Multimodal μ CT/ μ MR based semiautomated segmentation of rat vertebrae affected by mixed osteolytic/osteoblastic metastases

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Title: Muti-modal μ CT / μ MR based Semi-Automated Segmentation of Rat Vertebra Affected by Mixed Osteolytic / Osteoblastic Metastases

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Abstract

Purpose: Multimodal micro-imaging in preclinical models is used to examine the effect of spinal metastases on bony structure, however the evaluation of tumour burden and its effect on microstructure has thus far been mainly qualitative or semi-quantitative. Quantitative analysis of multi-modality imaging is a time consuming task, motivating automated methods. As such, this study aimed to develop a low complexity semi-automated multimodal μ CT/ μ MR based approach to segment rat vertebral structure affected by mixed osteolytic/osteoblastic destruction.

Methods: Mixed vertebral metastases were developed via intracardiac injection of Ace-1 canine prostate cancer cells in **three** 4-week-old rnu/rnu rat. μ CT imaging (for high resolution bone

visualization), T1-weighted μ MR imaging (for bone registration) and T2-weighted μ MR imaging (for osteolytic tumour visualization) were conducted on **one L1, three L2, and one L3 vertebrae** (excised). **One sample (L1-L3) was processed** for undecalcified histology and stained with Goldner's trichome. The μ CT and μ MR images were registered using a 3D rigid registration algorithm with a mutual information metric. The vertebral micro-architecture was segmented from the μ CT images using atlas-based demons deformable registration, levelset curvature evolution, and intensity based thresholding techniques. The μ CT based segmentation contours of the whole vertebrae were used to mask the T2-weighted μ MR images, from which the osteolytic tumour tissue was segmented (intensity based thresholding).

Results: Accurate registration of μ CT and μ MRI modalities yielded accurate and precise segmentation of whole vertebrae, trabecular centruns, individual trabeculae, and osteolytic tumour tissue. While the algorithm identified the osteoblastic tumour attached to the vertebral pereosteal surfaces, it was limited in segmenting osteoblastic tissue located within the trabecular centruns.

Conclusions: This semi-automated segmentation method yielded accurate registration of μ CT and μ MRI modalities with application to the development of mathematical models analyzing the mechanical stability of metastatically involved vertebrae and in preclinical applications evaluating new and existing treatment effects on tumour burden and skeletal micro-structure.

Introduction

The skeleton is one of the most common sites of metastatic disease; bone metastasis accounts for skeletal lesions in 40 times as many patients as those affected by all other forms of bone cancer combined¹. Skeletal lesions most commonly appear in the spine² and can present as osteolytic (bone destroying), osteoblastic (excess bone deposition), or mixed osteolytic/ osteoblastic. Modern cancer therapies have resulted in a significant increase in the occurrence of mixed vertebral lesions with more diffuse patterns of involvement^{3, 4}. Understanding the structural implications of complex patterns of metastatic involvement in the spine is an important step towards the development and appropriate use of interventions to prevent fracture and associated neurologic complications^{5, 6}.

Preclinical models are commonly used to represent metastatic disease in the skeleton^{7, 8, 9, 10, 11, 12}. Widespread access to high resolution imaging modalities has made it possible to visualize the pattern of metastatic disease within the skeleton in preclinical models on a micro-scale. Such image data can be used towards building accurate mathematical models to quantify the impact of bone quality (including architecture and material properties), and tumour burden on bone stability and to predict the risk and location of fracture. Development of such models requires accurate segmentation of the vertebral structure including bone, marrow and both osteolytic and osteoblastic tumour tissue. However, the amount of data contained in these high resolution scans motivates the development of automated segmentation methods.

In previous works^{13, 14}, semi-automated CT-based methods have been developed for quantification of metastatic disease in human vertebral bodies. In this work, the quantification scheme was based on conventional CT in which osteolytic destruction and osteoblastic deposition appear as low and high intensity voxels respectively. Conventional CT is however not

71 suitable for the examination of micro-structural effects. We further extended this work and
72 developed a μ CT based automated method to segment the full healthy vertebrae (including the
73 posterior elements) and vertebrae with osteolytic metastatic involvement in a rat model ¹⁵. This
74 approach was able to accurately segment whole vertebrae, the trabecular centrum, and the
75 individual trabecular mesh. In this work tumour burden was evaluated as the volume of
76 osteolytic destruction; both tumour tissue and bone marrow appear as low intensity voxels in the
77 μ CT images, however, and thus the true tumour burden was not quantified.

78 A solution to determine the true extent of tumour burden within bone is to employ multi-modal
79 imaging. MRI yields robust images of soft tissue structures and is able to differentiate tumour
80 burden (higher intensity) from bone marrow. However, registration of this data with the bone
81 information generated from the μ CT image data is necessary to spatially resolve the relative
82 distributions of the soft and hard tissues.

83 Previous works have introduced different methods for multi-modal registration of medical
84 images ^{16 - 19}. All of these works use a mutual information metric to perform the registration,
85 however the techniques used to assure accuracy in the registration differ. Chen et al. employed
86 extensive preprocessing to eliminate noise and enhance the quality of the images before starting
87 the registration process ¹⁶, while Lu et al. use the method of joint histogram estimation to predict
88 the distribution of the fused image ¹⁷. Other work performed by Eldib et al. uses the concept of
89 surface matching to register the two image modalities ¹⁸, and finally Piella et al. ¹⁹ use a
90 sophisticated multi-resolution wavelet-based approach to register two images. None of the
91 mentioned algorithms however have been employed on high resolution images. The utilization of
92 high resolution data may greatly affect the computational time and memory requirements
93 considering the extensive computations used in all of these algorithms.

The objective of this study was to develop a low complexity highly-automated multimodal approach to segment the rat vertebral structure and quantify metastatic tumour burden within bone using a combination of μ CT and μ MR modalities. We hypothesize that semi-automated multi-modal analysis applied to 3D μ CT and μ MRI reconstructions will allow registration of whole vertebrae affected by mixed osteolytic/osteoblastic metastases and yield accurate and repeatable segmentation of both bone and soft tissue elements within the bone.

Materials and Methods

Animal Model: Osteolytic/osteoblastic spinal metastasis were developed using canine Ace-1 prostate cancer cells in **three** 3 week old rnu/rnu **rats**. Ace-1 cells were previously transfected with the luciferase gene to enable bioluminescent image monitoring of tumour growth within the rodent model. Cells were cultured for 7 days using a standard protocol (DMEM/F12 media with 10% FBS and 1% antibiotics). One and a half million cells in 200 μ l of media were injected into the left ventricle of anaesthetized rats (2% isofluorane/oxygen). The **rats were** weighed, marked and monitored until fully awake.

Bioluminescence imaging (IVIS Imaging System, Caliper Life Science, Hopkinton, MA) was performed on day 14 following intracardiac cancer cell injection to confirm the establishment of metastases. Luciferin (Xenogen Corp., Alameda, CA) was dissolved in 0.9 % NaCl solution at a concentration of 30mg/ml and 60 mg/kg injected intra-peritoneally to anaesthetized animals (2% isofluorane/oxygen). After five minutes, the bioluminescent signal was acquired with a 1 minute integration time. The signal was captured as the absolute total flux (# of photons emitted per steradian cm^2) and analyzed using Living image software with the bioluminescence image overlaid on a plain photograph. Following subsequent bioluminescent confirmation of vertebral metastatic involvement at day 21, the **rats were** sacrificed and their L1-L3 vertebral levels were

excised. **The L1-L3 motion segment of one rat was** immediately fixed in 4% paraformaldehyde
8, 20.

Image Acquisition:

μCT: First to third lumbar vertebrae were dissected and scanned at $(14\ \mu\text{m})^3$ isotropic resolution using a *μCT* scanner using an x-ray source at 90 mA and 80 kV, with 907 views covering 360° of rotation. The scan time was 2.5 hours. (GE Explore Locus, General Electric Company, Fairfield, USA). The 3D volume (with intensities converted to Hounsfield units) was reconstructed using the GE Explore Reconstruction Utility. The *μCT* was able to display cortical and trabecular bone and well as osteoblastic tumour as high intensity voxels. Bone marrow and osteolytic tumour appeared as low intensity values, which were not distinguishable from each other (Figure 1a).

μMR: MRI used a 7 Tesla pre-clinical Biospec system (Bruker, Ettlingen, Germany) with a quadrature birdcage coil tuned to the ¹H frequency (~300.3 MHz), custom-built by Stark Contrast MRI Research, Germany. This coil was specifically designed for good signal to noise ratio in imaging ex vivo bone. The coil was fixed tuned to an average load as load change is not essential for small sizes. The inner diameter of the coil was 17mm, to fit a 15 cc Falcon tube, and the outer diameter was set to 60mm, to fit the B-GA6S gradient coil insert of the Bruker system. For imaging with this coil, the spine was embedded in agarose gel within a 15 cc Falcon tube. Imaging then proceeded using 30.8 mm field-of-view along the spine length, and 15.4 mm field-of-view in each transverse plane. Spine anatomy was then resolved to a $(60\ \mu\text{m})^3$ voxel size using a 512x256x256 matrix with 13.5 hour acquisitions.

The first scan presented a T1-weighting to accentuate bone ultrastructure and ensure accurate *μCT* and *μMRI* registration. Data acquisition parameters included an echo time (TE) of 7.6 ms,

repetition time (TR) of 500 ms, RARE factor of 2, and 3 signal averages. Using these parameters, the boundaries of the trabecular structure were clearly visible, though bone marrow and osteolytic tumour could not be visualized distinctly (Fig. 1b).

The second scan presented a T2-weighting to accentuate soft tissue contrast and clearly differentiate lower intensity bone marrow from higher intensity tumour tissue (Fig. 1C). Data acquisition parameters included a TE of 48 ms, TR of 3000 ms, RARE factor of 4, and 1 signal average.

Histological confirmation of osteolytic/osteoblastic tumours: Following μ CT / μ MR imaging, **one** sample (**L1-L3**) was processed for undecalcified histology. Five μ m sections were stained using Goldner's Trichrome, which differentiates between mineralized bone (green/blue) and unmineralized new bone formation (osteoid; red/orange) (Figure 1).

Figure 1. (a) Sagittal histology slice of L1 vertebra stained with Goldner's Trichrome, which differentiates between mineralized bone (green/blue) and unmineralized, new bone formation (osteoid; red/orange) (b) Inset shown at 6.8x magnification.

In histological analysis osteoblastic tumour can be qualitatively characterized as a disorganized arrangement of osteoblastic cells, however, in μ CT images the intensity of the voxels corresponding to osteoblastic tumour is equivalent to that of non-pathologic newly formed bone.

Semi-Automated Multimodal Segmentation

Rigid Registration: All μ MR and μ CT images were resampled to $(34.9 \mu\text{m})^3$ and manually aligned to a global axis (AmiraDEV 4.1, Visage Imaging, USA). The aligned μ CT scan was further registered to the bone μ MRI data using a 3D rigid registration algorithm (AmiraDEV 4.1). In this registration, the μ CT was chosen as the moving image and the μ MRI was chosen as the fixed image. A quasi Newton optimizer²¹. was used with an initial optimizer step of $(10^{-3} \times \text{bounding box})$ and final optimizer step of $(10^{-7} \times \text{voxel size})$ for translation. The values for rotation, scaling and shearing were chosen appropriately by the algorithm. A normalized mutual information metric was utilized. The histogram range for both the fixed and the moving image were chosen as the full range of gray level values for each image. The contrast μ MRI was then registered to the bone μ MRI using the rigid registration algorithm with the same parameters. For this registration bone μ MRI was used as the fixed image and the contrast μ MRI was used as the moving image. This resulted in acceptable registration of all 3 scans based on qualitative evaluation (Figure 2).

Figure 2: (a) Sagittal view of a μ CT slice of a rat spine with mixed osteolytic /osteoblastic metastatic involvement secondary to Ace-1 cancer cell injection, (b) Corresponding slice in the bone μ MR after the rigid registration, (c) Corresponding slice in the contrast μ MR after the rigid registration.

Segmentation

μ CT: An extended version of a previously developed algorithm¹⁵ was used to segment the whole vertebrae of interest from the initial μ CT scans at $(14\mu\text{m})^3$ isotropic voxel size. The algorithm uses atlas-based demons deformable registration along with level set curvature evolutions to segment the whole vertebra. Mixed metastatic involvement in the vertebra resulted in growth of osteoblastic tumour on the outer cortical shell of the vertebra along with large osteolytic lesions within the trabecular centrum and compromising the cortical shell. This heterogeneous distribution of bone required an increase in the number of pyramid levels of the demons deformable registration (to 30) in order to achieve good accuracy in the segmentation of the pathologic vertebrae (Figure 3a). Additional iterations of the level set algorithm were also

used with the inverse speed image to contract/smooth the segmentation boundaries yielding a clear segmentation of the trabecular centrum (Figure 3b).

Figure 3: μ CT scan of (a) axial view of a segmented whole vertebra using the automated segmentation algorithm, (b) axial view of the segmented trabecular centrum and the cortical shell using the automated segmentation algorithm, (c) segmentation of the osteoblastic tumour growth on the outside of the vertebra.

As validated by histology the osteoblastic tumour was predominantly attached to the periosteal surface of the cortical shell (Figures 1 and 3b). To segment the osteoblastic deposition, the trabecular centrum was removed from the whole vertebral segmentation, leaving the outside band of the vertebra including the cortical shell and osteoblastic tumour. Intensity-based histogram analysis was performed on this region. Based on concurrency with manual segmentation, a threshold was chosen as a function of the mean (μ) and the standard deviation (σ) to segment the osteoblastic tissue, with an upper bound of $(\mu + \sigma)$ and a lower bound of $(\mu - \sigma/4)$ for L1, $(\mu - \sigma/2)$ for L2 and L3. (Figure 3c).

The images were then edge enhanced and resampled from $(14\mu\text{m})^3$ to $(8.725\mu\text{m})^3$ prior to the next step of the segmentation. In contrast to earlier work done on rat vertebrae affected by osteolytic destruction alone¹⁵, the presence of osteoblastic tumour resulted in a larger variation in intensity values within the trabecular centrum. This prevented the utilization of a constant intensity based thresholding technique to segment the individual trabecular structure. Rather, a

threshold value equal to the mean intensity value of all the voxels present in the trabecular centrum was employed to effectively segment the complex trabecular structure within these rat vertebrae (Figure 4).

Figure 4: (a) Histogram of the intensity values in the μ CT of the trabecular centrum with the mean of the distribution indicated, (b) Axial view of the segmentation of the trabecular mesh from the μ CT image.

μ MRI: The whole vertebral segmentation output from μ CT scan was downsampled to $(34.9\mu\text{m})^3$ and further wrapped around the contrast μ MR which was previously registered to the μ CT scan. An intensity-based threshold equal to the mean of the segmented region plus half of one standard deviation ($\mu + \sigma/2$) was chosen to define the osteolytic tissue (Figure 5).

Figure 5: (a) Axial view of the μ MR image segmented using the segmentation boundaries obtained from the μ CT image. (b) Segmentation of the osteolytic tumour within the whole vertebra.

The accuracy of the automated segmentations was evaluated by comparing the outputs to manually refined segmentations (representing the gold standard) using a volumetric concurrency (VC) measure. (equation 1)

$$VC = \frac{\frac{Vol_{intersection}}{Vol_{manual}} + \frac{Vol_{intersection}}{Vol_{automatic}}}{2} \quad (1)$$

Where $Vol_{intersection}$ represents the volume of the overlapping region in the manual and automatic segmentations and Vol_{manual} and $Vol_{automatic}$ correspond to the individual volumes of the manual and automated segmentations respectively.

Results

The automated segmentation of the whole vertebrae yielded 91% concurrency in L1, **91.9% $\pm$ 2.8%** concurrency in L2 and 92% concurrency in L3 when compared to manually refined segmentations. The segmentation of the trabecular centrum yielded 93%, **93.8% $\pm$ 1.2%**, and 92% concurrency in L1, L2 and L3 respectively when compared to the manually refined

segmentations. The output of the segmentation of the individual trabecular network did not require any manual refinement. The osteoblastic tissue segmentation on the outside of the cortical shell yielded 80% to **87%** concurrency when compared to manually refined segmentations. Although based on histological analysis osteoblastic tumour was primarily concentrated on the outside of the trabecular centrum, in a few slices they were also detected inside the trabecular centrum. Despite several attempts, including histogram based approaches applied inside the trabecular centrum (with and without the growth plate), we were not able to distinguish newly formed healthy bone from osteoblastic tumour tissue in these vertebrae using the μ CT or μ MR image data, as they possess the same tomographical attitude.

The customized μ MR coil enabled good quality image acquisition of the bony structure as well as good quality images visualizing the non-bone soft tissue structures (bone marrow and osteolytic tumour). Using the contrast μ MRI, we were able to accurately segment the osteolytic tumour with no need for further manual refinement.

Discussion

In this work we have presented a semi-automated multimodal approach to segment rat vertebrae affected by mixed osteolytic / osteoblastic tumour. The whole bone vertebral segmentation algorithm was able to segment metastatic vertebrae despite extensive osteolytic destruction and osteoblastic deposition caused by the aggressive behavior of tumour cells, with very limited user interaction (~1minute). The algorithm was even more robust in segmenting the trabecular centrum, and individual trabecular network clearly accounting for the boundaries of the bone and non bone structures (92-95% concurrencies with manually refined segmentations).

The algorithm was limited in segmenting the osteoblastic voxels (lower volumetric concurrency ~80%) as they appeared as the same intensity as newly formed bone. Future work on older rats

may provide a more sensible osteoblastic deposition pattern that would be more amenable to histogram or feature based segmentation.

A customized μ MR coil was necessary to achieve sufficient resolution in imaging the bony vertebral structure. With a quick change of parameters the coil was also capable of generating robust soft tissue images to allow clear differentiation between bone marrow and osteolytic tumour tissue within the bone.

Except for the initial manual alignment of the images to the global axes of the Amira visualization software and identification of the vertebral levels to be analyzed (~1 minute in total), the remainder of the algorithm was executed automatically, making it a precise and repeatable approach. Rigid registration was shown to be a fast and easy way to register multimodal vertebral images. It was able to consistently register the μ CT and μ MR volumes despite the resampling carried out prior to the registration.

Bone μ MRI was observed to be required as a localizer for registration of the μ CT to the contrast μ MRI, however it did not yield sufficient resolution and contrast (in comparison to the μ CT data) to be used for accurate quantification of the bony structure. Future work post-processing such images may be undertaken in an effort to obtain similar bone and soft tissue segmentation using μ MRI alone.

Achieving sufficient resolution on images remains a concern when attempting to accurately quantify microstructure. We have previously shown that sufficient resolution for accurate segmentation/ analysis of micro-structure of 21 day old rnu/rnu rats is $(8.725 \mu\text{m})^3$ ¹⁵. Upsampling of μ CT images prior to trabecular segmentation can provide sufficient contrast from lower resolution scans $(14 \mu\text{m})^3$. In other applications, however, such as finite element and micro

finite element analysis (FEA and μ FEA), lower resolutions have been shown to be sufficient to yield accurate analysis of vertebral biomechanical behaviour under load ^{23, 24}.

The highly automated registration and segmentation algorithm presented in this study has demonstrated its ability to quantify skeletal structure and metastatic involvement in the spine. Such information could be used to develop mathematical models of vertebrae in order to analyze strain patterns generated through finite element modeling or the comparison of loaded and unloaded 3D images, to estimate ultimate failure loads and to predict fracture locations. Moreover, it could readily be used to analyze micro-structural parameters of metastatic vertebra in preclinical applications looking at quantitative evaluation of new and existing treatments aimed at spinal lesions.

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