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Validation of marrow fat assessment using noninvasive imaging with histologic examination of human bone samples

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Abstract

Purpose—The marrow composition throughout the body is heterogeneous and changes with age. Due to the heterogeneity, invasive biopsies of the iliac crest do not truly represent the complete physiological status, impeding the clinical effectiveness of this method. Therefore, we aim to provide verification for an *in vivo* imaging technique using co-registered histologic examinations for assessment of marrow adiposity.

Methods—Five recently expired (i.e. < 24 hours) human cadavers were scanned with a dual source CT (DECT) scanner in order to measure marrow fat in the lumbar vertebrae. These donors were also imaged using water-fat MRI (wfMRI) which was used to estimate the fraction of yellow marrow. After imaging, lumbar columns were excised and the superior and inferior aspects of 21 vertebrae were removed. The remaining center section was processed for histological examination to find the ratio of adipocyte volume per tissue volume (AV/TV).

Results—Results of DECT and wfMRI had a high correlation ($r = 0.88$). AV/TV ranged from 0.18 to 0.75 with a mean (SD) of 0.36 (0.18). Inter-evaluator reliability for AV/TV was $r > 0.984$. There were similar correlations between AV/TV and the imaging modalities, DECT-derived MF and wfMRI ($r = 0.802$ and 0.772 , respectively).

Conclusions—A high MF variation was seen among the 25 vertebrae imaged. Both DECT and wfMRI have a good correlation with the histologic adipocyte proportion and can be used to measure MF. This makes longitudinal studies possible without painful, less-effective, invasive biopsies

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Keywords

DECT; Water-Fat MRI; marrow verification; imaging co-registration

INTRODUCTION

Cancellous bone of a single vertebral body is heterogeneous, with density varying by as much as 20% [1]. Common *in vivo* methods of assessing cancellous BMD (e.g. DXA, Quantitative Computed Tomography) integrate the density of calcified tissue with marrow density. However, marrow composition can vary from higher density hematopoietic red marrow to less dense adipocytic yellow marrow. Increased marrow fat can also alter microstructure heterogeneity of the trabecular matrix and has also been shown to influence vertebral failure patterns [2–4]. Most bone histopathology studies were limited in focus to studying osseous structures [5–7] or specific pathologic abnormalities in cancellous bone [8–10]. To our knowledge, there has been no study performed to correlate *in vivo* imaging of bone and bone marrow with coregistered histologic quantification of marrow fat.

We present here a study on the correlation between histologic examination of marrow fat fraction and clinical imaging by means of Dual Energy Computed Tomography (DECT) and Magnetic Resonance Imaging (MRI). DECT has the ability to identify marrow composition through use of basis material decomposition techniques [11]. We have already developed water-fat MRI in a previous study to measure the fat fraction signal in bone marrow [12].

METHOD AND MATERIALS

1. Subjects

Donors were accepted into this study as part of a larger investigation into the heterogeneity of cancellous bone. Approval for study was given by the Anatomy Bequest Program at the University of Minnesota. The five donors were female with a mean age of 56.8 ± 8.2 years. 4 of the 5 subjects were diagnosed with cancer and last treated with radiation or chemotherapy within the past 6 months. The diagnoses included cancer of the cervix, breast, and epiglottis and Chronic Lymphocytic Leukemia. Potential subjects were excluded with a history of knee, hip, or shoulder replacement, obesity, or were ever treated with a bisphosphonate. Specific skeletal sites with diagnosed metastatic disease were avoided. Donors were received by the University of Minnesota Anatomy Bequest Program.

2. Imaging

Within 24 hours postmortem, fully intact bodies were brought to the Radiology department at the University of Minnesota Medical Center, Fairview in Minneapolis, MN. Each body was first imaged using a Siemens Somatom Definition Flash DECT scanner (Siemens Medical Solutions USA, Inc., Malvern, PA, USA). The scan utilized Dual source CT at energies of 80kVp and at 140kVp with an additional tin filter for accurate image co-registration. Each donor was scanned cephalic caudal with 1mm slices for both energies. Lack of acceptable resolution resulted with only 20 lumbar vertebra imaged with MRI.

Vertebral body excision also reduced the number of vertebral bodies examined by both imaging modalities and H&E examination to 17 (Table 1).

For DECT, yellow marrow volume fractions (YMF) were calculated from basis material composition estimates for bone ROIs relative to the manufacturer-reported basis material compositions for the CT calibration phantom. Average basis material composition (ρ_{H_2O} , $\rho_{K_2HPO_4}$) was estimated for an ROI placed within matching regions of in CT data sets acquired at 80 kVp and 140 kVp using the following equations:

$$\rho_{H_2O} = \frac{1}{D} \cdot [\beta_{K_2HPO_4,140} \cdot (CT_{80} - C_{80}) - \beta_{K_2HPO_4,80} \cdot (CT_{140} - C_{140})] \quad (\text{Equation 1})$$

$$\rho_{K_2HPO_4} = \frac{1}{D} \cdot [\beta_{H_2O,80} \cdot (CT_{140} - C_{140}) - \beta_{H_2O,140} \cdot (CT_{80} - C_{80})] \quad (\text{Equation 2})$$

$$D = \beta_{H_2O,80} \cdot \beta_{K_2HPO_4,140} - \beta_{H_2O,140} \cdot \beta_{K_2HPO_4,80} \quad (\text{Equation 3})$$

where CT_{80} and CT_{140} are the average CT values within the defined ROIs and $\beta_{H_2O,80}$, $\beta_{H_2O,140}$, $\beta_{K_2HPO_4,80}$, $\beta_{K_2HPO_4,140}$ are calibration slopes determined from CT calibration phantom measurements.

These basis material compositions were interpreted relative to water and a theoretical prediction of the basis material composition of yellow marrow obtained from energy-dependent mass attenuation coefficients calculated using an openly available tool (XCOM) [13, 14], atomic composition data from ICRU Publication 46 and a density of 0.93 g/cm³ [15]. Patient measurements were projected onto the line in the basis material space connecting the basis material composition of yellow marrow and that of water using a minimum distance projection, P, parameterized such that the position P=100% corresponded to yellow marrow and the position P=0% corresponded to water. Since red bone marrow is more dense than water, values of P can range from -66% to 100%. The CT-derived estimate of MF was then normalized to the MRI scale without affecting correlation using: $YMF = 0.6 * P + 0.396$.

After DECT imaging, the donor was imaged on a 3 Tesla MRI scanner (Tim TRIO, Siemens Medical Solutions, Malvern, PA, USA) using a three-point Dixon technique [16–18] as used previously for a in vivo pelvic study [12]. Images were acquired with the donor in supine position using the vendor's spine array receive coil in combination with an anterior body matrix coil centered over the lumbar spine. After localizer scans, a series of 3D gradient echo images were acquired with TR = 9 ms, flip = 1°, matrix 448×266, 256 slices, 1.5 mm slice thickness, acquisition time 225 s. Three consecutive images were acquired with TE = 2, 3, and 4 ms. The raw image data were reconstructed offline in Matlab (MathWorks, Natick MA) using the method of Berglund et al. [18], as implemented in the ISMRM Fat-Water Toolbox [19], and using a 9-peak model for the lipid spectral peaks [20]. The water and fat images produced by the reconstruction were used to calculate the signal fat fraction (sFF), defined as $sFF = \text{fat} / (\text{fat} + \text{water})$. The sFF maps were exported in DICOM format for analysis. ROIs were recorded in order to ensure accurate co-registration with DECT imaging.

3. Excision, registration technique

After clinical imaging, vertebral samples were removed by the University of Minnesota Bequest program staff. Vertebral bodies were separated from the pedicles using a diamond tipped bone saw and placed in formalin. 21 individual Lumbar vertebrae were then cleaned of soft tissue to expose cortical bone.

The vertebral bodies were cut with a DELTA PEC bench band saw (Chang Type, Taiwan) to remove superior and inferior aspects and keep a 5mm section. Taking a histologic section from the center of the vertebral body ensured that the AV/TV was taken from a representative section of the imaging ROI. These sections were decalcified (Formical-2000, Decal Chemical Corp., New York, USA) (20x volume per sample) until the cortical bone was completely demineralized. The sections were cut mid-sagittally to make left and right halves. Each vertebral half was then embedded in paraffin wax and stained by the H&E staining method. Eight vertebral body midsections were processed whole instead of being cut in half so that matching ROIs could be analyzed (Figure 1).

4. Histology image tile procedure

A full digital image of each vertebrae half was produced by image tiling with a Nikon AZ100M Macro Fluorescence Scope (Nikon Corporation, Tokyo, Japan) with a color camera at 4x magnification. The cellular components of the produced images were then analyzed using ImageJ [21]. The scale was calibrated to 512 pixels/mm and the images were converted to an 8-bit gray scale. An approximately 2 mm diameter region of interest in each vertebra half was selected and areas of high artifact were avoided. A threshold was applied to identify adipocytes as void spaces remaining from the H&E staining process. Non-adipocytic void spaces, commonly seen as artifacts in H&E staining, were excluded from this selection process at the discretion of each user. Adipocyte volume per tissue volume (AV/TV) was calculated for each half. The AV/TVs for both halves were averaged together.

In order to test intra-sample variability, histologic examinations were also performed at 0.5cm superior and 0.5cm inferior to the middle in three vertebral bodies. Also, two investigators examined the histology images for the marrow fat ratio in order to test inter-user variability using the intraclass correlation coefficient.

RESULTS

DECT-derived yellow marrow fraction estimations were calculated for all 25 lumbar vertebrae in the five donors. YMF ranged from 0.07 to 0.91 for the lowest and highest fat signal, respectively. For wfMRI-derived signal fat fraction, values could only be determined for 20 lumbar vertebrae; one subject had poor signal to noise ratio above L5 and another had a poor signal to analyze L1. Values ranged from 0.22 for less fatty marrow to 0.79 for the highest sFF.

The AV/TV of 21 lumbar vertebrae ranged from 0.18 to 0.75 with a mean (SD) of 0.36 (0.18). Vertebral bodies taken from the same cadaver showed much less diversity as seen by standard deviations of AV/TV between 0.03 and 0.07. For the three vertebrae that were analyzed at three levels within 1 cm, the average coefficient of variation between the

sections was 0.08. There was a high correlation between the two users as seen by an intraclass correlation coefficient of $r = 0.984$.

17 vertebral bodies were scanned with both DECT and wfMRI and then processed for H&E examination. There were moderate correlations between the AV/TV and the results of DECT and wfMRI ($r = 0.80$ and 0.77 , respectively) (Figure 2a). A higher correlation was seen between the two imaging modalities with similar ROIs than with histology ($r = 0.88$) (Figure 2b).

DISCUSSION

Current clinical practice of marrow fat assessment typically involves an invasive and painful bone marrow biopsy of the posterior iliac crest. However, this method does not give an accurate picture of the heterogeneity that is present in bone marrow throughout the body. For the first time, AV/TV quantifications taken from vertebral bodies have been correlated with results from dual energy CT (DECT) and wfMRI modalities. Both DECT and wfMRI were able to estimate yellow marrow fat fractions as validated by AV/TV in 17 coregistered samples.

Although bone marrow adipocyte parameters have been used to quantify marrow fat from trephine biopsies of the iliac crest [22–25], it is not commonly used on H&E stains of intact vertebral body cross-sections. In our small sample size, we found that inter-subject AV/TV can vary by nearly 60%, and even different intra-subject sites varied by as much as 40%. This high variability could be due to the diverse treatment effects each individual received prior to death or just a reflection of the true heterogeneous makeup of bone marrow within individuals. The technique used to estimate AV/TV is highly reproducible if proper instruction is given for estimating adipocyte grayscale threshold.

1. Clinical implications

With the increased commercial availability of dual energy CT scanners and recent proof of acceptable dose exposure [14], DECT is becoming more prevalent in hospitals and treatment centers. The ability to measure marrow fat at any cancellous bone site could provide a pathway for studies to monitor changes, *in vivo*, using DECT. It may also be useful in radiation therapy treatment planning where targeting of active bone marrow would reduce the radiation treatment exposure to healthy tissue. DECT can easily be adapted in treatment facilities (e.g. radiation oncology departments) where whole body PET/CTs are commonplace and single energy CT imaging is commonly performed to monitor cancer survivors.

2. Limitations

The subjects in this study were all given cytotoxic treatments within the last 6 months before death which is known to affect the bone marrow composition. For donors who underwent radiation therapy, treatment fields and specific absorbed dose were not known for each skeletal site. Also, investigations into the true variation in the average human body would need this examination repeated with a more controlled population. However, this study was focused on validating marrow fat fraction estimation from *in vivo* imaging techniques.

Decomposition processes may introduce some bias into the data. However, these processes are likely to be negligible, as bone marrow from recently deceased individuals is commonly transplanted to immunocompromised patients.

3. Conclusion

In conclusion, cancellous bone regions were examined by histology and co-registered *in vivo* clinical imaging. Marrow fat fraction intensities taken from imaging were validated with histologic quantification of adipocyte prevalence. This may be able to monitor bone health of individuals who have undergone cytotoxic treatment, especially the cancer survivor.

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Figure 1.

Multi-Modality Co-registration between histologic examination and *in vivo* imaging. Two vertebral bodies analyzed: top row is representative case of lower marrow fat and bottom row is higher marrow fat. A. & B. axial section of whole vertebral body with H&E staining. Void spaces of adipocytes were contoured by threshold and artifacts were removed at the discretion of the investigator. C. & D. water-fat MRI images of lumbar vertebrae and example of ROI placement. E. & F. DECT images reconstructed to highlight marrow fat intensities. For imaging modalities, red represents high marrow fat concentration.

Figure 2.

Marrow fat estimation from histology and *in vivo* imaging modalities. A) 17 vertebral bodies were imaged with DECT and MRI and then processed for H&E examination. Results of the imaging are compared with histologic adipocyte volume per tissue volume (AV/TV). B) Correlation between the marrow fat estimation results from two non-invasive imaging techniques.

Table 1

Donor demographics and lumbar vertebrae selection

Donor	Age	Body Mass Index	Disease	Lumbar Vertebrae Examined*		
				Excised	DECT	MRI
1	53	22.9	Cervical Ca	L2-L4	All 5	L2-L5
2	47	20.7	Breast Ca	L1-L4	All 5	All 5
3	62	22.8	Epiglottic Supraglottic Tumor	All 5	All 5	L5
4	68	23.5	CLL	All 5	All 5	All 5
5	54	22.1	Hepatitis	L1-L4	All 5	All 5
Average:	56.8	22.4	Count:	21	25	20

* 17 vertebral bodies were imaged with DECT, MRI, and H&E histology