EFFECT OF AGE, SEX, AND ANATOMICAL SITE ON THE MICROSTRUCTURAL ABNORMALITY IN HUMAN PROXIMAL FEMUR

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INTRODUCTION: Accurate prediction of bone fragility fractures remains a major challenge in managing postmenopausal and elderly patients, who are particularly susceptible to these complications. The current gold standard is to measure bone mineral density (BMD) and compare it against population-based benchmarks to assess the degree of bone mass loss and its associated fracture risk. However, bone fragility fractures are not solely attributable to bone mass loss; changes in trabecular bone microstructure also play a critical role in determining bone strength at anatomical sites prone to fractures, such as the proximal femur and vertebral body. Although various methods have been proposed to incorporate microstructural factors into fracture risk prediction, most focus on selected microstructural features and fail to establish a clear criterion for defining microstructural normality. Our recent studies revealed that trabecular bone microstructure from young donors tended to follow inherently invariant probability distributions [1], and deviations from these invariants may compromise bone mechanical competence. These findings suggest that these invariants could serve as intrinsic benchmarks for direct evaluation of microstructural abnormalities in bone. Based on this concept, the present study aimed to demonstrate the feasibility of using these benchmarks to assess microstructural abnormalities at different anatomical locations in human proximal femurs.

METHODS: Thirty six human cadaver proximal femurs were procured for this study, including sex-matched young (20-35 years of age), mid-aged (40-60 years of age), and old (>70 years of age) groups, with each subgroup containing six donors (N = 6). Each proximal femur was scanned using a high-resolution micro-CT system (SkyScan 1173, 35µm resolution) and reconstructed into a digital model. Subsequently, individual bone spheres were dissected from these digitized femurs (**Fig. 1**), aligned with their primary axes, and dissected into bone cubes measuring $6 \times 6 \times 6$ mm³ [2]. Approximately, 150 to 200 cubes per femur were acquired from head, neck, and intertrochanter regions in each femur. Using Individual Trabeculae Segmentation (ITS) software [3, 4], the size, location, and orientation of each trabecula within each cube were analyzed. Based on these ITS results, probability distributions were calculated for the following microstructural parameters for each cube: nominal plate area (PA), plate thickness (PT), rod length (RL), rod diameter (RD), and nearest neighbor distances between plate-to-plate (NNDpp), plate-to-rod (NNDpr), rod-to-rod (NNDrr), and rod-to-plate (NNDrp) (**Fig. 2**). The average probability distributions for bone cubes from young donors were computed and used as benchmark distributions, representing baseline microstructural patterns. These benchmarks were then pairwise compared with the probability distributions of each bone cubes to assess its conformity to these invariants using a pairwise m/n bootstrap Kolmogorov–Smirnov (K–S) tests [1]. Cubes whose probability distributions were statistically indistinguishable from these benchmarks were considered conforming to the invariants. If the probability distributions of one cube was statistically indistinguishable from the benchmarks, this cube would be considered as conforming to these invariants. The ratio of conforming cases to total comparisons was calculated for each anatomical site (femoral head, neck, and intertrochanteric region) and defined as the conforming ratio, expressed as a percentage (%), with 100% indicating complete conformity at a given site. This ratio was used to evaluate the extent of microstructural abnormality at each anatomical site. Multivariate ANOVA followed by post hoc (Bonferroni) tests was performed to assess the effects of age, sex, and anatomical location on microstructural abnormality and to examine statistical differences between test groups.

RESULTS: Multivariate ANOVA analyses (**Table 1**) indicated that aging had significant effects on conforming ratio in all microstructural parameters (PA, PT, RL, RD, NNDpp, NNDpr, NNDrr, and NNDrp), whereas sex demonstrated significant effects only on NNDrp. Anatomical location showed significant effects on conforming ratio in plate area (PA) and thickness (PT) and rod-to-rod as well as rod-to-plate nearest neighbor distances (NNDrr and NNDrp). Cross-effects among these three factors were reflected mainly in PA and NNDrp, with weak or no effects on other microstructural parameters. *Post hoc* (Bonferroni) comparisons exhibited that conforming ratio in PA, and NNDrp decreased with age, whereas significant differences were observed in PT, NNDpp, NNDpr, RL, RD, and NNDrr only between young bones and those from the other two age groups. In addition, significant differences in conforming ratio were observed only in PA, PT, NNDrr, and NNDrp between anatomical sites, showing these differences mainly manifested between intertrochanter and other two sites (femoral head and neck).

DISCUSSION: The results of this study demonstrate that age, sex, and anatomical location significantly affect the microstructural integrity of the human proximal femur. Age-related microstructural abnormalities were observed across all features, whereas sex-dependent differences were limited to the rod-to-plate arrangement within the bone. Furthermore, anatomical location mainly influenced microstructural integrity in PA, PT, and NNDrr, and NNDrp. Considering the adverse effect of microstructural non-conformity on bone mechanical competence, these findings align well with clinical observations of increased fracture risk in the elderly population and postmenopausal women. However, further research is needed to translate these novel benchmarks into clinical practice.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates the feasibility of using a set of novel and intrinsic benchmarks (probability invariants) to evaluate local microstructural abnormality at different anatomical sites in human proximal femurs. These benchmarks can be used to improve risk assessment of bone fragility fractures in addition to BMD and other influencing factors.

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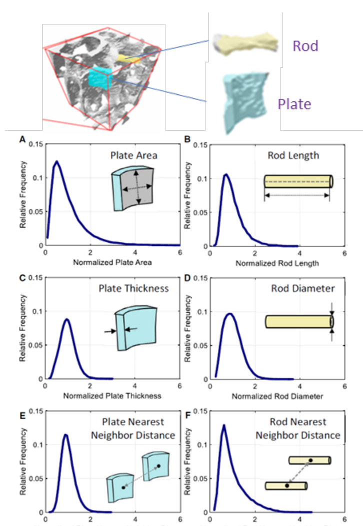

Fig. 2 Trabecular size and spatial arrangement and their probability distributions in bone

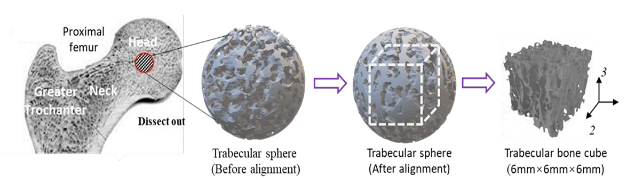

Fig. 1 Dissection of trabecular bone cubes from human cadaver proximal

Table 1. ANOVA Table (*p* values)

	PA	PT	NNDpp	NNDpr	RL	RD	NNDrr	NNDrp
Age	<0.001	0.001	0.002	<0.001	0.018	0.025	<0.001	<0.001
Sex	NS	NS	NS	NS	NS	NS	NS	<0.001
Anatomical location	0.018	0.009	NS	NS	NS	NS	0.03	0.002
Age*Sex	0.005	0.026	NS	NS	NS	NS	<0.001	<0.001
Age*Anatomical location	0.01	NS	NS	0.015	NS	NS	NS	NS
Sex*Anatomical location	0.017	NS	0.048	NS	NS	NS	NS	NS
Age*Sex*Anatomical location	<0.001	NS	NS	NS	NS	NS	0.045	NS