Original Study

Castration-Resistant Prostate Cancer Tissue Acquisition From Bone Metastases for Molecular Analyses

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Abstract

We analyzed 115 iliac crest bone marrow biopsy specimens from 101 patients with metastatic castration-resistant prostate cancer, divided into a test (n = 57) and a validation (n = 58) set. We developed a score based on computed tomography Hounsfield units and lactate dehydrogenase levels, which were associated with a positive biopsy result. The score can be used to select patients for whom a bone marrow biopsy will provide tissue for molecular characterization.

Background: The urgent need for castration-resistant prostate cancer molecular characterization to guide treatment has been constrained by the disease's predilection to metastasize primarily to bone. We hypothesized that the use of clinical and imaging criteria could maximize tissue acquisition from bone marrow biopsies (BMBs). We aimed to develop a score for the selection of patients undergoing BMB. **Materials and Methods:** A total of 115 BMBs were performed in 101 patients: 57 were included in a derivation set and 58 were used as the validation set. The clinical and laboratory data and prebiopsy computed tomography parameters (Hounsfield units [HUs]) were determined. A score for the prediction of biopsy positivity was developed from logistic regression analysis of the derivation set and tested in the validation set. **Results:** Of the 115 biopsy specimens, 75 (62.5%) were positive; 35 (61.4%) in the test set and 40 (69%) in the validation set. On univariable analysis, hemoglobin ($P = .019$), lactate dehydrogenase ($P = .003$), prostate-specific antigen ($P = .005$), and mean HUs ($P = .004$) were selected. A score based on the LDH level ($\geq$ 225 IU/L) and mean HUs ($\geq$ 125) was developed in multivariate analysis and was associated with BMB positivity in the validation set (odds ratio, 5.1; 95% confidence interval, 1.9%-13.4%; $P = .001$). The area under the curve of the score was 0.79 in the test set and 0.77 in the validation set. **Conclusion:** BMB of the iliac crest is a feasible technique for obtaining tumor tissue for genomic analysis in patients with castration-resistant prostate cancer metastatic to the bone. A signature based on the mean HUs and LDH level can predict a positive yield with acceptable internal validity. Prospective studies of independent cohorts are needed to establish the external validity of the score.

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Introduction

Prostate cancer is currently the second most common cancer in men, accounting for 15% of male cancer cases. Prostate cancer is the fifth leading cause of death in men worldwide (6.6% of total deaths) and is a major cause of morbidity.[1] Death from this disease follows the development of metastatic castration-resistant prostate cancer (mCRPC), for which no validated predictive molecular biomarkers to aid treatment selection are available to date. The low cost and high throughput evaluation of tumor genomes and transcriptomes is, nevertheless, rapidly enabling unprecedented opportunities to pursue the study of putative predictive tumor biomarkers. This is especially critical as the intra- and interpatient heterogeneity of the prostate cancer genome is described.[2,3]

We have previously described how the optimal evaluation of novel agents for the treatment of mCRPC requires the pursuit of a pharmacologic audit trail.[4-6] The pharmacologic audit trail involves the study of putative predictive biomarkers for patient selection, the evaluation of pre- and post-treatment normal tissue, and tumor biopsy evaluation of target modulation by medication, and reanalysis of the tumor at disease progression after a response to determine the mechanisms of resistance. Critical to this is access to tumor tissue, although it is hoped that the molecular characterization of circulating biomarkers such as messenger RNA,[7] circulating tumor DNA,[8-10] and/or circulating tumor cells[11-13] will also have clinical utility.

Up to 90% of patients with advanced prostate cancer will have disease metastatic to the bone, with most having disease involving the pelvis. Assessment of disease in the bone, which is commonly performed by bone scintigraphy, is, at best, suboptimal. Scintigraphy currently provides no qualitative information on the activity of the lesions, and progression is determined exclusively by the appearance of new tracer uptake. Technological advances in the processing of tissue from bone biopsies has enabled the performance as a valid approach for tissue acquisition from these patients.[14] Moreover, DNA and RNA sequencing from bone biopsy specimens is now technically feasible.[15] Such biopsies are being increasingly undertaken and even mandated in clinical trials. We hypothesized that the yield of CRPC tissue from bone biopsies could be increased by routine and inexpensive, nonsimultaneous imaging guidance using computed tomography (CT) and clinical parameters. A previous report on iliac crest CRPC bone biopsies yielded 25% positive samples without imaging guidance, with lower hemoglobin, greater alkaline phosphatase, and greater lactate dehydrogenase (LDH) levels associatinzg with increased yield.[16] A more recent report evaluating the effect of abiraterone acetate on androgen signaling in bone metastases had a positive yield in 47% of bone biopsies undertaken.[17] Studies evaluating bone biopsies performed under simultaneous CT guidance reported a positive yield of $\leq$ 67%.[15] Differences in bone density parameters on pelvic CT scans (Hounsfield units [HUs]), indicating sclerotic bone reaction associated with malignant infiltration, have also been reported.[15]

In the present study, we evaluated the association of clinical and radiologic factors with bone marrow biopsy (BMB) positivity. We propose a model that can predict the success rate and maximize tumor tissue acquisition for biomarker evaluation and molecular characterization in developmental therapeutic agents for CRPC.

Materials and Methods

Patient Population

Patients with mCRPC who undergone a BMB from October 2011 to November 2014 at the Royal Marsden National Health Services Foundation Trust (Sutton, UK) were retrospectively identified. The criteria for inclusion in the present study were CRPC, age $\geq$ 18 years, and evidence from imaging studies (CT, bone scan, or magnetic resonance imaging) of bone metastases from prostate cancer. Patients with a CT scan of the pelvis performed $>$ 6 weeks before the biopsy were excluded. The clinical and imaging parameters were retrospectively collected from the electronic patient records. All patients provided informed consent before undergoing biopsy. The method for image acquisition (CT scanner) remained consistent throughout the study.

Tissue Acquisition and Analysis

Tissue was collected using a bone trephine biopsy from the right or left posterior iliac crest. No image guidance was used for tissue acquisition. Biopsies were performed using 8-gauge (3.05-mm) needles. The biopsy specimens were sealed in a container with a 10% parafilm solution and fixed at room temperature for 24 to 30 hours with agitation. After fixing the samples, they were briefly rinsed in distilled water, placed in a container of ethylenediaminetetraacetic acid (EDTA) solution, sealed, and incubated for about 48 hours at 37°C. The EDTA solution was prepared by (1) dissolving 50 g of sodium hydroxide in 3500 mL of distilled water; (2) adding EDTA; and (3) stirring until the solution cleared. The pH of the solution was checked and adjusted to 7.0 each day the solution was used. Next, 2-μm-thick sections were stained with hematoxylin-eosin (Figure 1) and analyzed by 1 pathologist (D.N.R.), who was unaware of the clinical and imaging data. Cases were considered negative when no intact tumor cells could be identified. Positive cases, with intact tumor cells identified, were classified into those showing $<$ 50 cells and those showing $\geq$ 50 cells.

Figure 1 Computed Tomography Parameters in the Posterior Iliac Crest