Bone metastasis is an important issue when treating patients with breast cancer. At postmortem examination, the incidence of bone metastasis in breast cancer patients was as high as 70% (1). In breast cancer, patients with metastasis confined to bones may have a prolonged clinical course. Pain and pathologic fracture are the major complications of bone metastasis and can significantly debilitate the life quality of patients (1, 2).

The complications of bone metastasis are believed to be associated with the disruption of the normal coupling between osteoblasts and osteoclasts (6, 7). This disruption is thought to be caused by tumor-derived humoral mediators produced by metastasized cancer cells within the bone marrow. In most breast cancer patients with bone metastasis were determined. These patients were treated and followed up as clinically indicated. Fifty-four healthy women were recruited as control. Serum TRACP5b activity, BAP activity, and NTX level of breast cancer patients with bone metastasis were significantly higher than those of normal controls. In normal subjects, serum TRACP5b activity and NTX level are significantly correlated ($P < 0.0001$). Neither was correlated with BAP activity. In breast cancer patients with bone metastasis, all marker pairs correlated to each other significantly ($P < 0.0001$). Biomarkers were examined repeatedly in 38 patients who were evaluable for treatment response. Based on clinical criteria, 20 patients were responders and 18 were nonresponders. In the 20 responders, serum TRACP5b activity and NTX level decreased significantly ($P < 0.0001$ and 0.0107, respectively) after treatment. In the 18 nonresponders, only NTX level showed significant increase ($P = 0.0342$) after treatment; TRACP5b and BAP were unchanged. By means of multiple logistic regression with stepwise selection, we determined that TRACP5b activity has a higher probability than NTX level to indicate treatment response as a function of percent change after treatment (18 times versus 12 times). Our data support the use of either TRACP5b activity or NTX level to follow up breast cancer patients with bone metastasis after treatment instead of the prevailing BAP activity. (Cancer Epidemiol Biomarkers Prev 2006;15(3):424–8)

**Introduction**

Bone metastasis is an important issue when treating patients with breast cancer. At postmortem examination, the incidence of bone metastasis in breast cancer patients was as high as 70% (1). In breast cancer, patients with metastasis confined to bones may have a prolonged clinical course. Pain and pathologic fracture are the major complications of bone metastasis and can significantly debilitate the life quality of patients (1, 2).

The complications of bone metastasis are believed to be associated with the disruption of the normal coupling between osteoblasts and osteoclasts (6, 7). This disruption is thought to be caused by tumor-derived humoral mediators produced by metastasized cancer cells within the bone marrow. In most cases, the osteoclasts are preferentially activated and result in bone destruction (8). Radiologically, bone metastasis from breast cancer is usually seen as a mixture of osteolytic and osteosclerotic lesions. Pathologic examination of a metastatic bony lesion typically reveals the presence of breast cancer cells accompanied by increased numbers of osteoclasts/osteoblasts in the vicinity (8). The activated osteoclasts may erode bone heavily; however, the efficiency of new bone formation by osteoblasts does not keep pace, resulting in net bone loss.

Conventionally, the diagnosis of bone metastasis in breast cancer patients starts from the appearance of clinical symptoms. It is then confirmed by roentgenography and/or bone scintigraphy (9). Biochemical markers of osteoblasts/osteoclasts, including bone alkaline phosphatase (BAP) activity and NH$_2$-terminal telopeptide of type 1 collagen (NTX), may provide greater diagnostic sensitivity (10, 11). Recently, tartrate-resistant acid phosphatase 5b (TRACP5b) has been recognized as a marker of osteoclasts (12, 13). There are two isoforms of type 5 TRACP in human serum, 5a and 5b (14). Serum TRACP5b is a proteolytically cleaved form with disulfide-linked polypeptide subunits of 16 and 23 kDa. One important difference between these two isoforms is the presence of sialic acid in 5a but not in 5b. Purified human osteoclastic TRACP is type 5b. Using immunometric assays to increase specificity, we and others were also able to show that TRACP5b is a sensitive and specific marker for bone metastasis in breast cancer patients (15, 16).

This study aims to evaluate the sensitivity of our immunoassay to serum TRACP5b activity in monitoring treatment results of bone metastasis in breast cancer patients compared with that of serum BAP activity and NTX levels.

**Materials and Methods**

Patients. Sixty-eight breast cancer patients with newly diagnosed bone metastasis seen in the Division of Hematology/Oncology of the Tri-Service General Hospital between December 2000 and July 2002 were studied after informed consent...
had been obtained. Patients ranged in age from 28 to 78 years (mean, 51 years). Clinical symptoms, radiological features, and \(^{99m}\text{Tc}\)-hydroxy-methylene-diphosphonate \((^{99m}\text{Tc}\text{-MDP})\) whole-body bone scintigraphy have determined the onset of bone metastasis. All breast cancer patients with bone metastasis received chemotherapy, hormone therapy, radiation therapy, or bisphosphonate therapy as clinically indicated. These patients were followed monthly with physical check-up. Tumor marker studies including carcinoembryonic antigen and CA 15.3 were determined every 3 months. Image studies including roentgenology, sonography, computed tomography, and bone scintigraphy were done as clinically indicated. Treatment responders must have obtained improvement of clinical symptoms of bone pain plus at least one of the following variables: >50% decrease in both serum carcinoembryonic antigen and CA 15.3 levels or return to normal if these markers were abnormally high before treatment; >50% decrease in tumor size in those patients with measurable tumors; and improvement of bone scintigraphic finding. For those patients whose bone scintigraphy showed worsening changes but the other clinical and biochemical variables showed improvement were still considered responders. All other patients were defined as nonresponders. Fifty-four healthy women of ages 21 to 77 years (mean, 49 years) were recruited as a control group after informed consent was obtained. The institutional review board of the Tri-Service General Hospital approved this study.

**Collection of Serum and TRACP5b Activity Assay.** Serum of each patient was collected monthly for 1 year after entering this study or until death. For normal healthy women, sera were collected once on entering the study. Venous blood was drawn, allowed to clot at room temperature for 30 to 60 minutes, then stored for no more than 4 hours at 4°C before centrifugation to collect serum. All sera were then stored at −80°C for no more than 1 month and thawed at room temperature immediately before TRACP5b activity was measured.

**Serum TRACP5b Activity Assay.** Osteoclastic TRACP5b activity was measured by a ligand capture immunosassay as previously reported (15). Briefly, this assay uses a TRACP5b-specific antibody (14G6) to immobilize serum TRACP. The bound TRACP5b activity is subsequently estimated using 4-nitrophenyl phosphate as substrate at pH 6.1. Results are reported as micromoles of substrate hydrolyzed per minute per liter of serum at 37°C (μmol/min/L). At this pH, the contribution by serum TRACP5a activity is minimized whereas that of TRACP5b remains high. With this assay, the biochemical specificity for TRACP5b is ~90% (12). The clinical specificity and sensitivity for both osteoporosis and extensive bone metastasis in breast cancer have previously been reported (17, 18). The analytic precision was estimated as the mean percent coefficient of variation (CV) for duplicate measurements of samples. The intraassay error was determined by assay of aliquots of six sera ranging in activity from 2.54 to 9.37 μmol/min/L. The average CV was calculated to be 3.9%. The intra-assay error was determined by simultaneous assay of eight duplicates of five sera ranging in activity from 2.50 to 11.0 μmol/min/L; the average CV was calculated to be 5.1% (18). The mean ± SD TRACP5b activity obtained from 427 breast cancer patients with bone metastasis was 2.58 ± 0.95 μmol/min/L (18).

**BAP Activity Assay and NTX Assay.** Serum BAP activity is an indicator of osteoblastic activity and was measured by a commercially available quantitative enzyme-linked immunoassay (METRA BAP ELA kit, Quidel Corp., San Diego, CA) in which serum BAP is immobilized by specific antibody and its activity measured using 4-nitrophenyl phosphate as substrate. Results are expressed as moles of substrate hydrolyzed per minute per liter of serum at room temperature (mol/min/L).

The ranges of the BAP activities in healthy women of ages >45 years were 14.2 to 42.7 mol/min/L with a median of 25 mol/min/L (provided by the assay producer). Serum NTX is a product of type I collagen degradation and an indicator of bone resorption. Serum NTX was measured by a commercially available quantitative competitive-inhibition ELISA (Osteomark NTX Serum, Ostex International, Inc., Seattle, WA). Results are expressed as nanomoles of bone collagen equivalents per liter of serum (nmol/L BCE/L). The ranges of the NTX serum levels in healthy women were 6.2 to 19 nmol/L BCE/L with a mean of 12.6 nmol/L BCE/L (provided by the assay producer).

**Statistical Analysis.** All descriptive data are expressed as median (range). Two nonparametric methods, Wilcoxon rank-sum test (PROC NPARIWAY, SAS 9.1) and Wilcoxon signed-rank test (PROC UNIVARIATE, SAS 9.1), were used to assess the independent groups (responder versus nonresponder) and treatment effects (before versus after) in each group, respectively. To avoid collinearity among the independent variables, collinearity diagnostic analysis was done with the following criteria: tolerance >0.4 or variance inflation <2.5, and condition number <10. There was not any collinearity among the independent variables.
Table 1. Age, duration of treatment, and concentrations between responders and nonresponders

<table>
<thead>
<tr>
<th>Variable</th>
<th>Responders (n = 20)</th>
<th>Nonresponders (n = 18)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>47.5 (30-78)</td>
<td>46.5 (40-61)</td>
<td>0.8112</td>
</tr>
<tr>
<td>Duration (d)</td>
<td>147.0 (34-910)</td>
<td>149.0 (30-995)</td>
<td>0.9957</td>
</tr>
<tr>
<td>TRACP5b before treatment (µmol/min/L)</td>
<td>6.5 (2.5-15.0)</td>
<td>4.7 (2.2-12.2)</td>
<td>0.0476</td>
</tr>
<tr>
<td>BAP before treatment (mol/min/L)</td>
<td>47.9 (19.0-271.2)</td>
<td>50.6 (15.8-257.7)</td>
<td>0.7779</td>
</tr>
<tr>
<td>NTX before treatment (nmol/L BCE/L)</td>
<td>19.5 (10.0-82.2)</td>
<td>13.4 (6.9-114.9)</td>
<td>0.0873</td>
</tr>
<tr>
<td>TRACP5b after treatment (µmol/min/L)</td>
<td>2.9 (1.7-9.4)</td>
<td>4.4 (2.3-16.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BAP after treatment (mol/min/L)</td>
<td>45.6 (17.3-475.1)</td>
<td>62.1 (12.3-226.4)</td>
<td>0.2765</td>
</tr>
<tr>
<td>NTX after treatment (nmol/L BCE/L)</td>
<td>16.1 (4.6-36.2)</td>
<td>19.5 (8.6-79.1)</td>
<td>0.0930</td>
</tr>
</tbody>
</table>

*Data were assessed by Wilcoxon rank-sum two-sample test with two-sided exact tests (PROC NPAR1WAY, SAS 9.1).

Results

Serum TRACP5b Activity, BAP Activity, and NTX Level in Breast Cancer Patients with Bone Metastasis and Healthy Women. The median of serum TRACP5b activity, BAP activity, and NTX level in 68 breast cancer patients with newly diagnosed bone metastasis were 5.301 µmol/min/L, 66.04 mol/min/L, and 23.60 nmol/L BCE/L, respectively. The median of serum TRACP5b activity, BAP activity, and NTX level in 54 healthy women were 2.680 µmol/min/L, 37.30 mol/min/L, and 10.16 nmol/L BCE/L, respectively. As shown in Fig. 1, the median values of all these three bone metabolic markers are significantly higher in breast cancer patients with bone metastasis than in healthy women (P < 0.0001). The TRACP5b activity in normal subjects is significantly correlated with the NTX level (P = 0.0021) but it is not correlated with BAP activity (P = 0.2713). The NTX level is not correlated with BAP activity (P = 0.4956). In the 68 breast cancer patients with bone metastasis before any treatment, TRACP5b activity is significantly correlated with the NTX level and BAP activity, respectively (P < 0.0001). The NTX level is significantly correlated with BAP activity as well (P < 0.0001).

Treatment Response of Serum TRACP5b Activity, BAP Activity, and NTX Level in Breast Cancer Patients with Bone Metastasis Before and After Treatment. Thirty-eight patients were evaluable for treatment response during the study period. The other 30 patients were not evaluable due to any of the following causes: early death, lost to follow-up, patient’s refusal, and end of the study. Evaluable patients received such systemic therapies as chemotherapy, hormone therapy, Herceptin infusion, and bisphosphonate therapy as clinically indicated. Twenty of the 38 patients were treatment responders and 18 were nonresponders. The TRACP5b activity, BAP activity, and NTX level of 38 paired serum samples, before and after treatment, were tested by Wilcoxon signed-rank test.

There were no significant differences in age, duration of measurements, and concentrations of BAP and NTX, either before or after treatment, between responders and nonresponders (P > 0.05; Table 1). Although the responders had significantly higher TRACP5b activity than nonresponders before treatment (median, 6.5 versus 4.7; P = 0.0476; Table 1), the responders had highly significant lower TRACP5b activity than nonresponders after treatment (median, 2.9 versus 4.4; P < 0.0001; Table 1).

Among the three biomarkers (TRACP5b, BAP, and NTX), TRACP5b and NTX were highly significant decreased after treatment in responders (P < 0.0001 and P = 0.0107, respectively; Table 2). The median percentage change of TRACP5b activity, BAP activity, and NTX level was −57%, 0%, and −48%, respectively, in treatment responders. In nonresponders, NTX was significantly increased after treatment (P = 0.0342; Table 2). However, there was not a significant increase in TRACP5b activity in nonresponders (P = 0.1415; Table 2). The median percentage change of TRACP5b activity, BAP activity, and NTX level was 10%, 19%, and 43%, respectively. The BAP activity did not show a significantly difference either in responders or nonresponders (P = 0.8124 and P = 0.0898, respectively; Table 2).

After adjusting for age, duration of measurement, and percent change of BAP, we found that patients who had decreased percent change of TRACP5b activity (% change of TRACP5b < 0) had 18 times probability to have a response as compared with nonresponders (% change of TRACP5b ≥ 0; odds ratio, 18.07; P = 0.0208; Table 3). In addition, patients who had decreased percent change of NTX level (% change of NTX level < 0) had almost 12 times probability to have a response as compared with nonresponders (% change of NTX level ≥ 0; odds ratio, 11.88; P = 0.0081; Table 3).

Discussion

Image studies such as plain radiography, bone scintigraphy, computerized tomography, and magnetic resonance are the most frequently used measures for the diagnosis and follow-up of bone metastasis in breast cancer patients (11, 19-23).

Table 2. The Wilcoxon signed-rank test for biomarker concentrations before and after treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Responders (n = 20), median (range)</th>
<th>Nonresponders (n = 18), median (range)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>TRACP5b (µmol/min/L)</td>
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<td>4.7 (2.17-12.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BAP (mol/min/L)</td>
<td>47.9 (19.0-271.2)</td>
<td>50.6 (15.8-257.7)</td>
<td>0.8124</td>
</tr>
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<td>NTX (nmol/L BCE/L)</td>
<td>19.5 (10.0-82.2)</td>
<td>13.4 (6.9-114.9)</td>
<td>0.0107</td>
</tr>
</tbody>
</table>

*Data were assessed by Wilcoxon signed-rank test (PROC UNIVARIATE, SAS 9.1).
However, each image measure has its own limitations and some of them are cost-ineffective. Moreover, the fear of radiation related injury has limited the frequent uses of repeated image studies. Some advantages of using biochemical markers over image studies for diagnosis and follow-up of bone metastasis are that (a) markers have potential for increased sensitivity; (b) markers relate to systemic events rather than local events; (c) markers respond more rapidly to treatment; (d) markers should discriminate between healing lesions and progressive lesions; and (e) markers should provide more information on the mechanisms and cellular dynamics of bone destruction.

Bone formation markers, including alkaline phosphatase, osteocalcin, COOH-terminal propeptide of type 1 procollagen, and NH2-terminal propeptide of type 1 procollagen, can be measured in the serum (11, 24-26). They all lack specificity and the data from cancer patients are scanty. On the bone resorption side, markers can be measured both in urine and in serum (11). Classic markers in urine include calcium and hydroxyproline (11, 27, 28). Such new markers as pyridinoline, deoxypyridinoline, NTX, COOH-terminal telopeptide of type 1 collagen, and free portions of both pyridinoline and deoxypyridinoline have been under investigation (29-32). However, to measure these markers from urine is tedious and not convenient in routine clinical practice. The most practical should be those potential resorption markers in the serum because serum is easy to collect in the clinic and easy to handle in the laboratory. Such serum bone resorption markers include TRACP5b, COOH-terminal cross-linked telopeptide of type 1 collagen, and NTX (12, 13, 16, 33, 34).

Recently, we and other investigators have shown that serum TRACP5b activity is a valuable marker of osteoclast and bone resorption (12, 13, 16, 18, 35). It has the added advantages that serum TRACP5b may not be affected by food intake or renal or hepatic disease. In addition, the clinical variation of TRACP5b is very low (36). Serum TRACP5b activity may be a useful marker in the detection and follow-up of breast cancer patients with bone metastasis. In this study, we have shown that serum TRACP5b activity, BAP activity, and NTX level are significantly higher in breast cancer patients with bone metastasis than normal subjects and are significantly correlated to each other. Their correlations were not altered by systemic therapies. Therefore, it is plausible to use any of these three markers to diagnose and monitor bone metastasis in breast cancer patients over time. However, in our further analysis using 76 paired sera from 38 patients who were evaluable for treatment, response revealed that TRACP5b activity is the most sensitive marker in monitoring treatment response of bone metastasis in breast cancer patients.

The limitation of this study is the relatively fewer number of patients who were evaluable for treatment response. This is a restriction in clinical research, especially for cancer patients. However, we would like to report this signal result for further study. Second, currently there is no standard criterion for evaluation of the treatment response on bone metastasis in breast cancer patients. Therefore, in this study, the responder has been defined as a patient who must have obtained improvement of symptoms of bone metastasis as a prerequisite and either one of the objective variables defined (i.e., decrease of tumor markers, reduction of tumor size, and improvement of bone scintigraphy). However, many of our patients had normal tumor markers on diagnosis of bone metastasis; some were without measurable tumors; and in a proportion of cases the follow-up bone scintigraphy showed controversial results with clinical findings. Therefore, we now do not have enough data to make a correlation between the changes in TRACP5b activities and tumor markers or the overall responses defined by the measuring tumor sizes. A prospective study aiming to compare the TRACP5b activities and quantitative bone scintigraphy is now under way to overcome the above-mentioned limitations by recruiting enough number of patients.

Nowadays, physicians commonly use alkaline phosphatase or BAP activity as a marker to detect or monitor bone metastasis in breast cancer patients. However, our results suggest that BAP may not be the most sensitive bone marker for this purpose. Our data support instead the use of either TRACP5b or NTX to follow up breast cancer patients with bone metastasis after treatment. TRACP5b is perhaps more sensitive than NTX for this purpose, as shown in this study, but further study with more patients is needed to confirm our current findings.

**Acknowledgments**

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**References**

Tartrate-Resistant Acid Phosphatase 5b Activity Is a Useful Bone Marker for Monitoring Bone Metastases in Breast Cancer Patients after Treatment

Yoke-Chun Chung, Chih-Hung Ku, Tsu-Yi Chao, et al.


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