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Biomechanical properties and microarchitecture parameters of trabecular bone are correlated with stochastic measures of 2D projection images

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Abstract

It is well known that loss of bone mass, quantified by areal bone mineral density (aBMD) using DXA, is associated with the increasing risk of bone fractures. However, bone mineral density alone cannot fully explain changes in fracture risks. On top of bone mass, bone architecture has been identified as another key contributor to fracture risk. In this study, we used a novel stochastic approach to assess the distribution of aBMD from 2D projection images of Micro-CT scans of trabecular bone specimens at a resolution comparable to DXA images. Sill variance, a stochastic measure of distribution of aBMD, had significant relationships with microarchitecture parameters of trabecular bone, including bone volume fraction, bone surface-to-volume ratio, trabecular thickness, trabecular number, trabecular separation and anisotropy. Accordingly, it showed significantly positive correlations with strength and elastic modulus of trabecular bone. Moreover, a combination of aBMD and sill variance derived from the 2D projection images ($R^2=0.85$) predicted bone strength better than using aBMD alone ($R^2=0.63$). Thus, it would be promising to extend the stochastic approach to routine DXA scans to assess the distribution of aBMD, offering a more clinically significant technique for predicting risks of bone fragility fractures.

Keywords

Trabecular bone; distribution of areal bone mineral density; experimental variogram; sill variance; DXA

Introduction

Osteoporotic fractures are a vital public health concern and create a great economic burden for our society [1, 2]. It is estimated that more than 2 million fractures occurred in the United States at a cost of \$17 billion each year [3]. It is critical that we identify those at highest risk in the population and reduce the number of osteoporotic fractures. The standard

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clinical measurement of areal bone mineral density (aBMD) by Dual-energy X-ray absorptiometry (DXA) gives only a rough estimate of fracture risk [4, 5]. Numerous studies have indicated that bone strength is only partially explained by aBMD [6, 7]. aBMD actually represents the amount of bone mass and indicates the quantity, not the quality of bone. However, both quantity and quality of bone are determinants of bone fragility. Bone quality is defined as the totality of features and characteristics that influence a bone's ability to resist fracture [8]. Among such features are ultrastructure, microarchitecture, microdamage, and remodeling rates in bone. Particularly, one of major contributors to bone fragility is the microarchitecture of trabecular bone.

Due to limitation of aBMD from DXA in assessing bone fracture risks, several approaches have been recently developed to provide supplemental information. One approach is to directly reconstruct 3D structure of trabecular bone using high resolution imaging modalities. The primary assumption is that bone strength is dependent on its architecture. The most common techniques are computed tomography and magnetic resonance imaging that can produce high resolution images to directly assess 3D microarchitecture of trabecular bones. High resolution peripheral quantitative computed tomography (HR-pQCT, also known as *in vivo* Micro-CT technique) is one of the most promising 3D imaging techniques. Numerous studies in both clinical settings [9] [10] [11, 12] and *in vitro* situations [13, 14] have shown the effectiveness of HR-pQCT in assessing microstructure of trabecular bone in human tibia and distal radius. HR-pQCT technique may also provide direct measurements of microarchitecture of trabecular bone *in vivo*. However, the general public has limited access to such facilities, with the affordability being a major concern. Such an imaging modality still remains a high end research tool [8].

Another approach is to extract the hidden geometric and microstructural features of bone from the existing 2D projection image modalities through imaging process techniques. Fractal analysis, one of such techniques, has been widely applied to high resolution 2D radiography images in both clinical and *in vitro* studies [15–24]. In clinical studies, fractal analyses of trabecular bone from calcaneus and distal radius radiographs have helped distinguish the patients with osteoporotic fractures from those in an age-matched control group [18, 23, 25, 26]. Previous study has shown that the fractal analysis of texture on calcaneus radiographs was able to discriminate osteoporotic patients with vertebral fracture from controls [25]. In *in vitro* studies, fractal analysis of radiographs has been used to predict 3D microarchitecture of trabecular bone [16, 19, 21, 22, 24, 27]. For instance, 2D fractal analyses of calcaneus and femoral neck from Micro-CT images [22, 27], and femoral head from Magnetic Resonance images [19] have assisted in predicting 3D microarchitecture parameters of the trabecular bones.

Fractal analysis has been used to identify the correlations between the so-called fractal dimension and microarchitectural features of trabecular bone in high resolution (e.g., high resolution radiographs), but not for 2D projection images with moderate resolutions (e.g., DXA scans). The reason is that fractal analysis requires a large projection surface and distinguishable textures [28] from high resolution images. However, limited resolution of DXA images does not fulfill such requirement. These constraints on fractal analysis make it unsuitable for analysis of small surface with moderate resolution, such as DXA images.

In this study, a stochastic method was proposed to examine the 2D projection images of trabecular bone and assess the heterogeneity of areal bone mineral density distribution. It has been debated that the image resolution required for clinical evaluations may be much less than that needed in basic research [8]. If useful information from bone microarchitecture, such as heterogeneity of spatial mineral distribution, can be extracted from the low resolution images, the improvement of imaging resolution would become less

important. The heterogeneity of mineral spatial distribution in bone has been shown to influence the resistance of bone to failure by both theoretical arguments and empirical data [29, 30]. Therefore, assessing such spatial heterogeneity in bone mineral density becomes clinically significant. In fact, variations of grey scale values in 2D projection images, such as DXA images, reveal the spatial distribution of areal bone mineral density. Additionally, the variation of bone mineral density distribution is statistically random as it results from numerous complex biological processes (e.g. mineralization, bone remodeling) in a highly non-linear and unsystematic fashion. Thus, we need to adopt stochastic approaches to examine the 2D projection images and quantitatively assess the heterogeneity of areal bone mineral density.

The objectives of this study were: (1) to develop a stochastic approach for assessment of aBMD distribution using 2D projection images of trabecular bone; (2) to identify the correlations of stochastic parameters of 2D projection images with the mechanical properties and microarchitecture of trabecular bone; (3) to examine whether it is feasible to extend such stochastic assessment to 2D projection images with a resolution comparable to DXA images; (4) to investigate whether a combination of aBMD and stochastic assessment of aBMD distribution would enhance the prediction of bone fragility than using aBMD alone.

Materials and methods

Microarchitecture parameters and biomechanical properties of trabecular bone specimens were obtained from 3D Micro-CT images and uniaxial compression tests on a mechanical testing system, respectively. Then, variogram-based stochastic analysis was derived and verified using simple 2D lattice structures to reveal the physical meaning of stochastic measures and their connections with microarchitecture of trabecular bone. Finally, this stochastic approach was used to analyze 2D projection images of trabecular bone samples.

Micro-CT images of trabecular bone specimens

3D Micro-CT images of trabecular bone specimens and their microarchitecture parameters were obtained from previous studies [31, 32]. Fifteen cylindrical specimens of trabecular bone with a diameter of 8 mm and a height of 10 mm were cored from the proximal tibias of six male human cadavers (48 ± 14 years old) that were free of bone diseases. These trabecular bone specimens were scanned by a high resolution Micro-CT system following established techniques [33, 34]. Raw images of trabecular bone specimens were reconstructed to produce 3D Micro-CT images with a voxel size of 50 μ m. A custom-written program was used to compute microarchitecture parameters from 3D Micro-CT images [32]. Bone volume fraction (BV/TV), bone surface-to-volume ratio (BS/BV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp) were calculated using stereology principles [35]. Anisotropy was calculated as the maximum to minimum mean intercept length ratio [35].

Biomechanical properties of trabecular bone specimens

Measurement of ultimate strength and elastic modulus of the trabecular bone specimens was also reported in the previous study [31]. Uniaxial compression test of these bone specimens was performed on a mechanical testing system (Model 8501, Instron Corp, Canton, MA) with an extensometer attached to the specimens. A thin layer of cyanoacrylate adhesive was used to glue bone specimens to brass end caps so that the effect of end artifacts can be eliminated [36]. The specimens were compressed to fracture with a strain rate of 0.1% per second. Ultimate strength and elastic modulus of the cylindrical specimens of trabecular bone were determined from the stress-strain curve as the maximum stress sustained by the

sample during the compression test and the slope of the linear region of the stress–strain curve, respectively [31].

Stochastic assessment of inhomogeneity through variograms

Stochastic assessments of inhomogeneity (variation of aBMD distribution in this study) can be described by experimental variograms, which are widely used in geosciences [37–39]. Previous studies have introduced experimental variograms to describe the inhomogeneity of bone properties [28, 40, 41]. The concept of experimental variograms is briefly described here. A semi-variance, $\gamma(\mathbf{h})$, is defined as the half of the expected squared differences of bone properties between any two data locations with a lag distance of $\mathbf{h}$.

$$\gamma(\mathbf{h}) = \frac{1}{2} E[\{Z(\mathbf{x}) - Z(\mathbf{x} + \mathbf{h})\}^2] \quad (1)$$

where $Z(\mathbf{x})$ is a function to describe the random field of bone properties; Both $\mathbf{x}$ and $\mathbf{h}$ are vectors; $\mathbf{x}$ is the spatial coordinates of the data location. Lag distance, $\mathbf{h}$, represents the Euclidean distance and direction between any two data locations.

The experimental variogram is calculated as an average of semi-variance values at different locations that have the same value of lag distance ($\mathbf{h}$).

$$\hat{\gamma}(\mathbf{h}) = \frac{1}{2m(\mathbf{h})} \sum_{i=1}^{m(\mathbf{h})} E[\{Z(\mathbf{x}_i) - Z(\mathbf{x}_i + \mathbf{h})\}^2] \quad (2)$$

where $m(\mathbf{h})$ is the number of data pairs for the observations with a lag distance of $\mathbf{h}$.

A simple mathematical function can be used to describe the underlying stochastic process of experimental variograms. Such a mathematical model, known as an authorized model, must satisfy the following conditions: an intercept on the ordinate (axis of semi-variance), a monotonically increasing section and conditional negative semi-definite [39]. Only functions that ensure non-zero variances may be used for variograms. Examples of simple authorized models are exponential, Gaussian and spherical models. The semi-variance (γ) with an exponential model can be represented by the following formula:

$$\gamma(\mathbf{h}) = c(1 - e^{-\mathbf{h}/L}) \quad (3)$$

where $\gamma(\mathbf{h})$ is the semi-variance of bone properties as a function of lag distance ($\mathbf{h}$); c , sill variance, is the converging value of semi-variance when lag distance ($\mathbf{h}$) approaches infinity; L , correlation length, is a distance parameter defining the spatial extent of the model. Correlation length is an important parameter to describe the spatial variation of a random field. A large correlation length implies a smooth variation whereas a small correlation length corresponds to rapid changes in the property over the spatial domain.

The stochastic measures, i.e. correlation length (L) and sill variance (c), can be obtained by fitting the aforementioned exponential model to the experimental variogram using the least square estimate.

Stochastic assessment of 2D lattice structures

To help better understand the meaning of stochastic parameters (i.e., correlation length and sill variance) and their connections with microstructural properties of trabecular bone, simple 2D lattice structures were constructed for stochastic assessment (Figs. 1 and 2). The 2D lattice structures with a dimension of 120×120 pixels consist of white and black stripes,

representing bone and marrow spaces, respectively. In these 2D lattice structures, bone and marrow spaces were changed to represent various combinations of volume fraction and trabecular separation. In Figure 1, the volume fraction was held at a constant of 30% while trabecular separation varied from 10 pixels (Fig.1a) to 20 pixels (Fig. 1c) and to 30 pixels (Fig. 1e). In Figure 2, trabecular separation was kept as a constant of 30 pixels whereas volume fractions varied from 10% (Fig.2a) to 20% (Fig.2c) and to 30% (Fig.2e).

Experimental variograms of these simple 2D lattice structures (Figs. 1 and 2) indicated that the semi-variance ascended from zero to 95% of the sill variance when the lag distance reached about three times of the correlation length. Periodic fluctuations of the semi-variance were observed afterwards, indicating the presence of periodicity within the simple lattice structures. Such periodic fluctuations were centered on the sill variance, suggesting that the semi-variance converged at the sill variance.

Great changes in the correlation length of variograms were observed when the volume fraction of simple lattice structures was fixed and trabecular separation varied (Fig 1). On the other hand, the sill variance was about the same for the simple lattice structures (Fig 1). As discussed earlier, the correlation length is related to the degree of smoothness or roughness in the lattice structure. Thus, a relatively larger correlation length implied a smooth variation (Figs. 1e and 1f), whereas a smaller correlation length corresponded to acute changes of bone properties over the spatial domain (Figs 1a and 1b). In addition, changes in both the correlation length and sill variance were demonstrated when the trabecular separation of the simple lattice structures was fixed and the volume fraction varied (Fig 2). The above results clearly demonstrate that the stochastic parameters (*i.e.* correlation length and sill variance) could sensitively reflect spatial variations of structures.

Stochastic assessment of 2D projection images

To achieve the first objective of this study, 2D projection images of trabecular bone were generated from 3D high resolution Micro-CT images by averaging the greyscale values of multiple imaging slices in a projection plane (Figs. 3a and 3b). In the plane, the greyscale value (Z) of each point (x, y) was determined by the following equation:

$$Z(x, y) = \frac{1}{N} \sum_{i=1}^N V(x, y, z) \quad (4)$$

where $Z(x, y)$ represents the greyscale value of the 2D projection image at the location (x, y); $V(x, y, z)$ is the greyscale value of the 3D Micro-CT image at the voxel location (x, y, z); N is the number of image slices in the projection direction (z).

Variations of the greyscale value in 2D projection images of the trabecular bone samples actually represent the spatial distribution of aBMD since the correlation between greyscale values in Micro-CT images and bone mineralization levels has been established from previous studies in the literature [42–44]. Spatial variations of aBMD in 2D projection images are random since they result from many complex biological processes (*i.e.*, bone remodeling) that are activated in a highly non-linear and unsystematic fashion. Thus, it becomes necessary to use stochastic approaches to assess the distribution of aBMD through experimental variograms.

Typical experimental variograms of 2D projection images of trabecular bone samples (Figs. 4c and 4f) indicated that semi-variance of aBMD in 2D projection images increased with increasing lag distance and reached a plateau, also known as the sill of variogram. In other word, as lag distance increased aBMD became more dissimilar on average until reaching a threshold value (the plateau). In comparison of the 2D projection images between two

trabecular bone specimens with distinct bone volume fractions (Figs.4a and 4d) and different variation of aBMD distribution (Fig.4b and 4e), the semi-variance in the variogram of the trabecular bone with a low bone volume fraction ($BV/TV = 0.13$) reached the plateau slowly (Fig.4c) whereas the other one ($BV/TV = 0.33$) arrived at the plateau relatively rapidly (Fig. 4f). In addition, the denser specimen (Fig.4f, $c = 4097$) had a higher sill variance than the more porous specimen (Fig.4c, $c = 1928$).

Stochastic parameters vs. microstructural and mechanical properties of trabecular bone

To achieve the second objective of this study, stochastic assessments were performed on high resolution (pixel size of $50\ \mu\text{m}$) 2D projection images of fifteen trabecular bone specimens. Experimental variograms were obtained and exponential models were then fitted on these experimental variograms. Stochastic measures (correlation length and sill variance) were obtained for the 2D projection image of each trabecular bone specimen. Simple linear regression analyses were performed to examine the relationship of stochastic measures, including sill variance and correlation length, with the 2D projection images with microarchitectural and biomechanical properties of trabecular bone from human tibias.

Efficacy of the stochastic approach in analyzing aBMD distribution using DXA images

To achieve the third objective of this study, raw images of trabecular bone specimens were reconstructed to produce 3D Micro-CT images with a voxel size of $300\ \mu\text{m}$. Then, 2D projection images with a pixel size of $300\ \mu\text{m}$ were created so that they were comparable to the currently available resolution of DXA images [45]. This would answer the clinically relevant question whether the novel stochastic assessment of 2D projection images could be appropriate for DXA images. Simple regression analyses were also conducted to study the relationship of stochastic assessment of 2D projection images of trabecular bone specimens with a pixel size of $300\ \mu\text{m}$ and their biomechanical properties and microarchitectures.

To achieve the fourth objective of this study, 3D Micro-CT images were also used to calculate aBMD of trabecular bone specimens since greyscale levels in Micro-CT images were an indication of CT attenuation values in bone specimens [46, 47]. First, 3D Micro-CT images of trabecular bone specimens were segmented to distinguish bone tissue from bone marrow by conducting a global threshold and maintaining the greyscale values of bone tissue [31]. After segmentation, nominal aBMD was obtained by projecting the 3D segmented images into the transverse plane and adding the greyscale values of all pixels in the transverse plane together and dividing such a sum by the total area of projected transverse plane [47]. Finally, multiple regression analyses were conducted to determine whether a combination of nominal aBMD and stochastic measures predicted the strength of trabecular bone better than using nominal aBMD alone.

Statistical analysis

All statistical analyses were performed on SPSS (IBM, Armonk, New York). In general, the linear regression analysis was performed to evaluate the correlation between the variables tested in this study. To test whether stochastic parameters from 2D projection images were uniquely correlated with the mechanical properties of trabecular bone, partial correlation analyses were performed by removing the effect of other factors (i.e. aBMD) on the correlation between the stochastic parameters and the mechanical behavior of bone from the analysis. The significance level was established when the p -value was less than 0.05.

Results

Stochastic analyses of high resolution 2D project images ($50\ \mu\text{m}$) of trabecular bone indicated that significant positive relationships were observed between sill variance and the

elastic modulus (Fig.5a, $R^2 = 0.81$, $p < 0.001$) and between sill variance and ultimate strength (Fig.5b, $R^2 = 0.82$, $p < 0.001$) of trabecular bone. Additionally, the sill variance of distribution of aBMD was correlated with microarchitecture parameters. Linear regression analyses indicated a significant positive relationship between sill variance and bone volume fraction (Fig.6a, $R^2 = 0.56$, $p = 0.001$). Similar relationships were also observed between the sill variance of distribution of aBMD and additional microarchitecture parameters, including bone surface-to-volume ratio (Fig.6b, $R^2 = 0.54$, $p = 0.002$), trabecular thickness (Fig.6c, $R^2 = 0.54$, $p = 0.002$), trabecular number (Fig.6d, $R^2 = 0.48$, $p = 0.004$), trabecular separation (Fig.6e, $R^2 = 0.50$, $p = 0.003$), and anisotropy (Fig.6f, $R^2 = 0.37$, $p = 0.02$). No significant relationships were found between the correlation length and biomechanical as well as microarchitectural properties of trabecular bone.

Stochastic analyses of 2D projection images of trabecular bone with a pixel size of 300 μm demonstrated that the sill variance of distribution of BMD also was significantly ($p < 0.001$) and positively correlated with both elastic modulus ($R^2 = 0.84$) and strength ($R^2 = 0.77$) of trabecular bone specimens (Figs. 7a and 7b). Moreover, the sill variance had significant correlations with microarchitecture parameters, including bone volume fraction (Fig.8a, $R^2 = 0.44$, $p = 0.007$), bone surface-to-volume ratio (Fig.8b, $R^2 = 0.39$, $p = 0.01$), trabecular thickness (Fig.8c, $R^2 = 0.40$, $p = 0.01$), trabecular number (Fig.8d, $R^2 = 0.34$, $p = 0.02$), trabecular separation (Fig.8e, $R^2 = 0.32$, $p = 0.03$), and anisotropy (Fig.8f, $R^2 = 0.30$, $p = 0.03$).

The combination of aBMD and sill variance from high resolution 2D projection images (50 μm) significantly improved the power of prediction of the trabecular bone strength from $R^2 = 0.63$ to $R^2 = 0.83$ (Table 1). The same was also observed for 2D projection images with a moderate resolution (pixel size of 300 μm), in which the R-Squared value increased from 0.63 to 0.85 when combining both aBMD and sill variance (Table 2).

The partial correlation analysis indicated that sill variance, as a structural measure of bone, was significantly ($p = 0.003$, Pearson correlation coefficient = 0.74) correlated with bone strength after eliminating the effect of aBMD, a quantity measure of bone.

Discussion

In this study, the theory of random field was used to extract stochastic measures of aBMD distribution from high resolution (pixel size of 50 μm) 2D projection images of trabecular bone. Sill variance, a stochastic measure of distribution of aBMD, showed significant correlations with strength and microarchitecture parameters of trabecular bone. Such relationships were consistent with observations reported in the literature that reduction of bone inhomogeneity contributes to bone fragility. Moreover, the results of this study indicate that such relationships could also be extended to 2D projection images with comparable resolutions (e.g. pixel size of 300 μm) of DXA scans. Thus, a combination of aBMD and stochastic assessment of distribution of aBMD may provide an enhanced estimate of bone fragility from routine DXA scans.

Stochastic assessments of this study revealed an inhomogeneous aBMD distribution (heterogeneity) in 2D projection images of trabecular bone. Sill variance actually determines the global variance of aBMD, which is referred to the converging value of semi-variance from experimental variograms when the lag approaches infinity. Significantly positive relationships between sill variance of 2D projection images and bone strength of trabecular bone in this study suggest that decreases in bone heterogeneity are associated with increases in bone fragility. It is consistent with reports in the literature that a decrease in bone tissue inhomogeneity has resulted in a reduction in the toughness of bone [30]. Additionally, the

inhomogeneous distribution of bone mineral density has been known to affect the mechanical properties of trabecular bone [48–52]. The underlying mechanism for the influence of tissue heterogeneity on bone fragility may be due to the spatial variation of tissue properties that thwarts crack growth and propagation in bone [53–55].

Significant relationships between sill variance of 2D projection images and microarchitecture parameters of trabecular bone were in agreement with the previous studies [28]. Pothuaud et al. introduced a new parameter, trabecular bone score (TBS), to characterize experimental variograms of 2D projection images of trabecular bone. TBS was calculated as the slope at the origin of the experimental variogram, describing the rate of changes of semi-variance with respect to lag distance. Significant correlations were also observed between TBS and microarchitecture parameters (e.g., bone volume fraction and trabecular number) of trabecular bone from human femurs and lumbar spines [28]. In fact, TBS is somewhat related with two stochastic measures (sill variance and correlation length) described in this study, by considering TBS as the ratio of sill variance to the correlation length. Thus, TBS may represent a coupled effect of the correlation length and sill variance. Our study further indicates that it is the sill variance, rather than correlation length, that correlates directly with microarchitecture parameters. One advantage of stochastic assessments of 2D projection images is that the sill variance and correlation length can be used in the stochastic modeling and simulation of aBMD distribution in bone based on the random field theory [41], which can provide further insights on how the inhomogeneous mass distribution in trabecular bone affects bone fragility.

It is feasible to extend the aforementioned stochastic approach to the low resolution 2D projection images from DXA scans to study the aBMD distribution. Our results from stochastic assessment of 2D projection images with a pixel size of 300 μm have confirmed the significant positive correlations of the sill variance with the strength (Fig.7a) and modulus (Fig.7b) of trabecular bone. Although the R-squared values were reduced when compared to high resolution 2D projection images (Fig.6), the statistically significant relationships between the sill variance and microarchitecture parameters of trabecular bone were still preserved (Fig.8). 2D projection images with a pixel size of 300 μm are comparable with the image resolution (312 μm) obtained from the current DXA techniques [45]. Consequently, the stochastic assessment of aBMD distribution from 2D projection images of DXA scans may offer an additional means to enhance the risk prediction of bone fragility fractures (Tables 1 and 2).

There are several limitations for this study. First, greyscale values were used in this study to represent aBMD for 2D projection images. The actual bone mineral density values can be obtained by placing a series of hydroxyapatite standards with various concentrations around bone samples when Micro-CT images of trabecular bone were scanned. Second, trabecular bone specimens used in this study consist of both high density and low density groups. The results of this study indicated that removal of high density group from the analysis resulted in a reduction of prediction power of the sill variance for 2D projection images with a pixel size of 300 μm . Therefore, the prediction power of sill variance in the low density group may be limited. Third, the DXA images were not obtained directly from trabecular bone specimens. Although 2D projection images of Micro-CT images with a pixel size of 300 μm may not be exactly the same as DXA images, they may at least offer a reasonable approximation to study the stochastic assessment of aBMD distribution. Finally, cylindrical bone samples do not represent irregular bone shape in clinical DXA scans. The irregular bone shape in the clinical setting could create a non-uniform distribution along the projection direction such that the inhomogeneity of the projected 2D bone image may partially result from the varying bone dimension along the projection direction.

In conclusion, the stochastic assessment of 2D projection images of trabecular bone has demonstrated that the sill variance of aBMD distribution was significantly associated with bone strength and microarchitecture parameters of trabecular bone. Such an observation can be extended to 2D projection images obtained from DXA scans. The combination of aBMD and stochastic assessment of aBMD distribution may enhance the prediction of bone fragility.

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Highlights

- aBMD distribution from 2D projection images of trabecular bone was assessed by a stochastic method.
- Sill variance, a stochastic measure of aBMD distribution, showed significantly positive correlations with strength and modulus of trabecular bone.
- A combination of aBMD and stochastic measures predicted bone strength better than using aBMD alone.

Fig. 1. Simple 2D lattice structures with the same volume fraction (30%) and different trabecular separation; Bone and marrow spaces were represented by white and black stripes, respectively; (a) bone space = 3, marrow space = 7; (b) variogram of the preceding lattice structure; (c) bone space = 6, marrow space = 14; (d) variogram of the preceding lattice structure; (e) bone space = 9, marrow space = 21; (f) variogram of the preceding lattice structure.

Fig. 2. Simple 2D lattice structures with the same trabecular separation and different volume fractions; Bone and marrow spaces were represented by white and black stripes, respectively; (a) bone space = 3, marrow space = 27; (b) variogram of the preceding lattice structure; (c) bone space = 6, marrow space = 24; (d) variogram of the preceding lattice structure; (e) bone space = 9, marrow space = 21; (f) variogram of the preceding lattice structure.

Fig. 3. Schematic representation of generating 2D projection images, which was obtained by projecting the 3D Micro-CT images into a plane perpendicular to the projection axis (i.e., the transverse plane). A 3D Cartesian coordinate system (x , y , z) was set up such that the projection axis was in the z direction.

Fig. 4.

Stochastic analysis of spatial distribution of areal bone mineral density in 2D projection images. (a) a slice of Micro-CT images with a low bone volume fraction (BV/TV) = 0.13; (b) 2D projection image of the specimen with low bone volume fraction; (c) the variogram of the trabecular bone specimen with low bone volume fraction; (d) a slice of Micro-CT images of a dense specimen with BV/TV = 0.33; (e) 2D projection image of the dense specimen; (f) the variogram of the dense specimen.

Fig. 5. Sill variance of aBMD distribution had significantly positive relationships with (a) elastic modulus ($R^2 = 0.81$, $p < 0.001$), and (b) ultimate strength ($R^2 = 0.82$, $p < 0.001$) of trabecular bone.

Fig. 6.

Sill variance of distribution of areal bone mineral density had significantly positive relationships with microarchitecture parameters (a) bone volume fraction; (b) bone surface-to-volume ratio; (c) trabecular thickness; (d) trabecular number; (e) trabecular separation; and (f) anisotropy of trabecular bone.

Fig. 7. Relationship between sill variance of aBMD distribution from 2D projection images with a pixel size of 300 μ m and biomechanical properties of trabecular bone: (a) elastic modulus ($R^2=0.84$, $p<0.001$), and (b) ultimate strength ($R^2=0.77$, $p<0.001$).

Fig. 8. Sill variance of aBMD distribution from 2D projection images with a pixel size of 300 μm had significant relationships with microarchitecture parameters (a) bone volume fraction; (b) bone surface-to-volume ratio; (c) trabecular thickness; (d) trabecular number; (e) trabecular separation; and (f) anisotropy.

Table 1

Association of trabecular bone strength with aBMD alone and with combination of aBMD and sill variance from 2D projection images with a pixel size of 50 μm .

Model	R Square	Adjusted R Square	p-value
Strength ~ aBMD	0.63	0.61	<0.001
Strength ~ aBMD + Sill Variance	0.83	0.80	<0.001

Table 2

Association of trabecular bone strength with aBMD alone and with the combination of aBMD and sill variance from 2D projection images with a pixel size of 300 μm .

Model	R Square	Adjusted R Square	p-value
Strength ~ aBMD	0.63	0.61	<0.001
Strength ~ aBMD + Sill Variance	0.85	0.82	<0.001