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ARTÍCULOS ORIGINALES / *Originals*

ASSESSMENT OF TEXTURE AND PREFERRED ORIENTATION OF TRABECULAR BONE OF HUMAN, DRY, CADAVERIC VERTEBRAL SLICES

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Summary

This paper reports an assessment of texture and preferred orientation of trabeculae in tomographic like images acquired from 1.5 mm thick midsagital slices taken from dry, cadaveric, human vertebrae with wedging lower than 8 degrees (sexagesimal). Slices were scanned and the digital images obtained assesed with the Autocorrelation Function (ACF, computed from the Fast Fourier Transform [FFT]). Trabecular thickness and average of medullar spaces areas per unit area were measured on the images. Finally, the slices were submitted to physical analysis (bone tissue volume, ash content). Decay of vertebral trabecular bone was assessed with Vmin/Vmax (ACF ellipticity, a parameter that ranges from aproximately 1,0 when the integrities of vertical and horizontal trabeculae are conserved to 0.3 when the loss of bone mass has ocurred mainly at the expense of horizontal trabeculae). Vmin/Vmax was significantly correlated with the fraction of vertebral volume occupied by bone tissue (BVS/TVS). The latter showed a significant and positive correlation with the vertebral ash density. Average trabecular thickness correlated with the BVS/TVS ratio and the average area of medullar spaces is inversely related with their number per unit area. The data support the hypothesis that the parameter Vmin/Vmax, obtained through the autocorrelation function computed from the FFT of 2D images, can be a surrogate of three dimensional assessment of the integrity of vertebral orthogonal trabecular lattice. Clinical application of this analysis must await the development of imaging techniques capable to examine the trabecular structure at high resolution with radiation levels compatible with clinical use.

Key words: *autocorrelation function, texture, preferred trabecular orientation, trabecular bone, vertebrae, bone morphometry.*

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Resumen

EVALUACIÓN DE LA TEXTURA Y ORIENTACIÓN PREFERIDA DEL TEJIDO TRABECULAR DE CORTES DE VÉRTEBRAS HUMANAS, SECAS, CADAVÉRICAS

Se presenta una evaluación de la textura y orientación preferida de las trabéculas óseas de imágenes obtenidas de cortes medio sagitales, de 1,5 mm de espesor de vértebras humanas, cadavéricas, secas, con acuñamiento inferior a 8 grados sexagesimales. Los cortes fueron escaneados y sus imágenes digitales evaluadas con la Función de Autocorrelación (ACF, computarizada a partir de la Transformada de Fourier [FFT]). El grosor de las trabéculas y el número y áreas de los espacios medulares fueron medidos en cada imagen. En cada corte se midieron el volumen de tejido óseo y el contenido de cenizas. La textura del tejido trabecular fue evaluada mediante el parámetro Vmin/Vmax (ACF, "ellipticity", cuyo valor varía entre 1,0, cuando la integridad de las trabéculas verticales y horizontales está conservada y 0,3 cuando ocurre la pérdida de masa ósea principalmente a expensas de las trabéculas horizontales). Este parámetro correlacionó significativamente con BVS/TVS, la fracción del volumen vertebral ocupada por tejido óseo. La variable BVS/TVS se correlacionó significativa y positivamente con la densidad mineral del tejido vertebral. El grosor trabecular promedio se encontró correlacionado con BVS/TVS. El área promedio de los espacios medulares mostró una correlación exponencial negativa con su número por unidad de área. Este trabajo sugiere que el parámetro Vmin/Vmax puede subrogar la evaluación tridimensional de la integridad de la trama ortogonal de las trabéculas vertebrales. La aplicación clínica de este análisis deberá aguardar el desarrollo de tecnologías capaces de examinar la estructura trabecular con alta resolución a niveles de radiación compatibles con su uso clínico.

Palabras clave: función de autocorrelación, transformada de Fourier, textura, orientación trabecular preferida, hueso trabecular, vértebra, morfometría ósea.

Introduction

Current diagnostic methods for vertebral osteoporosis focus on measurement of bone mineral density (BMD). The architecture of vertebral trabecular bone change as bone loss progresses.[1]

We became interested in the quantification of trabecular architecture and decided to explore the utility of the Autocorrelation Function (ACF, computed from the Fourier Transform) to produce images that might alert the clinician on the probability of vertebral collapse, The tecnique was first suggested by Cleek et al. [2] working with two human vertebral bone slices and checked with aluminum foam slices. This paper confirms that trabecular bone architecture and preferred orientation of trabeculae can be assessed by this technique. The work was performed with human, dry, cadaveric vertebrae.

Material y Methods

Identification and preparation of vertebrae.

The vertebrae employed in this study were obtained at the Anatomy Museum of this medical school. We had access to 25 incomplete skeletal packages: 12 males and 13 females. Sex and age were assigned through several attributes of crania and pelvis.[3] All skeletons were of subjects over 50 years of age. We selected one vertebra per subject, either thoracic or lumbar, without macroscopic superficial signs of damage. Wedging of vertebral bodies (see below) was, in all cases, inferior to eight degrees.[4] The set of vertebrae under study was composed as follows. Males: four lumbar (L1-L4) and seven thoracic (T10-T12) vertebrae. Females: eight lumbar (L1-L4) and five thoracic (T11-T12) vertebrae.

The neural arch of each specimen was removed with a hand saw. The arch was sectioned through the pedicles, close to the vertebral body. The antero-inferior border of each end plate was marked for consistency in orientation.

Wedging of the vertebral body
This variable was assessed measuring (in mm) the anterior (A) and posterior heights (B) and the antero-posterior diameter (C) of vertebral body, with the formula:

$$\phi = 2 \sin^{-1} [(B-A) / 2C]$$

Mid-sagital slices of vertebral bodies
These were obtained with a 0.5 mm thick, low-speed diamond saw (Isomet, Buehler). After removal of the neural arch, midsagital slices (1.5 mm thick) were obtained. Cutting was done at the middle of the large, irregular aperture where the basi-vertebral veins exit from the vertebral body. Actual thickness of the slices (mm) was measured with a micrometer, to a 0.1 mm precision.

Dry weight of slices.
After drying at 105 °C for one hour, slices were weighed with an analytical balance and their weights expressed in mg.

Bone volume of section (BVS, mm^3)
The volumen of trabecular plus cortical bone was measured by application of the Achimede´s Principle. The slices were weighed while submerged in ethanol (density: 0.821). The following formula was used:
BVS (mm^3) = (weight in air, mg) – [(weight submerged in ethanol, mg)/0.821]

Ethanol was used instead of distilled water because its low surface tension avoided the formation of bubbles within bone trabeculae.

Total volume of section (TVS, mm^3)
This variable was estimated by the product of the area of section (mm^2, measured on the digital image with the image analysis program) multiplied by its thickness (mm).

Bone volume as a fraction of total bone of section (BVS/TVS, %)
This variable was calculated as the ratio between the volume of bone tissue (trabecular + cortical) and total volume of the section, expressed a % of the latter. The two independent estimates of BVS/TVS (application of the Archimede´s Principle) and morphometric assessment of digital images were found significantly correlated ($p<0.0001$).

Weight of ashes (mg)
Slices were incinerated at 500ºC during 10 hours. The weight of ashes was recorded in mg.

Ash Density (AD) is reported as mg of ashes per mm^3 of trabecular + cortical bone

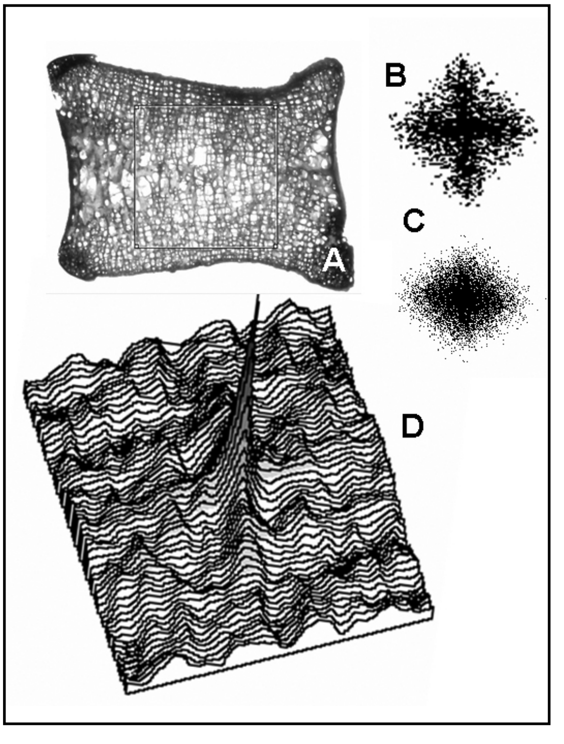

Figure 1. A. Digital image of a midsagital section of a vertebral body. **B.** FFT of the area marked in A. **C.** Autocorrelation Function image of B. **D.** Surface plot of C.

Trabecular bone texture analysis

The following definition of bone texture was adopted:[5] a feature used to partition images into regions of interest and/or to classify those regions and/or to compare regions or samples and/or to extract image features.

Texture analysis was performed through the Autocorrelation Function Analysis of Fast Fourier Transform.[6]

a) Digital image of slices were obtained by transparency (as if they were slides) with a scanner (Genius, HR6V2) at 300 dpi.

b) The images were converted to 256 gray levels by the transforming the RGB planes with the NIH Image J program.[7] A ROI of 512 x 512 pixels was selected and moved to the center of image. The ROI area covers approximately 70% of the vertebral section (Figure 1A). The image of the FFT (Figure 1B) and of the autocorrelation function of the FFT were then obtained (Figure 1C).

c) The surface plot of the latter was then be obtained (Figure 1D). Thresholding is set at the 32nd, 63rd, 96th, 128th, 160th and 192nd levels of gray. The program produces the images of slices at the most informative zone (low frequencies, peak in Figure 1d) at the indicated levels of gray. They are copied to a new file labelled "slices" ("particles" in the language of the program). The program then measures the length of the minor (Vmin) and major (Vmax) axes of particles (Figure 2). The ratio Vmin/Vmax (ACF ellipticity; mean±standard error) as shown below, is a function of the bone loss at the expenses of horizontal trabeculae.

d) The angle formed by those axes (reported in sexagesimal degrees) indicate the preferred orientation of trabecular structure.

Morphometric analysis

These analysis were done as a check of the ratio BVS/TVS. A key step in the morphometric analysis of bone digital images is the segmentation of the greyscale images into a bone phase and a marrow phase. Automated global thresholding was done according to Otsu´s method [8] available as a plug-in in the Image J program.[7] Measurements were done on the upper, middle and lower thirds of each image. Sampling of the digital images was done within a ROI close to 10^5 pixels (184 x 560 pixels). The thickness of horizontal trabeculae were measured tracing a line from the frontal to caudal sides of the images [excluding cortical bone] at the center of the upper, middle and lower third of each image. Average overall trabecular thickness is reported in microns and qualified as "apparent" on account of the section thickness. The number of medullar spaces and their individual areas were measured within the ROI. Trabecular bone is reported as a percentage of the ROI not occupied by medullar spaces.

Statistical analysis

Conventional statistical techniques were used for the assessment of the data.[9,10] Data are reported as mean±SEM, and Student´s "t" tests and ANOVA were performed to assess the significance of differences: $p<0.05$. Linear regressions were used with the exception of a one component exponential decay function to fit the data displayed in Figure 5B:

$$Y = Y_{max} \times (1 - e^{-kt})$$

The best fit of the data to a function was performed with the aid of a computer program whose algorithm proceeds by interations until the difference between two consecutive sums of squares is smaller than 0.01%.

Results

The data reported in this paper was obtained studying 25 selected dry vertebrae (thoracic and lumbar) from 12 male and 13 female adult subjects. Analyses were performed on midsagital, 1.26±0.16 mm thick slices. As expected, the areas of slices were significantly greater for men (742±33 mm^2) than for female (532±13 mm^2, p=0.0002) subjects. No significant difference (p=0.587) was observed in ash density between thoracic (1.90±0.24) and lumbar vertebrae (1.82±0.45). No significant difference in the wedging of vertebrae [4] was observed between sexes (males: 3.9±1.5°, females: 3.4±1.7°; $p=0.9121$).

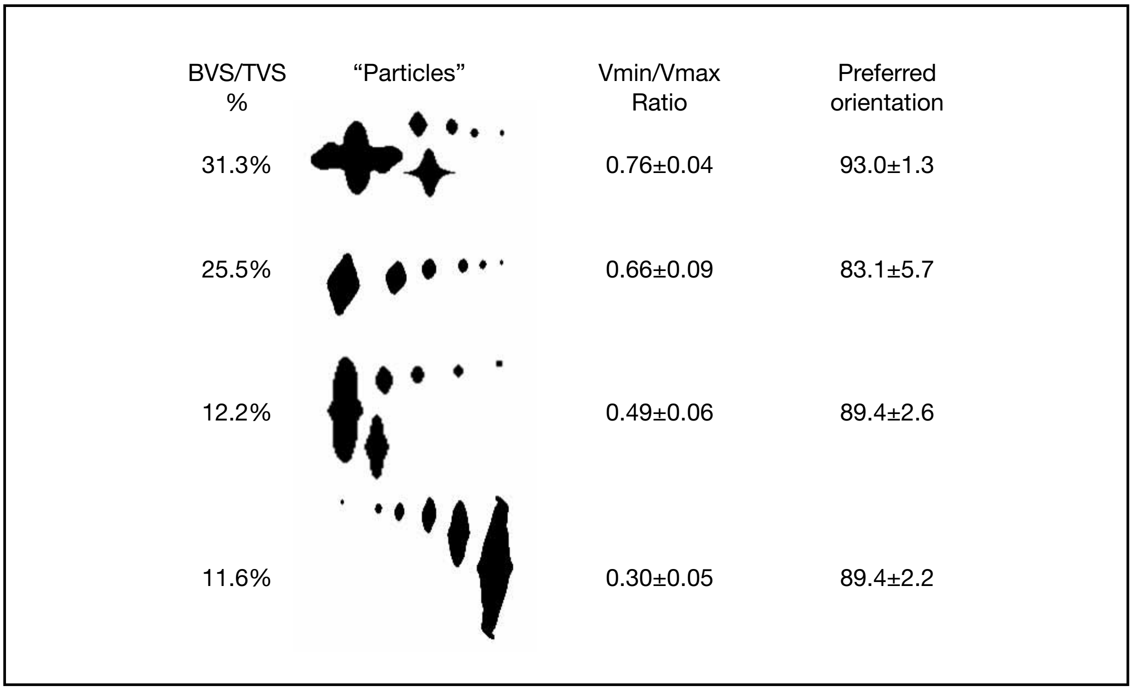

Figure 2. Four examples of Autocorrelation Function data. From left to right: fraction of bone (trabecular+cortical) occupying the midsagital section of vertebrae, sections of the central peak shown in Figure 1D at six gray levels, at the peak of lower frequencies, the Vmin/Vmax ratio of the latter and the preferred orientation of trabeculae. The reader should note that the image of FFT is rotated 90º in the plane of the image. Therefore, the horizontal and vertical axes of particles correspond to craneo-caudal and antero-posterior trabeculae, respectively.

Data obtained through the Autocorrelation Function of FFT

Figure 2 illustrates four examples of the vertebrae investigated in this work. Trabecular bone volume as a percentage of total section volume ranged from 31.3% to 11.6% of total bone volume (similar to that reported by Mosekilde[11]), together with data furnished by the autocorrelation function: a) the images of six virtual slices obtained from the peak of the surface plot, at six different levels of grays (see M&M), b) the ratio between axes (Vmin /Vmax) and c) the preferred orientation of trabeculae. The reader should note that the image of FFT is rotated 90º in the plane of the image. Therefore, the horizontal and vertical axes of particles correspond to craneo-caudal and antero-posterior trabeculae, respectively. With the progress of age, the former trabeculae deteriorates more rapidly than the latter. In agreement, a significant relationship between the ratios Vmin/Vmax and BVS/TVS were observed ($R^2 = 0.7765$, $p<0.0001$, Figure 3).

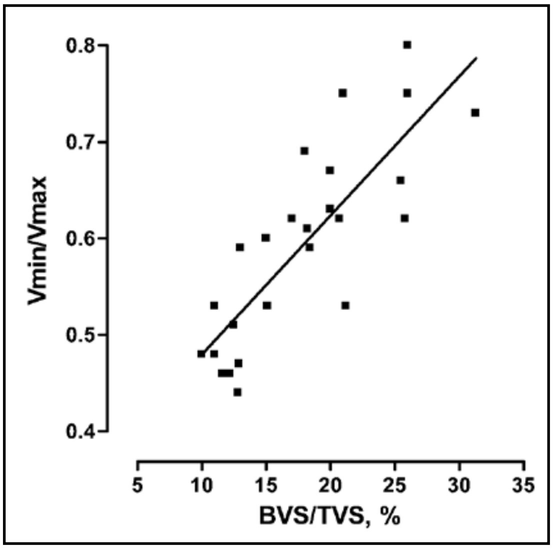

Figure 3. Average Vmin/Vmax as a function of the ratio BVS/TVS (the fraction of vertebral tissue volume occupied by bone tissue).

Relationships between physical and morphometric analyses

The highly significant correlations reported below between BVS/TVS and morphometric data, support the notion that they are complementary to each other.

Ash density per unit of bone tissue or vertebral bone volume are close to those reported by Mosekilde et al.[12] The ash densities values per cm^3 of vertebral tissue volume were positively correlated with BVS/TVS (Figure 4; r=0.738, p=0.0001).

Analyses of thresholded images showed that bone trabeculae ocuppied a fraction of the middle third of images (0.43±0.02) which was significantly lower than in the upper (0.58±0.04, p=0.0024) or lower third of slices (0.53±0.05, p=0.0076). No significant differences were observed comparing the upper vs. the lower thirds of images (p=0.439).

The overall apparent trabecular thickness of vertical trabeculae was directly related with the bone mass of slices (p<0.0001, Figure 5A). The number of medullar spaces per unit area of 10^5 pixels was inversely related with their average area. The data fitted a 1-component exponential decay function (R^2=0.801, p<0.0001; Figure 5B).

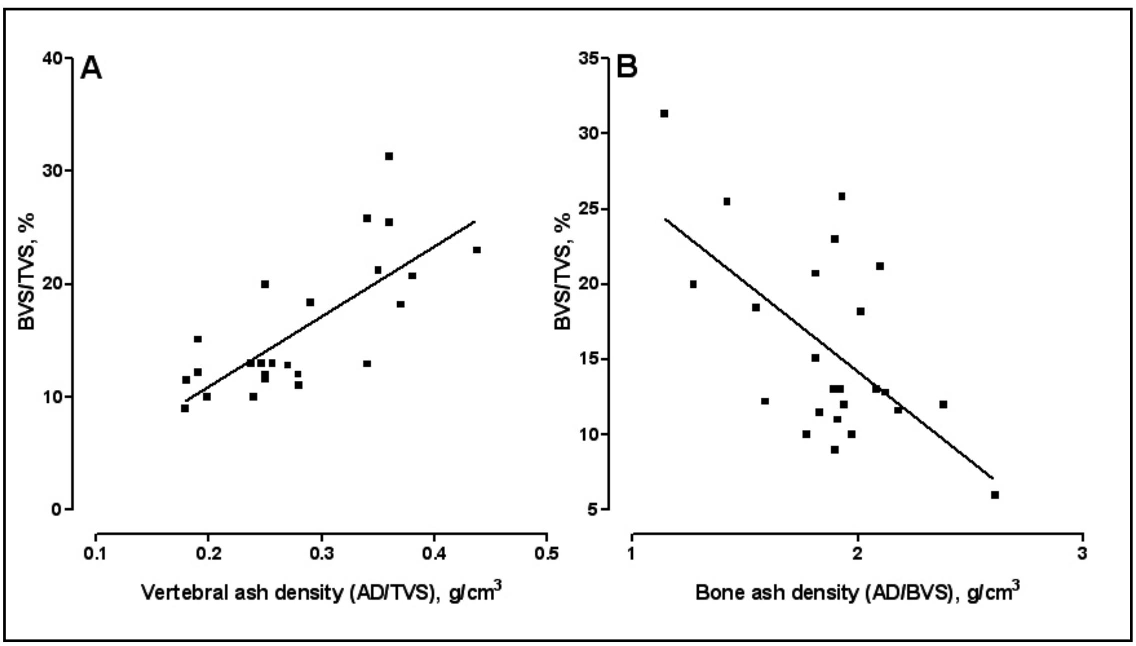

Figure 4. A. Vertebral ash density in g/cm^3 as a function of vertebral tissue volume occupied by bone. **B.** Bone ash density (AD/BVS) in g/cm^3 as a function of vertebral tissue volume occupied by bone (BVS/TVS).

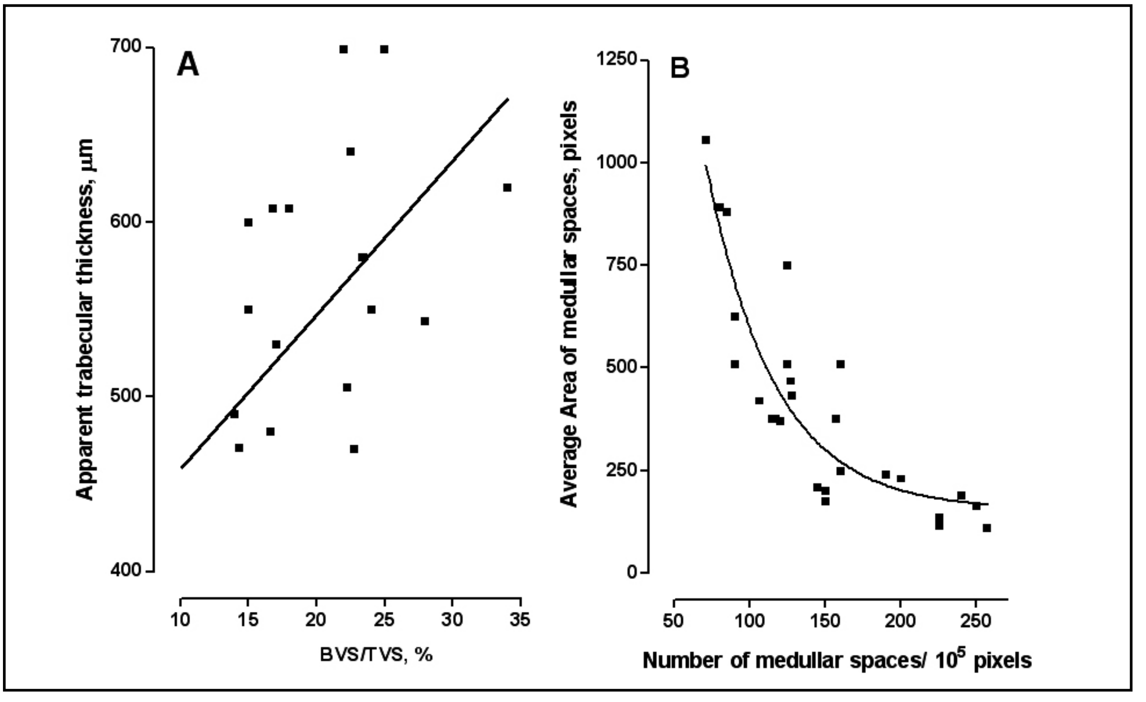

Figure 5. Morphometric measurements were performed on the upper, middle and lower third of vertebral sections. **A.** Average overall apparent thickness of vertical trabeculae are plotted as a function of vertebral volume occupied by bone. **B.** Relationship between the average area of medullar spaces as a function of their number.

Preferred orientation of trabecular

No significant difference between sexes was found in the preferred trabecular orientation (males: 88.3±0.8°; females 88.4±1.3°; p=0.921). The preferred orientation of trabeculae, other variable furnished by the autocorrelation function, did not differ from 90° in agreement with the selection of vertebrae. The data obtained suggests that changes in the preferred orientation of trabeculae may be expected after the loss of two thirds of healthy vertebral bone mass.

Discussion

Texture analysis of vertebral trabecular lattice

This paper presents a study done on 25 human, dry, cadaveric vertebrae, obtained from skeletons of men and women over 50 years of age.

Current diagnostic methods for vertebral osteoporosis focus on measurement of bone mineral density (BMD). The latter do not fully account for the effect of changes in other aspects of bone quality such as architecture, tissue properties and levels of microdamage. The vertebral trabecular architecture decays as bone loss progresses.[11] It can withstand normal daily loads in the cranio-caudal axis but is more susceptible to failure from unusual off-axis loads.[12,13]. There is also evidence that the decay is a significant factor in determining fracture risk as the trabecular structure adapts to compensate for continuing bone loss.[14,15] Figure 2 exhibits a summary of the data furnished by the ACF associated with the vertebral bone mass. The preferred orientation of trabecular lattice from all individuals investigated agrees with the fact that wedging of vertebral bodies were within normal values.[4]

As stated in Material and Methods, the ratio Vmin/Vmax is a function of the bone loss (Figure 3), mainly at the expense of horizontal trabeculae (Figure 2). With the progress of age and the disappearance of the horizontal sustaining trabeculae,[16] the slenderness ratio of the remaining trabeculae increases. When this ratio reaches a critical size (about 100:1),[11,16] (presumed to occur at BVS/TVS below 10%) structural collapse may occur, most likely due to elastic buckling and bending than to sudden compressive forces. The integrity of the three dimensional orthogonal trabecular lattice, an important qualitative property, can be assessed by the Vmin/Vmax. Early diagnosis of vertebral architecture is instrumental for the prevention of fractures. Current evidence indicates that once the trabecular latice is disrupted, it cannot be restored with therapeutic drug treatments.[17-19]

As expected, a significant correlation was observed between vertebral ash density and the BVS/TVS ratio (Figure 4). This agrees with several studies [11,16,21-23] reporting that vertebral body ash density correlates negatively with the progress of age (plus a series of contributing factors: nutrition, physical activity, sun exposure, etc). Morphometric analysis of the lattice gave supporting complementary data. Apparent trabecular thickness is correlated with the BVS/TVS ratio (Figure 5A) and the average area of medullar spaces is inversely related with the number of such spaces per unit area (Figure 5B).

Concludind remarks

Early diagnosis of vertebral architecture is instrumental for the prevention of fractures. This paper shows that a parameter (Vmin/Vmax) obtained through the autocorrelation function computed from the FFT of 2D images, can be a surrogate of three dimensional assessment of vertebral orthogonal trabecular lattice.

The data obtained suggests that changes in the preferred orientation of trabeculae may be expected after the loss of two thirds of healthy vertebral bone mass. Finally, a sour note. The reader should note that the quality of digital images obtained with the technique indicated in M&M is comparable to that attained with µCT scanners. The development of imaging techniques capable to examine the trabecular structure at high resolution (in the order of 20 µm) with radiation levels compatible with clinical use will provide one avenue through which the effect of changes in bone quality may be better understood.

Conflict of interest

The authors have no conflict of interest to declare.

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