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Research Paper

Strategies for obtaining bone biopsy specimens from breast cancer patients – Past experience and future directions



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ABSTRACT

Background: Cancer and its treatment can have multiple effects on the bone. Despite the widespread use of in vivo and in vitro models, it is still necessary to understand these effects in humans. Obtaining human bone biopsies is technically challenging and in this article we review the experiences from the Ottawa Bone Oncology Program.

Methods: A series of bone biopsy studies in breast cancer patients with and without bone metastasis have been performed. We reviewed the results of these studies and present them in a descriptive manner. We discuss lessons learned from each project and how they have affected future directions for research.

Results: Since 2009, 5 studies have been performed accruing 97 breast cancer patients. Study endpoints have ranged from comparing the yield of malignant cells from CT-guided versus standard iliac crest biopsies, to studies assessing the feasibility of micro-CT analysis on Jedhadi trephines to evaluate bisphosphonate effects on bone micro-architecture. More recently, we have assessed the feasibility of performing repeat bone biopsies in the same patient as well as evaluating the practicality of obtaining bone tissue at the time of orthopaedic surgery.

Conclusion: Human bone tissue is an important biological resource. Our experience suggests that obtaining bone biopsies is feasible and can yield adequate amount of tumour cells for many studies. However, these remain technically challenging specimens to obtain and given the rapid advances in cancer therapeutics and the use of potent adjuvant bone-targeted agents, more centres need to be involved in these types of studies.

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1. Introduction

Over the last few decades bone oncology research has tended to focus on the mechanisms of bone destruction when tumour cells are present [1–3]. The realisation of the important interplay between the tumour cell, the bone microenvironment and the osteoclast in particular led to the rapid expansion of clinical studies with bone-targeting agents, such as bisphosphonates and denosumab [4,5]. However, with the advent of more effective anti-cancer therapies, as well as studies demonstrating alterations in estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) status between primary

and metastatic sites, there has been increasing interest in evaluating the actual biological effects of cancer and its treatment on the bone in patients themselves [6,7]. Indeed, the expanding role of adjuvant bisphosphonates would suggest that more in vivo studies in patients are actually needed [8,9].

Despite the growing knowledge about the bone micro-environment, it is clinically evident that the information so far derived from the use of animal models and cell lines has not been consistently predictive of benefit in patients [10–12]. For example, despite models suggesting significant direct and indirect anti-tumour effects of bone-targeted agents, to date their effects on bone response rates, progression free or overall survival in patients with metastatic disease has been modest [13–15]. In addition, the issue around the adjuvant use of bisphosphonates has become clinically challenging; initial animal models suggested bisphosphonates were most effective in a high bone turnover environment, however a recent meta-analysis would suggest that the clinical benefit

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is limited and only seen in postmenopausal patients [8]. Subsequently animal models that mimic a low estrogen/postmenopausal environment have been developed and recent data suggest that the combination of bisphosphonate and a low estrogen environment can inhibit tumour growth, an effect that is not seen in the premenopausal/high estrogen environment. This finding gives a biological rationale for the clinical results seen with adjuvant bisphosphonates in the postmenopausal patient [12].

Given the limitation of current *in vitro* and *in vivo* animal models, human bone biopsy tissue represents a valuable resource for further research efforts and for guiding clinical care [15]. Unfortunately, bone remains one of the more technically difficult areas to biopsy. In this paper we will discuss a series of studies that have been performed by the Ottawa Bone Oncology Program (OBOP) outlining the types of studies we have performed and the challenges of performing such studies. We will evaluate future directions where we feel studies of human bone metastasis tissue could potentially yield the most benefits to patients.

2. Methods

Since 2009, a series of studies were conducted at The Ottawa Hospital Cancer Centre. Each study received local Research Ethics Board approval and evaluated a range of different endpoints. We have reviewed the results of these studies and present them in a descriptive manner. We also discuss some of the challenges faced by each project and how we have tried to incorporate these lessons into subsequent projects (Table 1).

2.1. Is the yield of metastatic tumour cells similar with CT-guidance and standard iliac crest trephine biopsy?

With significant hormone status discordance between primary and metastatic sites in breast cancer patients having been reported [16–18], acquisition of metastatic tissue may have important implications in planning subsequent treatments. Amir et al. reported that biopsy of any site of metastatic recurrence at the time of first metastases led to change in management of 14% of women with breast cancer (95% CI, 8.4% to 21.5%) [19], as a result of change in the expression of ER, PR and Her2 receptors [19]. While tissue acquisition for visceral and nodal sites can be relatively straight forward, acquisition of metastatic tissue from patients with bone-only sites of recurrence can lead to additional challenges. In this situation, there are two acceptable methods of bone tissue acquisition: bone marrow aspiration and biopsy from the iliac crest; or CT-guided bone biopsy. Bone marrow trephine/aspiration is traditionally performed in the outpatient clinic using Jamshidi bone biopsy needles. While CT-guided biopsies are performed in the radiology suite by an interventional radiologist, who will

choose the safest skeletal site to biopsy. There are important cost and logistical issues associated with each of these techniques with CT-guided biopsies being significantly more expensive. Once tissue is obtained, samples can be analysed by microscopy, immunohistochemistry [20] and if sufficient tumour cells are obtained, gene expression profiling can be conducted [9]. Success of such analysis depends on the quality and source of the specimen but in one study we showed that the analysable yield of sufficient RNA for microarray analysis was 60% from bone metastasis core needle biopsies and 80% from bone marrow aspirate specimens [9].

In a single arm feasibility study to compare the two types of biopsy, Hilton et al. assessed whether bone marrow trephine/aspiration biopsy can be utilised in place of CT-guided biopsy of bone metastases in patients with metastatic breast cancer [20]. Patients underwent a CT-guided bone biopsy followed by a standard outpatient bone marrow aspirate and trephine performed from the posterior iliac crest. Forty patients entered the study and tumour cells were identified at similar rates from both the iliac crest bone biopsies (19/39 patients, 48.8%) and the CT-guided biopsy samples (16/34 patients, 47%). The rate of receptor discordance between the primary and metastatic tumours (53.8%) was similar to that reported in the literature [16]. The acquired tissue through bone marrow biopsies were also of sufficient quality to permit routine molecular sequencing [20]. Given the similarity in yield of malignant cells with the two procedures and that CT-guided biopsies are considerably more expensive and resource intensive, our future studies chose bone marrow trephine/aspiration biopsy when studying bone metastatic bone disease [20].

Lessons learned:

1. When obtaining consent for obtaining bone biopsies it is important to consider what future studies might be performed on these specimens so that appropriate consent can be obtained.
2. Standard operating procedures are needed for tissue handling as different studies required different storage media (e.g. if specimen is for IHC or genomics).
3. The clinical research associate (CRA) should be present when biopsies are performed. Due to the many different staff members performing the biopsies the CRA ensured that all patients had consented, that the correct storage media was used and that there was effective communication with the pathology department to ensure that the appropriate tests were performed.

2.2. Can Jamshidi bone biopsy needles be used to assess the effects of cancer and its treatment on bone homeostasis, quality, and architecture in breast cancer patients?

Traditionally studies designed to assess bone quality in biopsy

Table 1
Lessons learned from the creation of the Ottawa Bone Oncology Program.

Study types	Lessons learned:
Issues affecting all studies	<ul style="list-style-type: none"> • Ensure consent covers future studies might be performed on these specimens. • Biopsies should be performed by a well-trained individual. • Standard operating procedures are needed for tissue handling.
Studies exploring bone quality Studies evaluating repeat biopsies	<ul style="list-style-type: none"> • The yield of tumour cells is relatively low. • Jamshidi biopsy needle can be used for the assessment of bone quality, however larger studies are needed. • Patients are often willing to undergo repeat bone biopsies. • Low tumour yields a significant issue • The number of specimens with tumour cells present from both pre- and post-treatment specimens in the same patient will be relatively low.
Studies obtaining specimens from surgical specimens	<ul style="list-style-type: none"> • Coordination between multiple teams is needed. • Advanced notification is desirable however if not possible specimen storage protocols are necessary. • The abundance of tumour available at open surgical procedures allows for multiple end uses.

specimens (e.g. in patients with osteomalacia) have used a trans-iliac bone biopsy with a 7 mm “Bordier” core needle [21]. In this study we examined whether or not it would be possible to use the 2 mm Jamshidi bone biopsy needle as a more practical and less invasive method to assess bone homeostasis, quality, and architecture in humans. This feasibility study was performed on three patients with advanced breast cancer, to evaluate metastatic specimens for bone microarchitecture, bone density, and histomorphometry.

Trans-iliac crest bone biopsy specimens were obtained from the posterior iliac crest using a Jamshidi bone biopsy trephine only (i.e. no Bordier biopsy was performed), samples were then stained and prepared for histomorphologic analysis [21]. Architectural measurements were made using three dimensional micro-computed tomography (3D microCT), while bone mineral density (BMD) of the core biopsies were analysed using a PIXIMUS bone densitometer. The quality of the samples obtained in this small study was sufficient for all three samples to be used for architectural measurement [21]. However, image analysis is a labour intensive process raising concerns about the practicality of this technique in future studies.

Lessons learned:

1. Jamshidi can be used for the assessment of bone quality. With the increased use of adjuvant bone-targeting agents this may offer a unique opportunity for future studies.
2. This technique may make the acquisition of bone tumour specimens more readily available for further immunohistochemical and genetic analysis, studies in this setting are required.

2.3. Will patients agree to repeat bone biopsies?

Much more so than visceral metastases where pre and post treatment biopsies are technically much easier to acquire, pharmacodynamics analyses of bone-specific therapeutics represents a unique challenge as patients may not be willing to undergo two separate bone biopsies. Whether or not bone-specific studies can be successfully performed on repeat bone biopsies was recently addressed in a study conducted by our research team [22]. Following animal work showing that doxycycline can result in decrease in tumour burden in a bone metastasis model of human breast cancer using MDA-MB-231 triple negative breast cell lines [23], we evaluated whether or not this effect could be demonstrated in patients with bone metastases from breast cancer [24].

In this phase II single arm, prospective study, 37 patients with breast cancer (of any ER, PR or Her2 receptor status) and bone metastases were enrolled and received doxycycline (100 mg orally, twice a day) in addition to their standard anticancer therapy. The primary end point was assessment of palliative benefit of adding doxycycline for 12 weeks to standard bone-targeted therapy as reflected by changes in validated pain questionnaire scores and markers of bone turnover. In conjunction, pre- and post- 2 mm posterior iliac crest bone biopsies were collected to evaluate the biological effects of doxycycline on bone microenvironment. Of 37 patients, 36/37 (97%) completed a baseline biopsy. One baseline biopsy was attempted but could not be completed for technical reasons. While 25/37 (68%) completed the repeat week 12 bone biopsy. Twelve patients (32%) did not complete the 12 week biopsy because of coming off study early and they therefore did not complete the 12 weeks of doxycycline administration (8 patients), 2 declined the repeat biopsy, and 2 biopsies were attempted but could not be completed due to patient body habitus.

Lessons learned:

1. For intervention trials patients are often willing to undergo repeat biopsies however attrition during the trial intervention is a

significant issue.

2. Biopsies should be performed by a well-trained individual. If the bone biopsy at baseline did not go smoothly the patient was unlikely to agree to undergo a second one.

2.4. Can sufficient bone metastasis cells be obtained from bone biopsies for molecular studies?

The mechanisms of how tumour cells metastasize to bone, and what happens to them when they get there, including their ability to respond to treatment and treatment effects on the bone microenvironment is not fully understood in humans. For this reason access to tumour bone metastases specimens pre and post treatment interventions would be an asset. While our study successfully obtained paired biopsy specimens (pre and post doxycycline treatment) for 25 patients, only 17% of baseline samples, and 12% of week 12 bone biopsies contained metastatic tumour cells [22]. Of these, only one patient had a paired sample containing tumour cells in both the baseline and the week 12 biopsy. Given the unfortunate yield of paired samples using this method, it would require significantly larger patient cohorts to obtain sufficient numbers of paired specimens for meaningful analysis of treatment effects directly on tumour cells. However, it should be noted that all samples obtained had sufficient bone resident cell populations, and as such, these methods would readily allow assessment of treatment effects on the bone microenvironment as a whole.

Lessons learned:

1. Given the relatively low yield of tumour cells in biopsies taken from the same patient at different times alternative techniques to increase the yield are required.

2.5. Obtaining bone biopsies at the time of orthopaedic surgery

It was evident from our previous studies that with iliac crest biopsy there often times were no visible tumour cells in the biopsy specimen and when they were there, their numbers were quite low. We therefore formed an organised program for collaboration with the local orthopaedic surgeons as most of the orthopaedic surgery for metastatic and impending fractures in Ottawa is performed at one of two hospitals. The rationale for this ongoing work is to create cells lines in addition to creating a bone metastatic tissue bank (bio-bank) from bone metastatic tissue from cancer patients undergoing orthopaedic surgery. Most patients are consented for this use of their tissue just prior to surgery in the emergent situation. This consent also allows access to archival surgical specimens of their primary tumour if it is available.

To date we have only been able to capture samples from patients who have been prescheduled for preventative orthopaedic surgery due to risk of fracture. We have had challenges capturing patients who are admitted for fracture through the emergency room or trauma service, as these individuals are often admitted and treated by a large cohort of surgeons. Additionally, we are limited to the types of tumours place patients at a higher risk of fracture. However from July 2014 to February 2016 we successfully collected 29 specimens from patients undergoing orthopaedic surgery. These specimens have come from patients with breast cancer (n=23), melanoma (n=1), lung cancer (n=1) and unknown primary (n=4).

Lessons learned:

1. Successful acquisition and processing of materials required co-ordinated efforts amongst team members from many different departments and physical locations.
2. Wherever possible, advanced notification allows laboratory staff to process specimens in a timely manner. When advance

warning is not possible, specimens can be stored in culture medium for short duration at 4 °C to allow for later processing of tumour.

- Given the abundance of tumour available at open surgical procedures, significant amounts of tumour and bone tissue samples can be obtained at time of orthopaedic surgery, which allows for multiple end uses.
- This access to bone metastases specimens is fairly restricted to tumour types that tend to induce risk of fracture, and as such access to other important bone metastasizing tumours such as prostate cancers are not readily available.

3. Discussion

Despite the availability of in vivo and in vitro models for bone metastasis behaviour there is still an ongoing need to understand the effects of cancer and its treatment on the bone in patients. Unfortunately obtaining human bone biopsy specimens is technically challenging and therefore our collaborative group OBOP has performed a series of trials to try and maximise the yield of bone metastatic tumour cells through multi-disciplinary collaboration. We have also attempted to prospectively ensure that these specimens are used for future research endeavours.

In this paper, we summarized studies in which we confirm the significant discordance in hormone receptor status between primary and metastatic bone disease at the time of metastasis [20]. We have also shown similar yields of tumour cells using the unguided bone marrow aspirate or the CT-guided technique, however the overall low yield of tumour cells raises questions around the feasibility of either technique for drug evaluation studies [20]. Subsequent studies confirmed that core biopsy needles could be used to evaluate the histomorphology of the bone from metastatic breast cancer [21]. This could prove particularly useful in future studies evaluating the effects of various adjuvant agents on the bone. In addition, one study confirmed that patients are willing to undergo serial bone biopsies [24]. More recently in collaboration with our orthopaedic colleagues, we were able to obtain 29 samples to initiate the establishment of a bone tissue bank. Although this technique is feasible, technically easy to perform and provides adequate amounts of tissue for many studies, it is only applicable in the subset of patients had with either acute or pending pathological fracture [25].

Clearly there are limitations to our studies. They are frequently of small size and in many of them the yield of viable tumour cells for further studies is limited. Alternative imaging techniques have been evaluated by others to increase the yield from bone specimens [26,27]. However, these will all be limited by cost and need of expert interventionists to perform it. Studies of circulating tumour cells (CTC) and disseminated tumour cells (DTCs) are ongoing as alternatives to bone biopsy [28,29]. However, there will still be a need for actual bone metastasis tissue as the properties of these cells may not be the same as the cells that actually lead to bone destruction. An interesting example is the identification of CTCs using Veridex as this is only tool clinically approved and is based on Epcam expression [30]. Lobular tumours of the breast that have a propensity for spreading to bone do not express Epcam and therefore might not be the most effective tool for guiding either research or patient care [31–33].

4. Future directions

Through local, national and international collaborations, we have developed a tissue acquisition program to ensure the ongoing evaluation of bone in cancer patients. We strive to ensure that

these valuable specimens are utilised to maximum return. Hopefully more centres will create similar programs to continue to evaluate the complex interplay of bone, the tumour micro-environment and metastatic tumour. Initial decisions about the most appropriate site and method of bone biopsy are crucial to enhance yield, and with improving imaging techniques, tumour directed biopsy is likely to provide optimal tissue for real time analysis and also tissue banking for future studies. Paired biopsies of bone metastatic disease, other metastatic sites and potentially circulating tumour cells may also provide further crucial information. Ultimately this collaborative work will improve the care of not only breast cancer patients but all bone metastatic cancer patients [11,34–36].

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