original article

Annals of Oncology 21: 33–39, 2010
doi:10.1093/annonc/mdp262
Published online 14 July 2009

Circulating tumor cells and bone metastases as detected by FDG–PET/CT in patients with metastatic breast cancer

U. De Giorgi[1,2,3], V. Valero[1], E. Rohren[4], M. Mego[1,2], G. V. Doyle[5], M. C. Miller[5], N. T. Ueno[1,6], B. C. Handy[7], J. M. Reuben[2], H. A. Macapinlac[4], G. N. Hortobagyi[1] & M. Cristofanilli[1]*

Departments of; [1]Breast Medical Oncology; [2]Hematopathology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; [3]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola, Italy; [4]Department of Nuclear Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; [5]Immunicon Corporation, Huntingdon Valley, PA, USA; Departments of; [6]Stem Cell Transplantation and Cellular Therapy and [7]Laboratory Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA

Received 26 March 2009; accepted 31 March 2009

Background: We evaluated the relationship between the detection and prognostic significance of circulating tumor cells (CTCs) and sites of metastases detected by 2-[fluorine-18]fluoro-2-deoxy-D-glucose–positron emission tomography/computed tomography (FDG–PET/CT) in patients with metastatic breast cancer (MBC).

Patients and methods: From May 2004 to January 2008, 195 patients with relapsed/progressive MBC underwent whole-body FDG–PET/CT and provided blood samples for assessment of CTC count.

Results: Higher CTC numbers were detected in patients with bone metastases relative to those with no bone lesions (mean 65.7 versus 3.3, $P = 0.0122$) and in patients with multiple bone metastases relative to those with one or two bone lesions (mean 77.7 versus 2.6, $P < 0.001$). CTCs predicted overall survival (OS) in 108 patients with multiple sites of metastases including bone ($P = 0.0008$) but not in 58 without bone metastases ($P = 0.4111$) and in 29 with bone involvement only ($P = 0.3552$). All 15 patients but one with human epidermal growth factor receptor 2 (HER-2) positive tumors who were treated with trastuzumab-based regimens had <5 CTCs at progression. In multivariate analysis, CTCs, but not bone metastases, remained a significant predictor of OS.

Conclusion: Presence of extensive bone metastases as detected by FDG–PET/CT is associated with increased CTC numbers in MBC.

Key words: bone metastases, breast cancer, circulating tumor cells, FDG–PET/CT, prognosis

introduction

The presence of circulating tumor cells (CTCs) in patients with metastatic breast cancer (MBC) has been associated with shorter survival time than when CTCs are absent [1]. Unlike soluble circulating tumor markers, such as CA15-3 and CA27-29, the number of CTCs seems not to simply reflect tumor bulk [1, 2]. A detailed analysis of tumor burden by the bidimensional sum of the metastatic lesions showed limited correlation between CTC levels and radiographic measurement of tumor load [3]. However, the detection of CTCs in patients with evidence of nonvisceral disease (including metastases in chest wall, lymph nodes, and bone) was of higher prognostic significance than in those with visceral disease [2, 4]. This observation based on standard imaging is of interest because metastases in chest wall, lymph node and bone are not commonly associated with a poor prognosis in patients with MBC and sometimes difficult to measure to assess treatment benefit [5].

2-[Fluorine-18]fluoro-2-deoxy-D-glucose (FDG)–positron emission tomography (PET) appears to be more sensitive than conventional imaging in the detection of breast cancer metastases at any site [6]. FDG–PET/computed tomography (CT) allows for fusion of functional and anatomic datasets, resulting in more accurate evaluation of disease [7], which has resulted in a better detection and reflection of the tumor activity in MBC, in particular of bone metastases [8]. It has been postulated that the number of CTCs may be proportional to the tumor's proliferation and metabolic activity [9]. FDG–PET/CT imaging thus represents a useful tool to better identify metastases in bone and other sites in patients with MBC and to evaluate the relationship between CTC levels and sites of metastases.

The objective of this study was to test the hypothesis that an increased level of CTCs is closely related to FDG–PET/CT-detected metastases in bone and/or other sites in patients diagnosed with relapsed/progressive MBC.

*Correspondence to: Dr M. Cristofanilli, Department of Breast Medical Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Tel: +1-713-792-8138; Fax: +1-713-794-1838; E-mail: mcristof@mdanderson.org

Table 1. Patient and disease characteristics (n = 195)

	n (%)
Age (years)	
Median (range)	54 (24–84)
Histology[a]	
Infiltrating ductal carcinoma	161 (83)
Infiltrating lobular carcinoma	14 (7)
Other	20 (10)
Estrogen receptor status	
Positive	122 (63)
Negative	71 (36)
Unknown	2 (1)
HER2 status	
Positive	45 (23)
Negative	146 (75)
Unknown	4 (2)
Setting of relapse/progression	
First relapse	78 (40)
≥Second relapse/progression	117 (60)
Last treatment before first relapse (n = 78)	
Follow-up	65 (83)
Adjuvant hormonal therapy[b]	13 (17)
Last treatment before ≥second relapse/progression (n = 117)	
Chemotherapy	48 (41)
Hormonal therapy ± CT	53 (45)
HER2 target ± CT ± HT[c]	16 (14)
Sites of disease	
Bone	137 (70)
Lymph nodes and/or chest wall[d]	131 (67)
Lung and/or pleura	67 (34)
Liver	60 (31)
Other abdominal[e]	17 (9)
CNS	7 (4)

[a]Tumors classified as infiltrating ductal carcinoma had no other invasive histologic types reported; likewise, infiltrating lobular carcinoma had only invasive lobular carcinoma; all other tumors were classified as 'other', including mixed infiltrating ductal/lobular carcinoma.
[b]All patients received adjuvant hormonal therapy within 3 months from relapse; one received trastuzumab also.
[c]Of these patients, 15 received trastuzumab, while one received lapatinib.
[d]Including primary tumor or recurrence, lymph node (any site), and/or chest wall metastases.
[e]Including peritoneum (n = 11), adrenal gland (n = 5), and kidney (n = 1).
CT, chemotherapy; HT, hormonal therapy; CNS, central nervous system; HER2, human epidermal growth factor receptor 2.

patients and methods

patients

This is a retrospective evaluation of patients with MBC that were evaluated with CTCs, as standard of care and radiological imaging for staging. The retrospective study was approved by our Institutional Review Board and a waiver of consent was granted. Before enrollment, all patients had to have been diagnosed with relapsed/progressive MBC based on the following staging procedures: clinical examination, chest X-ray, bone scan, abdominal ultrasound, and contrast-enhanced CT and/or magnetic resonance imaging. Upon diagnosis of relapse/progression, consenting patients underwent whole-body FDG–PET/CT scans and provided blood samples for CTC analysis. Clinical and pathological data were collected from the Breast Medical Oncology Database.

FDG–PET/CT acquisition and evaluation

FDG–PET/CT scans were carried out using a Discovery ST camera [General Electric (GE) Medical Systems, Waukesha, WI] combined with an eight-slice Light-Speed Ultra CT scanner (GE Medical Systems) [10]. Patients were imaged 60 min after i.v. administration of 10- to 15-mCi FDG. PET studies were acquired at 3–5 min per bed position, depending upon the patient's weight and body habitus, for a total of six or seven bed positions.

Interpretation of the dual PET–CT images was carried out by a nuclear medicine physician/radiologist trained in PET–CT. Lesions with standardized uptake value (SUV) of >2.5 were considered malignant [11]. A region of interest was drawn at each pathologic site of tracer uptake, and the SUVs were calculated automatically by the computer using the body weight method: SUV_decay-corrected activity (kBq)/tissue (ml) injected FDG dose (kBq)/body weight (g). Maximum SUV was measured at every site of metastases, at the primary tumor (if present), and at each of the respective regional and distant nodal groups [12].

PET and CT images obtained in all standard planes were reviewed on an Advantage workstation (GE Medical Systems). Two reviewers visually and quantitatively analyzed the images and recorded their findings after they reached a consensus. For visual analysis, abnormal FDG uptake was defined as substantially greater activity in the tissue than in the aortic blood on attenuation-corrected images. When abnormal FDG uptake was present in bone, the exact anatomic location of the abnormal uptake was identified on the CT images [13].

isolation and enumeration of CTCs

At diagnosis of relapse/progression, patients provided blood samples for CTC analysis. Blood sample collection was done as previously described [2]. All CTC evaluations were done in our central laboratory. The CellSearch System (Veridex LLC, Raritan, NJ) was used for the isolation and enumeration of CTCs. The system consisted of a semiautomated sample preparation system and the CellSearch epithelial cell kit to immunomagnetically enrich cells expressing the epithelial cell adhesion molecule [14]. Isolated cells were then fluorescently labeled with the nucleic acid dye 4′,6-diamidino-2-phenylindole and labeled mAbs specific for leukocytes (CD45-allophycocyanin) and epithelial cells (cytokeratin 8,18,19-phycoerythrin). CTCs are defined as nucleated cells lacking CD45 and expressing cytokeratin [15]. Identification and enumeration of CTCs was done using the CellSpotter Analyzer (Immunicon Corporation, Huntingdon Valley, PA) by trained operators blinded to patient outcomes as previously reported [2]. For CTCs, a threshold of 5 CTCs/7.5 ml blood was used to evaluate results, with poor prognosis indicated by ≥5 CTCs/7.5 ml blood and good prognosis defined as <5 CTCs/7.5 ml blood [2].

statistical analysis

Student's t-test and Fisher's exact test were used to test for statistically significant differences in the number of patients with <5 or ≥5 CTCs. Overall survival (OS) was defined as the elapsed time between the date of blood sampling and the date of either death or the last follow-up (if death did not occur during the follow-up period). Kaplan–Meier survival plots were generated on the basis of CTC count at baseline and metastatic sites of disease, and the curves were compared using log-rank testing. Cox proportional hazards regression was used to determine univariate and multivariate hazard ratios for selected potential predictors of OS. Statistical significance was defined as a P value of <0.05.

results

patients' characteristics

From May 2004 to January 2008, 195 patients who were diagnosed with relapsed/progressive MBC underwent FDG–PET/CT scans and provided blood samples for CTC analysis. One hundred seventeen (60%) patients had received prior treatment of MBC with hormonal therapy (53 cases), chemotherapy with or without hormonal therapy (48 cases), or human epidermal growth factor receptor 2 (HER2)-targeted therapies combined with chemotherapy and/or hormonal therapy (16 cases), whereas 78 (40%) had new diagnoses with MBC. The clinical characteristics of the 195 patients evaluated are detailed in Table 1. In this cohort of 195 patients, 103 (53%) had <5 CTCs at relapse/progression and 92 (47%) had ≥5 CTCs. Patients treated with HER2-targeted therapies (15 trastuzumab and one lapatinib) had lower CTC counts with only one case with ≥5 CTCs at progression during trastuzumab (number of CTCs = 17).

associations with disease sites and disease extent

Of the 92 patients with ≥5 CTCs, 83 (90%) presented with bone metastases; furthermore, of 50 cases with ≥21 CTCs, 48 (96%) had bone metastases (Table 2A). Of 137 patients with bone metastases at relapse/progression, 83 (61%) had ≥5 CTCs, while 54 (39%) had <5 CTCs ($P = 0.0122$). Higher CTC numbers were detected in patients with bone metastases alone and patients with metastases in bone plus other sites relative to those with no bone metastases (Figure 1A). Of the 137 patients with bone metastases, higher CTC numbers were detected in the patients with more extensive bone metastases relative to those with one or two bone lesions (Table 2B and Figure 1B).

Among patients with no bone metastases, lower CTC counts were observed in patients with lymph node and/or chest wall metastases ($n = 23$; mean 1.4 ± 2.0 CTCs) and those with lung and/or pleural metastases ($n = 25$; mean 2.9 ± 8.1 CTCs), with or without lymph node and/or chest wall metastases, while patients with also liver metastases presented with higher CTC count ($n = 9$; mean 8.9 ± 16.4 CTCs). Seven patients had central nervous system (CNS) metastases at relapse/progression. Five of these presented <5 CTCs, while the other two had ≥5 CTCs, which were associated with either bone metastases (CTCs = 7) or liver metastases (CTCs = 11). Of 17 patients with abdominal metastases in sites other than the liver [peritoneum ($n = 11$), adrenal glands ($n = 5$), and kidney ($n = 1$)], only two did not present metastases in either bone or liver and both cases had <5 CTCs.

Of 15 HER2-positive patients treated with trastuzumab, six (40%) had extensive bone involvement with or without liver metastases (including the only case with ≥5 CTCs), four (27%) liver metastases without extensive bone involvement, three (20%) lung metastases, and two (13%) CNS.

associations with metabolic activity

All 137 patients with bone metastases but seven had increased FDG uptake within one or more lesions. Of these seven cases, four had <5 CTCs; of the remaining three with ≥5 CTCs, two also had liver metastases with elevated FDG uptake (CTCs = 143 and 25), while one presented with primary tumor with elevated FDG uptake (CTCs = 75). Of 60 patients with liver metastases, two had no increase in FDG uptake. Both of these patients had bone metastases and ≥5 CTCs. Among 67 patients with lung metastases, 17 presented with lung lesions suspected of being metastases at the time of CT but without FDG uptake; of these, nine patients had <5 CTCs (including one with bone metastases without FDG uptake), while eight patients presented with ≥5 CTCs. Either bone or liver metastases were present in all these eight cases. All 131 patients with lymph node and/or chest wall metastases had at least one lesion with FDG uptake indicating malignancy.

associations with survival

Median OS was 14 months (range 1–45+) for all patients. At the time of analysis, 121 of the 195 (62%) patients were still alive. CTC levels predicted OS in all these 195 patients (Figure 2A). In patients with bone and other sites of MBC, the OS was significantly worse than in patients with bone metastases only and/or absence of bone involvement (Figure 2B). CTC levels were not able to predict OS in 58 patients without bone metastases ($P = 0.4111$) (Figure 3A) and in 29 with bone involvement only ($P = 0.3552$) (Figure 3B) whereas predicted OS in 108 with bone and other sites of MBC ($P = 0.0008$) (Figure 3C).

Table 2. CTC number according to the presence/absence of bone metastases from breast cancer (A) and skeletal tumor burden in patients with bone metastases (B)

		CTC number (%)			CTC count	
		<5	≥5			
			5−20	≥21	Mean ± SD	Median (range)
A	195 cases, *n* (%)					
Bone metastases	137 (70)	54 (39)	35 (26)	48 (35)	65.7 ± 187.9	10 (0–1780)
No bone metastases	58 (30)	49 (84)	7 (12)	2 (4)	3.3 ± 8.7	1 (0–51)
B	137 cases, *n* (%)					
Extensive bone metastases[a]	115 (84)	36 (31)	31 (27)	48 (42)	77.7 ± 202.9	13 (0–1780)
Limited bone metastases[b]	22 (16)	18 (82)	4 (18)	0	2.6 ± 5.2	0 (0–16)

[a]Defined as having at least three bone lesions.

[b]Defined as until two single bone lesions (e.g. one vertebra and one rib).

CTC, circulating tumor cells; SD, standard deviation.

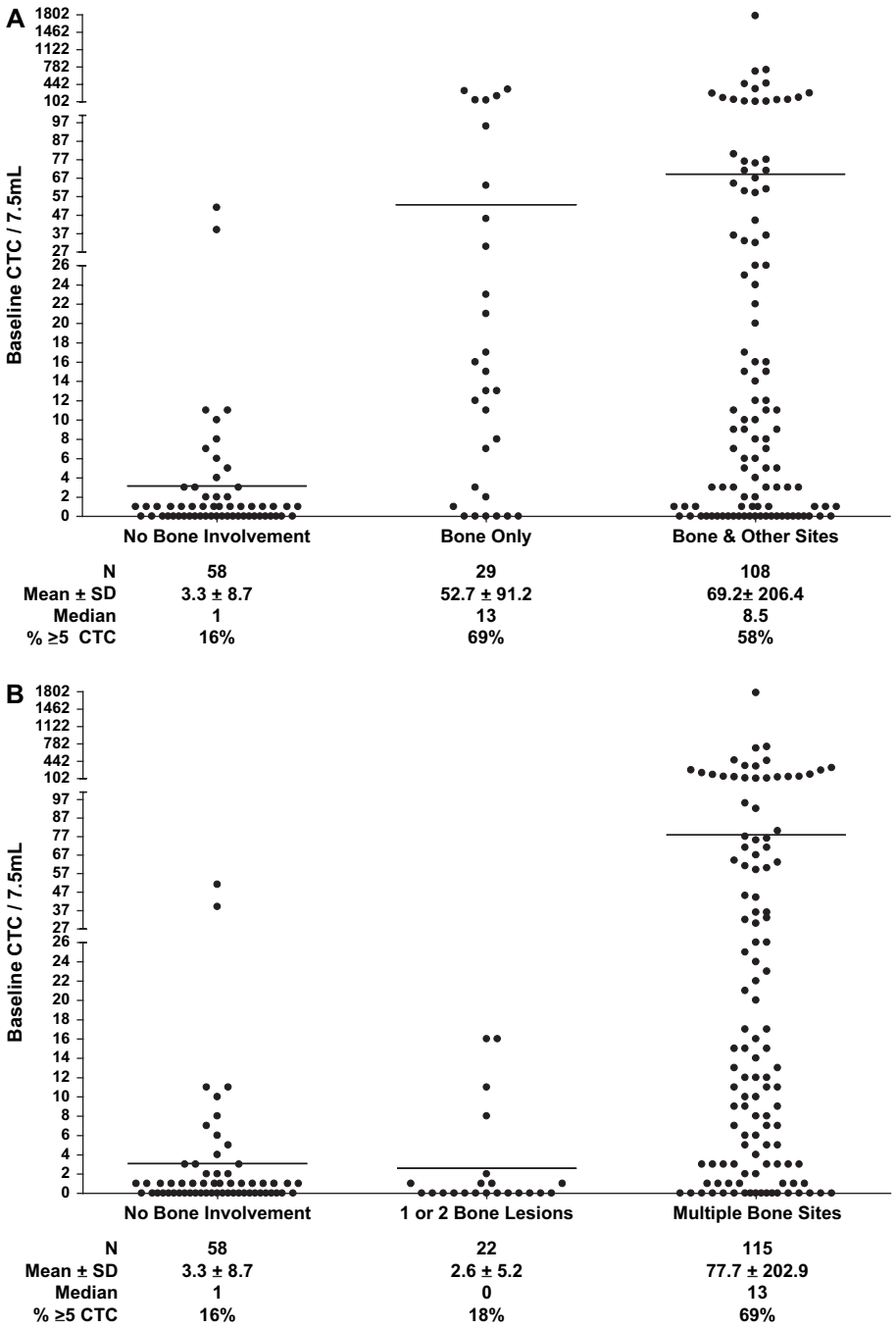

Figure 1. Circulating tumor cell (CTC) count relationship with bone metastases. Association of CTCs with the presence of bone metastases with or without other sites of disease (**A**) and extension of bone involvement (**B**).

Univariate analysis showed that baseline CTCs, HER2 status, line of therapy, and lung, liver, abdominal, CNS, and bone metastases were associated with OS. In multivariate analysis, CTCs, but not bone metastases, remained significant as predictor of OS after adjustment for the univariately significant factors (Table 3).

discussion

Metastatic spread is a 'kinetic phenomenon' requiring a series of sequential events primarily at the level of gene expression in the various populations of cancer cells (e.g. epithelial versus progenitor cells). Furthermore, the interaction between the cancer cell (the seed) and the microenviroment is essential in determining the fate of those cells and the selection of metastatic sites [16–18]. Our study provides evidence of a difference in the frequency and number of CTCs in breast cancer patients with bone metastases relative to metastases at other sites. Patients with bone metastases had significantly higher numbers of CTCs than patients without bone metastases, whereas patients with no bone metastases rarely presented with ≥21 CTCs (Table 2A). When bone metastases were present, higher CTC numbers were described particularly

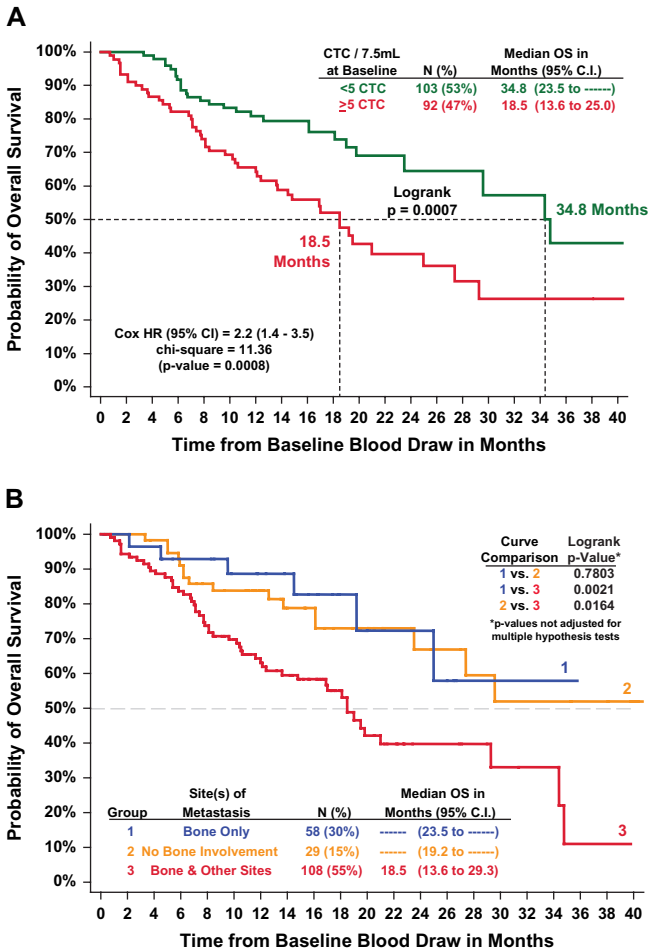

Figure 2. Ability of baseline circulating tumor cell (CTC) levels (**A**) and sites of metastases (**B**) to predict overall survival in 195 metastatic breast cancer patients.

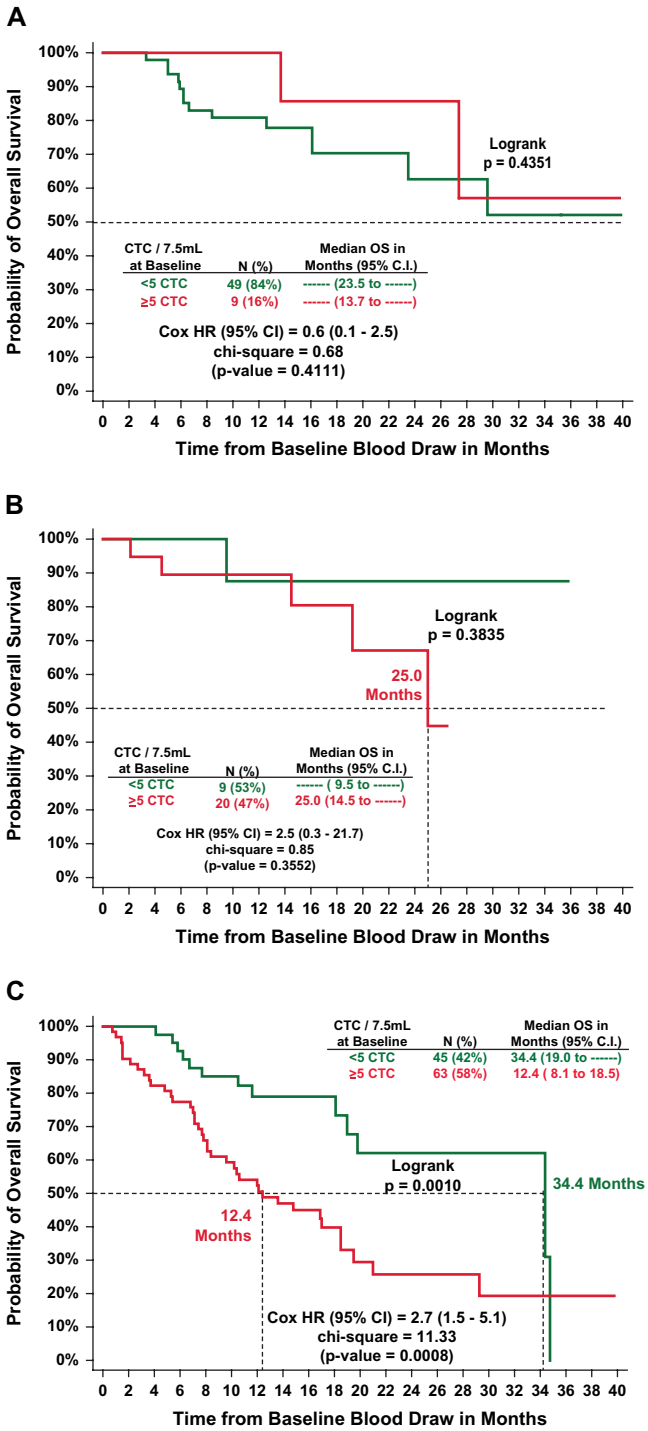

Figure 3. Ability of baseline circulating tumor cell (CTC) levels to predict overall survival in different breast cancer metastatic patterns. (**A**) Patients with no bone involvement ($n = 58$). (**B**) Patients with bone metastasis only ($n = 29$). (**C**) Patients with bone metastases and other metastatic sites ($n = 108$).

in cases of diffuse skeletal disease, whereas those with one or two bone lesions had sharply lower CTC values (Table 2B). Moreover, reduced CTC counts were observed in patients with soft tissue metastases and those with lung and/or pleural metastases compared with patients with liver metastases. Thus, this result indicates that the shedding and circulation of cancer cells is quite different not only in the single patients but also in relation to the site of recurrent disease. Considering that CTCs have been shown to be a strong, independent prognostic factor in MBC and that we demonstrated that patients with bone metastases have the higher percentage and number of positive CTCs, we must conclude that those patients may have worse outcome. This finding is quite significant and contradicts the common belief that bone disease is typically more indolent. What are the biological significance and the clinical implications of those findings? It is possible that patients with more extensive bone involvement have also developed bone marrow infiltration and therefore, the detection of CTCs is the reflection of such advanced status [19].

Our results show a close association between high levels of CTC and extensive bone involvement (Figure 1B); even in multivariate analysis, CTCs, but not bone metastases, remained significant as predictor of OS (Table 3). In early prospective studies on CTCs in MBC, bone metastases were studied with standard imaging and were generically included in the 'nonvisceral disease' group, representing from 12% to 19% of all assessable cases [2, 4]. In consequence, the effect of bone metastases on CTC levels could have been underestimated in these trials but could contribute to explain the poor survival

Table 3. Multivariate Cox regressions analysis for overall survival of prognostic factors that were statistically significant after univariate analysis

	Categories		OS risk	
Variable	Positive	Negative	HR (95% CI)	*P*
CTC number	≥5	<5	1.79 (1.06−3.00)	0.029
HER2 status	3+/FISH+	0−2+	0.55 (0.29−1.08)	0.084
Line of therapy	Continuous (1−2+)		1.83 (1.09−3.07)	0.023
Lung/pleura metastases	Continuous		1.89 (1.15−3.10)	0.012
Liver metastases	Continuous		1.65 (0.98−2.79)	0.062
Other abdominal metastases[a]	Continuous		2.09 (1.07−4.07)	0.030
CNS metastases	Continuous		2.92 (1.09−7.86)	0.034
Bone metastases	Continuous		1.36 (0.73−2.51)	0.334

[a]Including patients with abdominal metastases in sites other than the liver [peritoneum ($n = 11$), adrenal glands ($n = 5$), and kidney ($n = 1$)].
OS, overall survival; HR, hazard ratio; CI, confidence interval; CTC, circulating tumor cell; CNS, central nervous system; HER2, human epidermal growth factor receptor 2.

rates for patients with nonvisceral disease. In another recent prospective study, a possible correlation between ≥5 CTCs at baseline and the presence of bone metastases as detected by whole-body bone scan was noted for the first time [20]. The superiority of FDG–PET/CT over bone scan in specificity and sensitivity for detection of bone metastases is well documented [6, 8, 13, 21, 22]. In the present study, we show that increased levels of CTC are strictly correlated with the presence of extensive but not limited bone disease as detected by FDG–PET/CT, indicating a possible relationship between CTC counts and the bone tumor burden and disease activity. Instead, in previous studies with standard imaging, increased CTC levels were not associated with tumor load measured by bidimensional radiography [3, 23].

In the present study, all 137 patients with bone metastases but seven had metabolically active lesions. Of these seven patients without FDG uptake in the bone lesions, three had ≥5 CTCs; therefore, the results on this series do not permit a comparative analysis between bone metastases with or without increased FDG uptake.

Notably, all patients but one with HER2-positive tumors who were treated with trastuzumab-based regimens had <5 CTCs at progression, indicating that the use of trastuzumab could selectively act against either CTCs or CTCs production. This property of trastuzumab and possibly other HER2-targeted therapies, if confirmed in prospective studies, could provide a means to better manage these patients, in whom disease progression inevitably occurs during or after completion of trastuzumab-based therapy for MBC [24, 25].

In conclusion, our study provides evidence of a strong correlation between a high number of CTCs and extensive bone metastases. This, together with the association between baseline CTC count and survival time, shows that this biomarker reflects the intrinsic biology of the tumor. Prospective studies designed around the CTC biomarker in specific clinical and therapeutic contexts will need to be conducted to assess the critical role CTCs play in the prognostic and therapeutic monitoring of MBC.

acknowledgements

The authors thank Evan Cohen and Meshaal Nadeem for their help in collecting clinical data.

disclosures

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in the study if they were not being evaluated as part of the investigation. Employment: MCM, Immunicon; leadership position: GVD, Immunicon; stock ownership: GVD, Immunicon and MC, Cellexicon; and other remuneration: GVD, Immunicon.

references

1. Gaforio JJ, Serrano MJ, Sanchez-Rovira P et al. Detection of breast cancer cells in the peripheral blood is positively correlated with estrogen-receptor status and predicts poor prognosis. Int J Cancer 2003; 107: 984–990.
2. Cristofanilli M, Budd GT, Ellis M et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med 2004; 351: 781–791.
3. Budd GT, Cristofanilli M, Ellis MJ et al. Circulating tumor cells versus imaging—predicting overall survival in metastatic breast cancer. Clin Cancer Res 2006; 12: 6403–6409.
4. Cristofanilli M, Hayes DF, Budd GT et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol 2005; 23: 1420–1430.
5. Solomayer EF, Diel IJ, Meyberg GC et al. Metastatic breast cancer: clinical course, prognosis, and therapy related to the first site of metastasis. Breast Cancer Res Treat 2000; 59: 271–278.
6. Hodgson NC, Gulenchyn KY. Is there a role for positron emission tomography in breast cancer staging? J Clin Oncol 2008; 26: 712–720.
7. Cohade C, Wahl R. Applications of positron emission tomography/computed tomography image fusion in clinical positron emission tomography—clinical use, interpretation methods, diagnostic improvements. Semin Nucl Med 2003; 33: 228–237.
8. Du Y, Cullum I, Illidge TM, Ell PJ. Fusion of metabolic function and morphology: sequential [18F]fluorodeoxyglucose positron-emission tomography/computed tomography studies yield new insights into the natural history of bone metastases in breast cancer. J Clin Oncol 2007; 25: 3440–3447.
9. Pachmann K, Camara O, Kavallaris A et al. Monitoring the response of circulating epithelial tumor cells to adjuvant chemotherapy in breast cancer allows detection of patients at risk of early relapse. J Clin Oncol 2008; 26: 1208–1215.
10. Mawlawi O, Podoloff DA, Kohlmyer S et al. Performance characteristics of a newly developed PET/CT scanner using NEMA standards in 2D and 3D modes. J Nucl Med 2004; 45: 1734–1742.
11. Young H, Baum R, Cremerius U et al. Measurement of clinical and subclinical tumour response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations—European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. Eur J Cancer 1999; 35: 1773–1782.
12. Ma BB, King A, Lo YM et al. Relationship between pre-treatment level of plasma Epstein-Barr virus DNA, tumor burden, and metabolic activity in advanced nasopharyngeal carcinoma. Int J Radiat Oncol Biol Phys 2006; 66: 714–720.

13. Tateishi U, Gamez C, Dawood S et al. Bone metastases in patients with metastatic breast cancer: morphologic and metabolic monitoring of response to systemic therapy with integrated PET/CT. Radiology 2008; 247: 189–196.
14. Rao CG, Chianese D, Doyle GV et al. Expression of epithelial cell adhesion molecule in carcinoma cells present in blood and primary and metastatic tumors. Int J Oncol 2005; 27: 49–57.
15. Kagan M, Howard D, Bendele T et al. A sample preparation and analysis system for identification of circulating tumor cells. J Clin Lig Assay 2002; 25: 104–110.
16. Suzuki M, Mose ES, Montel V, Tarin D. Dormant cancer cells retrieved from metastasis-free organs regain tumorigenic and metastatic potency. Am J Pathol 2006; 169: 673–681.
17. Pestalozzi BC, Zahrieh D, Mallon E et al. Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: combined results of 15 International Breast Cancer Study Group Clinical Trials. J Clin Oncol 2008; 26: 3006–3014.
18. Wu JM, Fackler MJ, Halushka MK et al. Heterogeneity of breast cancer metastases: comparison of therapeutic target expression and promoter methylation between primary tumors and their multifocal metastases. Clin Cancer Res 2008; 14: 1938–1946.
19. Meads MB, Hazlehurst LA, Dalton WS. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. Clin Cancer Res 2008; 14: 2519–2526.
20. Nolé F, Munzone E, Zorzino L et al. Variation of circulating tumor cell levels during treatment of metastatic breast cancer: prognostic and therapeutic implications. Ann Oncol 2008; 19: 891–897.
21. Ohta M, Tokuda Y, Suzuki Y et al. Whole body PET for the evaluation of bony metastases in patients with breast cancer: comparison with 99Tcm-MDP bone scintigraphy. Nucl Med Commun 2001; 22: 875–879.
22. Fuster D, Duch J, Paredes P et al. Preoperative staging of large primary breast cancer with [18F]fluorodeoxyglucose positron emission tomography/computed tomography compared with conventional imaging procedures. J Clin Oncol 2008; 26: 4746–4751.
23. Cristofanilli M, Broglio KR, Guarneri V et al. Circulating tumor cells in metastatic breast cancer: biologic staging beyond tumor burden. Clin Breast Cancer 2007; 7: 471–479.
24. Slamon DJ, Leyland-Jones B, Shak S et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344: 783–792.
25. Nahta R, Yu D, Hung MC et al. Understanding resistance to HER2-targeted therapy in human breast cancer. Nat Clin Pract Oncol 2006; 3: 269–280.