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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.

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 microenvironment, 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	• 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. • The yield of tumour cells is relatively low.
Studies exploring bone quality	• Jamshidi biopsy needle can be used for the assessment of bone quality, however larger studies are needed.
Studies evaluating repeat biopsies	• 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	• 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.