Serial Plasma Osteopontin Levels Have Prognostic Value in Metastatic Breast Cancer

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Abstract

Purpose: Osteopontin is a malignancy-associated protein measurable in blood and tumor tissue. To evaluate its prognostic value in advanced disease, we conducted a prospective clinical study measuring serial osteopontin plasma levels in women with metastatic breast cancer throughout the course of their disease.

Experimental Design: One hundred fifty-eight women with newly diagnosed metastatic breast cancer were enrolled in the study. Plasma osteopontin was measured using our validated ELISA, at baseline and every 3 to 12 weeks during and after therapy until death. Multivariate time-dependent survival analyses were conducted using models that right censored patient outcomes 3, 6, and 12 months after the last known osteopontin measurement.

Results: Osteopontin was measured in 1,378 samples (median, 9 per patient). Ninety-nine patients had elevated baseline osteopontin (median, 177 ng/mL; range, 1-2,648 ng/mL). In univariate analysis, elevated baseline osteopontin was associated with short survival ($P = 0.02$). In a multivariate model incorporating standard prognostic factors, baseline osteopontin was significantly associated with survival duration (relative risk, 1.001; $P = 0.038$). Metastasis-free interval, visceral metastases, and Eastern Cooperative Oncology Group status 2 to 4 also retained significance. In a multivariate model incorporating standard prognostic factors and changes in sequential osteopontin levels, an osteopontin increase of >250 ng/mL at any time was the variable with the most prognostic value for poor survival (relative risk, 3.26; $P = 0.0003$), and poor Eastern Cooperative Oncology Group status also retained significance.

Conclusions: This is the first study to show that in women with metastatic breast cancer, increases in osteopontin levels over time are strongly associated with poor survival. Sequential monitoring of osteopontin may have use in making treatment decisions for these patients.

Remarkable progress has been made in elucidating the genetics and molecular biology of breast cancer. Since the early 1990s, there are clear indications of a trend toward decreased breast cancer mortality, mainly attributable to mammography screening and better treatments for early breast cancer (1, 2). Unfortunately, many women still go on to develop distant metastases, which are the leading cause of death.

Multiple systemic therapies are now available for women with metastatic breast cancer. These do not cure the disease but are often used in sequence to improve symptoms and prolong survival while maintaining maximal quality of life. Clinical features of the disease (short disease-free interval, visceral disease, large tumor burden, lack of response to prior therapy) are most often used to forecast aggressive tumor behavior (3, 4). In this setting, early, intensive chemotherapy, and/or novel treatments, possibly accompanied by greater toxicities, may be indicated. Markers assayed in the primary tumor, such as estrogen and progesterone receptors and HER-2/*neu*, have prognostic and predictive value (3, 4) and have provided the rationale for specific drug targeting based on marker expression (5).

Tumor markers that reliably forecast aggressive behavior and poor survival, assayed in accessible tissues, such as blood, at the time of metastasis or serially during treatment (6–9), would have considerable value in identifying those women who might benefit from early intensive treatments and in suggesting the need to alter therapy if a patient's tumor is not responding. Given the many systemic therapy options, it is important to rapidly identify successful treatments and discard those that are ineffective. Although some sites of metastatic disease

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(e.g., nodes, liver and skin) are monitored relatively easily by clinical examination, other metastatic sites are more challenging (e.g., diffuse bony metastases, pleural effusions, intra-abdominal seeding, and pulmonary lymphangitis). A blood marker that is associated with response and closely follows the course of the disease (decreasing with regression and increasing with progression/relapse) would be of substantial value in managing such patients (6–8).

At present, no single blood marker has such properties. In breast cancer, the most commonly used are carcinoembryonic antigen and products of the *MUC-1* gene, such as CA15.3 or CA27.29 (6). The extracellular domain of the HER-2 receptor (ECD/HER-2) can be shed and also measured in serum or plasma (5). Tumor cells shed into the circulation from primary or metastatic cancers also may have use as future blood tumor markers (10, 11). In 1996, the American Society of Clinical Oncology convened an expert panel to create clinical practice guidelines (updated in 1997 and 2000) for the use of tumor marker tests in breast cancer (12–14). Although routine use of serum carcinoembryonic antigen, CA15.3, or CA27.29 tests were not recommended for monitoring response to treatment, in the 2000 guideline update, a caveat was added to indicate that in the absence of readily measurable disease, increasing levels of these markers might be used to suggest treatment failure. Thus, there remains a need for development and evaluation of blood markers that are prognostic for patient survival and/or are associated with response to therapy, both for use at the time of diagnosis of metastatic breast cancer and to monitor therapy over time. Here, we have tested the potential use of measuring serial blood osteopontin levels in this setting.

Osteopontin is a secreted, integrin-binding protein that has been associated with cancer and other pathologies (15–21). Osteopontin is found in blood, urine, and other body fluids (16, 22, 23), as well as in tumor tissue (24–27). Osteopontin also seems to play a functional role in breast cancer progression (17, 19, 20, 28). We developed and validated the first ELISA able to measure osteopontin in blood (23, 29–31) and found, in a cohort of 70 patients with metastatic breast cancer (29), that osteopontin plasma levels were significantly elevated, relative to healthy women or women on well follow-up after treatment of primary breast cancer. In addition, we found that higher osteopontin levels were associated with shorter survival and with larger numbers of sites of involvement.

Here, we have followed up on that study and have conducted a prospective clinical study in metastatic breast cancer, designed to evaluate whether (*a*) baseline plasma levels of osteopontin at the time of diagnosis of metastatic disease and (*b*) changes in plasma osteopontin levels over time are prognostic for survival, compared with clinical prognostic factors, in a cohort of 158 women with newly diagnosed metastatic breast cancer. The study was also designed to address the usefulness of changes in osteopontin in monitoring response to therapy, and these results will be presented in a second article.

Materials and Methods

Study population. Women with documented metastatic breast cancer, who had any site of metastasis outside the locoregional (ipsilateral breast, chest wall, axilla) area, were eligible for the study. Patients who had received previous systemic therapy for metastatic disease were not enrolled, although patients could have received previous adjuvant chemotherapy/hormone therapy. A baseline blood sample for osteopontin assessment was required within 8 weeks of the definitive diagnosis of metastases, before initiation of systemic treatment. A further baseline sample was taken immediately before initiation of systemic treatment, if >28 days had elapsed from the initial sample. Although bisphosphonates and palliative irradiation were permitted throughout the study, neither was considered a systemic treatment. The study was approved by the University of Western Ontario Health Sciences Research Ethics Board, and all women gave informed consent.

Trial design. The type of systemic treatment for metastatic disease was determined by the patient's oncologist. Follow-up visits were scheduled as clinically necessary but were usually every 3 to 4 weeks for women on active chemotherapy and every 6 to 12 weeks for patients after chemotherapy or on hormone therapy. History and physical examination were recorded at each visit. Blood for complete blood count (hemoglobin, white count and differential, platelets), biochemical screen (calcium, total protein, albumin, creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin), and osteopontin assessment was also obtained at each visit.

Overall survival was defined as the time interval between obtaining the first baseline osteopontin sample and death, and all 158 patients entered in the study were included in this analysis. Data also were collected on the type of first systemic treatment for metastatic disease and response to therapy.

Sample collection and osteopontin plasma assay. Blood samples were collected at baseline, at each visit during first systemic treatment, and every 6 to 12 weeks thereafter until death. Blood aliquots were withdrawn into tubes with EDTA anticoagulant and subsequently centrifuged at 2,000 rpm for 15 minutes at room temperature to generate plasma samples. The plasma was aliquoted into 1.5 mL Eppendorf tubes and centrifuged at 10,000 rpm for 3 minutes at 4°C to remove any white cells and debris. The plasma was transferred to fresh tubes and stored at −80°C.

Plasma samples were analyzed for osteopontin using the ELISA assay we have developed and validated (23, 29–31), as previously described. The upper limit of normal for our osteopontin assay has not been established definitively. However, in an earlier study (29), we reported (*a*) in 35 healthy women, the median plasma osteopontin level was 47 ng/mL (range, 22-122 ng/mL) and (*b*) in 44 patient controls (women on well follow-up following treatment for early breast cancer, with a minimum of 6 months following completion of all primary treatment and with no evidence of disease), the median osteopontin level was 60 ng/mL (range, 15-117 ng/mL). Similarly, in a small group of healthy men, we found that the median osteopontin level was 92 ng/mL (range, 58-123 ng/mL; ref. 30). Thus, in determining the number of patients with elevated osteopontin levels in the current study, we have considered 123 ng/mL to represent the upper limit of normal and levels above 123 ng/mL as being elevated.

Statistical analysis. A power calculation was done with the statistical package EGRET (Statistics and Epidemiology Research Group, 909 Northeast 43rd Street, Suite 202, Seattle, WA 98105) using the particular target population's known hazard function and censoring distribution. We have previously shown that osteopontin tumor positivity carries a relative risk (RR) of 2.1 for subsequent decreased survival in a sample of 154 women being treated for lymph node–negative breast cancer (26). Because the mortality rate for a population of patients being treated for metastatic breast cancer is more frequent in early follow-up than for lymph node–negative tumor patients, a study of 150 patients provides 90% power to be able to detect an increased RR of 2.0.

The initial analysis involved a univariate investigation of the relationship between baseline osteopontin levels and overall survival time. Initial investigations were conducted using a comparison of Kaplan-Meier survival curves with the Cox proportional hazards model incorporating osteopontin levels as a continuous variable. A number of

standard prognostic factors for women with metastatic breast cancer were examined in univariate analysis. These included (*a*) metastasis-free interval (time from definitive surgery for early-stage disease to diagnosis of metastases) expressed as a continuous variable; (*b*) presence of visceral metastases; (*c*) metastatic burden, using the surrogate of number of metastatic sites dichotomized as 1 versus >1; (*d*) Eastern Cooperative Oncology Group (ECOG) performance status dichotomized as 0 to 1 versus 2 to 4; (*f*) and (*g*) estrogen and progesterone receptor status dichotomized as positive versus negative. The effects of these confounding baseline variables on the relationship between baseline plasma osteopontin and outcome were investigated using a multivariate proportional hazards model (32).

To assess the prognostic value of serial measurements of plasma osteopontin in women with metastatic breast cancer, a modification of the proportional hazards model proposed by Gail (33) was used. This modification of Cox regression, which allows for the investigation of changes in blood tumor marker levels over time, can be used to determine the best predictive threshold level of change and can be used to control for confounding baseline variables (34). To protect ourselves from the problems of multiple comparisons while searching for the best predictive threshold level of change, we determined *a priori* only to undertake this investigation if our initial analysis showed that a change in osteopontin over time was predictive of outcome. Thus, after we determined that *a change* in osteopontin was predictive of outcome, we asked the question, *what level of change* was most predictive. Furthermore, we restricted this investigation to 10 levels of change.

To account for the possible effect of "time decay" (35) on the prognostic value of the serial osteopontin measurements, three distinct time-dependent models were assessed. The first model assumed that changes in osteopontin levels may only have a short-term prognostic ability; thus, patient outcomes were right censored at 3 months after the last available osteopontin measurement. To investigate medium-term and longer-term prognostic ability, two additional models were created with right censoring of patient outcomes at 6 and 12 months after final osteopontin measurement, respectively. The baseline osteopontin values and serial changes in osteopontin were found to have significant prognostic value in all three models; thus, only the longer-term model (right censoring at 12 months) is reported throughout.

Effect of potential bias due to missing values. To assess potential bias arising in multivariate models due to missing covariates, primary outcomes for patients with missing values were compared with primary outcomes of patients without missing values. These comparisons were made on a covariate-by-covariate basis (e.g., direct comparison of patients with complete ECOG status to patients with missing ECOG status). If patients with missing values are found to have systematically different outcomes, it can be concluded that missing values are likely not a random event; thus, the models may be biased. If outcomes are not systematically different, we conclude that it is reasonably unlikely that missing values have biased the mulitvariate models.

Table 1. Study population: patient characteristics at breast cancer diagnosis

Stage	
I	17 (11%)
II	78 (49%)
III	14 (9%)
IV	35 (22%)
Missing*	14 (9%)
Histology	
Invasive ductal	137 (87%)
Invasive lobular	18 (11%)
Other	3 (2%)
ER/PR	
Both positive	80 (51%)
Either positive	26 (16%)
Both negative	24 (15%)
Unknown	28 (18%)
Treatment of primary cancer	
Surgery	158
Partial mastectomy ± node dissection	47 (30%)
Mastectomy ± node dissection	88 (55.5%)
Biopsy only	23 (14.5%)
Radiotherapy (adjuvant) †	55 (35%)
Breast ± nodes	33 (21%)
Chest wall ± nodes	20 (13%)
Nodes only	2 (1%)
Chemotherapy (adjuvant) †	54 (34%)
CMF	27 (17%)
CEF	17 (11%)
AC	3 (2%)
Other	7 (4%)
Hormone (adjuvant) †	55 (35%)
Tamoxifen	48 (30.5%)
MA-12 trial (tamoxifen vs placebo)	5 (3%)
MA-14 trial (tamoxifen ± Octreotide)	2 (1%)

Abbreviations: PR, progesterone receptor; ER, estrogen receptor; CMF, cyclophosphamide/methotrexate/fluorouracil; CEF, cyclophosphamide/epirubicin/fluorouracil; AC, cyclophosphamide/doxorubicin.
*Six missing T value, five missing N value, three missing both T and N value.
† Some patients received more than one of these treatments.

Results

Study population. Between July 1997 and November 1999, 158 women with metastatic breast cancer seen at the London Regional Cancer Program were recruited to our study. Their median age was 61 years (range, 20-84 years), and 138 (87%) were postmenopausal. Thirty-five women (22%) were found to have metastases at first presentation of breast cancer. The remaining 123 women (78%) had a previous diagnosis of early-stage breast cancer and subsequently developed metastases. The median time from definitive diagnosis of breast cancer to first metastasis was 36 months (range, 0-397 months). Data on initial stage, histology, receptor status, and previous treatment (for primary disease) are summarized in Table 1.

At study registration (baseline), all patients had at least one site of distant metastasis, and 17 (11%) had concurrent locoregional recurrence. Table 2 summarizes the patient characteristics at baseline, at the time of diagnosis of metastatic disease. The database was closed for analysis in July 2003. At this time, 26 women (16.5%) were still alive, but all patients had completed their first systemic treatment. The types of first systemic treatment delivered for metastatic disease are outlined in Table 3. First systemic treatment for this group of patients was hormone therapy in 111 (70%) of cases and chemotherapy in 43 (27%). Concurrent bisphosphonates were given to 51 women (32%). Four women declined all systemic treatment. Most patients received a number of subsequent palliative chemotherapy and/or hormone therapies between the time of registration until death. Median survival from date of registration was 20 months.

Table 2. Study population: characteristics at time of metastatic disease

ECOG performance status	
0-1	87 (55%)
2-4	46 (29%)
Missing	25 (16%)
Presence of visceral disease*	
Yes	116 (74%)
No	41 (26%)
No. organ sites involved	
1	78 (49%)
2	42 (27%)
3	27 (17%)
≥4	11 (6%)
Distribution of disease	
Local recurrence	17
Metastatic sites	
Bone	105
Liver	44
Lung	40
Nodes	37
Effusion/ascites	33
Skin	16
Brain	4
Bone marrow	3
Other	3

*Any of lung, liver, brain, pleural effusion, ascites (one mssing data).

Osteopontin plasma samples and results. Throughout the course of the study, a total of 1,378 blood samples were obtained from 157 patients (one patient withdrew). The median number of samples per patient was 9 (range, 1-26), and the interquartile range was 3 to 13. Only a baseline blood sample was obtained from 15 patients because of early death (1-8 weeks) in eight, and loss to follow-up in seven. In patients with multiple samples, the median interval between samples was 57 days (range, 3-337 days), and the interquartile range was 31 to 79 days. The median time interval from first osteopontin measurement to last was 13 months (range, 1-61 months), and the interquartile range was 3 to 30 months. The median time interval from last osteopontin measurement to death was 2 months (range, 1-35 months), and the interquartile range was 1 to 4 months. Baseline osteopontin levels ranged from 1 to 2,648 ng/mL with a median value of 177 ng/mL, and 99 (63%) women had elevated levels (above 123 ng/mL).

In univariate analysis, baseline levels of osteopontin RR [1.001; 95% confidence interval (95% CI), 1.000-1.001; P = 0.02] were inversely and significantly associated with duration of survival, right censored at 12 months from final osteopontin measurement. Of the clinical prognostic factors investigated in univariate analysis, short metastasis-free interval (RR, 0.966; 95% CI, 0.935-0.997; P = 0.032), the presence of visceral metastases (RR, 2.012; 95% CI, 1.418-2.856; P = 0.0001), ECOG status 2 to 4 (RR, 1.984; 95% CI, 1.348-2.919; P = 0.0005), and metastatic burden (more than one metastatic site; RR, 1.761; 95% CI, 1.246-2.489; P = 0.0014) were significantly associated with outcome.

The relationship between baseline osteopontin values and survival is shown graphically in Fig. 1. Patients were divided into those whose baseline osteopontin values were elevated relative to the upper level of normal values (123 ng/mL; refs. 29, 30) versus those whose baseline osteopontin values were below this value. Patients with elevated baseline osteopontin plasma values had significantly poorer survival than those whose initial osteopontin values were not elevated (P = 0.0012).

In a multivariate model incorporating all standard baseline prognostic factors, baseline osteopontin level was a significant independent prognostic factor for survival duration (RR, 1.001; 95% CI, 1.000-1.001; P = 0.038) in this group of women with metastatic breast cancer (Table 4). Although a RR increase of 1.001 may seem small, every unit (ng/mL) difference in osteopontin between patients at baseline results in a compounded 0.1% relative increase in hazard. Because baseline osteopontin ranges from 1 to 2,648 ng/mL between patients, this compounded increase in risk can be considerable. Metastasis-free interval (RR, 0.95; 95% CI, 0.901-0.999; P = 0.048), the presence of visceral metastases (RR, 2.55; 95% CI, 1.489-4.350; P = 0.0006), and ECOG performance status of 2 to 4 (RR, 1.94; 95% CI, 1.169-3.213; P = 0.01) also retained significance as independent prognostic factors.

We went on to examine the association between sequential osteopontin levels and survival duration. Figure 2 illustrates that most patients showed steadily increasing osteopontin levels over the last five samples taken before death. The median time from the collection of the final osteopontin sample to death was 2.3 months (range, 0.2-35.5 months). Formal analysis of changes in osteopontin over time, controlling for the initial baseline osteopontin level, showed that any increase in osteopontin levels between subsequent readings conferred an increased hazard to survival duration (RR, 1.58; 95% CI, 1.044-2.399; P = 0.03).

We then went on to examine the use of the magnitude of changes in osteopontin values at sequential samplings. Exploratory regression models examining osteopontin increases of at least 50, 100, 150, 250, 300, 350, 400, 450, 500, and 1,000 ng/mL showed that an osteopontin increase of at least 250 ng/mL showed the strongest association with survival (RR, 4.98; 95% CI, 2.737-9.059; P = 0.0001). Fifty-three patients experienced an osteopontin increase of >250 ng/mL at some time during the study period, and this cutoff was used in the subsequent multivariate models.

When all 109 patients with all known prognostic variables were offered to a maximal model (Table 5), osteopontin

Table 3. First systemic treatment for metastatic breast cancer

First systemic treatment	158
Hormone	111 (70%)
Antiestrogen	78 (49%)
Aromatase inhibitor	32 (20%)
Progestogen	1
Chemotherapy	43 (27%)
Anthracycline combination	32 (20%)
Taxane	5 (3%)
Other	6 (4%)
None	4 (3%)

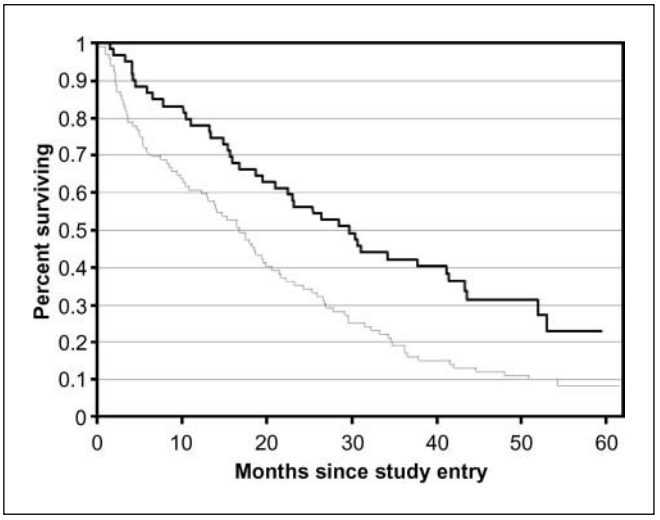

Fig. 1. Product limit survival curve for baseline plasma osteopontin. Kaplan-Meier product limit survival curves for the entire study population by plasma osteopontin level at study entry (baseline osteopontin). Black line, baseline osteopontin <123 ng/mL. Gray line, baseline osteopontin >123 ng/ml.

increase of >250 ng/mL proved to be the variable with the most prognostic value for poor survival (RR, 3.26; 95% CI, 1.716-6.198; P = 0.0003). Poor ECOG performance status was the only other variable retaining significance (RR, 1.99; 95% CI, 1.034-3.843; P = 0.039). The median time from the first recorded osteopontin increase of >250 ng/mL to death was 9.3 months (range, 0.4-52 months).

Effect of potential bias due to missing values. Of the 157 patients eligible for assessment in various models, 16 patients had missing metastasis-free intervals, 25 had missing ECOG status, 22 had missing estrogen receptor status, and 28 had missing progesterone receptor status. There were no significant differences, or trends towards significant differences, in survival duration between patients with missing variables and complete data sets.

Discussion

Since the first description of a transformation-specific secreted phosphoprotein produced by transformed cell lines in culture (36), which subsequently became known as osteopontin, increasing evidence has accumulated for its role in tumorigenicity and metastasis, both as a functional contributor and as a potential tumor marker (17, 19, 25). Several studies have shown that osteopontin levels may be elevated in cancer, both in tumor tissue (24–27, 37–39) and in patients' blood (22, 29–31, 40, 41). Some studies also have examined the relationship between osteopontin and patient outcome and have presented evidence that elevated blood osteopontin of patients with various tumors and in their primary tumor tissue may be associated with poor prognosis and reduced survival in breast and other tumors.

In 1997, we used the osteopontin ELISA that we had developed previously (23) and reported that plasma osteopontin levels were elevated in a series of 70 women with metastatic breast cancer (median, 142 ng/mL; range, 138-1,312 ng/mL), relative to a group of 35 healthy women (median, 47 ng/mL; range, 22-122 ng/mL), and patient controls (women who were 6 months posttreatment for primary breast cancer, with no evidence of disease at the time of sampling; median, 60 ng/mL; range, 15-117 ng/mL; P < 0.001; ref. 29). In that study, we found that elevated osteopontin was associated with shorter survival in this group of patients with metastatic breast cancer, when they were grouped into terciles for osteopontin levels (P < 0.001). Furthermore, when plasma osteopontin was considered as a continuous variable in a Cox proportional hazards model, there was a strong association between increased osteopontin and decreased survival (P < 0.0001).

Osteopontin blood levels have also been reported to be elevated by others in various cancers. Fedarko et al. (40) reported elevated serum levels of osteopontin in breast, lung, and prostate cancers, although not in colon cancers (studying 20 cases for each cancer). Similarly, Kim et al. (41) reported that plasma osteopontin levels were significantly higher (P < 0.001) in 51 patients with epithelial ovarian cancer (487 ng/mL) compared with 107 healthy controls (147 ng/mL), and Saeki et al. (42) reported similar findings in 30 cases of multiple myeloma. In a series of 100 men with hormone-refractory prostate cancer, our group (30) has shown that osteopontin plasma levels correlate independently and negatively with overall survival (P = 0.029). The median osteopontin level in patients that study was 198.5 ng/mL (range, 15-2,363 ng/mL). Plasma osteopontin level was also found to be an independent prognostic factor for survival in 54 patients with squamous carcinomas of the head and neck (43). Recently, our group reported that osteopontin plasma levels were elevated in 72 patients with transitional cell carcinoma of the bladder and were associated with disease stage (31).

Here, we measured plasma osteopontin in a cohort of 157 women with newly diagnosed metastatic breast cancer. Baseline osteopontin was measured within 8 weeks of diagnosis of metastatic breast cancer and before initiation of systemic treatment. We also measured sequential osteopontin plasma levels in these patients, at each clinic visit during systemic therapy and subsequently every 6 to 12 weeks until death. We have confirmed and extended data from our initial pilot study (29) of 70 unselected women with metastatic breast cancer. Median osteopontin levels in our current and the previous study (29) were 177 ng/mL (range, 1-2,648) and 142 ng/mL (range, 138-1,312 ng/mL), respectively. The higher baseline

Table 4. Multivariate analysis (109 patients) exploring the relationship between baseline osteopontin level and duration of survival in women with metastatic breast cancer

Variable	RR of death (95% CI)	*P*
Baseline osteopontin value	1.001 (1.000-1.001)	0.0375
Visceral metastases	2.545 (1.489-4.350)	0.0006
ECOG status 2-4	1.938 (1.169-3.213)	0.0103
Metastasis-free interval	0.949 (0.901-0.999)	0.0474
Metastatic burden	0.920 (0.546-1.551)	0.7544
PR positive	0.794 (0.451-1.397)	0.4237
ER positive	0.623 (0.365-1.066)	0.0843

Abbreviations: PR, progesterone receptor; ER, estrogen receptor.

median level and range in our current study may reflect a higher burden of disease before patients started on systemic therapy for metastatic cancer. In contrast, all patients in our pilot study were receiving treatment for metastatic disease.

In multivariate survival analyses, we were able to confirm the prognostic value, in patients with established metastatic breast cancer, of clinical features, such as metastasis-free interval, presence of visceral metastases, and ECOG performance status (3, 4); in addition, baseline osteopontin levels were inversely and significantly associated with overall survival. Furthermore, measurement of osteopontin levels over time identified a significant relationship between increasing osteopontin levels and risk of death, with a rapid increase observed in the final few months of life. In exploratory multivariate analysis, an increase in osteopontin level of >250 ng/mL proved to be the strongest prognostic factor for overall survival, with an increase in risk of death of 3.26 and a median duration of survival of only 9 months.

There is limited and often conflicting information on the prognostic value of blood markers in women with metastatic breast cancer (12–14, 44, 45), and the most convincing data relate to ECD/HER2. In common with ECD/HER2, there is a sound biological rationale supporting a potential role for osteopontin as a blood marker of poor outcome in patients with cancer (17, 19, 20). As for ECD/HER2, our study has shown that measurement of baseline osteopontin levels in patients with metastatic breast cancer provides prognostic information beyond conventional clinical features. In addition, blood osteopontin measurement has potential application in other cancers (30, 31, 41, 43).

There are limitations of this current study that warrant consideration. First, it is important to note that not all enrolled patients could be included in the multivariate analysis. A total of 31 patients were missing key prognostic factors (e.g., ECOG performance status and estrogen receptor and progesterone receptor status) that were essential covariates in the multivariate model. In our study, we obtained these measures retrospectively in that we relied upon the primary clinician responsible for the care of the patient to record these factors, either at the time of primary diagnosis (estrogen receptor and progesterone receptor) or at baseline assessment for metastatic disease (ECOG performance status). Although analysis of patients with missing values did not reveal any systematic differences in outcomes, and thus their loss from the multivariate model was not likely to cause bias, we would recommend that future observational studies attempt to obtain key prognostic factors prospectively whenever possible. Next, it has been noted that not all patients returned at scheduled time intervals to submit subsequent plasma samples for osteopontin. This can be a problem with all observational studies conducted in populations of unwell patients and may increase when patients become more unwell. One of the strengths of the analytic approach we employed is that it does not assume that all patients submitted samples at equal time intervals. In this respect, the results of our analysis may be more robust in that the clinical application of our conclusions may not require rigidly scheduled clinic visits.

Table 5. Multivariate analysis (109 patients) exploring factors prognostic for duration of survival in women with metastatic breast cancer, including osteopontin increase of >250 ng/mL

Variable	RR of death (95%CI)	*P*
Osteopontin increase of >250 ng/mL	3.261 (1.716-6.198)	0.0003
Baseline osteopontin value	1.000 (0.999-1.001)	0.8171
ECOG status 2-4	1.993 (1.034-3.843)	0.0394
Visceral metastases	1.656 (0.822-3.337)	0.1585
Metastasis-free interval	1.001 (0.938-1.069)	0.9661
Metastatic burden	1.010 (0.480-2.127)	0.9791
PR positive	0.861 (0.434-1.712)	0.6703
ER positive	0.620 (0.321-1.197)	0.1546

Abbreviations: PR, progesterone receptor; ER, estrogen receptor.

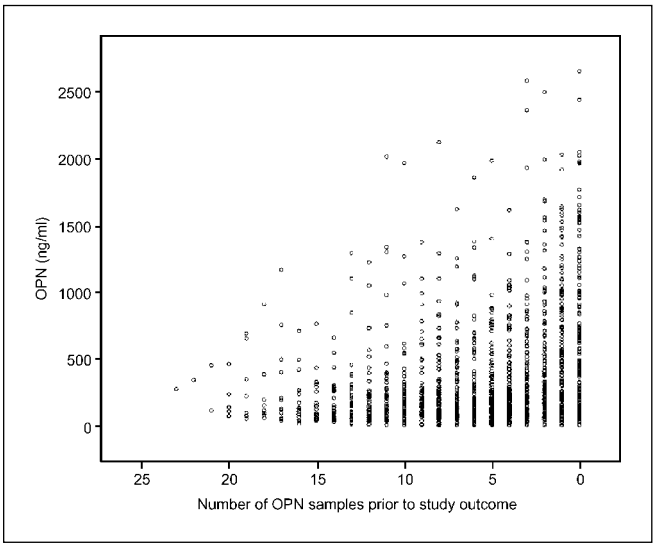

Fig. 2. Increase in osteopontin (OPN) level before mortality. This plot shows increasing plasma osteopontin levels over time in patients who died during the study. Sample number is indexed to the time of death (0 = last sample obtained before death).

Our data show that serial measurements of osteopontin over time in women with metastatic breast cancer enhance its prognostic value. Further work is needed to determine whether this can translate into a test that has clinical relevance based on the tumor marker use grading system described by Hayes et al. (46, 47). The results presented here support the hypothesis that osteopontin plasma levels have potential use in metastatic breast cancer, with initial levels at the time of diagnosis being associated with patient survival. Furthermore, our study is the first to show that increases in osteopontin plasma levels over time are strongly prognostic (RR, 3.26; for any increase of >250 ng/mL between clinic visits) for poor survival. This finding suggests that sequential monitoring of osteopontin plasma levels in patients being treated for metastatic breast cancer may have use in making treatment decisions for these patients. Due to the potential clinical use, we strongly recommend this novel finding be confirmed in subsequent studies.

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References

1. National Cancer Institute of Canada. Canadian cancer statistics 2005. [cited; http://www.cancer.ca/stats/].

2. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365:1687–717.

3. Winer EP, Morrow M, Osborne CK, Harris JR. Malignant tumors of the breast. In: DeVita VT, Hellman S, Rosenberg SA, editors. Principles and practice of oncology. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2001. p. 1651–717.

4. Chang J, Clark GM, Allred DC, Mohsin S, Chamness G, Elledge RM. Survival of patients with metastatic breast carcinoma: importance of prognostic markers of the primary tumor. Cancer 2003;97:545–53.

5. Yamauchi H, Stearns V, Hayes DF. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J Clin Oncol 2001;19:2334–56.

6. Stearns V, Yamauchi H, Hayes DF. Circulating tumor markers in breast cancer: accepted utilities and novel prospects. Breast Cancer Res Treat 1998;52:239–59.

7. Duffy MJ. Evidence for the clinical use of tumour markers. Ann Clin Biochem 2004;41:370–7.

8. Seregni E, Coli A, Mazzucca N. Circulating tumour markers in breast cancer. Eur J Nucl Med Mol Imaging 2004;31 Suppl 1:S15–22.

9. Bast RC, Jr., Lilja H, Urban N, et al. Translational crossroads for biomarkers. Clin Cancer Res 2005;11:6103–8.

10. Muller V, Pantel K. Bone marrow micrometastases and circulating tumor cells: current aspects and future perspectives. Breast Cancer Res 2004;6:258–61.

11. 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;23:1420–30.

12. Clinical practice guidelines for the use of tumor markers in breast and colorectal cancer. Adopted on May 17, 1996 by the American Society of Clinical Oncology. J Clin Oncol 1996;14:2843–77.

13. 1997 update of recommendations for the use of tumor markers in breast and colorectal cancer. Adopted on November 7, 1997 by the American Society of Clinical Oncology. J Clin Oncol 1998;16:793–5.

14. Bast RC, Jr., Ravdin P, Hayes DF, et al. 2000 update of recommendations for the use of tumor markers in breast and colorectal cancer: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol 2001;19:1865–78.

15. Oates AJ, Barraclough R, Rudland PS. The role of osteopontin in tumorigenesis and metastasis. Invasion Metastasis 1997;17:1–15.

16. Rittling SR, Denhardt DT. Osteopontin function in pathology: lessons from osteopontin-deficient mice. Exp Nephrol 1999;7:103–13.

17. Rittling SR, Chambers AF. Role of osteopontin in tumour progression. Br J Cancer 2004;90:1877–81.

18. Sodek J, Ganss B, McKee MD. Osteopontin. Crit Rev Oral Biol Med 2000;11:279–303.

19. Furger KA, Menon RK, Tuck AB, Bramwell VH, Chambers AF. The functional and clinical roles of osteopontin in cancer and metastasis. Curr Mol Med 2001;1:621–32.

20. Tuck AB, Chambers AF. The role of osteopontin in breast cancer: clinical and experimental studies. J Mammary Gland Biol Neoplasia 2001;6:419–29.

21. Gravallese EM. Osteopontin: a bridge between bone and the immune system. J Clin Invest 2003;112:147–9.

22. Senger DR, Perruzzi CA, Gracey CF, Papadopoulos A, Tenen DG. Secreted phosphoproteins associated with neoplastic transformation: close homology with plasma proteins cleaved during blood coagulation. Cancer Res 1988;48:5770–4.

23. Bautista DS, Saad Z, Chambers AF, et al. Quantification of osteopontin in human plasma with an ELISA: basal levels in pre- and postmenopausal women. Clin Biochem 1996;29:231–9.

24. Brown LF, Papadopoulos-Sergiou A, Berse B, et al. Osteopontin expression and distribution in human carcinomas. Am J Pathol 1994;145:610–23.

25. Agrawal D, Chen T, Irby R, et al. Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. J Natl Cancer Inst 2002;94:513–21.

26. Tuck AB, O'Malley FP, Singhal H, et al. Osteopontin expression in a group of lymph node negative breast cancer patients. Int J Cancer 1998;79:502–8.

27. Coppola D, Szabo M, Boulware D, et al. Correlation of osteopontin protein expression and pathological stage across a wide variety of tumor histologies. Clin Cancer Res 2004;10:184–90.

28. Wai PY, Kuo PC. The role of osteopontin in tumor metastasis. J Surg Res 2004;121:228–41.

29. Singhal H, Bautista DS, Tonkin KS, et al. Elevated plasma osteopontin in metastatic breast cancer associated with increased tumor burden and decreased survival. Clin Cancer Res 1997;3:605–11.

30. Hotte SJ, Winquist EW, Stitt L, Wilson SM, Chambers AF. Plasma osteopontin: associations with survival and metastasis to bone in men with hormone-refractory prostate carcinoma. Cancer 2002;95:506–12.

31. Ang C, Chambers AF, Tuck AB, Winquist E, Izawa JI. Plasma osteopontin levels are predictive of disease stage in patients with transitional cell carcinoma of the bladder. Br J Urol Int 2005;96:803–5.

32. Katz MH, Hauck WW. Proportional hazards (Cox) regression. J Gen Intern Med 1993;8:702–11.

33. Gail MH. Evaluating serial cancer marker studies in patients at risk of recurrent disease. Biometrics 1981;37:67–78.

34. Fisher LD, Lin DY. Time-dependent covariates in the Cox proportional-hazards regression model. Annu Rev Public Health 1999;20:145–57.

35. Altman DG, De Stavola BL. Practical problems in fitting a proportional hazards model to data with updated measurements of the covariates. Stat Med 1994;13:301–41.

36. Senger DR, Wirth DF, Hynes RO. Transformed mammalian cells secrete specific proteins and phosphoproteins. Cell 1979;16:885–93.

37. Chambers AF, Wilson SM, Kerkvliet N, O'Malley FP, Harris JF, Casson AG. Osteopontin expression in lung cancer. Lung Cancer 1996;15:311–23.

38. Rudland PS, Platt-Higgins A, El-Tanani M, et al. Prognostic significance of the metastasis-associated protein osteopontin in human breast cancer. Cancer Res 2002;62:3417–27.

39. Bellahcene A, Castronovo V. Increased expression of osteonectin and osteopontin, two bone matrix proteins, in human breast cancer. Am J Pathol 1995;146:95–100.

40. Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW. Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. Clin Cancer Res 2001;7:4060–6.

41. Kim JH, Skates SJ, Uede T, et al. Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA 2002;287:1671–9.

42. Saeki Y, Mima T, Ishii T, et al. Enhanced production of osteopontin in multiple myeloma: clinical and pathogenic implications. Br J Haematol 2003;123:263–70.

43. Le QT, Sutphin PD, Raychaudhuri S, et al. Identification of osteopontin as a prognostic plasma marker for head and neck squamous cell carcinomas. Clin Cancer Res 2003;9:59–67.

44. Ali SM, Leitzel K, Chinchilli VM, et al. Relationship of serum HER-2/*neu* and serum CA 15–3 in patients with metastatic breast cancer. Clin Chem 2002;48:1314–20.

45. Lipton A, Ali SM, Leitzel K, et al. Serum HER-2/*neu* and response to the aromatase inhibitor letrozole versus tamoxifen. J Clin Oncol 2003;21:1967–72.

46. Hayes DF. Designing tumor marker studies: will the results provide clinically useful information. Arch Pathol Lab Med 2000;124:952–4.

47. Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456–66.