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Histomorphometric study of femoral heads in hip osteoarthritis and osteoporosis

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Summary. During the period from 2000 to 2003, ninety eight samples of femoral heads were collected. In these pieces, two zones were analyzed: a high-load zone (the hard core of the head) and a low-load zone (the round *ligamentum teres* zone). As control group, 6 femoral heads (3 of women and 3 of men), proceeding from autopsy in peoples without pathological antecedents and youngs, were studied. After the samples had been embedded in methylmethacrylate and stained, they were subjected to an histomorphometric study. By means of histomorphometry, trabecular bone volume (TBV) and osteoid substance (OSV) was determined. Statistically significant differences were found as for peripheral osteoid volume (low-load zone) (p=0.036) and trabecular bone volume, both peripheral and central. Both volumes decreased in osteoporotic samples and in those from women (p=0.000), in comparison with control group. Regarding the relationship between the high-load and low-load zone, significant data were obtained. The high-load zone had a greater trabecular bone volume than the low-load zone, regardless of the pathology and sex, but this increase was more pronounced in the arthrosic samples and in those from men. Additionally, this trabecular bone volume in the high-load zone decreased with increasing age of the donor (p=0.037), when the control group is compared. In sum, we observed a reduction in the formation of TBV and OSV in osteoporosis but also a decrease in the arthrosic, in samples from older subjects, in women, and in the low-load zone of the samples, when the control group is compared. These data suggest the coexistence of both pathologies, which is more pronounced in older subjects and women.

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Introduction

Advances in medicine have made it possible for people to live longer than before and this has led to greater out-patient attention being necessary for such individuals. In turn, this has sparked another series of problems, among them an increase in bone disease. Nevertheless, it should be recalled that hip fractures and osteoarthritis, (OA), both of which are linked to old age, cannot be completely prevented, which has led to an increase in their incidence and severity and an increase in public spending on surgery and rehabilitation (Pierron et al., 1990).

Affectation of the coxofemoral joint is one of the most frequent conditions in medicine. There are more than 100 nosological entities, but those causing the most socioeconomic problems are osteoarthritis and osteoporosis (the essential cause of hip fractures in the elderly).

Osteoporosis (OP) is characterized by the loss of bone mineral and a destructuring of the bone microarchitecture, leading to a loss of bone resistance and an increased risk of fracture (Ray et al., 1997; Melton, 1993). Hip fractures are considered to be a clinical endpoint for the diagnosis of osteoporosis. The disease affects mainly the elderly (senile osteoporosis) and women (post-menopausal osteoporosis) (Melton, 1993).

OA is a degenerative illness that affects all the joints of the body and it has been defined (Croft et al., 1990) as an alteration between the synthesis and degradation of cartilage and subchondral bone. From the clinical point of view, osteoarthritis is the second cause of invalidity after cardiovascular disease and it affects 10% of the population older than 60.

Different methods are used for the diagnosis of OP. Bone densitometry (Kanis et al., 1996), based on the density of bone mineral, is the one most widely used. Simple radiology (Singh et al., 1970) and magnetic resonance (Link et al., 2003) are also used. Histomorphometry has been used for the assessment of the microarchitecture of bone tissue. Parfitt et al. (1987) described a series of parameters for carrying out quantification of this tissue. Later, other authors (Fazzalari et al., 1992; Morini et al., 1996; Fazzalari and Parkinson, 1998) used this method to gain further insight into osteoporosis and OA.

The samples chosen for study of these pathologies have been femoral heads (Fazzalari et al., 1992; Morini et al., 1996; Grynpas et al., 1991) and the iliac crest (Healey et al., 1985; Crane et a., 1990; Fazzalari et al., 1992). The latter is considered to be a zone of bone tissue that is readily accessible and where the removal of samples is innocuous. Nevertheless, the femur head is the most reliable sampling zone since it is the anatomical zone most affected by the pathologies in question.

In 1972, Foss and Byers published their report confirming observations made by orthopedic surgeons on the relative absence of osteoarthitic changes in excised femoral heads from patients who had had hip fracture.

Increasing bone mineral density (BMD) has been found in several studies in patients with OA (Hochberg et al., 2004). Therefore, the simultaneous occurrence of OP and OA is denied by many clinicians. Drees et al. (2005) cannot support the hypotheses that OA prevents OP. Moreover, the occurrence of OP in this study reflected the incidence of OP in the average female and was astonishingly high in the male population; this does not support the hypothesis that the two conditions are mutually exclusive.

There is current open discussion whether a known BMD should influence the decision for a cemented or uncemented prosthesis.

Speculation has been that weight-bearing activities, which are beneficial to the attainment and preservation of peak bone mass, also increase the risk of damage to articular cartilage leading to OA in lower extremity joints. Another explanation offered has been that high body mass index, which is associated with higher BMD, confers a detrimental biomechanical load to weight-bearing joints, thus leading to OA (Amin, 2002).

In the present study, a histomophometric assessment of femoral heads, extracted during prosthesis implantation, from subjects who had a hip fracture or who had OA, was performed.

The aim was to quantify the bone tissue in both pathologies and note the interrelationship between the two illnesses and the load effect.

Materials and methods

From January of 2000 to December of 2003, ninety-eight patients undergoing primary arthroplasty of the hip through the posterolateral approach, were selected. Out of this sample we excluded individuals affected by rheumatoid arthritis, hip dysplasia and avascular necrosis, choosing those who had intracapsular hip fractures (26 patients) and primary osteoarthritis (56 patients). The mean age of the subjects was 74.6 years (59-98), 53% being women and the rest men.

Once the femoral heads had been removed, they were preserved in 90° alcohol until processing for histological studies.

Following this, the heads were cut, taking a central slice in a sagittal section, including the round ligament (A) and the hard core (B), as examples of the low-load and high-load zones, respectively. The cut lines were delineated from 1 cm of lateral surface and the opposite face of the fovea capitis femoris (Fig. 1).

Once both zones had been removed they were embedded in methacrylate for bone processing without decalcification, using a polymerization time of 21 days in an oven at 32°C.

Five measurements taken at intervals of 2 mm in each zone were studied. Five-micron thick sections were collected with a Micro-HS® microtome (Carl Zeiss, Germany) for staining with Goldner trichrome (Luna, 1992) and Von Kossa (Kossa, 1901) staining; the techniques used for the histological and histomorphometric analyses. Histological analyses consisted of assessment of the samples under a light microscope and digitization using a Nikon Coolpix FXA® digital color camera (Tokyo, Japan).

The histomorphometric analyses was accomplished using the Eclipse Net program for Nikon Camera (Tokyo, Japan) installed on an Intel®Pentium® 4 CPU computer, 53 GHz AT/AT compatible 1.048.048 KB of RAM (Silicon Valley, CA. USA).

The static morphometric parameters assessed were the osteoid volume (OSV) and the trabecular volume (TBV) of the peripheral zone (A) and of the central zone (B).

The data obtained were processed with the SPSS 11.0 statistical program for Windows (Microsoft Corporation, USA). The Chi-square statistical test was used, with a level of significance of $p<0.05$.

Results

Histological results

Osteoporosis

The main finding was a quantitative and qualitative minor trabecular tissue in comparison with the control group and the group of OA. The trabeculae were thinner in the two zones studied (high-load and low-load zone). Additionally, the trabeculae displayed a smaller number of connections with one another (Fig. 2A). At the periphery we observed the presence of cartilaginous tissue, which conserved its thickness but not structural or cellular organization (Fig. 2B). Regarding osteoid material, this was minor in all samples studied compared with control group and OA group.

Hip osteoarthritis

As the main characteristics, the samples with an arthrosic pathology exhibited alterations in the joint cartilage and subchondral bone. The main change was the considerable extent of cartilage degradation, leading to the disappearance of the cartilage-subchondral bone interface (Fig. 3). In the zone where the cartilage tissue had disappeared, the subchondral bone displayed a degeneration of granulomatous tissue. In advanced stages, this had induced cystic cavities with mixoid content (geodes) (Fig. 4). Similarly, a major trabecular thickness was observed in both zones studied. This was more prominent in the low-load zone (Fig 5). In most samples analysed, a major thickness of osteoid substance was observed, mainly in the low-load zone, when was compared with control group and OA group.

Histomorphometric results

The variables considered for the histomorphometric analyses were OSV and TBV. An OSV was considered pathological when the values were less than 0.1 μm^3 and normal when they were above this; a TBV was considered pathological at values below 0.20 μm^3 and normal at values higher than this (Table 1).

A statistical study was performed using Chi-squared multivariate analysis ($p<0.05$) with respect to the sex, age and illness variables.

The OSV (low-load zone) showed statistically significant differences with regards to sex ($p=0.036$).

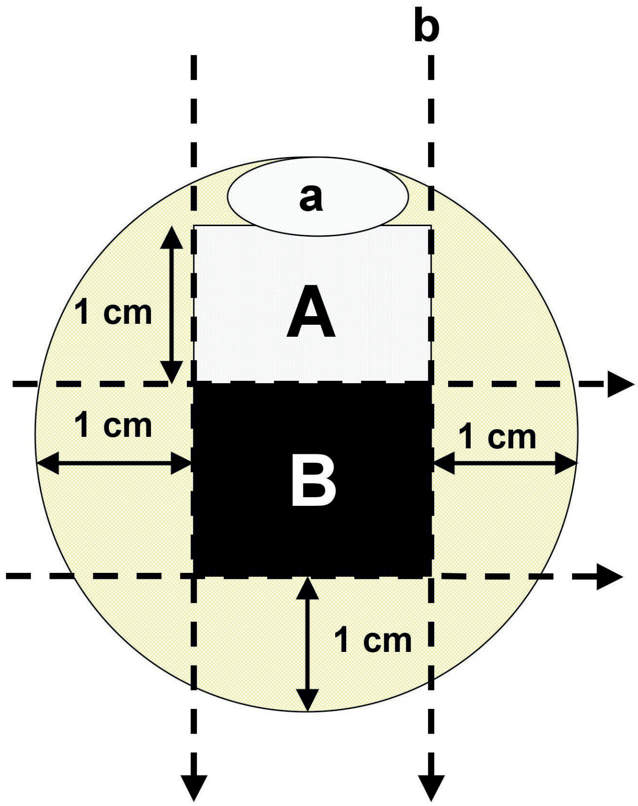

Fig. 1. Schematic distribution of study's zones of the femoral head. a: area of the fovea capitis femoris. **A:** Area of low-load. **B:** Area of high-load. b: Line of cut, delineated from 1 cm of the lateral side and opposite side of the fovea capitis femoris.

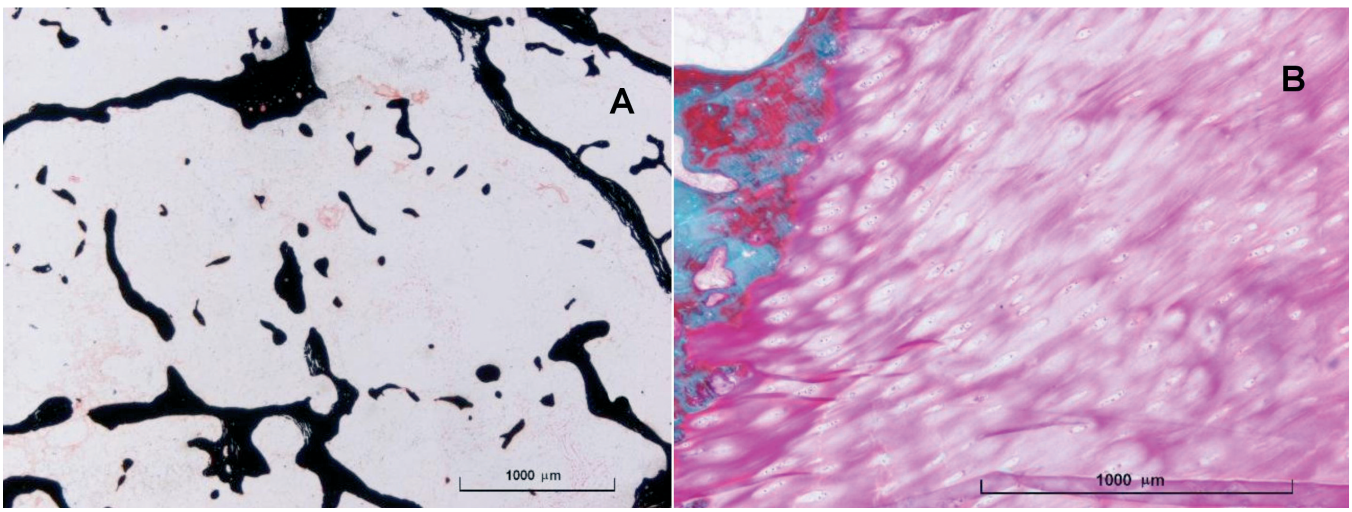

Fig. 2. Osteoporotic woman of 80 years old. Note the decrease in bone tissue, in thickness and in trabecular connections. Von Kossa stain. Bar 1000 μm. **B:** Osteoporotic woman of 70 years old. The micrograph show the joint cartilaginous tissue conserved, with alteration of the cellular and fibrillary organization. Goldner trichrome. Bar: 1000 μm .

Table 1. Means and Standard Deviations of OSV (mm3) and TBV (mm3) in the sample.

Pathology	Nº cases	OSV A	OSV B	TBV A	TBV B
Women					
osteoporosis	20	0.016±0.02	0.02±0.02	7.71±3.15	12.51±5.21
osteoarthritis	24	0.04±0.04	0.026±0.03	19.01±12.67	22.57±8.86
Men					
osteoporosis	6	0.032±0.02	0.026±0.03	5.44±1.11	12.77±3.93
osteoarthritis	32	0.05±0.05	0.021±0.03	21.64±8.15	22.86±9.25

Table 2. Percentage of samples showing normal and pathological OSV and TBV as a function of the variables (pathology, sex and age).

	Pathology		Sex		Age		
	Osteoporosis	Osteoarthritis	Women	Men	≤ 65	65-75	> 75
OSV A							
Pathological	33.8*	66.2*	59.2*	40.8*	N.S.	N.S.	N.S.
Normal	0*	100*	22.2*	77.8*	N.S.	N.S.	N.S.
OSV B							
Pathological	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Normal	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
TBV A							
Pathological	42.1***	57.9***	64.9***	35.1***	N.S.	N.S.	N.S.
Normal	0***	100***	31.8***	68.2***	N.S.	N.S.	N.S.
TBV B							
Pathological	50***	50***	65.2*	34.8*	45.5**	49**	85**
Normal	2.9***	97.1***	41.2*	58.8*	54.5**	51**	15**

* $P < 0.05$, ** $P < 0.03$, *** $P < 0.01$

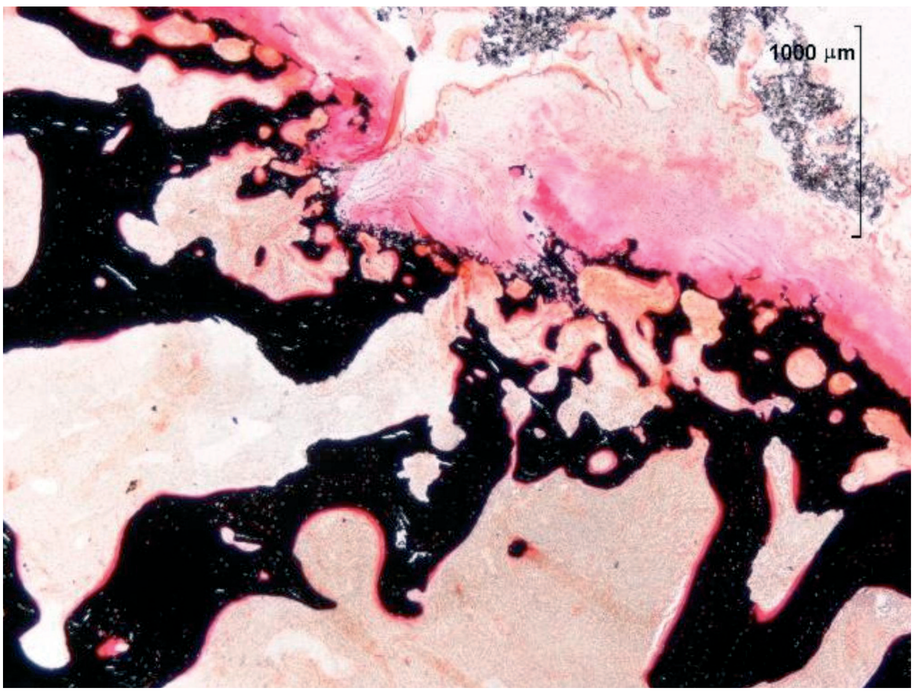

Fig. 3. Osteoarthritic man of 65 years old. Micrograph showing the disappearance of the cartilage interface. Von Kossa stain. Bar: 1000 µm.

Most of the samples hade a pathological OSV (93%), most being from women (59.2%). A normal OSV (7%) was found in 77.8% of men as compared with 22.2% of women. All the osteoporotic samples had a pathological OSV but, in contrast, only 85.4% of the samples were pathological in the arthrosic samples (p=0.037) (Table 2). In the OSV (High-load zone), no statistically significant differences were found for any of the

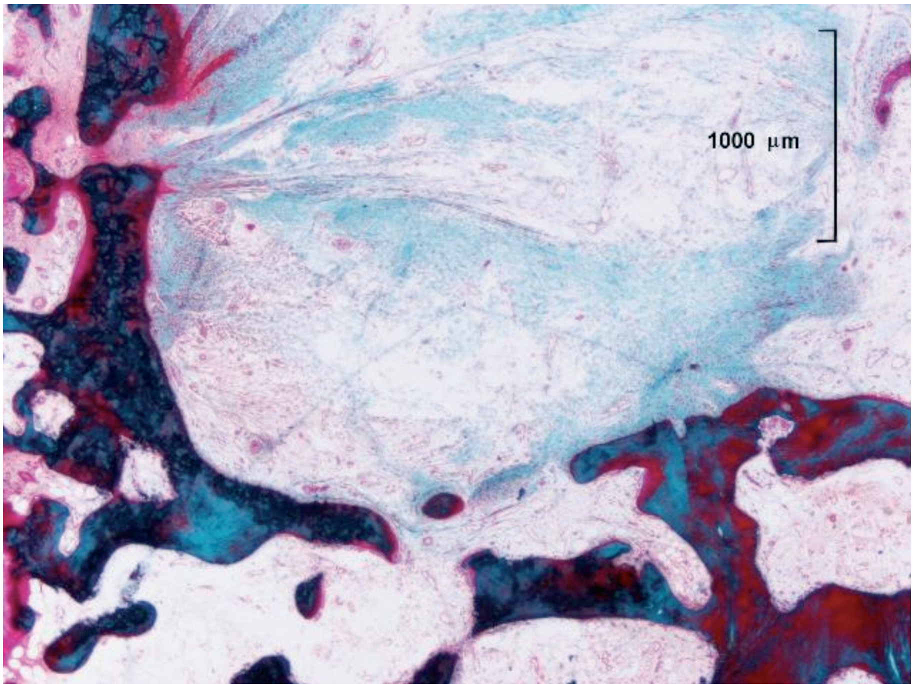

Fig. 4. Osteoarthritic woman of 75 years old. Note large central geode surrounded by trabecular bone. Goldner trichrome. Bar: 1000 μm.

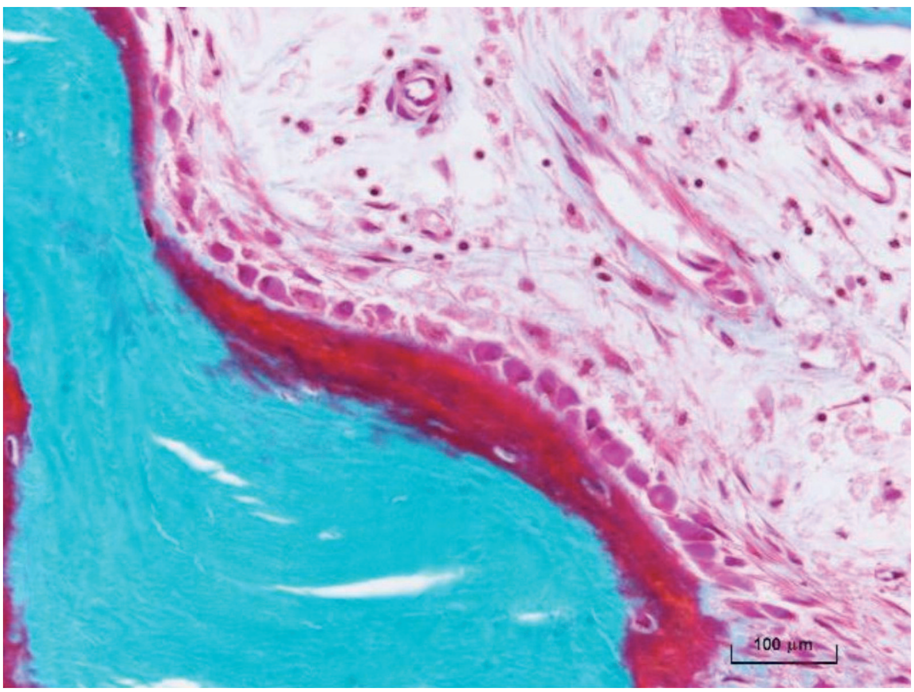

Fig. 5. Osteoarthritic man of 70 years old. Note large osteoid rim, synthesized by the osteoblasts line with bone-forming activity. Goldner trichrome. Bar: 100 μm.

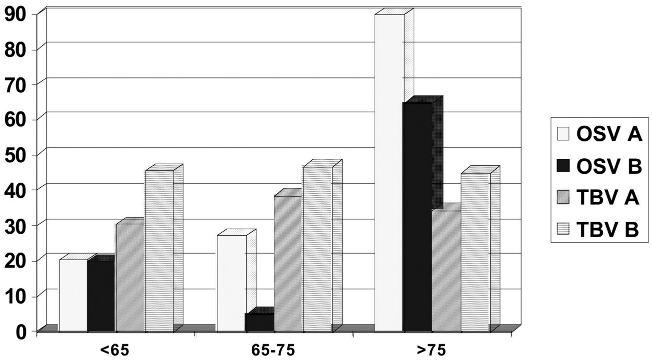

Fig 6. Histomorphometrics data in Patients (%) according to age. Osteoid volume (OSV). Trabecular bone volume (TBV). Non-load zone (A) and load zone (B).

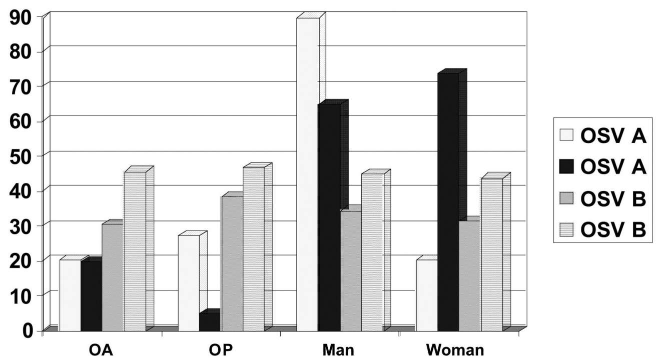

Fig. 7. Histomorphometrics data in Patients (%) according to disease and gender. Osteoid volume (OSV). Trabecular bone volume (TBV). Non-load zone **(A)** and load zone **(B)**.

variables studied.

Pathological TBV A values were found in a higher percentage in the women (64.9%) than in the men (35.1%))p=0.008). Similar percentages were found with respect to TBV B (65.2% vs. 34.8%) (p=0.033). Regarding illness, a value of p=0.000 was found for both TBV A and TBV B. All patients with osteoporosis had a pathological TBV as compared with the arthrosic subjects, in which the TBV was pathological in only 41.1% of cases. On assessing the samples with respect to age, statistically significant differences were found (p=0.016) for TBV B but not for TBV A. Eighty five per cent of the patients older than 75 had a pathological TBV, as compared with 45% of the younger subjects (59-75 years) (Table 2).

Discussion

The aim of this study was to determine histomorphometric differences between OA and OP in the proximal end of the femur since this is a load-bearing bone and is more intensely affected in these pathologies. The proposals found in the literature correspond to use of the iliac crest (Singh et al., 1970; Healey et al., 1985; Crane et al., 1990; Fazzalari et al., 1992) and femoral head (Crane et al 1990; Pierron et al., 1990, Fazzalari and Parkinson, 1998; Grynpas et al., 1991) The comparative study of Fazzalari (Fazzalari et al., 1992) between samples of iliac crest and femoral head corroborated the use of the femoral head as a more representative sampling point for the analysis of such illnesses.

The zones of the femoral head studied in this work were the zone of the *ligamentum teres femori* (A) and that corresponding to the hard core of the head (B) (Fig. 1). The reason for this was that a comparison between a low-load zone (A) and a zone of high-load (B) was wished (Morini et al., 1996; Fazzalari and Parkinson, 1998). Another factor in this choice was the ease of recognizing the same site in all the samples, taking into account the deformity of the piece, above all in the case of arthrosic pathology. In this way it was possible to guarantee sampling reproducibility.

The formation of OSV was greater in the arthrosic samples than in the osteoporotic ones (Chai and Fang, 1993) and greater in men than in women (Table 2).

Also, the same significant differences were found with respect to TBV (Morini et al., 1996, Fazzalari and Parkinson, 1998). This increase was more marked in the high-load zone with respect to the low-load zone, and both showed a decrease with the rise in the subjects' age (Oettmeier et al., 1989). The same results were reported by Fazzalari (Fazzalari et al., 1992), although in that work the high-load zone chosen did not correspond to the hard core.

The TBV A and TBV B was significantly minor in women than in men, in both OA (Fazzalari and Parkinson, 1998) and OP individuals. In previous studies (Crane et al., 1990; Morini et al., 1996) the same results were observed but only in the low-load zone.

OA is considered to be a cartilage disease, with preserved bone mass (Foss and Byers, 1972; Carlsson et al., 1979). As bone rigidity increases in OA, the capacity to sustain load impacts decreases, leading to an alteration of the cartilage. In contrast, a decrease in rigidity secondary to the loss of bone mass, as occurs in osteoporosis, would result in an attenuation for load impacts and would protect the cartilage (Radin and Paul, 1970). Considering that OP appears with a decreased percentage of mineral (Li and Aspden, 1997; Moreschini et al., 1995), this would support the hypothesis that OP is inversely correlated with OA. The results obtained in the present work support the notion of the coexistence of OP and OA. This relationship can be explained in terms of the minor TBV in both pathologies, mainly in women and increasing with age (Oettmeier et al., 1989; Fazzalari and Parkinson, 1998). Those OP and OA could coexist, but to a certain extent the dynamics of OA could also slow down the evolution of the OP. If this is indeed so, OA would be a negative risk factor for generalized

OP (Radin and Paul, 1970; Moreschini et al., 1995), although not for local OP due to age (Dunstan et al., 1990).

In conclusion, in the present work a minor bone formation in the low-load zone of the femoral head was observed; this was more marked in women and OP pathology. A pathological trabecular volume was observed in both zones of the femoral head in OP samples, although this was more striking in the low-load zone and in women. However, in OA samples trabecular volume was normal in the high-load zone.

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