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# BONE BIOPSY IN THE DIAGNOSIS OF RENAL OSTEODYSTROPHY

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A b s t r a c t: When renal disease develops, mineral and vitamin D homeostasis is disrupted, resulting in diverse manifestations in bone cells and structure as well as the rate of bone turnover. In ESRF when patients require chronic maintenance dialysis, nearly all of them have abnormal bone histology named renal osteodystrophy (ROD). On the other hand, survival rates of patients on dialysis have increased with improved dialytic therapy and the resultant increased duration of dialysis has led to a rise in renal osteodystrophy. Because this metabolic bone disease can produce fractures, bone pain, and deformities late in the course of the disease, prevention and early treatment are essential. Serum PTH levels are commonly used to assess bone turnover in dialyzed patients. However, it is found that serum PTH levels between 65 and 450 pg/ml seen in the majority of dialysis patients are not predictive of the underlying bone disease. To date, bone biopsy is the most powerful and informative diagnostic tool to provide important information on precisely the type of renal osteodystrophy affecting patients, the degree of severity of the lesions, and the presence and amount of aluminum and strontium deposition in bone. Bone biopsy is not only useful in clinical settings but also in research to assess the effects of therapies on bone. Although considered as an invasive procedure, bone biopsy has been proven as safe and free from major complications besides pain, haematoma or wound infections, but the operator's experience and skill is important in minimizing morbidity. Alternatives to bone biopsy continue to be pursued, but the non-invasive bone markers have not been proven to hold sufficient diagnostic performance related to the bone turnover, mineralization process and bone cell abnormality. At present however, the transiliac bone biopsy remains the golden standard in the diagnosis of renal osteodystrophy.

**Key words:** bone biopsy, renal osteodystrophy, renal failure.

## *Introduction*

Renal failure produces changes in mineral metabolism that affect bone structure, turnover, and cellular characteristics. When patients reach end-stage renal failure and require chronic maintenance dialysis, nearly all of them have abnormal bone histology (1). On the other hand, survival rates of patients on dialysis have increased with improved dialytic therapy. However, the resultant increased duration of dialysis has led to a rise in renal osteodystrophy (ROD). Because this metabolic bone disease can produce fractures, bone pain, and deformities late in the course of the disease, prevention and early treatment are essential. Serum PTH levels are commonly used to assess bone turnover in dialyzed patients. However, it is found that serum PTH levels between 65 and 450 pg/ml seen in the majority of dialysis patients are not predictive of the underlying bone disease (2). To date, bone biopsy is the most powerful and informative diagnostic tool to provide important information on precisely the type of renal osteodystrophy affecting patients, the degree of severity of the lesions, and the presence and amount of aluminum and strontium deposition in bone (3, 4). Bone biopsy is not only useful in clinical settings but also in research to assess the effects of therapies on bone (5). Alternatives to bone biopsy continue to be pursued, but they have not been proven to have the same specificity or sensitivity to effectively determine the potential value of a specific therapeutic regimen.

## *Bone biopsy*

*Double tetracycline labelling – DTCL*. The first prerequisite for an informative bone biopsy is proper in vivo labelling with specific, nontoxic, time-spaced bone markers before the biopsy. Antibiotics from the tetracycline family are used because they have spontaneous fluorescence and bind to actively forming bone surfaces. With labelling, the level of bone turnover and rate of bone formation can be determined, and possible mineralization abnormalities can be identified. A double labelling technique is best, although the schedule is somewhat more complicated for patients than one prolonged single administration. In most cases, the first label is administered for 2 days followed by an 8–15 day free interval. Anything fewer than 8 days lessens the distinction between labels, particularly in the case of a mineralization defect. Anything more than 15 days may artificially increase the number of single labels because the number of forming sites being completed or started will probably increase. During the 2–4 days after the free interval, the patient takes a second course of antibiotics. Bone biopsy is then performed 4-6 days after the last administration of tetracycline. During this delay, the tetracycline becomes slightly buried within mineralized

osteoid and thus does not leach out. In an emergency, labelling can be shortened to a 1-day-on, 6-days-off and 1-day-on schedule with a single dose of oral tetracycline per day (1.0–1.5 g of tetracycline or 600–900 mg of Declomycin®). Gastrointestinal side effects may be greater with this approach. Using two labels with different colors assures accurate assessment of the mineralization rate. Tetracycline hydrochloride has a light yellow and demeclocycline hydrochloride (Declomycin®) a yellow-orange fluorescence. With the use of only one type of tetracycline, the two labels can merge in states of low bone turnover and be unrecognizable as a double label. For patients with normal renal function, the dosages of tetracycline hydrochloride and Declomycin® are usually 500 mg and 300 mg t.i.d., respectively. For patients with impaired renal function, dosages should be reduced to 500 mg and 300 mg b.i.d.

*Procedure* – Bone biopsies could be performed using a few different instruments (e.g. Bordier-Meunier, Jamshidi needle, etc.) with an internal diameter from 5 to 7 mm. The biopsy set consists of 4 instruments (Fig. 1): a poin-

Fig. 1. Bone biopsy needle set

ted obturator (A), a stabilising sleeve with sharp serrated edges (B), a trephine biopsy needle with a flared handle (C) and a blunt metal rod to push the specimen out of the biopsy needle (D).

Most bone biopsies are performed under local anesthesia. The transiliac bone biopsy site is 2 cm posterior and 2 cm inferior to the anterior iliac spine (Fig. 2). It is the generally preferred site owing to its accessibility and high bone turnover rate (6). Iliac crest biopsies result in cores with a single cortical surface, while transiliac biopsies yield cores with two cortical surfaces (6). The specimen is cut into two cylinders. The largest part is used for histological examination and the second part is weighed directly after sampling and used for bulk analysis by means of electrothermal atomic absorption (7, 8). The different steps of the manual technique are shown in Fig. 3.

Fig. 2. The transiliac bone biopsy site
(By courtesy of Dirk De Weerdt, Dept.
of Nephrology Antwerp, Belgium)

Fig. 3. Bone biopsy procedure: steps 1–4
(by courtesy of Dirk De Weerdt)

The patient lies on a firm table in the supine position with the side on which biopsy is planned placed along the edge of the table. 1% lidocain solution is used for local infiltration of the skin where 1.5 cm incision is made using a scalpel. A pointed obturator (Fig. 3A) is introduced into the guide sleeve (Fig. 3B) and both are then inserted through the skin incision. The obturator is penetrated gently until its tip comes into contact with the ileum and the guide sleeve is advanced over the obturator until its serrated edge touches the ileum in direction towards the opposite shoulder. Then, the obturator is withdrawn, the trephine biopsy needle (Fig. 3C) is inserted into the guide sleeve and rotated manually with firm pressure until it cuts successively through all layers of the bone. After penetration of the inner cortical table, the biopsy trephine should be rotated by 360 degrees about 20 times and then withdrawn, using a slow rotating motion. Removal of the biopsy from the trephine is accomplished by introducing the blunt extractor (Fig. 3D), gently pushing the specimen out (16).

*Complications* from bone biopsies can include pain, haematoma, wound infection, and rarely neuropathy. However, studies show that horizontal or transiliac and vertical or superior biopsies of the anterior iliac crest result in very low morbidity and no mortality as a result of the procedure (9). The operator's experience is important in minimizing morbidity and in obtaining an adequate specimen. Use of recently developed techniques should further decrease any complications. Patient reports of pain range from none to moderate and rarely severe.

*Qualitative and quantitative evaluations* of the bone sample constitute the final steps in processing a bone biopsy. Qualitative assessment consists of such factors as the suitability of a biopsy for morphometric analysis, the amount of sample needed, where histologic structures should be measured, and what elements to evaluate. With quantitative evaluation, numerical values are assigned to the various elements constituting bone (10). Potential differences between groups of patients or normal individuals or changes occurring after treatment can then be statistically evaluated.

Histomorphometric analysis was performed on 5 μm Goldner stained undecalcified, methylmetacrylate embedded bone sections. For the detection and localization of aluminum the sections were stained with Aluminon®. Unstained sections (7 μm) were used for the evaluation of tetracycline labels by fluorescence microscopy.

Semi-quantitative automated image analyzers are available greatly reducing the time required to evaluate bone slides. Although they assess parameters of bone structure, they are not reliable in discriminating such elements as cellular details, detecting woven vs. lamellar bone, and recognizing erosion surface. However, complete automatic analysis of bone may be possible in the future, with improved video cameras, staining techniques, and computerized image-analysis capabilities.

Based on histomorphometric findings two main groups of renal bone disease could be classified: low (LTO) and high bone turnover (HTO) (1,5). Adynamic bone disease is characterized by a decreased number of osteoblasts and osteoclasts with a low bone formation rate (BFR) approaching zero values. The second type of LTO – osteomalacia (OM) has a superimposed mineralization defect producing a great amount of unmineralized osteoid. HTO bone disease includes mild and severe (osteitis fibrosa) hyperparathyroid bone disease (HPTH), characterized by an excessive number of osteoclasts and osteoblasts and a high rate of bone formation and resorption. Mixed uremic osteodystrophy (Mx) possesses the combined features of both HPTH and OM.

A lot of uncertainty still exists with these standard histological techniques for the altered biological activity or mechanisms of the disease at cellular level which are necessary for understanding the nature and course of the disease. The recent advances in the development of sensitive techniques including *in situ* hybridization (ISH) and *in situ*-reverse transcriptase-PCR (IS-RT-PCR) has introduced a new era in the study of various aspects of biomedical research including the field of renal osteodystrophy.

*Clinical application* – Nephrologists must determine a patient's level of bone turnover to apply the correct therapy. Serum PTH levels measured with radioimmunometric assay are commonly used to assess bone turnover in dialyzed patients. Although these levels have been found to be more sensitive than the previously employed radioimmunoassay, at present there is no consensus regarding the serum level of PTH that reflects normal bone turnover in ESRD patients. Because bone biopsies provide a sensitive measurement of bone changes, they more accurately determine the type of renal osteodystrophy and can indicate potential aluminum and strontium accumulation in dialyzed patients. Bone biopsies also allow tailored therapeutic measures. The extent of aluminum deposits at the bone-osteoid interface and the level of bone turnover determine the optimal duration of chelating therapy.

If the biopsy shows no significant deposits of aluminum, the degree of bone turnover will help the practitioner determine the route, aggressiveness, and length of calcitriol therapy. Severe hyperparathyroidism with marked bone marrow fibrosis is an indication of high doses of intravenous calcitriol if the calcium × phosphorus product can be controlled. Additionally, a bone biopsy can predict whether there will be high resistance to intravenous calcitriol at the needed massive doses. In this case, parathyroidectomy may be necessary. The severity of the effect of secondary hyperparathyroidism on the bone may also indicate the extent of the post-parathyroidectomy "hungry bone syndrome" and allow preventive measures such as the preoperative injection of calcitriol. In patients with a mild to moderate increase in bone turnover with or without mineralization defect, doses of intravenous or preoperative calcitriol and duration

of therapy may be adjusted to avoid the development of ABD. In the case of ABD, calcitriol therapy is not desirable because of the risk of inducing hypercalcemia, extraosseous calcifications and further suppression of the parathyroid glands' activity. Moreover, the use of a low calcium dialysate is recommended, as well as a lesser daily intake of calcium in the diet, lowering also the treatment with calcium containing phosphate binding agents. A recent study with lanthanum carbonate as a new phosphate binder, has shown its safety and effectiveness as well as the promotion of low towards the states with higher bone turnover (5).

Patients with osteomalacia in the absence of aluminum and strontium should be treated with vitamin D and calcium supplements if needed.

A bone biopsy establishes the precise relationships between the serum indices of calcium metabolism and bone lesions (11). This enhances the interpretation of longitudinal follow-up of noninvasive parameters while the patient is undergoing a particular therapy.

*Own results* – Our bone biopsy study in 84 ESRD patients revealed 62% of the predialysis population to have abnormal bone histology (1). ABD was found to be the most frequent bone lesion observed in 23% and HPTH (mild form) was diagnosed in only 9% of the patients. The distribution of ROD in our study differs considerably from the ROD spectra reported previously in non-dialysed renal failure patients (12–15), allowing tailored specific therapeutic measures for each ROD entity.

As a part of multicentric, prospective, double bone biopsy study (baseline and after a year of treatment) we compared the effect of lanthanum carbonate (LC) and calcium carbonate (CC) on the evolution of renal osteodystrophy in dialysis patients (5). LC treated dialysis patients showed almost no evolution towards low bone turnover over a year while CC treatment promoted development of ABD in half of the patients.

Our experience of more than 150 transiliac bone biopsies performed showed no evidence of serious complications besides a few patients who experienced moderate pain at the site of the bone biopsy. So, we can conclude this method as a safe and valuable diagnostic tool in the diagnosis of renal osteodystrophy.

## *Conclusions*

Bone biopsies are presently much more widely used for diagnosis and research than they have been in the past. However, traditional constraints continue to be perceived because of the procedure's invasiveness and cost, potential pain for the patient, delays between the biopsy and pathology reports, lack of specialized centres with expertise to interpret bone samples, lack of technical training, and limited understanding of the information provided by the results.

Efforts to minimize these constraints have included improved instrument design and biopsy techniques and more intensive and detailed training of clinicians and pathologists. Advances in bone sample processing have resulted in a faster turnaround time between the bone biopsy and availability of histologic results. This has enhanced the value of bone biopsy in routine patient care. Also, bone morphometrists have now a better understanding in bone biopsy results.

Alternatives to bone biopsy continue to be pursued. The search for non-invasive serum or bone markers that predict bone turnover, mineralization status, bone aluminum or strontium accumulation, and cellular abnormalities has resulted in improved methods to determine serum levels of various calcitropic hormones, isolation of proteins and enzymes from bone, and development of commercially available assays. However, these alternatives have not yet proven to be specific or sensitive enough to effectively replace the golden standard of bone biopsy.

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Резиме

## ДИЈАГНОЗА НА РЕНАЛНАТА ОСТЕОДИСТРОФИЈА СО КОСКЕНА БИОПСИЈА

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Со намалувањето на бубрежната функција доаѓа до каскада на промени во метаболизмот на минералите и витаминот Д, кои имаат реперкусија врз целуларните елементи и структурата на коскеното ткиво, како и врз динамиката на коскената преградба. Во фаза на терминална бубрежна инсуфициенција скоро сите пациенти исполуваат некој тип на коскена болест наречена ренална остеодистрофија. Подобрувањето на квалитетот на дијализниот режим го зголемува бројот на пациенти со долготраен дијализен стаж,

односно ја зголемува инциденцата на реналната остеодистрофија. Бидејќи метаболните коскени заболувања можат во некоја подоцнежна фаза да продуцираат фрактури, коскена болка и деформитети, неопходна е превенција, односно третман на пациентите во раната фаза од болеста. Серумската концентрација на паратхормонот (ПТХ) се смета за маркер за процена на коскената преградба. Но, вредностите на ПТХ меѓу 65 и 450 пг/мл најдени кај најголемиот број дијализни пациенти, не се предиктивни за типот на коскеното заболување. Трансилијачната коскена биопсија се смета за најмоќно дијагностичко орудие за добивање на прецизна информација за типот на реналната остеодистрофија, степенот на оштетување како и присуството на депозити на алуминиум или стронциум. Овој тип на дијагностика е корисен не само за клиниката туку и за истражувањата во однос на терапевтскиот ефект врз промените на коската. Иако инвазивна процедура, коскената биопсија не резултира со посериозни компликации освен болка, хематом или инфекција на раната, но потребна е стручност и искуство на лекарот што ја изведува. И понатаму продолжува потрагата за алтернативна дијагностика со неинвазивни коскени маркери кои би имале висока предиктивна вредност во однос на коскената преградба, процесот на минерализација и клеточните коскени абнормалности, но засега коскената биопсија останува како златен стандард во дијагностиката на реналната остеодистрофија.

**Клучни зборови:** коскена биопсија, ренална остеодистрофија, бубрежна слабост.

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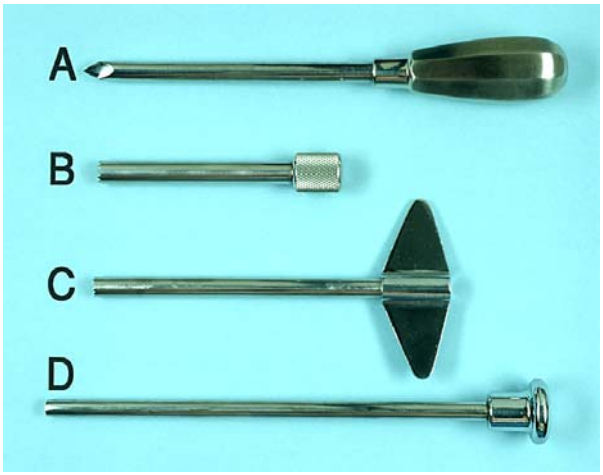



**Fig. 1. Bone biopsy needle set**

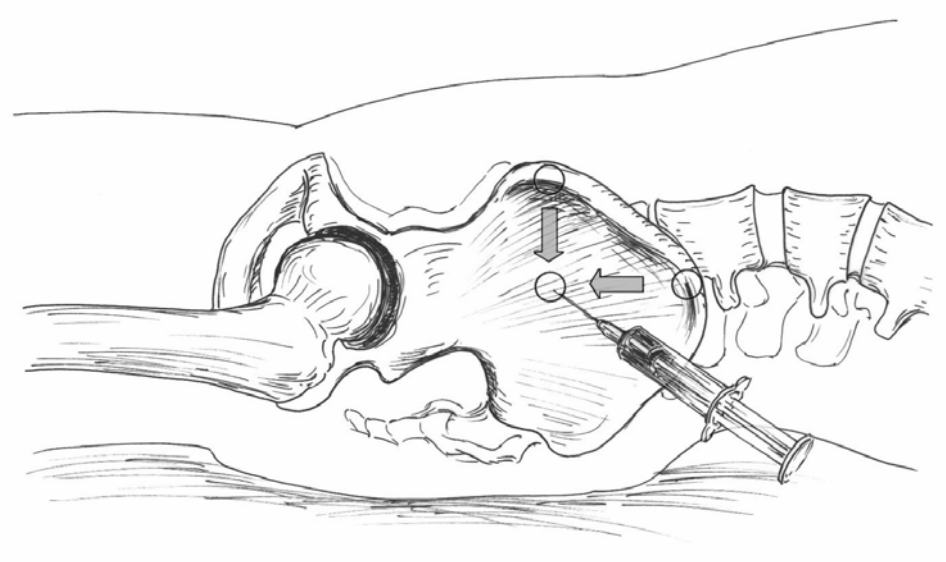


**Fig. 2. The transiliac bone biopsy site**
(By courtesy of Dirk De Weerdt, Dept. of Nephrology Antwerp, Belgium)

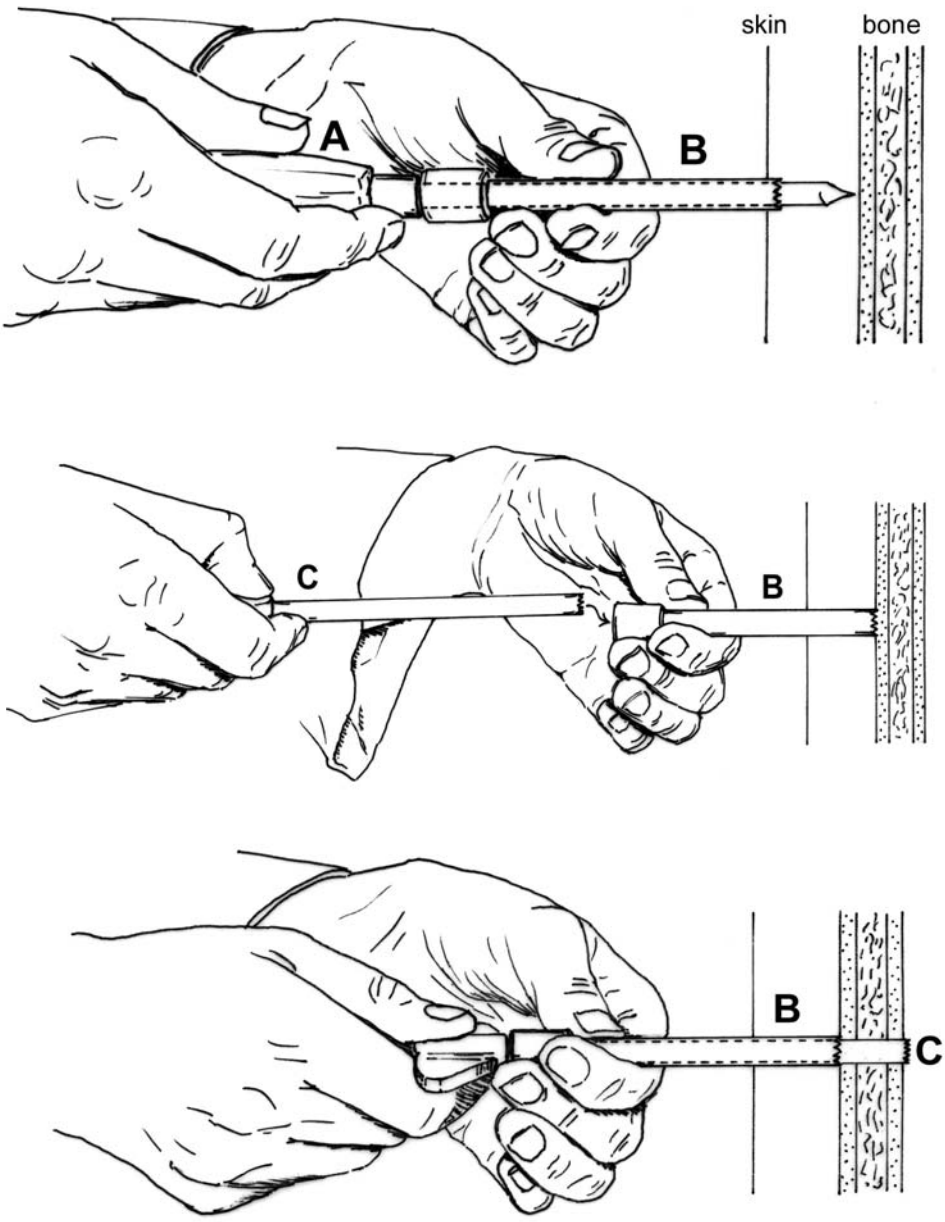
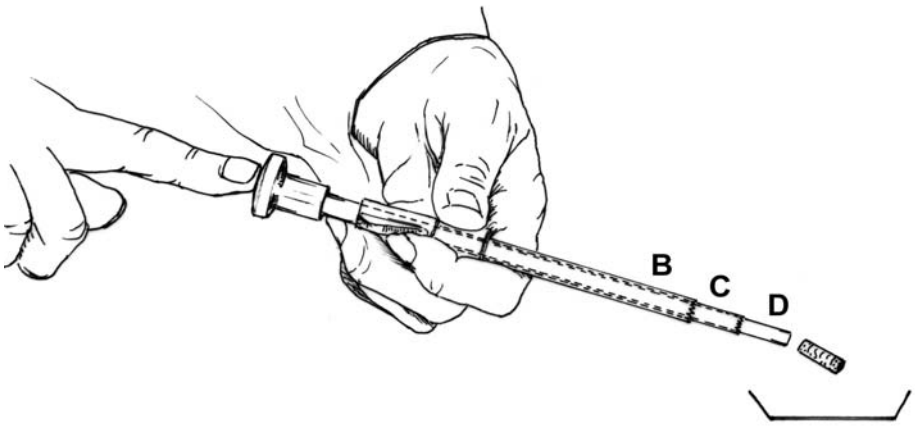



**Fig. 3. Bone biopsy procedure: steps 1–4**
(by courtesy of Dirk De Weerdt)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