

¹⁸F-FDG PET/CT Findings and Circulating Tumor Cell Counts in the Monitoring of Systemic Therapies for Bone Metastases from Breast Cancer

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Our objective was to compare the predictive significance of ¹⁸F-FDG PET/CT findings and circulating tumor cell (CTC) count in patients with bone metastases from breast cancer treated with standard systemic therapy. **Methods:** Breast cancer patients with progressive bone-only metastatic disease without visceral metastases starting a new line of systemic therapy underwent ¹⁸F-FDG PET/CT and had CTC counts determined before and during treatment. Disease status was reassessed by CTC count (≥ 5 vs. < 5 CTC/7.5 mL of blood) and ¹⁸F-FDG PET/CT approximately 2–4 mo after initiation of the new systemic therapy. **Results:** CTC counts at follow-up agreed with the ¹⁸F-FDG PET/CT assessment in 43 (78%) of the 55 evaluable patients. Of the 12 patients with discordant CTC and ¹⁸F-FDG PET/CT results, 8 (66%) had ≥ 5 CTCs, with no evidence of progressive disease at the time of the ¹⁸F-FDG PET/CT study, whereas 4 (33%) had < 5 CTCs, with evidence of progressive disease by ¹⁸F-FDG PET/CT. ¹⁸F-FDG PET/CT findings and follow-up CTC counts were found to be significantly associated with both progression-free survival ($P = 0.02$ and $P < 0.0001$, respectively) and overall survival ($P = 0.02$ and $P = 0.01$, respectively). In multivariate analysis, the ¹⁸F-FDG PET/CT assessment remained as the only predictive factor for progression-free survival ($P < 0.0001$), whereas estrogen receptor status was the only predictive factor for overall survival ($P = 0.01$). **Conclusion:** ¹⁸F-FDG PET/CT is a useful tool for therapeutic monitoring in patients with bone metastases from breast cancer. Prospective studies are needed to define the role of ¹⁸F-FDG PET/CT and CTC in the setting of response discordance to establish bone-dominant disease as a tumor-response measurable disease.

Key Words: PET/CT; circulating tumor cells; therapeutic monitoring; bone metastases; breast cancer

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Bone metastases occur during the course of metastatic breast cancer (MBC) in nearly two thirds of patients and represent the first site of metastasis in about one third of patients (1–4). Patients with bone metastases from breast cancer have a life expectancy of several years, and both local (radiotherapy and surgery) and systemic (hormone therapy, chemotherapy, trastuzumab, and bisphosphonates) modalities are available to treat bone metastases (5). How best to monitor the response of bone metastases from breast cancer to systemic therapy represents a critical issue in clinical practice and in phase II–III trials.

Both ¹⁸F-FDG PET/CT and circulating tumor cell (CTC) counts have been studied in patients with bone metastases from breast cancer. ¹⁸F-FDG PET/CT is more sensitive than conventional imaging for detecting MBC (6) and has improved anatomic correlations, resulting in more accurate measurement of bone metastases in MBC (7,8). The presence of CTC before treatment is an independent predictor of progression-free survival (PFS) and overall survival (OS) in patients with MBC (9). ¹⁸F-FDG PET/CT and CTC measurement showed a high sensitivity in the detection of bone relapse or progression of breast cancer (6,10), and we previously found that the presence of extensive bone metastases as detected by ¹⁸F-FDG PET/CT was associated with increased CTC numbers in MBC (10). Specifically, we showed that CTC numbers were higher in patients with bone metastases than in those with no bone lesions (mean,

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65.7 vs. 3.3, $P = 0.0122$) and higher in patients with 3 or more bone metastases than in those with fewer bone lesions (mean, 77.7 vs. 2.6, $P < 0.001$) (10). Furthermore, we found that CTC counts were lower in patients with lymph node or chest wall metastases without bone lesions (mean, 1.4 ± 2.0 CTCs) (10).

In this study, we sought to determine the predictive significance of CTC counts and ^{18}F -FDG PET/CT findings in patients with bone metastases from breast cancer treated with standard systemic therapies. We also sought to determine the correlation between CTC counts and ^{18}F -FDG PET/CT findings in such patients.

MATERIALS AND METHODS

Patients

This study was conducted using the MD Anderson Cancer Center Breast Medical Oncology database. We identified all patients who had received systemic treatment for bone metastases from breast cancer—including patients with intrathoracic lymph node or chest wall metastases in addition to bone metastases—from December 2004 to May 2008. Patients with visceral metastases were excluded. All patients underwent ^{18}F -FDG PET/CT scans and CTC enumeration within 3 wk before starting a new treatment as the standard of care. Metabolically active disease by ^{18}F -FDG PET/CT was identified in all patients. Disease status was reassessed by ^{18}F -FDG PET/CT and CTC count at approximately 2–4 mo after the initiation of the new systemic treatment, depending on treatment type and schedule. Fifty-five patients met these criteria and were included in the study. The retrospective protocol was approved by the Institutional Review Board of the MD Anderson Cancer Center, with a waiver of the requirement for informed consent.

Response Evaluation by ^{18}F -FDG PET/CT

^{18}F -FDG PET/CT scans were obtained using a multislice PET/CT camera (Discovery ST, STE, or RX, with 8-, 16-, or 64-slice CT; GE Healthcare) using a standard clinical protocol (11). PET and CT images were reconstructed and reviewed on an Advantage workstation (GE Healthcare). In a region of interest, only the lesions that exhibited the most substantial ^{18}F -FDG uptake were selected as the target lesions for evaluating response to therapy.

There is no accepted standard for ^{18}F -FDG PET/CT response for bone metastases from breast cancer; however, progressive disease (PD) versus nonprogressive disease (non-PD) has been validated as a predictor of survival in patients with MBC, including patients with bone metastases, and showed a 75% overall agreement with CTC counts in a previous study (12). Thus, in the current study, increased ^{18}F -FDG uptake in a target lesion that was also enlarged on CT (i.e., $\geq 20\%$ increase in the longest diameter) or a new metabolically active lesion was considered indicative of PD, whereas substantially unchanged or decreased ^{18}F -FDG uptake without substantial increase in lesion size was considered indicative of non-PD. Because progressive sclerosis on CT is also a marker of response in bone metastases, the increase of bone lesions without a clear evidence of ^{18}F -FDG uptake has not been considered indicative of progression.

Detection and Enumeration of CTC

The CellSearch System (Veridex LLC) was used for the enumeration of CTCs (13). Blood samples were collected and

CTCs enumerated as previously described (9). Patients were categorized according to CTC counts as having a favorable (< 5 CTC/7.5 mL of blood) or unfavorable (≥ 5 CTC/7.5 mL of blood) outcome of systemic therapy (9).

Statistical Analysis

PFS was defined as the time elapsed between the date of baseline CTC blood sampling and the date of clinical disease progression or death or, if neither progression nor death occurred during follow-up, the date of the last follow-up visit. OS was defined as the time elapsed between the date of baseline CTC blood sampling and the date of either death or, if death did not occur during the follow-up period, the last follow-up visit. Student t and Fisher exact tests were used to test for statistically significant differences between the patients with < 5 CTCs/7.5 mL of blood and those with ≥ 5 CTC/7.5 mL of blood. Kaplan–Meier survival plots were generated by CTC count and ^{18}F -FDG PET/CT assessment (non-PD or PD), and the curves were compared using log-rank testing. Cox proportional hazards regression was used to test the statistical significance of selected potential prognostic factors for PFS and OS. A P value of less than 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Between December 30, 2004, and May 31, 2008, 55 patients received systemic therapies for either bone metastases only ($n = 26$) or bone metastases and intrathoracic lymph node or chest wall metastases ($n = 29$) and underwent both CTC and PET/CT evaluation. Thirty-six patients (65%) had received prior treatment for MBC with hormone therapy (23 cases), chemotherapy with or without hormone therapy (9 cases), or *HER2*-targeted therapies combined with chemotherapy or hormone therapy (4 cases). Nineteen patients (35%) had newly diagnosed MBC. The clinical

TABLE 1. Clinical Characteristics of Study Population ($n = 55$)

Variable	Sample size (n)	% Patients
ER	55	
Negative	12	21.8
Positive	43	78.2
<i>HER2</i>	55	
Nonamplified	46	83.6
Amplified	9	16.4
Line of therapy	55	
First line	19	34.5
Later	36	65.5
Metastatic site	55	
Bone only	26	47.3
Bone plus soft tissue	29	52.7
Baseline CTC count	55	
< 5	19	34.5
≥ 5	36	65.5
Follow-up CTC count	55	
< 5	33	60
≥ 5	22	40
PET/CT follow-up assessment	55	
Nonprogression	38	69.1
Progression	17	30.9

characteristics of the 55 patients evaluated are summarized in Table 1. In this cohort, 36 patients (65%) at baseline and 22 patients (40%) at follow-up had ≥ 5 CTC/7.5 mL of blood. The median CTC count per 7.5 mL of blood was 13 (range, 0–694) at baseline and 3 (range, 0–760) at follow-up. The average time \pm SD between baseline and follow-up blood sampling was 16.9 ± 9.1 wk (range, 4.3–38.7 wk) and between baseline and follow-up ^{18}F -FDG PET/CT was 16.9 ± 8.3 wk (range, 8.1–35.7 wk).

Relationship Between CTC Count and Disease Progression as Determined by ^{18}F -FDG PET/CT

CTC count at follow-up (< 5 CTC/7.5 mL of blood or ≥ 5 CTC/7.5 mL of blood) agreed with ^{18}F -FDG PET/CT assessment (non-PD or PD) in 43 patients (78%). Among the remaining 12 patients, 4 (33%) with fewer than 5 CTCs at follow-up were found to have evidence of PD by ^{18}F -FDG PET/CT, whereas 8 (67%) with persistent CTCs (≥ 5) at follow-up did not.

Among the 4 patients with < 5 CTCs at follow-up and PD by ^{18}F -FDG PET/CT, 3 had < 5 CTCs at baseline and received chemotherapy only (2 cases) or chemotherapy plus trastuzumab (1 case); 1 patient had a baseline CTC count of 262 CTC/7.5 mL of blood that was undetectable (CTC = 0), despite PD with liver metastases after 5 mo of hormone therapy.

Among the 8 patients with a persistence of ≥ 5 CTCs at follow-up without evidence of PD by ^{18}F -FDG PET/CT, 5

patients had impending tumor progression at a median follow-up of 56 d (range, 24–92 d), 1 experienced PD after 7 mo, and 2 remained progression-free at 24 and 36 mo. The latter 2 patients received chemotherapy in addition to hormone therapy. Among the 6 patients with evidence of PD by ^{18}F -FDG PET/CT, 5 patients—including 4 with estrogen receptor (ER)-positive tumors—were treated with chemotherapy only and the sixth was treated with chemotherapy in addition to hormone therapy.

CTC and ^{18}F -FDG PET/CT Ability to Predict Survival

In all 55 patients, the mean PFS was 10.5 ± 7.2 mo (range, 1.9–33.8 mo), and the mean OS was 18.1 ± 6.7 mo (range, 3.1–36.8 mo). At the time of analysis, 7 patients (13%) were considered progression-free—with an average follow-up time of 18.0 ± 8.3 mo (range, 10.0–33.8 mo)—and 18 patients (33%) had died.

Follow-up CTC count (< 5 CTC/7.5 mL of blood or ≥ 5 CTC/7.5 mL of blood) and ^{18}F -FDG PET/CT assessment (non-PD or PD) were found to be significantly associated with both PFS ($P = 0.02$ and $P < 0.0001$, respectively) and OS ($P = 0.02$ and $P = 0.01$, respectively). The baseline CTC count was not a significant predictor for either PFS ($P = 0.78$) or OS ($P = 0.74$).

Median PFS was 13 mo in patients with both < 5 CTCs and ^{18}F -FDG PET/CT nonprogression, 6 mo in patients with < 5 CTCs or ^{18}F -FDG PET/CT nonprogression but not both, and 5 mo in patients with neither < 5 CTCs nor

TABLE 2. Factors Associated with PFS

Variable	Total no. of . . .			Univariate analysis			Multivariate analysis		
	Patients	Patients with progressive disease	Median survival (mos)	<i>P</i>	Hazard ratio	95% confidence interval for hazard ratio	<i>P</i>	Hazard ratio	95% confidence interval for hazard ratio
ER*									
Negative	12	12	6.78	—	1.00	—			
Positive	43	36	8.54	0.06	0.52	0.26–1.02			
HER2									
Nonamplified	46	39	8.35	—	1.00	—			
Amplified	9	9	8.08	0.38	1.40	0.67–2.91			
Line of therapy									
First line	19	17	15.21	—	1.00	—			
Later	36	31	7.11	0.09	1.67	0.92–3.06			
Metastatic site									
Bone only	26	22	8.35	—	1.00	—			
Bone plus soft tissue	29	26	8.08	0.92	0.97	0.55–1.72			
Baseline CTC count									
< 5	19	15	8.08	—	1.00	—			
≥ 5	36	33	8.35	0.78	1.09	0.59–2.02			
Follow-up CTC count*									
< 5	33	28	14.42	—	1.00	—			
≥ 5	22	20	5.32	0.02	2.02	1.11–3.65			
PET/CT follow-up assessment*									
Nonprogression	38	31	14.42	—	1.00	—	—	1.00	—
Progression	17	17	4.53	< 0.0001	7.14	3.25–15.70	< 0.0001	7.14	3.25–15.70

*Factors with *P* value < 0.10 from univariate analysis are included in multivariate analysis.

¹⁸F-FDG PET/CT nonprogression ($P < 0.00002$). Median OS was not reached (>31 mo) in patients with both <5 CTCs and ¹⁸F-FDG PET/CT nonprogression, was 24 mo in patients with <5 CTCs or ¹⁸F-FDG PET/CT nonprogression but not both, and was 18 mo in patients with neither <5 CTC nor ¹⁸F-FDG PET/CT nonprogression ($P = 0.02$).

Tables 2 and 3 summarize factors associated with PFS and OS estimates, respectively. Follow-up CTC counts and ¹⁸F-FDG PET/CT assessment (PD vs. non-PD) were significantly associated with PFS (Fig. 1), but ¹⁸F-FDG PET/CT assessment remained significant only in a multivariate analysis (Table 2). ER status was significantly associated with OS (Fig. 2) and was the only variable that remained significant in a multivariate analysis (Table 3).

DISCUSSION

The therapeutic monitoring of bone metastases from breast cancer represents a critical issue in clinical practice and experimental trials. Results of the present study showed that follow-up CTC levels and ¹⁸F-FDG PET/CT assessment (PD vs. non-PD) are significantly associated with both PFS and OS. However, in multivariate analysis, ¹⁸F-FDG PET/CT assessment (PD vs. non-PD) remained the only predictive factor for PFS, whereas ER status remained the only predictive factor for OS. In addition, CTC counts at follow-up agreed with the ¹⁸F-FDG PET/CT assessment in 43 (78%) of the 55 patients. Of the 12 patients with discordant CTC and ¹⁸F-FDG PET/CT results, 8 patients

(66%) had a persistence of ≥ 5 CTCs. Interestingly, among these 8 patients, 5 hormone receptor–positive patients treated with chemotherapy only ($n = 4$) or chemotherapy and hormone therapy ($n = 1$) experienced impending tumor progression, with a median follow-up of 56 d (range, 24–92 d). Two hormone receptor–positive patients treated with chemotherapy and hormone therapy were progression-free at 24 and 36 mo. Finally, follow-up CTC count was significantly associated with PFS (Fig. 1), whereas ER status was significantly associated with OS (Fig. 2).

Our study had some limitations. It was retrospective, with patients under different systemic therapies. However, these data indicate that ¹⁸F-FDG PET/CT could be useful for monitoring response to systemic therapies in patients with bone metastases, even if a role for CTC cannot be excluded in selected cases. Moreover, several authors have previously reported ER expression, *HER2* gene amplification, and other markers by CTC, despite the lack of expression or gene amplification in the primary tumor (14–16). Further molecular characterization of CTC might help to select effective targeted therapies, monitor their efficacy, and improve knowledge about the biology of bone metastases from breast cancer.

In addition, our results confirm a possible relationship between high levels of CTC and extensive bone involvement as detected by ¹⁸F-FDG PET/CT in patients with relapsed or progressive MBC. In our series, which included patients with MBC with bone metastases but not visceral

TABLE 3. Factors Associated with OS

Variable	Total no. of . . .		Median survival (mos)	Univariate analysis			Multivariate analysis		
	Patients	Patient deaths		P	Hazard ratio	95% confidence interval for hazard ratio	P	Hazard ratio	95% confidence interval for hazard ratio
ER*									
Negative	12	7	13.08	—	1.00	—	—	1.00	—
Positive	43	11	—	0.01	0.30	0.12–0.79	0.01	0.30	0.12–0.79
HER2									
Nonamplified	46	18	24.54	—	1.00	—	—	—	—
Amplified	9	0	—	0.99	—	—	—	—	—
Line of therapy									
First line	19	6	—	—	1.00	—	—	—	—
Later	36	12	—	0.93	1.11	0.42–2.97	—	—	—
Metastatic site									
Bone only	26	7	24.74	—	1.00	—	—	—	—
Bone plus soft tissue	29	11	—	0.49	1.40	0.54–3.63	—	—	—
Baseline CTC count									
<5	19	5	—	—	1.00	—	—	—	—
≥ 5	36	13	24.74	0.74	1.19	0.42–3.35	—	—	—
Follow-up CTC count*									
<5	33	7	—	—	1.00	—	—	—	—
≥ 5	22	11	23.49	0.02	2.99	1.16–7.75	—	—	—
PET/CT follow-up assessment									
Nonprogression	38	9	—	—	1.00	—	—	—	—
Progression	17	9	18.96	0.01	3.29	1.28–8.48	—	—	—

*Factors with P value < 0.10 from univariate analysis are included in multivariate analysis.

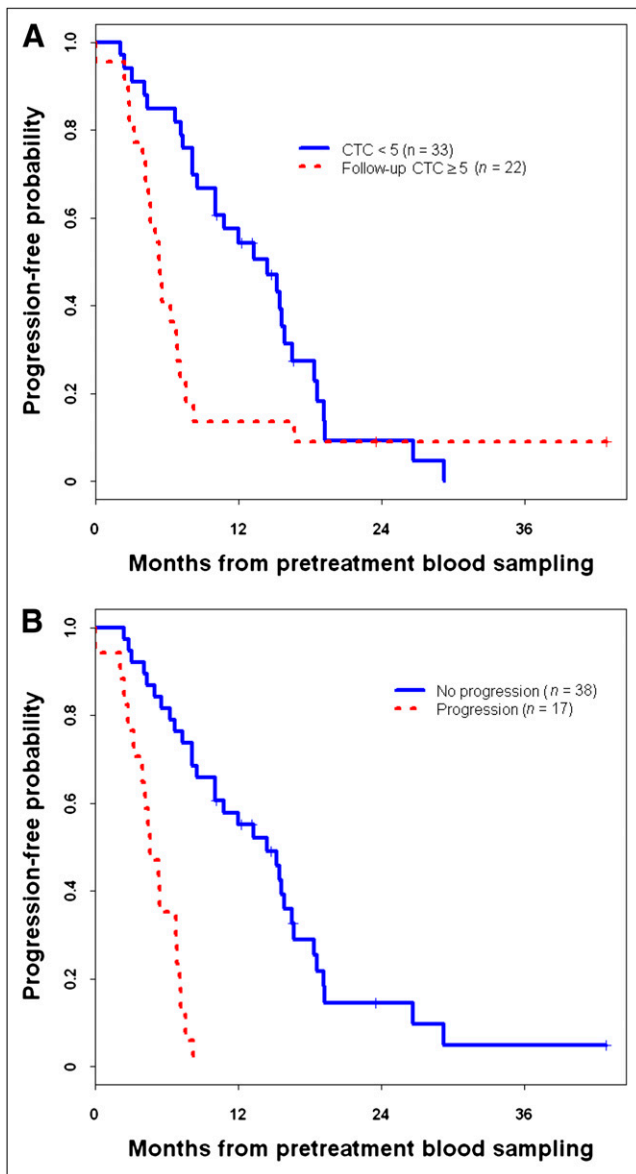


FIGURE 1. Kaplan–Meier estimates of PFS by follow-up CTC count (A) and ¹⁸F-FDG PET/CT response (B). (A) For patients with follow-up CTC count < 5: patients at risk = 33 at 0 mo, 17 at 12 mo, and 2 at 24 mo and survival rate = 100% at 0 mo, 54% at 12 mo, and 14% at 24 mo. For patients with follow-up CTC count > 5: patients at risk = 22 at 0 mo, 3 at 12 mo, 1 at 24 mo, and 1 at 36 mo and survival rate = 100% at 0 mo, 14% at 12 mo, 8% at 24 mo, and 8% at 36 mo. (B) For ¹⁸F-FDG PET/CT nonprogression patients: patients at risk = 38 at 0 mo, 20 at 12 mo, 3 at 24 mo, and 1 at 36 mo and survival rate = 100% at 0 mo, 55% at 12 mo, 17% at 24 mo, and 6% at 36 mo. For ¹⁸F-FDG PET/CT progression patients: patients at risk = 17 at 0 mo and 6 at 6 mo and survival rate = 100% at 0 mo and 35% at 6 mo.

metastases, 5 or more CTCs were evident in 65% of patients at baseline and 40% at follow-up. In contrast, in other series with unselected patients with MBC, 5 or more CTCs were apparent in 45%–50% of patients at baseline and 25%–30% at follow-up (9,16). Other reports also

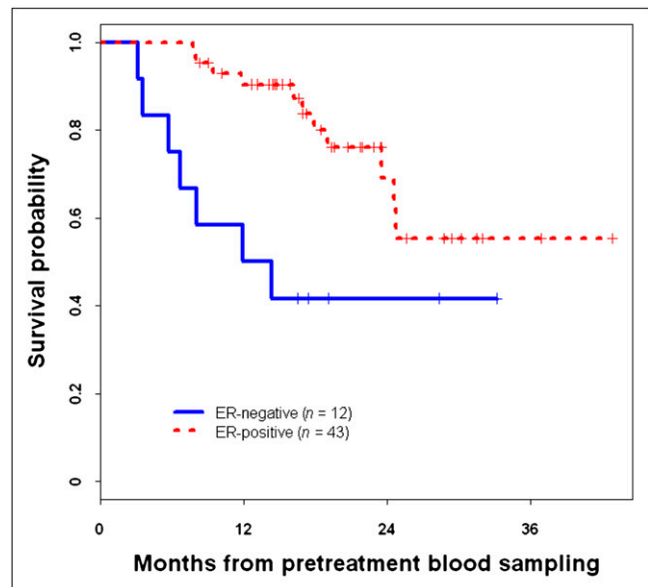


FIGURE 2. Kaplan–Meier estimates of OS by ER status. For ER-negative patients: patients at risk = 12 at 0 mo, 6 at 12 mo, and 2 at 24 mo and survival rate = 100% at 0 mo, 50% at 12 mo, and 39% at 24 mo. For ER-positive patients: patients at risk = 43 at 0 mo, 36 at 12 mo, 10 at 24 mo, and 2 at 36 mo and survival rate = 100% at 0 mo, 90% at 12 mo, 73% at 24 mo, and 52% at 36 mo.

showed that CTC counts both at baseline and at follow-up were higher in patients with MBC with bone metastases than in patients with MBC in general, using either bone scanning or ¹⁸F-FDG PET/CT (10,17). In contrast, in all early and 1 recent prospective study on CTC in MBC, bone metastases were studied with standard imaging techniques. Because patients with bone metastases were included in a generic no-visceral-metastases group, the effect of bone metastases on CTC levels could have been underestimated; the inclusion of patients with bone metastases could help explain the poor survival rates in these early trials for patients with nonvisceral disease (9,18–20). A major limitation of the use of CTC counts for therapeutic monitoring in patients with MBC is that nearly 50% of such patients do not have increased levels of CTC at progression (9); however, CTC counts seemed higher in MBC cases with bone metastases. Moreover, the PET classification of response that we used (non-PD vs. PD) might be optimized, and such optimization might lead to a better predictive value of ¹⁸F-FDG PET/CT with respect to PFS and OS (7,8,12). The integrated use of ¹⁸F-FDG PET/CT and CTC might lead to a significant improvement in monitoring the response of bone metastases from breast cancer to systemic therapy, and this should be prospectively investigated.

CONCLUSION

To our knowledge, this is the first study to assess the role of ¹⁸F-FDG PET/CT and CTC counts in the therapeutic

monitoring of bone metastases in patients with breast cancer. The multivariate analysis indicated that ^{18}F -FDG PET/CT was the only predictive sign; however, the combination of FDG PET/CT and CTC might be a useful tool to monitor response to therapy in patients without measurable extraosseous disease, especially in patients with elevated CTC at baseline. The discordance of ^{18}F -FDG PET/CT assessment and CTC count needs to be evaluated in a prospective study to determine the value of ^{18}F -FDG PET/CT and CTC individually and in combination. A prospective study could validate the benefit of these 2 approaches used separately and in combination in determining prognosis, monitoring response, and establishing bone-dominant disease as a tumor response-measurable disease.

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