Baseline serum NTx levels are prognostic in metastatic breast cancer patients with bone-only metastasis

S. M. Ali¹*, L. M. Demers², K. Leitzel², H. A. Harvey², D. Clemens³, N. Mallinak³, L. Engle², V. Chinchilli², L. Costa⁴, C. Brady⁵, J. Seaman⁵ & A. Lipton²

¹VA Medical Center, Lebanon, PA; ²The M. S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA; ³Ostex International, Seattle, WA; ⁴Novartis Pharmaceutical Corp., East Hanover, NJ, USA; ⁵Unidade de Oncologia, Hospital de Santa Maria, Lisbon, Portugal

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Background: There is significant heterogeneity in survival of patients with metastatic breast cancer who have bone-only metastasis. We studied the correlation of serum N-telopeptide (NTx), a marker of bone resorption, and its correlation with clinical outcomes in patients with metastatic breast cancer with bone-only or bone plus soft tissue metastasis.

Patients and methods: Serum was taken from 250 metastatic breast cancer patients with bone-only or bone plus soft tissue metastasis who participated in two similar randomized studies of second-line hormone therapy. An enzyme-linked immunosorbent assay specific for NTx of type I bone collagen was used to detect serum levels.

Results: Sixty patients (24%) had elevated serum NTx levels, using the mean + 2 standard deviations (26 nanomoles Bone Collagen Equivalents per liter) of healthy women as a cut-off. The median duration of clinical benefit was significantly shorter in the group with elevated serum NTx levels compared with the group that had normal serum NTx levels (P = 0.0004). Time to progression (TTP) was also significantly shorter in the patients with elevated serum NTx at 139 days compared with 220 days (P = 0.0006). Median survival was also significantly shorter in patients with elevated baseline serum NTx levels at 663 days compared with 941 days (P <0.0001).

Conclusion: In this study, breast cancer patients with bone-only or bone plus soft tissue metastasis and elevated serum NTx levels have a shorter duration of clinical benefit, TTP and overall survival.

Key words: bone-only metastasis, bone plus soft tissue metastasis, metastatic breast cancer, serum NTx, survival

Introduction

There is significant heterogeneity in the survival of patients with metastatic breast cancer. In one study, breast cancer patients who develop liver metastasis have the shortest survival [1]. In another report, patients with visceral metastasis have the worst survival [2]. It has generally been felt that patients with bone-only metastasis have a better prognosis than patients with visceral metastasis [3]. However, there is also considerable heterogeneity in patients with bone-only metastasis, with a median survival of 20 months and a 20% survival at 5 years [4].

A number of reports have shown that the urine and blood levels of the bone remodeling markers are elevated in a significant number of patients with bone metastasis [5–8]. Markers of bone resorption have been evaluated in cancer patients in a variety of ways. They have been evaluated for the early detection of bony metastasis, for assessing tumor burden in bone, for predicting response to therapy, and as a surrogate marker for determining response to therapy. The first generation of bone resorption markers were not specific for type I bone collagen. In the search for greater specificity of these bone collagen breakdown products, monoclonal antibodies were developed against the telopeptide fragments that are associated with the pyridinium cross-links in bone collagen [9]. Assays are currently available that can measure the telopeptide fragment from either the N-terminal or C-terminal end of type I bone collagen fibrils. The N-terminal telopeptide assays, termed NTx, utilize an antibody that was raised against the α-2 chain of type I bone collagen fibrils. The NTx monoclonal antibody does not recognize the attached pyridinium cross-link. NTx has been evaluated in relation to other markers of bone resorption and is reported to be highly specific for bone [10].

We elected to evaluate this marker in a group of patients with metastatic breast cancer with bone-only or bone plus soft tissue metastasis. These patients participated in two randomized parallel studies of second-line hormonal therapy, comparing the second generation aromatase inhibitor fadrozole to megestrol acetate. Patients with visceral metastasis were excluded from this analysis. The baseline levels of serum NTx were correlated with clinical benefit (complete response, partial response and stable disease for
>24 weeks), duration of clinical benefit, time to progression (TTP) and overall survival (OS).

**Patients and methods**

**Patient population**

Baseline sera were obtained from patients with metastatic breast cancer 2 weeks before entry into two clinical trials of second-line hormone therapy for metastatic breast cancer. The two trials compared the second-generation aromatase inhibitor fadrozole with megestrol acetate [11]. The subset of patients who had bone-only or bone plus soft tissue metastasis were eligible for this study. Three-hundred and ten patients were enrolled in the studies with bone-only or bone plus soft tissue disease. Serum was available for 250 of 310 (81%) of these patients, and only the patients with sera were included in this analysis.

Patients in both studies were between 36 and 92 years of age and had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 3. All patients were required to be postmenopausal as defined according to one of the following criteria: women aged ≥50 years who had not menstruated during the preceding 12 months, or had castrate follicle-stimulating hormone levels (≥40 IU/l); those <50 years of age who had castrate follicle-stimulating hormone levels; or those who had undergone a bilateral oophorectomy. All patients had received one prior hormone therapy for metastatic disease. Patients could have received one prior adjuvant chemotherapy or one prior adjuvant hormonal therapy. Patients were enrolled in the two studies from February 1989 to September 1992. The study was conducted in the pre-bisphosphonate era and patients did not receive bisphosphonates as part of the study.

**Efficacy assessments**

Tumor response in each study was assessed by appropriate measurements or X-rays every 3 months. Complete response (CR) required the complete disappearance of all disease for at least 4 weeks. Partial response (PR) was defined as a ≥50% decrease in the sum of the product of two diameters of all measurable lesions and at least stabilization of all non-measurable disease maintained for a minimum of 4 weeks. Stable disease (SD) consisted of a <50% decrease in the sum of the products of two diameters of all measurable lesions that was maintained for at least 26 weeks. Progressive disease (PD) was defined as a >25% increase in the sum of the product of two diameters of one or more measurable tumors, or an unequivocal increase in the number of new lesions. Patients with clinical benefit were defined as those responding (CR and PR) plus those with SD for at least 26 weeks.

TTP and survival were calculated from the date of randomization. TTP represented the time to objective disease progression or death. Duration of response in patients who achieved CR, PR or SD for ≥26 weeks was also defined as the time from randomization to the time of first observation of objective progression. Survival was the time from the date of randomization to death.

**Serum preparation**

Blood was drawn by forearm venipuncture within 14 days prior to the initiation of second-line hormone therapy. Blood was centrifuged at 500 g for 10 min at room temperature, and serum was collected, aliquoted and stored at −70°C for NTx analysis.

**NTx ELISA assay**

A competitive-inhibition, enzyme-linked immunoabsorbent assay (ELISA) was used to measure serum levels of NTx using reagents obtained from Ostex International (Seattle, WA). This assay uses a microtiter plate format with adsorbed NTx in direct competition with sample NTx for binding to NTx antibody labeled with horseradish peroxidase. Assay values are generated in nanomoles of Bone Collagen Equivalents per liter (nm BCE). Interassay variability was established at 6.9% [10].

**Statistical methods**

A serum NTx level of 26 nM BCE (mean + 2 standard deviations) was used as a cut-off. This cut-off value was suggested by the manufacturer of serum NTx ELISA based on the studies conducted in healthy postmenopausal women not receiving bisphosphonate therapy. Two-way frequency tables were constructed to describe the categorical variables such as elevated serum NTx and response status. The chi-square test was applied to ascertain statistically significant differences. Variables used in the logistic regression for binary outcomes and proportional hazards regression analysis for the time-to-event outcomes were: age, serum NTx level (≤26 versus >26 nm BCE), receptor status [estrogen receptor (ER)⁺ or progesterone receptor (PR)⁺ versus ER⁺ and PR⁺, unknown versus ER⁺ and PR⁺⁺], disease-free interval (<2 years versus ≥2 years, none versus ≥2 years), performance status (1 versus 0, 2 versus 0) and adjuvant chemotherapy. Bonferroni adjustments were made for multiple comparisons for those predictor variables with more than two levels.

Kaplan–Meier survival curves were plotted graphically to visually compare time-to-event variables, such as time to disease progression and survival time for both the elevated and non-elevated groups. The log-rank test was used to compare survival curves. All calculations were performed using PROC FREQ, PROC LOGISTIC, PROC LIFETEST, PROC PHREG and PROC TTEST in version 8.2 of SAS (SAS Institute, Cary, NC). A level of $P < 0.05$ was considered statistically significant.

**Results**

**Patient characteristics**

One-hundred and ninety-six breast cancer patients with bone-only metastasis and 54 patients with metastatic disease involving bone and soft tissue were included in this study. Sixty of 250 patients with bone-only metastatic breast cancer had elevated serum NTx levels (24%), while 190 patients had levels within the normal range (76%). There were no significant differences between the elevated NTx and normal NTx groups according to disease-free interval, site of metastasis and ER/PR status. Patients in the elevated NTx group were significantly older, however, weighed significantly less and differed in ECOG status (Table 1).

**Response to treatment**

Clinical benefit to second-line endocrine therapy was determined to be 45% overall in this group of 250 patients. The clinical benefit (CR plus PR plus SD) to endocrine therapy was 47.9% (91 of 190 patients) in patients who had normal serum NTx levels as compared with 37% (22 of 60 patients) in patients with elevated serum NTx levels ($P = 0.13$). The duration of clinical benefit was shorter in patients who had elevated serum NTx levels (345 versus 538 days; $P = 0.0004$).

We studied this observation in a logistic regression model that included receptor status, disease-free interval, performance status, age, adjuvant chemotherapy and serum NTx levels. Hormone receptor status was the only significant predictive factor for clinical benefit to hormonal therapy in the multivariate model (Table 2).
Time to progression

Median TTP for patients with elevated serum NTx was significantly shorter than that for patients with non-elevated serum NTx (139 days versus 220 days, \( P = 0.0004 \)). A Kaplan–Meier plot of TTP is presented in Figure 1. Elevated baseline serum NTx levels continued to be associated with shorter TTP in the multivariate model. The only other significant factor in the multivariate model was receptor status (Table 2). Patients who expressed both ER and PR had a longer TTP compared with patients who were either ER+ or PR+ (Table 2).

Overall survival

Survival was defined as the period from the start of second-line hormone treatment with either megestrol acetate or fadrozole until death. One-hundred and fifty-seven of 250 patients (63%) had died by the end of the follow-up period. Median survival for all 250 patients was 863 days.

Baseline serum NTx level was a prognostic factor in patients for survival with bone-only or bone plus soft tissue metastasis when using the dichotomous cut-off of 26 nm BCE (663 versus 941 days) \( (P < 0.0001) \) (Figure 2). The data were also analyzed using

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**Table 1. Distribution of serum NTx by clinical characteristics**

<table>
<thead>
<tr>
<th>Prognostic factor</th>
<th>NTx below cut-off (( n = 190 ))</th>
<th>NTx above cut-off (( n = 60 ))</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean in years)</td>
<td>64 (37–92)</td>
<td>68 (41–92)</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72 (40–124)</td>
<td>67 (38–110)</td>
<td>0.02</td>
</tr>
<tr>
<td>Disease-free interval (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No interval</td>
<td>21 (11%)</td>
<td>8 (13.3%)</td>
<td>0.85</td>
</tr>
<tr>
<td>&lt;2 years</td>
<td>39 (20.5%)</td>
<td>11 (18.3%)</td>
<td></td>
</tr>
<tr>
<td>( \geq )2 years</td>
<td>130 (68.4%)</td>
<td>41 (68.3%)</td>
<td></td>
</tr>
<tr>
<td>ER/PR status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both ER+ and PR+</td>
<td>116 (61%)</td>
<td>29 (48.3%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Either ER+ or PR+</td>
<td>55 (29%)</td>
<td>22 (36.7%)</td>
<td></td>
</tr>
<tr>
<td>Both ER and PR unknown</td>
<td>18 (9.5%)</td>
<td>9 (15%)</td>
<td></td>
</tr>
<tr>
<td>Site of metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft tissue/bone</td>
<td>41 (21.6%)</td>
<td>13 (21.7%)</td>
<td>0.99</td>
</tr>
<tr>
<td>Bone</td>
<td>149 (78.4%)</td>
<td>47 (78.3%)</td>
<td></td>
</tr>
<tr>
<td>ECOG performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>93 (48.9%)</td>
<td>16 (27.6%)</td>
<td>0.02</td>
</tr>
<tr>
<td>1</td>
<td>67 (35.3%)</td>
<td>30 (51.7%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>30 (15.8%)</td>
<td>12 (20.7%)</td>
<td></td>
</tr>
</tbody>
</table>

One ER- and PR-negative patient, and two patients with an ECOG performance status of 3 were excluded from the analysis.

**Table 2. Multivariate analysis for clinical benefit, time to progression (TTP) and overall survival (OS)**

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Clinical benefit</th>
<th>TTP</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P )-value</td>
<td>Odds ratio</td>
<td>( P )-value</td>
</tr>
<tr>
<td>Age</td>
<td>NS</td>
<td>NS</td>
<td>0.04</td>
</tr>
<tr>
<td>NTx &gt;26 versus ( \leq )26 nM BCE</td>
<td>NS</td>
<td>0.0008</td>
<td>1.76</td>
</tr>
<tr>
<td>ER+ or PR+ versus ER+ and PR+</td>
<td>0.0006</td>
<td>0.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Unknown versus ER+ and PR+</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Disease-free interval</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>( &lt; )2 years versus ( \geq )2 years</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>None versus ( \geq )2 years</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ECOG 1 versus 0</td>
<td>NS</td>
<td>NS</td>
<td>0.004</td>
</tr>
<tr>
<td>ECOG 2 versus 0</td>
<td>NS</td>
<td>NS</td>
<td>0.006</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td>0.08</td>
<td>0.57</td>
<td>NS</td>
</tr>
</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group.

\( P \)-values of >0.1 have been reported in this table as non-significant (NS).
quartiles of serum NTx. There was a significant effect ($P < 0.001$) for increasing serum NTx quartiles to predict for shortened survival. Serum NTx level was further evaluated as a prognostic marker in the multivariate model. In this model we compared NTx levels with clinical and demographic parameters that have been reported to be prognostic in metastatic breast cancer patients. Serum NTx continued to be a significant prognostic factor in a multivariate model, with an odds ratio of 1.71 and a $P$-value of 0.003 (Table 2). The other prognostic factors were age, performance status and adjuvant chemotherapy.

**Discussion**

It is well accepted that there is significant heterogeneity in the survival of breast cancer patients. This fact was highlighted by Rahman et al. who reported that patients with favorable characteristics had a significantly longer survival time when compared with patients with unfavorable characteristics [12]. There are a number of reports that have studied prognostic factors and correlated them with response rate, TTP and OS. The factors that have been shown to be predictive of response in patients receiving hormone therapy are receptor status, dominant site of metastasis and menopausal status [2, 13]. In addition, other variables such as age, number of metastatic sites, performance status and disease-free interval have been shown to be independent prognostic factors for OS [2, 14].

Metastatic breast cancer patients with osseous metastasis have significant heterogeneity in survival [4]. Times to disease progression and response rates are difficult to assess in patients with osseous metastasis due to the difficulty in objectively assessing bony metastasis. Coleman et al. have looked at a more rigorous clinical end point of OS. The median survival after the first recurrence of breast carcinoma in bone is 20 months, with 20% of the patients alive at 5 years. This is in marked contrast to the survival of those whose first recurrence of breast carcinoma is in the liver (3 months). In a later publication, Coleman and his colleagues reported that patients who developed visceral metastasis at a later date did poorly as compared with patients who continue to have bone-confined metastasis [15]. In this cohort of patients with bone-only or bone plus soft tissue disease, coincident with the initial presentation of their breast carcinoma, a lower histological grade, an ER+ status, a long disease-free interval (>3 versus <3 years) and premenopausal status were good prognostic factors. These baseline patient and disease characteristics are similar to the prognostic factors that have been reported earlier for all patients with metastatic breast cancer.

Bony lesions are evaluable but not measurable according to currently available radiological studies. Most studies do not report on the number of bone segments involved, but evaluate bone as a site only. Markers of bone resorption may serve as a surrogate to radiological imaging when assessing different aspects of bony involvement in patients with metastatic disease. Metabolic breakdown products of bone collagen have long been the focus of study as potential markers of bone remodeling. Bone is predominantly composed of type I bone collagen. Mature type I bone collagen contains cross-linking amino acids that stabilize the collagen fibrils in bone, thus adding tensile strength and stability to bone [16]. Peptides in the cross-linking domain of type I bone collagen are termed telopeptides. Assays have been developed that can measure the NTx and C-terminal telopeptide (ICTP, CTX) [17] in serum and urine. The NTx assay utilizes an antibody that was raised against the $\alpha$-2 chain of type I bone collagen fibrils. The NTx monoclonal antibody does not recognize the attached pyridinium cross-link. The NTx assay is commercially known as the Osteomark assay and can determine both serum and urine NTx as a specific marker of bone collagen breakdown [10].

The relationship of bone metastasis and bone markers to disease burden in patients with skeletal metastases has also been evaluated. Demers et al. [18] evaluated patients with bone metastasis and correlated the number of skeletal segments involved with the blood and urine level of markers of bone resorption and bone formation. Deoxypyridinoline (DPD), NTx, ICTP and bone-specific alkaline phosphatase (BAP) levels were correlated with the number of skeletal segments with metastatic disease in this cohort of cancer patients. Both the levels of BAP and urine NTx were significantly correlated with the number of skeletal sites involved.
with metastasis. These results suggest that the bone marker level can be used to estimate the extent of bone metastasis.

Elevated baseline serum NTx levels were associated with shorter TTP and duration of clinical benefit and survival. NTx levels continued to be a significant factor for TTP and OS when adjusted for other variables in the multivariate model. In our study, baseline serum NTx levels did not correlate with clinical benefit. The only factors predictive of clinical benefit in this group of patients were ER and PR status. In a univariate analysis, patients who expressed both ER and PR had a response rate of 52% as compared with 25% in patients who expressed ER or PR only. Our study confirms the importance of ER/PR expression in patients who have bone metastasis.

Markers of bone resorption are reflective of the surface area of bone being actively resorbed and the rate of bone turnover. A number of studies have suggested that serum NTx levels may correlate with the extent of disease in bone. Disease burden has been shown to correlate with clinical outcomes in patients with metastatic breast cancer. In this study we have shown that patients who have increased disease burden in the bone do poorly compared with those with low disease burden.

These were international, multi-center studies conducted in the pre-bisphosphonate era. The results of this retrospective analysis should be interpreted with caution. These second-line hormone therapy studies were not specifically designed to examine skeletal end points such as radiation therapy or pathological fractures. However, this is the large study and generates the interesting theory that high tumor burden as measured by serum NTxs has a negative effect on clinical outcome. Baseline serum NTx levels should be used to stratify patients in the current generation of trials studying bone metastasis. The only other way of measuring disease burden in bone is by counting bony lesions. Serum NTx levels are a convenient way of quantifying bone disease burden.

We conclude that elevated baseline serum NTx levels predict for shorter TTP and worse survival in patients with metastatic disease to bone. Serum NTx levels should be evaluated further in prospective studies. Additional bone-specific stratification factors are needed to improve our understanding of the impact of bone-targeted therapies on clinical end points like response rate, TTP and OS.

References