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Research Article

Histomorphometric and microarchitectural analyses using the 2 mm bone marrow trephine in metastatic breast cancer patients–preliminary results

M. Fralick [a], N. Bouganim [b], R. Kremer [b], N. Kekre [c], S. Robertson [d], L. Vandermeer [c], I. Kuchuk [c], J. Li [e], M. Murshed [e], M. Clemons *,[c]

[a] Department of Internal Medicine, University of Toronto, Toronto, Canada
[b] Department of Medicine, McGill University Health Center, McGill University, Montreal, Canada
[c] Department of Medicine, The Ottawa Hospital Cancer Centre, Ottawa, University of Ottawa, Ottawa, Canada
[d] Department of Pathology, The Ottawa Hospital Cancer Centre, Ottawa, and Department of Medicine, University of Ottawa, Ottawa, Canada
[e] Department of Medicine and Faculty of Dentistry, Shriners Hospital for Children, McGill University, Montreal, Canada

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ABSTRACT

Background: Bone-targeted agents are widely used for the treatment of osteoporosis, the prevention of cancer-therapy induced bone loss, and for reducing the risk of skeletal related events in patients with metastatic disease. Despite widespread use, relatively little is known about the in vivo effect of these agents on bone homeostasis, bone quality, and bone architecture in humans. Traditionally bone quality has been assessed using a transiliac bone biopsy with a 7 mm "Bordier" core needle. We examined the possibility of using a 2 mm "Jamshidi" core needle as a more practical and less invasive method to assess bone turnover and potentially other tumor effects.

Methods: A pilot study on the feasibility of assessing bone quality and microarchitecture and tumor invasion using a 2 mm bone marrow trephine was conducted. Patients underwent a posterior trans-iliac trephine biopsy and bone marrow aspirate. Samples were analyzed for bone microarchitecture, bone density, and histomorphometry. The study plan was to accrue three patients with advanced breast cancer to assess the feasibility of the study before enrolling more patients.

Results: The procedure was well tolerated. The sample quality was excellent to analyze bone trabecular microarchitecture using both microCT and histomorphometry. Intense osteoclastic activity was observed in a patient with extensive tumor burden in bone despite intravenous bisphosphonate therapy.

Discussion: Given the success of this study for assessing bone microarchitecture, bone density, and histomorphometry assessment using a 2 mm needle the study will be expanded beyond these initial three patients for longitudinal assessment of bone-targeted therapy.

1. Introduction

Bone-targeted agents aimed at reducing osteoclastogenesis including bisphosphonates and denosumab are widely used in cancer patients. Their uses include; the treatment of osteoporosis, prevention of cancer-therapy induced bone loss, reducing the risk of skeletal related events, reducing pain, and improving quality of life in patients with metastatic disease [1]. In cancer patients these agents are administered systemically at high and frequent, often monthly, doses and for extended periods of time relative to their use in the treatment of osteoporosis. Despite this there is a paucity of data about the in vivo effect of these agents on bone homeostasis, bone quality, and microarchitecture in humans. This topic has gained increasing attention recently with the awareness of long term toxicities of bisphosphonates such as osteonecrosis of the jaw (ONJ) and atypical fractures.[2,3].

Traditionally bone strength has been viewed as an integration of bone quantity and bone quality [4]. Clinically, bone strength and susceptibility to fracture is based on bone mineral density (BMD) as reflected through dual energy X-ray absortiometry (DEXA) scans [5]. This, however, has only modest sensitivity/specificity [5,6], and only a small proportion of fracture risk reduction is actually explained by bone density increases [7,8].

Clinical and laboratory evidence suggest mechanical properties, in addition to BMD, play an important role in bone strength [9–11]. However, assessing bone quality requires invasive testing, traditionally with a 7–8 mm "Bordier" trephine of transiliac bone [12].

* Correspondence to: Division of Medical Oncology, The Ottawa Hospital Cancer Centre, 501 Smyth Road, Ottawa, Canada. Tel.: +1 613 737 7700x70170; fax: +1 613 247 3511.
E-mail address: mclemons@toh.on.ca (M. Clemons).

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The size of the needle poses two problems. First, it is uncomfortable and painful for the patient. Second, it is difficult technically in achieving an adequate sample in cancer patients on bone-targeted agents as anecdotally the procedure is more challenging than in patients with osteoporosis [13]. Previous data from cadavers has demonstrated that the 2 mm trephine provides comparable results to a 7 mm trephine [14]. A review of the literature revealed no studies comparing the two trephine sizes in living cancer patients.

Given the widespread use of outpatient posterior iliac crest bone marrow biopsies in hematology and oncology using a 2 mm Jamshidi needle, we wanted to see if this would allow a more practical, technically easier sampling amenable to routine screening of bone status in cancer patients.

2. Methods

2.1. Subjects and design

This study was a pilot feasibility study performed in three patients prior to starting a larger prospective, single-arm, non-interventional feasibility study using a 2 mm trephine to assess bone quality in breast cancer patients. Patients were required to have: advanced breast cancer, with or without bone metastases with or without bisphosphonate treatment, and an ECOG ≤ 2. All patients enrolled underwent a bone mineral density assessment using DEXA. The study was approved by the research ethics committee at the Ottawa Hospital Cancer Centre, and all patients provided written informed consent.

2.2. Bone specimen collection

Trans-iliac crest bone biopsy specimens were obtained from the posterior iliac crest using a Jamshidi bone biopsy trephine (diameter 2 mm) (Cardinal Health, Dublin, Ohio, United States of America) (see Appendix 1). The samples underwent histomorphometric analysis, microarchitectural analysis, and routine pathologic assessment.

2.3. Histological and histomorphometrical analysis of bone

Hematoxyline and Eosin (HE), von Kossa and van Gieson (VKVG), and tartrate-resistant acid phosphatase (TRAP) staining of plastic sections of posterior iliac bone samples from three patients were performed on plastic sections. As described elsewhere, for plastic sectioning, bone samples fixed in 4% PFA/PBS were embedded in methyl methacrylate, and sectioned (7-μm thickness), and von Kossa and van Gieson staining was applied [15]. Unmineralized bone sections were analyzed using Osteomeasure software (Osteometrics, Inc.). Images were taken at room temperature using a light microscope (DM200; Leica) with a $20\times$ (numerical aperture of 0.40) or $40\times$ (numerical aperture of 0.65) objective. All histological images were captured using a camera (DP72; Olympus), acquired with a DP2-BSW software (XV3.0; Olympus), and processed using Photoshop (Adobe).

2.4. Three dimensional micro-computed tomography (3D microCT) of bone samples

The core biopsy samples were scanned wet in 70% ethanol by micro-CT at 40X magnification with a SkyScan 1072 (Skyscan, Aartselaar, Belgium) and analyzed with a bone analysis software (ver2.2f, Aartselaar, Belgium). Trabecular bone structure was measured. Parameters were acquired with a rotation of 0.9° between each picture and the x-ray source set at 100 kV and 98 μA. The segmentation of the image was made by a global threshold and a voxel size of $21.90\times 21.90\times 21.90\ \mu m^3$; the same threshold setting was used for all the samples. Architectural measurements were made as previously described [16,17].

2.5. Bone mineral density measurements by DEXA

Core biopsies were subjected to bone mineral density (BMD) analysis using a PIXIMUS bone densitometer (PIXIMUS TM, GE medical systems, Schenectady, NY, USA). A quality control phantom was used to calibrate the densitometer prior to each experiment. All patients also underwent DEXA scanning.

2.6. Statistical analysis

The primary endpoint of this pilot study was to assess the feasibility of obtaining sufficient tissue using bone marrow aspirates and trephine biopsy for histomorphometry. The secondary endpoint was comparison between current bone mineral density and pathological analysis of bone marrow trephine biopsy looking at histomorphometry, and micro-architecture analysis. Given this was a pilot study simple descriptive statistics were used.

3. Results

Between January and July 2011 three patients consented and underwent outpatient biopsies. Patient number one and number two received two biopsies from separate areas of the posterior iliac crest. Patient three received a single biopsy. The demographic characteristics of each patient can be found in Table 1. Notably, all patients were post-menopausal, had metastatic disease, and two were on long-term bisphosphonate therapy.

4. 3D microCT data

Bone volume (BV/TV) ranged from 10.8 to 13.5% consistent with the published literature [18]. Trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N) and bone

Table 1
Demographic data of study patients.

	Patient 1	Patient 2	Patient 3
Age	64	56	59
Menopause status	Postmenopausal	Postmenopausal	Postmenopausal
BMI (kg/m^2)	34.0	19.5	27.9
Smoking history	None	None	None
BMD (*t*-score)	−1.9 [L1–L2]	−1.3 [L1–L4]	−2.2 [L1–L4]
Breast cancer	Metastatic to soft tissues	Extensive bone metastases	Limited bone metastases
Current therapy	Aromatase inhibitor	Aromatase inhibitor	Aromatase inhibitor
Bisphosphonate use and duration	Nil	Yes (3 years)	Yes (2 years)

BMI = body mass index, BMD = bone mineral density and L = lumbar spine (i.e. L1 = first lumbar vertebrae).