Failed lumbar fusion leads to increased markers of accelerated biologic aging

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Introduction: Due to an aging population, the need for lumbar fusion in the United States is increasing. Pseudoarthrosis due to failure of bone healing is the most common reason for revision surgery and can cause disability, pain, and decreased patient satisfaction. Although prior work has evaluated the negative impact that pseudoarthrosis can have on various postoperative outcomes, there is minimal data regarding the negative pathophysiologic impact that pseudoarthrosis may have at the cellular- and organism-levels. While bone healing is an inherently inflammatory process, failure of bony healing can lead to an ongoing cascade of acute inflammatory cell recruitment, fibrotic tissue changes, and ultimately the negative sequelae of chronic inflammation—all of which can lead to accelerated biologic aging. Biologic age is measured by a variety of validated biomarkers that change reliably age. Accelerated biologic aging refers to an increase in biologic age compared to a cell or organism's chronologic age. Accelerated biologic age, as measured by metrics such as DNA methylation and telomere length, has recently been shown to be correlated with traditional frailty indices and complication rates amongst patients undergoing spinal deformity surgery. However, the impact that spine surgery itself may have on aging acceleration has not been studied. The **objective** of this study was to utilize a rat model of posterolateral fusion to determine if failed fusion demonstrated accelerated biological aging compared to nonoperative controls and successful fusion.

Methods: Animal studies were approved by the Thomas Jefferson University Institutional Animal Care and Use Committee (IACUC; protocol #02436). A total of 79, 21–24-month-old male Brown Norway rats were randomly assigned to one of three groups; 1) non-surgery control (n = 26), 2) Successful fusion (n=27), or 3) Pseudoarthrosis (n=26). Groups 2 and 3 both underwent our previously published L4-L5 posterolateral fusion surgery, but group 2 received demineralized bone matrix allograft (DBM) after transverse process decortication, whereas group 3 received bone wax. DBM is a well-described augment of fusion, whereas bone wax hinders fusion. Whole blood was collected via gingival vein preoperatively (on day of surgery) and on postoperative days 4, 14, and 28. On postoperative day 42 (end-term), cardiac puncture was used to collect whole blood, mice were sacrificed, and spines were harvested. Animals were approximately 23 to 26 months at end-term. Whole blood cytokine levels were quantified through V-PLEX assays. Manual palpation of spines (n=14 in BW and n = 20 in DBM) were scored by two blinded assessors at end-term using a grading scale with a score of 0 for "not fused", 1 for "unilateral fusion with evidence of bridging bone", and 2 for "bilateral fusion". The nucleus pulposus (NP) cells of the disc at L4-L5 were harvested and RNA was isolated from each group (n = 3) prior to RNA sequencing. RNA sequencing analysis was conducted using Gene Set Enrichment Analysis (GSEA) using the Hallmark, Reactome, and GO databases.

Results: Manual palpation scoring showed that all rats in the BW group presented with un-fused spines. In the DBM group, 14/20 rats displayed fused spines, 3/20 were partially fused, and 3/20 were unfused. On day 4 post surgery, cytokine analysis of whole blood showed that rats in the DBM group had significantly higher levels of IFN-γ ($p = 0.0325$), IL-1β ($p = 0.0012$), IL-6 ($p<0.001$), and KC/GRO ($p = 0.0148$) compared to controls. The BW group had significantly elevated IL-6 ($p = 0.0012$) compared to controls on day 4. At day 42, IL-1β ($p = 0.032$) and TNF-α ($p = 0.0098$) were significantly elevated in the BW group only, compared to control (**Figure 1**). When comparing the DBM group to controls, RNA sequencing analysis revealed 1640 differentially expressed genes (Log2FC cut-off of 1.5-fold and FDR<0.05, 510 upregulated and 1130 downregulated). When comparing the BW group to controls, 212 genes were significantly differentially expressed (174 upregulated and 37 downregulated). Analysis of the DBM group to the BW group revealed 335 differentially expressed genes (297 upregulated and 38 downregulated). GSEA analysis demonstrated that both DBM and BW groups were associated with significant downregulation of pathways associated metabolism and mitochondrial function including 'oxidative phosphorylation' and 'aerobic' and 'cellular respiration' when compared to control groups (GOBP). Additionally, the BW group also displayed downregulation of 'chromatin remodeling' pathways (GOBP). Importantly, when comparing the BW group to the DBM group, the BW group showed significant downregulation of pathways associated chromatin remodelling, DNA repair, ECM organization, transcription, and telomere maintenance (GOBP and Reactome) (**Figure 2**).

Discussion: This is the first study to investigate the impact of pseudoarthrosis on biologic aging. Additionally, the animals that underwent surgery were at a clinically relevant age (24-month-old rats=60 years of age in humans). Early in the postoperative period, both DBM and BW groups had increased pro-inflammatory cytokines (IL-6) supporting that surgery instigates an inflammatory state. However, we also observed a late inflammatory state in the BW, characterized by enhanced IL-1β and TNF-α cytokine levels at end-term, which was not present in the DBM group. RNA sequencing analysis of nucleus pulposus cells from the BW group demonstrated an increase in pathways associated with well-documented hallmarks of aging, including epigenetic alterations, genomic instability, and telomere attrition, when compared to animals in the DBM group.

Significance/Clinical Relevance: Our findings show failed fusion promotes systemic inflammation and may accelerate biological aging of nucleus pulposus cells in aged rats. Our ongoing work aims to identify novel therapeutic strategies that target one or multiple markers of aging during spine fusion surgery. Such an approach could further promote bone formation and spine fusion and prevent pseudoarthrosis, a major cause of disability in older adults.

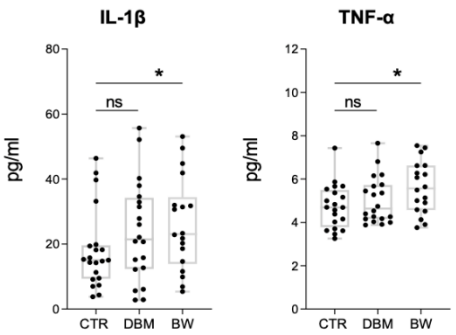

Figure 1. Cytokine concentration (pg/mL) in whole blood at postop day 42. * denotes $p < 0.05$

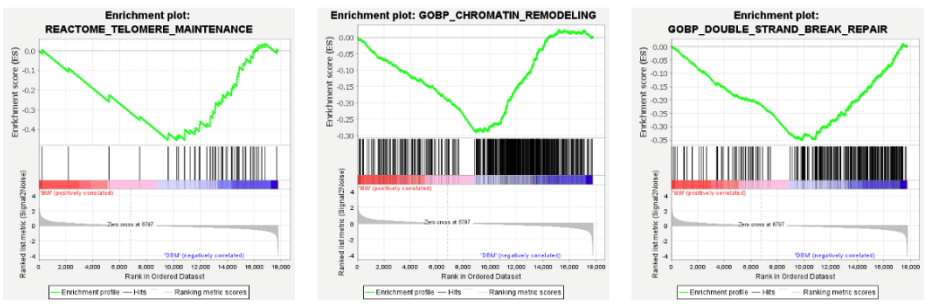

Figure 2. Each of these are plots of negatively enriched pathways (a) "telomere maintenance" (Reactome), (b) "chromatin remodeling" (GOBP), and (c) "double strand break repair" (GOBP) in the BW group compared to DBM