Spinal Alignment Device Enables Detection of Sagittal Curvature Changes in a Mouse Disc Degeneration Model

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Disclosures: The authors have no potential conflicts to this work.

Introduction: Sagittal spinal alignment is a key determinant of spinal function, and its disruption is linked to pain and disability in patients with intervertebral disc (IVD) degeneration (IVDD) and paraspinal muscle dysfunction [1]. Small-animal models provide valuable opportunities to study mechanisms of spinal pathology, but few in vivo preclinical studies assess sagittal alignment, IVD height, or paraspinal muscle changes after IVD injury. Prior work has shown that paraspinal muscle injury can induce sagittal spinal deformity [2] and that IVD injury can cause paraspinal muscle pathology [3], yet these studies did not quantify sagittal curvature or motion segment wedge angles. Whether quadrupeds exhibit alignment changes comparable to those seen in human spine pathology remains unclear, and the small size of mice poses technical challenges for reproducible measurement. To address this gap, we aimed to (1) develop a standardized alignment device to precisely measure sagittal spinal curvature in mice, and (2) evaluate the effects of lumbar IVD puncture injury on these parameters. We assessed lumbar Cobb angle, kyphotic Cobb angle, IVD wedge angle, and IVD height in a mouse IVDD model.

Methods: This study is approved by IACUC. Two alignment device prototypes were developed to standardize lateral positioning for in vivo rodent X-ray imaging using a Faxitron system. Prototype 1 consisted of a cylindrical device with cube supports (**Fig. 1D**), while Prototype 2, the final design, featured an alignment surface with limb-specific grooves elevated by stands for consistent positioning (**Fig. 1E**). The study included two parts: a reproducibility assessment in n=20 four-month-old naïve C57BL/6J mice (10 male, 10 female) imaged at baseline with no device, Prototype 1, and Prototype 2, and an evaluation in a lumbar disc injury model in four-month-old mice divided into Sham, Mild Injury, and Severe Injury groups (n = 3–4 per group, female) undergoing anterior spinal surgery. The injury study focused on female mice due to sexual dimorphism in spinal deformity [4], as females exhibit greater curvature changes; male data are being processed in ongoing work. Lateral X-rays were acquired at 8 weeks post-injury with Prototype 2 or no device. Sagittal spinal curvature parameters—including lumbar Cobb, kyphotic Cobb, IVD angle, and IVD height—were quantified using a custom MATLAB script. Users sequentially selected vertebral IVD levels and defined anterior, posterior, and central landmarks using perpendicular reference lines to ensure repeatable orientation [5]; the script computed anterior, posterior, and central IVD heights (mm) and IVD angle [6]. One-way repeated measures ANOVA with Tukey's post hoc test compared prototype effects in naïve (**Fig. 2A, C**) and with injury (**Fig. 3A-D**). Two-way repeated measures ANOVA with Tukey's post hoc test assessed prototype effects across spinal levels (**Fig. 2B, D**).

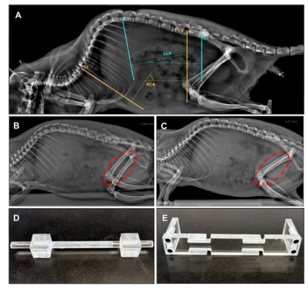

Figure 1. Alignment devices and sagittal curvature parameters in mice: (A) sagittal spinal angles (lumbar Cobb and kyphotic Cobb), (B) lateral X-ray with cylindrical device (Prototype 1), (C) cylindrical device, (D) lateral X-ray with limb holder device (Prototype 2), and (E) limb holder device.

Results: In naïve mice (combined sexes), one-way ANOVA revealed a significant effect of device on lumbar Cobb angle (p=0.02) but not kyphotic Cobb angle (p=0.89). Prototype 2 demonstrated reduced measurement variance (SEM=23.8) compared with Prototype 1 (SEM=35.8) and non-device conditions (SEM=36.8), indicating improved precision (**Fig. 2A, C**). Prototype 2 also improved consistency of disc angle (no device: SEM=59.3, Prototype 2: SEM=26.5) and IVD height measurements across lumbar levels L1/L2 through L4/L5 (no device: SEM=7.97, Prototype 2: SEM=6.74), although variability at L5/L6 and L6/S1 was observed (**Fig. 2B, D**). In female injured C57BL/6J mice at 8 weeks post-injury, Prototype 2 showed a trend toward decreased lumbar Cobb angle with increasing injury severity (p=0.05) and a non-significant decrease in kyphotic Cobb angle (p=0.25) compared with Sham; mean angles in injured mice were lower than Naïve control values (**Fig. 3A–D**). IVD angle measurements showed an overall decrease in lordosis with injury, with individual levels deviating from this trend. IVD height also decreased with injury, consistent with degeneration.

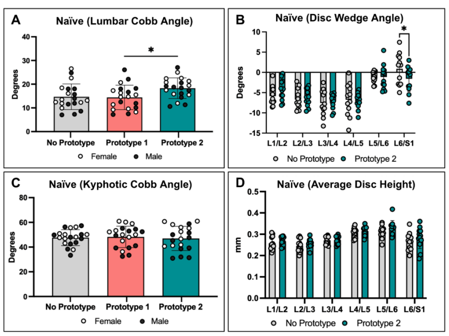

Figure 2. Prototype 2 reduced variability in sagittal spinal angles in naïve C57BL/6J mice (n=20): (A) lumbar cobb angle, (B) disc wedge angle, (C) kyphotic Cobb angle, and (D) average disc height, across no prototype, Prototype 1, and Prototype 2.

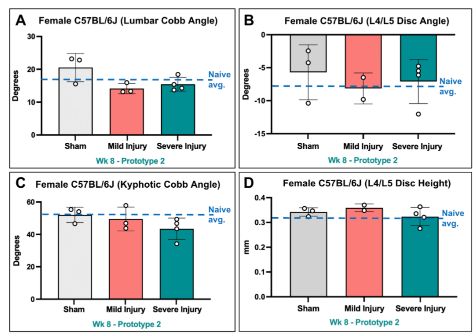

Figure 3. Sagittal spinal deformity at 8 weeks post-injury in C57BL/6J mice: (A) lumbar cobb angle, (B) disc wedge angle, (C) kyphotic Cobb angle, and (D) disc height, with Prototype 2.

Discussion: This study developed and validated a custom alignment device to standardize in vivo sagittal Faxitron X-ray imaging in a mouse model of intervertebral disc degeneration (IVDD) and quantified sagittal spinal curvature parameters, including lumbar Cobb angle, kyphotic Cobb angle, IVD wedge angle, and IVD height. Prototype 2, which secured the fore- and hindlimbs during anesthesia, improved reproducibility and precision compared with Prototype 1, which aligned the spine without limb support, and with no-device imaging. Specifically, Prototype 2 reduced variance in lumbar Cobb angle, IVD wedge angle, and IVD height measurements. Injury in three adjacent lumbar IVDs, when measured with Prototype 2, exhibited statistical trends toward decreased lumbar Cobb angle and IVD height. These changes are consistent with values expected from IVD injury, suggesting that mice exhibit changes in sagittal balance to have an important parallel with human IVDD conditions. Ongoing studies are increasing sample sizes to clarify statistical findings. Segment-specific variability at L5/L6 and L6/S1 highlights inherent anatomical differences and the potential influence of osteophyte formation and other vertebral defects observed at the lower lumbar levels of some injured animals. We postulate that sagittal angulation changes result from interacting effects of IVDD and paraspinal muscle remodeling, and ongoing histological analyses are assessing fatty infiltration and fibrosis in the multifidus. Overall, this study demonstrates that a spinal alignment device improves sagittal curvature measurements in mice and may be applicable to other species, if scaled for animal size. Future work will expand to larger cohorts, track longitudinal changes in spinal alignment and paraspinal muscle remodeling to further validate the alignment device and sagittal measurements as a tool to link structural and functional outcomes in preclinical IVD injury models.

Significance: We established a reproducible and precise method to quantify sagittal spinal alignment in rodent models of IVD injury. The device enables longitudinal assessment of structural changes, suggesting that quadrupeds and humans both exhibit sagittal curvature changes following IVDD. This device and animal model provide a platform to investigate relationships between spinal deformity and paraspinal muscle remodeling in preclinical research.

References: [1] Lafage+ Spine, 2008. [2] Noonan + Sci Rep, 2023. [3] Davies + NASSJ, 2021. [4] Bailey+ J Anat, 2016. [5] Pfirrman+ J Orthop Res, 2006. [6] Hu+ GSJ, 2021.

Acknowledgements: Supported by NIH R01AR080096 and R01AR078857.