could be a key factor in progression of *S*-IVDD, as increased degradative enzyme activity can result in increased tissue breakdown, resulting in decreased capacity of the tissue to resist normal loads, and increased tissue inflammation. It is not clear whether this difference in TIMP release by the tissues from patients with *S*-IVDD are driving symptom development, or a result of factors related to symptom development. However, the significant shift in the regulation of degradative enzyme activity by the tissues of the IVD during *S*-IVDD indicates that regulation of degradative enzyme activity is an important differentiating factor for patients who develop *S*-IVDD from those who develop *A*-IVDD.

The data from this study also indicated a potential role for PDGF-AA and VEGF in the development of *S*-IVDD, and the progression of IVDD during *A*-IVDD. The release of PDGF-AA and VEGF by *S*-NP tissues was significantly higher than the release of these growth factors by *A*-NP tissues at T/P-Grades 2/3 and 4. This finding indicates that there may be increased attempts to vascularize the NP during the development of *S*-IVDD that does not occur during the development of *A*-IVDD. Interestingly, the release of PDGF-AA and VEGF by *A*-NP tissues was significantly higher at T/P-Grade 5 compared to T/P-Grade 2/3. This indicates that increased signaling related to vascularization of the NP tissue may also occur at later stages of IVDD with or without symptom development. Therefore, another key factor in differentiating between patients who develop *S*-IVDD and those who develop *A*-IVDD may be the increased production of PDGF-AA and VEGF by the NP during the earlier stages of IVDD development.

LIMITATIONS

As with any study, there are numerous limitations to consider when interpreting the data from this study. The determination of asymptomatic for lower back pain was not based on direct communication with the diseased donor and was based on information obtained from relative that consented the patient to be a tissue donor. Therefore, it is possible that some of the patients did suffer from lower back pain and did not report this to the consenting person prior to tissue donation. However, it is unlikely that the donors would experience a similar level of disabling lower back pain as the patients in the *S*-IVDD, who required surgery to address their pain, without their close family members knowledge. Additionally, there was a notable disparity in the number of *A*-IVDD versus *S*-IVDD patients, and multiple samples were analyzed from each *A*-IVDD tissue donor. However, due to the inherent difficulty in obtaining tissues from patients with *A*-IVDD, it would be difficult to recover tissues from a similar number of patients with *A*-IVDD as were obtained from patients with *S*-IVDD who were undergoing surgery to treat their disease. Further, assessing samples from multiple levels of the same patient, which may have significantly different grades of IVDD, may better reflect the variability in IVD pathobiology observed in clinical patients with *A*-IVDD.

Additionally, there was a disparity in the distribution of the levels of the lumbar spine analyzed in the *A*-IVDD and *S*-IVDD patients. For the majority of the patients in the *A*-IVDD cohort, IVD tissues were recovered from lumbar (L1-L5) levels. However, the clinical distribution of *S*-IVDD levels was concentrated in L4-L5 and L5-S1, potentially presenting unique biochemical changes that reflect the increased biomechanical load experienced in the lower lumbar levels.[84,85] While analysis could

have been focused on just these two levels of the lumbar spine, the significant decrease in sample size could have resulted in a significant decrease in study power. On going studies aimed at increasing the number of *A*-IVDD patients could allow for analysis of the data that includes IVD level as a factor or focused on specific levels of the lumbar spine. The data from this study will be used to help guide the analysis of the samples in these future studies.

Regarding gross/radiographic IVD assessments, it was not financially feasible to acquire MRI images for donor segments, therefore, this study relied on the comparison of T-grading system to assess gross IVDD in the *A*-IVDD. While it is possible that the T-grading and P-grading of IVDD performed in this study were not assessing similar level of IVDD, a previous study reported a Cohen's kappa of .61 ($p<.001$) for the two systems across grades, and 92% of disagreements were by a difference of 1 grade (usually T grade 1 IVDs were scored as P grade 2).[41] Therefore, since the T-grades and P-grades of the IVDs in this study were mostly ≥3, it is likely that the two measures were assessing similar levels of IVDD in the two patient populations.

Finally, the timeframe for recovering tissues from *A*-IVDD patients and *S*-IVDD patients and processing and culturing the tissues in the lab were different. Tissues recovered from *S*-IVDD surgical patients recovered from the patient and processed for culture in the laboratory within an hour of surgery, while tissues recovered from the *A*-IVDD patients were recovered from the deceased tissue donor and processed in the laboratory within 24 hours. It is possible that the delay in the *A*-IVDD cohort could have significantly affected the viability the IVD and the release of proteins from the tissues during culture. However, the protocol for tissue recovery used for the *A*-IVDD patients is

the same as the protocol used to recover osteochondral allograft tissues used clinically, and a significant reduction in tissue viability is not observed in this timeframe in osteochondral allograft tissues.

CONCLUSIONS

With these limitations in mind, the data from this study indicates potentially important differences in the inflammatory and degradative environment of the IVD during the development and progression of IVDD for patients with for patients who develop *S*-IVDD compared to patients who develop *A*-IVDD. The most consistent differences between *S*-IVDD and *A*-IVDD tissues were the significant increase in RANTES release, and significant decrease in TIMPs released by *S*-IVDD tissues. This indicates that activation of specific inflammatory pathways and loss of degradative enzyme regulation may be significant factors in the development of *S*-IVDD clinically. Additionally, the data indicates that increased inflammatory signaling in the NP, shifts in the production of specific MMPs (increased MMP-2 and MMMP-13, decreased MMP-3 and MMP-8), and increased VEGF and PDGF-AA during the early stages of IVDD are also important in differentiating patients who *S*-IVDD from patients who develop *A*-IVDD.

The data also indicates changes in tissue biomarker release that may be related to progression of IVDD regardless of symptom development. Of the proteins that were released at significantly higher levels by the *S*-IVDD cohort compared to the *A*-IVDD cohort, the release of MMP-2, MMP-13, PDGF-AA, and VEGF increased significantly by *A*-IVDD tissues with a T/P-Grade of 5 compared to tissues with a T/P-Grade of 2/3. Therefore, the increase level of these proteins in the IVD tissue may occur as a consequence of IVDD development, but the increased levels at the lower levels of IVDD may be important for symptom development in patients with *S*-IVDD.

Additional studies are needed to determine the effect of these proteins on the between the biochemical environment of an IVD, the role of these proteins on the development and progression of IVDD in patients, and the development of *S*-IVDD. Identifying the factors that contribute to these changes in IVD tissue biomarker release, and the potential role of these proteins in the development and progression of *S*-IVDD may allow for the development of novel diagnostic, prognostic, and treatment modalities to improve outcomes for patients with *S*-IVDD.

FIGURES

Patient Characteristics

	S-IVDs	*A*-IVDs
Sex		
Male	79	10
Female	105	10
Total	184	20
Mean Age	55.8 ± 15.2	51.8 ± 14.2
<30 kg/m^2	82	9
>30 kg/m^2	102	9
Sample Characteristics		
Identified Levels		
L1	5	14
L2	14	15
L3	35	17
L4	74	14
L5	75	7
Total	203	67
T/P Grades		
2/3	64	39
4	99	19
5	40	9
Total	203	67

Table 7: Demographics, levels, T/P-grades in *S*-IVDD and *A*-IVDD patients. BMI information was not available for n=2 *A*-IVDD patients.

S vs A Model Results

Biomarker	AF (p-values)			NP (p-values)		
	2/3	4	5	2/3	4	5
GRO-α	0.338	0.228	**0.028***	0.089	0.065	**0.003***
IL-1RA	**0.001***	0.087	0.319	**0.026***	0.055	**0.003***
IL-6	0.182	0.571	0.082	**0.045***	0.090	**0.012***
IL-8	0.715	0.884	0.055	**0.004***	0.205	**0.002***
MCP-1	0.123	**0.003***	**0.011***	0.179	0.335	**0.001***
MCP-3	0.098	0.225	0.138	0.390	**0.003***	0.156
MIP-1α	0.324	0.108	0.138	**0.016***	**0.038***	**0.001***
MIP-1β	0.233	0.162	0.259	**0.041***	0.081	**0.001***
RANTES	**0.001***	**0.001***	**0.001***	**0.001***	**0.001***	**0.001***
TNF-α	0.062	0.093	0.098	0.108	0.341	**0.001***
MMP-1	0.899	0.517	0.338	0.001*	0.085	**0.001***
MMP-2	**0.001***	**0.008***	0.225	**0.001***	**0.002***	0.371
MMP-3	**0.001***	**0.010***	0.883	**0.023***	0.057	0.805
MMP-7	0.337	0.063	0.366	0.241	0.053	0.477
MMP-8	**0.001***	**0.001***	**0.001***	**0.001***	**0.001***	**0.007***
MMP-9	0.593	0.645	0.150	0.520	0.811	0.755
MMP-13	**0.001***	**0.019***	**0.037***	**0.001***	**0.003***	0.506
TIMP-1	**0.013***	0.055	0.226	**0.001***	**0.001***	0.510
TIMP-2	**0.001***	**0.001***	**0.003***	**0.001***	**0.001***	**0.049***
TIMP-3	**0.001***	**0.023***	**0.009***	**0.001***	**0.004***	**0.019***
TIMP-4	**0.001***	**0.001***	**0.001***	**0.001***	**0.001***	**0.001***
PDGF-AA	**0.003***	0.081	0.153	**0.001***	**0.022***	0.059
VEGF	0.151	0.133	0.413	**0.041**	**0.042***	0.161
FGF2	**0.001***	0.410	0.363	0.603	0.178	0.287

Table 8: Results of *S*-IVDD versus *A*-IVDD cohort ANOVAs for ex vivo biomarker release with adjustment for age, sex, and obesity status. * Indicates $p<0.05$ within each T/P-grade.

Ex vivo inflammatory cytokine/chemokine release by *S*-IVDs versus *A*-IVDs

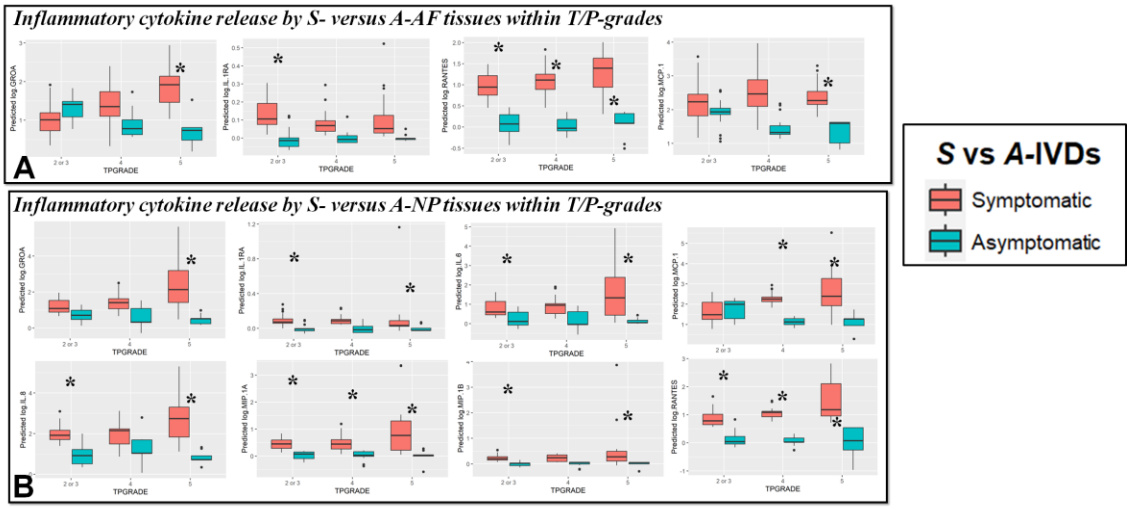


Figure 2: Significant ($p<0.05$) ANOVA results for inflammatory cytokine/chemokine release by *S*-IVDs versus *A*-IVDs within T/P-grade groups 2/3, 4, or 5 while accounting for age, sex, obesity, and their interactions.

Ex vivo MMP release by *S*-IVDs versus *A*-IVDs

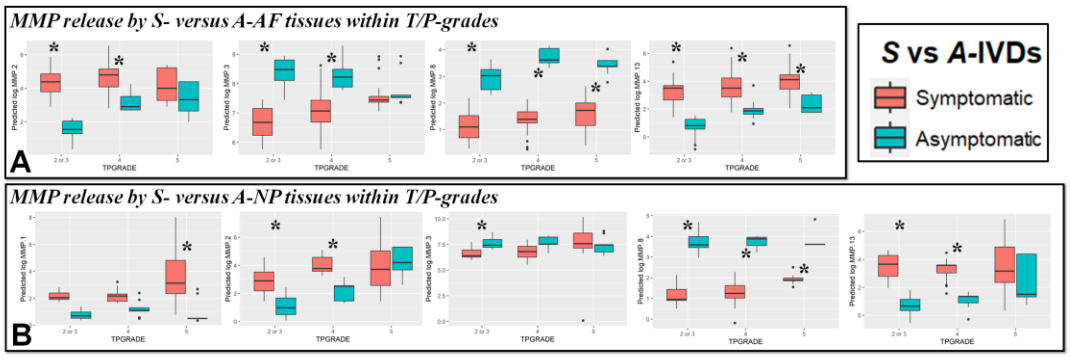


Figure 3: Significant ($p<0.05$) ANOVA results for MMP release by *S*-IVDs versus *A*-IVDs within T/P-grade groups 2/3, 4, or 5 while accounting for age, sex, obesity, and their interactions.

Ex vivo TIMP and Growth Factor release by *S*-IVDs versus *A*-IVDs

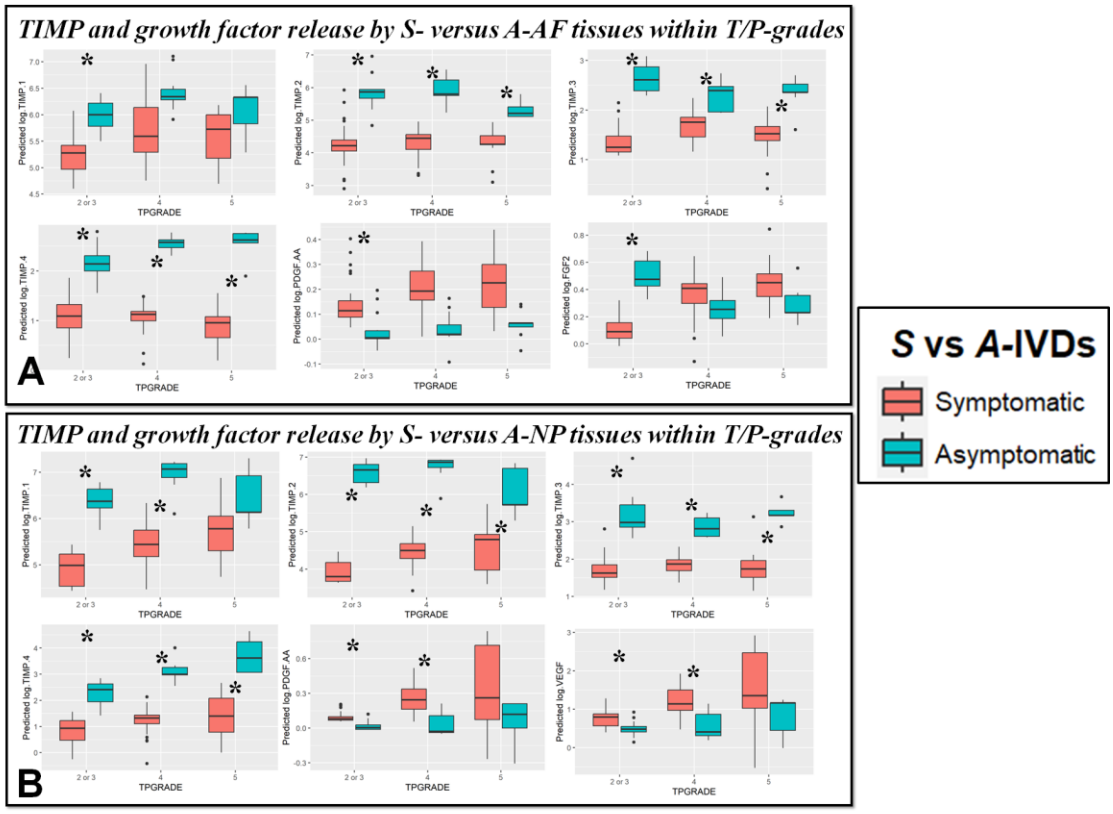


Figure 3: Significant ($p<0.05$) ANOVA results for TIMP and growth factor release by *S*-IVDs versus *A*-IVDs within T/P-grade groups 2/3, 4, or 5 while accounting for age, sex, obesity, and their interactions.

S or A T/P-Grade ANOVA Results (p-values)

Biomarkers	***S-AF***	***A-AF***	***S-NP***	***A-NP***
GRO-α	**0.026***	0.177	0.050	0.351
IL-1RA	0.802	0.693	0.712	0.704
IL-6	**0.027***	0.252	0.183	0.461
IL-8	0.080	0.351	0.332	0.155
MCP-1	0.303	**0.020***	**0.017***	**0.001***
MCP-3	0.361	0.527	0.093	0.815
MIP-1α	0.421	0.618	0.071	0.530
MIP-1β	0.544	0.705	0.073	0.561
RANTES	0.177	0.633	**0.045***	0.177
TNF-α	0.676	0.390	**0.031***	0.214
MMP-1	0.188	0.933	0.056	0.224
MMP-2	0.202	**0.007***	**0.049***	**0.001***
MMP-3	**0.042***	**0.041***	0.410	0.650
MMP-7	0.141	0.252	0.772	0.205
MMP-8	**0.036***	**0.014***	**0.031***	0.964
MMP-9	**0.015***	0.066	0.181	0.358
MMP-13	0.260	**0.006***	0.926	**0.009***
TIMP-1	0.237	0.335	0.174	0.079
TIMP-2	0.902	0.162	0.224	0.398
TIMP-3	0.147	0.516	0.813	0.447
TIMP-4	0.306	0.149	0.074	**0.001***
PDGF-AA	0.244	0.404	**0.026***	**0.004***
VEGF	0.081	0.081	0.063	**0.027***
FGF2	**0.026***	**0.040***	0.766	0.366

Table 4: Results of ANOVAs for ex vivo release of inflammatory cytokines/chemokines, MMPs, TIMPs, and growth factors *S*- or *A*-AF or -NP tissues while accounting for age, sex, and obesity status.

S or A Tukey HSD Post-Hoc Results

S-AF			*A-AF*			*S-NP*			*A-NP*		
Biomarkers	***Comparison***	***P-value***	**Biomarkers**	***Comparison***	***P-value***	**Biomarkers**	***Comparison***	***P-value***	**Biomarkers**	***Comparison***	***P-value***
GRO-α	5 > 2/3	0.020*	*MCP-1*	5 < 2/3	0.047*	*MCP-1*	5 > 2/3	0.021*	*MCP-1*	4-5 < 2/3	0.001*
IL-6	5 > 2/3	0.020*	*MMP-2*	4-5 > 2/3	0.019*	*RANTES*	5 > 2/3	0.037*	*MMP-2*	4-5 > 2/3	0.029*
MMP-3	5 > 2/3	0.035*	*MMP-8*	4 > 2/3	0.015*	*TNF-α*	5 > 4	0.031*	*MMP-13*	5 > 2/3	0.004*
MMP-8	5 > 2/3	0.029*	*MMP-13*	4-5 > 2/3	0.012*	*MMP-2*	4 > 2/3	0.040*	*TIMP-4*	4-5 > 2/3	0.024*
MMP-9	5 > 2/3	0.012*	*FGF2*	4 < 2/3	0.030*	*MMP-8*	5 > 2/3	0.029*	*PDGF-AA*	5 > 2/3	0.004*
FGF2	5 > 2/3	0.029*				*PDGF-AA*	5 > 2/3	0.037*	*VEGF*	5 > 2/3	0.032*

Table 5: Significant ($p<0.05$) biomarker results of pairwise post-hoc analyses between T/P-grade groups within *S*-IVDs or *A*-IVDs.

Significant ex vivo Biomarkers in S-AF Tissues

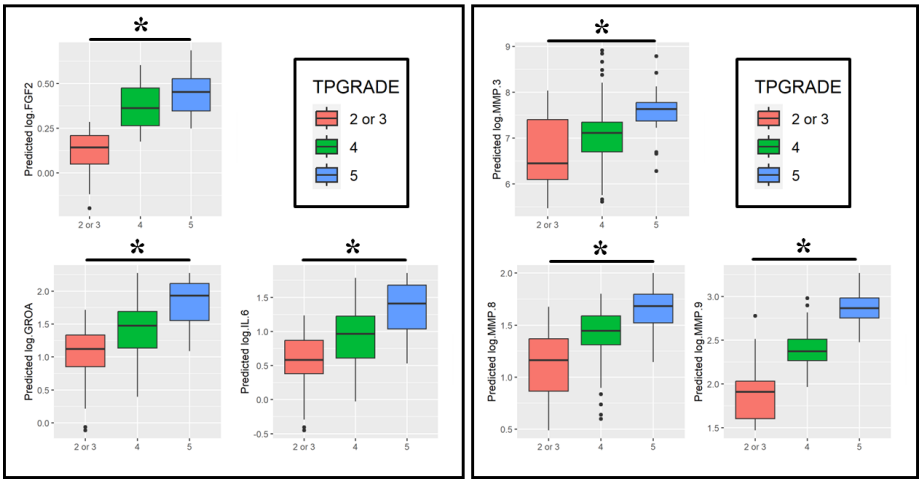


Figure 4: Significant ($p<0.05$) ANOVA and Tukey post-hoc results for ex vivo release growth factors, IL-6, and MMPs between P-Grades within *S*-AF tissues.

Significant ex vivo Biomarkers in A-AF Tissues

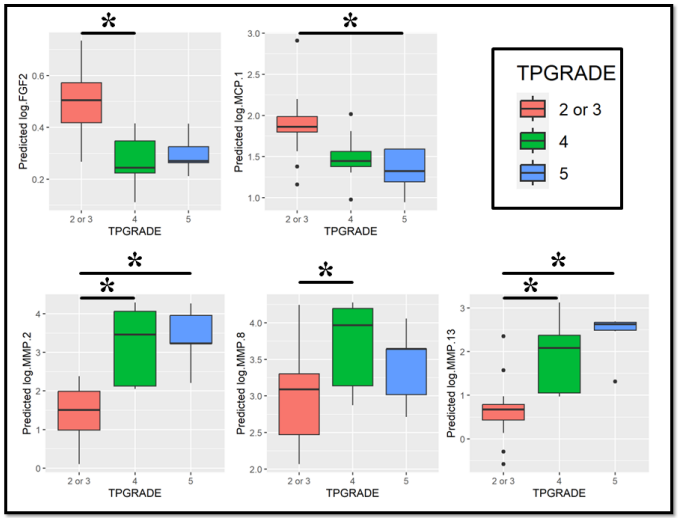


Figure 5: Significant ($p<0.05$) ANOVA and Tukey post-hoc results for ex vivo release of FGF2 and MMPs between T-Grades within *A*-AF tissues.

Significant ex vivo Biomarkers in S-NP Tissues

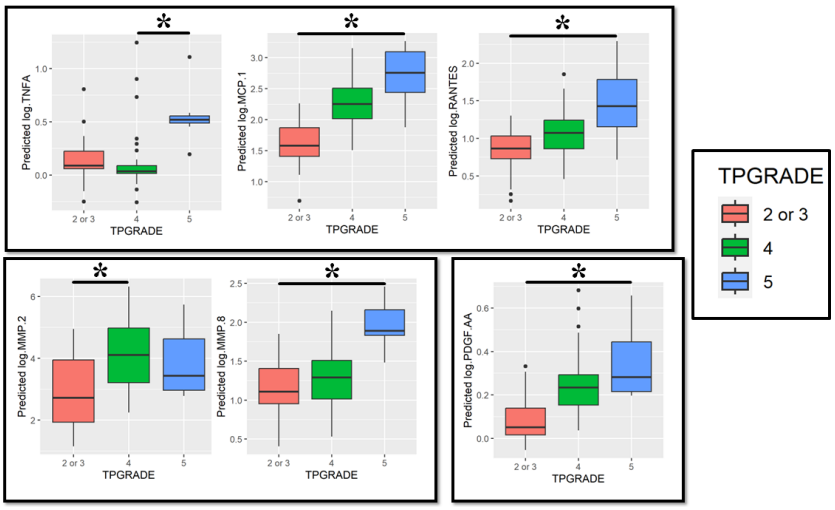


Figure 6: Significant ($p<0.05$) ANOVA and Tukey post-hoc results for ex vivo release of inflammatory cytokines/chemokines, MMPS, and PDGF-AA between P-Grades within *S*-NP tissues.

Significant ex vivo Biomarkers in A-NP Tissues

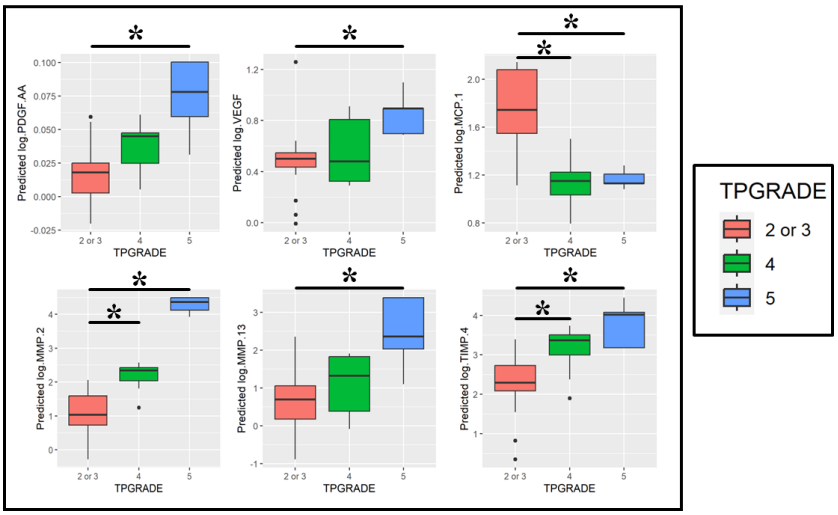


Figure 7: Significant ($p<0.05$) ANOVA and Tukey post-hoc results for ex vivo release of growth factors, MCP-1, MMPs, and TIMP-4 between T-grades within *A*-NP tissues.

REFERENCES

1. Wu A, March L, Zheng X, et al. Global low back pain prevalence and years lived with disability from 1990 to 2017: estimates from the Global Burden of Disease Study 2017. *Ann Transl Med*. 2020;8(6):299. doi:10.21037/atm.2020.02.175

2. Fujii K, Yamazaki M, Kang JD, et al. Discogenic Back Pain: Literature Review of Definition, Diagnosis, and Treatment. *JBMR Plus*. 2019;3(5). doi:10.1002/jbm4.10180

3. Berry JA, Elia C, Saini HS, Miulli DE. A Review of Lumbar Radiculopathy, Diagnosis, and Treatment. *Cureus*. 11(10). doi:10.7759/cureus.5934

4. Kalichman L, Cole R, Kim DH, et al. Spinal stenosis prevalence and association with symptoms: the Framingham Study. *Spine J*. 2009;9(7):545-550. doi:10.1016/j.spinee.2009.03.005

5. Al Qaraghli MI, De Jesus O. Lumbar Disc Herniation. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK560878/

6. Bydon M, Alvi MA, Goyal A. Degenerative Lumbar Spondylolisthesis: Definition, Natural History, Conservative Management, and Surgical Treatment. *Neurosurg Clin N Am*. 2019;30(3):299-304. doi:10.1016/j.nec.2019.02.003

7. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine (Phila Pa 1976)*. 2001;26(17):1873-1878. doi:10.1097/00007632-200109010-00011

8. Yu LP, Qian WW, Yin GY, Ren YX, Hu ZY. MRI Assessment of Lumbar Intervertebral Disc Degeneration with Lumbar Degenerative Disease Using the Pfirrmann Grading Systems. *PLoS One*. 2012;7(12):e48074. doi:10.1371/journal.pone.0048074

9. Brinjikji W, Luetmer PH, Comstock B, et al. Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations. *AJNR Am J Neuroradiol*. 2015;36(4):811-816. doi:10.3174/ajnr.A4173

10. Belavy DL, Brisby H, Douglas B, et al. Characterization of Intervertebral Disc Changes in Asymptomatic Individuals with Distinct Physical Activity Histories Using Three Different Quantitative MRI Techniques. *J Clin Med*. 2020;9(6):1841. doi:10.3390/jcm9061841

11. Rahyussalim AJ, Zufar MLL, Kurniawati T. Significance of the Association between Disc Degeneration Changes on Imaging and Low Back Pain: A Review Article. *Asian Spine J*. 2020;14(2):245-257. doi:10.31616/asj.2019.0046

12. Thompson JP, Pearce RH, Schechter MT, Adams ME, Tsang IK, Bishop PB. Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. *Spine (Phila Pa 1976)*. 1990;15(5):411-415. doi:10.1097/00007632-199005000-00012

13. Pękala P, Taterra D, Krupa K, et al. Correlation of morphological and radiological characteristics of degenerative disc disease in lumbar spine: a cadaveric study. *Folia Morphol (Warsz)*. 2022;81(2):503-509. doi:10.5603/FM.a2021.0040

14. An HS, Masuda K, Inoue N. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthop Sci*. 2006;11(5):541-552. doi:10.1007/s00776-006-1055-4

15. Wuertz K, Haglund L. Inflammatory Mediators in Intervertebral Disk Degeneration and Discogenic Pain. *Global Spine J*. 2013;3(3):175-184. doi:10.1055/s-0033-1347299

16. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol*. 2014;10(1):44-56. doi:10.1038/nrrheum.2013.160

17. Maitre CLL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *The Journal of Pathology*. 2004;204(1):47-54. doi:10.1002/path.1608

18. Li Y, Zhang T, Tian W, et al. Loss of TIMP3 expression induces inflammation, matrix degradation, and vascular ingrowth in nucleus pulposus: A new mechanism of intervertebral disc degeneration. *The FASEB Journal*. 2020;34(4):5483-5498. doi:10.1096/fj.201902364RR

19. Pratsinis H, Kletsas D. Growth factors in intervertebral disc homeostasis. *Connect Tissue Res*. 2008;49(3):273-276. doi:10.1080/03008200802147951

20. Phillips KLE, Cullen K, Chiverton N, et al. Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master regulator of catabolic processes. *Osteoarthritis and Cartilage*. 2015;23(7):1165-1177. doi:10.1016/j.joca.2015.02.017

21. Bachmeier BE, Nerlich A, Mittermaier N, et al. Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. *Eur Spine J*. 2009;18(11):1573-1586. doi:10.1007/s00586-009-1031-8

22. Visse R, Nagase H. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Circulation Research*. 2003;92(8):827-839. doi:10.1161/01.RES.0000070112.80711.3D

23. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA, Kang JD. Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration. *Spine J*. 2013;13(3):331-341. doi:10.1016/j.spinee.2012.02.027

24. Basaran R, Senol M, Ozkanli S, Efendioglu M, Kaner T. Correlation of matrix metalloproteinase (MMP)-1, -2, -3, and -9 expressions with demographic and radiological features in primary lumbar intervertebral disc disease. *Journal of Clinical Neuroscience*. 2017;41:46-49. doi:10.1016/j.jocn.2017.03.001

25. Vergroesen PPA, Kingma I, Emanuel KS, et al. Mechanics and biology in intervertebral disc degeneration: a vicious circle. *Osteoarthritis and Cartilage*. 2015;23(7):1057-1070. doi:10.1016/j.joca.2015.03.028

26. Stone WL, Leavitt L, Varacallo M. Physiology, Growth Factor. In: *StatPearls*. StatPearls Publishing; 2023. Accessed April 27, 2023. http://www.ncbi.nlm.nih.gov/books/NBK442024/

27. Masuda K, An HS. Prevention of disc degeneration with growth factors. *Eur Spine J*. 2006;15(Suppl 3):422-432. doi:10.1007/s00586-006-0149-1

28. Kennon JC, Awad ME, Chutkan N, DeVine J, Fulzele S. Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration. *Biomol Concepts*. 2018;9(1):43-52. doi:10.1515/bmc-2018-0003

29. Ali R, Maitre CL, Richardson S, Hoyland PJ, Freemont A. Connective tissue growth factor expression in human intervertebral disc: implications for angiogenesis in intervertebral disc degeneration. *Biotechnic & Histochemistry*. 2008;83(5):239-245. doi:10.1080/10520290802539186

30. Zhang W, Gong Y, Zheng X, et al. Platelet-Derived Growth Factor-BB Inhibits Intervertebral Disc Degeneration via Suppressing Pyroptosis and Activating the MAPK Signaling Pathway. *Front Pharmacol*. 2022;12:799130. doi:10.3389/fphar.2021.799130

31. Verheijde JL, Rady MY, McGregor JL. The United States Revised Uniform Anatomical Gift Act (2006): New challenges to balancing patient rights and physician responsibilities. *Philos Ethics Humanit Med*. 2007;2:19. doi:10.1186/1747-5341-2-19

32. Le Maitre CL, Hoyland JA, Freemont AJ. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1beta and TNFalpha expression profile. *Arthritis Res Ther*. 2007;9(4):R77. doi:10.1186/ar2275

33. Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human Intervertebral disc degeneration. *Arthritis Research & Therapy*. 2005;7(4):R732. doi:10.1186/ar1732

34. Park JY, Kuh SU, Park HS, Kim KS. Comparative expression of matrix-associated genes and inflammatory cytokines-associated genes according to disc degeneration: analysis of living human nucleus pulposus. *J Spinal Disord Tech*. 2011;24(6):352-357. doi:10.1097/BSD.0b013e3181fee4df

35. Wang WJ, Yu XH, Wang C, et al. MMPs and ADAMTSs in intervertebral disc degeneration. *Clinica Chimica Acta*. 2015;448:238-246. doi:10.1016/j.cca.2015.06.023

36. Deng B, Ren JZ, Meng XQ, et al. Expression profiles of MMP-1 and TIMP-1 in lumbar intervertebral disc degeneration. *Genet Mol Res*. 2015;14(4):19080-19086. doi:10.4238/2015.December.29.16

37. Le Maitre C, Freemont A, Hoyland J. Human disc degeneration is associated with increased MMP 7 expression. *Biotechnic & Histochemistry*. 2006;81(4-6):125-131. doi:10.1080/10520290601005298

38. Rutges JPHJ, Kummer JA, Oner FC, et al. Increased MMP-2 activity during intervertebral disc degeneration is correlated to MMP-14 levels. *J Pathol*. 2008;214(4):523-530. doi:10.1002/path.2317

39. Rastogi A, Kim H, Twomey JD, Hsieh AH. MMP-2 mediates local degradation and remodeling of collagen by annulus fibrosus cells of the intervertebral disc. *Arthritis Research & Therapy*. 2013;15(2):R57. doi:10.1186/ar4224

40. Fearing BV, Hernandez PA, Setton LA, Chahine NO. Mechanotransduction and cell biomechanics of the intervertebral disc. *JOR Spine*. 2018;1(3). doi:10.1002/jsp2.1026

CHAPTER 5: RELATIONSHIPS BETWEEN HISTOLOGICAL IVDD TO SIGNALING PEPTIDES AND MATRIX AFFECTING ENZYMES WITHIN AND BETWEEN ASYMPTOMATIC AND SYMPTOMATIC POPULATIONS

Jacob S. Kramer[1,2], Aaron M. Stoker[1,2], Theodore J. Choma[2], Emily V. Leary[1,2], Jinpu Li[1,2], Don K. Moore[2], James L. Cook[1,2]

[1]Thompson Laboratory for Regenerative Orthopaedics, University of Missouri, Columbia, MO

[2]Department of Orthopaedic Surgery, University of Missouri, Columbia, MO

INTRODUCTION

Back pain is one of the most common sources of disability and medical costs worldwide.[1,2] There are numerous suspected etiologies of back pain, though symptoms resolve without the isolation of a specific cause in most cases.[2,3] However, the degeneration of lumbar intervertebral discs (IVDD) is commonly linked to the development of acute and/or chronic back pain.[4–6] Lumbar IVDD describes the deterioration of osseus (bony) and hyaline (cartilaginous) endplates, the highly collagenous annulus fibrosus (AF), and the hydrophilic nucleus pulposus (NP), which reduces the available space and alignment in the lumbar spine.[7,8] IVD degeneration can lead to the NP rupturing through the AF (herniation), narrowing of nerve passageways (stenosis), and instability (spondylolisthesis).[3,9,10] Magnetic resonance imaging (MRI) radiographs are the standard method for assessing IVDD in symptomatic, clinical patients (*S*-IVDD); however, radiographic IVDD, herniations, stenosis, and spondylolisthesis are often discovered incidentally in people without associated symptoms (*A*-IVDD).[11–13] Therefore, radiographic IVDD does not represent all relevant biological factors affecting the development of *S*-IVDD.[14] Other factors including microstructural (histologic) changes and the biochemical environment of the IVD have also been associated with the development of *S*-IVDD and *A*-IVDD.[14–20]

While practically and clinically useful, radiographic measurements of IVDD are not sensitive to smaller scale tissue changes that are known to occur during *S*-IVDD and *A*-IVDD.[11,21] Therefore, standardized histopathological grading systems have been developed with the goal of characterizing IVDD changes that are inhomogeneous throughout the disc.[14] A grading system was published by Boos et al. in 2002, which

assigned a total histologic degeneration score (HDS) of 0-22 based on presence and/or severity of 11 features of histological IVDD in sagittal *A*-IVD sections or *S*-IVD explants.[14] The original histological scoring system published by Boos et al. has been modified 4 histological category scores that showed the greatest agreement in their original study: AF/NP cell morphology (0-6), mucous degeneration in the AF (0-3), tear and cleft formation (0-3), and granular changes in the NP (0-3), resulting in total HDS scores ranging from 0-15.[15] This system is sometimes further modified to include cell death scoring (0-4), which raises the highest possible total HDS to 19.[14,22] Increased scores in each category indicate AF/NP cell proliferation and aggregation (cell morphology), loss of AF fiber demarcation and excessive proteoglycan staining (mucous degeneration), ruptures in the AF/NP matrix (tear and cleft formation), collections of eosinophilic staining proteins in the NP matrix (granular changes), and the presence of apoptotic bodies and/or ruptured cells (cell death).[15]

Despite the widespread use of the system published by Boos et al., the relationship between histological IVDD and *S*-IVDD development has not yet been clarified in any more meaningful way than the relationship between *S*-IVDD and radiographic IVDD progression.[23,24] Most published studies compare IVDs based on total HDS rather than between *S*-IVDD versus *A*-IVDD cohorts, which does not account for the observation that *S*-IVDs and *A*-IVDs can have similar total HDS.[24,25] This highlights the need to identify other factors, such as the concentration of specific inflammation related and degradation related biomarkers in the IVD tissues, that may be more representative of the fundamental differences between *S*-IVDs and *A*-IVDs with similar levels of histological IVDD.

A complex and sensitive molecular balance between inflammation, anabolism, and catabolism is required to maintain IVD cells and tissues.[19,26–31] These functions are in part regulated by the expression and production of inflammatory cytokines and chemokines, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors.[17,31,32] Determining how changes in the production of these biomarkers by IVD tissues during IVDD, and in the context of *S*-IVDD and *A*-IVDD development may provide important insight into the factors that contribute to the development and progression of *S*-IVDD in patients.

Inflammatory cytokines and chemokines can attract and stimulate immune cells to produce more cytokines, chemokines, or other molecular agents intended to maintain homeostasis.[33] The most commonly studied secreted inflammatory cytokines and chemokines related to the development and progression of *S*-IVDD and *A*-IVDD are tissue necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, IL-6, IL-8, IL-17, interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1 (CCL2), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES, CCL5), and MCP-3 (CCL7)..[17] An imbalance in the production of these inflammatory mediators has been found to induce variable cellular effects including autophagy, apoptosis, and senescence in AF and NP cells, which may affect the progression of *S*- versus *A*-IVDD.[17,34] Shamji et al. reported significantly increased immunohistochemical (IHC) staining of IL-17 in *S*- versus *A*-IVD tissues. Additionally, Weiler et al. reported significant, positive correlations between TNF-α IHC staining, histological IVDD, and age in *S*-IVD and *A*-IVD tissues.[35,36] *S*-IVDs have also been shown to express increasing levels of chemokines MCP-1, MCP-3, MIP-1α, MIP-1β,

RANTES, and IL-8 with increasing degeneration.[37–39] It is well recognized that an aberrant inflammatory cascade contributes to the development and progression of IVDD, though the exact role of inflammation in *S*-IVDD versus A-IVDD has not been fully established.[17,40]

The degradation of extracellular matrix (ECM) components by MMPs is another important component of IVD tissue maintenance.[41] These proteases can be subdivided into collagenases (MMP-1, 8, 13), gelatinases (MMP-2, 9), stromelysins (MMP-3, 10), and matrilysin (MMP-7).[42] Most of these enzymes are produced in an inactive (latent) form that must be modified in order to become active.[43] Additionally, MMPs are regulated by the production of tissue inhibitors of matrix metalloproteinases (TIMPs), which bind directly to the MMP to block the protease activity of the enzyme.[44,45] A cycle of inflammation and imbalances in MMP/TIMP levels in the IVD are thought to contribute to excessive degradation and fibrotic tissue formation.[32,41,46] Within *S*-IVDs, Weiler et al reported significant, positive correlations between IHC staining for MMP-1, MMP-2, MMP-3, and total HDS.[41] Le Maitre et al. additionally reported no observable MMP-3 or MMP-13 IHC staining in histologically normal IVDs, though the data was assessed based on symptomatic status of the IVD, and *S*-IVDs and *A*-IVDs had a similar range of total HDS scores in this study.[32] Roberts et al. reported significantly increased MMP-3 and MMP-7 IHC staining with increasing *S*-IVDD, more consistent TIMP-2 staining in *S*-IVDs compared to *A*-IVDs, and TIMP-1 staining was observed in *S*-IVD samples but not in *A*-IVDs.[47] However, Deng et al. found no significant difference in the expression TIMP-1 in *S*-IVD versus *A*-IVD tissues.[48] Further, a previous study found that *S*-IVD tissues with higher total HDS had significantly higher expression levels of TIMP-

1 and TIMP-2 compared to *S*-IVD tissues with lower total HDS.[49] Taken together, the current literature characterizing the role of MMP/TIMP balance in the development of *S*-IVDD and *A*-IVDD is incomplete and sometimes conflicting.[47–50]

The controlled growth of cells and tissues is an important component of tissue maintenance.[51] Growth factors are able to activate immune cells, promote or inhibit cell proliferation and matrix synthesis, and stimulate the production of inflammatory cytokines, chemokines, and MMPs.[51] The expression and production of growth factors including insulin growth factor (IGF-1), fibroblastic growth factor (FGF), bone morphogenic protein (BMP), growth differentiation factor (GDF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF) have been observed in IVDD tissues.[20,52] However, how specific growth factors affect *S*- IVDD versus *A*-IVDD development is not known.[19,52,56–58]

While the role of growth factors in IVDD are poorly understood, studies that focus on the potential of growth factors as therapeutic agents for the treatment of *S*-IVDD may provide insight into potential roles for growth factors during the development and progression of IVDD clinically.[20] Paglia et al. reported significantly decreased cell death and matrix degradation following PDGF intradiscal injection in a preclinical rabbit model of degenerative disc disease (DDD).[53] In a bovine IVDD model testing the effects of FGF-2, Li et al. reported suppression of excessive proteoglycan production and dose dependent modulation of MMP-13 expression and production.[54] VEGF has been investigated due to its role in the proliferation of blood vessels, which has may be a significant factor affecting IVD nutrient access.[55] Therefore, growth factor production by

the IVD tissue during IVDD may be associated with the tissues attempt to decrease tissue ECM degradation.

Together, current literature indicates that the characterization of inflammatory cytokines/chemokines, MMPs, TIMPs, and growth factors in *S*-IVDD and *A*-IVDD is incomplete, and additionally that few studies considered the symptomatic state of the IVD when assessing changes in IVD biomarker levels based on total HDS. An increased understanding in the relationship between histopathological changes to IVD tissues structures and differences in tissue protein concentration levels based on the symptomatic status of the IVD is needed to develop patient and stage specific therapies for the mitigation or prevention of *S*-IVDD.

Therefore, this study aimed to identify significant differences in the *ex vivo* production of inflammatory cytokines/chemokines, MMPs, TIMPs, and growth factors by AF and NP tissues recovered from patients with *S*-IVDD and *A*-IVDD, based on the level of histopathological changes to the tissues associated with IVDD. Further, this study aimed to identify significant relationships between the *ex vivo* production of these proteins by the IVD tissues and level of histopathological changes to the tissues associated with IVDD within the *S*-IVDD and *A*-IVDD cohorts. An increased understanding of the individual and biochemical differences of *S*-IVDD and/or *A*-IVDD patients and tissues is needed to develop patient and stage specific therapies for the mitigation or prevention of *S*-IVDD. Therefore, within total HDS and category scores, it was hypothesized that *S*-IVD tissues would release significantly ($p<0.05$) increased concentrations of inflammatory cytokines/chemokines, MMPs, and significantly decreased concentrations of TIMPs and

growth factors compared to *A*-IVD tissues during ex vivo culture while adjusting for age, sex, obesity of the patient. Additionally, it was hypothesized that the release of inflammatory and degradative biomarkers during *ex vivo* culture would significantly increase with increasing total HDS and category scores in each tissue group (*S*-AF, *A*-AF, *S*-NP, *A*-NP).

METHODS

S-IVDD Population: IRB approval (IRB#2010692), and informed patient consent, spine tissues were recovered from patients (*S*-IVDD patients, n=202, mean age 55.3y, 118F) undergoing surgery to treat *S*-IVDD disorders such as herniation, stenosis, spondylolisthesis, and DDD. *S*-IVD tissues were not recovered from tumor or infection related cases. Patient/donor age was restricted to 17-95 years.

A-IVDD Population: With consent as recorded in a legal permit under the Uniform Anatomical Gift Act[59], IVD tissues from qualified tissue donors (*A*-IVDD patients, n=25, mean age 53.4y, 12F) were recovered by the Midwest Transplant Network. Medical history was evaluated during a Donor Risk Assessment Interview (DRAI). *A*-IVDD patient tissues testing positive for pathogens or diseases including HIV/AIDS, hepatitis, HTLV-I/II, Chagas' disease, hepatitis, malaria, cancers, Ebola virus, and Zika virus were excluded from this study. Additionally, IVD tissues were not recovered from patients with a history of discogenic back pain or spinal surgery were not recovered. Patient age was restricted to 17-95 years.

S-IVD Tissue Collection and culture: *S*-IVD issues recovered from clinical procedures (n=207 specimens) were suspended in sterile phosphate buffered saline (PBS) within one hour of surgery completion. A 6 mm diameter explant from *S*-IVD tissues were created for ex vivo tissue cultures. Tissue explants were cultured for 3 days in supplemented DMEM (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium). Media were collected after culture and stored at -20°C.

A-IVD Tissue Collection and culture: Spine segments were recovered from the deceased and transported to the lab within 24 hours of death. IVDs were recovered from all available lumbar levels (n=72) of each received lumbar spinal column (n=36). A 6 mm diameter explant from AF and NP were created for *ex vivo* tissue cultures. Tissue explants were cultured separately for 3 days in supplemented DMEM (L-ascorbic acid, L-glutamine, penicillin/streptomycin/amphotericin B, sodium pyruvate, non-essential amino acids, insulin-transferrin-selenium). Media were collected after culture and stored at -20°C.

Histological Assessment: A portion of each cultured *S*-IVD specimen and uncultured, sagittal/transverse sections in the median plane of each *A*-IVD were formalin fixed, decalcified in 10% EDTA, paraffin-embedded, and tissue sections were stained using H&E, Toluidine blue, and Picrosirius red. For *S*-IVD tissues, histological classification of AF or NP tissue was performed, and tissues found to contain a mixture of AF, NP, cartilage endplate, and/or bone were excluded from analysis in this study. A modified IVD scoring system based on the scheme published by Boos et al.[60] was used to evaluate each tissue by one blinded pathologist. *S*- and *A*-IVD tissues were assessed for total HDS (TOTAL HDS, 0-19) comprised of categories including cell morphology (CELL-M, 0-6), mucous degeneration in the AF (MUCOUS, 0-3), cell death (DEATH, 0-4), tear and cleft formation (TEAR, 0-3), and granular changes in the NP (GRAN, 0-3). A category followed by a number will indicate a score within that category i.e., “GRAN 3” refers to granular change scores of 3.

Media biomarker analysis: Using commercially available Luminex multiplex magnetic bead assays, *S*-IVD and *A*-IVD tissue culture media were tested for inflammatory

cytokines/chemokines: GRO-α, MCP-1, MCP-3, TNF-α, IL-1RA, IL-6, IL-8, MIP-1α, MIP-1β, RANTES (Millipore); matrix metalloproteinases: MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13 (R&D Systems); tissue inhibitors of matrix proteinases: TIMP-1, TIMP-2, TIMP-3, TIMP-4 (Millipore); and growth factors: PDGF-AA, PDGF-AB/BB, VEGF, and FGF-2 (Millipore).

Statistical analysis: In this report, ":" will be used to refer to an interaction between patient characteristics (e.g., "Age:Obesity"" indicates the interaction between age and obesity). In order to account for patient characteristics (i.e., age, sex, and BMI) as factors, BMI was dichotomized as obese/not obese (<30/>30 kg/m^2). Due to low frequency, tissues with scores of "0" and "1" were combined within CELL-M, MUCOUS, DEATH, and TEAR categories. Additionally, samples with TOTAL HDS of 2-6 and 15-19 were combined for analysis.

Media biomarker concentrations were standardized to the wet weight of each explant and natural log transformed. An analysis of variance (ANOVA) was then created for each biomarker within each TOTAL HDS and category score, and *S*-IVDs were compared to *A*-IVDs with adjustment for patient characteristics (age, sex obesity status) and their interactions. Then, ANOVAs were created within *S*- or *A*-IVDD cohorts, and biomarker release was compared between TOTAL HDS or category scores. Two-sided significance was set at $\alpha=0.05$.

RESULTS

Donor/Patient Characteristics:

From 202 *S*-IVDD patients, tissues from 207 IVDs were collected from L1-L5. From 25 *A*-IVDD patients, 72 IVDs were recovered from L1-L5 (Table 1). *S*-IVDD patient age ranged from 19-85 years with 87 younger patients (<55 years) and 115 older (>55 years) patients. *A*-IVDD patient age ranged from 17-74 with 12 younger and 13 older individuals. In the *S*-IVDD cohort, 89 patients were classified as not obese (<30 kg/m^2) and 113 as obese (>30 kg/m^2). In the *A*-IVDD cohort, 13 individuals were classified as not obese (<30 kg/m^2) and 10 as obese (>30 kg/m^2), and BMI information was unavailable for two *A*-IVDD patients.

Histological Assessment

S-IVD tissues were most frequently assigned TOTAL HDS 7-12, DEATH and TEAR 1-3, CELL-M and MUCOUS 2-3, and GRAN 0-2 (Table 2). *S*-AF and *S*-NP tissues were assigned similar TOTAL HDS, though S-AF tissues tended to have higher CELL-M and TEAR scores than *S*-NP tissues. *A*-IVD tissues were most frequently assigned TOTAL HDS 9-13, CELL-M 3-4, and DEATH, MUCOUS, and TEAR 2-3 (Table 3). *A*-AF tissues tended to have TOTAL HDS 9-13, while *A*-NP tissues tended to have TOTAL HDS 7-11.

Ex vivo protein release by S- versus A-AF or -NP tissues within Total HDS

Inflammatory Cytokines/Chemokines

Within TOTAL HDS scores (in parentheses), the *ex vivo* release of GRO-α (TOTAL HDS 7-8), IL-1RA (7, 10, 13), MCP-1 and TNF-α (10-12), MIP-1α (8-10), and MIP-1β (8-9) were significantly ($p \leq 0.039$) increased by *S-IVDs* versus *A*-IVDs (Table 4). The release of RANTES was significantly ($p \leq 0.048$) increased by *S*-IVDs versus *A*-IVDs within TOTAL HDS 2-13 (Figure 1). For TOTAL HDS, the release of inflammatory cytokines and chemokines was more frequently increased significantly by *S*-NP than *S*-AF tissues when compared to *A*-NP and *A*-AF, respectively.

MMPs

S-IVDs versus *A*-IVDs significantly ($p \leq 0.048$) increased the release of MMP-1 (TOTAL HDS 2-6, 9-11) from NP tissues, MMP-2 (9-13) from AF and NP tissues, and MMP-13 (7, 9-10, 13) from AF tissues (Table 5). *A*-IVDs significantly ($p \leq 0.032$) increased the release of MMP-3 (8-10) from AF tissues and MMP-8 (2-13) from AF and NP tissues (Figure 2).

TIMPs and Growth Factors

A-AF and *A*-NP tissues consistently released significantly ($p \leq 0.045$) increased concentrations of TIMP-2 (TOTAL HDS 2-11), TIMP-3 (7-10), and TIMP-4 (2-6, 8-13) compared to *S*-IVD tissues (Table 6). *S*-IVDs versus *A*-IVDs released significantly ($p \leq 0.045$) increased concentrations of growth factors PDGF-AA (9-11, 13), VEGF (7, 10-11, 13) from AF and NP tissues, and FGF2 (10) from NP tissues (Figure 3).

Ex vivo protein release by S-* versus *A-AF or -NP tissues within category scores

CELL-M

Because the majority of the scores for CELL-M in both the *S*-IVDD and *A*-IVDD cohorts were 3 and 4, the focus of the discussion will be on the differences between the groups within these scores for CELL-M. (Table 7). Significant differences within CELL-M demonstrated a similar protein release pattern that was observed for TOTAL HDS. The *S*-IVD tissues released significantly higher levels of GRO-α (3), MCP-1 (3,4), TNF-α (3), IL-1RA (3,4), IL-6 (3), IL-8 (3), MIP-1α (3,4), MIP-1β (3,4), RANTES (3,4), MMP-1 (3,4), MMP-2 (3,4), MMP-9 (3,4), MMP-13 (3,4), PDGF-AA(3,4), and VEGF (3,4) compared to the *A*-IVD tissues. The *A*-IVD tissues released significantly higher levels of MMP-3 (3,4), MMP-8 (3,4), TIMP-1 (3,4), TIMP-2 (3,4), TIMP-3 (3,4), and TIMP-4 (3,4) compared to the *S*-IVD tissues.

DEATH

The majority of significant differences in protein release between tissues from the *S*-IVDD and *A*-IVDD cohorts based on the DEATH score were observed for tissues with a score of 2 (Table 8). The *S*-IVD tissues released significantly higher levels of GRO-α (2), MCP-1 (2), TNF-α (3), IL-1RA (2), IL-6 (2), IL-8 (2), MIP-1α (2,3), MIP-1β (2), RANTES (0-3), TNF-α (2), MMP-1 (2), MMP-2 (1,2), MMP-7 (2), MMP-9 (2,3), MMP-13 (0-2), PDGF-AA(2,3), and VEGF (2,3) compared to the *A*-IVD tissues. The *A*-IVD tissues released significantly higher levels of MMP-3 (2,3), MMP-8 (0-4), TIMP-1 (2), TIMP-2 (0-3), TIMP-3 (2,3), and TIMP-4 (0-4) compared to the *S*-IVD tissues.

MUCOUS

Significant differences in protein release were consistent across MUCOUS scores, but were primarily observed in MMPs, TIMPs, and growth factors (Table 9). The *S*-IVD

tissues released significantly higher levels of IL-1RA (3), MCP-1 (2,3), RANTES (0-3), TNF-α (3), MMP-2 (0-3), MMP-9 (2,3), MMP-13 (0-3), PDGF-AA(2,3), and VEGF (3) compared to the *A*-IVD tissues. The *A*-IVD tissues released significantly higher levels of MMP-3 (2,3), MMP-8 (0-3), TIMP-1 (2), TIMP-2 (0-3), TIMP-3 (0-3), and TIMP-4 (0-3) compared to the *S*-IVD tissues.

TEAR

The number of significant differences in protein release were greatest within TEAR 2-3 (Table 10). The *S*-IVD tissues released significantly higher levels of GRO-α (2,3), MCP-1 (2,3), IL-1RA (0-3), IL-6 (2,3), IL-8 (2,3), MIP-1α (2,3), MIP-1β (2), RANTES (0-3), TNF-α (2), MMP-1 (3), MMP-2 (0-3), MMP-7 (2,3), MMP-9 (2), MMP-13 (0-3), PDGF-AA(2,3), and VEGF (2,3) compared to the *A*-IVD tissues. The *A*-IVD tissues released significantly higher levels of MMP-3 (3), MMP-8 (0-3), TIMP-1 (2,3), TIMP-2 (0-3), TIMP-3 (2,3), and TIMP-4 (0-3) compared to the *S*-IVD tissues.

GRAN

Significant differences in protein release were present throughout GRAN scores (Table 11). The *S*-IVD tissues released significantly higher levels of GRO-α (2,3), MCP-1 (2,3), IL-1RA (0-3), IL-6 (2,3), IL-8 (2,3), MIP-1α (2,3), MIP-1β (2), RANTES (0-3), TNF-α (2), MMP-1 (0-3), MMP-2 (1-3), MMP-7 (0), MMP-13 (0-3), PDGF-AA(0-3), and VEGF (0,2/3) compared to the *A*-IVD tissues. The *A*-IVD tissues released significantly higher levels of MMP-3 (0), MMP-8 (0-3), TIMP-1 (0-3), TIMP-2 (0-3), TIMP-3 (0-3), and TIMP-4 (0-3) compared to the *S*-IVD tissues.

Ex vivo protein release by S-AF, S-NP, A-AF or A-NP tissues between Total HDS

Within each tissue type, there were fewer significant differences between total histology scores than was observed between the *S*-IVD and A-IVD tissues. For *S*-AF tissues, the release of MIP-1β, TNF-α, and MMP-1 by tissues from IVDs with a TOTAL HDS of 15-18 was significantly ($p \leq 0.029$) higher than *S*-AF tissues with a TOTAL HDS of 2-11 (Table 12). For *A*-AF tissues, the release of VEGF by tissues from IVDs with a TOTAL HDS of 7 was significantly ($p = .001$) higher than *A*-AF tissues with a TOTAL HDS of 9-13. For *S*-NP tissues, the release of IL-1RA and MCP-3 by tissues from IVDs with a TOTAL HDS of 12 were significantly ($p \leq 0.034$) higher than *S*-NP tissues with a TOTAL HDS of 8, 9, and/or 10. Further, the release of MCP-1, MMP-2, and RANTES by *S*-NP tissues from IVDs with a TOTAL HDS of 2-6 were significantly ($p \leq 0.034$) lower than for *S*-NP tissues with a TOTAL HDS of 10 or 12. For *A*-NP tissues, the release of GRO-α, IL-6, RANTES, and MMP-9 by tissues from IVDs with a TOTAL HDS of 8 was significantly ($p \leq 0.034$) lower than for *A*-NP tissues with a TOTAL HDS of 9 or 11. Further, the release of PDGF-AA by *A*-NP tissues from IVDs with a TOTAL HDS of 2-6 was significantly ($p = 0.005$) higher than for *A*-NP tissues with a TOTAL HDS of 7-13. Finally, the release of IL-1RA by *A*-NP tissues from IVDs with a TOTAL HDS of 2-6 and 12 was significantly ($p \leq 0.034$) higher than for *A*-NP tissues with a TOTAL HDS of 7-11.

Ex vivo protein release by S-AF, S-NP, A-AF or A-NP tissues between category scores

When each tissue cohort was separated by the scores for each histological category, the majority of differences observed between tissues was observed for the CELL-M and DEATH scores. For *S*-AF tissues, the release of GRO-α, IL-6, IL-8, MIP-1α, MIP-1β, TNF-α, MMP-1, MMP-2, and MMP-13 by tissues with a CELL-M score of

6 was significantly ($p \leq 0.028$) higher than *S*-AF tissues with lower CELL-M scores. Further, the release of GRO-α, IL-6, IL-8, MCP-1, MIP-1α, MIP-1β, MMP-1, MMP-2, and MMP-13 by *S*-AF tissues with a Death score of 4 was significantly ($p \leq 0.031$) higher than *S*-AF tissues with lower Death scores. Finally, the release TIMP-1, TIMP-2, and TIMP-4 by *S*-AF tissues with a Mucous score of 0/1 was significantly ($p \leq 0.001$) lower than *S*-AF tissues with higher Mucous scores.

For *S*-NP tissues, the release of GRO-α, IL-1RA, IL-8, MCP-1, MCP-3, MIP-1β, TNF-α, MMP-1, MMP-9, and MMP-13 by tissues with a CELL-M ($p \leq 0.028$) score of 6 was significantly higher than *S*-NP tissues with lower CELL-M scores. Further, the release of GRO-α, IL-1RA, IL-8, MCP-1, MCP-3, MIP-1α, MIP-1β, TNF-α, MMP-1, MMP-13, and VEGF by *S*-NP tissues with a Death score of 4 was significantly ($p \leq 0.031$) higher than *S*-NP tissues with lower Death scores.

For *A*-AF and *A*-NP tissues, there were fewer proteins with significant differences between groups based histological categorical scores than were observed for the *S*-AF and *S*-NP tissues. For *A*-AF tissues, the release of MMP-9 and VEGF by tissues with a CELL-M score of 5 or 6, respectively, were significantly ($p \leq 0.043$) higher than *A*-AF tissues with lower CELL-M scores. Further, the release of MCP-1 and VEGF by *A*-AF tissues with a Death score of 3 or 4, respectively, were significantly ($p \leq 0.017$) higher than *S*-AF tissues with lower Death scores. For *A*-NP tissues, the release of PDGF-AA, MMP-2, MMP-9, and MMP-13 by tissues with a CELL-M ($p \leq 0.041$) score of 5 was significantly higher than *A*-NP tissues with lower CELL-M scores. Further, the release of IL-1RA, PDGF-AA, and VEGF by *A*-NP tissues with a Death score of 0/1 was significantly ($p \leq 0.031$) higher than *A*-NP tissues with higher Death scores. Finally, the

release GRO-α, IL-6, MCP-1, and MMP-7 by *A*-NP tissues with a GRAN score of 2/3 was significantly ($p \leq 0.025$) higher than *A*-NP tissues with lower GRAN scores.

DISCUSSION

In agreement with numerous previous studies, the data from this study indicates that the level of degradation of the IVD as assessed by the modified Boos histological scoring system does have a significant effect on the release of inflammatory and degradative enzymes related biomarkers by IVD tissues recovered from patients with *S*-IVDD and *A*-IVDD during *ex vivo* culture.[29,35,40,61,62] Further, MMP expression and production have also been found in some studies to increase with increasing histological *S*-IVDD severity.[32,49] However, few if any studies directly compare *S*-IVD and *A*-IVD inflammatory cytokine/chemokine or MMP release while additionally accounting for patient characteristics and histological IVDD severity. The data from this study indicate similar difference in release of inflammatory and degradative enzyme related biomarkers as observed in chapter 3 and 4. However, the data from this study indicates that changes in some biomarkers may be associated with specific changes in IVD tissue structure and composition. Therefore, these data have the potential to determine how specific changes in IVD tissue structure relate to changes in tissue biomarker production by IVD tissues between patients with *S*-IVDD and *A*-IVDD, which may be indicative of clinically important differences in IVD tissue metabolism related to the development and progression of *S*-IVDD and *A*-IVDD in patients.

The data from this analysis continues to support the concept that the development and progression of *S*-IVDD is related to increased localized inflammatory signaling in the IVD, and a shift in the balance for degradative enzyme activity towards catabolism. When *S*-IVDD and *A*-IVDD tissues were compared based TOTAL HDS, RANTES release was consistently higher, and MMP-8, TIMP-2, TIMP-3, and TIMP-4 release was consistently

lower, by tissues from the *S*-IVDD cohort compared to the *A*-IVDD cohort. Further, the release of MMP-1, MMP-2, MMP-13, PDGF-AA, and VEGF were found to be significantly higher by tissues from the *S*-IVDD cohort compared to the *A*-IVDD cohort with TOTAL HDS of 7-13. Because of the complexity of the factors that comprise the TOTAL HDS score, looking at differences between groups based on the categorical scores that comprise the TOTAL HDS may provide more detailed information regarding how the changes in tissue histological structure related to IVDD relates to the release of proteins by the IVD tissues during ex vivo culture.

When the tissues from the *S*-IVDD cohort were compared to the *A*-IVDD cohort based on the categorical scores of the TOTAL HDS, many potentially clinically important differences between the cohorts were identified. These data indicate that CELL-M scores of 3-4, DEATH scores of 2, TEAR scores of 2-3, and GRAN scores of 0-1 appear to represent key changes to tissue IVD tissue structure that are associated with significant increases in localized inflammatory signaling for patients with *S*-IVDD compared to patients with *A*-IVDD. Further, these data indicate that CELL-M scores of 3-5, DEATH scores of 2-3, MUCUS scores of 2-3, TEAR scores of 2-3, and GRAN scores of 0-3 appear to represent key changes to tissue IVD tissue structure that are associated with significant shift in the regulation of degradative enzymes towards increased tissue catabolism for patients with *S*-IVDD compared to patients with *A*-IVDD. and degradative distinction between *S*- IVDs and *A*-IVDs. It is intriguing to note that mucous changes to the AF were primarily associated with significant differences in the release of MMPs, TIMPs, and growth factors by the tissue. This indicates that mucosal changes to the AF associated with *S*-IVDD may be due to shifts in degradative enzyme activity and not differentiated from

patients with *A*-IVDD by the level of inflammation in the tissue. Further, the data appears to indicate that how the IVD responses to changes in cell morphology (CELL-M) and cell death (DEATH) in the tissue may be a significant contributor to determining if a patient develops *S*-IVDD or *A*-IVDD. Together, these data indicate that a complex combination of tissues changes are related to shifts in the production of the biomarkers by the IVD tissues during IVDD, and using the TOTAL HDS may hinder the ability to identify changes in the levels of specific biomarkers related to specific histological changes to the IVD related to the development and progression of *S*-IVDD clinically.

When the tissues from the *S*-IVDD and *A*-IVDD cohorts were analyzed based on changes between histological scores within each tissue type, the data indicated that the release of inflammatory and degradative biomarkers by tissues in the *S*-IVDD cohort were affected more by the changes in tissue structure related to IVDD than the tissues in the *A*-IVDD. Further, the data from this study indicates that the release of inflammatory biomarkers by the *S*-IVD tissues were significantly affected by increases in the CELL-M and DEATH scores. Additionally, the release of the MMP-1, MMP-2, and MMP-13 the *S*-AF was significantly higher by *S*-AF tissues with high CELL-M and DEATH scores. This further indicates that changes in the cellular composition of the IVD is a significant factor in the development and progression of *S*-IVDD in clinical patients. Interestingly, GRAN changes to the NP from *A*-IVDD patients appeared to have a larger effect on the release of inflammatory and degradative biomarkers by the tissue than was observed for the NP from *S*-IVDD patients. This indicates that changes to the NP during *A*-IVDD patients may have unique effects on the inflammatory and degradative environment of the IVD.

LIMITATIONS

As with any study, there are numerous limitations to consider when interpreting the data from this study. The determination of asymptomatic for lower back pain was not based on direct communication with the diseased donor and was based on information obtained from relative that consented the patient to be a tissue donor. Therefore, it is possible that some of the patients did suffer from lower back pain and did not report this to the consenting person prior to tissue donation. However, it is unlikely that the donors would experience a similar level of disabling lower back pain as the patients in the *S*-IVDD, who required surgery to address their pain, without their close family members knowledge. Additionally, there was a notable disparity in the number of *A*-IVDD versus *S*-IVDD patients, and multiple samples were analyzed from each *A*-IVDD tissue donor. However, due to the inherent difficulty in obtaining tissues from patients with *A*-IVDD, it would be difficult to recover tissues from a similar number of patients with *A*-IVDD as were obtained from patients with *S*-IVDD who were undergoing surgery to treat their disease. Further, assessing samples from multiple levels of the same patient, which may have significantly different grades of IVDD, may better reflect the variability in IVD pathobiology observed in clinical patients with *A*-IVDD.

Additionally, there was a disparity in the distribution of the levels of the lumbar spine analyzed in the *A*-IVDD and *S*-IVDD patients. For the majority of the patients in the *A*-IVDD cohort, IVD tissues were recovered from lumbar (L1-L5) levels. However, the clinical distribution of *S*-IVDD levels was concentrated in L4-L5 and L5-S1, potentially presenting unique biochemical changes that reflect the increased biomechanical load experienced in the lower lumbar levels.[13,40] While analysis could

have been focused on just these two levels of the lumbar spine, the significant decrease in sample size could have resulted in a significant decrease in study power. On going studies aimed at increasing the number of *A*-IVDD patients could allow for analysis of the data that includes IVD level as a factor or focused on specific levels of the lumbar spine. The data from this study will be used to help guide the analysis of the samples in these future studies.

Further, the timeframe for recovering tissues from *A*-IVDD patients and *S*-IVDD patients and processing and culturing the tissues in the lab were different. Tissues recovered from *S*-IVDD surgical patients recovered from the patient and processed for culture in the laboratory within an hour of surgery, while tissues recovered from the *A*-IVDD patients were recovered from the deceased tissue donor and processed in the laboratory within 24 hours. It is possible that the delay in the *A*-IVDD cohort could have significantly affected the viability the IVD and the release of proteins from the tissues during culture. However, the protocol for tissue recovery used for the *A*-IVDD patients is the same as the protocol used to recover osteochondral allograft tissues used clinically, and a significant reduction in tissue viability is not observed in this timeframe in osteochondral allograft tissues.

Additionally, there were differences in type and timing of histological assessments for *S*-IVD and *A*-IVD tissues. However, these differences are due to the innate differences in tissue recovery between the two cohorts. Because the entire IVD was recovered from tissue donors in the *A*-IVDD cohort, it was possible to assess the entire IVD histologically for this study. However, removal of the entire IVD *en bloc* is not standard of practice for the surgical treatment of patients with *S*-IVDD. Therefore, IVDs in the *S*-IVDD cohort

would not be assessed as whole sagittal sections like IVDs in *A*-IVDD cohort. Further, the tissue samples recovered from surgical patients were pooled tissue fragments from IVD, and it was not possible to differentiate between AF and NP tissues prior to culture. Therefore, histological assessment of the cultured IVD explant was performed to ensure accurate identification of the tissue that was cultured. Since AF and NP tissues were easily distinguishable prior to culture in the *A*-IVDD cohort, histological assessment was not required to determine tissue type of the cultured explant. Therefore, it is possible that more significant associations were observed between in the *A*-IVDD cohort and patient demographics in this study because the entire IVD was assessed histological, and only one tissue explant was assessed in the *S*-IVDD cohort.

CONCLUSIONS

With these limitations in mind, the data from this study indicates potentially important differences in the inflammatory and degradative environment of the IVD during the development and progression of IVDD for patients with for patients who develop *S*-IVDD compared to patients who develop *A*-IVDD. In agreement with the data from previous chapters, the most consistent differences between *S*-IVDD and *A*-IVDD tissues were the significant increase in RANTES release, and significant decrease in TIMPs released by *S*-IVDD tissues. This indicates that activation of specific inflammatory pathways and loss of degradative enzyme regulation may be significant factors in the development of *S*-IVDD clinically.

The data from this study also indicates that the response of the IVD tissues to changes in the cellular composition of the IVD (CELL-M and DEATH scores) may be a critical differentiating factor between patients with *S*-IVDD and *A*-IVDD. These scores were consistently associated with differences in the release of inflammatory and degradative enzyme related proteins by the IVD tissues between *S*-IVDD and *A*-IVDD cohorts, and between scores within the *S*-IVDD cohort. Future studies are aimed at determining how the changes in biomarker release identified in this study relate to the clinical development and progression of *S*-IVDD. Determining the relationship between the changes in biomarker identified in this study to the clinical development of *S*-IVDD and *A*-IVDD may allow for the development of novel prognostic, diagnostic, and therapeutic methods to improve outcomes for patients with LBP.

FIGURES

Patient Characteristics

	S-IVDD	*A*-IVDD
Sex		
Male	84	13
Female	118	12
Total	202	25
Mean Age	55.3 ± 15.4	53.4 ± 14.7
<30 kg/m^2	89	13
>30 kg/m^2	113	10

Sample Characteristics

Identified Levels		
L1	4	16
L2	14	15
L3	36	19
L4	76	14
L5	77	8
Total	207	72

Table 9: Demographics, levels, T/P-grades in *S*-IVDD and *A*-IVDD patients. BMI information was not available for n=2 *A*-IVDD patients.

S-IVD Histology Total and Category Score Distributions (% of tissues)

SCORE	TOTAL		CELL-M		DEATH		MUCOUS	TEAR		GRAN
	AF	*NP*	*AF*	*NP*	*AF*	*NP*	*AF*	*AF*	*NP*	*NP*
0	0 (0)	0 (0)	7 (4.9)	0 (0)	1 (0.7)	0 (0)	1 (0.7)	6 (3.1)	1 (1.1)	42 (44.7)
1	0 (0)	0 (0)	7 (4.9)	0 (0)	24 (16.7)	6 (6.4)	14 (9.7)	54 (27.8)	13 (13.8)	26 (27.7)
2	0 (0)	0 (0)	23 (16)	6 (6.4)	51 (35.4)	43 (45.7)	45 (31.3)	71 (36.6)	57 (60.6)	22 (23.4)
3	0 (0)	0 (0)	69 (47.9)	48 (51.1)	52 (36.1)	36 (38.3)	84 (58.3)	13 (6.7)	23 (24.5)	4 (4.3)
4	2 (1.4)	0 (0)	17 (11.8)	17 (18.1)	16 (11.1)	9 (9.6)	0 (0)	0 (0)	0 (0)	
5	5 (3.5)	1 (1.1)	6 (4.2)	13 (13.8)						
6	5 (3.5)	5 (5.3)	15 (10.4)	10 (10.6)						
7	10 (6.9)	17 (18.1)								
8	21 (14.6)	14 (14.9)								
9	26 (18.1)	19 (20.2)								
10	25 (17.4)	16 (17.0)								
11	19 (13.2)	7 (7.4)								
12	13 (9.0)	11 (11.7)								
13	8 (5.6)	2 (2.1)								
14	5 (3.5)	0 (0)								
15	4 (2.8)	1 (1.1)								
16	0 (0)	1 (1.1)								
17	0 (0)	0 (0)								
18	0 (0)	0 (0)								
19	0 (0)	0 (0)								

Table 10: Score distributions for TOTAL HDS, CELL-M, DEATH, MUCOUS, TEAR, and GRAN in *S*-IVDs.

A-IVD Histology Total and Category Score Distributions (% of tissues)

SCORE	TOTAL		CELL-M		DEATH		MUCOUS	TEAR		GRAN
	AF	*NP*	*AF*	*NP*	*AF*	*NP*	*AF*	*AF*	*NP*	*NP*
0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	24 (32.9)
1	0 (0)	0 (0)	0 (0)	0 (0)	3 (4.2)	3 (4.1)	5 (6.9)	6 (8.3)	7 (9.6)	22 (30.1)
2	0 (0)	0 (0)	1 (1.4)	1 (1.4)	43 (59.7)	44 (60.3)	27 (37.5)	41 (56.9)	41 (56.2)	13 (17.8)
3	0 (0)	0 (0)	39 (54.2)	40 (54.8)	25 (34.7)	25 (34.2)	40 (55.6)	25 (34.7)	25 (34.2)	14 (19.2)
4	0 (0)	0 (0)	25 (34.7)	25 (34.2)	1 (1.4)	1 (1.4)				
5	0 (0)	0 (0)	6 (8.3)	6 (8.2)						
6	1 (1.4)	3 (4.1)	1 (1.4)	1 (1.4)						
7	2 (2.8)	10 (13.7)								
8	4 (5.6)	15 (20.5)								
9	15 (20.8)	13 (17.8)								
10	10 (13.9)	12 (16.4)								
11	16 (22.2)	10 (13.7)								
12	11 (15.3)	3 (4.1)								
13	10 (13.9)	7 (9.6)								
14	3 (4.2)	0 (0)								
15	0 (0)	0 (0)								
16	0 (0)	0 (0)								
17	0 (0)	0 (0)								
18	0 (0)	0 (0)								
19	0 (0)	0 (0)								

Table 11*:* Score distributions for TOTAL, CELL-M, DEATH, MUCOUS, TEAR, and GRAN in *A*-IVDs.

Ex vivo inflammatory cytokine/chemokine release by *S*-IVDs versus *A*-IVDs within TOTAL HDS

Biomarker	2-6	*p*	7	*p*	8	*p*	9	*p*	10	*p*	11	*p*	12	*p*	13	*p*
GRO-α			*S*-NP	0.039	*S*-NP	0.039										
IL-1RA			*S*-AF	0.001					*S*-AF	0.038					*S*-AF	0.033
IL-6																
IL-8					*S*-NP	0.010										
MCP-1									*S*-NP	0.016	*S*-NP	0.013	*S*-AF	0.019		
MCP-3	*S*-NP	0.013														
MIP-1α					*S*-NP	0.027	*S*-NP	0.039	*S*-AF	0.037						
MIP-1β					*S*-NP	0.010	*S*-NP	0.042								
RANTES	*S*-AF	0.048	*S*-AF	0.003	*S*	≤0.03	*S*	≤0.001	*S*	≤0.001	*S*	≤0.004	*S*	≤0.029	*S*	≤0.010
TNF-α									*S*-NP		*S*-AF	0.01	*S*-AF	0.044		

Table 4: Significant (p<0.05) ANOVA results for inflammatory cytokine/chemokine release by *S*-IVDs versus *A*-IVDs within total HDS 2-6, 7, 8, 9, 10, 11, 12, and 13 while accounting for age, sex, obesity, and their interactions. Within each score column, groups with significantly (p<0.05) increased biomarker release are indicated. "*S"* or *"A"* alone indicate that a significant in both AF and NP tissues. "*S*-AF/NP" indicates a significant increase within AF or NP tissues only.

Ex vivo MMP release by *S*-IVDs versus *A*-IVDs within TOTAL HDS

Biomarker	2-6	*p*	7	*p*	8	*p*	9	*p*	10	*p*	11	*p*	12	*p*	13	*p*
MMP-1	*S*-NP	0.027					*S*-NP	0.036	*S*-AF	0.044	*S*-NP	0.013				
MMP-2							*S*	≤0.029	*S*	≤0.018	*S*-AF	0.031	*S*-AF	0.019	*S*-AF	0.018
MMP-3					*A*-NP	0.032	*A*-AF	0.007	*A*-AF	0.027						
MMP-7			*A*-AF	0.049			*S*-NP	0.004			*A*	≤0.048				
MMP-8	*A*	≤0.030	*A*	≤0.001	*A*-NP	0.001	*A*	≤0.001	*A*	≤0.001	*S*	≤0.003	*A*-AF	0.003	*A*	≤0.014
MMP-9											*S*-AF	0.011				
MMP-13			*S*-NP	0.015			*S*-AF	0.001	*S*-AF	0.001					*S*-AF	0.001

Table 5: ANOVA results for MMP release by *S*-IVDs versus *A*-IVDs within total HDS 2-6, 7, 8, 9, 10, 11, 12, and 13 while accounting for age, sex, obesity, and their interactions. Within each score column, groups with significantly (p<0.05) increased biomarker release are indicated. "*S"* or *"A"* alone indicate that a significant in both AF and NP tissues. "*S*-AF/NP" indicates a significant increase within AF or NP tissues only.

Ex vivo TIMP and growth factor release by S-IVDs versus A-IVDs within TOTAL HDS

Biomarker	2-6	*p*	7	*p*	8	*p*	9	*p*	10	*p*	11	*p*	12	*p*	13	*p*
TIMP-1					*A*-NP	0.002									*A-NP*	0.032
TIMP-2	*A*-AF	0.050	*A*-AF	0.003	*A*	≤0.046	*A*	0.001	*A*-NP	0.001	*A*	≤0.007			*A*	≤0.045
TIMP-3			*A*-AF	0.008	*A*	≤0.010	*A*	0.016	*A*-NP	0.011						
TIMP-4	*A*	≤0.029			*A*	≤0.017	*A*	0.001	*A*	≤0.023	*S*	≤0.0.001	*A*-NP	0.008	*S*-AF	0.002
PDGF-AA							*S*-AF	0.035	*S*-AF	0.039	*S*	≤0.026			*S*-NP	0.035
VEGF			*S*-AF	0.035					*S*-NP	0.045	*S*-NP	0.003			*S*-AF	0.014
FGF2									*S*-NP	0.034						

Table 6: ANOVA results for TIMP and growth factor release by *S*-IVDs versus *A*-IVDs within total HDS 2-6, 7, 8, 9, 10, 11, 12, and 13 while accounting for age, sex, obesity, and their interactions. Within each score column, groups with significantly (p<0.05) increased biomarker release are indicated. "*S"* or *"A"* alone indicate that a significant in both AF and NP tissues. "*S*-AF/NP" indicates a significant increase within AF or NP tissues only.

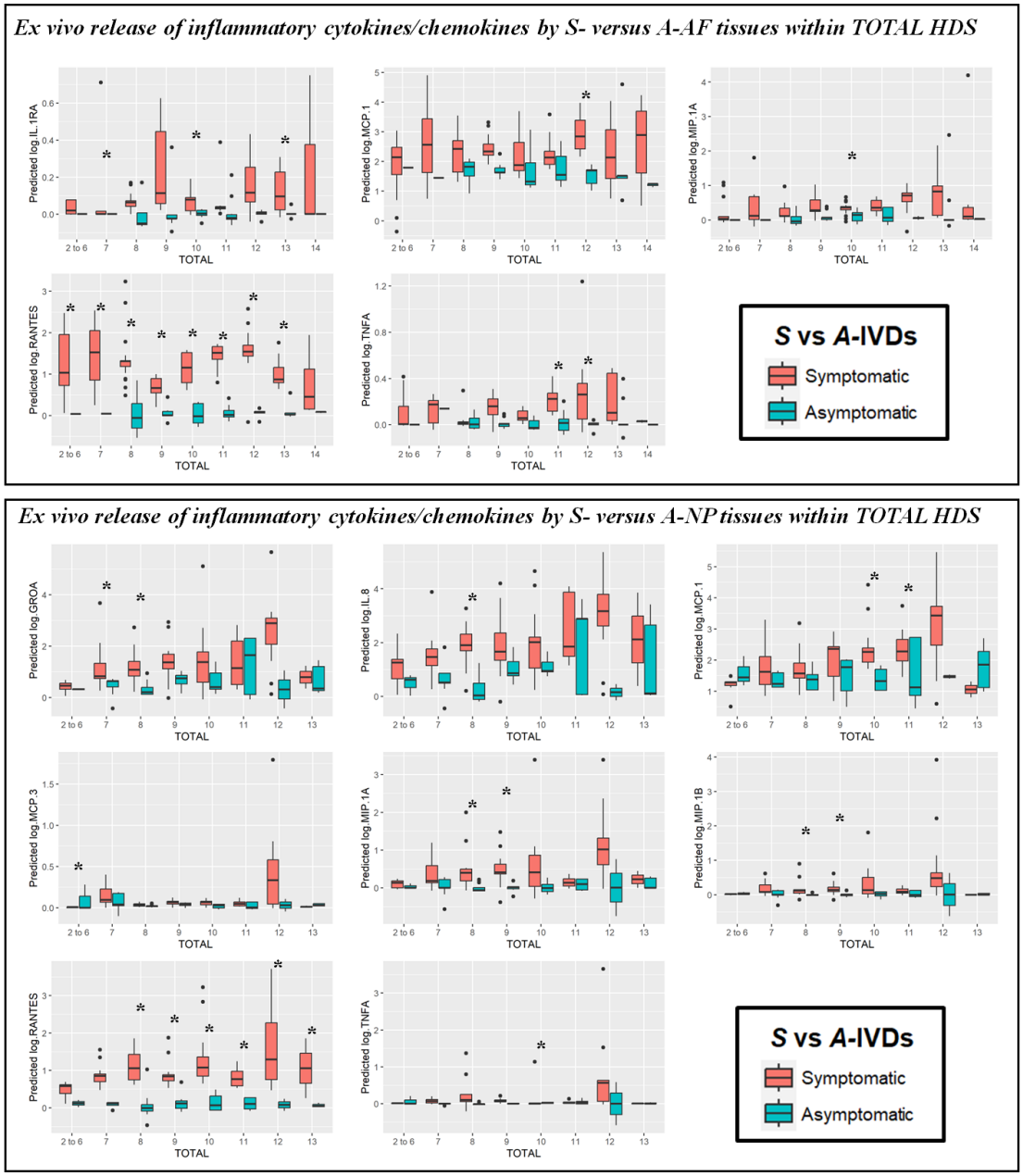
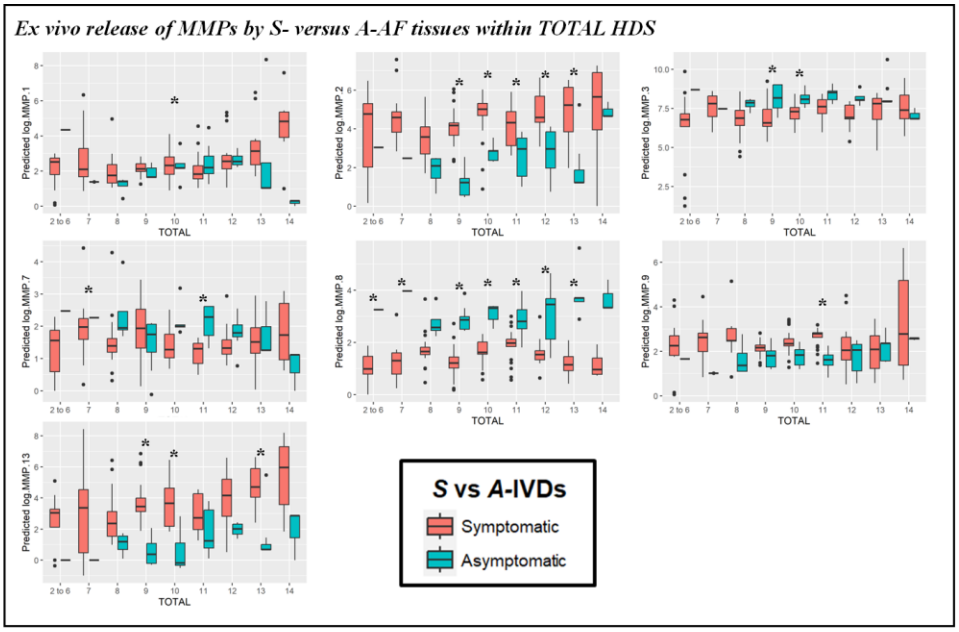


Figure 4: Significant (* = p<0.05) differences in ex vivo inflammatory cytokine/chemokine release by *S*- versus *A*-AF or NP tissues within TOTAL HDS while accounting for age, sex, and obesity status



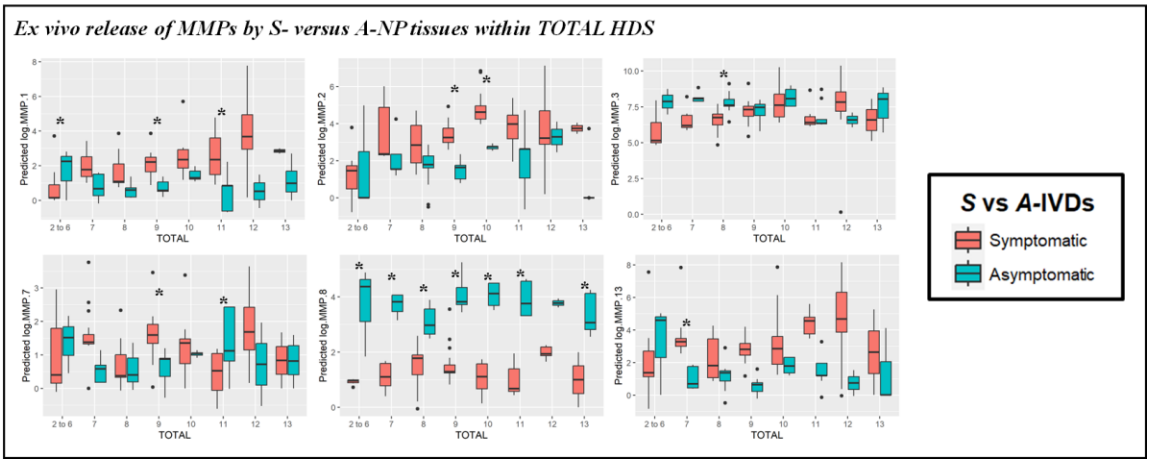


Figure 5: Significant (* = $p<0.05$) differences in ex vivo MMP release by *S*- versus *A*-AF or NP tissues within TOTAL HDS while accounting for age, sex, and obesity status

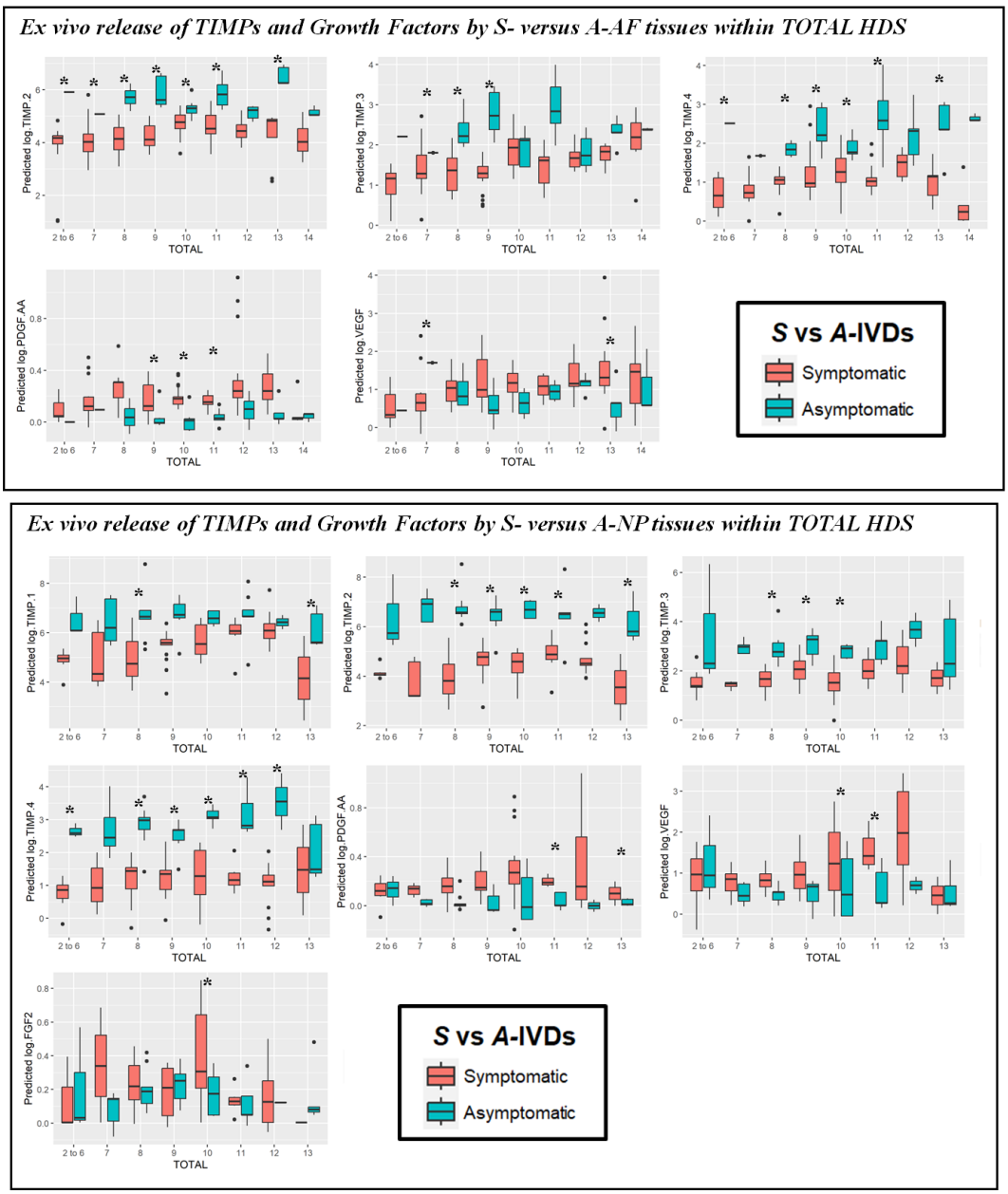


Figure 6: Significant (* = $p<0.05$) differences in ex vivo TIMP and growth factor release by *S*- versus *A*-AF or NP tissues within TOTAL HDS while accounting for age, sex, and obesity status.

Ex vivo protein release by *S*-IVDs versus *A*-IVDs within CELL-M

Biomarker	*WITHIN CELL-M SCORES*							
	3		4		5		6	
	Group	*p-value*	*Group*	*p-value*	*Group*	*p-value*	*Group*	*p-value*
GRO-α	*S*-NP	0.020						
IL-1RA	*S*	≤0.019	*S*	≤0.028	*S*-NP	0.040		
IL-6	*S*-NP	0.034						
IL-8	*S*-NP	0.012			*S*-NP	0.040	*S*-AF	0.046
MCP-1	*S*-AF	0.046	*S*-AF	0.006	*S*-AF	0.019		
MCP-3								
MIP-1α	*S*-NP	0.001	*S*-NP	0.032				
MIP-1β	*S*-NP	0.007	*S*-NP	0.036				
RANTES	*S*	≤0.001	*S*	≤0.001	*S*	≤0.009		
TNF-α	*S*	≤0.026						
MMP-1	*S*-NP	0.008	*S*-NP	0.007	*S*-NP	0.046	*S*-AF	0.037
MMP-2	*S*	≤0.001	*S*	≤0.001	*S*-AF	0.003		
MMP-3	*A*	≤0.027	*A*	≤0.042				
MMP-7			*S*-NP	0.026				
MMP-8	*A*	≤0.001	*A*	≤0.001	*A*	≤0.001	*A*-AF	0.001
MMP-9	*S*-AF	0.002			*A*-AF	0.049		
MMP-13	*S*	≤0.001	*S*	0.006	*S*-AF	0.001	*S*-AF	0.031
TIMP-1	*A*	≤0.001	*A*-NP	0.001				
TIMP-2	*A*	≤0.001	*A*	≤0.001	*A*-NP	0.046		
TIMP-3	*A*	≤0.001	*A*	≤0.001	*A*-NP	0.019		
TIMP-4	*A*	≤0.001	*A*	≤0.001	*A*-NP	0.002	*A*-AF	0.001
PDGF-AA	*S*	≤0.002	*S*	0.034	*A*	≤0.044		
VEGF	*S*-AF	0.032	*S*-NP	0.005				
FGF2					*A*-AF	0.049		

Table 7: Significant ($p<0.05$) results of ANOVA comparing ex vivo protein release by *S*-IVD versus *A*-IVD tissues within CELL-M scores with adjustment for age, sex, and obesity status. The "Group" column indicates the group(s) that is significantly increased. "*S*" or "*A*" with no tissue type indicates significant increases in both AF and NP tissues.

Ex vivo protein release by *S*-IVDs versus *A*-IVDs within DEATH

Biomarker	WITHIN DEATH SCORES							
	0/1		2		3		4	
	Group	*p-value*	*Group*	*p-value*	*Group*	*p-value*	*Group*	*p-value*
GRO-α			*S*-NP	0.003				
IL-1RA			*S*	≤0.012				
IL-6			*S*-NP	0.003				
IL-8			*S*-NP	0.002				
MCP-1			*S*	≤0.035				
MCP-3			*S*-AF	0.008				
MIP-1α			*S*	≤0.009	*S*-NP	0.034		
MIP-1β			*S*	≤0.004				
RANTES	*S*-AF	0.017	*S*	≤0.001	*S*	≤0.001		
TNF-α			*S*	≤0.031				
MMP-1			*S*-NP	0.001	*A*-NP			
MMP-2	*S*-AF	0.010	*S*	≤0.001	*S*	≤0.013		
MMP-3			*A*	≤0.016	*A*-AF			
MMP-7			*S*-NP	0.040	*A*-AF			
MMP-8	*A*	≤0.037	*A*	≤0.001	*A*	≤0.001	*A*-AF	0.001
MMP-9			*S*-AF	0.047	*S*-AF			
MMP-13	*S*-AF	0.011	*S*	≤0.001			*A*-AF	0.018
TIMP-1			*A*	≤0.003				
TIMP-2	*A*-AF	0.032	*A*	≤0.001	*A*	≤0.001		
TIMP-3			*A*	≤0.001	*A*	≤0.001		
TIMP-4	*A*-AF	0.030	*A*	≤0.001	*A*	≤0.001	*A*	≤0.040
PDGF-AA			*S*	≤0.002	*S*	≤0.025		
VEGF			*S*-NP	0.034	*S*-NP			
FGF2								

Table 8: Significant ($p<0.05$) results of ANOVA comparing ex vivo protein release by *S*-IVD versus *A*-IVD tissues within DEATH scores with adjustment for age, sex, and obesity status. The "Group" column indicates the group(s) that is significantly increased. "*S*" or "*A*" with no tissue type indicates significant increases in both AF and NP tissues.

Ex vivo protein release by *S*-IVDs versus *A*-IVDs within MUCOUS

Biomarker	*WITHIN MUCOUS SCORES*					
	0/1		2		3	
	Group	*p-value*	*Group*	*p-value*	*Group*	*p-value*
GRO-α						
IL-1RA					*S*-AF	0.001
IL-6						
IL-8						
MCP-1			*S*-AF	0.04	*S*-AF	0.001
MCP-3						
MIP-1α						
MIP-1β						
RANTES	*S*-AF	0.002	*S*-AF	0.001	*S*-AF	0.001
TNF-α					*S*-AF	0.003
MMP-1						
MMP-2	*S*-AF	0.009	*S*-AF	0.001	*S*-AF	0.001
MMP-3			*A*-AF	0.004	*A*-AF	0.001
MMP-7						
MMP-8	*A*-AF	0.009	*A*-AF	0.001	*A*-AF	0.001
MMP-9			*S*-AF	0.041	*S*-AF	0.035
MMP-13	*S*-AF	0.031	*S*-AF	0.001	*S*-AF	0.001
TIMP-1			*A*-AF	0.010		
TIMP-2	*A*-AF	0.002	*A*-AF	0.001	*A*-AF	0.001
TIMP-3	*A*-AF	0.016	*A*-AF	0.001	*A*-AF	0.001
TIMP-4	*A*-AF	0.004	*A*-AF	0.001	*A*-AF	0.001
PDGF-AA			*S*-AF	0.002	*S*-AF	0.001
VEGF					*S*-AF	0.023
FGF2						

Table 9: Significant ($p<0.05$) results of ANOVA comparing ex vivo protein release by *S*-IVD versus *A*-IVD tissues within MUCOUS scores with adjustment for age, sex, and obesity status. The "Group" column indicates the group(s) that is significantly increased. "*S*" or "*A*" with no tissue type indicates significant increases in both AF and NP tissues.

Ex vivo protein release by *S*-IVDs versus *A*-IVDs within TEAR

Biomarker	*WITHIN TEAR SCORES*					
	0/1		2		3	
	Group	*p-value*	*Group*	*p-value*	*Group*	*p-value*
GRO-α			*S*-NP	0.01	*S*-NP	0.014
IL-1RA	*S*-NP	0.046	*S*	≤0.026	*S*	≤0.014
IL-6			*S*-NP	0.015	*S*-NP	0.034
IL-8			*S*-NP	0.007	*S*-NP	0.018
MCP-1			*S*	≤0.014	*S*	≤0.023
MCP-3						
MIP-1α			*S*	≤0.029	*S*-NP	0.01
MIP-1β			*S*-NP	0.014	*S*-NP	0.037
RANTES	*S*-AF	0.002	*S*	≤0.001	*S*	≤0.001
TNF-α			*S*	≤0.031		
MMP-1					*S*-NP	0.001
MMP-2	*S*-AF	0.012	*S*	≤0.001	*S*-NP	0.002
MMP-3			*A*-AF	0.001		
MMP-7			*S*-NP	0.001	*S*-NP	0.044
MMP-8	*A*	≤0.001	*A*	≤0.001	*A*	≤0.001
MMP-9			*S*-AF	0.049		
MMP-13	*S*-AF	0.022	*S*	≤0.001	*S*	≤0.014
TIMP-1			*A*	≤0.016	*A*-NP	0.008
TIMP-2	*A*	≤0.003	*A*	≤0.001	*A*	≤0.001
TIMP-3	*A*	≤0.038	*A*	≤0.001	*A*	≤0.014
TIMP-4	*A*	≤0.006	*A*	≤0.001	*A*	≤0.001
PDGF-AA			*S*	≤0.004	*S*	≤0.004
VEGF			*S*	≤0.049	*S*	≤0.021
FGF2						

Table 10: Significant ($p<0.05$) results of ANOVA comparing ex vivo protein release by *S*-IVD versus *A*-IVD tissues within TEAR scores with adjustment for age, sex, and obesity status. The "Group" column indicates the group(s) that is significantly increased. "*S*" or "*A*" with no tissue type indicates significant increases in both AF and NP tissues.

Ex vivo protein release by *S*-IVDs versus *A*-IVDs within GRAN

Biomarker	*WITHIN GRAN SCORES*					
	0		1		2/3	
	Group	*p-value*	*Group*	*p-value*	*Group*	*p-value*
GRO-α	*S*-NP	0.010	*S*-NP	0.009		
IL-1RA			*S*-NP	0.021		
IL-6	*S*-NP	0.009	*S*-NP	0.021		
IL-8	*S*-NP	0.003	*S*-NP	0.007		
MCP-1	*S*-NP	0.019	*S*-NP	0.040		
MCP-3						
MIP-1α	*S*-NP	0.013	*S*-NP	0.012	*S*-NP	0.014
MIP-1β			*S*-NP	0.018	*S*-NP	0.024
RANTES	*S*-NP	0.001	*S*-NP	0.001	*S*-NP	0.001
TNF-α						
MMP-1	*S*-NP	0.014	*S*-NP	0.003	*S*-NP	0.001
MMP-2			*S*-NP	0.001	*S*-NP	0.001
MMP-3	*A*-NP	0.030				
MMP-7	*S*-NP	0.044				
MMP-8	*A*-NP	0.001	*A*-NP	0.001	*A*-NP	0.001
MMP-9						
MMP-13	*S*-NP	0.007	*S*-NP	0.036	*S*-NP	0.001
TIMP-1	*A*-NP	0.018	*A*-NP	0.001	*A*-NP	0.004
TIMP-2	*A*-NP	0.001	*A*-NP	0.001	*A*-NP	0.001
TIMP-3	*A*-NP	0.003	*A*-NP	0.001	*A*-NP	0.001
TIMP-4	*A*-NP	0.001	*A*-NP	0.001	*A*-NP	0.001
PDGF-AA	*S*-NP	0.038	*S*-NP	0.024	*S*-NP	0.002
VEGF	*S*-NP	0.023			*S*-NP	0.018
FGF2						

Table 11: Significant ($p<0.05$) results of ANOVA comparing ex vivo protein release by *S*-IVD versus *A*-IVD tissues within GRAN scores with adjustment for age, sex, and obesity status. The "Group" column indicates the group(s) that is significantly increased. "*S*" or "*A*" with no tissue type indicates significant increases in both AF and NP tissues.

Ex vivo inflammatory cytokine/chemokine release by *S*-IVDs or *A*-IVDs between TOTAL HDS

Biomarker	Increased ex vivo Protein Release between TOTAL HDS Scores							
	S-AF	***p***	***A-AF***	***p***	***S-NP***	***p***	***A-NP***	***p***
GRO-α							11 > 8	0.049
IL-1RA					12 > 8-9	0.021	2-6, 12 > 7-11	0.003
IL-6							11 > 7-10	0.014
IL-8								
MCP-1					12 > 2-6	0.0161		
MCP-3					12 > 8-10	0.026		
MIP-1β	15-18 > 2-11	0.004						
RANTES	11 > 9	0.016			12 > 2-6	0.0344	9 > 8	0.038
TNF-α	15-18 > 2-10	0.029						
MMP-1	15-18 > 2-11	0.001						
MMP-2					10 > 2-6	0.017		
MMP-9							11 > 8	0.011
PDGF-AA							2-6 > 7-13	0.005
VEGF			7 > 9-13	0.001				

Table 12: Significant ($p<0.05$) results of ANOVAs within tissue groups comparing ex vivo protein release by *S*-IVD or *A*-IVD tissues between TOTAL HDS with adjustment for age, sex, and obesity status. Significant increases in ex vivo protein release between TOTAL HDS within each tissue group are listed.

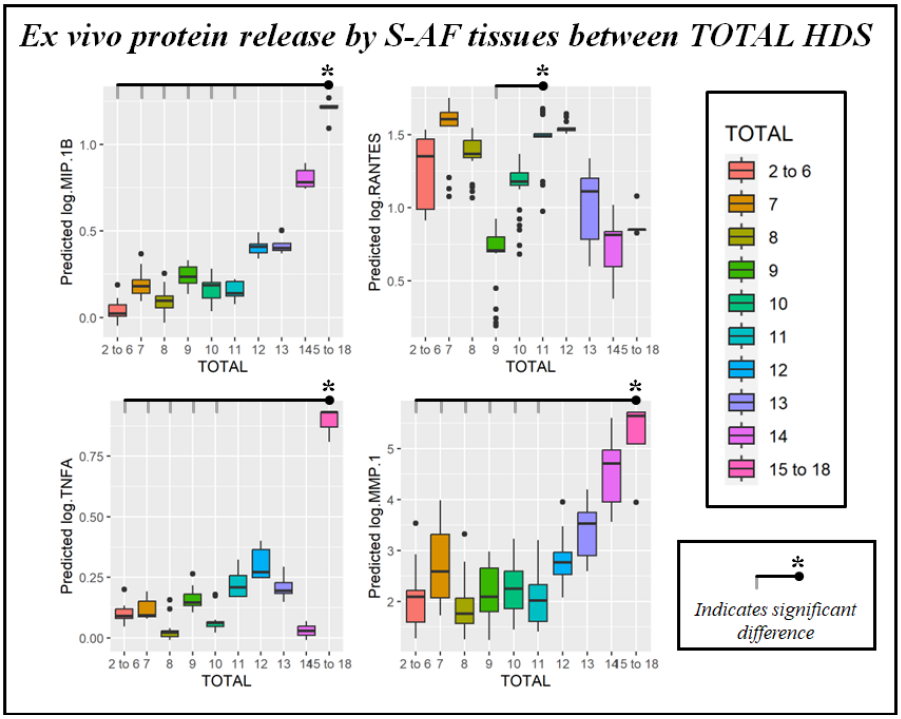


Figure 7: Significant ($p<0.05$) differences in inflammatory cytokine and MMP-1 release by *S*-AF tissues between TOTAL HDS while accounting for age, sex, and obesity status.

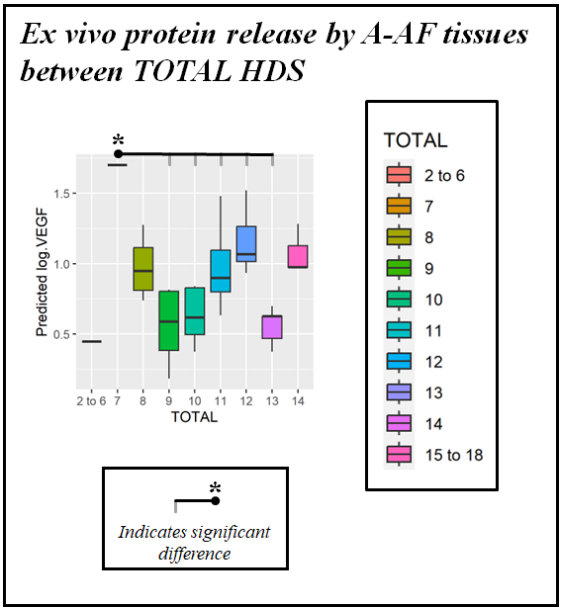


Figure 8: Significant ($p<0.05$) differences in VEGF release by *A*-AF tissues between TOTAL HDS while accounting for age, sex, and obesity status.

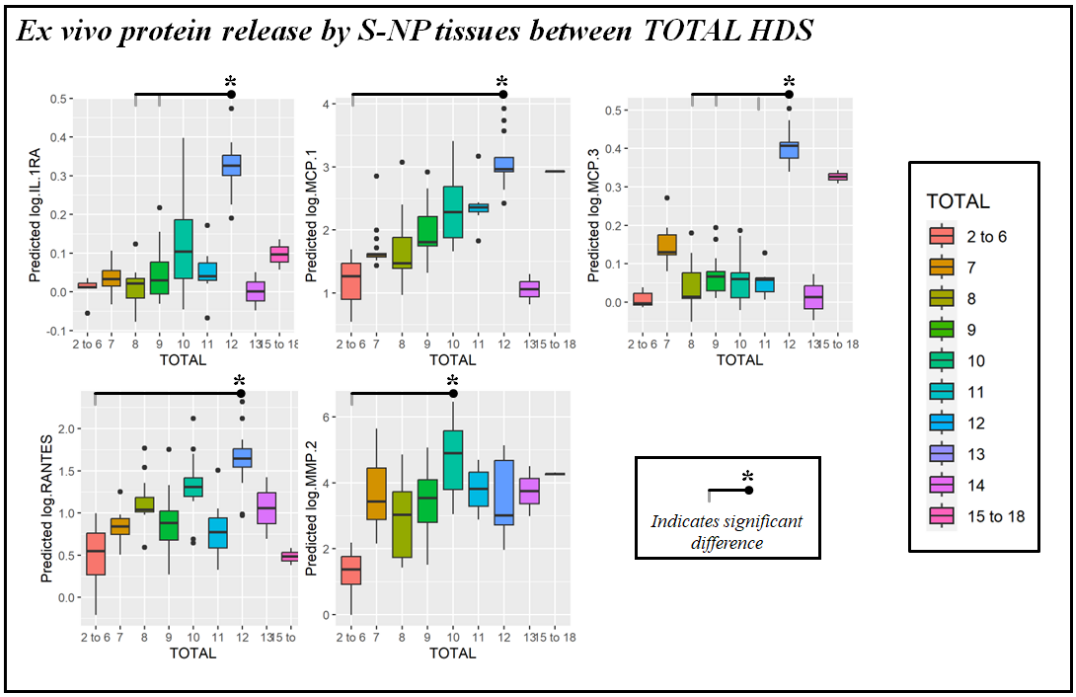


Figure 9: Significant (p<0.05) differences in inflammatory cytokine/chemokine and MMP-2 release by *S*-NP tissues between TOTAL HDS while accounting for age, sex, and obesity status.

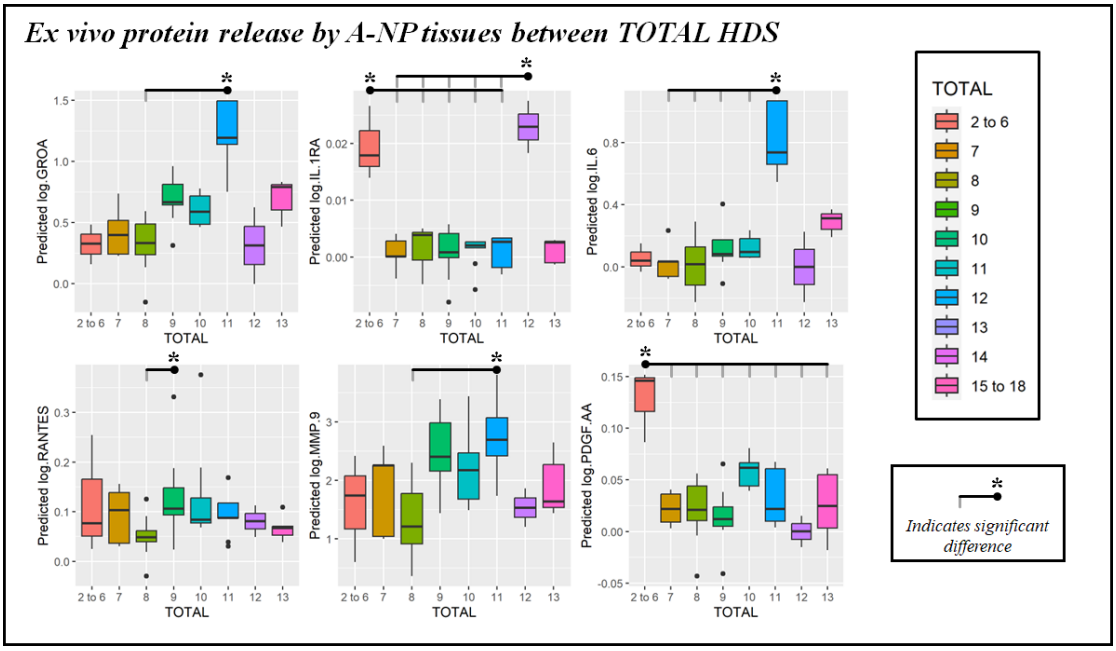


Figure 7: Significant (p<0.05) differences in inflammatory cytokine/chemokine, MMP-9, and PDGF-AA release by *A*-NP tissues between TOTAL HDS while accounting for age, sex, and obesity status.

Ex vivo protein release by *S*-IVDs or *A*-IVDs between CELL-M scores

Biomarker	CELL-M (0-6)							
	S-AF	*p*	*A-AF*	*p*	*S-NP*	*p*	*A-NP*	*p*
GRO-α	6 > 0-5	0.001			6 > 2-4	0.012		
IL-1RA					6 > 2-5	0.005		
IL-6	6 > 0-5	0.001						
IL-8	6 > 0-5	0.001			6 > 2-4	0.01		
MCP-1					6 > 2-5	0.001		
MCP-3					6 > 3-5	0.004		
MIP-1α	6 > 1-4	0.001						
MIP-1β	6 > 0-5	0.001			6 > 2-5	0.003		
RANTES								
TNF-α	6 > 3	0.028			6 > 3-4	0.012		
MMP-1	6 > 0-5	0.001			6 > 2-4	0.001		
MMP-2	6 > 2	0.008					5 > 3-4	0.02
MMP-3								
MMP-7								
MMP-8								
MMP-9			5 > 3	0.043	6 > 3	0.043	5 > 3-4	0.041
MMP-13	6 > 0-3	0.001			6 > 2-4	0.001	5 > 3-4	0.020
TIMP-1								
TIMP-2								
TIMP-3								
TIMP-4	5 > 0-3 > 6	0.001			4-5 > 2	0.005		
PDGF-AA							5 > 3-4	0.006
VEGF			6 > 3, 5	0.021				
FGF2								

Table 15: Significant ($p<0.05$) results of ANOVA and Tukey post-hoc tests comparing ex vivo protein release by *S*-IVD or *A*-IVD tissues between CELL-M scores with adjustment for age, sex, and obesity status.

Ex vivo protein release by *S*-IVDs or *A*-IVDs between DEATH scores

Biomarker	DEATH (0-4)							
	S-AF	*p*	*A-AF*	*p*	*S-NP*	*p*	*A-NP*	*p*
GRO-α	4 > 0-3	0.001			4 > 2-3	0.018		
IL-1RA					4 > 0-3	0.001	0/1 > 2-3	0.006
IL-6	4 > 0-3	0.001						
IL-8	4 > 0-3	0.001			4 > 2-3	0.042		
MCP-1	4 > 2-3	0.022	3 > 2	0.017	4 > 2-3	0.001		
MCP-3					4 > 0-3	0.001		
MIP-1α	4 > 0-3	0.001			4 > 0-2	0.014		
MIP-1β	4 > 0-3	0.001			4 > 0-3	0.001		
RANTES								
TNF-α					4 > 2/3	0.003		
MMP-1	4 > 0-3	0.001			4 > 2/3	0.031		
MMP-2	4 > 2-3	0.003						
MMP-3								
MMP-7	0/1 > 3	0.005						
MMP-8	3 > 4	0.007						
MMP-9								
MMP-13	4 > 2-3	0.001			0/1, 4 > 2-3	0.002		
TIMP-1								
TIMP-2								
TIMP-3								
TIMP-4	0-3 > 4	0.015						
PDGF-AA							0/1 > 2-3	0.001
VEGF			4 > 2-3	0.004	4 > 2	0.018	0/1 > 2	0.021
FGF2								

Table 16: Significant ($p<0.05$) results of ANOVA and Tukey post-hoc tests comparing ex vivo protein release by *S*-IVD or *A*-IVD tissues between DEATH scores with adjustment for age, sex, and obesity status.

Ex vivo protein release by *S*-IVDs or *A*-IVDs between MUCOUS scores

Biomarker	MUCOUS (0-3)			
	S-AF	*p*	*A-AF*	*p*
GRO-α				
IL-1RA				
IL-6				
IL-8				
MCP-1				
MCP-3				
MIP-1α				
MIP-1β				
RANTES				
TNF-α				
MMP-1				
MMP-2				
MMP-3	2-4 > 0/1	0.015		
MMP-7				
MMP-8				
MMP-9				
MMP-13			3 > 2	0.004
TIMP-1	3 > 2 > 0/1	0.001		
TIMP-2	2-4 > 0/1	0.001		
TIMP-3				
TIMP-4	3 > 0/1	0.001		
PDGF-AA				
VEGF				
FGF2				

Table 17: Significant ($p<0.05$) results of ANOVA and Tukey post-hoc tests comparing ex vivo protein release by *S*-IVD or *A*-IVD tissues between MUCOUS scores with adjustment for age, sex, and obesity status.

Ex vivo protein release by *S*-IVDs or *A*-IVDs between TEAR scores

Biomarker	TEAR (0-3)							
	S-AF	*p*	*A-AF*	*p*	*S-NP*	*p*	*A-NP*	*p*
GRO-α								
IL-1RA								
IL-6								
IL-8								
MCP-1								
MCP-3							0/1 > 2-3	0.001
MIP-1α								
MIP-1β								
RANTES								
TNF-α								
MMP-1								
MMP-2					2-3 > 0/1	0.001		
MMP-3								
MMP-7								
MMP-8								
MMP-9								
MMP-13								
TIMP-1								
TIMP-2								
TIMP-3								
TIMP-4	2-3 > 0/1	0.012						
PDGF-AA								
VEGF	3 > 0/1	0.024						
FGF2								

Table 18: Significant ($p<0.05$) results of ANOVA and Tukey post-hoc tests comparing ex vivo protein release by *S*-IVD or *A*-IVD tissues between TEAR scores with adjustment for age, sex, and obesity status.

Ex vivo protein release by *S*-IVDs or *A*-IVDs between GRAN scores

Biomarker	GRAN (0-3)			
	S-NP	*p*	*A-NP*	*p*
GRO-α			2/3 > 0-1	0.009
IL-1RA				
IL-6			2/3 > 0	0.018
IL-8				
MCP-1			2/3 > 0-1	0.001
MCP-3				
MIP-1α				
MIP-1β				
RANTES				
TNF-α				
MMP-1				
MMP-2			0 > 2/3	0.046
MMP-3				
MMP-7			2/3 > 0	0.025
MMP-8				
MMP-9				
MMP-13				
TIMP-1				
TIMP-2				
TIMP-3				
TIMP-4				
PDGF-AA			0 > 1-3	0.002
VEGF				
FGF2				

Table 18: Significant ($p<0.05$) results of ANOVA and Tukey post-hoc tests comparing ex vivo protein release by *S*-IVD or *A*-IVD tissues between GRAN scores with adjustment for age, sex, and obesity status.

REFERENCES

1. Wu A, March L, Zheng X, et al. Global low back pain prevalence and years lived with disability from 1990 to 2017: estimates from the Global Burden of Disease Study 2017. *Ann Transl Med*. 2020;8(6):299. doi:10.21037/atm.2020.02.175

2. Casiano VE, Sarwan G, Dydyk AM, Varacallo M. Back Pain. In: *StatPearls*. StatPearls Publishing; 2023. Accessed May 8, 2023. http://www.ncbi.nlm.nih.gov/books/NBK538173/

3. Bagley C, MacAllister M, Dosselman L, Moreno J, Aoun SG, El Ahmadieh TY. Current concepts and recent advances in understanding and managing lumbar spine stenosis. *F1000Research*. 2019;8:F1000 Faculty Rev-137. doi:10.12688/f1000research.16082.1

4. Takatalo J, Karppinen J, Niinimäki J, et al. Does Lumbar Disc Degeneration on Magnetic Resonance Imaging Associate With Low Back Symptom Severity in Young Finnish Adults? *Spine*. 2011;36(25):2180. doi:10.1097/BRS.0b013e3182077122

5. Cheung KMC, Karppinen J, Chan D, et al. Prevalence and Pattern of Lumbar Magnetic Resonance Imaging Changes in a Population Study of One Thousand Forty-Three Individuals. *Spine*. 2009;34(9):934. doi:10.1097/BRS.0b013e3181a01b3f

6. Dydyk AM, Khan MZ, Singh P. Radicular Back Pain. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK546593/

7. Waxenbaum JA, Reddy V, Futterman B. Anatomy, Back, Intervertebral Discs. In: *StatPearls*. StatPearls Publishing; 2022. Accessed April 11, 2022. http://www.ncbi.nlm.nih.gov/books/NBK470583/

8. Inoue N, Espinoza Orías AA. Biomechanics of Intervertebral Disc Degeneration. *Orthop Clin North Am*. 2011;42(4):487-499. doi:10.1016/j.ocl.2011.07.001

9. Al Qaraghli MI, De Jesus O. Lumbar Disc Herniation. In: *StatPearls*. StatPearls Publishing; 2022. Accessed August 30, 2022. http://www.ncbi.nlm.nih.gov/books/NBK560878/

10. Bydon M, Alvi MA, Goyal A. Degenerative Lumbar Spondylolisthesis: Definition, Natural History, Conservative Management, and Surgical Treatment. *Neurosurg Clin N Am*. 2019;30(3):299-304. doi:10.1016/j.nec.2019.02.003

11. Pfirrmann CW, Metzdorf A, Zanetti M, Hodler J, Boos N. Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine*. 2001;26(17):1873-1878. doi:10.1097/00007632-200109010-00011

12. Tamagawa S, Sakai D, Nojiri H, Sato M, Ishijima M, Watanabe M. Imaging Evaluation of Intervertebral Disc Degeneration and Painful Discs—Advances and Challenges in Quantitative MRI. *Diagnostics*. 2022;12(3):707. doi:10.3390/diagnostics12030707

13. Brinjikji W, Luetmer PH, Comstock B, et al. Systematic Literature Review of Imaging Features of Spinal Degeneration in Asymptomatic Populations. *AJNR Am J Neuroradiol*. 2015;36(4):811-816. doi:10.3174/ajnr.A4173

14. Boos N, Weissbach S, Rohrbach H, Weiler C, Spratt KF, Nerlich AG. Classification of age-related changes in lumbar intervertebral discs: 2002 Volvo Award in basic science. *Spine*. 2002;27(23):2631-2644. doi:10.1097/00007632-200212010-00002

15. Weiler C, Lopez-Ramos M, Mayer HM, et al. Histological analysis of surgical lumbar intervertebral disc tissue provides evidence for an association between disc degeneration and increased body mass index. *BMC Res Notes*. 2011;4:497. doi:10.1186/1756-0500-4-497

16. Le Maitre CL, Dahia CL, Giers M, et al. Development of a standardized histopathology scoring system for human intervertebral disc degeneration: an Orthopaedic Research Society Spine Section Initiative. *JOR Spine*. 2021;4(2):e1167. doi:10.1002/jsp2.1167

17. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol*. 2014;10(1):44-56. doi:10.1038/nrrheum.2013.160

18. Kozaci LD, Guner A, Oktay G, Guner G. Alterations in biochemical components of extracellular matrix in intervertebral disc herniation: role of MMP-2 and TIMP-2 in type II collagen loss. *Cell Biochem Funct*. 2006;24(5):431-436. doi:10.1002/cbf.1250

19. Ali R, Maitre CL, Richardson S, Hoyland PJ, Freemont A. Connective tissue growth factor expression in human intervertebral disc: implications for angiogenesis in intervertebral disc degeneration. *Biotech Histochem*. 2008;83(5):239-245. doi:10.1080/10520290802539186

20. Masuda K, An HS. Prevention of disc degeneration with growth factors. *Eur Spine J*. 2006;15(Suppl 3):422-432. doi:10.1007/s00586-006-0149-1

21. Thompson JP, Pearce RH, Schechter MT, Adams ME, Tsang IK, Bishop PB. Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. *Spine*. 1990;15(5):411-415. doi:10.1097/00007632-199005000-00012

22. Lee NN, Kramer JS, Stoker AM, et al. Canine models of spine disorders. *JOR Spine*. 2020;3(4):e1109. doi:10.1002/jsp2.1109

23. Roberts S, Evans H, Trivedi J, Menage J. Histology and Pathology of the Human Intervertebral Disc. *JBJS*. 2006;88(suppl_2):10-14. doi:10.2106/JBJS.F.00019

24. Histology and Pathology of the Human Intervertebral Disc : JBJS. Accessed September 21, 2022. https://journals.lww.com/jbjsjournal/Abstract/2006/04002/Histology_and_Pathology_of_the_Human.3.aspx

25. Le Maitre C, Freemont A, Hoyland J. Human disc degeneration is associated with increased MMP 7 expression. *Biotech Histochem*. 2006;81(4-6):125-131. doi:10.1080/10520290601005298

26. Fernando HN, Czamanski J, Yuan TY, Gu WY, Abdi S, Huang CYC. Mechanical Loading Affects the Energy Metabolism of Intervertebral Disc Cells. *J Orthop Res Off Publ Orthop Res Soc*. 2011;29(11):1634-1641. doi:10.1002/jor.21430

27. Grunhagen T, Wilde G, Soukane DM, Shirazi-Adl SA, Urban JPG. Nutrient Supply and Intervertebral Disc Metabolism. *JBJS*. 2006;88(suppl_2):30. doi:10.2106/JBJS.E.01290

28. Iatridis JC, MacLean JJ, Roughley PJ, Alini M. Effects of Mechanical Loading on Intervertebral Disc Metabolism In Vivo. *J Bone Joint Surg Am*. 2006;88(0 2):41-46. doi:10.2106/JBJS.E.01407

29. Molinos M, Almeida CR, Caldeira J, Cunha C, Gonçalves RM, Barbosa MA. Inflammation in intervertebral disc degeneration and regeneration. *J R Soc Interface*. 2015;12(104). doi:10.1098/rsif.2014.1191

30. Lu P, Takai K, Weaver VM, Werb Z. Extracellular Matrix Degradation and Remodeling in Development and Disease. *Cold Spring Harb Perspect Biol*. 2011;3(12). doi:10.1101/cshperspect.a005058

31. Taylor JR. Growth of human intervertebral discs and vertebral bodies. *J Anat*. 1975;120(Pt 1):49-68.

32. Maitre CLL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J Pathol*. 2004;204(1):47-54. doi:10.1002/path.1608

33. Sun Z, Zhang M, Zhao XH, et al. Immune cascades in human intervertebral disc: the pros and cons. *Int J Clin Exp Pathol*. 2013;6(6):1009-1014.

34. ZHANG F, ZHAO X, SHEN H, ZHANG C. Molecular mechanisms of cell death in intervertebral disc degeneration (Review). *Int J Mol Med*. 2016;37(6):1439-1448. doi:10.3892/ijmm.2016.2573

35. Shamji MF, Setton LA, Jarvis W, et al. Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum*. 2010;62(7):1974-1982. doi:10.1002/art.27444

36. Weiler C, Nerlich AG, Bachmeier BE, Boos N. Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. *Spine*. 2005;30(1):44-53; discussion 54. doi:10.1097/01.brs.0000149186.63457.20

37. Kepler CK, Markova DZ, Dibra F, et al. Expression and Relationship of Proinflammatory Chemokine RANTES/CCL5 and Cytokine IL-1β in Painful Human Intervertebral Discs. *Spine*. 2013;38(11):873. doi:10.1097/BRS.0b013e318285ae08

38. Ahn SH, Cho YW, Ahn MW, Jang SH, Sohn YK, Kim HS. mRNA Expression of Cytokines and Chemokines in Herniated Lumbar Intervertebral Discs. *Spine*. 2002;27(9):911-917.

39. Kawaguchi S, Yamashita T, Katahira G ichiro, Yokozawa H, Torigoe T, Sato N. Chemokine profile of herniated intervertebral discs infiltrated with monocytes and macrophages. *Spine*. 2002;27(14):1511-1516. doi:10.1097/00007632-200207150-00006

40. Wuertz K, Haglund L. Inflammatory Mediators in Intervertebral Disk Degeneration and Discogenic Pain. *Glob Spine J*. 2013;3(3):175-184. doi:10.1055/s-0033-1347299

41. Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 SSE Award Competition in Basic Science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. *Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Sect Cerv Spine Res Soc*. 2002;11(4):308-320. doi:10.1007/s00586-002-0472-0

42. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem*. 2003;253(1-2):269-285. doi:10.1023/a:1026028303196

43. Nagase H. Activation mechanisms of matrix metalloproteinases. *Biol Chem*. 1997;378(3-4):151-160.

44. Visse R, Nagase H. Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Circ Res*. 2003;92(8):827-839. doi:10.1161/01.RES.0000070112.80711.3D

45. Vo NV, Hartman RA, Yurube T, Jacobs LJ, Sowa GA, Kang JD. Expression and regulation of metalloproteinases and their inhibitors in intervertebral disc aging and degeneration. *Spine J Off J North Am Spine Soc*. 2013;13(3):331-341. doi:10.1016/j.spinee.2012.02.027

46. Rastogi A, Kim H, Twomey JD, Hsieh AH. MMP-2 mediates local degradation and remodeling of collagen by annulus fibrosus cells of the intervertebral disc. *Arthritis Res Ther*. 2013;15(2):R57. doi:10.1186/ar4224

47. Roberts S, Caterson B, Menage J, Evans EH, Jaffray DC, Eisenstein SM. Matrix Metalloproteinases And Aggrecanase: Their Role in Disorders of the Human Intervertebral Disc. *Spine*. 2000;25(23):3005.

48. Deng B, Ren JZ, Meng XQ, et al. Expression profiles of MMP-1 and TIMP-1 in lumbar intervertebral disc degeneration. *Genet Mol Res*. 2015;14(4):19080-19086. doi:10.4238/2015.December.29.16

49. Bachmeier BE, Nerlich A, Mittermaier N, et al. Matrix metalloproteinase expression levels suggest distinct enzyme roles during lumbar disc herniation and degeneration. *Eur Spine J*. 2009;18(11):1573-1586. doi:10.1007/s00586-009-1031-8

50. Wang WJ, Yu XH, Wang C, et al. MMPs and ADAMTSs in intervertebral disc degeneration. *Clin Chim Acta*. 2015;448:238-246. doi:10.1016/j.cca.2015.06.023

51. Stone WL, Leavitt L, Varacallo M. Physiology, Growth Factor. In: *StatPearls*. StatPearls Publishing; 2023. Accessed April 27, 2023. http://www.ncbi.nlm.nih.gov/books/NBK442024/

52. Pratsinis H, Kletsas D. Growth factors in intervertebral disc homeostasis. *Connect Tissue Res*. 2008;49(3):273-276. doi:10.1080/03008200802147951

53. Masuda K, An HS. Prevention of disc degeneration with growth factors. *Eur Spine J*. 2006;15(Suppl 3):422-432. doi:10.1007/s00586-006-0149-1

54. Kennon JC, Awad ME, Chutkan N, DeVine J, Fulzele S. Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration. *Biomol Concepts*. 2018;9(1):43-52. doi:10.1515/bmc-2018-0003

55. Zhang W, Gong Y, Zheng X, et al. Platelet-Derived Growth Factor-BB Inhibits Intervertebral Disc Degeneration via Suppressing Pyroptosis and Activating the MAPK Signaling Pathway. *Front Pharmacol*. 2022;12:799130. doi:10.3389/fphar.2021.799130

56. Paglia DN, Singh H, Karukonda T, Drissi H, Moss IL. PDGF-BB Delays Degeneration of the Intervertebral Discs in a Rabbit Preclinical Model. *Spine*. 2016;41(8):E449. doi:10.1097/BRS.0000000000001336

57. Li X, An HS, Ellman M, et al. Action of fibroblast growth factor-2 on the intervertebral disc. *Arthritis Res Ther*. 2008;10(2):R48. doi:10.1186/ar2407

58. Fournier DE, Kiser PK, Shoemaker JK, Battié MC, Séguin CA. Vascularization of the human intervertebral disc: A scoping review. *JOR Spine*. 2020;3(4):e1123. doi:10.1002/jsp2.1123

59. Verheijde JL, Rady MY, McGregor JL. The United States Revised Uniform Anatomical Gift Act (2006): New challenges to balancing patient rights and physician responsibilities. *Philos Ethics Humanit Med PEHM*. 2007;2:19. doi:10.1186/1747-5341-2-19

60. Classification of Age-Related Changes in Lumbar Intervertebr... : Spine. Accessed August 24, 2022. https://journals.lww.com/spinejournal/Fulltext/2002/12010/Classification_of_Age_Related_Changes_in_Lumbar.2.aspx

61. Le Maitre CL, Hoyland JA, Freemont AJ. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1β and TNFα expression profile. *Arthritis Res Ther*. 2007;9(4):R77. doi:10.1186/ar2275

62. Phillips KLE, Chiverton N, Michael AL, et al. The cytokine and chemokine expression profile of nucleus pulposus cells: implications for degeneration and regeneration of the intervertebral disc. *Arthritis Res Ther*. 2013;15(6):R213. doi:10.1186/ar4408

63. An HS, Masuda K, Inoue N. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthop Sci*. 2006;11(5):541-552. doi:10.1007/s00776-006-1055-4

64. Fearing BV, Hernandez PA, Setton LA, Chahine NO. Mechanotransduction and cell biomechanics of the intervertebral disc. *JOR Spine*. 2018;1(3). doi:10.1002/jsp2.1026

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

The development of *S*-IVDD is multifactorial; relevant factors and their relative importance to *S*-IVDD development are difficult to determine due to the patient-to-patient variability in clinical profiles and ubiquity of *A*-IVDD.[1,2] Currently there is not a treatment that restores the tissues structure and composition of the IVD after the development of *S*-IVDD, and treatments are currently reactive in nature and limited to medications, physical therapy, injections, and surgery.[3] Understanding the fundamental differences between *S*-IVDD and *A*-IVDD patients and tissues may be an avenue towards isolating and addressing likely contributors to pain. Therefore, this dissertation research investigated the relationships between radiographic/gross IVDD, histologic IVDD, the development of pain, protein release, and patient factors including age, sex, and obesity in *S*-IVDD and *A*-IVDD populations. The overall goal for this research was to determine which combination of macroscopic, microscopic, molecular, and/or patient factors are associated with the development of *S*-IVDD such that biomarker and clinical profiles may be developed to eventually provide patient and stage-specific prognoses and treatments.

The relationships between age, histologic IVDD, radiographic IVDD, and *S*-IVDD development are likely affected by a number of factors potentially including sex, obesity, lifestyle, and genetic factors.[1,2,4,5] These factors may also interact with each other in ways that could affect the manifestation of *S*- or *A*-IVDD. Chapter II investigated the relationships between macroscopic and histological IVDD and the development of symptoms, but also which patient characteristics and interactions were associated with IVDD progression within each cohort. Those data revealed significantly increased

macroscopic IVDD in *S*- versus *A*-IVDs, and further that obesity may affect the age-related progression of macroscopic *S*-IVDD, causing more advanced IVDD progression at earlier ages. These findings agree with previous studies that identify increased risk of back pain and IVD disorders in obese individuals and could indicate obesity as an important factor in IVD health.[6] Histologically, male *A*-IVDD patients had significantly increased TOTAL HDS if they were classified as obese, though this finding could reflect the difference in histological methods used for *S*-IVDs and *A*-IVDs. Together, these data point towards obesity as a potentially important modifier of the typical age-related progression of IVDD which may increase the likelihood of *S*-IVDD development.

While it is widely considered that patient characteristics including age, sex, and BMI likely influence the development of *S*-IVDD and *A*-IVDD, they have not been sufficient to predict the development of symptoms. Many studies support that the aberrant AF and NP cell production of inflammatory cytokines/chemokines, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and growth factors are also involved in the development of *S*-IVDD.[7–10] Chapter III presented novel data and analyses comparing *S*- and *A*-IVD ex vivo protein release based on *S*- and *A*-IVDD patient characteristics. This study revealed significant increases in ex vivo inflammatory protein release by, specific increases in degradative enzyme release by each cohort, and significantly increased release of degradation inhibitors by *A*-IVDs. Additionally, obesity was associated with significantly increased cytokine release and decreased TIMP release in *A*-IVDs, whereas increasing age was associated with significantly decreased MMPs release and increased growth factor release by *S*-IVDs.

Taken together, these data could indicate that obesity modifies the molecular behavior of *S*- and *A*-IVDs, though obesity alone remains insufficient to predict the occurrence of pain.

Many biological factors other than radiographic IVDD are thought to contribute to the transition of *A*-IVDD to *S*-IVDD, though their specific roles and timings are not known.[11] Therefore, comparing biological factors between similarly radiographically degenerative *S*- and *A*-IVDs is critical in determining potential sources of *S*-IVDD development. Previous human IVD tissue and cell culture studies have primarily aimed to characterize *S*-IVD matrix metabolism and to develop models for potential *S*-IVDD therapies.[12–14] Additionally, previous studies have compared IVDs at different radiographic degenerative stages (i.e. degenerative versus non-degenerative comparisons). However, few if any studies have characterized differences in ex vivo protein production by *S*-IVDs versus *A*-IVDs with similar levels of macroscopic degeneration.[15,16] Chapter IV demonstrated a clear, fundamental difference in *ex vivo* protein release by *S*-IVDs versus *A*-IVDs that indicated significantly increased inflammation in *S*-IVDs in all T/P-grades. While increased inflammatory protein expression by *S*-IVDs has been demonstrated in previous studies, few studies include the content or release of matrix degrading enzymes and their inhibitors, which illustrated distinct release patterns by *S*-IVDs and *A*-IVDs in the present studies. The increased release of MMP-, MMP-8, and TIMPs 1-4 indicate these degradative regulators as important for the maintenance of *A*-IVDs. Conversely, the increased release of MMP-1, MMP-2, and MMP-13 by *S*-IVDs could represent targets for inhibition.

Despite the widespread use of the histological grading scheme published by Boos et al., the relationship between histological IVDD and *S*-IVDD development has not yet

been clarified in any more meaningful way than the relationship between *S*-IVDD and radiographic IVDD progression.[17,18] "Unfavorable cost effectiveness and missing therapeutic consequences" have led to a "growing informal consent in the surgical community to stop doing [histological analyses of excised surgical tissues]".[19] Weiler, Boos, and Nerlich additionally proposed that "histological examination of [excised] disc tissue allows a proper assessment of histo-degenerative changes and serves as a document for medicolegal purposes and quality control".[19] Chapter V of this dissertation revealed significant differences in *S*- versus *A*-IVD protein release that appeared to be influenced differently when comparing within each histological category. While these results may reflect the distribution of histological category scores, they could indicate that *S*- and *A*-IVD protein release profiles are each uniquely and intricately related to changes in cell morphology and matrix properties. It appears that, despite similar histological IVDD severity, *A*-IVDs are capable of diminishing or preventing IVDD related pain responses. Therefore, therapies focused on shifting *S*-IVD protein release profiles towards the *A*-IVD protein release profile described in this study may similarly mitigate pain stemming from the mechanobiological and nociceptive consequences of damaged IVD cells and tissues.

REFERENCES

1. Ganesan S, Acharya AS, Chauhan R, Acharya S. Prevalence and Risk Factors for Low Back Pain in 1,355 Young Adults: A Cross-Sectional Study. *Asian Spine J*. 2017;11(4):610-617. doi:10.4184/asj.2017.11.4.610

2. Elfering A, Semmer N, Birkhofer D, Zanetti M, Hodler J, Boos N. Risk factors for lumbar disc degeneration: a 5-year prospective MRI study in asymptomatic individuals. *Spine*. 2002;27(2):125-134. doi:10.1097/00007632-200201150-00002

3. Berry JA, Elia C, Saini HS, Miulli DE. A Review of Lumbar Radiculopathy, Diagnosis, and Treatment. *Cureus*. 11(10). doi:10.7759/cureus.5934

4. Ala-Kokko L. Genetic risk factors for lumbar disc disease. *Ann Med*. 2002;34(1):42-47. doi:10.1080/078538902317338634

5. Seidler A, Bolm-Audorff U, Siol T, et al. Occupational risk factors for symptomatic lumbar disc herniation; a case-control study. *Occup Environ Med*. 2003;60(11):821-830. doi:10.1136/oem.60.11.821

6. Sheng B, Feng C, Zhang D, Spitler H, Shi L. Associations between Obesity and Spinal Diseases: A Medical Expenditure Panel Study Analysis. *Int J Environ Res Public Health*. 2017;14(2):183. doi:10.3390/ijerph14020183

7. Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol*. 2014;10(1):44-56. doi:10.1038/nrrheum.2013.160

8. Maitre CLL, Freemont AJ, Hoyland JA. Localization of degradative enzymes and their inhibitors in the degenerate human intervertebral disc. *J Pathol*. 2004;204(1):47-54. doi:10.1002/path.1608

9. Li Y, Zhang T, Tian W, et al. Loss of TIMP3 expression induces inflammation, matrix degradation, and vascular ingrowth in nucleus pulposus: A new mechanism of intervertebral disc degeneration. *FASEB J*. 2020;34(4):5483-5498. doi:10.1096/fj.201902364RR

10. Pratsinis H, Kletsas D. Growth factors in intervertebral disc homeostasis. *Connect Tissue Res*. 2008;49(3):273-276. doi:10.1080/03008200802147951

11. An HS, Masuda K, Inoue N. Intervertebral disc degeneration: biological and biomechanical factors. *J Orthop Sci*. 2006;11(5):541-552. doi:10.1007/s00776-006-1055-4

12. Du J, Pfannkuche J, Lang G, et al. Proinflammatory intervertebral disc cell and organ culture models induced by tumor necrosis factor alpha. *JOR Spine*. 2020;3(3):e1104. doi:10.1002/jsp2.1104

13. Stern S, Lindenhayn K, Perka C. Human intervertebral disc cell culture for disc disorders. *Clin Orthop*. 2004;(419):238-244. doi:10.1097/00003086-200402000-00039

14. Le Maitre CL, Hoyland JA, Freemont AJ. Studies of Human Intervertebral Disc Cell Function in a Constrained In Vitro Tissue Culture System. *Spine*. 2004;29(11):1187.

15. Wuertz K, Haglund L. Inflammatory Mediators in Intervertebral Disk Degeneration and Discogenic Pain. *Glob Spine J*. 2013;3(3):175-184. doi:10.1055/s-0033-1347299

16. Deng B, Ren JZ, Meng XQ, et al. Expression profiles of MMP-1 and TIMP-1 in lumbar intervertebral disc degeneration. *Genet Mol Res*. 2015;14(4):19080-19086. doi:10.4238/2015.December.29.16

17. Roberts S, Evans H, Trivedi J, Menage J. Histology and Pathology of the Human Intervertebral Disc. *JBJS*. 2006;88(suppl_2):10-14. doi:10.2106/JBJS.F.00019

18. Histology and Pathology of the Human Intervertebral Disc : JBJS. Accessed September 21, 2022. https://journals.lww.com/jbjsjournal/Abstract/2006/04002/Histology_and_Pathology_of_the_Human.3.aspx

19. Weiler C, Lopez-Ramos M, Mayer HM, et al. Histological analysis of surgical lumbar intervertebral disc tissue provides evidence for an association between disc degeneration and increased body mass index. *BMC Res Notes*. 2011;4:497. doi:10.1186/1756-0500-4-497

VITA

Jacob Kramer was born shortly following a sister and brother on March 7, 1996, in Casper, Wyoming. One year later, the family of five moved to La Verne, California. Jacob was reserved and serious from a young age, though he had a few close friends. In 2004, Jacob's mother, Robin, relocated her family to Defiance, Missouri for her work. Robin has always been Jacob's source of fire and heart, insisting relentlessly on the powers of patience and compassion. In 2005, Jacob's sister, Hailey, suffered lasting neurological trauma, which drove him forward in a number of ways.

Jacob began work on a Bachelor of Science in Biochemistry in 2014 at the University of Missouri. Following his first year, he joined the Biomodulatory Materials Laboratory in the College of Chemical Engineering and ran assays for the first time.

In 2018, Jacob was due to graduate from the university, but had no plan whatsoever for his time afterward. It was during this time that Jacob found the Thompson Laboratory for Regenerative Orthopaedics, and with some difficulty, became a graduate student. The lab became a new home, and its members became a new family. During the final year of his PhD, Jacob was graciously offered a post-doctoral position in the Thompson Lab, which he happily accepted.

Throughout five years, Jacob met Anna, who also became a graduate student in the Thompson lab. They were engaged in the final months of Jacob's PhD. They live together with their dog, Baxter, and cat, Rhodes. Jacob and Anna often exercise in the garage while Baxter and Rhodes lay together in the sun.