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

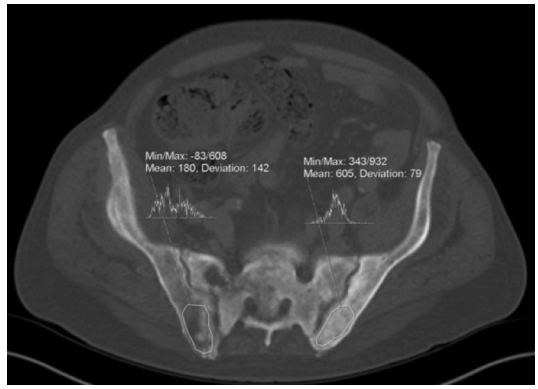
Patient Population

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



Imaging Studies

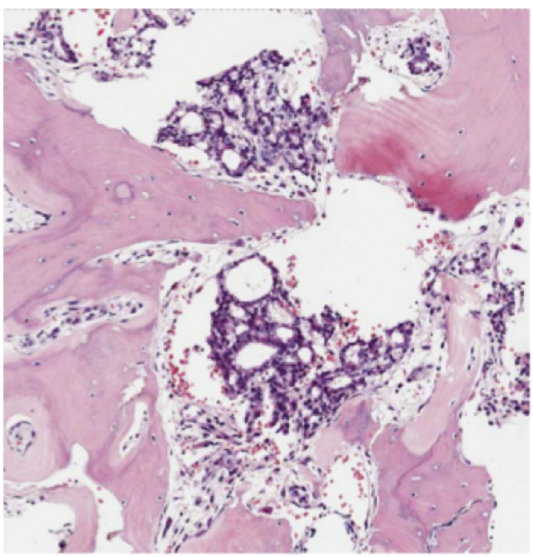
Patients with a CT scan of the pelvis performed > 6 weeks before the biopsy were excluded from the analyses. The images were analyzed by an experienced radiologist (N.T.) specializing in the field of prostate cancer. An area with a diameter of 0.8 to 1 cm (depending on the patient's anatomy) was drawn in the posterior aspect of the iliac crest in a region thought to be representative of the biopsied area; the location was equivalent for all patients. The mean HU of the biopsy site (left or right) was determined in 3 consecutive slices (5 mm thickness), and the average value was used in the analyses (Figure 2). The bone scans were reviewed for the presence of metastatic disease in the iliac crests and to estimate the bone tumor burden, classified as < 5 bony sites, 5 to 20 bone metastases, or > 20 metastases, indicating widespread disease.

Statistical Analysis

A descriptive analysis of the baseline laboratory and imaging features was performed, and the median and interquartile range (IQR) are reported. Random assignment algorithms were used to allocate biopsies to the test or the validation group. The test group was used to obtain a model for the prediction of positivity in BMBs. The dependent variable of the model (bone marrow positivity) was defined as the presence of tumor in the processed tissue. The cutoff values for dichotomous variables were established from the test set. Those that presented with greater receiver operating characteristic (ROC) area under the curve (AUC) values were selected for development of the predictive model, which was validated in the second, validation group. The mean values of the baseline parameters between the groups were compared using the Student *t* test.

Univariable analyses were performed using logistic regression models with only 1 covariate. Variables with a statistically significant association to the dependent variable ($P < .05$) were selected for inclusion in a multivariable logistic regression model, with bone marrow positivity as the dependent variable. Internal validity of the model was tested by establishing the ROC AUC in the test set (Figure 3). External validity was established by determining the ROC AUC in the validation set (Figure 3). Statistical significance was determined by testing the obtained AUCs against a null hypothesis of 0.5. The sensitivity, specificity, and positive and negative predictive values of the model were determined in the test and validation sets. The observed positivity rate of the biopsy specimens in the whole cohort was used as the prevalence value for the calculation of the predictive values. The score was then tested for its association with bone marrow positivity, defined as biopsy specimens yielding ≥ 50 tumor cells using logistic regression modeling. All statistical procedures were performed using SPSS Statistics, version 20 (IBM Corp., Armonk, NY).

Figure 2 Hematoxylin and Eosin Staining of a Positive Bone Marrow Biopsy



Results

Samples and Patient Characteristics

A total of 115 biopsies in 101 patients were performed from October 19, 2011 to November 11, 2014. Overall, 75 biopsies (65.2%) were positive. Of these, 20 biopsies (26.7%) yielded < 50 cells and 55 biopsies (73.3%) > 50 cells. The biopsy cores had a median length of 17 mm (IQR, 12-22 mm). Of the 115 biopsies, 67 (58.3%) were acquired from the right pelvis and 48 (41.7%) from the left pelvis. The median interval from the CT scan to the performance of the biopsy was 14 days (IQR, 4-28 days). Of the 101 patients, 83 (72.2%) had received previous docetaxel and 80 (69.6%) had received previous abiraterone. Details of the last treatment before the biopsy are summarized in Table 1. In 34 biopsies (29.6%), the patients had undergone previous radiotherapy to the pelvis, and in 33 biopsies (28.7%), the patients had received previous bone targeting agents (Table 1). In total, 27 patients (23.5%) were using opioids for the treatment of bone metastatic pain at biopsy and 70.3% of patients had been revealed to have > 20 bone metastases on the bone scan.

Of the 115 biopsy specimens, 57 were included in the test set and 58 were included in the validation set. The baseline laboratory and CT (mean HU) parameters in the test and validation sets are listed in Table 2. Of the 57 biopsy specimens in the test set and 58 in the validation set, 35 (61.4%) in the test set and 40 (69%) in the validation set were positive; with no significant differences between the 2 groups ($P = .395$). The test and validation cohorts had similar prognostic baseline laboratory and CT parameter distributions, with no statistically significant differences.

Uni- and Multivariable Analysis (Test Set)

Of the 57 biopsy specimens in the test set, 35 (61.4%) were classified as positive for tumor content. The variables were first tested as continuous variables (Table 3). Only the baseline LDH ($P = .006$) and baseline prostate-specific antigen ($P = .006$) levels were significantly associated with positive biopsy results. Continuous variables were dichotomized and tested in univariable logistic regression models (Table 4). The type of previous anticancer treatment ($P = .705$), use of previous pelvic radiotherapy ($P = .120$), and previous bisphosphonate use ($P = .975$) were not associated with biopsy positivity. Low hemoglobin levels (≥ 11.5 g/dL vs. < 11.5 g/dL; $P = .019$), high LDH levels (≥ 225 IU/L vs. < 225 IU/L;

Figure 3 Receiver Operating Characteristic Curve Analysis of the Test and Validation Sets

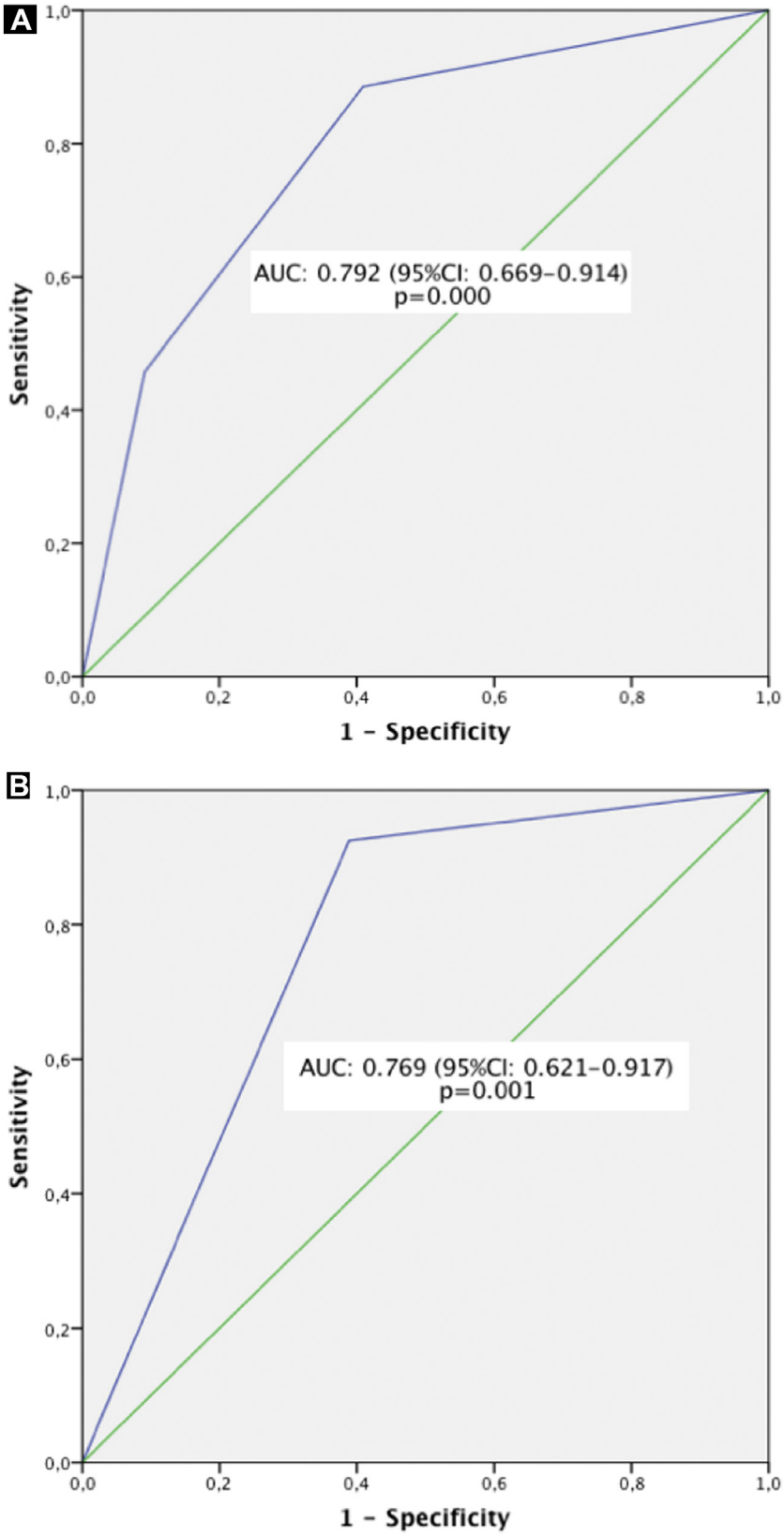


Table 1 Clinical Characteristics

Characteristic	n (%)
Total patients	115 (100)
Last treatment before BMB	
Hormonal agents[a]	70 (60.9)
Chemotherapy[b]	28 (24.3)
Other (investigational agents; phase I/II clinical trials)	17 (14.8)
Previous bone targeting agents	
None	82 (71.3)
Bisphosphonates	27 (23.5)
Radium-223	1 (0.9)
Strontium	3 (3.6)
Cabozantinib	1 (0.9)
Samarium	1 (0.9)
Previous RT to pelvis	
Yes	35 (30.4)
No	80 (69.6)
Pain requiring opioids	
Yes	27 (23.5)
No	88 (76.5)

Abbreviation: RT = radiotherapy.
[a]Abiraterone, enzalutamide, bicalutamide, goserelin, and dexamethasone.
[b]Docetaxel, cabazitaxel.

$P = .003$), PSA levels ($\geq$ 225 vs. $<$ 225 ng/mL; $P = .005$), high alkaline phosphate levels ($\geq$ 100 vs. $<$ 100 IU/L; $P = .025$), and high mean HUs on CT ($\geq$ 125 HU vs. $<$ 125 HU; $P = .004$) were significantly associated with a positive BMB and were selected for multivariable analysis. On multivariable analysis, only mean HUs $\geq$ 125 (odds ratio [OR], 3.85; 95% confidence interval [CI], 1.06-13.94; $P = .036$) and elevated LDH $\geq$ 225 IU/L (OR, 8.7; 95% CI, 1.68-45.11; $P = .003$) were significantly associated with BMB positivity (Table 5).

Predictive Score: Performance in Test and Validation Sets

From the results of the multivariable analysis in the test set, a score (BMB score) was developed by assigning 1 point to each of the parameters (0 points if neither the HUs were $\geq$ 125 nor the LDH was $\geq$ 200; 1 point if either the HU was $\geq$ 125 or LDH was $\geq$ 200; and 2 points if both the HUs were $\geq$ 125 and the LDH was $\geq$ 200). The score was significantly associated with bone marrow positivity in both the test (OR, 5.4; 95% CI, 2.1-13.7; $P < .001$) and validation (OR, 5.1; 95% CI, 1.9-13.4; $P = .001$) sets. In the validation set, the score was associated with a positive result, independent of other parameters (Tables 6 and 7). In the test set, only 23.5% of the biopsies with a score of 0 were positive compared with 77.5% of the biopsies with a score of 1 to 2 ($P < .001$). Similarly, in the validation set, only 21.4% of the biopsies with a score of 0 were positive for tumor content compared with 84.1% of biopsies with a score of 1 to 2 ($P < .001$). The AUC of the BMB score was 0.79 (95% CI, 0.67-0.91; $P < .001$) in the test and 0.77 (95% CI, 0.59-0.88; $P < .001$) in the validation set.

Table 3 Univariate Analysis (Test Set) Results: Continuous Variables

Variable	HR (95% CI)	*P* Value
Hemoglobin	0.53 (0.14-1.95)	.340
Platelets	0.75 (0.2-2.75)	.663
Neutrophils	2.44 (0.57-10.5)	.231
Lymphocytes	1.06 (0.36-3.12)	.922
NLR	1.3 (0.52-3.2)	.575
LDH	32.4 (2.69-391.6)	.006[a]
ALP	1.52 (0.77-3.02)	.231
Albumin	0.89 (0.76-1.03)	.113
PSA	1.92 (1.2-3.04)	.006[a]
Mean HU	1.01 (0.57-2.11)	.78

Hemoglobin, platelets, neutrophils, lymphocytes, NLR, LDH, ALP, PSA, and mean HUs were log-transformed.
Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; HR = hazard ratio; HUs = Hounsfield units; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; PSA = prostate-specific antigen.
[a]Statistically significant.

Sensitivity, Specificity, and Predictive Values

We established the sensitivity, specificity, and predictive values of each of the parameters in the model. The global positivity rate (65.2%) was used to calculate positive and negative

Table 2 Baseline Laboratory and Computed Tomography Parameters

Variable	All Biopsies (n = 115)	Test Set (n = 57)	Validation Set (n = 58)	*P* Value[a]
Hemoglobin (g/L)	11.3 (10.7-12.8)	11.6 (10.8-12.8)	11.3 (10.6-12.8)	.868
Platelets	220 (176-270)	220 (169-276)	220 (181-269)	.911
Neutrophils	3.8 (3-5.1)	3.8 (3-5.1)	3.8 (2.9-5.2)	.906
Lymphocytes	1.1 (0.8-1.4)	1.1 (0.8-1.5)	1.1 (0.8-1.4)	.817
NLR	3.6 (2.4-6.1)	3.6 (2.1-6.3)	3.2 (2.4-6.1)	.685
ALP (IU/L)	172 (96-423)	205 (95-345)	167 (105-450)	.546
Albumin (g/L)	36 (33-38)	36 (33-38)	36 (33-37)	.268
LDH (IU/L)	196 (166-255)	198 (165.5-265.5)	195.5 (168-252)	.310
PSA (ng/mL)	212 (94-500)	212 (96.5-609)	205 (85-455)	.215
Mean HU	136.5 (27.5-235.8)	144 (42-241)	114 (5-230.5)	.282

Data presented as mean (range).
Abbreviations: ALP = alkaline phosphatase; HUs = Hounsfield units; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; PSA = prostate-specific antigen.
[a]Student *t* test for equivalence of mean values.

Table 4 Univariate Analysis (Test Set) Results: Categorical Variables (Cutoff Values)

Variable	Positive (%)	OR (95% CI)	*P* Value
Hemoglobin		0.25 (0.08-0.8)	.019[a]
<11.5	77.8 (21/27)		
≥11.5	46.7 (14/30)		
Platelets		0.97 (0.32-2.93)	.953
<200	61.9 (13/21)		
≥200	61.1 (22/36)		
Neutrophils		2.03 (0.69-6)	.200
<3.5	52 (13/25)		
≥3.5	68.8 (22/32)		
Lymphocytes		1.41 (0.48-4.17)	.534
<1	56.5 (13/23)		
≥1	64.7 (22/34)		
NLR		2.08 (0.68-6.35)	.197
<3	50 (10/20)		
≥3	67.6 (25/37)		
LDH		11.3 (2.27-56)	.003[a]
<225	44.4 (16/36)		
≥225	90.5 (19/21)		
PSA		5.75 (1.72-19.3)	.005[a]
<225	43.3 (13/30)		
≥225	81.5 (22/27)		
ALP		4.03 (1.2-13.6)	.025[a]
<100	37.5 (6/16)		
≥100	70.7 (29/41)		
Albumin		0.44 (0.13-1.47)	.441
<34	73.7 (14/19)		
≥34	55.3 (21/38)		
Mean HU		5.78 (1.76-18.93)	.004[a]
<125	35 (7/20)		
≥125	75.7 (28/37)		
Treatment before biopsy		0.87 (0.42-1.81)	.705
Hormonal	62.5 (20/32)		
Chemotherapy	64.7 (11/17)		
Other	50 (4/8)		
Previous pelvic RT		0.4 (0.12-1.27)	.120
Yes	47.1 (8/17)		
No	67.5 (27/40)		
Bisphosphonates		0.98 (0.31-3.1)	.975
Yes	61.5 (24/39)		
No	61.1 (11/18)		
Strong opioids		1.29 (0.27-6.16)	.751
Yes	66.7 (7/12)		
No	57.8 (26/45)		

Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OR = odds ratio; PSA = prostate-specific antigen; RT = radiotherapy.
[a]Statistically significant.

Table 5 Multivariate Analysis[a] (Test Set) Results

Variable	OR (95% CI)	*P* Value[a]
Hemoglobin	0.68 (0.15-3.02)	.610
LDH	8.7 (1.68-45.11)	.003[b]
ALP	2.06 (0.47-9.03)	.336
PSA	2.79 (0.7-11.12)	.144
Mean HU	3.85 (1.06-13.94)	.036[b]

Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OR = odds ratio; PSA = prostate-specific antigen.
[a]Backward stepwise logistic regression, with *P* values calculated according to change in log-likelihood.
[b]Statistically significant.

predictive values. The mean HU number had greater sensitivity (0.80 in the test set; 0.88 in the validation set) and the LDH level had greater specificity (0.90 in the test and 0.78 in the validation set). The BMB score (0 vs. 1-2) showed a high sensitivity (0.89 in the test and 0.93 in the validation sets), with relatively low specificity (0.59 in the test set and 0.61 in the validation set; Table 8).

Ability of the BMB Score to Predict Biopsy Yield of ≥ 50 Cells

The biopsy specimens were further classified into those yielding ≥ 50 cells and < 50 cells, because of previous reports of

Table 6 Bone Marrow Biopsy Score: Uni- and Multivariable Analysis Results

Variable	OR (95% CI)	*P* Value
Univariate analysis (validation set)		
BMB score	5.07 (1.9-13.4)	.001[a]
Hemoglobin	0.34 (0.11-1.08)	.068
Platelets	0.93 (0.29-3)	.900
Neutrophils	1.20 (0.39-3.69)	.751
Lymphocytes	0.47 (0.14-1.57)	.470
NLR	1.53 (0.5-4.68)	.458
PSA	3.18 (0.95-10.6)	.060
ALP	1.54 (0.42-5.59)	.513
Albumin	0.42 (0.1-1.69)	.220
Previous pelvic RT	1.63 (0.44-5.98)	.465
Bisphosphonates	1.45 (0.34-6.16)	.613
Strong opioids	1.43 (0.38-5.48)	.598
Multivariate analysis (validation set)		
BMB score	4.18 (1.55-11.25)	.005
Hemoglobin	0.55 (0.14-2.06)	.372
ALP	1.17 (0.25-5.39)	.844
PSA	2.05 (0.53-7.99)	.300

Abbreviations: ALP = alkaline phosphatase; BMB = bone marrow biopsy; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase; NLR = neutrophil-to-lymphocyte ratio; OR = odds ratio; PSA = prostate-specific antigen; RT = radiotherapy.
[a]Statistically significant.

Table 7 BMB Score: Categorical Analysis Results for Test and Validation Sets

BMB Results	Test Set			Validation Set		
	Positive BM (%)	OR (95% CI)[a]	*P* Value	Positive BM (%)	OR (95% CI)[a]	*P* Value
Any positive cells						
0	4/17 (23.5)	—	—	3/14 (21.4)	—	—
1	15/22 (68.2)	7 (1.7-171.2)	.008	21/25 (84)	19.3 (3.6-101.7)	< .001
2	16/18 (88.9)	20 (4.1-165.1)	.001	16/19 (84.2)	19.6 (3.3-115.4)	.001
Total	35/57 (61.4)	—	—	40/58 (69)	—	—
≥50 Cells						
0	1/17 (5.9)	—	—	2/14 (14.3)	—	—
1	12/22 (54.5)	19.2 (2.15-171.5)	.008	16/25 (64)	10.7 (1.9-58.7)	.007
2	10/18 (55.6)	20 (2.16-184.9)	.008	14/19 (73.7)	16.8 (2.7-102.9)	.002
Total	23/57 (40.4)			26/58 (55.2)		

Abbreviations: BM = bone marrow; BMB = bone marrow biopsy; CI = confidence interval; OR = odds ratio.
[a]BMB score of 0 used as a reference for logistic regression analysis.

phosphatase and tensin homolog status and survival in CRPC BMB samples. In those studies, biomarker status had only been considered in those biopsy specimens containing ≥ 50 cells.[18] In our studies, 23 biopsy specimens (40.4%) in the test set and 32 (55.2%) in the validation set contained ≥ 50 cells. The BMB score was associated with positivity (≥ 50 cells) in both the test (OR, 3.1; 95% CI, 1.41-6.84; $P = .005$) and the validation (OR, 3.7; 95% CI, 1.6-8.4; $P = .002$) sets. The AUC of the BMP score was 0.72 (95% CI, 0.58-0.85) in the test set and 0.73 (95% CI, 0.59-0.86) in the validation set. In the validation set, only 2 biopsy specimens (14.3%) with a score of 0 had ≥ 50 cells but 30 (68.2%) of those with a score of 1 to 2 were positive.

Table 8 Sensitivity, Specificity, and Predictive Values

Variable	Estimate (95% CI)	
	Test Set	Validation Set
BMB score (0 vs. 1-2)		
Sensitivity (%)	88.6 (74-95.5)	92.5 (80.1-97.4)
Specificity (%)	59.1 (38.7-76.7)	61.1 (38.6-79.7)
Positive predictive value (%)	78.3 (68.3-85.8)	79.9 (68.8-82.8)
Negative predictive value (%)	75.6 (53.7-89.3)	83 (60.8-93.9)
Mean HU ≥ 125		
Sensitivity (%)	80 (64.1-90)	87.5 (73.9-94.5)
Specificity (%)	59.1 (38.7-76.7)	66.7 (43.7-83.7)
Positive predictive value (%)	75.7 (59.8-86.6)	85.4 (71.6-93.2)
Negative predictive value (%)	65 (43.3-81.8)	70.6 (46.9-86.7)
LDH ≥ 225 IU/L		
Sensitivity (%)	54.3 (38.2-69.5)	45 (30.7-60.2)
Specificity (%)	90.1 (72.2-97.5)	77.8 (54.8-91)
Positive predictive value (%)	90.5 (71.1-97.4)	81.8 (61.5-92.7)
Negative predictive value (%)	55.6 (39.6-70.5)	38.9 (24.8-55.1)

Abbreviations: BMB = bone marrow biopsy; CI = confidence interval; HU = Hounsfield unit; LDH = lactate dehydrogenase.

Discussion

With the advent of novel agents for the treatment of CRPC and the improved understanding of the molecular biology mechanisms driving disease progression beyond castration, the improvement of mechanisms for tissue acquisition and molecular analysis has become of paramount importance. Up to 89% of patients with mCRPC might harbor clinically actionable genomic aberrations.[19] Furthermore, despite significant interpatient heterogeneity, the alterations in known oncogenic drivers have been highly concordant within the individual's metastatic sites. Assessing single metastasis through soft tissue biopsies or BMBs could therefore provide a reasonable assessment of the oncogenic landscape and prove informative for treatment selection.[3]

The propensity to spread to the bones (in many cases, the only metastatic site) is a distinct characteristic of prostate cancer. Thus, a large proportion of patients do not have soft tissue metastases amenable for biopsy. A number of studies published in the past decade have reported variable rates of positive BMBs ranging from 25% to 50% for nonimaging-guided biopsies[16,17,20] and increasing to 67% to 77% when performed under direct CT guidance.[15,21] Our cohort, with biopsies performed without direct CT guidance, had a bone biopsy positivity rate of 62.5%, consistent with the findings from previous reports.

Previous studies have established associations among the clinical, analytical, and CT parameters with BMB positivity.[16,21] The present study, however, is the first study to establish the value of the widely used CT and analytical parameters and develop a score with direct applicability in the clinical setting, with validation of these results in a separate control group. We have proved the predictive potential of a simple score that can help select patients likely to provide enough tissue for molecular analyses such as exome and transcriptome next-generation sequencing, which is now becoming embedded in many of our therapeutic trials in CRPC. In a recently published multi-institutional CRPC genomic sequencing project,[19]

29% of all sequenced tissue was from bone metastases, highlighting the importance of adequate patient selection for the performance of BMBs.

The high sensitivity of the BMB score supports its use for the identification of patients with a low likelihood of a positive result. We would therefore recommend not performing the procedure in patients with a score of 0 (ie, if the bone density of the iliac crest does not exceed a HU of 125 and the LDH levels are < 225 IU/L). In such cases, the probability of achieving a negative result (negative predictive value) is about 76% to 83% compared with a 78% to 79% chance of a positive result (positive predictive value) if the score is > 0. Extrapolating our findings to the validation set, excluding patients with a score of 0 would have "saved" 11 patients (18.9%) from undergoing biopsy with negative results and would have only "missed" 3 (5.2%) biopsies with positive results, increasing the positive yield from 69% to 84.1%. The model presents high internal validity, as determined by the AUC model obtained when testing the ROC AUC in the test and validation sets, which had very similar AUC values.

Our study had a number of limitations. The variety of treatments received by the patients could have made our data set less homogeneous than that of other cohorts of biopsies performed in the setting of clinical trials.[16] Furthermore, our patient population represented patients with advanced, CRPC and a high burden of bone metastases. It remains unclear whether our BMB score would be valid for patients with earlier disease stages. Finally, because all biopsies were performed in a single center, validation of the score is needed in independent centers for external validity of the score to be established. The high consistency of the results between the test and validation sets does, nevertheless, suggest the potential applicability in other centers that regularly perform BMBs.

Our BMB score was developed by defining positive BMBs as those with any evidence of tumor cells found after hematoxylin-eosin staining. The heterogeneity of the data set, which included patients participating in different studies over several years, precluded the association of the score with the successful determination of specific molecular biomarkers. However, previous studies reporting an association of phosphatase and tensin homolog status (determined in soft tissue biopsies and BMBs) and survival had restricted evaluable samples to those with ≥ 50 tumor cells.[18] We have shown that our score is capable of discriminating those patients likely to yield > 50 cells. In the validation set, the exclusion of patients with a score of 0 would have increased the positivity yield from 55.2% to 68.2%.

Conclusion

Performing serial BMBs in patients with mCRPC is a feasible and valid approach for the acquisition of cancer tissue for molecular analysis. We have presented a BMB score that demonstrates how the use of imaging and laboratory parameters can help select patients and increase the rate of positive biopsy specimens.

Clinical Practice Points

- Up to 90% of patients with advanced prostate cancer have disease metastatic to the bone, which is, in many cases, the only site of metastatic disease.
- The development of circulating and tissue-based predictive biomarkers such as AR-V7 splice variants or genomic aberrations of DNA repair genes has been proposed for treatment selection in advanced prostate cancer.
- Previous reports have established the yield of non–image-guided positive BMB specimens in 25% to 47% of cases.
- Using a score based on the CT HUs (mean HU > 125) and LDH level (> 225 IU/L) can help select patients with an increased likelihood of having a positive BMB specimen from the iliac crest.
- Patients with a score of 0 (mean HUs < 125 and LDH < 225 IU/L) will have a very low BMB yield and should not be selected for the procedure.
- Optimization of the methods for patient selection for a fresh biopsy procedure could help in molecular stratification and adequate treatment selection for patients with mCRPC.

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Disclosure

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