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DETERMINING THE MINERALIZED VOLUME FRACTION OF CANCELLOUS BONE

L. Zou, R.D.Bloebaum, J.G. Skedros
Bone and Joint Research Laboratory, VA Medical Center and The Division of Orthopedic Surgery
University of Utah School of Medicine, Salt Lake City, UT 84148

INTRODUCTION: Studying the interrelationship between structure, mineral content, orientation and amount of cancellous bone can greatly improve our understanding of the biological and load carrying function of this tissue [1]. Calibrated high resolution backscattered electron (BSE) imaging can be used to conduct correlated mineral content and histometric analyses so that the interrelationship between the mineral content and amount of bone (volume fraction) can be determined. Since BSEs sample only 1 to 5 microns of tissue depth, BSE imaging is more accurate than conventional light microscopic and microradiographic methods [2]. The use of BSE imaging technology to determine the mineralized volume fraction of bone tissue is based on Delesse's principle, which states that the volume of a porous material can be closely approximated by analyzing an appropriate number of sectional area measurements [3]. The main problem confronting orthopaedic researchers who are interested in determining volume fraction of cancellous bone from sectional area measurements is the lack of data establishing the sample size required for cancellous bone specimens from specific anatomical locales and the number of analyzed sections required in a given specimen of known size and shape.

The objective of the present investigation is to determine the number of transverse and vertical sections needed to approximate the mineralized volume fraction to within 95% of the true values in cancellous bone specimens of known size and shape from three species. Additionally, sample sizes required in population studies of each of the three species will be determined.

MATERIALS & METHODS: Cancellous bone specimens were obtained from condyles of greyhound dog, cow and human femora that had been cut perpendicular to their long axis into 5mm thick slices. From each species, four cylindrical cores (specimens), 7.5mm in diameter and 5mm in height, were drilled from these slices. After being embedded in polymethyl methacrylate, the specimens were ground, polished and coated with gold in preparation for BSE imaging in scanning electron microscope (SEM).

The specimens were imaged at 50X magnification. In each specimen, bone area measurements were made at seven transverse levels (sections) and ten vertical sections. Pilot studies had demonstrated that a greater number of vertical sections would be needed. The area fraction of mineralized bone and ratio of the cumulative areas of mineralized bone to the total of imaged areas, were quantified using image analysis equipment interfaced to the SEM. Mineralized volume fraction of bone in each specimen was approximated from the cumulated transverse section or vertical section area measurement data.

A Bootstrap statistical method was used because of the small number of bone specimens available for analysis [4]. Using this method assumes that the true value of the volume fraction can be determined from the cumulated area measurements when all possible sections are analyzed in either transverse or vertical directions in a given porous specimen. Bootstrap replications were conducted for 1000 of the possible permutations of the number of sections. Using these replications it is possible to estimate the appropriate number of sections needed when using area measurements to approximate the volume fraction of bone within 95% of true value. The sample size (i.e., number of cores, hence number of animals) required was determined using the statistical technique of sample percentiles, where the number of sections required in each specimen represents an estimate of the population percentile of bone specimens in each species [5].

RESULTS: Using four cancellous specimens (cores) from each of three species, mineralized volume fraction can be approximated to 95% of true value in human and dog specimens when seven transverse levels were analyzed (Fig. 1). However, more than seven transverse levels are required for the bovine specimens. Using the same histometric and statistical methods, more than ten sections would be needed when analyzing vertically cut cores from all three species. In this experiment the maximum number of sections obtained from the four specimens from each species represents only the 87.5th percentile of the population. To estimate the 95th percentile of a population distribution, it was calculated that ten specimens would be necessary.

DISCUSSION: The present study provides the statistical basis for a practical sampling technique that can be used to determine the appropriate number of sections needed when bone area measurements are used to estimate the volume fraction of a cancellous bone specimen in the three common species investigated by orthopaedic researchers. The appropriate number mentioned here is considered to be the minimum number of sections that must be analyzed in order to determine the volume fraction of cancellous bone within 95% of the true value in a cylindrical core measuring 7.5mm in diameter and 5mm in height. However, both the sample size (n=10) and number of transverse sections per cancellous core (n=7) exceed the corresponding numbers typically used in studies that have used similar techniques to determine volume fraction of human and canine cancellous bone in similar anatomic locales [6]. Consequently the present study provides useful guidelines when using BSE imaging to analyze the volume fraction of cancellous bone cores. The results herein are relevant in the histometric analysis of bone biopsies, cored specimens used in mechanical testing and the analysis of the amount of cancellous bone adjacent to orthopaedic implants.

The greater number of sections required when cores were examined in a vertical (axial) plane demonstrates how preferred orientation of cancellous bone, if not recognized, can influence the number of sections that may be needed in such analyses. The relatively greater number of transverse sections required in a bovine cores may reflect the relatively higher volume fraction of cancellous bone found in this species, but may also be influenced by preferred trabecular orientations. Future studies will be required to determine if random cuts in cancellous specimen cores from the three species can reduce the number of sections needed to closely approximate their volume fractions from area fraction measurements.

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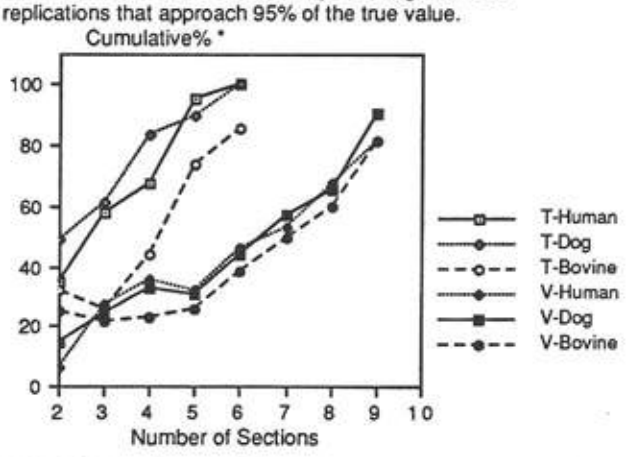
Figure 1: The plot for determining the number of sections in the transverse plane (T) and vertical plane (V) for all three species.
* The cumulative% denotes the percentage in 1000 replications that approach 95% of the true value.



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L. Zou, Bone and Joint Research Laboratory, VA Medical Center (151F), 500 Foothill Blvd., Salt Lake City, Utah 84148.