

Bone histomorphometry: a concise review for endocrinologists and clinicians

Histomorfometria óssea: uma revisão concisa para endocrinologistas e clínicos

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SUMMARY

Bone histomorphometry is a quantitative histological examination of an undecalcified bone biopsy performed to obtain quantitative information on bone remodeling and structure. Labeling agents taken before the procedure deposit at sites of bone formation allowing a dynamic analysis. Biopsy is indicated to make the diagnosis of subclinical osteomalacia, to characterize the different forms of renal osteodystrophy and to elucidate cases of unexplained skeletal fragility. Bone histomorphometric parameters are divided into structural and remodeling subgroups, with the latter being subdivided into static and dynamic categories. Metabolic bone disorders such as osteomalacia, hyperparathyroidism, hypothyroidism, osteoporosis and renal osteodystrophy display different histomorphometric profiles. Antiresorptive and anabolic drugs used for the treatment of osteoporosis also induce characteristic changes in the bone biopsy. Bone histomorphometry is an important research tool in the field of bone metabolism and provides information that is not available by any other investigative approach. *Arq Bras Endocrinol Metab.* 2010;54(2):87-98

Keywords

Bone histomorphometry; metabolic bone diseases; bone biopsy; bone structure; osteoporosis drugs

SUMÁRIO

Histomorfometria óssea é uma avaliação histológica quantitativa de uma biópsia óssea calcificada realizada para obter informação sobre a remodelação e a estrutura óssea. Uma análise dinâmica é possível quando substâncias que fazem a marcação do osso são tomadas antes do procedimento e se depositam no local de formação óssea. A biópsia é indicada para diagnóstico de osteomalácia, diferentes formas de osteodistrofia renal e nos casos não explicados de fragilidade esquelética. O preparo e a análise das amostras necessitam de um laboratório especializado. A histomorfometria avalia parâmetros estruturais e de remodelação óssea, sendo o último subdividido em estático e dinâmico. Doenças osteometabólicas como osteomalácia, hiperparatireoidismo, hipoparatireoidismo, osteoporose e osteodistrofia renal apresentam parâmetros histomorfométricos distintos. Medicamentos antirreabsortivos e anabólicos usados no tratamento da osteoporose também induzem alterações características na biópsia óssea. A histomorfometria óssea é uma ferramenta importante no metabolismo ósseo e oferece informação que não é possível por nenhum outro método diagnóstico. *Arq Bras Endocrinol Metab.* 2010;54(2):87-98

Descritores

Histomorfometria óssea; biópsia óssea; microestrutura óssea; doenças osteometabólicas; terapias para osteoporose

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INTRODUCTION

In the technique of bone histomorphometry, a histological examination of undecalcified transilial bone

biopsy specimens is performed to obtain quantitative information on bone remodeling and structure. It is considered a valuable and well-established clinical and

research tool for studying the pathogenesis of metabolic bone diseases as well as for defining mechanisms by which drugs affect the bone (1-4).

Histomorphometry has traditionally been assessed in two dimensions by means of histology, where the structural and remodeling parameters are measured on sections, and the third dimension is extrapolated using standard stereology theory (5). In the last two decades, there have been significant advances in histomorphometric techniques, such that semi automated and automated images analysis coupled to sophisticated stereology software have largely substituted the manual techniques (6).

Remarkable advances in bone histomorphometry were made in the 1950's and 60's due the two major discoveries. First, there was the advent of plastic embedding allowing high quality histologic sections of mineralized bone (7). Second there was the use of labeling fluorochromes, such as tetracyclines which incorporate at the mineralization front, leading to a better understanding of the dynamic process of bone formation (8).

Bone metabolism falls into two main categories: modeling and remodeling. Both processes are performed by the same effector cells, but the end result differs fundamentally. Modeling is responsible for changes in bone shape and mass during growth, whereas the main effect of remodeling is to renew existing bone. Bone remodeling occurs in two distinct phases: resorption of the existing mineralized bone matrix by osteoclasts followed by formation of new bone by osteoblasts. The process occurs at spatially discrete foci and the group of cells involved is referred to as the basic multicellular unit (BMU). The number of active BMUs and the relative amounts of bone resorbed and formed within individual BMUs determine the rate of bone turnover (9-10). This review describes the indications for bone biopsy, the variables that are measured; and how they differ among the major metabolic bone diseases. Furthermore, as bone histomorphometry has been a key tool in this regard we will also discuss the alterations seen in bone structure and remodeling indices in response to osteoporosis therapies.

STEPS BEFORE HISTOMORPHOMETRY ANALYSIS

Indications for bone biopsy

In clinical practice, bone biopsy is most often performed to exclude or confirm a diagnosis of subclinical osteomalacia and to characterize the different forms of

renal osteodystrophy (2,3). In addition, bone biopsy is useful in patients with skeletal disease presenting with excessive fragility or bone pain, young individuals without secondary causes of osteoporosis and unexplained low bone mass or fractures. Although bone biopsy is a valuable research tool in osteoporosis, the indications in clinical practice are limited. Firstly because it is unpractical to perform bone biopsy on the many patients who have this disease, and secondly due to the large intraindividual and interindividual variability in cancellous bone volume, there is substantial overlap between bone volume in normal postmenopausal women and those with osteoporosis.

Tetracycline labeling

When tetracycline is taken before the biopsy, it deposits labels at the sites of new bone formation allowing these regions to be visualized and quantitatively analyzed. Tetracycline schedules vary according to different laboratory protocols. In general, 3 weeks before the scheduled biopsy, tetracycline (1,000 mg/day) is administered twice for 2 or 3 days with a drug free interval of 10 to 14 days between the two courses. Bone biopsy should be performed at least 3-5 days following the last day of tetracycline administration. Tetracycline is incorporated into the bone at sites of new bone formation, binding irreversibly to hydroxyapatite at the mineralization front. A double label is formed when bone formation at a particular site was ongoing during the entire labeling sequence (Figure 1). A single label is deposited if formation either started or ended during the interval between the uses of the two courses of tetracycline administration (8).

Biopsy procedure and specimen processing

The iliac crest is the preferred site for bone biopsy and the ideal biopsy should contain inner and outer cortical plates with intervening cancellous bone. The biopsy is performed as an outpatient minor surgery and it is safe and generally well tolerated (2,11). After the specimen is obtained, a specialized laboratory prepares undecalcified bone specimens and performs histomorphometric analysis. See reference number 2 for a detailed description of surgical procedure, biopsy processing and analysis.

Methods of measurement

The sections are analyzed morphometrically, according to standard stereologic principles, using computer-



Figure 1. (A) Bone-forming site with a team of osteoblasts lining an osteoid seam. (B) Resorption cavity containing osteoclasts. (C) Double tetracycline labels at a site of active bone formation. (B: mineralized bone; O: osteoid; M: marrow; Ob: osteoblasts; Oc: osteoclasts; RC: resorption cavity). (C: 1 first label, 2 second label).

aided planimetry. The image analysis system consists of a digitizing tablet, a high resolution digital color video camera mounted on a microscope with UV capability, digital drawing tablet and a computer equipped with a customized image analysis software program.

Histomorphometric parameters

Histomorphometric variables are derived from primary measurements made at the microscope, such as area, perimeter and thickness. Nomenclature, mathematical derivations and units have been standardized by the American Society of Bone and Mineral Research (12). The revised system expresses all data in terms of source (the structure on which the measurement is made), the measurement and the referent, expressed as follows: source-measurement/referent.

Histomorphometric parameters are generally divided into two categories: structural and remodeling, with the latter being subdivided into static and dynamic parameters. Standard bone histomorphometry in clinical settings is typically limited to the analysis of cancellous bone; however, the analysis can also be done within cortical bone as well as endocortical and periosteal surfaces providing important information on special situations such as the study of growing individuals (13) and the response to anabolic therapy (14).

Structural parameters

Structural parameters provide information about bone mass and structure. These parameters are related to the three dimensional geometry of the bone and are calculated from measurements of the total bone area and the total bone perimeter. The assessment of bone structure is important due its relationship with bone strength (15).

Cancellous bone volume (BV/TV, %): percent of total marrow cavity that is occupied by cancellous bone (both mineralized and non-mineralized). When the ratio BV/TV is low, this indicates a bone deficit in cancellous bone mass.

Trabecular width (Tb.Wi) or thickness (Tb.Th): mean distance across individual trabeculae, given in micrometers.

Trabecular number (Tb.N): number of trabecular plates per unit distance.

Trabecular separation (Tb.Sp): mean distance between trabeculae, given in micrometers.

Cortical width (Ct.Wi): average width of both inner and outer cortices. In growing individuals; however, in

whom there are differences in bone cell activity at the internal and external cortices, they are recorded separately.

Wall width or thickness (W.Th): mean distance from the cement line to the marrow space of completed trabecular bone osteons or packets.

Remodeling parameters

The following parameters are classified as “static parameters” and provide information about the amount of unmineralized bone (osteoid) and extent of resorption cavities (Howships’ lacunae).

Osteoid volume (OV/BV, %): percent of a given volume of bone tissue that consists of unmineralized bone (osteoid).

Osteoid surface (OS/BS, %): percent of bone surface covered in osteoid.

Osteoid thickness (O.Th): mean thickness, given in micrometers for osteoid seams.

Eroded surface (ES/BS, %): percent of bone surface occupied by resorption cavities (Howships lacunae), with or without osteoclasts. Hook-shaped resorption cavity is a large cavity which is characteristic to hyperparathyroidism.

Osteoblast surface (Ob.S/BS): percent of bone surface occupied by osteoblasts
Osteoclast surface (Oc.S/BS): percent of bone surface occupied by osteoclasts (Figure 1).

Dynamic remodeling parameters

These parameters yield information on bone formation rate and can only be measured when patients have been tetracycline-labeled prior to biopsy. They are measured on the unstained sections, viewed under UV light. The basic parameters are:

Mineralizing surface (MS/BS, %): percent of bone surface that displays a tetracycline label reflecting active mineralization. It is calculated as the double-labeled surface plus one half of the single-labeled surface and is expressed as a function of total bone surface. It is a measure of the proportion of bone surface upon which new mineralized bone was being deposited during the period of tetracycline labeling.

Mineral apposition rate (MAR $\mu\text{m}/\text{day}$): measurement of the linear rate of new bone deposition. It is the mean distance between the double labels, divided by the time interval between them.

Bone formation rate (BFR/BS, $\mu\text{m}^3/\mu\text{m}^2/\text{day}$): amount of new bone formed in unit time per unit of

bone surface. It is calculated by multiplying the mineralizing surface by the mineral apposition rate.

Adjusted apposition rate (Aj.AR): indicates the amount of new bone being made, per unit surface of osteoid, per unit time (i.e., bone formation rate averaged over the entire osteoid surface).

Mineralization lag time (Mlt/day): This index represents the average time interval between osteoid formation and its subsequent mineralization and is calculated by dividing the osteoid width by the apposition rate.

Activation frequency (Ac.F): provides an estimate for bone remodeling rate. It is calculated dividing the BFR/BS by wall width. The value generated represents the probability that a new remodeling cycle will be initiated at any point on the bone surface, providing a measure of the frequency at which two successive remodeling cycles are initiated at the same time on bone surface.

NORMAL VALUES FOR BONE HISTOMORPHOMETRY

There are few studies evaluating bone histomorphometry in normal healthy subjects, in different populations (16-20) because of the obvious practical difficulties in obtaining material. Recker and cols. (17) analyzed bone biopsies from 34 postmenopausal healthy white American women in order to establish reference values for static and dynamic histomorphometric variables for this population (17). BV/TV, MAR, wall thickness and osteoid thickness declined significantly with age. In addition, high variability was observed in the dynamic parameters among these healthy individuals. Studies comparing Afro- and White- Americans have also been published; demonstrating some racial differences (18-19). In general, Afro-Americans display lower bone formation rate and mineralizing surface than White-Americans. Furthermore, a longer total formation period was observed in Afro-Americans. A *post mortem* bone histomorphometry study was conducted in 125 Brazilian men and women of different ages and races in order to establish normal values for static histomorphometric parameters (20). The authors demonstrated differences in structural and remodeling parameters depending on gender, race and age.

In summary, bone histomorphometry findings may vary widely among healthy individuals which makes it difficult to establish normal values. Features such as age, gender and race have an important influence.

HISTOMORPHOMETRIC FINDINGS IN CLINICAL DISORDERS

Osteomalacia

Osteomalacia is a generic term that describes defective mineralization of the organic matrix of bone. It is essentially a histological diagnosis and may exist in the absence of biochemical and radiological abnormalities (21). It is characterized by an impairment of bone mineralization. Most of the time, it is caused by a decrease in circulating calcium versus phosphate product, which in turn, can be due to several pathogenetic mechanisms. The characteristic histomorphometric findings of this disease are an accumulation of osteoid reflected by increased osteoid thickness, surface, and volume (Figure 2) (22-23). Cancellous bone volume is normal in osteomalacia. Osteoblasts continue to synthesize and secrete the matrix, but it does not mineralize. Normally, the analysis of dynamic parameters may reveal a range of severity in mild disease characterized by reduced or undetectable distance between double labels to no tetracycline uptake whatsoever, reflecting absence of mineralization in the most severe cases (23). In the latter case, there is a decrease of MAR, mineralizing surface as well as bone formation rate along with a prolonged mineralization lag time greater than 100 day. Increased bone turnover due to secondary hyperparathyroidism is usually present in the early stages of osteomalacia where the elevated remodeling rate coupled with the mineralization defect accelerate the deposition of unmineralized matrix. However, turnover decreases as the osteoid seams get thicker, making it harder for the osteoclasts to gain access to calcified bone surface.

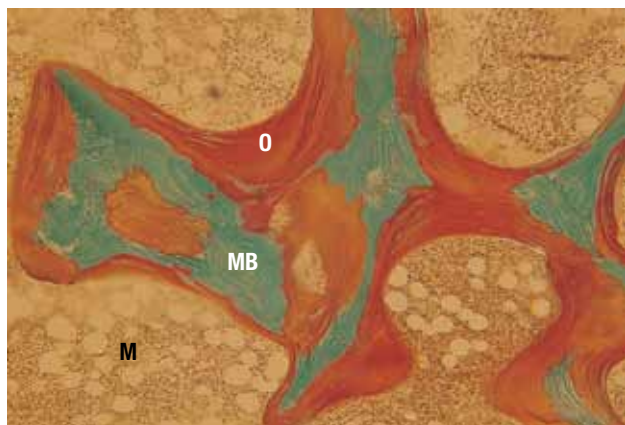


Figure 2. Iliac crest biopsy from a patient with osteomalacia. There is a marked increase in osteoid volume as a result of increased extent and thickness of osteoid seams. (MB: mineralized bone; O: osteoid).

Postmenopausal osteoporosis

Osteoporosis in postmenopausal women is mainly characterized by a reduction in cancellous bone volume resulting from a progressive loss of entire trabeculae leading to reduced trabecular connectivity, and to a lesser extent resulting from a trabecular thinning (24-26) (Figure 3). A decrease in cortical thickness with trabecularization (cancellization) of the endocortical border along with an increase in remodeling activity in this area is usually seen (4). However, in cancellous bone the dynamic histomorphometric indices vary widely, making it difficult to stratify the postmenopausal osteoporosis into high, normal and low turnover. In a bone histomorphometry study of 50 postmenopausal women with untreated osteoporosis, two subsets of patients were identified: one with normal bone turnover and the other with high turnover accounting for 30% of the women. However, this conclusion was based on the osteoid surface only, because the tetracycline-based bone formation rate demonstrated a normal distribution in this group of patients (24). Another study classified untreated postmenopausal women with osteoporosis according to their turnover status. When bone formation rate was used as the discriminant variable, 19% had high turnover, 72% normal turnover and 9% had low bone turnover (25). Furthermore, two other studies with postmenopausal women with osteoporosis demonstrated the same wide variation in turnover status among these patients, leading to the conclusion that there were no important subsets of patients with postmenopausal osteoporosis (26,27). In general, women with postmenopausal osteoporosis are characterized by a wide heterogeneity of bone turnover at the tissue level and probably by decreased bone formation at the cellular level. However, it is important to note that in most cases bone biopsy is performed when the disease is in an advanced stage. Thus, probably the disturbances of bone metabolism that led to the reduction in bone mass took place several years before the time of the biopsy and are no longer evident. Therefore, based on heterogeneity of bone turnover frequently seen in postmenopausal osteoporosis the biopsy is an impractical way to determine turnover status in clinical practice. It is likely that biochemical markers of bone resorption and formation will be used increasingly for this purpose.

Hyperparathyroidism

The histomorphometric profile in primary hyperparathyroidism ranges from severe to milder cases of oste-

itis fibrosa cystica. However, more than 80% of patients have reduced cortical width, slightly increased cancellous bone volume, and accelerated bone turnover, demonstrated by increases in eroded, osteoid and mineralizing surfaces as well as in the numbers of osteoclasts and osteoblasts and in the activation frequency (28-30). Despite the rapid remodeling state in primary hyperparathyroidism, the balance between resorption and formation in the cancellous compartment is conserved because cancellous bone volume is normal or may even be increased, and trabecular connectivity is preserved (29). There is a reduction of MAR despite the increase in the mineralizing surface which reflects a decreased rate of organic matrix production by the osteoblasts. However, the extension of mineralizing surface overcompensates for the decrease in MAR, so the overall bone formation rate is still increased.

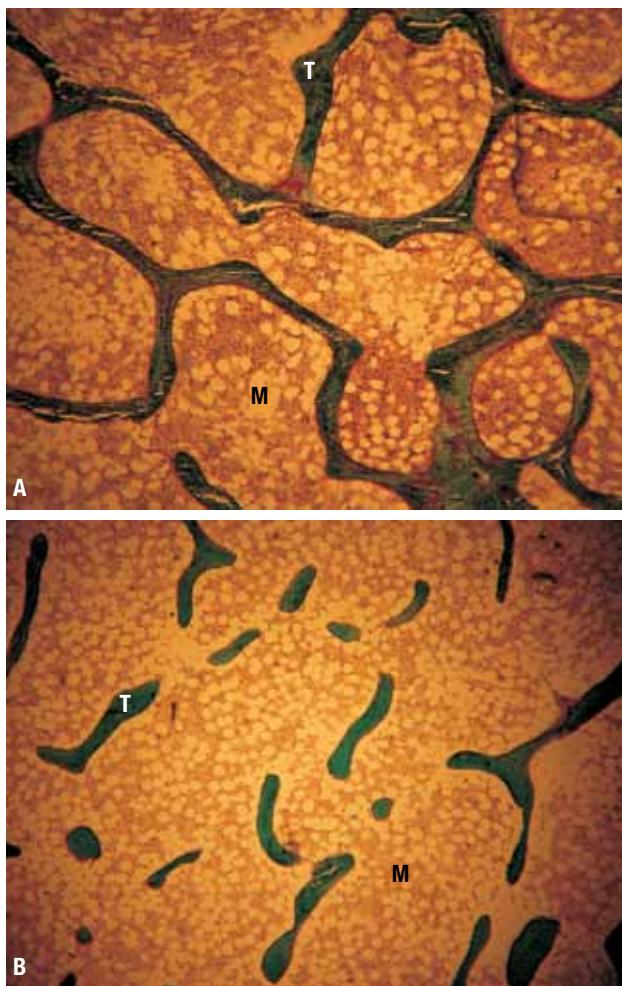


Figure 3. (A) Trabecular bone in a normal postmenopausal woman. Note well preserved trabecular connectivity. (B) Trabecular bone in a subject with postmenopausal osteoporosis demonstrating marked reduction in cancellous bone volume and loss of connectivity. (T: trabeculae; M: marrow).

In sharp contrast to the preservation of cancellous bone in primary hyperparathyroidism there is a significant deficit in cortical bone. Cortical porosity is typically increased with prominent intracortical erosion cavities containing multiple osteoclasts. Subperiosteal resorption bays filled with osteoclasts, fibroblasts, and loose connective tissues stroma are occasionally noted. An increase in eroded surface on endocortical bone is frequently seen and because the cavities are deep and coalescent they lead to a reduction in cortical width. In severe cases of hyperparathyroidism, in addition to all the findings described above, there is an increased deposition of immature (woven) bone and marrow fibrosis (Figure 4).

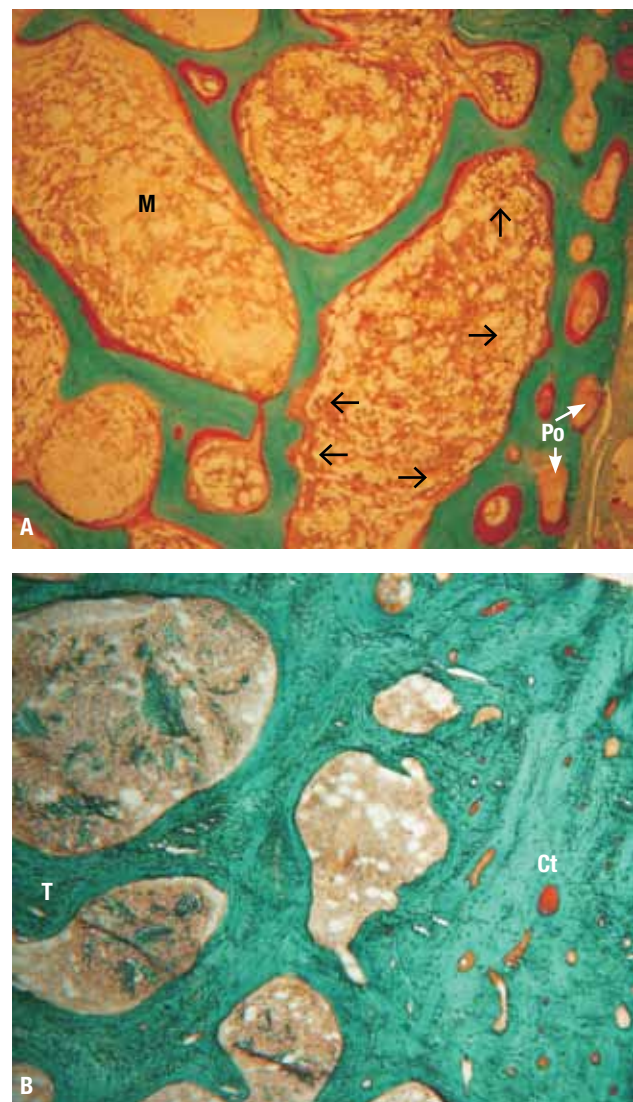


Figure 4. (A) Iliac crest biopsy from a patient with primary hyperparathyroidism showing extended resorption surface (black arrows) and thin cortex (white arrows) with increased porosity. (B) Cancellous bone in hypoparathyroidism demonstrating thick, well-connected trabeculae and thick cortex. (T: trabeculae; Ct: cortical; Po: porosity).

An association between hyperparathyroidism and vitamin D deficiency has been frequently described. Thus, it is important to note that the histomorphometric appearance of the bone biopsy when these two conditions are concurrent may be altered dramatically (31).

Hypoparathyroidism

Few studies have assessed bone metabolism in hypoparathyroidism patients and all of them have demonstrated that in absence of the parathyroid hormone (PTH) bone remodeling is reduced (32,33). Histomorphometric findings confirmed the low turnover state and also show an increase in cancellous bone volume (Figure 4). Rubin et al, in a study with 33 subjects with hypoparathyroidism demonstrated they had greater cancellous bone volume, trabecular width and cortical width than control subjects (33). Furthermore, analyses of cancellous, endocortical and intracortical surfaces showed significantly reduced osteoid width (O.Wi) as well as osteoid surface (OS). In addition, the percentage of bone surface that was mineralizing, mineral apposition rate (MAR) and bone formation rate (BFR) was also significantly lower in all 3 envelopes. However, bone resorption rate (BRs.R) was significantly lower in the hypoparathyroid subjects in both cancellous and endocortical compartments and tended to be lower in the intracortical compartment. All these findings are compatible to suppression in the skeletal dynamic indices.

Renal osteodystrophy

Renal osteodystrophy (ROD) is a heterogeneous group of metabolic bone diseases that accompanies progressive chronic kidney disease (CKD) (34-38). Recently, the Kidney Disease Improving Global Outcomes (KDIGO) working group proposed that the term ROD should be limited to the specific changes in bone histology and defined according to histomorphometric criteria. In addition, uniform terminology for bone histomorphometry reports of CKD patients based on assessment of turnover, mineralization, and volume was suggested (35). The metabolic bone diseases frequently seen in CKD patients are: secondary hyperparathyroidism (HPT) (from mild to severe), osteomalacia, aluminum bone disease, adynamic bone disease and mixed uremic osteodystrophy (36). In general, patients with secondary HPT present higher levels of PTH than those observed in patients with pri-

mary HPT. Therefore, they display a more exuberant increase in remodeling parameters, frequently accompanied by depositions of woven osteoid and variable amounts of peritrabecular marrow fibrosis (Figure 5). In addition, the normally sharp junction between cortical and cancellous bone may be completely obliterated by both endocortical resorption bays and an increase in cancellous bone area. Patients with osteomalacia present the same histomorphometric findings that are observed in bone biopsies from patients with osteomalacia due to other causes than CKD. Mixed uremic osteodystrophy is characterized histologically by features of both hyperparathyroidism and osteomalacia. Adynamic bone disease is characterized by decreased bone formation and normal or reduced osteoid. It is important to point out that a patient with ROD can transition from one histologic form to another induced by pharmacologic agents (such as calcimimetic and vitamin D analogs) or to disease progression (37). Additionally,

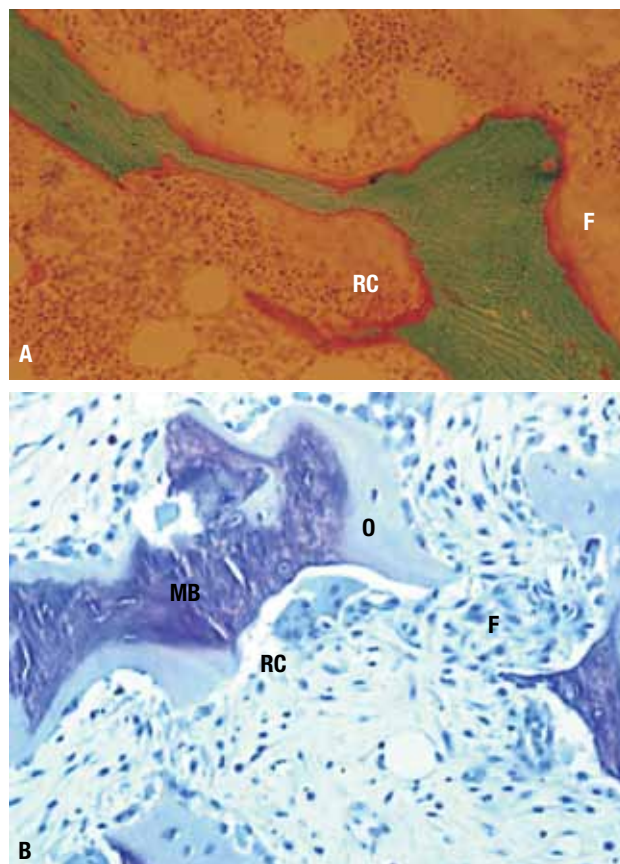


Figure 5. (A) Biopsy from a patient with secondary hyperparathyroidism demonstrating a large resorption cavity and peritrabecular fibrosis. (B) Biopsy showing mixed uremic disease with an increased number and extent of osteoid seams (osteomalacia) and resorption cavities, and fibrosis (hyperparathyroidism) (MB: mineralized bone; RC: resorption cavity; O: osteoid; F: fibrosis).

because osteoporosis is a prevalent form of metabolic bone disease in all populations it also affects CKD patients further impairing their bone quality (38). In this regard, two forms of ROD are particularly important to discriminate from osteoporosis: adynamic bone disease and osteomalacia. The reason for is that antireabsorptive osteoporosis therapies are contraindicated in low bone turnover renal diseases.

Histomorphometric findings of the main bone active agents

A wealth of information is available on the effects of osteoporosis drugs on iliac crest, mainly because regulatory agencies require biopsies to be performed to assess the safety of new therapeutic agents. This has contributed to a better understanding of the mechanism of action of these drugs at cellular and structural levels. The drugs are considered under two categories: anticatabolic, also known as antiresorptive, and anabolic.

Anticatabolic therapies

Anticatabolic therapies suppress bone resorption by decreasing the number, activity and life span of osteoclasts and consequently by reducing bone turnover rate.

Hormone therapy

There have been several reports on effects of hormone therapy (HT) on iliac bone in postmenopausal women, primarily demonstrating evidence of suppression of bone turnover (39-42). Decreases in eroded surface, resorption cavity size and resorption rate are the most common findings both with oral or transdermal formulations. In addition, conventional doses of HT result in inhibition of bone formation, which is reflected by reduced osteoid and mineralizing surfaces and bone formation rate with no change or a decrease in wall width (39-40). However, in a cross-sectional prevention study in women given long-term, high dose, subcutaneous estrogen, bone biopsies revealed an increase in wall width and a decrease of eroded cavity area (41). Consistent with this observation, a longitudinal study with paired biopsies evaluating the effect of subcutaneous HT (75 mg E₂ 6 monthly plus 5 mg of oral medroxyprogesterone acetate for 10 days in each calendar month) for 6 years revealed significantly increased cancellous bone volume due to an increase in trabecular thickness and number (42). Wall width was also increased and bone turnover was suppressed. The

authors suggested that this increase in wall thickness is evidence of an anabolic action achieved by the stimulation of osteoblastic activity, which increased bone formation at the cellular level leading to a positive balance in the bone remodeling unit.

Selective Estrogen Receptor Modulator (SERMs)

Raloxifene

There are fewer reports on the effects of SERMs on iliac bone biopsies than for other anticatabolic therapy (43-44). The MORE study, Multiple Outcomes of Raloxifene Evaluation trial, demonstrated that 60 mg of raloxifene reduced the bone formation rate, but there were no changes in eroded surface of osteoclast number. Bone structure was preserved with no change in cancellous bone volume, trabecular thickness and cortical width (43). Another study compared the effects of raloxifene with oral HT, EEC 0.25 mg plus acetate medroxyprogesterone 2.5 mg daily. After 1 year of treatment, HT significantly reduced activation frequency and bone formation rate but raloxifene did not (44). There is only one report of the effects of another SERM, tamoxifen, on iliac bone. This study evaluated pre- and postmenopausal women with breast cancer who had undergone mastectomy and received 33 months of tamoxifen or placebo. Tamoxifen treatment resulted in a longer remodeling period, smaller resorption cavity area, and reduced bone formation rate (45).

Bisphosphonates

Alendronate

The effects of alendronate have been investigated in patients with postmenopausal or glucocorticoid-induced osteoporosis, where biopsies were taken from the treatment and placebo groups at the end of the study period (46,47). The most common histomorphometric findings in patients with postmenopausal osteoporosis treated with alendronate (5,10, or 20:5 mg/day) were reductions in osteoid surface and thickness, mineralizing surface, bone formation rate and activation frequency. All these findings confirm decrease in bone remodeling. Mineral apposition rate was unchanged and this coupled with the decrease in osteoid thickness indicates that alendronate suppresses bone turnover without inhibition of bone mineralization during 2 or 3 years of treatment. Although alendronate induced a marked reduction of biochemical markers of bone resorption lit-

tle, if any effect, on eroded surface, osteoclast number and erosion depth was observed. Following 2 years of alendronate treatment, there was a significant increase in wall width of cancellous bone packets accompanied by a trend toward a decrease in erosion depth, which resulted in a positive bone balance. However these effects were not seen in patients treated for 3 years. No difference in cancellous bone volume between placebo and alendronate treated groups was observed (46).

Despite the fact that the pathogenesis of glucocorticoid-induced osteoporosis is quite different from that of postmenopausal osteoporosis, alendronate had quite similar effects on both conditions (47).

Risedronate

A paired biopsy design was used to study the effects of risedronate treatment (5 mg/day) on bone histomorphometry (48). Similar to the actions of alendronate on iliac bone, 3 years of treatment with risedronate in postmenopausal women with osteoporosis caused a moderate reduction in bone turnover as evidenced by decreased mineralizing surface, bone formation rate, and activation frequency. In addition, normal bone mineralization was demonstrated by unchanged osteoid thickness and mineralization lag time, and a trend toward an improvement of bone balance. There was a significant decrease in the resorption rate after risedronate treatment, but no changes in eroded surface and depth were observed. No changes on cancellous bone structure were observed by conventional histomorphometry.

Ibandronate

Bone histomorphometry was performed on a subgroup of women participating in the BONE study in order to assess bone quality and architecture (49). Patients were randomized to receive one of the following: placebo, continuous oral daily ibandronate (2.5 mg/day) or intermittent oral ibandronate (20 mg every other day for 12 doses every 3 months) and they were randomly assigned to undergo transiliac bone biopsy at either Month 22 or Month 34 of treatment. Quantitative assessment demonstrated no impairment in mineralization of bone matrix: osteoid thickness tended to be similar or slightly lower in the ibandronate groups versus the placebo group. A modest reduction in bone turnover assessed by activation frequency and bone formation rate with daily regimen relative to placebo was

observed. A significant increase in trabecular number and decrease in trabecular separation were observed with intermittent ibandronate relative to placebo when results from 22 and 34 months were pooled. More recently, bone quality and micro-architecture was assessed by bone histomorphometry in a subset of patients following two years of intravenous ibandronate 2 mg every 2 months or 3 mg every 3 months or placebo. Primary mineralization of new bone remained normal as indicated by the slightly lower osteoid thickness and osteoid volume with normal mineral apposition rate (MAR). However, bone formation rate and other parameters of dynamic remodeling were decreased when compared with controls to a greater degree in the patients who received the dose of 3 mg (50).

Zoledronate

The effects of intravenous zoledronic acid on bone structure and remodeling were evaluated in a subgroup of 152 patients of the Horizon trial after 1 year of medication or placebo (51). Histomorphometric indices of bone remodeling such as activation frequency, mineralizing surface and bone formation rate (volume referent) were reduced by a median of 63% in the treated group when compared to placebo. Furthermore, osteoid volume and thickness were also significantly lower in the patients treated with zoledronate. A slight increase in the mineral apposition rate (MAR) was reported in the treated group, which raised the possibility of the presence of an anabolic effect of zoledronate. However, this issue is controversial because missing values were assigned for MAR in patients without double labels. Potentially, this could have biased the MAR average in the zoledronic acid-treated group.

Anabolic therapy

Anabolic therapies work by a fundamentally different mechanism of action than anticatabolic agents. Instead of reducing the activation frequency of bone remodeling, they increase it. In each BMU, the amount of new bone formed exceeds the amount that was removed resulting in an increase of bone mass, rather than simply maintaining bone microarchitecture.

Teriparatide

The main histomorphometric findings seen after treatment with teriparatide were an increase in mineral apposition rate, and in bone formation, as revealed by

increased osteoblast, osteoid, and mineralizing perimeters, mineral apposition rate, and bone formation rate (52-54). Moreover, increased osteoclast number and eroded surface were seen as early as 28 days of treatment suggesting enhanced activation of bone remodeling units at this early stage of treatment. Teriparatide treatment also demonstrated an ability to increase the length of individual forming units which may have been achieved by extending bone formation to quiescent surfaces adjacent to the original resorption cavity (14).

There was an early concern that teriparatide therapy might have a deleterious effect on cortical bone. In fact, a decrease in BMD at predominantly cortical regions in patients treated with teriparatide suggested that transiently increased bone remodeling could induce a loss of cortical bone. However, both animal and human biopsy demonstrated an increase in cortical thickness and stimulation of bone formation on the endosteal surface. In addition, there is now histomorphometric evidence based on tetracycline uptake and insulin-like growth factor expression for stimulation of bone formation at the periosteal surface of the ilium following teriparatide treatment (14,52-54). This effect of teriparatide on periosteal bone formation might induce an increment of bone area which could improve bone strength.

Strontium ranelate

It was shown in preclinical studies that strontium ranelate can stimulate bone formation and decrease bone resorption (55). However, the mechanism of action of strontium in humans is not completely understood. A histomorphometric study demonstrated a slight 9% increment in mineral apposition rate along with a significant 38% increase in osteoblast surface in the treated group when compared to the placebo (56). Osteoid thickness was significantly lower in the strontium ranelate group on cancellous, cortical and endocortical envelopes. Bone formation rate was not different between the groups and no significant differences between groups regarding the resorption parameters, eroded surface, osteoclast surface and activation frequency were observed. Recently, a comparative study of teriparatide and strontium ranelate on bone histomorphometry based on a single transiliac bone biopsy after a 6-month treatment was published. The authors concluded that the effects of strontium ranelate on bone remodeling and cell activity were modest (57).

CONCLUSIONS

Bone histomorphometry is a powerful tool for the assessment of bone metabolism providing information that is not available by any other investigative approach. In addition, it provides invaluable information on skeletal safety of new pharmacological interventions in clinical trials.

With the exception of CKD, the indication for bone biopsy is limited in clinical practice, knowledge of the histomorphometric alterations seen in the various metabolic bone disease as well as the changes induced by the active-bone drugs contribute to better management of patients with osteoporosis and other bone metabolic diseases.

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