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**TRABECULAR BONE MICROARCHITECTURE
IS RELATED TO THE NUMBER OF RISK FACTORS AND ETIOLOGY
IN OSTEOPOROTIC MEN**

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Short running title: bone architecture and number of risk factors (44 characters)

ABSTRACT:

Microarchitecture of trabecular bone is a very important component of bone quality in osteoporosis and a determinant of vertebral fracture in men with low bone mineral density (BMD). In contrast to women, male osteoporosis is, in most cases, secondary. The relationships between microarchitecture and different risk factors have never been evaluated in men. 152 men with low BMD at the lumbar spine or hip (BMD, T-score < -2.5) were included in this study. Risk factors were: age, BMI, alcohol intake, corticosteroid therapy, hypogonadism, chronic diseases. Transiliac bone biopsies were obtained and histomorphometry was done on an image analyzer; the following parameters were measured: cortical thickness (Ct.Th), trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), separation (Tb.Sp) and number (Tb.N), Interconnectivity Index (ICI), Star Volume of the bone marrow, and strut analysis with Node and Free-end count. The 50 men with 2 risk factors had a lower BMD, lower Ct.Th and a significant higher Star volume than those with one factor or idiopathic osteoporosis. The 26 men with at least 3 risk factors, had a lower BMD, a reduction of BV/TV and Ct.Th and a marked disorganization of the trabecular network (increased Tb.Sp, ICI, Star volume and Free-end to Free-end struts). The prevalence of vertebral fractures was higher in these patients. When the main risk factor was considered, a marked decrease in trabecular bone connectivity was observed in hypogonadic men. In osteoporotic men, higher the number of risk factors, lower the connectivity of trabecular network and higher the vertebral fracture risk.

249 words

Key words: Osteoporosis, Microarchitecture, Bone quality, Vertebral Fractures, Risk Factors.

81 characters

INTRODUCTION

Osteoporosis is now defined as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk (Anonymous, 1993). Several epidemiologic studies have shown that about 20 to 25 percent of hip fractures occurred in men and it has been estimated, in the USA, that the lifetime risk of hip fracture was about 6 percent, and the risk of vertebral fracture was about 5 to 8 percent in 50- year-old white men (Melton et al., 1992). As a result of a higher prevalence of concomitant disease, the mortality associated with hip fractures in elderly men is at least twice as great in women (Kellie and Brody, 1990; Melton et al., 1992).

In men, some densitometric studies demonstrate that the risk of fragility fractures increases as bone mineral density (BMD) declines (Gardsell et al., 1990; Nguyen et al., 1996; Aoyagi et al., 2000). However there is a considerable overlap in BMD measurements between controls and fractured patients and it has been suggested that BMD alone does not explain the fracture risk (Legrand et al., 1999). The microarchitecture of trabecular bone is an important component of what is now termed “bone quality” (Felsenberg and Boonen, 2005). We have extensively developed new algorithms for image analysers that can be used to measure trabecular microarchitecture on histological bone sections (Chappard et al., 1999; Chappard et al., 2001). In a previous study, we have found, by logistic regression analysis, that several microarchitectural descriptors were significant predictors of vertebral fractures. In other words, histomorphometry indicated that trabecular bone connectivity is an independent determinant of vertebral fracture and can be used to determine the fracture risk in clinical studies (Legrand et al., 2000).

However, in contrast to women, male osteoporosis is, in most cases, secondary. Causes such as prolonged corticosteroid therapy, alcohol abuse, hypogonadism and gastrointestinal disorders are now clearly recognized. The impact of a low Body Mass Index (BMI), cigarette smoking, hypercalciuria and thyrotoxicosis is more controversial but they seem to constitute real risk factors of bone loss (Orwoll and Klein, 1995; Seeman, 2002). The main problem to study the histological impact of these factors is that most osteoporotic men have several conditions (e.g. alcohol abuse *and* smoking *and* hypogonadism) which contribute together to bone loss (Orwoll and Klein, 1995). To our knowledge, the relationships between the trabecular microarchitecture and the different risk factors have never been evaluated in men. Furthermore, only few studies have analyzed bone remodelling. They suggest that bone formation is reduced in men with alcoholic osteoporosis (De Vernejoul et al., 1983;

Chappard et al., 1991), hypercalciuria (Zerwekh et al., 1992), corticosteroid therapy (Weinstein et al., 1998; Dalle Carbonare et al., 2001) or primary osteoporosis (Jackson et al., 1987; Francis et al., 1989). Bone resorption is more difficult to assess, but seems to be increased in hypogonadism-induced osteoporosis (Jackson et al., 1987).

The aim of the present study was to evaluate the relationships between trabecular bone microarchitecture (evaluated on transiliac bone biopsy) and the number of etiologic factors for osteoporosis and vertebral fractures in men with a low BMD.

MATERIAL AND METHODS

Patients

Three hundred and fifty-five men were referred in our unit (by their general practitioner or rheumatologist) in order to measure BMD because they had risk factors for osteoporosis, apparent osteopenia or vertebral fractures on X-ray films. After clinical and biological evaluation, patients with malignant tumours, multiple myeloma, mastocytosis, hyperparathyroidism, severe renal failure or treated for osteoporosis, were not included in this study.

One hundred and fifty two men with significant reduction in bone mineral density (BMD more than 2.5 SD below the normal value for young adults on the spine or the hip) gave informed consent. They were included in a transversal but prospective study to examine the relationships between (a) the microarchitectural changes of trabecular bone, (b) the spine and hip BMD, (c) the clinical risk factors for osteoporosis, (d) the presence of vertebral fractures. Ages ranged from 19 to 79 years (mean 52.6 years).

Assessment of aetiology and risk factors for bone loss

An extensive medical history (especially alcohol and medication use) and physical examination were obtained for each subject. Serum levels of calcium, phosphate, alkaline phosphatase, gamma glutamyl-transferase, testosterone, LH, 25-OH vitamin D, parathyroid hormone and thyroid hormones were measured. Urinary excretion of calcium and phosphate was also measured. Finally, the risk factors were checked:

- Age and Body Mass Index: age > 65 years or BMI < 19 kg/m² were considered as risk factors for bone loss
- Tobacco smoking: at least 15 cigarettes per day for at least 10 years

- Alcohol abuse: alcohol intake more than 80 g per day for at least 10 years
- Use of corticosteroids: at least 7.5 mg of prednisone (or equivalent) per day for at least 1 year
- Hypogonadism: repeated low serum testosterone and confirmation by dynamic tests
- Chronic disease associated with bone loss: gastrointestinal disorders, liver or lung disease, hypercalciuria, hyperthyroidism.

Bone Densitometry

We measured BMD (areal density in g/cm^2) with a dual energy X-ray absorptiometer (DXA) operating in fan beam mode (Hologic QDR 2000, Hologic Inc. Waltham, MA, USA). Quality control scans were performed daily, using anthropomorphic spine phantom supplied by the manufacturer; long-term coefficient of variation (<2 years) was 0.40%. Lumbar BMD was assessed from L2 to L4 in postero-anterior incidence and fractured vertebrae were excluded from analysis. At upper left femur, total hip BMD was taken into account.

Radiographic Assessment

Anteroposterior and lateral spinal X-ray films were performed at the time of the bone biopsy. They were analyzed independently by two trained investigators who were unaware of the patient status. A patient was classified as having a vertebral fracture if both readers independently found a definite fracture. He was classified as normal if both readers independently found that the films were normal. When the readers disagreed, the films were reviewed in conference between both investigators. Vertebral fracture was defined as a reduction of at least 20 percent in the anterior, middle or posterior vertebral height with the following criteria: (a) in anterior wedge deformity, ratio of anterior/posterior height < 80% (b) in concavity deformity, ratio of middle/posterior height < 80% (c) in compression deformity, ratio of posterior height/posterior height of the adjacent vertebra < 80%.

Bone Biopsies

Transiliac bone biopsies were obtained under local anaesthesia with a trephine having a 7 mm internal diameter (Model LHEA, Commecca-Commed, Beaucoz , France), 2 cm below the iliac crest and 2 cm behind the antero-superior iliac spine. Specimens were fixed in an alcohol based fluid and were embedded undecalcified in methylmethacrylate. For each patient, six undecalcified non serial sections (7 μm thick, separated by 100 μm) were cut dry on a heavy duty microtome, parallel to the axis of the

bone core. All biopsies were complete and unbroken (i.e., with two cortices and no artefactual disruption of the trabecular network). Sections were stained with a modified Goldner's trichrome.

Histomorphometric analysis

The histomorphometric analysis was done on a Leica Quantimet Q570 image analyzer (Leica France, Rueil Malmaison, France) as previously reported (Chappard et al., 1996; Chappard et al., 1999). In that way, basic histomorphometric measurements can be obtained. Cortical thickness (Ct.Th) is the mean of external + internal cortical thickness, expressed in μm . Trabecular bone volume (BV/TV) is the amount of trabecular bone within the cancellous space (expressed in %). BV/TV is derived from measurements of bone area (B.Ar) and cancellous tissue area (T.Ar) and expressed as:

$$\text{BV/TV} = 100 * \text{B.Ar/T.Ar}$$

In addition, four stereological methods were used in order to appreciate the spatial distribution of trabeculae and their connectivity.

1) Trabecular thickness, number and separation.

The following trabecular characteristics: mean thickness of the trabeculae (Tb.Th, in μm), trabecular number (Tb.N, in /mm) and mean separation between trabeculae (Tb.Sp, in μm) were derived from measurements of trabecular perimeter (B.Pm) and B.Ar according to Parfitt's formulae (Parfitt et al., 1983). All these parameters are based on the assumption that trabecular bone has a microarchitecture made of parallel plates connected by transverse rods.

2) *Inter Connectivity Index of marrow spaces (ICI)*

The connectivity of the marrow cavities was measured by skeletonization of their profiles on binary images. Skeletonization is a process of peeling off a pattern as many pixels as possible, without affecting the general shape of the pattern. It reduces foreground regions to a skeletal remnant that preserves the extent and connectivity of the original region while throwing away most of the original foreground pixels; however, the skeleton often contains insignificant branches that are removed by a pruning algorithm. On the pruned skeleton, the total number of nodes, node-to-node branches, node-to-free end branches were determined (Fig. 1A). Also, the number of “trees” was obtained, a tree being the structure composed of interconnected node(s) with node-to-node and/or node-to-free end branch(es). The InterConnectivity Index of the bone marrow cavities combines these parameters as previously shown {Chappard, 1999 #11}. The higher the level of connectivity of the marrow cavities, the higher the fragmentation of the bone trabecular network.

3) *Characterization of the trabecular network.*

The binary images of the trabeculae were skeletonized and pruned with the same algorithms as described for ICI determination (Mellish et al., 1991; Croucher et al., 1996; Chappard et al., 1999). On the skeleton, nodes (N), free-end (F) and the various types of trabeculae were measured: Node-to-Node struts (NN) and Free-end-to-Free-end struts (FF) (Fig. 1B). Measurements of strut lengths were expressed as a percentage of the total strut length (TSL).

4) *Star volume of the bone marrow*

The star volume of the marrow space was determined according to the chord length distribution method described for porous glasses or cements (Levitz and Tchoubar, 1992). As previously described by our group, this method is faster and more adapted to image analyzer than the original manual technique proposed by Vesterby (Vesterby, 1993). A series of grids of parallel lines (with angles from π to 2π), was used to explore the marrow cavities ((Fig. 2). The cubed length of each over imposed chord l_o^3 was measured and the star volume was defined as:

$$V_{m.space}^* = \pi/3 * \overline{l_o^3}$$

ICI and other last parameters are measured independently of B.Ar and T.Ar without assumption about the “plate and rod” model hypothesis.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS release 10.1, Spss Inc, Chicago). All results are expressed as mean \pm one standard deviation. The nominal significance level was set at 0.05. Correlations were searched between parameters: linear correction analysis used Pearson's r based on the model $y = ax + b$ where y is the dependent variable and x the predictor variable. Comparison between subgroups of men for BMD measurements, histological parameters and vertebral fracture prevalence were tested by multivariate analysis of variance (MANOVA) with post-hoc Scheffé's test. For comparing the effect of microarchitectural parameters with the number of risk fractures and the number of fractures, variables were standardized by subtracting the mean, then dividing by the standard deviation. "Standardization" is a special case of transformation since transformed variables have no units; it is used to compare variables that are originally measured in different units.

RESULTS

Clinical, biological and radiographic evaluation

Among the 152 men, 28 (18.4%) were older than 65 years, 11 (7.2%) had a BMI $< 19 \text{ kg/m}^2$, 35 (23%) took corticosteroids, 28 (18.4%) had an alcohol abuse, 70 (46.1%) were smoking. Clinical and biological tests showed a hypogonadism in 14 men (9.2%), an additional chronic disease associated with bone loss in 46 men (30.3%).

Most osteoporotic men had several pathological factors which might have, together, contributed to bone loss. So, the patients were divided according to the number of risk factors per patient:

- 28 men had no risk factors (idiopathic osteoporosis and age < 65 years and BMI $> 19 \text{ kg/m}^2$),
- 48 had a sole risk factor (e.g. hypogonadism or corticosteroid therapy),
- 50 men had two risk factors (e.g. hypogonadism *and* alcohol abuse)
- 26 patients had at least 3 risk factors (e.g. hypogonadism *and* alcohol abuse *and* corticosteroid therapy).

Anteroposterior and lateral spinal radiographs evidenced at least one vertebral crush fracture in 84 patients and none in the remaining 68 others. Among fractured patients, 25 patients had only one vertebral fracture while 45 men had 2 to 4 fractures. Fourteen patients had severe osteoporosis with 5 to 10 vertebral fractures.

Relationships between BMD, microarchitectural parameters and aetiology of osteoporosis

The histological findings were examined taking into account the main aetiology of bone loss in patients. Most osteoporotic men had several pathological factors which could together contribute to bone loss but we have the opportunity to compare the BMD and the microarchitectural parameters in 4 particular subgroups of patients:

- 28 men with strictly idiopathic osteoporosis,
- 30 men with corticosteroid therapy as the sole risk factor,
- 21 were heavy drinkers,
- 11 men with hypogonadism as the sole risk factor.

As seen in Table I, spine BMD was quite similar among groups but hip BMD was lower in corticosteroid, alcohol or hypogonadism-induced osteoporotic patients. Histomorphometric analysis showed that corticosteroid-induced osteoporosis was characterized by a lower Tb.Th. However, there was no significant difference between men with primary OP and men with corticosteroid -induced osteoporosis for any connectivity parameter.

In contrast, a significant decrease in trabecular bone connectivity was noted in men with hypogonadism: Tb.Sp, ICI, Free-end to Free-end struts and $V_{m.space}^*$ were significantly higher whereas Tb.N and Node to Node struts were lower in these patients.

Relationships between BMD, microarchitectural parameters and number of risk factors for bone loss per patient

As seen in Table II, the 50 men with 2 risk factors had a lower hip BMD, a lower Ct.Th and a significant higher $V_{m.space}^*$ which indicate a decrease in trabecular connectivity. Furthermore the 26 men, with at least 3 risk factors, had a lower hip BMD, a reduction of both BV/TV and Ct.Th and a marked disorganisation of the trabecular network with an increase in Tb.Sp, ICI, $V_{m.space}^*$ and Free-end to Free-end struts. The prevalence of vertebral fractures was higher in these patients. In the same way, regression analysis showed a significant relationship between the risk factors number per patient and total hip BMD ($r = -0.38$, $p = 0.01$), ICI ($r = 0.19$, $p = 0.02$), Free-end to Free-end struts ($r = 0.21$, $p = 0.01$) and $V_{m.space}^*$ ($r = 0.23$, $p = 0.005$). Hip BMD was found correlated with BV/TV ($r = 0.20$, $p = 0.02$) and architectural parameters such as ICI ($r = -0.32$, $p = 0.001$), $V_{m.space}^*$ ($r = -0.22$, $p = 0.02$) and FFS

($r = -0.24$, $p = 0.07$). Microarchitectural deterioration increased with the number of risk factors as seen on Figure 4 after standardization of variables.

DISCUSSION

In the present study, the relationships between BMD, trabecular bone microarchitecture and risk factors for bone loss were examined in a large cohort of men suffering from osteoporosis. As previously shown by others, clinical and biological tests revealed that osteoporosis was associated, in 77% of these men, with risk factors or medical conditions which could explain bone loss (Nguyen et al., 1996). Furthermore, taking account into an age over 65 years and a low BMI ($<19 \text{ kg/m}^2$) as risk factors, only 18.4% of men can be classified as suffering from idiopathic osteoporosis. Despite a similar lumbar BMD, the vertebral fracture prevalence was significantly lower in men with idiopathic osteoporosis, suggesting some differences in bone quality between idiopathic and secondary osteoporosis.

In this large cohort, trabecular bone microarchitecture was explored according to the main aetiology of bone loss. Corticosteroids are known to depress bone formation by a direct effect on osteoblasts and by promoting apoptosis of osteocytes and osteoblasts (Weinstein et al., 1998; Weinstein and Manolagas, 2005). The total amount of bone replaced in each remodelling cycle is reduced by about 30 % (Dalle Carbonare et al., 2001) leading to progressive thinning of plates (Chappard et al., 2007). This can explain the lower Tb.Th observed in the 30 men undergoing corticosteroid therapy, without alcohol abuse or biological hypogonadism. In addition, a marked alteration of in the connectivity of trabecular bone was observed in patients with hypogonadism. This was also found to be associated with an elevated number of fractures per patient. The significant reduction in trabecular node number and the concordant increase in Free-end to Free-end struts, $V_{m.space}^*$ and ICI indicate that trabecular microarchitecture was strongly altered in these men when compared with the idiopathic osteoporosis subgroup. These results agree with animal studies in rats which have shown that orchidectomy significantly altered connectivity parameters only after 2 weeks while differences in BMD appeared only after 16 weeks (Libouban et al., 2002). The rapid increase in osteoclast number and the higher bone remodelling occurring after sex hormone deprivation explain this marked loss of trabecular connectivity. This is in accordance with the higher incidence of fragility fractures in men with hypogonadism, especially in androgen deprivation therapy for prostate cancer (Oefelein et al., 2001). It is likely that an analysis taking account of the accumulation of risk factors (per patient) is more appropriated in men with osteoporosis. In a number of animal studies, an association of factors has

been proposed to obtain a severe bone loss in the rat. Castration has been associated with hemichordotomy (Okumura et al., 1991), sciatic neurectomy (Iwamoto et al., 2002; Iwamoto et al., 2003), gastrectomy (Surve et al., 2001), local paralysis with botulinum toxin (Blouin et al., 2007) or corticosteroid treatment or a low calcium diet (Lill et al., 2002). In all these reports, bone loss was more pronounced when two factors were combined than when a single factor was used.

Recently, clinical research has been focused on the respective role of bone mass and bone quality in the pathogenesis of osteoporotic fractures. Despite the clear relationship between low BMD and fracture risk, there is a considerable overlap in BMD measurements between controls and fracture patients. In addition, several studies show that, even after adjustment for BMD, a history of previous vertebral fracture increases considerably the fracture risk in men and women (Ross et al., 1991). Furthermore, antiresorptive therapies, such as raloxifene or bisphosphonates, reduce the vertebral fracture risk by nearly 50% whereas BMD increases by only 3 to 10%. So BMD increase explained only 4 to 25% of the reduction in fracture risk (Faulkner, 2000). These findings contribute to demonstrate that bone density has limitations as a surrogate for bone strength and fracture risk. Compelling evidence indicates that bone tissue quality influences fracture risk. Bone quality depends on many factors including the architecture of cortical bone, the degree of mineralization, the quality of collagen and non collagen proteins, the bone turnover rate, the mean degree of mineralization of the matrix and microarchitectural changes with trabecular bone connectivity (Felsenberg and Boonen, 2005). The importance of trabecular disorganization has been stressed by several modelling studies: a small number of perforations can considerably reduce the mechanical resistance (Silva and Gibson, 1997) and plate perforation is now recognized to have a considerable effect on bone strength (Liu et al., 2006). Furthermore, several groups have shown that the relationships between microarchitecture and bone mass were not linear, due to the fractal nature of the trabecular network (Chappard et al., 2001; Thomsen et al., 2002). Finally, the main influence of trabecular microarchitecture in the pathogenesis of vertebral or hip fractures has been recently underlined by several authors. Oleksik et al., have investigated bone microarchitecture in transiliac biopsy specimens from 88 post menopausal osteoporotic women (Oleksik et al., 2000). The 26 women with at least one vertebral fracture, had a lower cortical thickness and significantly altered strut parameters. In the same way, we have previously shown that trabecular bone connectivity is a major and independent determinant of vertebral fracture in men with osteoporosis (Legrand et al., 2000). Recently, Ciarelli et al., have examined the 3D architecture of trabecular bone in patients with a hip fracture; they found that fractured patients had a more anisotropic structure with fewer transverse trabecular rods to the load

axis (Ciarelli et al., 2000). At the present time, despite the exciting progress in imaging bone microarchitecture such as MRI or peripheral computerized tomography, bone quality can not be measured with non invasive methods in routine clinical practice due to the cost of these equipments. In the same way, the use of microcomputed tomography, which is now extensively popularized, does not provide sufficiently confident measurements in 3D. This method overestimates the microarchitectural descriptors because the algorithms used in 3D are influenced by the shape of the measured objects (Chappard et al., 2005; Chappard et al., 2007). Evaluation of bone microarchitecture by 2D histomorphometry remains actually a method of choice in the diagnosis of metabolic and malignant bone diseases.

The results of this study show a clear relationship between clinical risk factors for osteoporosis and BMD, as previously shown in the young (Lofthus et al., 2006). In addition, our results strongly suggest that relationships exist between the number of clinical risk factors and trabecular microarchitecture alterations. Dividing the patients in four subgroups according the number of risk factors, a gradual and pathological increase in $V_{m.space}^*$, ICI and Free end to Free-end struts were observed. In the men with at least 3 clinical risk factors for bone loss, a reduction in femoral BMD and also a marked decrease in trabecular connectivity were observed. These findings were associated with higher vertebral fracture prevalence and a significant increase in the fracture number. To assess fracture risk and to take decisions about therapy is difficult in men. Despite the concordance between our results and those of animal studies, relationships between microarchitectural parameters and the main aetiology of osteoporosis have to be analysed cautiously. The number of men was low in the subgroups, particularly for men with hypogonadism. In addition, chronic disease associated with corticosteroid therapy (e.g. asthma) could play a role in the pathogenesis of bone loss. In the same way, a part of these patients were smoking and a possible interaction of this risk factor interacts with alcohol, steroids or hypogonadism cannot be excluded. Notwithstanding these limitations, the results of this study strongly suggest that in men with low BMD, higher the number of clinical risks factors, lower the connectivity of trabecular network and probably higher the vertebral fracture risk.

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Table I: Comparison between patients with primary OP, corticosteroid-induced OP, alcohol-induced OP and hypogonadism tested by multivariate analysis of variance for age, BMI, BMD and histological parameters.

	Primary OP (n = 28)	Corticosteroid therapy (n = 30)	Alcohol abuse (n = 21)	Hypogonadism (n = 11)
Clinical characteristics				
Age (years)	46.1 ± 12.6	49.9 ± 14.2	50.2 ± 11.6	60.6 ± 14.5 ^a
BMI (kg/m ²)	24.4 ± 2.6	23.4 ± 3.1	25.3 ± 5.4	26.1 ± 3.7 ^a
Spine BMD (g/cm ²)	0.74 ± 0.09	0.72 ± 0.12	0.74 ± 0.08	0.71 ± 0.09
Hip BMD (g/cm ²)	0.85 ± 0.08	0.77 ± 0.19	0.74 ± 0.11 ^{aa}	0.73 ± 0.09 ^{aa}
% of fractured men	39.2	46.0	68.1 ^a	78.3 ^a
Number of fractures per patient	0.7	1.3 ^a	2.1 ^a	2.3 ^a
Histomorphometric parameters				
Ct.Th (μm)	869 ± 227	802 ± 437	835 ± 257	699 ± 209
BV/TV (%)	13.9 ± 3.5	11.8 ± 4.7	13.9 ± 5.8	11.1 ± 5.2 ^a
Tb.Th (μm)	106 ± 29	83 ± 24 ^{aa}	109 ± 34 ^{bb}	108 ± 34 ^b
Tb.N (/μm)	1.34 ± 0.26	1.44 ± 0.49	1.20 ± 0.27 ^b	1.01 ± 0.21 ^{aa bc}
Tb.Sp (μm)	669 ± 158	699 ± 318	767 ± 251	928 ± 224 ^{aa b}
ICI	2.5 ± 1.1	3.0 ± 2.3	3.4 ± 3.1	5.1 ± 3.6 ^{aa b}
Star Volume (mm ³)	14.6 ± 8.1	21.2 ± 20.2	21.2 ± 17.5	26.6 ± 14.6 ^{a b}
FF/TSL (%)	16.9 ± 10.4	21.3 ± 13.6	19.9 ± 14.9	33.6 ± 19.3 ^{a a bc}
NN/TSL (%)	23.0 ± 9.7	28.1 ± 17.3	23.7 ± 15.0	14.4 ± 10.1 ^{aa bc}

^a p < 0.05, ^{aa} p < 0.01 versus primary OP^b p < 0.05, ^{bb} p < 0.01 versus corticosteroid therapy^c p < 0.05 versus alcohol abuse

Table II Comparison between patients with 0 to at least 3 risk factors (RF) for bone loss, tested by multivariate analysis of variance for BMD, histological parameters and vertebral fracture prevalence.

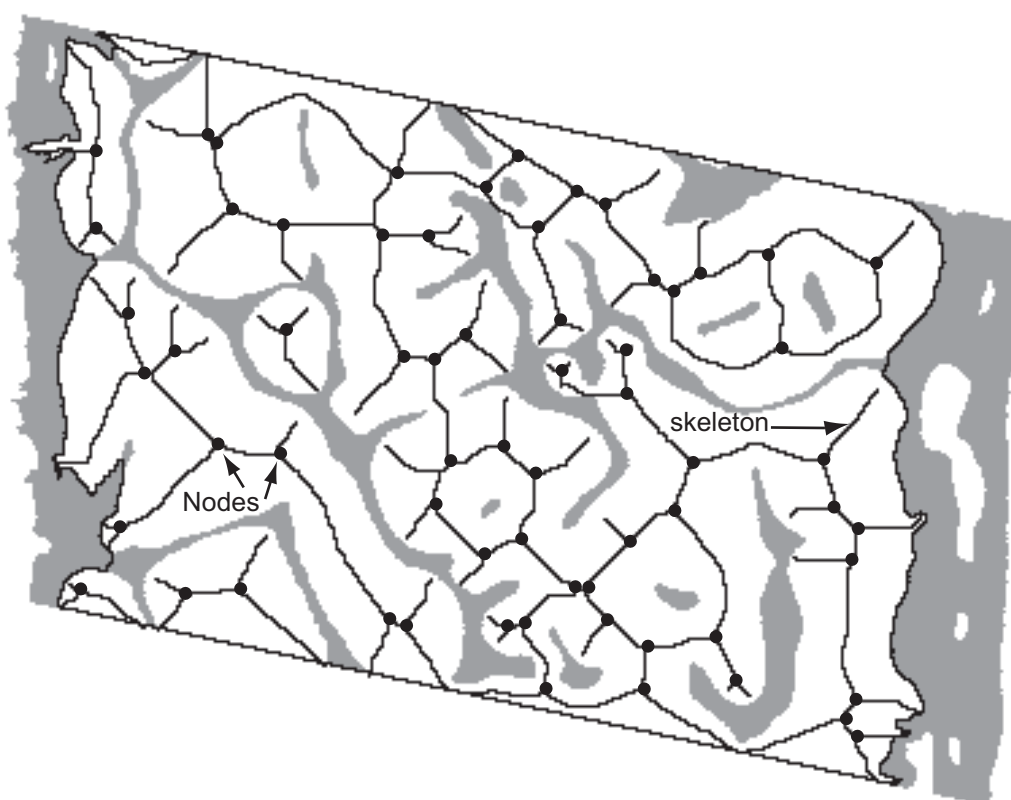
	No risk factor (RF) (n = 28)	One RF (n = 48)	Two RF (n = 50)	At least 3 RF (n = 26)
Clinical characteristics				
Spine BMD (g/cm ²)	0.74 ± 0.09	0.72 ± 0.08	0.71 ± 0.09	0.72 ± 0.09
Hip BMD (g/cm ²)	0.85 ± 0.08	0.81 ± 0.10	0.74 ± 0.13**	0.70 ± 0.13**
% of fractured men	39.2	64.5*	48	69**
Number of fractures per patient	0.75	1.6*	1.76*	1.88**
Histomorphometric parameters				
Ct.Th (μm)	869 ± 227	727 ± 214*	785 ± 358*	770 ± 301*
BV/TV (%)	13.9 ± 3.5	13.0 ± 4.2	13.2 ± 5.4	11.0 ± 3.7**
Tb.Th (μm)	105 ± 29	105 ± 28	99 ± 31	97 ± 38
Tb.N (/μm)	1.34 ± 0.26	1.23 ± 0.30	1.32 ± 0.43	1.19 ± 0.38
Tb.Sp (μm)	669 ± 158	763 ± 247	736 ± 286	825 ± 279*
ICI	2.5 ± 1.1	3.3 ± 3.0	3.0 ± 2.6	4.2 ± 2.9**
Star Volume (mm ³)	14.6 ± 8.1	19.3 ± 16.5	19.9 ± 11.8*	24.0 ± 20.2*
FF/TSL (%)	16.9 ± 10.4	21.4 ± 14.6	19.5 ± 14.9	26.9 ± 13.8*
NN/TSL (%)	23.0 ± 9.7	22.3 ± 9.8	27.3 ± 18.1	20.6 ± 11.3

* p < 0.05, ** p < 0.01 versus men without risk factors

Legends of Figures:

- Figure 1: Measurement of trabecular microarchitecture on digitized histological section from a patient with male osteoporosis. A) the interconnectivity index is determined after skeletonization of marrow spaces (cortical and trabecular bone are in grey). The ● represent the nodes on the skeletons. B) strut analysis after allows the identification of the different types of struts. The ○ represent the nodes on the skeleton of the trabeculae.
- Figure 2: Measurement of trabecular microarchitecture on a digitized histological section from a patient with male osteoporosis by the star volume method based on the overimposition of grids (A: 60°, B: 120°) on the marrow spaces.
- Figure 3: Low magnification (x 6) of bone biopsies from patients with male osteoporosis, undecalcified bone section, Goldner's trichrome. The combined effects of risk factors is illustrated. A) idiopathic male osteoporosis, B) one risk factor, C) 2 risk factors, D) 3 risk factors.
- Figure 4: Additional effects of risk factors on the microarchitectural deterioration of trabecular bone and number of fractures. Results are obtained after standardization of parameters. ICI and star volume of marrow spaces increase when trabeculae become perforated. On the contrary, Tb.N decreases when trabeculae are perforated by osteoclasts.

A Interconnectivity index of marrow spaces (ICI)



B Strut analysis

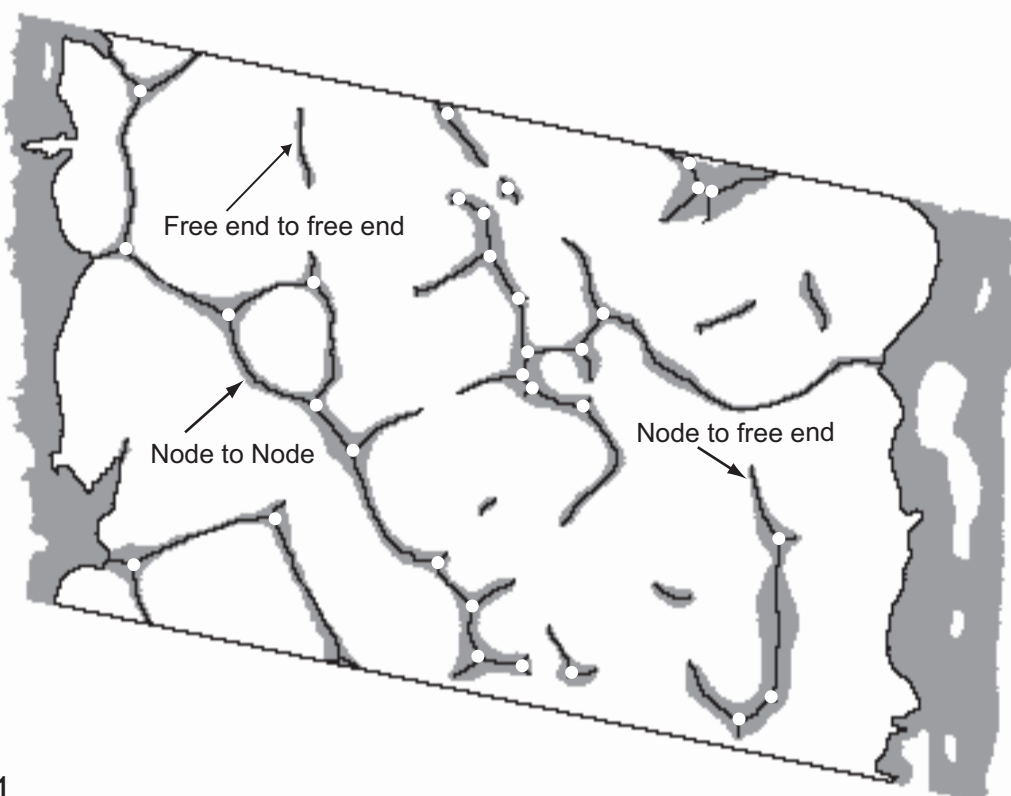


Figure 1

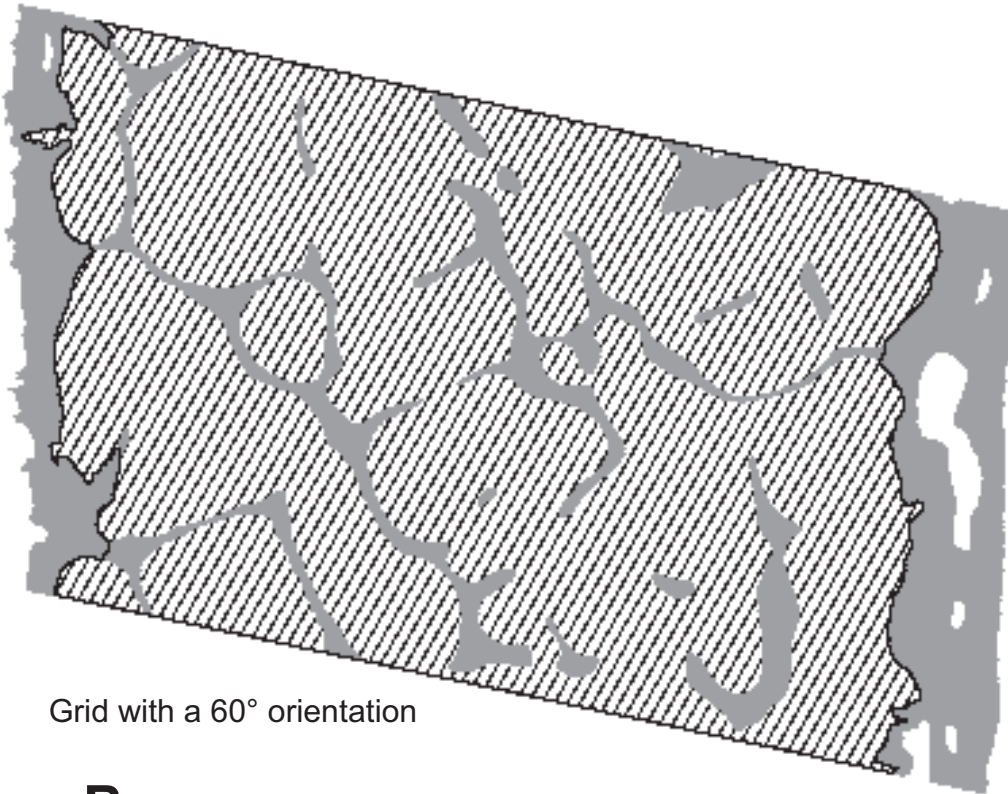
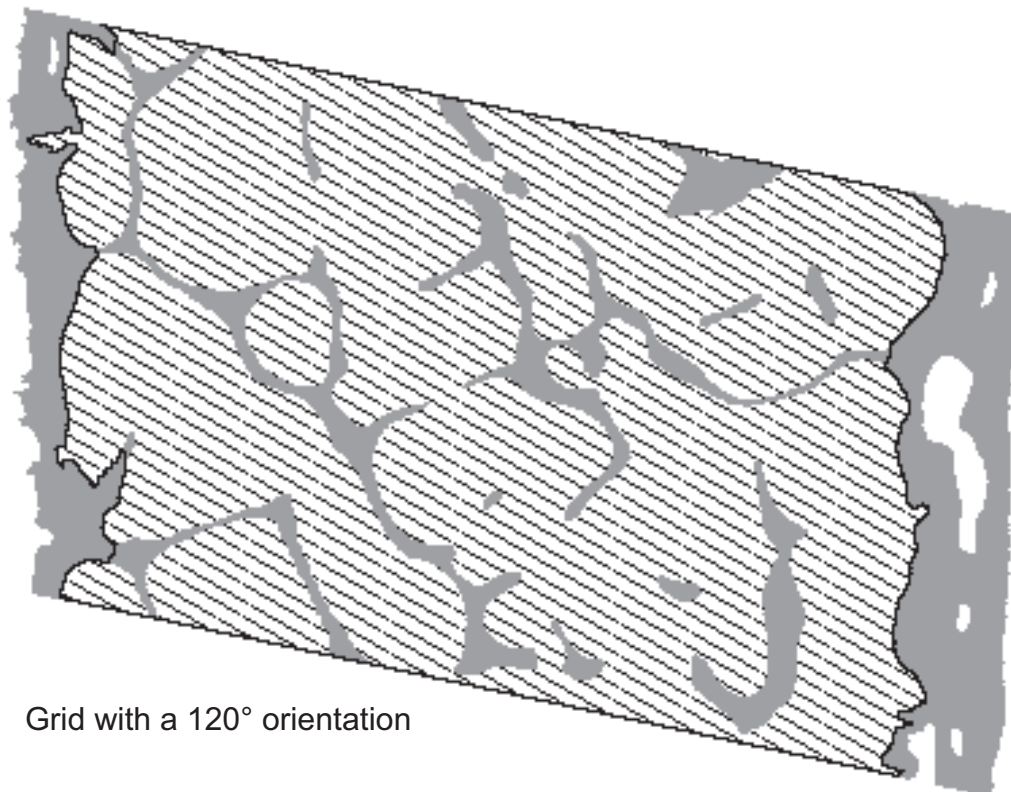
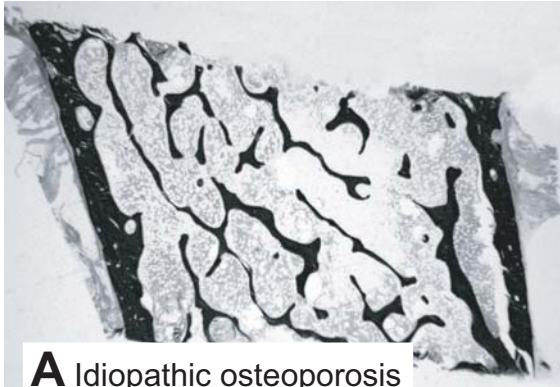
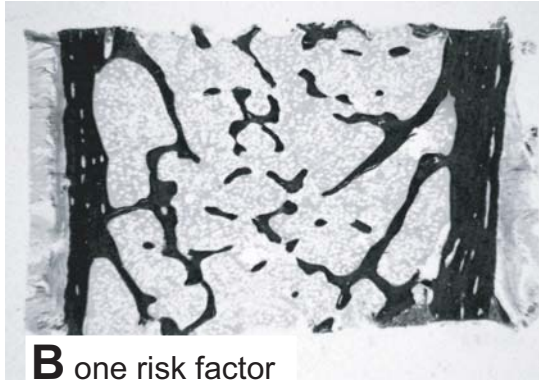
AStar volume of marrow spaces $V_{m.space}$ **B**

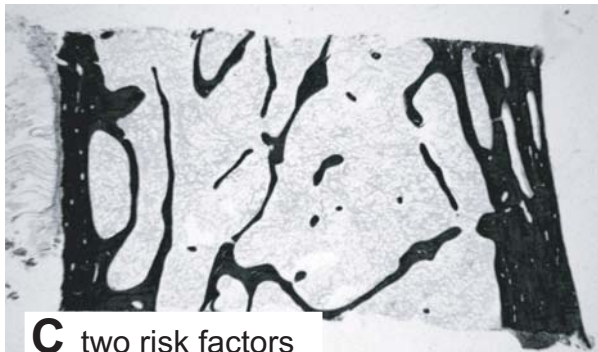
Figure 2



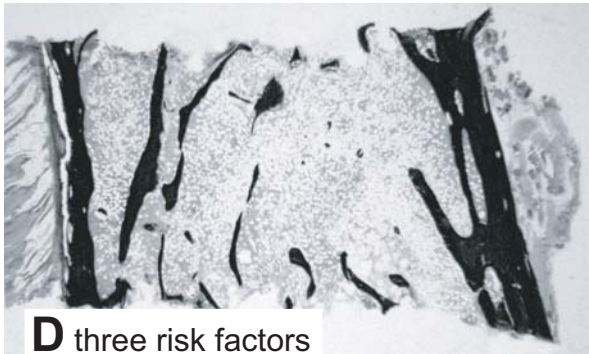
A Idiopathic osteoporosis



B one risk factor



C two risk factors



D three risk factors

Figure 3

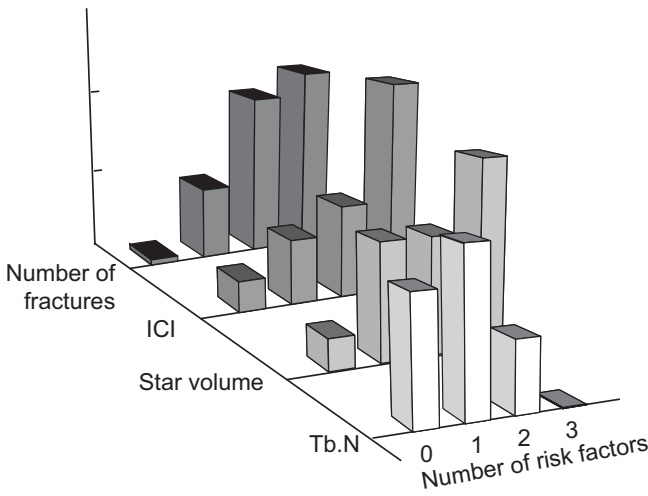


Figure 4