

Automated Quantitative Analysis of Metastatic Disease in Rat Spine: The Effects of Stereological Model and Spatial Resolution

⁺Hojjat, S. P.; ²Whyne, C. M.;

⁺University of Toronto, Toronto, ON, Canada ²Sunnybrook Health Sciences Centre, Toronto, ON, Canada
cari.whyne@sunnybrook.ca

INTRODUCTION:

Preclinical models are widely used in evaluation of new and existing treatments for spinal metastases. Micro-imaging is often utilized in a qualitative or semi-quantitative manner to examine treatment effects on bony architecture; automated quantification of such analyses could significantly improve such analyses and reduce the time required to process large data sets. Accurate assessment of changes in vertebral architecture may depend both on the images acquired and the models used to represent the structural data. This study aims to examine the effect of image resolution on architectural differences between healthy and metastatically involve vertebrae and to compare the capabilities of two different structural models, Parfitt's Plate Model² and Hilderbrand's model³ in accurate and automated evaluation of trabecular thickness.

METHODS:

Lumbar vertebrae (L1 – L3) of healthy (n = 6) and metastatically involved (n = 6) mu / mu rats were utilized for this study. Osteolytic vertebral metastases were developed via intracardiac injection of human MT1 breast cancer cells into the rats and analyzed at 21 days post-injection. Confirmation of spinal metastasis was achieved using bioluminescence imaging. The healthy and metastatically involved spines were μ CT scanned ex vivo at 14 μ isotropic spatial resolution.

An automated algorithm was used to segment the μ CT images¹. The algorithm used atlas based demons deformable registration followed by level set curvature evolutions to segment the whole vertebrae. A subsequent iteration of level set was used to yield a segmentation of the trabecular centrum. The individual trabecular network was further segmented using intensity based thresholding.

Architectural parameters were computed from the segmented μ CT images: Cortical Bone Volume (CBV), Trabecular Bone Volume (TBV), Trabecular Bone Surface Area and the degree of anisotropy based on Mean Intercept Length (MIL)¹. Trabecular Thickness (TbTh), Trabecular Number (TbN) and Trabecular Separation (TbS) were calculated based on the formulae derived from the bone volume and surface area measures using the Parfitt Model². TbTh was calculated separately from the images using the Hilderbrand model³. The degree of anisotropy was automatically assessed by measuring MIL in X, Y, and Z directions utilizing a binary shift/subtraction approach. Trabecular thickness using the two models and MIL were calculated for each image (generated through up/downsampling) at 8.725 (high), 17.45 (medium) and 34.9 (low) μ m³ isotropic spatial resolutions.

RESULTS:

Microstructural parameters were calculated using Parfitt's and Hilderbrand's models and MIL for healthy and metastatic vertebrae calculated at the highest resolution (Table 1). Parfitt's plate model showed a significant decrease in TBV, TbN and CBV and a significant increase in TbS in the metastatic vertebrae in comparison to the healthy group at the highest resolution. In both Hilderbrand's and Parfitt's models at the highest resolution there was no significant difference in TbTh between the healthy and metastatic groups. In both models, TbTh and TbS values rose while TBV and TbN decreased as the resolution was lowered. Significant reductions were observed only in TbTh between the healthy and metastatic vertebrae at the medium and low resolutions (Table 2). In all cases, the Hildebrand model yielded lower values of TbTh than the Parfitt model.

Table 1. Microstructural parameters of the healthy and metastatic vertebrae calculated at 8.725 (μ m³) resolution. (*p<0.05)

Attribute	Healthy		Metastatic	
	TbV * (%)	55.92 \pm 3.2		50.37 \pm 2.5
TbTh (μ m)	Parfitt	Hilderbrand	Parfitt	Hilderbrand
	100 \pm 17	87 \pm 21	109 \pm 8	100 \pm 13
TbS * (μ m)	78 \pm 13		108 \pm 6	
TbN*(#/mm ²)	5.7 \pm 0.77		4.6 \pm 0.19	
CBV * (cm ³)	0.0411 \pm 0.006		0.0293 \pm 0.007	

Structural anisotropy remained consistent in all groups at all resolutions, with ~3x greater MIL in the superior/inferior (Z) direction. In comparing

the healthy and metastatic groups, MIL values were lower in the healthy group at the highest resolution, similar in both groups at the medium resolution and lower in the metastatic group at the lowest resolution (Table 3).

Table2: Trabecular Thickness measured in (μ m) at varying resolutions

Resolution (μ m ³)	Healthy		Metastatic	
	Parfitt	Hilderbrand	Parfitt	Hilderbrand
8.725	100 \pm 21	87 \pm 21	109 \pm 8	100 \pm 13
17.45	138 \pm 7	130 \pm 15	118 \pm 4	104 \pm 4
34.9	271 \pm 15	261 \pm 20	210 \pm 13	171 \pm 19

Table 3: MIL measured in (μ m³) at different resolutions

Direction	Healthy			Metastatic		
	8.725	17.45	34.9	8.725	17.45	34.9
X	113 \pm 18	107 \pm 9	151 \pm 27	190 \pm 18	120 \pm 9	133 \pm 13
Y	119 \pm 16	111 \pm 7	157 \pm 29	197 \pm 23	124 \pm 11	137 \pm 15
Z	298 \pm 72	308 \pm 27	483 \pm 93	495 \pm 35	309 \pm 24	373 \pm 45

DISCUSSION:

This work presents an automated method for quantification of the microstructural parameters of whole rat vertebrae and its application to evaluated differences between healthy and metastatic bone. The analysis demonstrates that the presence of metastatic disease causes reductions in both trabecular and cortical bone volume, but only at the highest resolution. At this resolution TbN is reduced while TbTh is maintained, suggesting primarily loss of trabeculae in the metastatic vertebrae, as opposed to trabecular thinning. In contrast, at lower resolutions, no difference was seen between the healthy and metastatic vertebrae in terms of TBV and only significant reduction in TbTh was observed in the metastatic group. This suggests that sufficient resolution is essential to accurately demonstrate structural differences in vertebrae due to the presence of diffuse osteolytic tumor.

TbTh values calculated using the Hilderbrand model demonstrated similar behaviour, but yielded lower absolute thickness estimates than Parfitt model at all resolutions. This is consistent with the assumption of trabecular plates utilized in the Parfitt model, whereas the Hildebrand model accounts for a trabecular structure composed of a mixture of plates and rods³. However, achieving robust automated results using the Hildebrand method was limited in the final stage of the segmentation as the model was found to be sensitive to the presence of small islands of negligible thickness in some μ CT slices. Including these small islands in Hilderbrand's thickness calculations results in an extreme drop in mean TbTh, as a thickness is assigned to every point in the segmentation. These islands do not cause a problem in Parfitt's model in which the TbTh is derived from total volume and surface area measures. The need for manual refinement to ensure removal of these islands without compromising the interconnected trabecular structure suggest that Hilderbrand's model may be less optimal for automated μ CT applications considering preclinical models.

Anisotropy in trabecular structure was observed in both groups as there were significant difference between the MIL values in the superior/inferior (Z) direction and the values in the transverse (X and Y) directions. The degree of anisotropy was consistent in both groups suggesting that the metastatic destruction does not have any directional preference. The degree of anisotropy was also consistent at all resolutions, however the individual MIL measurements varied greatly between the highest and lowest resolution analyses.

The presented work suggests that automated use of Parfitt's plate model along with the MIL method can be used to yield quantitative analyses demonstrating differences in vertebral microstructure due to metastatic involvement. However the sensitivity of these architectural parameters to resolution, motivates the need for high resolution scanning in future preclinical applications.

REFERENCES:

- Hojjat S. P. et al. Journal of Neurosurgery: Spine 2009 (Submitted) 2.
- Parfitt A.M. et al. Bone and Mineral. 1987. 3.
- Hilderbrand T. et al. Journal of Microscopy 1997. 4.
- Odgaard A. et al., Bone 1997.