Asymmetric Vertebral Growth Contributes to Junctional Kyphosis in the Skeletally Immature Swine Model.

Matthew A. Halanski, MD[1]; Brittney Kokinos, MS[2]; Cameron Jeffers, MS[1]; Tom Crenshaw, PhD[2]
1. University of Arizona, 2. University of Wisconsin-Madison

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Introduction: Junctional Kyphosis is an unwanted secondary sagittal deformity that occurs at vertebral levels adjacent to spinal instrumentation. While most common at the proximal end of posterior based spinal constructs junctional issues may occur proximally or distally. The causes of junctional kyphosis are multifactorial and may include poor bone quality, pre-existing sagittal imbalance, implant failure, excessive soft tissue destabilization during index procedure, and poor selection of instrumentation levels. In this work, we instrumented the apical segments of non-surgically induced kyphotic swine, using a six-level posterior based vertebral body tether construct that stopped short of the first lordotic disc space. Correction of the apical kyphosis and the development of junctional kyphosis was monitored to understand how vertebral growth factors in the development and progression of junctional kyphosis.

Methods: This study was approved by institutional IACUC**.** Six, eight to nine-week-old hyper-kyphotic swine(28), underwent multi-level posterior compressive tethering with high (N=3, 25.6N) or low (N=3, 5N) tension applied equally to each vertebral level of tethered. The apex of the deformity was confirmed on radiographs and Wiltse style approach was used to minimize any mid-line or soft tissue stripping that might increase the likelihood of junctional kyphosis. Swine were harvested at four weeks (~13 weeks of age) imaged and tissues processed. As swine may have different numbers of vertebrae, for the purposes of this study, we defined the most distal instrumented disc space (D1) and the most proximal instrumented disc space (D5), adjacent un-instrumented disc spaces were labeled Dd (distally) and P1 and P2 (proximally). Pulsed fluorochrome labeling was performed by administering Alizarin Red 12-14 days prior to harvest and Oxytetracycline on the day of harvest. Vertebrae were coronally sectioned into ~3 mm thick slabs utilizing an Isomet Precision saw (Buehler Isomet 2000; Lake Bluff, IL), and the entire spinal segment was visualized using a Nikon NiE upright microscope (Nikon Instruments; Melville, NY) set-up for epifluorescence at 4X magnification. A single central vertebral slab was evaluated for regional growth. Regional growth rate (μm/day) measurements were performed by measuring the distance between fluorochrome labels using a custom validated image analysis program and dividing this distance by the time between fluorochrome label administration, defining ***% Growth Modulation***, defined as (anterior ¼ growth-posterior ¼ growth)/Mean vertebral growth rate. This equation evaluates the differences in growth rates (anterior versus posterior) and normalizes the differences by the overall vertebral physeal growth rates to account for different rates of growth in individual animals. Thus, in our kyphotic model, a positive % Growth Modulation is indicative of (therapeutic) growth modulation (anterior>posterior growth) whereas negative % growth modulation is indicative of worsening kyphosis**.** To quantify junctional kyphosis, lateral digital radiographs (XJet, C.F.D. Devices, Scanzorosciate, Italy) at the time of harvest was performed. Sagittal Cobb angles encompassing the last tethered disc and the first untethered disc (Sante dicom viewer (Santesoft, Nicosia, Cypress), both proximally and distally were measured. Decalcified sections were submitted to the Translational Research Initiatives in Pathology (TRIP) laboratories (UW-WIMR) for paraffin embedding, sectioning, and staining with Hematoxylin and Eosin and Masson's Trichrome. Slides were imaged using the Aperio AT2 at 0.5 μm/pixel resolution at 20x magnification. Scanned images (.svs) were then analyzed using BioQuant software (BioQuant Image Analysis Corporation, Nashville, TN). In BioQuant Software, an area was drawn and then calculated based on user specifications. The specification of interest was the average thickness of any given area (i.e., Hypertrophic, proliferative, reserve zone, etc. The summation of the hypertrophic zone and proliferative zone were the focus and what comprised of the 'total thickness' (area of focus). The percentage of epiphyseal ossification was measured at each disc space using the scanned micrograph images and Bioquant software. The distance (A/P) of the ossified epiphysis was divided by the total distance (A/P) of the entire epiphysis including the unossified, cartilaginous regions for each epiphysis and multiplied by 100. The mean epiphyseal ossification for each disc level was then calculated from the proximal and distal epiphysis at each disc space. The location of the sagittal geometric center of the nucleus pulposis (NP), was defined using the Bioquant software, at all available levels. The distance of the geometric NP center was located relative to the posterior to anterior edges of the ossified epiphysis or the overall vertebra. These were recorded as a percentage, posterior to anterior of the ossified epiphysis or the overall vertebra (i.e. 50% would indicate the geometric NP center was at the midpoint of the ossified epiphysis or vertebral body, <50% would indicate a posterior position, and >50% anterior). Growth rates, epiphyseal ossification, regional growth rates and % growth modulation between the instrumented and uninstrumented levels were then made.

Results: Increased overall kyphotic Cobb angle was found between at the discs outside the tethering construct versus the last instrumented disc space (p=0.00005), Figure 1a. A severe example of junctional kyphosis is demonstrated in **Figure 1b**. No significant differences were found in the physeal thickness or mean growth rates between the end-instrumented and the adjacent uninstrumented disc spaces (**Figure 1c, d**) However, decreased epiphyseal ossification was found at the uninstrumented levels compared with the instrumented levels (**Figure 1e**). While no significant differences in mean vertebral growth rates were observed between the instrumented and uninstrumented levels, the regional distribution of that growth was significantly different as the instrumented levels demonstrated greater anterior and less posterior growth (p<0.0001) than the adjacent uninstrumented levels resulting in opposite and significant vertebral growth modulation (p<0.0001), **Figure 1f, g**. Examples of the difference in regional growth between instrumented **(a)** and uninstrumented disc space **(b)** can be found in **Figure 2**.

Discussion: These data demonstrate that junctional kyphosis occurs following posterior "flexible" instrumentation in the kyphotic swine model. Similar to clinical practice, failing to instrument to the first lordotic disc space, may initiate the deformity, however, it does appear that once the sagittal imbalance is established, asymmetric growth contributes to the progression of the deformity.

Significance: While Junctional Kyphosis in the skeletally immature patient may be initiated by a host of patient or surgical technical factors, these data suggest that asymmetric vertebral growth contributes to junction kyphosis progression.