Tartrate-Resistant Acid Phosphatase 5b is a Useful Serum Marker for Extensive Bone Metastasis in Breast Cancer Patients

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ABSTRACT

Purpose: Previous studies showed that serum tartrate-resistant acid phosphatase 5b (TRACP5b) activity was increased in 70% to 94% of breast cancer (BC) patients with bone metastasis (BM). This study aims to determine whether serum TRACP5b is useful for identifying limited or extensive BM in BC patients.

Experimental Design: Serum TRACP5b activity was measured in 168 BC patients, including 81 who were newly diagnosed with early BC, 20 with extraosseous metastasis, 24 with limited BM, and 43 with extensive BM. Serum TRACP5b activity was also measured monthly in 151 patients with early BC until they developed BM. Four hundred and twenty-seven (427) healthy women ages 18 to 90 served as control. One-way ANOVA was used to compare serum TRACP5b among groups. The sensitivity and specificity of serum TRACP5b as a marker for BM were estimated by receiver operator characteristic (ROC) curves.

Results: Serum TRACP5b increased with age in healthy women (P < 0.0001). It was significantly elevated in patients with extensive BM compared with all other groups (P < 0.0001). ROC analysis established a cutoff value of 4.026 units/L to identify patients with extensive BM with a specificity of 98% and a sensitivity of 93% (area under the curve = 0.9807; 95% CI = 0.9698-0.9915). Among the 151 patients with early BC, 6 developed limited BM and 2 developed extensive BM during the follow-up period. Serum TRACP5b remained below the cutoff value in patients with limited BM, but became significantly increased in those whose BM became extensive.

Conclusion: Serum TRACP5b activity is a useful diagnostic marker for extensive BM in patients with BC.

INTRODUCTION

Tartrate-resistant acid phosphatase 5b (TRACP5b) is normally secreted by osteoclasts during bone resorption (1, 2). Its activity can be specifically measured in serum by immunoassays and has been proposed as a marker of bone resorption (3–6). Like other bone resorption markers such as pyridinoline, deoxypyridinoline, COOH-terminal and NH2-terminal telopeptides of cross-linked collagen type I (CTX, NTX), serum TRACP5b activity becomes increased after menopause in response to the deprivation of estrogen effect on bones (7, 8). TRACP5b may be as useful as these collagen markers, and it has lower preanalytic variability (9). Serum TRACP5b activity is also increased in several pathologic conditions including Paget’s disease, Gaucher disease, primary and secondary hyperparathyroidism, severe osteoporosis, multiple myeloma, and bone metastasis (BM) originated from breast and other cancers (10–16). In some of these pathologic states, bone destruction may proceed by variable degrees involving cathepsin K and matrix metalloproteinase pathways by which some markers may become increased although others may not (17, 18). Several investigators have shown that serum TRACP5b activity is elevated in breast cancer (BC) patients with BM compared with those with early BC or normal individuals (10, 19–23). Serum TRACP5b activity decreased after effective treatment with antiresorptive bisphosphonates and increased again after such treatment failed (20–22). Therefore, serum TRACP5b activity has been proposed as a useful diagnostic marker to monitor BM in BC. However, several important aspects of TRACP expression in cancer have not been addressed. It has been known for a long time that total TRACP activity is increased in prepubertal children and in postmenopausal women due to increased bone growth and resorption rates in these groups, respectively (24, 25). However, a detailed consideration of the effect of age on serum osteoclastic TRACP5b or its impact on interpretation of serum TRACP5b activity measurements in BC has not been made (9, 22, 23, 26). Also, the effects of adjuvant hormonal therapy, chemotherapy, or radiotherapy for BC without BM have not been determined. Finally, because most studies report a sensitivity ranging from 70% to 94% for detection of BM, it is not clear if the serum TRACP5b activity was increased only in patients with late, extensive metastases (22, 23, 26, 27). Recently, extremely elevated serum TRACP5b activity has been shown in patients with autosomal dominant osteopetrosis (28, 29). This seemingly contradictory observation of an increased resorption marker in the face of net bone formation has led to the notion that TRACP5b reflects the number of osteoclasts, functional and dysfunctional, rather than bone resorption. In this regard, we wonder if the serum TRACP5b activity is elevated only in the later stage of BM and not when bone involvement is limited.
and osteoclast numbers have not increased above a certain
threshold.

In this study, we measured the serum TRACP5b activity in
cancer-free, healthy Chinese women from 18 to 90 years of age,
and in BC patients without and with BM; aiming to clarify the
role of serum TRACP5b activity in diagnosis and treatment of
BC with BM. Three goals were undertaken. First, we sought to
establish the effect of age on TRACP5b activity in a large
number of cancer-free, healthy females, and to determine the
impact of this effect on the use of TRACP5b as a marker for BM
in a population of women also at risk for postmenopausal
osteoporosis. Second, we set out to determine if BC with or
without visceral metastasis had any effects on serum TRACP5b
activity. Finally, we wished to assess the value of TRACP5b as
an early biochemical marker for BM.

PATIENTS AND METHODS
Patients and Serum Collection
All patients with pathologically proven BC seen in the
Division of Hematology/Oncology of the Tri-Service General
Hospital in Taipei were invited to participate in this study.
Patients included those with early and metastatic BC diseases.
Early BC was defined as stage I to III diseases by the American
Joint Committee on Cancer staging system. All AJCC stage IV
patients with BM had not been treated previously with
bisphosphonates. Patients were deemed to have BM according
to either of the following criteria: (a) pathologically proven BM
by bone or bone marrow biopsy, or (b) in those who did not
receive biopsy, the presence of both classic clinical symptoms/
signs of BM and definitive evidence by image studies including
roentgenography, computerized tomography, magnetic reso-
nance, \( ^{99m} \text{Tc} \)-hydroxymethylenediphosphonate (\( ^{99m} \text{Tc} \)-MDP,
10 mCi) whole-body bone scintigraphy, or positron emission
computed tomography. Additional auxiliary diagnostic tests included
the serum tumor markers CEA and CA 15.3 and serum calcium
level. Limited BM was defined as three or less skeletal metastatic
sites in image studies. All other patients with bone involvement
were considered to have extensive metastasis. Patients without
BM received no treatment or standard postoperative adjuvant
chemotherapy, radiation therapy, and hormonotherapy as
clinically indicated. No bisphosphonate was used in patients
without BM. All patients with BM received various treatments
including chemotherapy, radiation therapy, hormonotherapy,
and bisphosphonates at the physician’s discretion. To establish
TRACP5b activity levels as a function of age, 427 healthy,
cancer-free female subjects from the ages of 18 to 90 years were
recruited as a control group after informed consent was obtained.
Venous blood was drawn monthly or before administration
of bisphosphonate in BC patients over a period of 24 months.
Venous blood was drawn once from control subjects. The blood
was allowed to clot at room temperature for 30 to 60 minutes,
and then stored for no more than 4 hours at 4°C before
centrifugation to collect serum. All sera were stored at −80°C
and thawed at room temperature immediately before TRACP5b
activity was measured. The TRACP5b activity was measured
within one month of blood collection. The Institutional Review
Board of the Tri-Serviced General Hospital approved this study.

Serum TRACP5b Activity Assay
Osteoclastic TRACP5b activity was measured by an
immunoassay as previously reported (6, 23). Isoform 5b activity
is defined as the type 5 TRACP captured by specific antibody
14G6 and measured at pH 6.1 (1). Briefly, biotinylated
monoclonal antibody 14G6 was used to coat duplicate avidin-
coated microwells. Serum samples were incubated in coated
wells overnight at 4°C. After washing, TRACP activity was
measured by adding 4-nitrophenyl phosphate as substrate in a
buffer of 100 mmol Na acetate/50 mmol Na tartrate (pH 6.1).
The result was expressed as units per liter. The interassay
analytic error (% CV) was determined by assay of aliquots of six
sera ranging from 2.54 to 9.37 units/L on six separate occasions;
the average CV was calculated to be 3.9%. The intraassay error
was determined by simultaneous assay of eight duplicates of five
sera ranging in activity from 2.50 to 11.0 units/L, the average
CV was calculated to be 5.1%.

Statistics
Simple linear regression was conducted to assess the
strength of the relationship between serum TRACP5b activity
and age in healthy study subjects (PROC REG, SAS v8.2).
ANOVA was done to compare mean serum TRACP5b activity in
different study groups of BC patients (PROC ANOVA, SAS
v8.2). A generalized linear model was applied to assess the
association of interests, as well as to adjust the potential
confounding factors (PROC GENMOD, SAS v8.2). To assess
the sensitivity and specificity of serum TRACP5b activity for
detection of BM, the receiver operator characteristic (ROC)
curve of TRACP5b activity was calculated from the raw
TRACP5b activities of all BC patients with BM and for those
with only extensive BM against the control group.

RESULTS
Serum TRACP5b Activity in Healthy, Cancer-Free Women
In the 427 cancer-free women ages 18 to 90, the mean
TRACP5b activity for the entire group was 2.58 ± 0.95 units/L.
The serum TRACP5b activity was significantly positively
correlated with age (Fig. 1; \( P < 0.0001 \)). For each 1-year
increment in age serum TRACP5b activity increased by
0.027 units/L. Age is a significant factor toward increase of
serum TRACP5b activity in healthy women. This is probably
due to the increasing proportion of women with postmenopausal
osteoporosis at each age increment, especially after age 45.

Serum TRACP5b Activity in Patients with BC
Serum TRACP5b activity was determined in 168 BC
patients, including 81 newly diagnosed with early BC, 20 with
extraosseous metastases, and 67 with BM. Extensive BM with
more than three skeletal lesions were found in 43 of the 67
patients, the other 24 had limited BM with three or less skeletal
lesions; 9 of the 43 patients with extensive BM and 8 of the 24
patients with limited BM received bone or bone marrow
biopsies. All of the other patients were diagnosed by clinical
symptoms plus image studies. ANOVA revealed the only group
to have significantly different serum TRACP5b activity was that
with extensive BM (Fig. 2; \( P < 0.0001 \)). No significant
differences were obtained among the other groups including
those with no metastasis, those with only visceral metastasis,
is a specific marker for bone disease. TRACP5b activity. Therefore, in BC patients, serum TRACP5b there were still no significant differences in mean serum those with no BM and those with extraosseous metastasis only, and those with limited BM. When patients were subgrouped into those with no BM and those with extraosseous metastasis only, there were still no significant differences in mean serum TRACP5b activity. Therefore, in BC patients, serum TRACP5b is a specific marker for bone disease.

Figure 3 shows the distribution of serum TRACP5b activities of the 67 patients with BM compared with those of other groups of patients as a function of age. After adjusting for age, patients with extensive BM had significantly higher serum TRACP5b activities than that of healthy women (Table 1; P < 0.0001). Serum TRACP5b activities in patients with early BC or those with limited BM were not different from that of healthy women (Fig. 3 and Table 1). In addition, the difference between patients with extensive BM and healthy women was significantly decreased with age (Fig. 3 and Table 1; P = 0.0410). The trends shown in Fig. 3 indicate that TRACP5b increases with age in healthy women, whereas it decreases with age in those with BC with extensive BM. The trends for rising serum TRACP5b with age in patients with early BC or limited BM were different from healthy women (Fig. 3 and Table 1). Therefore, although serum TRACP5b is increased with age in the general healthy population, age is not a confounding variable when using TRACP5b as a marker for BM among BC patients.

Sensitivity and Specificity of Serum TRACP5b Activity as a Marker for BM in BC

The ROC curves plotted for serum TRACP5b activity in patients with BM versus 427 control subjects are shown in Fig. 4. The area under the curve generated from the raw serum TRACP5b activities was 0.8553 (95% CI, 0.7999-0.9108) for all 67 patients with BM (Fig. 4, top). The area under the curve increased to 0.9807 (95% CI, 0.9698-0.9915) for the 43 patients with extensive metastasis only (Fig. 4, bottom). The set point of serum TRACP5b activity as age increases.

Fig. 1 The distribution of the serum TRACP5b activities of 427 normal adult women of different ages. Line, trend of rising serum TRACP5b activity as age increases.

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(1 mg daily) due to persistent elevated CEA. Her CEA and CA 15.3 tumor markers continued to increase and chemotherapy with UFUR was started in March 2002. She developed malignant pleural effusion in May 2002, and the serum TRACP5b activity rose to 7.48 units/L. A chest X-ray showed diffuse osteoblastic changes. She then received further palliative chemotherapy with vinorelbine and 5-fluorouracil. Serum TRACP5b activity rose to 12.25 units/L in August 2002 and monthly i.v. pamidronate therapy was begun. Serum TRACP5b activity continued to climb to 16.10 units/L in November 2002. She died of progressive disease in January 2003.

**Case 2.** The patient was 49 years old when diagnosed to have right BC in January 2000, with initial stage T2N2M0 disease. She received modified radical mastectomy and axillary dissection as the primary treatment. Postoperative chemotherapy with standard AC (cyclophosphamide, doxorubicin) regimen and radiation therapy were given. She entered this study in September 2001, and the baseline serum TRACP5b activity was 3.1 units/L. As shown in Fig. 6 (bottom), her serum TRACP5b activity rose over the next 2 months to exceed the threshold level at 4.484 units/L. She denied bone pain and a bone mineral density examination by dual-energy X-ray absorptiometry was normal. An X-ray survey revealed no evidence of BM. Her TRACP5b activity soon decreased to 2.969 units/L in January 2002. She started to complain of right anterior chest pain in March 2002, with a pain numerical analogue score of 3 (scale from 0 for no pain to 10 for maximum). Her serum CEA and CA 15.3 were normal. A whole-body bone scan showed increased uptake over right ninth costochondral junction. Her pain increased with time, although serum TRACP5b activity remained normal at 2.623 units/L in June 2002. A repeated whole-body bone scan and chest CT scan were done, but showed no changes. Serum TRACP5b activity became elevated at 6.38 units/L in August 2002, when she suffered severe pain (numerical analogue score, 9) over anterior chest wall and right iliac region. She was then hospitalized after a positron emission tomography scan showed multiple bony metastases. On admission, she had hypercalcemia of 14 mg/dL. Bone marrow biopsy showed metastatic adenocarcinoma. A magnetic resonance examination showed diffuse spine metastasis and X-ray studies showed an osteolytic lesion over left fifth rib. She then received a short course of palliative chemotherapy with vinorelbine and docetaxel for 5 months and monthly pamidronate (90 mg i.v.) until she died of progressive disease with extensive skeletal involvement.

**DISCUSSION**

Serum TRACP5b activity is a marker of osteoclast activity and number (1, 8, 28, 30). It may have advantages over other resorption markers of collagen degradation, because it is less affected by preanalytic variables including day to day variability.
Table 2  Characteristics of the six early BC patients who developed limited BM during follow-up

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Metastatic sites</th>
<th>Evidences of BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>61</td>
<td>thoracic spine (T4)</td>
<td>S/S, MR, bone scan, X-ray</td>
</tr>
<tr>
<td>B</td>
<td>36</td>
<td>lumbar spine (L4), pubic bone, patella</td>
<td>S/S, MR, bone scan, X-ray</td>
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<tr>
<td>C</td>
<td>44</td>
<td>thoracic spine (T8)</td>
<td>S/S, bone scan, X-ray</td>
</tr>
<tr>
<td>D</td>
<td>47</td>
<td>right humerus</td>
<td>S/S, bone scan, bone biopsy</td>
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<tr>
<td>E</td>
<td>59</td>
<td>sternum</td>
<td>S/S, bone scan, bone biopsy</td>
</tr>
<tr>
<td>F</td>
<td>49</td>
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<td>S/S, bone scan, MR</td>
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</table>

NOTE. S/S, symptoms/signs.

Several reports have shown that serum TRACP5b activity is increased in BC with BM compared with that of normal control subjects. However, not all patients with BM had elevated TRACP5b; sensitivity for BM detection in published studies ranges from 70% to 94%. This caused us to ask whether serum TRACP5b activity was related to the extent of metastasis. This is particularly valid because recent studies of TRACP5b in osteopetrosis and secondary hyperparathyroidism suggest that TRACP5b is related as much or more to osteoclast number than to bone resorption. In this paper, we showed that the serum TRACP5b activity is elevated only in patients with skeletal metastasis with four or more sites in image studies. The threshold value of 4.026 units/L has been defined as the detection limit of extensive metastasis by ROC curve analysis with 93% sensitivity and 98% specificity. The specificity dropped markedly to 64% if all patients with BM were included. Because most BC patients are above the age of 45, an age at which mean TRACP5b significantly increases in the otherwise healthy population, it is not necessary to convert the measured serum TRACP5b activity to age-corrected Z score for clinical application. However, in younger patients, age-corrected Z score may be needed for proper interpretation of serum TRACP5b activity as an osteoclast marker.

Considerable evidence from cross-sectional studies now indicates that mean TRACP5b is definitely elevated in BC patients with BM, but might be limited by its lower sensitivity in detecting minimal bone disease. The usefulness of serum TRACP5b activity as a sensitive marker in longitudinal studies for early diagnosis of BM in BC patients has not been clarified. We hypothesized that serum TRACP5b activity would be a more sensitive marker than the conventional image studies to detect early or minimal BM. In this study, we followed 151 BC patients without BM by monthly measurement of serum TRACP5b activity. Eight of them developed BM during follow up. In the six patients with limited BM, serum TRACP5b activity was examined over time, with the results showing that the threshold value of 4.026 units/L was exceeded in all cases. The specificity dropped to 64% if all patients with BM were included. Because most BC patients are above the age of 45, an age at which mean TRACP5b significantly increases in the otherwise healthy population, it is not necessary to convert the measured serum TRACP5b activity to age-corrected Z score for clinical application. However, in younger patients, age-corrected Z score may be needed for proper interpretation of serum TRACP5b activity as an osteoclast marker.

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Fig. 5 Change of the serum TRACP5b activity over time in six patients with early BC who developed limited BM in the study period. The last point of each line represents the time BM was diagnosed.

Fig. 6 Change of serum TRACP5b activities over time in Case 1 and Case 2. Arrows, times when BM was ensured.
activities did not become significantly elevated. After they were diagnosed to have BM, they all received appropriate treatment and their serum TRACP5b activities remained normal throughout the follow-up period. In some of them, the serum TRACP5b activity even decreased before being found to have BM.

However, serum TRACP5b activity may be a useful marker for early detection of extensive metastasis. In Case 1 with extensive metastasis (Fig. 6), serum TRACP5b activity became increased above 4.026 units/L at the 180th day after entering the study. However, BM was not confirmed until X-ray studies on the 520th day revealed the appearance of osteoblastic changes in ribs. In Case 2, her serum TRACP5b activity once rose to 4.484 units/L without clinical symptoms or X-ray evidence of BM at that time. The real cause of that elevation of TRACP5b activity is unknown. The TRACP5b activity decreased to below the threshold level 1 month later. Another 2 months later, limited BM may have developed over the right sternum when she started to suffer from anterior chest pain and the bone scan showed radio-uptake over the right ninth costochondral junction for the first time. Her serum TRACP5b activity did not increase above 4.026 units/L until 4 months later. Finally, a positron emission tomography scan and magnetic resonance confirmed that she had extensive BM. Because we had not established 4.026 units/L as the threshold level until after the study period, we simply monitored these two cases closely until the appearance of clinical symptoms. From the above findings, we conclude that serum TRACP5b activity may not be sensitive enough to detect early, limited BM. However, it is a sensitive marker for extensive BM. When a patient is found to have a significantly elevated serum TRACP5b activity, above the threshold level of 4.026 units/L in our laboratory, a concerted search for BM is strongly recommended.

What is the meaning of the finding of this study? If activity of TRACP5b is elevated only in cases with extensive metastases, determination of TRACP5b activity is not useful for diagnosis of early BM. In our previous studies of end-stage renal disease, we found that TRACP5b activity correlates well with the number of osteoclasts in bone (32). This is also true in in vitro studies of mouse osteoclasts and in Albers-Schonberg disease (28, 35). Therefore, the elevated activity of TRACP5b in BC patients would indicate an increased number of osteoclasts in those with extensive BM, as opposed to cases with limited BM. If the therapeutic effects of bisphosphonates are to reduce the number of osteoclasts through apoptosis, determination of TRACP5b activity will be useful to show the effectiveness of antiosteoclastic therapy. At present, our data on longitudinal changes in TRACP5b in BC with BM are too few to predict or to make treatment recommendations. Our illustrative cases, however, do indicate a need for follow-up studies because they do seem to indicate that decreasing levels of TRACP5b correlate with an improvement, at least in pain and life quality. It would also be good to know if the bisphosphonate therapy in BC actually has biological effects so that decisions can be made whether to continue this therapy in light of renal toxicity. Meanwhile, to make interpretations valid on longitudinal changes in TRACP5b, it was important for us to investigate other possible confounding variables of TRACP5b such as age, visceral metastasis, and antitumor therapy. These variables can now be ruled out with confidence, and further analysis of TRACP5b levels in patients with BC and BM will be simplified.

Our study has several limitations. First, because patients in this study may have had extensive BM for various times prior to enrollment, those with late extensive BM may have extremely high TRACP5b activities at baseline. On the other hand, those patients with early extensive BM may have lower TRACP5b activity at baseline. This may have had an impact on the threshold value of TRACP5b activity for diagnosis of extensive BM. Second, because those patients with limited BM, once diagnosed, started to receive appropriate treatment including bisphosphonates, we have difficulty knowing whether extensive BM is derived from limited BM or the two settings have different natural histories. Furthermore, the number of patients with limited BM were relatively small and only a few patients’ disease evolved from limited BM to extensive BM. Similarly, only a small proportion of early BC patients developed BM during follow-up in this study. Therefore, further prospective studies of more patients over a longer time period are needed to address whether the serum TRACP5b activity is indeed a reliable and sensitive marker of disease progression or for monitoring early BC patients and patients with limited BM. In all, we have achieved two of our three goals set at the initiation of this study. We have established in detail the relationship between age and serum TRACP5b in healthy adult Chinese women which will be highly useful to others conducting studies of bone markers in cancer. We have determined with considerable certainty that the presence of BC, with or without visceral metastasis and antitumor therapies, has no effect on serum TRACP5b levels before BM occurs. These results show that TRACP5b is a specific marker for metastatic bone disease in BC and will facilitate the design of longitudinal studies to address the value of TRACP5b and other biochemical bone markers as means to monitor therapy in patients with BM.

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REFERENCES


