

Correlation of MRI and Histological Examination of Physeal Bars in a Rabbit Model

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Introduction. The formation of a bony bridge, or bar, across an open physeal growth plate is frequently observed in pediatric disorders. Bone bar formation may lead to limb-length discrepancy and/or angular deformity [1]. Bar formation may be evaluated using plain radiographs, but image interpretation is difficult due to the irregular three-dimensional (3D) shape of the physis [2]. Magnetic resonance imaging (MRI) and computed tomography (CT) are also used to image the physis. MRI utilizes multi-plane image acquisition; however, the shape of the 3D physis must still be interpreted from planar images. Previous investigators have used 3D MR image reconstruction and analysis to estimate physeal bar areas in humans [2, 3]. Animal models have also been used to correlate the MR appearance of the physis and bar with histological morphology [4,5], but no study to date has validated indirect bar area measurements from MRI with direct histological measurements. The purpose of this study was to create a physeal bar, using a radiofrequency (RF) ablation technique, and validate the indirect measurement of bar area from MR images, using a prototype reconstruction algorithm, with direct histological measurements in an animal model.

Materials and Methods. All methods were approved by the local IACUC. To date, 10 of 18 10-week-old male New Zealand white rabbits have been evaluated. **Surgical Method:** The rabbits were anesthetized, and a 0.045" K-wire was used to create a track from the lateral bony cortex of the tibiae to approximately 1/2 to 1/3 of the way from the lateral edge of the physis, under fluoroscopic guidance. The K-wire was removed and a RF needle inserted into the same position. RF ablation was performed at 90°C for 4 minutes (2 consecutive 2-minute sessions). The rabbits were allowed free cage movement post-op and were sacrificed 6 weeks thereafter. MR imaging of the excised tibiae was performed on the same day. **Image Acquisition:** Imaging was performed using a 3T clinical MRI system and a prototype ($\phi=2\text{cm}$, length=7cm) birdcage coil. A 3D T1-weighted fat-suppressed spoiled gradient-recalled echo (SPGR) sequence was used to generate a volumetric dataset for physeal segmentation and physeal bar area quantification. Imaging parameters were: TR: 21.1ms, TE: 4.6ms, FOV: 6cm, flip angle: 10°, slice thickness: 0.2mm, matrix 512x512, receiver bandwidth: $\pm 31.25\text{kHz}$. These parameters displayed the physeal growth plate as hyper-intense voxels. Approximately 92 images were acquired for each tibia. **Image Analysis:** Custom written software (GE Healthcare, Waukesha, WI) was used for semi-automated segmentation of the normal physes. A 3D representation of the segmented image data set was constructed for each knee, the physeal bar was then defined and its area quantified. The bar area was also expressed as a percentage of a flattened projection of the whole physis [6]. **Histology:** Following MR imaging, the tibiae were fixed, decalcified, cut in half sagittally and embedded in paraffin blocks. Sagittal histological slices (stained with hematoxylin-eosin) were prepared from each block, with two consecutive 5 μm slices taken at 220 μm intervals to cover the entire area of the bar. The linear length of physeal disruption on each slice was measured with reference to a known calibrated 1mm standard. The lengths of the physeal disruption at each 5 μm interval location were averaged, and the total physeal bar area calculated as the summation of a series of trapezoids thus formed (Fig. 2). This method of calculating the histological bar area is independent of the side-to-side alignment of the slices, as the size of the individual trapezoids is dependent only on the length of the sides and its height. **Statistical Analysis:** A paired t-test was performed to detect differences of MRI and histological bar areas. The Pearson correlation coefficient (r) was calculated between the physeal bar areas derived from MRI and histology.

Results. From the 10 rabbits evaluated, the mean bar area from MRI measurements was $40.3 \pm 26.3\text{mm}^2$ (mean \pm st.dev.), and the mean bar area from histology measurements was $29.7 \pm 20.0\text{mm}^2$. One tibia was too fragmented for histological measurement and was excluded from statistical analysis. Representative MR and histological images are shown in Figure 1. The bar comprised $22.2 \pm 13.8\%$ of the total physis in the MR analysis. The bar area from histological measurements was $26.3 \pm 23.9\%$ smaller than the corresponding MR measurements; however, this difference was not significant ($p=0.18$). A strong correlation, $r=0.82$ ($p=0.006$), was found between the area measurements (Fig. 3).

Discussion. This study used MR images to quantify ablated areas of physis, and correlated the indirect MR measurements with direct histological measurements. A good correlation was found between the two measurement methods and the small differences encountered were likely due to the effects of histological sample preparation. Segmentation of the MR images enabled a 3D representation of the physis and bar to be created. The reconstructions (Fig. 2) provided a quantitative and objective method to evaluate the local bar and overall physis area. The irregular and undulating pattern of the physis was clearly visible in these reconstructions. Viewing the physis in 3D may avoid errors in interpretation or localization of physis and bar, as compared to traditional single plane imaging methods. Furthermore, the 3D MRI dataset was reformatted to increase the accuracy of defining the lateral borders of the ablated/damaged area, a known difficulty in physeal imaging [2]. The results of this study provide the foundation for quantitative evaluation of in-vivo human physes in future studies and development of predictive models for limb length discrepancy.

References. [1]. Ogden JA, JBJS, 1987. [2]. Sailhan F, et al., Eur Radiol. 2004;14:1600-8. [3]. Craig JG, et al., Radiology, 1999. [4]. Jaramillo D, et al., Radiology 1990. [5]. Cheon JE, et al. Invest Radiol 2003. [6]. Gering DT, inventor; General Electric Company, assignee. US patent app. 20080049991.

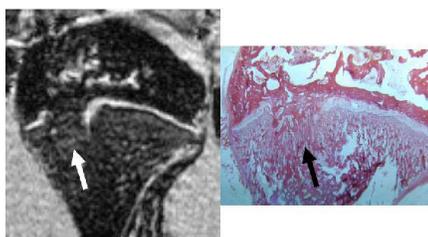


Figure 1. Representative images of an ablated rabbit physis. Left – A sagittal SPGR MR image. The ablated portion of the physis (bar) is indicated by the white arrow. Right – A sagittal histological section corresponding to the MR image. The ablated portion of the physis is indicated by the black arrow.

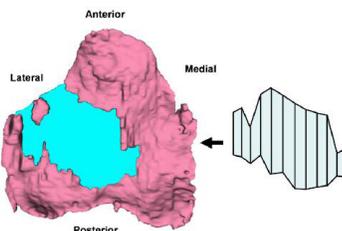


Figure 2. Left – Post-operative intact rabbit physis (pink) and ablated physeal bar (blue), as reconstructed from MR imaging. Right – Physeal bar area was calculated as the summation of a series of trapezoids covering the ablated area using histological images.

Acknowledgements. The authors would like to acknowledge Drs. Roger Widmann and Scott Rodeo in their assistance with the histology.

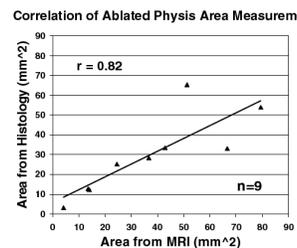


Figure 3. Correlation of bar measurements from MRI with histologic bar measurements.