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Histomorphometric Quantification of Human Pathological Bones from Synchrotron Radiation 3D Computed Microtomography

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ABSTRACT

Conventional bone histomorphometry is an important method for quantitative evaluation of bone microstructure. X-ray computed microtomography is a noninvasive technique, which can be used to evaluate histomorphometric indices in trabecular bones (BV/TV, BS/BV, Tb.N, Tb.Th, Tb.Sp). In this technique, the output 3D images are used to quantify the whole sample, differently from the conventional one, in which the quantification is performed in 2D slices and extrapolated for 3D case. In this work, histomorphometric quantification using synchrotron 3D X-ray computed microtomography was performed to quantify pathological samples of human bone. Samples of human bones were cut into small blocks (8mm x 8mm x 10 mm) with a precision saw and then imaged. The computed microtomographies were obtained at SYRMEP (Synchrotron Radiation for MEdical Physics) beamline, at ELETTRA synchrotron radiation facility (Italy). The obtained 3D images yielded excellent resolution and details of intra-trabecular bone structures, including marrow present inside trabeculae. Histomorphometric quantification was compared to literature as well.

1. INTRODUCTION

Degradation of bone is related to the decrease of trabeculae in the interior of the bone and to a lesser extent to the loss of cortical bone leading to the disease known as osteoporosis. This is a systemic skeletal disease characterized by low bone mass and microarchitectural changes of the bone tissue resulting in decreased bone stability and an increase in fracture risk [1].

Although bone strength is mainly determined by bone mineral density, the crucial role of trabecular bone architecture for predicting fracture risk has been proven in recent years.

Acknowledging the importance of trabecular bone microstructure, new tools that allow a three-dimensional (3D) non-invasive investigation of the bone microstructure and a quantitative analysis of trabecular architecture have been developed [2]. Among these, synchrotron X-ray computed microtomography (SR-μCT) is an important and still relatively new technique in the fields of biomedical engineering and materials science to visualize internal and external geometrical structures, as trabecular bone, directly in three dimensions. In recent years, SR-μCT has been applied as a nondestructive, high spatial resolution method to image and quantify the trabecular microarchitecture [3].

A key advantage offered by μCT is the ability to quantify microstructure in 3D. The final product of 3D microtomographic process is a volume of scalar data that represents the test body through its attenuation coefficients. On biology and medicine, it is common to analyze histological slices using stereology and histomorphometry. Histomorphometric analysis tries to relate values obtained from the histological slices with the 3D structure of the sample. The first method adapting the conventional procedure of histomorphometry to digital images was presented by Feldkamp [4].

To assess bone microarchitecture, 3D stereological indices are extracted according to the standard definitions used in histomorphometry: bone volume (BV), bone surface (BS), bone volume to total volume (BV/TV), surface to volume of the sample (BS/BV), connection thickness (Tb.Th), connection number (Tb.N) and connection separation (Tb.Sp). These parameters have been examined for different anatomical sites and physiological conditions of trabecular bone [5, 6].

In this context, the aim of this work was to perform histomorphometric quantification of trabecular bone microstructures in pathological bones of different parts of human skeleton.

2. METHODOLOGY

2.1. Bone Specimens

A total of seven bone biopsies, including cortical and trabecular bone, were collected from different skeletal sites in post-mortem men and women. The characteristics of each analyzed sample are shown in Table 1. Samples were classified by pathologists as having some kind of disorder (pathological condition). The pieces were fixed with 10% formaldehyde neutral buffered solution. The specimens were then carefully cleaned of non-osseous tissues and were allowed to air dry for 72 hours. The bone parallelepipeds, 8 mm per side and a length of approximately 10 mm, were prepared using a precision circular saw.

Table 1 - Bone samples characteristics

Sample	Skeletal site	Condition	Patient
1	Tibia	Pathological	A
2	Fibula	Pathological	B
3	Femur	Pathological	C
4	Cuboid	Pathological	D
5	Calcaneus	Pathological	D
6	Femur	Pathological	E
7	Humerus	Pathological	G

2.2. Image Acquisition

All specimens were imaged using synchrotron radiation μCT developed on the Synchrotron Radiation for Medical Physics (SYRMEP) beamline which is one of the ELETTRA bending magnets of ELETTRA Synchrotron Radiation Facility. The horizontal acceptance of the source, covered by the front-end light-port is 7 mrad. The beamline provides, at a distance of about 23 m from the source, a monochromatic, laminar-section X-ray beam with a maximum area of about 160x5 mm^2 at 20 keV. The monochromator, that covers the entire angular acceptance of the beamline is based on double Si(111) crystals working in Bragg configuration. The useful energy range is 8.3 - 35 keV. The intrinsic energy resolution of the monochromator of 10^{-4} is reduced to about 10^{-3} because of the natural divergence of the beam. Typical flux measured at the sample position at 17 keV is about $1.6x10^8$ photons/mm^2.s, with 300 mA, at 2 GeV (Abrami, 2005) [7].

The μCT system is based on 3D parallel tomographic acquisition. This set-up consists of a high precision rotary table mounted upon high-accuracy translators and cradles. This allows precise alignment of the rotation axis of the sample with the detector pixels. Three-dimensional imaging was performed by taking two-dimensional radiographs as the specimens were rotated through 180^o in 0.2º increments. The energy was set to 21 keV. The detector used was a water cooled CCD camera (Photonic Science X-ray Hystar, 2048 × 2048 full frame, 16 bit, pixel size = 14 μm, field of view 28 × 28 mm^2) coupled to a Gadolinium Oxysulphide scintillator placed on a straight fiber optic coupler (Fig. 1).

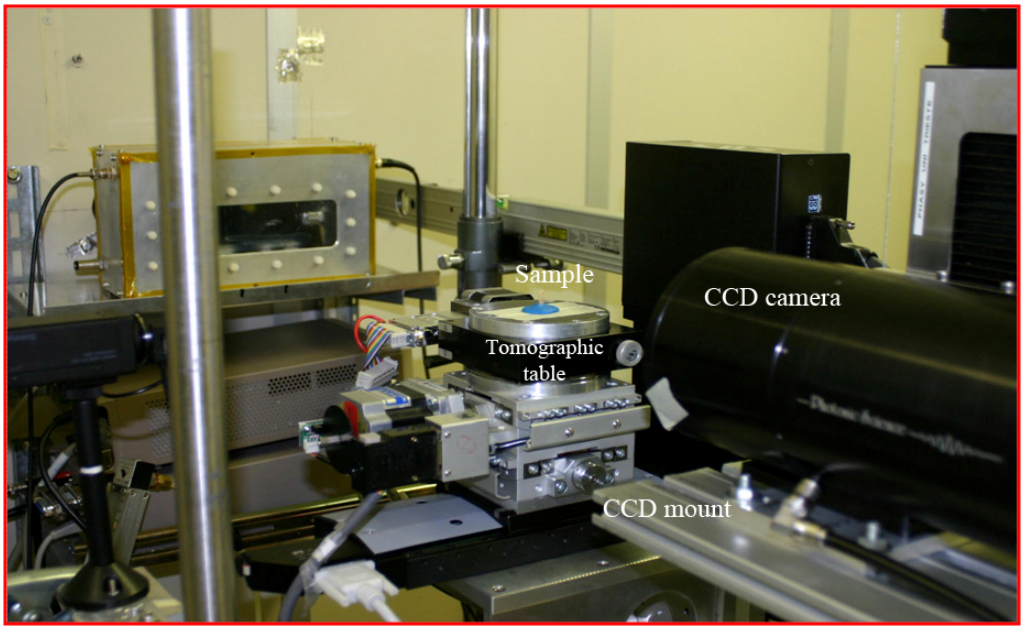

Figure 1. Microcomputed tomography system at SYRMEP beamline

The computed tomographies were reconstructed one slice at a time using a convolution back projection algorithm with the Shepp–Logan filter [8]. The reconstructed slices can be visualized as stacks of 2D images, or 3D views of the sample can be obtained by volume rendering procedures.

2.3. Image Processing

The 3D reconstructed data are a collection of coefficients distributed regularly into the space. Each set of eight coefficients (seen as pixels), displaced as a cube, forms a voxel. Once 3D map of the bone specimens is achieved using μCT, the specimen is fragmented into voxels, each one representing a single solid itself, following the same procedure established to compute the 3D histomorphometric parameters. For conventional technique and the Feldkamp's procedure [4], all parameters are evaluated from slices. The 3D histomorphometry is more compatible with the morphologic parameters: volume and surface. The obtained results reveal the potentials in using 3D tomographies to extract and evaluate histomorphometric parameters compared to 2D analysis since it avoids extrapolations [9].

The 3D tomography gives all the spatial information needed to evaluate the parameters BV/TV and BS/BV directly from the volume. The proposed model uses a voxel representation of the volume of microstructure. The total volume (TV) is the number of voxels contained on the volume data file. The total surface and the volume of the microstructure are counted summing the areas and volumes of each individual model found in the data volume. From the total surface (BS) and the total sample volume (BV), the other parameters can be calculated [10]:

$$\mathrm{Tb.Th} = \frac{2\mathrm{BS}}{\mathrm{BV}} \tag{1}$$

$$\mathrm{Tb.N} = \frac{\mathrm{BS}}{2\mathrm{TV}} \tag{2}$$

$$\mathrm{Tb.Sp} = \frac{2(\mathrm{TV} - \mathrm{BV})}{\mathrm{BS}} \tag{3}$$

3. RESULTS AND DISCUSSIONS

In order to analyze only trabecular region, a centered region of interest (ROI) was selected in each slice, so that cortical bone is eliminated from the images. For that, a bounding box was determined with the ImageJ® software. Figure 2 illustrates the ROI selected on the raw reconstructed images, of a given sample. As quantification has to be performed on 3D bone microarchitecture, it was necessary to select a trabecular bone volume of interest (VOI) within each sample. Three-dimensional tomographic reconstructions of a subvolume from a given specimen are shown in Figure 3.

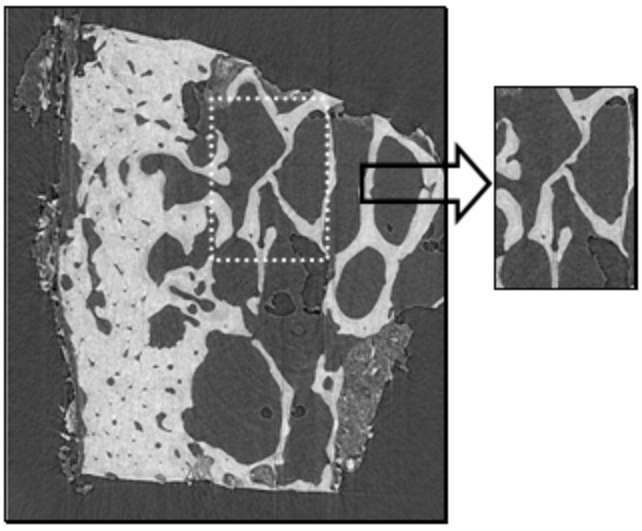

Figure 2. ROI containing trabecular bone for quantification

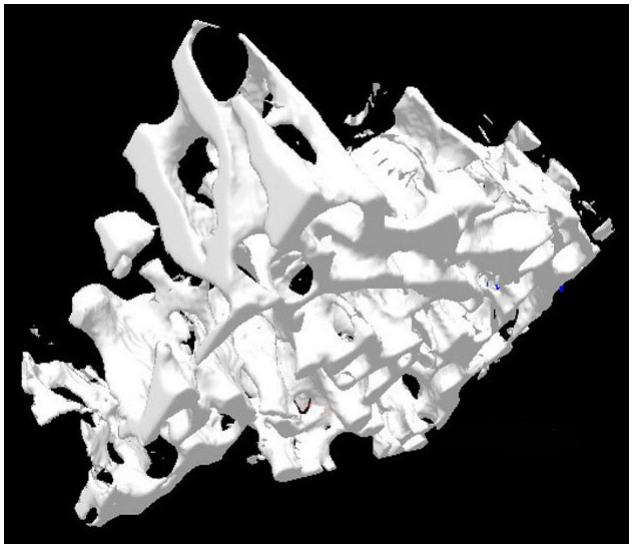

Figure 3. 3D reconstructed image of a given specimen

Prior to segmentation, a median filter was applied to remove noise. The 3D reconstructed volumes were segmented using a global threshold based on [11], above which all voxels are classified as bone and below which all voxels are classified as non-bone. Due to the very good image contrast and high signal-to-noise ratio, gray-level histograms were bimodal (Fig. 4), what makes the segmentation more trustful [12].

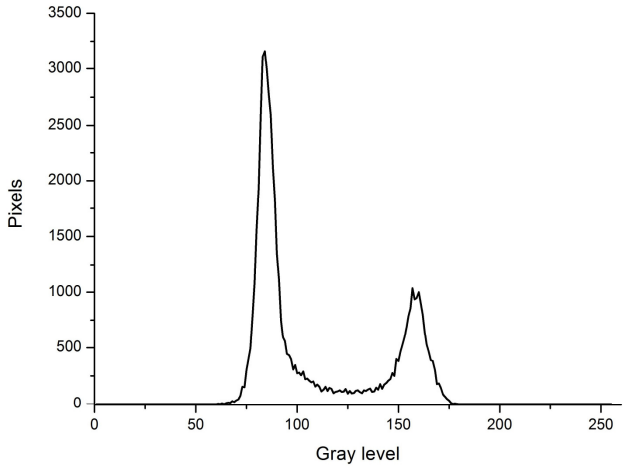

Figure 4. Histogram of a ROI

Trabecular bone consists of a three-dimensional lattice of trabecular plates and fluid-like bone marrow forming a structure with variable porosity and density. For both 3D images groups, all trabecular networks gave the appearance of having fenestrated plate-like structures

interconnected by rod-like struts, although there was considerable variation in geometric structure among specimens.

The quantification of trabecular structures includes the extraction of parameters from the 3D trabecular bone images which were obtained. A special purpose code for histomorphometric analysis [13] was utilized to compute the morphological parameters directly from μCT images that had been segmented following reconstruction. Five morphological parameters were examined: bone volume fraction (BV/TV), bone surface-to-volume ratio (BS/BV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp).

When comparing our results with other authors' results found in literature, concerning bones with some pathology, it can be seen that trabecular bone volume fraction (BV/TV) can reveal noticeable differences between samples. In the femur, the variation is greater than in other sites. In tibia site, bone volume fraction presented a great difference from the results found in literature (64%). However this parameter (BV/TV) alone does not completely characterize bone microarchitecture.

The other parameters also showed large variations between samples. It happens mainly because even when quantification is performed in the same kind of bone, there are differences in microarchitecture from one region to another inside this bone.

Simple regression analyses were used to determine the correlations of BV/TV and Tb.Th, Tb.Sp and Tb.N obtained for pathological bones from our work and other authors' work. In our work, there was a good correlation between bone volume fraction and 3D μCT measured parameters (trabecular number, thickness and spacing). The significant coefficients of determination (R^2) ranging from 0.44 to 0.85 suggest that the measurements of trabecular structure are accurate and reliable, while other authors' coefficients of determination ranged from 0.10 to 0.93 (Figures 5, 6 and 7)

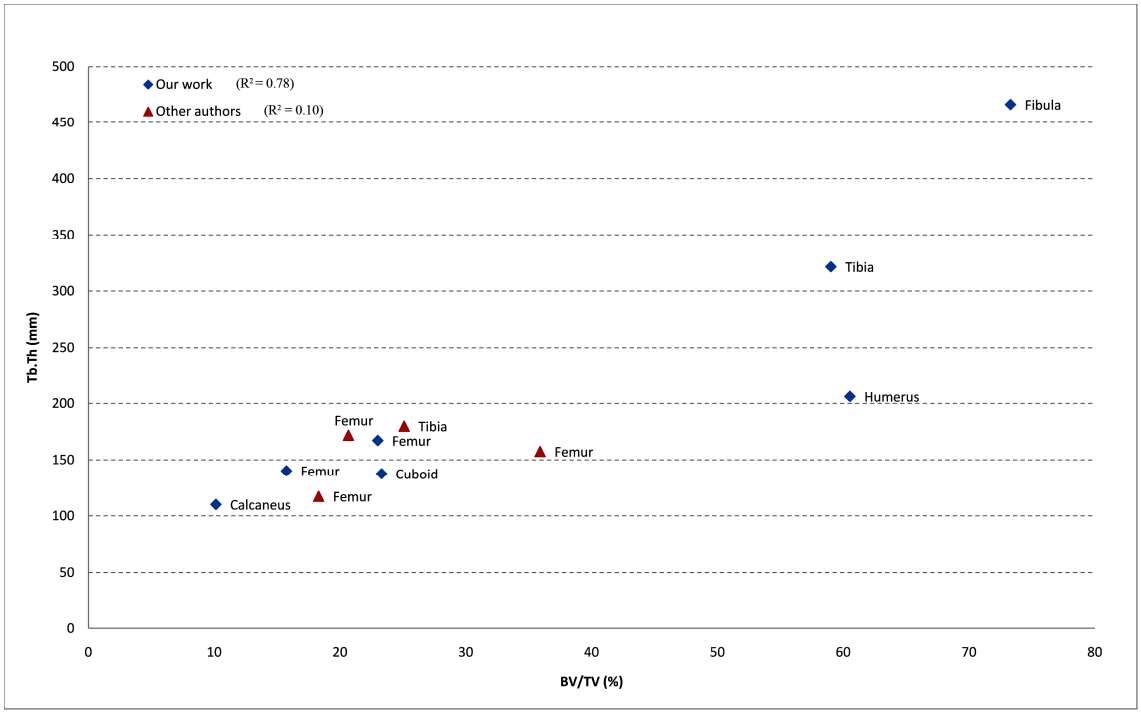

Figure 5. Correlation between BV/TV and Tb.Th of pathological bones from our work and other authors' work [14, 15, 16, 17]

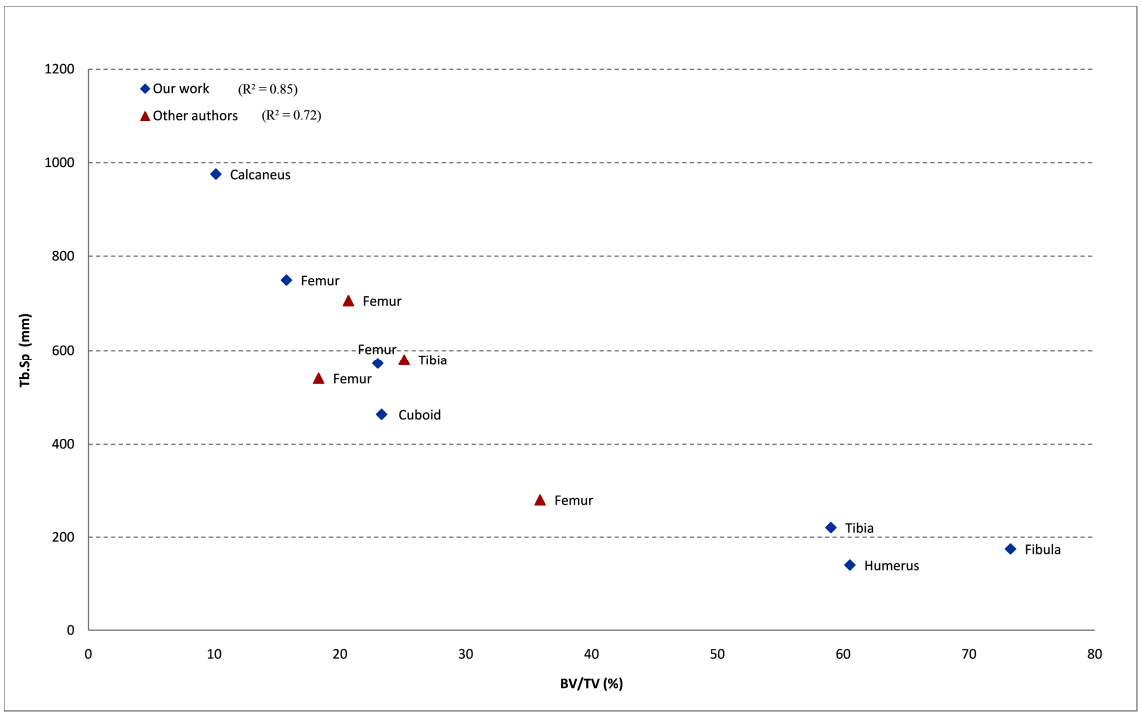

Figure 6. Correlation between BV/TV and Tb.Sp of pathological bones from our work and other authors' work [14, 15, 16, 17]

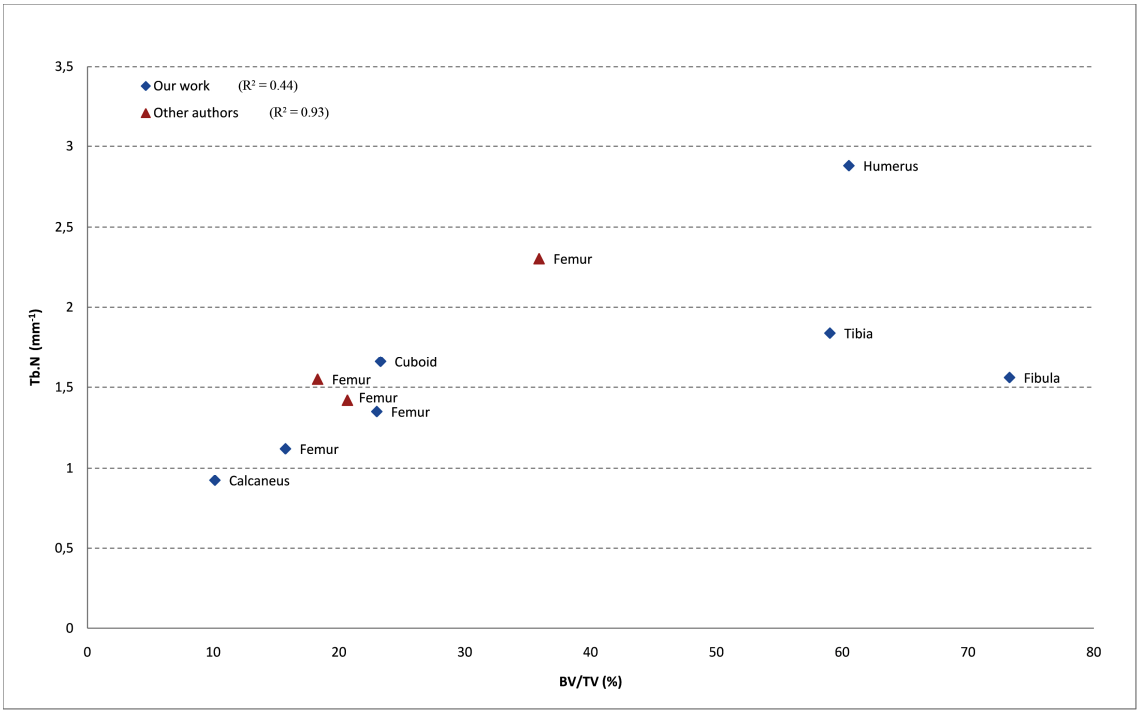

Figure 7. Correlation between BV/TV and Tb.N of pathological bones from our work and other authors' work [14, 15, 16]

4. CONCLUSIONS

In this work, the assessment of bone microarchitecture using SR-μCT was performed and the resulting images showed excellent resolution, mainly due to monochromatic characteristic of the beam (non beam-hardening effects) and the parallel geometry (less approximation in the reconstruction algorithms) which are key points for an accurate binarization and thus, quantification.

The histomorphometric quantification for human specimens yielded coherent results with the literature [14, 15, 16, 17, 18, 19, 20]. Concerning the obtained results for pathological bones from same skeletal site, in comparison to literature, a certain declining bone volume fraction (BV/TV) occurred. Nevertheless, there was good correlation between BV/TV and the other structural parameters.

The major objective of this study was to present a set of measurements of histomorphometric indices on bone microstructures from different pathological bone sites. The high quality of the tomographic images allowed accurate quantification in different kind of bones. This is just part of a greater work, which includes normal and pathological samples from various sites of human bones.

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