

Original Article

Association of microstructural and mechanical properties of cancellous bone and their fracture risk assessment tool scores

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Abstract: This study is to investigate the association between fracture probabilities determined by using the fracture risk assessment tool (FRAX) and the microstructure and mechanical properties of femoral bone trabecula in osteoporosis (OP) and osteoarthritis (OA) patients with hip replacements. By using FRAX, we evaluated fracture risks of the 102 patients with bone replacements. Using micro CT scanning, we obtained the analysis parameters of microstructural properties of cancellous bone. Through morphometric observations, fatigue tests and compression tests, we obtained parameters of mechanical properties of cancellous bones. Relevant Pearson analysis was performed to investigate the association between the fracture probability and the microstructure and mechanical properties of femoral bone trabecula in patients. Fifteen risk factors in FRAX were compared between OP and OA patients. FRAX hip fracture risk score and major osteoporotic in OP and OA patients were significantly different. FRAX was associated with tissue bone mineral density and volumetric bone mineral density. Our study suggests that the probabilities of major osteoporotic and hip fracture using FRAX is associated with bone mass but not with micro bone quality.

Keywords: Microstructural, cancellous bone, fracture risk assessment

Introduction

Osteoporotic fracture is a common metabolic bone disease, which is characterized by a decrease of bone mass and the degenerative change of bone microstructure, resulting in easy occurrence of fracture [1]. Osteoporotic fracture caused by a minor injury in the activities of daily life often occurs in cancellous bone-rich zone, with increasing disability and lethality rates, which may seriously affect the quality of life of older persons [2]. Thus, the prevention of osteoporotic fracture has become one of the most popular topics.

To evaluate the possibility of patient's fracture, WHO recommends the use of fracture risk assessment tool (FRAX) [3-6]. FRAX is developed via a serial of large sample studies using bone mineral density (BMD) and multiple frac-

ture risk factors, such as femoral neck (FT) T-score [6]. Kanis' study suggested that FRAX is superior to using BMD alone or simply combining it with one or more fracture risk factors [7]. The previous studies have shown that most of the factors involved in FRAX are closely related to the bone mass and bone quality. For an example, in the investigation of the relation between the age and the change of bone microstructure, it was found that as age increases, BV/TV, trabecular number and connectivity density decline, while the structure model index increases along with the discoid trabecula changes into the rod-like trabecula [8-10]. During aging process, the bone structure has significant difference between male and female [10]. The study of radial bone also showed that in female, the distal radius bone connectivity is poor and the aperture of the bone trabecular structure is larger [11]. The study on vertebral

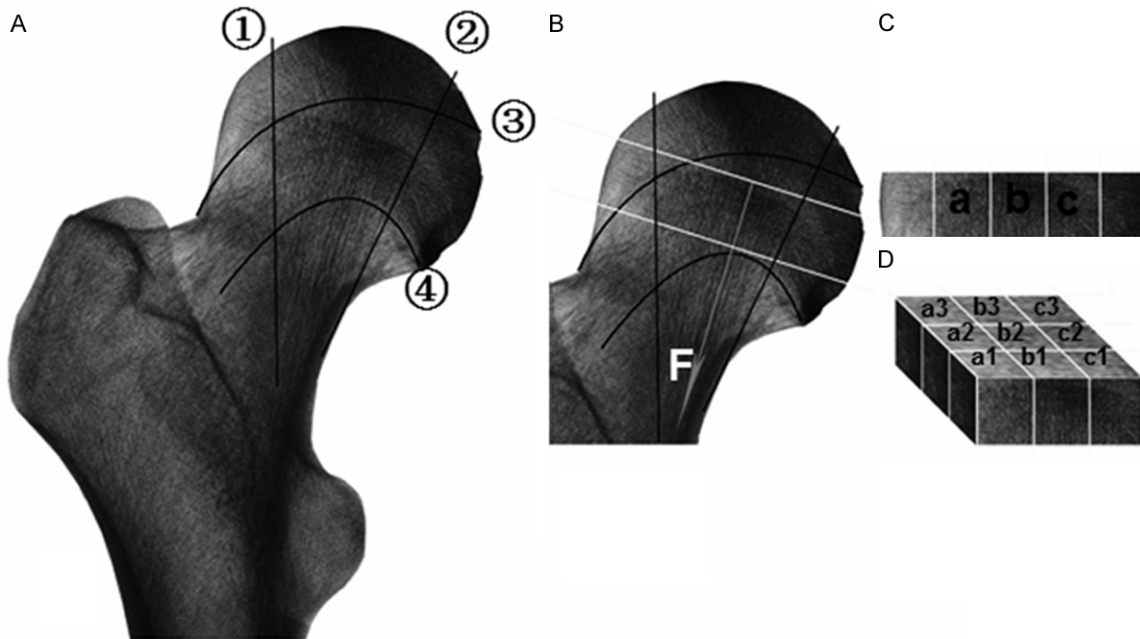


Figure 1. Schemes of sample preparation. A. Femoral head was placed in the position as the living body and positioning markers were made. Primary compressive group was between line ① and ②. Primary tensile group was between line ③ and ④. B. A 7 mm thick bone block layer within the maximum diameter vertical to the direction of stress F of femoral head was made. C. A total of 9 cancellous bones of 6 mm × 6 mm × 7 mm in the plane direction vertical to the bone block layer were taken. D. Before incision, accurate positioning with the calcar femorale as the base point was marked, the bones were coded respectively according to the position in the figure so as to guarantee that the number of specimen collected each time had the consistent position.

bone trabecula also showed an obvious difference between males and female in many aspects, including BV/TV, trabecular number, connectivity density, trabecular separation and structure model index [12].

FRAX used many fractures-related factors, which have significant impacts on bone quality, such as Age and gender on microstructure of cortical and trabecular bone at the human femoral neck [13], family's genetic factors on BMD [14-16], and habits like smoking and drinking on the microstructure of bone trabecula and bone reconstruction [17-20]. Besides, age is another essential factor. Aging leads to an increase in the number and volume fraction, a decrease in bone trabecula and connectivity density, an increase in structure model index and the change of discoid trabeculae into rod-like trabeculae [8, 9]. In addition, adrenal cortex hormones also show impacts. It may induce the apoptosis of osteoblasts and bone cells, suppress the formation of bone tissue, and destruct the structure and mechanical properties of bone tissue [21, 22]. Therefore the hor-

monone treatment to rheumatic arthritis may lead to the decline of BMD, resulting in a patient's secondary osteoporosis [23]. Last but not the least, deterioration of bone quality is an internal factor of fracture. In osteoporotic patients, bone reconstruction capacity diminishes, leading to the accumulation of micro-damage, thus the fracture occurs [24].

We speculate that FRAX integrated those essential risk factors may also reflect the quality of bone. Changes in the microstructure and mechanical properties of bone trabecula have great impacts on the occurrence and development of osteoporotic fracture [25-27]. So far, the association between the FRAX and microscopic bone quality was seldom reported. Our study investigates the association between the fracture probability obtained from the FRAX and microstructure and mechanical properties of femoral bone trabecula in osteoporosis (OP) and osteoarthritis (OA) patients with hip replacement. Our results may shed some lights on predicting osteoporotic fracture of population in the future.

Materials and methods

Study of osteoporotic fractures

The approval of the local ethical committee was obtained for the study. Human femoral heads were obtained from 77 OP donors (average age = 65.9 ± 11.4 years, range from 55 to 87 years, 29 males vs. 48 females), and 25 OA donors (average age = 63.3 ± 11.2 years, range from 56 to 78 years, 10 males vs. 15 females) who underwent total hip arthroplasty for either osteoporotic, subcapital hip fracture, or osteoarthritis. Each patient provided his/her history of present illness, past medical history and other clinical related medical records.

Prior written and informed consent was obtained from every patient and the study was approved by the ethics review board of Central South University.

Sample preparation

We placed femoral heads as the position in living body and made positioning mark. Then we took a 7 mm specimen perpendicular to the stress direction of caput femoris within the maximum diameter using circulating precise diamond wire cutting machine (Shenyang Kejing Instrument Co., Ltd., model: SXJ-2), then took 9 cancellous bone blocks with the size of 6 mm × 6 mm × 7 mm from the same plane (**Figure 1**). We ensured the same sampling positions, and coded them individually according to position. We continuously poured normal saline during the whole procedure. Then, samples were placed in alcohol solution with concentration of 40%, and store in refrigerator with temperature of 4°C for future use.

Structural analysis

We carried out micro CT scanning for samples using GE Explore Locus Sp Specimen Scanner (GE Health Care Co., London, Ont.) [28]. We placed specimen in the specimen holder vertically along the long axis, and then filled some medical gauze strip around to prevent specimen from drifting during the scanning process. We then added alcohol solution (concentration of 40%) as scanning medium and fully immersed the specimen and gauze strip. The specimen holder was placed in a vacuum box of 70 KPa for 20 minutes to ensure no bubble interfering.

The scanning parameters used were as follows: voltage of 80 kv, current of 80 μ A, scanning mode of 360° revolution, scanning time of 270 min, average frame of 4, angle increment of 0.4, exposure time of 3000 ms, and resolution ratio of 14.0 μ m × 14.0 μ m × 14.0 μ m voxel. Meanwhile, we scanned standard phantom to prepare for calibration of CT value. After completion of scanning, we manually calibrated rotating center and CT value, and completed overall structure reconstruction of scanning area with isotropy resolution ratio of 41.0 μ m × 41.0 μ m × 41.0 μ m.

We selected bone tissue in the center of specimen (4.3 mm × 4.3 mm × 4.3 mm) as the region of interest (region of interest, ROI) to perform three-dimensional reconstruction of 14.0 μ m × 14.0 μ m × 14.0 μ m voxel. Image information was drawn from the threshold automatically generated by computer to complete image binarization. We then selected bone trabecula within ROI to perform three-dimensional visualization using MicroView2.1.1 + Adance Bone Analysis software (GE Health Care Co.) with the system to perform quantitative analysis. The analysis parameters include: volumetric BMD (vBMD), tissue BMD (tBMD), bone volume fraction (BV/TV), bone area density (BS/BV), trabecular thickness (Tb. Th.), trabecular separation (Tb. Sp.), trabecular number (Tb. N.), structure model index (SMI), connectivity density (Conn. D.) and degree of anisotropy (DA).

Morphometric observation

After completion of micro CT scanning for specimens, we immersed them in basic Fuchsin solution with concentration of 80%, 90% and 100% for gradient immersing and colored each sample for 6 hours respectively, washed twice with 100% alcohol (once for 1 hour), then immersed in dimethylbenzene for 24 hours for transparent treatment, and then performed plastic embedding. Afterwards, samples were respectively immersed in three solutions under 4°C environment for 2 days (solution I, II and III respectively with methacrylic acid vinegar, phthalic acid dibutyl ester and benzoyl peroxide) [29]. Then we placed solution III in a glass bottle at 42°C for three day polymerization. After successfully made the specimen, we placed specimen on the bottom and added freshly prepared solution III for overnight in room temperature. Then, we placed it at 42°C

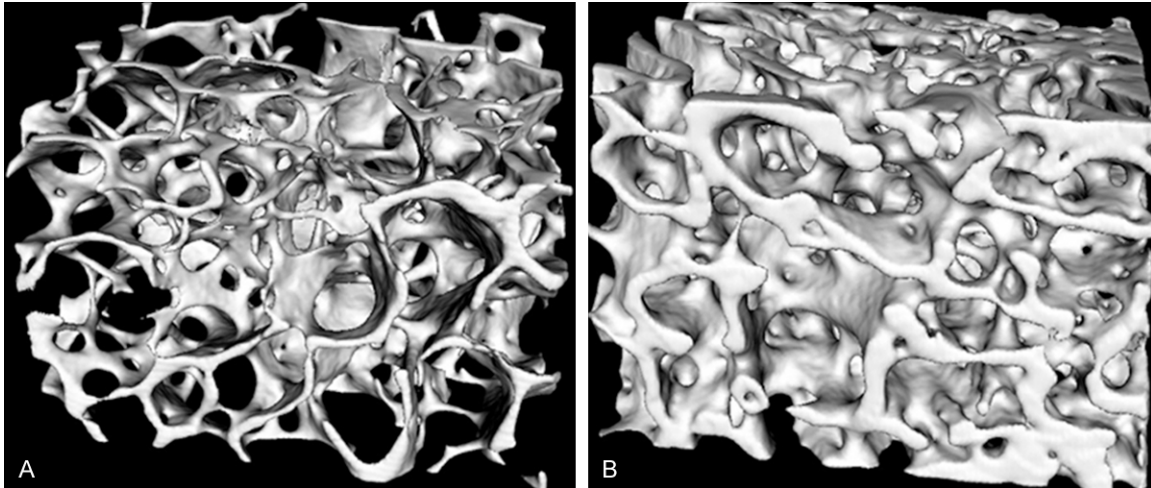


Figure 2. Comparison of three-dimensional visual imagings in cancellous bone patients between OP and OA. In OP patients (A), trabecular bones osteopenia appeared, trabecular bones volume tapered and trabecular bones amount reduced. Connection points among trabecular bones reduced and the strength of trabecular bones was less than OA patients (B).

to polymerize for three days. After successful embedding, we polished the embedded bag to blade, to obtain osteocoma with thickness of 60-80 μm , and then carried out mounting and drying for neutral resins. After observing tiny damages of osteocoma using Leica DMLA microscope imaging and analyzing system (Leica Corporation, Wetzla, Germany), we performed calculation. We also observed mean microcrack length (Cr.Le, μm), Microcrack density (Cr. Dn) and Microcrack surface density (Cr.S.Dn, $\mu\text{m}/\text{mm}^2$) under light microscope ($\times 200$). The computational formulas for various observation indexes of microcrack as follows: Mean microcrack length = total length of microcrack/total sum of microcrack; Microcrack density = total sum of microcrack/bone trabecula area percentage; and Microcrack surface density = total length of microcrack/bone trabecula area percentage.

Fatigue test

We took No. 2 specimen from 40% alcohol solution and measured its height using micrometer [30]. With approximate 7 mm distance, we wrapped up the surrounding of bone blocks with gauze strip immersed in normal saline and placed it in the fatigue test machine (Institution of Metabolism and Endocrinology of Central South University and Changchun Kexin Co., Ltd., model: RDS-36). The basic parameter settings in computer software were as follows:

maximum force of 60 N and descending speed of beam of 5 mm/min, starting with 10 N as initial force and 10000 cycle number as ending. During the whole damaging process, specimens were in room temperature of 28°C. After completion of fatigue test, we measured the height again with micrometer and calculated different height (DH) before and after fatigue test.

Compression test

We took No. 3 specimen from 40% alcohol solution and placed it on the bench of mechanical test machine vertically (manufactured by Changchun Kexin Co., Ltd., model: WDW3100) with the superior-inferior diameter of approximate 7 mm [30]. The basic parameter settings in computer operational software were as follows: descending speed of beam of 25 mm/min and compression degree of 30%. We used 10 N as initial force and stopped when force peak value occurred in the compression curve with obvious descending trend.

We then took load-deformation curve and recorded the maximum force when specimen was compressed 30%. The maximum load/area of thrust surface = maximum stress (MS, N/ mm^2). The elasticity load/area of thrust surface = elasticity stress (ES, N/ mm^2). The elasticity load/elasticity change = modulus of elasticity (EM, N/mm). Those factors reflected the anti-compression capacity of bone trabecula.

Fracture risk assessment tool scores

Table 1. Cancellous bone mass and microstructure parameter of total trabecular region in the femoral head in OP and OA patients

Parameters	OP (n = 77)	OA (n = 26)	P
vBMD (mg/mm ³)	193.15 ± 30.03	295.61 ± 21.14	0.001*
tBMD (mg/mm ³)	535.11 ± 60.03	598.01 ± 70.05	0.001*
Tb. Th (µm)	175.88 ± 44.31	220.83 ± 20.61	0.003*
Tb. SP	0.77 ± 0.18	0.63 ± 0.19	0.061
Tb. N (mm ³)	1.48 ± 0.23	1.50 ± 0.26	0.06
BV/TV (%)	20.62 ± 0.64	32.81 ± 0.85	0.005*
BS/BV (mm ⁻¹)	14.16 ± 2.55	10.36 ± 2.26	0.221
SMI	1.13 ± 0.71	1.95 ± 2.71	0.351
Conn.D (mm ⁻³)	3.15 ± 2.08	4.15 ± 5.01	0.50
DA	1.61 ± 0.22	1.48 ± 0.58	0.001*
MS (N/mm ²)	2.86 ± 0.95	4.61 ± 1.55	0.025
ES (N/mm ²)	1.92 ± 0.86	3.78 ± 1.85	0.006*
EM (N/mm)	59.27 ± 20.45	108.02 ± 50.91	0.013*
DH (mm)	1.93 ± 1.96	1.33 ± 1.36	0.216
Cr.S.Dn (µm/mm ²)	6.75 ± 2.96	5.64 ± 3.13	0.538

Note: vBMD, volume bone mineral density; tBMD, tissue bone mineral density; Tb. Th, Trabecular thickness; Tb. SP, Trabecular separation; Tb. N, Trabecular number; BV/TV, Bone area density; BS/BV, Bone volume fraction; SMI, Structure model index; Conn.D, connectivity density; DA, degree of anisotropy; MS, Maximum stress; ES, Elastic stress; EM, Elastic modulus; DH, Difference of height; Cr.S.Dn, Microcrack surface density. t-test was used to compare OP and OA groups. *P ≤ 0.05 was considered as statistically significant.

Table 2. The probability of major osteoporotic and hip fracture in OP and OA patients

Parameter	OP (n = 77)	OA (n = 26)	P
Major osteoporotic (%)	8.31 ± 2.89	3.81 ± 1.19	0.003 [#]
Hip fracture (%)	4.93 ± 1.97	1.74 ± 0.57	0.001 [#]

Note: t-test was used to compare OP and OA groups. [#]P ≤ 0.05 was considered as statistically different.

Statistical methods

All statistical analyses were completed using SPSS16.0 software package (SPSS Inc, Chicago, Illinois, USA). Results were expressed by Means ± SD using independent sample T-test and Pearson correlation analysis. P < 0.05 is considered as statistically significant difference.

Results

Fifteen risk factors in FRAX are compared between OP and OA patients

To better understand the microstructure of OP patients, we compared 15 factors which are

parameters in FRAX between OP and OA patients. For total trabecular region, the average levels of volumetric BMD (vBMD), tissue BMD (tBMD), trabecular thickness (Tb. Th.) and bone volume fraction (BV/TV) were significantly lower (**Figure 2**), whereas the degree of anisotropy (DA) was statistically higher in OP patients compared with OA patients (P < 0.05). However, no significant difference in the average bone area density (BS/BV), trabecular separation (Tb. Sp.), average values of structure model index (SMI), trabecular number (Tb. N.) and the average connectivity density (Conn.D.) were observed. The average maximum stress (MS), elastic stress (ES), elastic modulus (EM) was statistically lower in OP patients compared with OA patients (P < 0.05). The average values of difference of height (DH) and the microcrack surface density (Cr. S.Dn) were not statistically significant either between both groups (**Table 1**). These results indicated that those factors with significant differences between the two groups can be important in future study of OP.

FRAX hip fracture risk score and major osteoporotic in OP and OA patients are significantly different

To further study the risk of fracture in OP patients, we used the FRAX score to evaluate the probabilities of major osteoporotic and hip fracture in OP patients, and then compared them to those in OA patients using t-test. Both scores were statistically greater in OP patients compared with those in OA patients (P < 0.05) (**Table 2**), confirming higher risk of fracture in OP patients.

FRAX is associated with tBMD and vBMD

As we understand the risk of fracture in OP patients, we speculated that the structural properties of trabecular bones may be associated with FARX score. From our results of linear correlation coefficients, the probability of Major

Fracture risk assessment tool scores

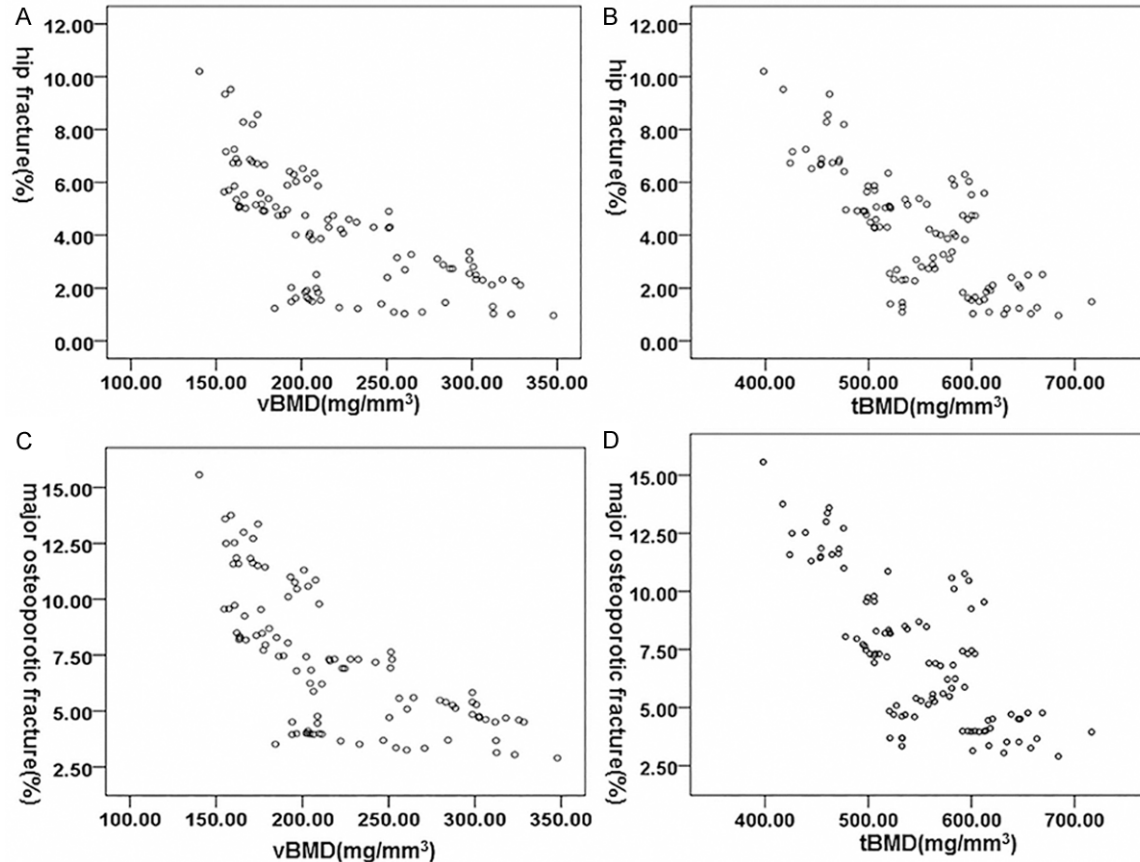


Figure 3. Correlation of the probability of hip and major osteoporotic fracture with vBMD and tBMD. A. Correlation of the probability of hip fracture with vBMD. B. Correlation of the probability of hip fracture with tBMD. C. Correlation of the probability of major osteoporotic fracture with vBMD. D. Correlation of the probability of major osteoporotic fracture with tBMD. *P* value of all studies are much less than 0.05.

osteoporotic and Hip fracture were correlated with tBMD and vBMD with *P* value much less than 0.05 (Figure 3). However, we did not identify statistically significant correlation between the FARX score and the other parameters (Table 3).

Discussion

Osteoporotic is a degenerative bone disease, which is characterized by the reduction of bone strength and increasing fracture risk. The bone mechanical strength is decided by bone structure as well as the intrinsic nature of the bone tissue itself [31, 32]. The changes of subtle spatial structure and mechanical properties of bone affect bone strength. From quantitative change to qualitative change in bone mass and bone quality, they decide bone strength [33-35].

In this study, we investigated the microstructural and mechanical properties of femoral

bone trabecula from 102 cases of hip replacement. We found that the bone mass and bone quality of femoral cancellous bone declined in OP group. The mechanical properties were poor and the degree of microdamage was higher compared to the OA group.

Previous study showed that osteoporotic fracture patients have perforating bone trabecula, resulting in less connectivity of bone trabecula, more bone fragility and fracture risk [36]. Besides, the plate-like bone trabecula can transform to the rod-like bone trabecula [36]. The study of Nazarian et al. also showed that the fracture of human vertebral cancellous bone is more likely to occur in some low BV/TV sites [37]. Microdamage is a bone fatigue process [38]. The type and shape of microdamage affect the nature and function of micro-structure of bone tissue [26, 39, 40]. The accumulation of micro-damage causes a decline of the elastic modulus [40], a reduction of the

Fracture risk assessment tool scores

Table 3. Linear correlation coefficients (r) between FRAX scores and structural properties of trabecular bone samples (n = 103)

	Major osteoporotic		Hip fracture	
	r	P	r	P
tBMD	-0.741 [#]	0.000	-0.751 [#]	0.000
vBMD	-0.701 [#]	0.000	-0.692 [#]	0.000
Tb. Th.	-0.112	0.625	-0.152	0.741
Tb. N	-0.362	0.435	-0.413	0.254
Tb. SP	0.212	0.544	0.182	0.346
BV/TV	-0.384	0.141	-0.404	0.173
BS/BV	0.119	0.251	0.137	0.211
Conn.D	-0.054	0.336	-0.078	0.414
SMI	0.043	-0.011	0.017	-0.145
DA	0.099	0.219	0.049	0.621
MS (N/mm ²)	-0.212	0.115	-0.185	0.268
ES (N/mm ²)	-0.054	0.375	-0.127	0.056
EM (N/mm)	-0.315	0.521	-0.253	0.759
DH (mm)	0.243	0.403	0.247	0.333
Cr.S.Dn (μm/mm ²)	0.022	0.471	0.034	0.437

Note: t-test was used to compare OP and OA groups. [#]P < 0.01.

mechanical properties of bone and increasing fracture risk [41], and thus degradation of the biological quality of the bone, increasing brittleness and increasing osteoporotic fatigue fractures.

We found that the probability of major osteoporotic and hip fracture in OP group is statistical higher than OA group using FRAX. Through the correlation analysis between the probability of major osteoporotic and hip fracture of 102 patients and the microstructural and mechanical properties of bone trabecula, we found that the probability of fracture was negatively correlated with vBMD and tBMD, but no obvious correlation was observed with other micro-structural parameters or mechanical test results. Our study suggests that the probabilities of major osteoporotic and hip fracture using FRAX is associated with bone mass and but surprisingly not with micro bone quality.

Our results may be partially explained by the fact that the race difference was not fully considered into the contribution values and thus influence our results in Chinese population. The limitation of FRAX may also impact our results due to its selection of related risk factors. Falling and osteoporosis are often considered

to be two main reasons for hip fracture and most of the patients we studied have falling experience. Thus, we consider adding minor trauma into the FRAX as a parameter to improve its accuracy. In addition, our study was limited by sample size. Besides, only hip replaced patients without rheumatoid arthritis were selected. We can hardly measure FRAX accuracy effectively.

Our study firstly tested the association of micro-structural and mechanical properties of cancellous bone and FRAX Score in Chinese OP patients. Our results suggested the limitation of FRAX and provided possible factor to be included as parameter. This study shed some interesting lights on the future study of fracture, especially in population.

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Disclosure of conflict of interest

None.

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