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# Bone Biopsy: Indications, Technique and Complications

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## Introduction

Bone biopsy is a minimally invasive procedure that remains the gold standard method to diagnose the type and severity of renal osteodystrophy (ROD).[1] Over the last 60 years, much was learned about the histological patterns of this disease. It is currently well established that histological changes are found in the bone of virtually all patients with end-stage kidney disease (ESKD)[2]. Despite advances in our understanding of ROD, we have yet to establish the optimal noninvasive method to determine bone turnover, mineralization status, bone aluminum accumulation, or cellular abnormalities. Knowledge of these parameters in patients with renal failure is important since they influence therapeutic decisions. Bone biopsy is also useful in many other areas of medicine, these include patients with osteoporosis, osteomalacia, osteogenesis imperfecta, Paget's disease and other metabolic bone diseases. Also, bone biopsy is utilized in increasing frequency in post-transplant patients[3]. In addition to its clinical use, bone biopsy is done routinely for research purposes to evaluate the effects of new therapies on bone. Recently, molecular histology such as immunohistochemistry (IHC) and *in situ* hybridization histochemistry (ISHH) have emerged as valuable tools to better understand the underlying pathophysiology of the various metabolic bone diseases[4].

## Clinical Indications

There are potentially many clinical indications to obtain a bone biopsy in a patient with ESKD (table 1). In patients with persistent and unexplained hypercalcemia, a bone biopsy can assess for the presence of aluminum, adynamic bone disease (ABD) or severe hyperparathyroidism (HPT), particularly if the PTH levels are not remarkably altered to either direction. Patients with persistent and unexplained hyperphosphatemia (i.e., no evidence of dietary non-compliance) may have significant bone resorption despite only moderately elevated serum PTH levels. These patients could benefit from parathyroidectomy (PTX). Symptomatic patients with unexplained bone pain or fractures could practically have any form of ROD. In these patients, if PTH levels are not remarkably elevated to suggest severe HPT, the underlying diagnosis that would explain the symptoms can be very difficult to establish without a bone biopsy. Similarly, asymptomatic patients with moderately elevated PTH levels (i.e., 200 – 400 pg/ml) may not necessarily have underlying hyperparathyroid bone disease that requires the use of vitamin D[5]. Whenever aluminum-related bone disease is suspected, a bone biopsy is particularly helpful in making the diagnosis and guiding the therapy by evaluating the extent of aluminum accumulation. Finally, prior to parathyroidectomy a bone biopsy can confirm the diagnosis and rule out aluminum accumulation, unless this was accomplished with great deal of confidence by the clinical and biochemical evaluation of the patient.

## Pre-Procedure

A thorough preparation for the bone biopsy is essential. As with any invasive procedure, a history and physical must be completed. Particular emphasis is placed on medications, allergies, and comorbid conditions that may need stabilization prior to the biopsy such as cardiopulmonary diseases. Routine pre-operation workup within 2-3 weeks of the procedure should include complete blood count with differential, comprehensive metabolic panel, prothrombin time, activated partial prothrombin time, electrocardiogram, and a chest x-ray. Hemodialysis (HD) patients should be dialyzed the day before the biopsy with very minimal or no heparin at all. They should not be dialyzed the day of the procedure and if possible, the day after the procedure as well. Heparin use ought to be minimized for one to two dialysis sessions thereafter. Peritoneal dialysis (PD) patients can perform an exchange the morning of the procedure but must have the biopsy done with an empty abdominal cavity. Coumadin, aspirin, and clopidogrel (Plavix®) are to be stopped one week prior to the biopsy and restarted one week after. Patients with potential bleeding disorders such as liver failure may require fresh frozen plasma and platelets prior to the procedure, depending on their coagulation studies.

A very important step in the preparation of bone biopsy is the *in vivo* labeling of bone. Strict compliance with the instructions of this step must be stressed to patients to ensure that the specimen is of excellent quality. Currently, labeling is done with antibiotics from the tetracycline family because they are nontoxic, bind to actively forming bone surfaces, and have spontaneous fluorescence. As part of the history, inquiry to the previous use of tetracycline is sought out. The recent use of tetracycline to treat an infection may hinder the pathologist in the evaluation of the specimen. Also, inadequate absorption of the medication during the labeling regimen, as might occur if the patient has a malabsorption syndrome or is taking the antibiotic with meals, dairy products, iron-containing medications, calcium supplements or an antacid, will also make histological interpretation difficult because of insufficient labeling. In the case of a malabsorption syndrome, increasing the dose of the medication is warranted. A double-labeling technique, in which a period of time is intercalated between two courses of antibiotics, is optimal. This technique provides the best information on mineralization rate and bone formation. In our center, we use a tetracycline regimen of *on* for two days, *off* for eight to fifteen days, *on* for four days, followed by the biopsy within four to six days from the last dose. The dose of tetracycline hydrochloride is 500 mg p.o. t.i.d. in patients with normal kidney function and 500 mg p.o. b.i.d. in those with renal failure. A shorter regimen can be used in cases where the bone biopsy is urgently needed. This regimen consists of a tetracycline dose of 1.0 to 1.5 g p.o. q.d, *on* for one day, *off* for six days, *on* for one day with the biopsy 24-48 hours after the last dose. Common side effects of tetracycline include photosensitivity, vomiting, diarrhea, and allergic reactions among others; the higher dose might result in increased gastrointestinal side effects. Another regimen for the double-labeling technique involves using tetracycline for the first label and demeclocycline for the second, with the time intervals remaining the same. Tetracycline fluoresces light yellow while demeclocycline a yellow-orange. This two-drug technique may allow for better assessment of the mineralization rate. The dose for the demeclocycline is 300 mg p.o. t.i.d. or 300 mg p.o. b.i.d. for patients with normal or impaired kidney function, respectively.

## The Procedure

The best possible and most commonly used site for the bone biopsy is the anterior iliac crest. This site is associated with the fewest postoperative complications, is easily accessible, and is not as invasive as other procedures such as obtaining a rib biopsy. In addition, rib biopsies in metabolic bone diseases have an inadequate amount of cancellous bone tissue. Some debate exists over which approach is the best to use in obtaining a bone biopsy from the iliac crest, the vertical versus the horizontal or "through-and-through" technique (Figure 1). We advocate the use of the vertical approach for various reasons. First, electric drill is used with the vertical approach, as opposed to the manual trochar used with the horizontal method, resulting in less physical pressure being applied to the bone and patient. This pressure differential potentially results in less damaged bone and less trauma. Second, hemostasis is easily accomplished with the vertical approach as it only results in one hole in the patient's iliac bone, which is readily accessible and compressible. The horizontal technique leaves two holes in the patient's pelvis, of which the internal hole is inaccessible for compression should bleeding occur. Third, the sample size is limited by the thickness of the iliac bone when using the horizontal method. In contrast, the vertical method yields a longer size sample, typically 2.5 to 3.5 cm long. This longer sample allows for the assessment of not only the cortical bone and subcortical cancellous bone, but also the deep cancellous bone that the horizontal specimen is unable to provide. Lastly, the two cortices of bone obtained with the horizontal technique have not been shown to provide any additional histological information.

The day of the bone biopsy the patient should be NPO for at least six hours prior to the procedure. Morning medications can be taken with just sips of water. An informed consent should have been obtained as part of the pre-operation process. The procedure is done in an outpatient operating room or in a procedure room setting, as maximum sterility and cardiovascular monitoring is important. In our center we utilize an outpatient operating room and provide general anesthesia. The anesthesiologist typically uses a laryngeal mask airway device for airway protection, since the procedure is relatively short. However, the biopsy can be easily done with conscious sedation in a regular procedure room.

The patient is placed in a supine position with the leg of the biopsy rotated outwards, and a supportive pad is placed under the hip to be biopsied. After locating the anterior iliac crest, the area is disinfected with betadine solution and a sterile field is created. Lidocaine, one percent without epinephrine, is infiltrated generously (10-15cc) to an area located about two to five centimeters behind the anterior iliac spine. The medial and lateral iliac walls, as well as the surrounding subcutaneous tissue, are also infiltrated with lidocaine. The periosteum should be infiltrated adequately. In the obese patient, the whole body can be tilted to one side so as to allow gravity to aid in exposing the biopsy site. If this is not feasible, the excess tissue can be moved aside with straps of two-inch tape. If the anterior iliac crest is still not accessible with the above measures, the posterior iliac crest can be used, just lateral to the sacral-iliac joint.

The electric drill we employ is a newer model (Straumann, Cambridge, MA; Figure 2) which works like the established electric drill but has the added feature of a one-step drilling and extraction process, therefore shortening the surgical time and making the procedure simpler. The drill is set up by first attaching the *ejector*, followed by the *extractor*, and finally either the *precutter* or *trephine*, depending on whether the drilling is for subcutaneous tissue or for bone, respectively (see below). The drill bits are disposable, which decreases the infection risk, ensure sharp tools, and eliminate potentially weakened drill bits that may occur as a result of frequent sharpening. While holding the iliac crest between two fingers, a longitudinal incision of half to one centimeter is made with a scalpel right over the anterior iliac crest. This is followed by blunt dissection of the subcutaneous tissue with a scissor or hemostat until the upper surface of the anterior iliac crest is exposed. Additional lidocaine to the periosteum with direct visualization might be helpful at this stage. The funnel (Figure 2) is now introduced through the incision and placed over the exposed iliac crest surface in the center between the medial and lateral edges. The axis of the funnel should be aligned with the axis of the underlying bone. This prevents the trephine from exiting through the pelvic bone during the drilling process.

The precutter is used first to remove the subcutaneous tissue and some of the periosteum. It is placed in the center of the funnel and the electric drill is engaged by pressing the first trigger (Figure 2, upper button). The precutter should not go into the bone. Next, the trephine is placed in the funnel in the same fashion. Drilling should be done with minimal pressure to avoid collapsing the cancellous bone. Some resistance can be expected while drilling, however, if a considerable amount of resistance is encountered, the trephine might be drilling into the sidewall of the cortex. If this is suspected, the trephine should be pulled out and the axis of the funnel readjusted to ensure adequate sampling of the cancellous bone. The drilling continues until the base of the drill just reaches the bottom of the funnel, without touching it. At this stage, the sleeve of the drill is pulled slowly upwards while continuing to drill. This process allows the extraction forceps to move over the bone core and capture the sample. The entire trephine can now be removed slowly upwards while the drill is still rotating.

As soon as the trephine has exited the body, a sterile medical wax plug in the form of a cylinder should be placed almost simultaneously into the cavity created by the trephine. This should minimize bleeding and prevent hematoma formation. The wax is neatly packed with the tip of a forceps wrapped in gauze to avoid adhesion of the wax to the metal of the forceps. Excess wax should be meticulously removed and the surface of the plug must be below the edges of the bone cavity to prevent contact with local tissue, which can lead to an inflammatory reaction. If excessive bleeding is noted, Gelfoam followed by the wax plug can be used, and if absolutely necessary, liquid thrombin can be injected through the wax plug and into the Gelfoam to further aid in hemostasis.

The bone core sample is ejected onto a sterile sheet while pressing the second button on the drill (Figure 2, lower button). An adequate specimen should be two to three centimeters in length and four millimeters in diameter, and it should consist of a layer of upper cortical bone with subcortical and deep cancellous bone (Figure 3). The sample should be picked up gently with forceps and placed in a 100% ethanol container.

Once a satisfactory specimen has been secured, the surgical area is thoroughly irrigated with a saline solution and the incision is closed in two layers, subcutaneous tissue with absorbable sutures and the skin using 0-3 Nylon. Antibiotic ointment is applied over the incision followed by an eye patch and Tegaderm. Ice is left over the area for one to two hours to ensure hemostasis and keep any swelling to a minimum. If the patient has an increased risk of bleeding, an abdominal circular binder can be used to put pressure over the biopsy site. The total time of the procedure from the first incision to the last suture generally takes approximately 15 to 20 minutes.

## Post-Procedure

Post-operation instructions to the patient include keeping the wound dry for at least 48 hours. The patient should not drive a car immediately after the procedure and should not do any heavy lifting, severe straining, or stair climbing for at least 4 days. Any sign of infection or bleeding should immediately be reported to the physician who preformed the biopsy. The patient, as well as the patient's primary care physician, should be informed that future x-rays of the pelvic bone may show artifact in the area of the bone biopsy and this is of no concern.

The bone biopsy specimen must pass through five steps in the bone histology lab: fixation, dehydration, embedding, sectioning, and staining. The 100% ethanol solution is the best fixative currently used for routine mineralized bone histology given that it preserves the tissue well and does not leach out the calcium. The dehydration step starts after the sample has been immersed in the 100% ethanol for 24 to 48 hours, depending on the size of the sample. This dehydration step is required since the embedding media is not miscible with water. Embedding is done with methyl methacrylate because it penetrates the specimen quickly, is nontoxic, does not cause artifacts such as bubbles, and has the necessary dissolving solvents. The next step, sectioning, is accomplished with special microtomes implanted with diamond knives or carbide edges. Staining is the last part of the preparation necessary. Various stains and techniques are available depending what is sought out, but their descriptions are beyond the scope of this chapter. On average, it takes at least 10 - 14 days before the biopsy slides are ready for interpretation.

## Complications

Potential complications include bleeding, hematoma, infection, superficial nerve injury, and pain. The overall frequency of complications is 0.52%, as reported by Duncan et al. based on a questionnaire sent to 18 different hospitals accounting for 14,810 biopsies.[6] The horizontal approach has a complication rate of 0.63%, while the vertical approach was 0.36%. Bleeding and hematoma are reduced to a minimum with the vertical technique and if all necessary precautions have been undertaken. Likewise, infection risk is not greater than one percent if sterile precautions are followed. If infections do occur, they are usually related to inappropriate postoperative wound care. More specific to this particular procedure is the potential formation of a granuloma as a result of wax debris that migrated from the bone cavity or was not adequately removed. Superficial nerve injury has been reported as a complication of the bone biopsy. It results when a superficial nerve, such as the nervus cutaneus femoralis, is accidentally lacerated. Because nerves of the peripheral nervous system can regrow, resolution of the symptoms created by the laceration can be expected. Lastly, pain associated with the bone biopsy varies, depending on the patient. Most patients will report "soreness" over the area lasting four to five days.

## Training Process

At the University of Kentucky, Division of Nephrology, Bone and Mineral Metabolism, over 30 practicing nephrologists from throughout the United States have been trained in the past two years to confidently and independently perform bone biopsies. The process involves spending at least one full day in the outpatient operating room with one of the experienced nephrologists. After observing one procedure, the trainee is observed and coached through as many biopsies as necessary to become proficient in the process. Usually, 2 or 3 biopsies are sufficient for adequate training. Once capable, the physician receives a certificate attesting to his/her proficiency in the procedure. To obtain temporary clinical privileges, the physician must hold a license to practice medicine in any State and must have a valid DEA registration.

## Conclusion

Bone biopsy remains the gold standard for the evaluation of ROD. Its role in the management of patients with ESKD is valuable and sometimes indispensable. The procedure is minimally invasive and is performed on an outpatient basis. It is well tolerated and very safe if carried out by an experienced and properly trained physician.

**Table 1: Potential Clinical Indications of Bone Biopsy in ESKD Patients***

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| Persistent and unexplained hypercalcemia |
| Persistent and unexplained hyperphosphatemia |
| Unexplained bone pain and fractures |
| Moderate hyperparathyroidism |
| Suspected aluminum-related bone disease |
| Suspected osteomalacia |
| Prior to parathyroidectomy |

*See text for discussion

**Figure 1:**

**Bone Biopsy Techniques**

Cross-section of the anterior iliac crest: (A) Vertical approach; (B) Horizontal approach (through-and-through)

**Figure 2:**

Bone biopsy drill and funnel

**Figure 3:**

Example of an adequate sample of extracted bone tissue

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