Protein Biomarkers Can Distinguish Histologically Degenerative Symptomatic from Asymptomatic Lumbar Intervertebral Discs

Jacob S. Kramer[1,2], Aaron M. Stoker[1,2], Abigail F. Baumann[1], Chantelle C. Bozynski[1,2], Emily V. Leary[1,2], Jinpu Li[1,2], Theodore J. Choma[2], Don 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

Jskgg7@mail.missouri.edu

Disclosures: Jacob S. Kramer (N), Aaron M. Stoker (1-MTF), Abigail F. Baumann (N), Chantelle C. Bozynski (N), Emily V. Leary (7B-BMJ Journals; 8-JISAKOS, J of Knee Surgery), Jinpu Li (N), Theodore J. Choma (3B – Medtronic Sofamor Danek, 4-Gentis, Inc.; 9-AO Spine NA, NASS, SRS), Don K. Moore (9-AAOS, LSRS, NASS), James L. Cook (1-Arthrex, MTF; 2-Arthrex; 3B- Arthrex, Bioventus, Collagen Matrix Inc, Trupanion; 5-Arthrex, AO Trauma, Collagen Matrix Inc, Cellularity, MTF, NIH, Organogenesis, Purina, Regenosine, SITES Medical; 7B-Thieme; 8-J of Knee Surgery; 9-MTN, MTF)

Introduction: Low back pain (LBP) is the most common cause of pain and disability, and its pathomechanisms may vary between individuals. While intervertebral disc degeneration (IVDD) is frequently noted in cases of chronic LBP, IVDD develops and progresses in patients who are not symptomatic for LBP. Individuals with otherwise similar clinical profiles and degrees of disc degeneration may experience different levels of pain or remain asymptomatic. It is not known which patient, tissue, cellular, and/or molecular factors contribute to the pathomechanisms that cause pain in patients with symptomatic IVDD. This study was designed to determine if differences in the *ex vivo* release of inflammation-, degradation-, and growth factor-related proteins by IVD tissues recovered from symptomatic (SYM) clinical patients and asymptomatic (ASYM) tissue donors could be identified for a given IVD histologic degenerative (HD) score. It was hypothesized that SYM IVD tissues would release significantly higher levels of pro-inflammatory and pro-degradative proteins compared to ASYM IVD tissues when matched for HD score.

Methods: ***Donor and Surgical Patient IVD Specimen Collection and Processing***: With IRB approval (IRB#2010692) and informed patient consent, IVD tissues were recovered from SYM patients undergoing surgery for IVDD (n=202 patients, mean age 55.3y, 118F). With consent recorded in a legal permit under the Uniform Anatomical Gift Act, IVD tissues were recovered from qualified ASYM tissue donors (n=25 patients, mean age 53.4y, 12F) without a reported history of back pain. Tissue explants for each SYM patient, and annulus fibrosus (AF) and nucleus pulposus (NP) tissue explants from ASYM donors were created and cultured for 3 days. On day 3, the media were collected for biomarker analysis, tissues were weighed, and approximately half of each SYM tissue explant was processed for histological assessment. ***Histological Assessment:*** A portion of each cultured SYM IVD specimen and uncultured, sagittal/transverse sections in the median plane of each ASYM IVD were formalin fixed, decalcified in 10% EDTA, paraffin-embedded, and tissue sections were stained using H&E, Toluidine blue, and Picrosirius red. For SYM 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 was used to evaluate each tissue by one blinded pathologist. SYM and ASYM IVD tissues were assessed for total HD score (0-19) comprised of categories including cell morphology (0-6), mucous degeneration in the AF (0-3), cell death (0-4), tear and cleft formation (0-3), and granular changes in the NP (0-3). Due to low frequency, HD scores ranging from 2 to 6 and HD scores ≥14 were separately aggregated. ***Media Analysis:*** Media samples were tested for IL-6, IL-8, IL-1RA, Gro-α, MCP-1, MCP-3, MIP-1α MIP-1β, RANTES, TNF-α, PDGF-AA, VEGF, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, and TIMP-4 using Luminex assays. ***Statistical analysis***: 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 SYM AF or NP tissues were compared to ASYM AF or NP tissues with adjustment for patient age, sex, obesity status, and their interactions. Two-sided significance was set at $p \leq 0.05$. Interaction plots of predicted biomarker values were used to interpret LM results.

Results: ***Inflammatory Cytokines/Chemokines*****:** SYM AF tissues released significantly ($p \leq 0.048$) higher levels of IL-1RA (HD scores 7, 10, 13), MCP-1 (HD score 12), MIP-1α (HD score 10), RANTES (HD scores 2-13), and TNF-α (HD scores 11-12) compared to ASYM AF tissues (Figure 1A). SYM NP tissues released significantly ($p \leq 0.042$) higher levels of GRO-α (HD scores 7-8), IL-8 (HD score 8), MCP-1 (HD score 10-11), MCP-3 (HD score group 2-6), MIP-1α and MIP-1β (HD scores 8-9), RANTES (HD scores 8-13), and TNF-α (HD score 10) (Figure 1B) compared to ASYMP NP tissues. ***MMPs:*** SYM AF tissues released significantly ($p \leq 0.044$) higher levels of MMP-1 (HD score 10), MMP-2 (HD scores 9-13), MMP-9 (HD score 11), and MMP-13 (HD scores 9-10, 13) compared to ASYM AF tissues (Figure 2A). ASYM AF tissues released significantly ($p \leq 0.049$) higher levels of MMP-3 (HD score 9-10), MMP-7 (HD scores 7, 11), and MMP-8 (HD scores 2-13, except 8) compared to SYM AF tissues. Similarly, SYM NP tissues released significantly ($p \leq 0.036$) higher levels of MMP-1 (HD scores 2-6, 9, 11), MMP-2 (HD scores 9-10), MMP-7 (HD score 9), MMP-13 (HD score 7), and significantly ($p \leq 0.046$) lower levels of MMP-3 (HD score 8), MMP-7 (HD score 11), and MMP-8 (HD scores 2-11, 13) compared to ASYM NP tissues (Figure 2B). ***TIMPs and Growth Factors:*** ASYM AF tissues released significantly ($p \leq 0.045$) higher levels of TIMP-2 (HD scores 2-11, 13), TIMP-3 (HD scores 7-9), TIMP-4 (HD scores 2-6, 8-11, 13), and significantly ($p \leq 0.039$) lower levels of PDGF-AA (HD scores 9-11) and VEGF (HD scores 7 and 13) compared to SYM AF tissues (Figure 3A). Similarly, ASYM NP tissues released significantly ($p \leq 0.045$) higher levels of TIMP-1 (HD scores 8, 13), TIMP-2 (HD scores 8-11, 13), TIMP-3 (HD scores 8-10), TIMP-4 (HD scores 2-6, 8-12), and significantly ($p \leq 0.039$) lower levels of PDGF-AA (HD scores 11 and 13), VEGF (HD scores 10-11), FGF2 (HD score 10) compared to SYM NP tissues (Figure 3B).

Discussion: The data from this study indicate that histologically degenerative, cultured disc tissues from symptomatic patients are associated with significantly higher release of inflammatory proteins and specific MMPs, and significantly lower release of TIMPs, compared to similarly histologically degenerative disc tissues from asymptomatic individuals. Specific proteins including RANTES, MMP-2, MMP-8, and TIMPs 2-4 are consistently upregulated by symptomatic or asymptomatic IVD tissues across a spectrum of histological degeneration, indicating potentially fundamental biochemical properties of symptomatic and asymptomatic discs that could be related to the development of pain in clinical patients. However, further study is required to determine if these changes in IVD protein release profiles are a cause or consequence of symptomatic IVDD. Ongoing studies in our lab are aimed at further characterization of symptomatic and asymptomatic IVDs to determine direct links to development of symptomatic IVDD that may serve as biomarkers for clinical application.

Significance: The quantification and comparison of tissue protein release profiles performed in this study indicate significant differences between IVD tissues recovered from symptomatic patients and asymptomatic donors that could be related to development and/or severity of symptomatic IVDD. Relating these differences to the development and progression of symptomatic IVD degeneration may allow for the development of novel diagnostic, preventative, and treatment methodologies for patients with debilitating lower back pain.