Regional Variations of Bone Quantity and Quality Impact Femoral Head Collapse

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Received: 10 November 2008 / Accepted: 30 July 2009 / Published online: 26 August 2009
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Abstract Osteonecrosis (ON) of the femoral head causes the bone to deteriorate, buckle, and collapse. As the vasculature is reportedly uniform in the femoral head, one would expect uniform susceptibility to ON; however, collapse typically occurs in the anterior region. We asked whether regional variations in bone quantity and/or quality could explain the bone's anterior susceptibility despite uniform vascularity. We examined seven femoral heads resected for primary osteoarthritis and three removed after femoral neck fracture. Each was cut into 4-mm-thick, 1.5 cm × 1.5-cm bone squares, processed for light microscopy, and sectioned twice. One section was stained with Gomori's trichrome and assessed by a computer-assisted microscope, which calculated trabecular area, a measure of bone quantity. The other was stained with hematoxylin and eosin and assessed by light microscopy to identify trabecular microfractures, a measure of bone quality. Bone quantity and quality were reduced in the fracture group as a whole; bone quantity was uniform in each femoral head, but the quality was reduced in the anterior portion. The quality was further reduced in the superior region of arthritic bone and in the lateral-inferior

regions of the fractured bones. Our findings suggest the anterior susceptibility is the result of bone loading and, as such, reinforcement of the femoral head in ON should focus on the anterior hemisphere.

Introduction

ON of the femoral head compromises cancellous bone and even may lead to buckling of the overlying articular surface, eventually causing collapse and degenerative changes [3, 17, 18]. The vascularity in the femoral head is reportedly uniform [15] and thus there is a presumed uniform susceptibility to have ON develop if caused strictly by vascular occlusion. However, it is the anterior portion of the bone that most often suffers the greater overall collapse. This observation suggests regional differences exist in the quantity and/or quality of femoral head bone and/or in stress distribution in the femoral head. Brown et al. postulated the trabecular bone "subjacent" to the subchondral plate in the weightbearing region initiates the collapse as a result of its poor quality [3], whereas Bergmann et al. [2] and Johnston [10] explained the greatest forces are applied to the anterior-superior face of the femoral head. To understand the pathophysiology of collapse, and even how to better manage precollapse stages, one should assess the quantity and quality of the entire femoral head and consider how it may relate and/or reflect contact pressure and stress distribution.

The presence of trabecular microfractures likely reflects bone quality and their existence, pathogenesis, locations, and clinical implications of these microfractures are well documented [4–6, 11, 14, 19]. A microfracture is a sub-clinical pathologic response to microtrauma in a trabecula of bone resulting from excessive load on normal bone or

Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

This study was performed at NYU Langone Medical Center.

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Fig. 1A–B H&E stained photomicrographs at (A) $\times 200$ magnification and (B) $\times 100$ magnification show a transverse section of a femoral head trabecula, which contains a trabecular microfracture surrounded by a trabecular microcallus and a cross-section of a trabecular microfracture, which contains a trabecular microcallus only.

normal load on insufficient bone. When a trabecula sustains a microfracture, a healing process inevitably ensues, which produces a trabecular microcallus. All microcalluses come from microfractures and all microfractures (except those in necrotic tissue) produce microcalluses. This mechanism is not completely understood but may involve inflammatory cytokines [5]. Histologically, the microcallus is a nodular lesion, appearing arched, angulated, fusiform, or rounded according to Fazzalari et al. [4]. Hahn et al. [7] explained the microcallus is immature woven bone. Koszyca et al. [11] further classified microfractures as being “nodular” composed mainly of woven bone, or “smooth” composed mainly of lamellar bone. These are acute (nodular) and chronic (smooth) manifestations of the same pathophysiology. Furthermore, we have observed an increased number of disorderly spaced cells in enlarged lacunae and creeping substitution with irregular (nonsmooth) contours (Fig. 1). A microfracture is a discontinuity measuring 4 to

10 μm ; thus, it rarely is present in the plane of a two-dimensional histologic section. However, a microcallus is an extensive zone of bone remodeling measuring approximately 500 μm [4]. Because a microcallus is larger, it more likely is present on a histologic section; thus, observing a microcallus is an indirect, yet appropriate, way of documenting a microfracture [4].

In contrast to trabecular microfractures that reflect bone quality, trabecular area may be considered a measure of bone quantity. Trabecular area is the percentage of area taken up by bony trabeculae in a given histologic section of bone; the remaining area is taken up by marrow.

We therefore asked: (1) Is there a relationship between quantity and quality of bone in femoral heads as a whole? (2) Do differences in bone quantity and quality exist between hemispheres? (3) Do differences in bone quantity and quality exist in quadrants?

Materials and Methods

We analyzed 10 human femoral heads removed from patients, who had undergone hip arthroplasties, for bone quality and quantity based on the histologic presence of microfractures and by the value of trabecular area. Three femoral heads were harvested from patients who sustained femoral neck fractures (fracture group; one male, two females; average age, 82.7 years; age range, 75–87 years). The remaining seven specimens were resected as a result of primary osteoarthritis (arthritic group; two males, five females; average age, 66.0 years; age range, 50–86 years). Laboratory and financial constraints prevented us from examining more femoral heads. All femoral heads first were cut in half with a band saw, separating the anterior from the posterior hemisphere. We then made serial coronal sections from each femoral head hemisphere producing 4-mm-thick bone slices (Fig. 2).

Each of the six to 10, 4-mm-thick bone slices from the 10 femoral heads was submerged in decalcifying solution (10% HCl in distilled water) until sufficiently soft. After decalcification, we cut each bone slice with a scalpel, separating the superior, inferior, medial, and lateral quadrants (Fig. 3). To ensure every bone slice was quartered with the same specifications, we centered each bone slice onto a cutting board specifically designed to align the quadrants. For the bone to fit inside a tissue cassette for processing, the bone quadrants were cut further, with a scalpel, into bone squares measuring 1.5 cm $\times$ 1.5 cm. The tissue then was fixed in 10% neutral-buffered formalin, processed for light microscopy, embedded in paraffin wax, and cut to a thickness of 4 μm with a microtome. We made a total of 750 paraffin-embedded bone samples, approximately 75 per femoral head. Two serial histologic sections

Fig. 2 This medial-to-lateral view of the proximal femur diagrams the anterior and posterior hemispheres and the 4-mm-thick bone slices in relation to the rest of the proximal femur. Ant = anterior; Post = posterior.

Fig. 3 This anterior-to-posterior view of the proximal femur diagrams the location of the superior (SQ), inferior (IQ), medial (MQ), and lateral quadrants (LQ) in relation to the rest of the proximal femur.

were made from each of the 750 paraffin-embedded bone squares. One section was stained with hematoxylin and eosin (H&E) for visual assessment, and the other was

Fig. 4A–B Photomicrographs with (A) H&E staining (Original magnification, $\times 5$) and (B) Gomori's trichrome staining (Original magnification, $\times 5$) show serial histologic sections from paraffin-embedded bone squares of a human femoral head.

stained with Gomori's trichrome for digital assessment (Fig. 4). The Gomori's trichrome provided a high contrast needed for the digital analysis.

One of us (CJZ) examined the H&E slides with a light microscope for the presence of microcalluses and/or microfractures based on the histologic descriptions mentioned previously. The observer was blinded to which population and specific area was being examined. The microfracture value is given as occurrences per histologic section, which measured $1.5 \text{ cm} \times 1.5 \text{ cm}$. The observer also blindly examined the Gomori's trichrome slides with an automated digital image analysis system outfitted with a computerized

scanning light microscope (BioGenex, San Ramon, CA) for trabecular area. The BioGenex software automatically calibrated the microscope to transparency, focus, and exposure for Gomori's trichrome; however, the software was calibrated manually for hue, saturation, and color intensity. These protocols were set onsite by sampling relevant trabeculae in Gomori's trichrome reference slides. We evaluated each experimental slide using the same digital protocol to objectively determine bone quantity in the histologic section, which measured $1.5\text{ cm} \times 1.5\text{ cm}$. The trabecular area value was given as a percent: [trabecular area/total area of histologic section]. In our study, the term "area" is used in place of the term "coverage," which is used frequently in quantitative pathology [1]. We coded all sections (H&E and Gomori's trichrome) according to the experimental group (fracture or arthritic), location (hemisphere and quadrant), and to the stain used (H&E or Gomori's trichrome).

We determined differences in bone quantity and quality between femoral heads of the fracture and arthritic groups using two-sample t-test with equal variances. In addition, we determined differences in bone quantity and quality between hemispheres of femoral heads using a two-sample t-test with equal variances. Finally, we determined differences in bone quantity and quality between quadrants of femoral heads using analysis of variance of multiple comparisons with Bonferroni correction. The mean, standard deviation, and exact probability values have been provided.

Results

Microfractures occurred more often ($p < 0.0001$) in femoral heads from the fracture group; however, trabecular area was greater ($p < 0.0001$) in femoral heads from the arthritic group (Table 1). Bone with less quantity has low quality.

The anterior hemispheres of the fracture and arthritic groups were greater ($p = 0.2389$ and 0.2612 , respectively) in trabecular area per 1.5 cm^2 of bone than their corresponding posterior hemispheres (Table 2). We observed no significant difference in bone quantity between hemispheres.

Table 1. Average trabecular microfractures and average value of trabecular area in femoral heads as a whole

	Fracture group	Arthritic group	Significance
MFxs per 1.5 cm^2	0.97	0.18	$p < 0.0001$
Standard deviation	1.52	0.65	
Area per 1.5 cm^2	10.28%	14.51%	$p < 0.0001$
Standard deviation	4.76	7.88	

The anterior hemispheres of the fracture and arthritic groups were greater in microfracture number per 1.5 cm^2 of bone than their complementary posterior hemispheres ($p = 0.0214$ and $p = 0.0354$) (Table 3). Bone quality was lower in the anterior hemisphere.

There were few differences in bone quantity between specific quadrants of the femoral head in each experimental group. In the fracture group, the inferior quadrant was greater ($p = 0.048$) in trabecular area per 1.5 cm^2 of bone compared with the lateral quadrant (Table 4). All other interregional differences were similar; the quantity of bone was similar. In the arthritic group, the superior quadrant was greater in trabecular area per 1.5 cm^2 of bone compared with the medial and lateral quadrants ($p = 0.002$ and 0.023 , respectively). All other interregional differences were similar; the quantity of bone was greatest in the superior quadrant but similar in all other quadrants.

There were differences in bone quality between specific quadrants of the femoral head in each experimental group. In the fracture group, the medial quadrant was greater ($p = 0.027$) in microfracture number per 1.5 cm^2 of bone compared with the lateral quadrant (Table 5). All other interregional differences were similar; the quality of bone was the lowest in the medial and lateral quadrants. In the arthritic group, the superior quadrant was greater in microfracture number per 1.5 cm^2 of bone compared with the inferior and lateral quadrants ($p = 0.049$ and 0.007 , respectively). All other interregional differences were similar; the quality was lowest in the superior quadrant but similar in all other quadrants.

Discussion

ON of the femoral head causes the bone to deteriorate, buckle, and eventually collapse. Although the bone reportedly has uniform vascularity and a presumably uniform regional susceptibility to develop ON, if caused strictly by vascular occlusion, femoral head collapse is seen clinically anteriorly. We sought an explanation for the difference in what is expected and what is seen. We asked: (1) Is there a relationship between quantity and quality of bone in femoral heads as a whole? (2) Do differences in bone quantity and quality exist between hemispheres? (3) Do differences in bone quantity and quality exist in quadrants?

Our main limitation was the number of specimens used. Laboratory and financial constraints prevented us from examining more femoral heads. We do not believe this jeopardized our conclusions because we found differences. When we examined for regional differences, our increased specificity of location resulted in lower power and thus lower statistical significance. Although the differences we

Table 2. Average value of trabecular area in femoral head hemispheres

	Fracture group, anterior hemisphere	Fracture group, posterior hemisphere	Significance	Arthritic group, anterior hemisphere	Arthritic group, posterior hemisphere	Significance
Area per 1.5 cm ²	10.36%	10.20%	p = 0.2389	14.89%	14.10%	p = 0.2612
Standard deviation	5.27	4.17		7.88	7.88	

Table 3. Average number of trabecular microfractures in femoral head hemispheres

	Fracture group, anterior hemisphere	Fracture group, posterior hemisphere	Significance	Arthritic group, anterior hemisphere	Arthritic group, posterior hemisphere	Significance
MFxs per 1.5 cm ²	1.19	0.73	p = 0.0241	0.25	0.13	p = 0.0354
Standard deviation	1.81	1.05		0.73	0.55	

Table 4. Average value of trabecular area in femoral head quadrants

	Fracture group, superior quadrant	Fracture group, inferior quadrant	Fracture group, medial quadrant	Fracture group, lateral quadrant
Area per 1.5 cm ²	10.33%	12.58%	10.85%	9.58%
Standard deviation	4.94	8.12	4.73	5.04
Significance				
Inferior	p = 0.201			
Medial	p = 1.000	p = 0.600		
Lateral	p = 1.000	p = 0.048	p = 1.000	
	Arthritic group, superior quadrant	Arthritic group, inferior quadrant	Arthritic group, medial quadrant	Arthritic group, lateral quadrant
Area per 1.5 cm ²	16.61%	14.82%	13.05%	13.67%
Standard deviation	9.37	7.39	7.20	6.40
Significance				
Inferior	p = 0.515			
Medial	p = 0.002	p = 0.401		
Lateral	p = 0.023	p = 1.000	p = 1.000	

found were not uniformly significant, the data had uniform trends. If we had more specimens, we could have increased regional specificity while achieving sufficient power. In addition, although we did not document trabecular size, number, strength, and orientation, we inferred from the cited data of others. Finally, when analyzing bone quantity, the computerized scanning light microscope was advantageous because of the high degree of reproducible accuracy.

There is a greater occurrence of microfractures and less trabecular area in the fracture group compared with the arthritic group. This suggests microfractures occur more frequently in bone with an overall reduced quantity. Bone quantity and quality are indeed related. These findings are consistent with the findings of prior studies, which

documented the likelihood that microfracturing becomes greater with less bone [4, 11, 14, 16, 19].

We found no differences in bone quantity between the anterior and posterior hemispheres in the fracture and arthritic groups. Their equal quantities of bone reflect the equal ability of each hemisphere to form bone. The vascular pattern, which persists throughout life once it is established at maturity, is reportedly homogeneous throughout the femoral head [15]. Because the quantity of bone is equal in both hemispheres, the quantity of bone cannot account for the anterior susceptibility of femoral head collapse in ON.

The anterior hemispheres of the fracture and arthritic groups have a greater number of microfractures. Given that both sets of hemispheres have equal quantities of bone, the

Table 5. Average number of trabecular microfractures in femoral head quadrants

	Fracture group, superior quadrant	Fracture group, inferior quadrant	Fracture group, medial quadrant	Fracture group, lateral quadrant
MFXs per 1.5 cm ²	0.90	1.22	0.58	1.40
Standard deviation	1.40	1.61	1.08	1.94
Significance				
Inferior	p = 1.000			
Medial	p = 1.000	p = 0.169		
Lateral	p = 0.512	p = 1.000	p = 0.027	
	Arthritic group, superior quadrant	Arthritic group, inferior quadrant	Arthritic group, medial quadrant	Arthritic group, lateral quadrant
MFXs per 1.5 cm ²	0.36	0.14	0.17	0.10
Standard deviation	0.94	0.50	0.55	0.56
Significance				
Inferior	p = 0.049			
Medial	p = 0.123	p = 1.000		
Lateral	p = 0.007	p = 1.000	p = 1.000	

lower quality in the anterior hemisphere is independent of quantity. Load patterns and/or trabecular size, number, and strength must account for this observed difference. According to Hodge et al. [8], Krebs et al. [12], and Yoshida et al. [20], the anterior hemisphere is subject to less total load but experiences higher peak load, because it continually osculates between being covered and uncovered by the acetabulum. We suggest these osculation-induced peak loads account for the lower bone quality in the anterior hemisphere and the increased susceptibility for it to collapse in ON. On the contrary, Yoshida et al. [20] explained the posterior hemisphere is subject to greater total load but experiences less peak load, because it is contained in the acetabulum.

In addition to peak loads, the individual trabecular size, number, and/or strength may account for the dichotomy in quality between hemispheres of equal quantity. According to Watson [19], as a collection, thinner trabeculae are equally strong as thicker trabeculae, but, as an individual, a thin trabecula is weaker. This suggests the anterior hemisphere contains a larger number of thinner, lightly stressed trabeculae, which fracture as a result of concentrated load, whereas the posterior hemisphere contains a smaller number of thicker, weightbearing trabeculae, which fracture from general fatigue.

We found no difference in bone quantity between quadrants of the fracture group, consistent with our prior findings, but only the superior quadrant of the arthritic group had a larger quantity of bone resulting from the accumulation of microcalluses, which according to Hahn et al. [7] may account for as much as 10% of bone quantity.

The difference in bone quality, between quadrants, reflects the bone pathology and load pattern. Homminga et al. [9] determined trabecular strength is greater when load is applied parallel to the main trabecular orientation and weaker when perpendicular. Furthermore, they hypothesized that when bone becomes anisotropic (over-adapted) to parallel loading, it becomes more fragile to perpendicular loading. In their study, it was the femoral heads from patients who had a femoral neck fracture that had anisotropic bone. When the anterior hemisphere articulates with the acetabulum, load is concentrated and applied in the perpendicular direction [2]. This correlated with the tendency (not significant) for microfracture location in the fracture group; inferior and lateral quadrants had more microfractures. They are also adjacent to the location of femoral neck fractures. Results from femoral heads of the arthritic group also correlated with their bone pathology and load. The superior and medial quadrants had more microfractures because they not only lack articular cartilage, but also they experience the greatest loading [10]. Although the inferior and lateral quadrants experience perpendicular loading, the arthritic bone is not anisotropic; thus, these quadrants have a lower microfracture number.

Compared with its corresponding posterior hemisphere, the anterior hemisphere of both experimental groups has an equal quantity of bone yet less quality. It has higher peak loads and may have overadapted, thinner, weaker individual trabeculae. We offer these observations as an explanation for the location and mechanism of femoral head collapse in ON, which most often occurs in the anterior and lateral margins [13, 18]. Because the vascular pattern of the femoral head is homogeneous, so is its

quantity of bone. Differences in bone quantity cannot account for the anterior susceptibility of the femoral head; thus, it is the load, concentrated by the perimeter of the acetabulum, which accounts for the pattern of collapse. The location of microfractures is indicative of this phenomenon and suggests core decompression and reinforcement of the necrotic femoral head may be more efficacious and necessary in the anterior hemisphere.

Acknowledgments We thank Tony Gallina for fabricating the cutting board and NYU School of Medicine faculty members, Mel Rosenfeld, Associate Dean for Curriculum and Assistant Professor of Cell Biology, and Joan Cangiarella, Associate Professor and Vice Chairman of Pathology, for histologic assistance; Brian West, Adjunct Professor of Pathology, for assistance calibrating the scanning microscope; and Michael Walsh, Assistant Professor of Environmental Medicine and Orthopaedic Surgery, for statistical support.

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