Prognostic value of serum tartrate-resistant acid phosphatase-5b for bone metastasis in patients with resectable breast cancer

MASAFUMI SHIMODA, YASUFUMI SATO, KAORI ABE, NANAE MASUNAGA, MASAMI TSUKABE, TETSUHIRO YOSHINAMI, YOSHIAKI SOTA, TOMOHIRO MIYAKE, TOMONORI TANEI and KENZO SHIMAZU

Department of Breast and Endocrine Surgery, Osaka University Graduate School of Medicine, Suita, Osaka 565-0871, Japan

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Abstract. Bone metastasis significantly affects the quality of life of patients with metastatic breast cancer, and can shorten overall survival. Identifying patients with early-stage breast cancer at high risk for bone metastasis and preventing bone metastasis may lead to a better quality of life and prolonged survival. The present study investigated whether serum tartrate-resistant acid phosphatase-5b (TRACP-5b), a bone turnover marker, can be a prognostic factor for bone metastasis. Female patients who underwent resectable breast surgery between May 2002 and August 2006 were consecutively investigated. A total of 304 patients with a median follow-up of 3,722 days were retrospectively analyzed. TRACP-5b levels in sera prepared from patients' blood drawn preoperatively without any presurgical treatments were measured using an enzyme-linked immunosorbent assay. The cutoff of TRACP-5b levels, in order to separate patients into high and low TRACP-5b groups, was set at median (347 mU/dl). The associations of clinicopathological factors, including TRACP-5b, with bone metastasis-free interval (BMFI), which was defined as the duration between surgery and the diagnosis of bone metastasis at any time point, were examined. Multivariate analysis of various clinicopathological features revealed that lymph node metastasis and histological grade were independent factors associated with BMFI (P=0.017 and 0.030, respectively). In patients with node-positive breast cancer (n=114), a high TRACP-5b level and a high grade were significantly and independently associated with worse BMFI (log-rank P=0.041 and 0.011, respectively). In conclusion, these findings indicated that TRACP-5b may predict bone metastasis in patients with node-positive breast cancer.

Correspondence to: Dr Masafumi Shimoda, Department of Breast and Endocrine Surgery, Osaka University Graduate School of Medicine, 2-2-E10 Yamadaoka, Suita, Osaka 565-0871, Japan
E-mail: mshimoda@onsurg.med.osaka-u.ac.jp

Abbreviations: TRACP-5b, tartrate-resistant acid phosphatase 5b; I-CTP, type I collagen-C-telopeptide; NTX, type I collagen cross-linked N-telopeptide; ER, estrogen receptor; PR, progesterone receptor; HER2, human epithelial growth factor receptor-2; HG, histological grade; CEA, carcinoembryonic antigen; CA15-3, cancer antigen 15-3; BMFI, bone metastasis-free interval; RFI, recurrence-free interval; mRFI, modified recurrence-free interval; B-CTx, type I collagen β C-terminal telopeptide

Key words: breast cancer, bone metastasis, TRACP-5b, bone turnover marker, osteoclast

Introduction

Among all cancer types, breast cancer, along with prostate cancer, has the highest frequency of bone metastasis. Bone metastases are observed in about half of the patients with metastatic breast cancer (1). Bone metastasis can cause severe pain, pathologic fractures, or nerve compression symptoms, which are detrimental to patients' quality of life. Bone metastases can now be manageable with radiation, bisphosphonate preparations, and anti-RANKL antibody (denosumab) administration, leading to easier control of the above symptoms without opioids. However, these treatments do not always benefit patients; due to limited radiation dose, the same lesion is only given one or two opportunities of palliative radiation. Bisphosphonates and denosumab have serious side effects, such as osteonecrosis of the jaw and hypocalcemia. Because of these unmet needs of patients with bone metastases, it is crucial to avoid bone metastases in patients with breast cancer.

To date, it is unclear whether the prevention of bone metastasis prolongs the prognosis of patients with cancer. However, weak but positive results are being obtained in breast cancer. A meta-analysis of 26 trials in which adjuvant bisphosphonates were administered in patients with early-stage breast cancer revealed that the risks of bone metastasis and breast cancer mortality were slightly but significantly reduced in postmenopausal patients (2). On the contrary, the risk of recurrence of cancer in organs other than the bone did not decrease. Similarly, adjuvant denosumab in addition to aromatase inhibitors not only decreases clinical fractures but also significantly prolongs disease-free survival in postmenopausal women with hormone receptor-positive early-stage breast cancer (3). In metastatic breast cancer, patients who developed bone metastasis at any time point have significantly shorter overall survival than patients who did not (4). Based on these findings, the identification of high-risk groups for bone metastasis may enable a more effective prevention of bone metastasis and eventual reduction in breast cancer mortality. To identify such

a population, a prediction tool for bone metastasis is essential. Nevertheless, no simple method for predicting bone metastasis in breast cancer has yet been developed.

Bone turnover markers reflect the status of bone metabolism and thus are in routine clinical use as biomarkers. For example, they are used as auxiliary diagnostics for osteoporosis and are used to monitor the effects of bone-modifying agents. They are classified as bone resorption markers and bone formation markers. Bone resorption markers, including tartrate-resistant acid phosphatase 5b (TRACP-5b), type I collagen-C-telopeptide (I-CTP), and type I collagen cross-linked N-telopeptide (NTX), are useful for the detection of bone metastasis in various cancer types, including breast cancer (5). Contrary to the aforementioned usage, bone turnover markers are not generally used as prognosticators of bone metastasis because the evidence on the predictive value of bone turnover markers is limited (6).

Among bone turnover markers, TRACP-5b has several notable properties as a reliable marker. TRACP-5b is specifically secreted by osteoclasts in high amounts in bone tissue, thereby correlating well with the degree of bone resorption (7). While TRACP-5b directly represents the osteoclast number, other bone resorption markers, such as I-CTP and NTX, only indirectly represent osteoclastic activity because they are by-products of bone remodeling. In addition, TRACP-5b is more sensitive than other bone turnover markers (8-10). Moreover, in patients with bone metastasis from breast cancer, the serum concentration of TRACP-5b reflects the degree of bone metastasis (11,12). TRACP-5b also has advantages in measurements, such as biochemical stability, less diurnal variation, and no perturbation by renal function (13,14). Thus, in this study, we investigated whether the serum concentration of TRACP-5b can be a prognosticator of bone metastasis.

Patients and methods

Patients. This retrospective cohort study investigated the association between preoperative serum TRACP-5b levels and breast cancer recurrence with a focus on bone metastasis. Patients who underwent resectable breast surgery between May 2002 and August 2006 were consecutively investigated. Eligible patients were as follows: Female patients diagnosed with stage I, II, or III breast cancer; aged ≥20 years; underwent surgery without any presurgical treatments; and had serum samples obtained before surgery. Patients who had been prescribed bone-modifying agents at the time of blood sampling were excluded.

Measurement of TRACP-5b. Sera were prepared from patients' blood drawn before surgery according to the standard procedure containing a centrifugation step. Serum samples were preserved at -80˚C until use. To measure the concentration of TRACP-5b in the patients' sera, the enzyme-linked immunosorbent assay for TRACP-5b (Osteolinks® TRAP-5b; SB Bioscience, Tokyo, Japan) was employed. The measurements of TRACP-5b were conducted at Nittobo's laboratory (Koriyama, Fukushima, Japan) without informing any patients' information. Quality control was done by drawing standard curves for each set of measurement. Reproducibility was checked by measuring serum samples with known TRACP-5b concentrations. TRACP-5b levels in 320 serum samples were measured successfully. Sixteen samples were excluded from the subsequent analysis because they were derived from patients with noninvasive breast cancer (9 samples) or patients taking bone-modifying agents for osteoporosis (7 samples).

Data collection and statistics. Patients' age at the time of surgery, menopausal status, tumor size, nodal status, estrogen receptor (ER) status, progesterone receptor (PR) status, human epithelial growth factor receptor-2 (HER2) status, tumor histological grade (HG), and preoperative serum concentrations of carcinoembryonic antigen (CEA) and cancer antigen 15-3 (CA15-3) were collected from each patient's medical record. The cutoff CEA, CA15-3, and TRACP-5b levels were set at the median values of all 304 cases (2 ng/ml, 11 U/ml and 347 mU/dl, respectively). Survival data, including date of surgery, date of diagnosis of breast cancer recurrence, date of diagnosis of bone metastasis, and date of death were collected from each patient's medical record. Bone metastasis-free interval (BMFI) was defined as duration between the surgery and diagnosis of bone metastasis at any time point. Events, including the diagnosis of the second malignancy, contralateral breast cancer, or death from causes other than breast cancer, were censored. Recurrence-free interval (RFI) was defined as described previously (15). To eliminate bone-only events, a modified RFI (mRFI) was applied, wherein bone-only recurrences were censored. Statistical testing was performed using JMP Pro 17 (JMP Statistical Discovery; Cary, NC, USA). Data in contingency tables were analyzed using Fisher's exact test. Kaplan-Meier curves among groups were compared with a univariate log-rank test and a multivariate Cox proportional hazard model. The correlation between TRACP-5b and CEA or CA15-3 was examined using Pearson's test. Survival curves were created using GraphPad Prism 6 (GraphPad Software; San Diego, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Nodal status and HG are associated with bone metastasis in all patient population. Initially, we aimed to determine the clinicopathological factors which are associated with bone metastasis in all patients with breast cancer included in the present study. The age at the time of surgery was 26-81 years old. The median follow-up was 3,722 days. Among the 304 patients with operable breast cancer, 46 developed bone metastases. The imaging modalities used to diagnose bone metastases were as follows: bone scintigram (n=27; 58.7%), positron emission tomography-computed tomography (CT) (n=7; 15.2%), magnetic resonance imaging (n=3; 6.5%), CT (n=6; 13.0%), X-ray (n=2; 4.3%), and unknown (n=1; 2.2%). Bone metastases noted on CT were confirmed on subsequent bone scintigrams in all but one patient. The baseline characteristics of patients who developed or did not develop bone metastases are shown in Table I. The ER-negative and HER2-positive populations were relatively small because patients receiving neoadjuvant chemotherapy, which is generally performed for patients with triple-negative breast cancer

Table I. Baseline characteristics of all patients who did or did not develop BM.

Characteristic	BM developed, n (%)	No BM developed, n (%)	Fisher's P-value
Menopausal status			0.147
Premenopausal	25 (8.2)	109 (35.9)	
Postmenopausal	21 (6.9)	149 (49.0)	
Tumor size			0.076
≤2 cm	15 (4.9)	123 (40.5)	
>2 cm	31 (10.2)	135 (44.4)	
Lymph node metastasis			0.013
Negative	21 (6.9)	169 (55.6)	
Positive	25 (8.2)	89 (29.3)	
ER			0.566
Negative	12 (3.9)	56 (18.4)	
Positive	34 (11.2)	200 (65.8)	
Unknown	0 (0.0)	2 (0.7)	
PR			0.104
Negative	25 (8.2)	103 (33.9)	
Positive	21 (6.9)	152 (50.0)	
Unknown	0 (0.0)	3 (1.0)	
HER2			0.101
Negative	33 (10.9)	200 (65.8)	
Positive	13 (4.3)	42 (13.8)	
Unknown	0 (0.0)	16 (5.3)	
Pathological type			0.745
IDC	40 (13.2)	237 (78.0)	
Others	3 (1.0)	16 (5.3)	
Unknown	3 (1.0)	5 (1.6)	
Histological grade			0.062
Grade 1 or 2	32 (10.5)	212 (69.7)	
Grade 3	11 (3.6)	33 (10.9)	
Unknown	3 (1.0)	13 (4.3)	
CEA			<0.001
Low (≤2 ng/ml)	32 (10.5)	89 (29.3)	
High (>2 ng/ml)	14 (4.6)	154 (50.7)	
Unknown	0 (0.0)	15 (4.9)	
CA15-3			0.107
Low (≤11 U/ml)	18 (5.9)	128 (42.1)	
High (>11 U/ml)	28 (9.2)	114 (37.5)	
Unknown	0 (0.0)	16 (5.3)	
TRACP-5b			1.000
Low (≤347 mU/dl)	23 (7.6)	131 (43.1)	
High (>347 mU/dl)	23 (7.6)	127 (41.8)	
Chemotherapy			0.010
No	17 (5.6)	148 (48.7)	
Yes	29 (9.5)	109 (35.9)	
Unknown	0 (0.0)	1 (0.3)	

BM, bone metastases; CA15-3, cancer antigen 15-3; CEA, carcinoembryonic antigen; ER, estrogen receptor; HER2, human epithelial growth factor receptor-2; IDC, invasive ductal carcinoma; PR, progesterone receptor; TRACP-5b, tartrate-resistant acid phosphatase 5b.

and HER2-positive breast cancer, were not included. Fisher's exact tests for 2 by 2 contingency tables showed that bone metastases were associated with positive nodal status, low serum CEA levels, and adjuvant chemotherapy (Table I). The serum calcium level is sometimes elevated in patients with bone metastasis (16). However, the baseline serum calcium levels were not increased in patients who developed bone metastasis as compared to those without bone metastasis (4.55±0.40 vs. 4.58±0.37 mEq/l; P=0.45), suggesting that patients who developed bone metastasis had no obvious bone metastasis at the time of blood sampling. Univariate log-rank testing revealed that positive nodal status (P=0.018) and a higher HG (P=0.032) were associated with worse outcomes of BMFI (Fig. 1A and B). In the multivariate analysis, both positive nodal status [P=0.017; hazard ratio 2.09 (95% confidence interval, 1.14-3.81)] and a higher HG [P=0.030; hazard ratio 2.15 (95% confidence interval, 1.08-4.28)] were independently associated with worse outcomes of BMFI. To elucidate whether nodal status and HG were associated with metastasis specifically to the bones, mRFI (intervals free of any recurrence except bone-only events) was analyzed. Both positive nodal status and a higher HG were associated with worse outcomes of mRFI (Fig. 1C and D), suggesting that these factors were prognostic not only for bone metastasis but also for metastasis in other organs. Log-rank testing of BMFI stratified by TRACP-5b level showed that the baseline TRACP-5b level was not associated with BMFI outcomes (P=0.684). TRACP-5b was weakly correlated with CEA (P=0.027) but was not correlated with CA15-3 (P=0.094) (Fig. S1).

TRACP-5b independently correlates with BMFI in patients with node-positive breast cancer. Given the previous findings, we aimed to determine whether TRACP-5b was associated with bone metastasis in patients with node-positive or high-grade breast cancer. The univariate log-rank testing demonstrated that a higher HG and a higher TRACP-5b level were associated with worse outcomes of BMFI (Table II; Fig. 2A and B). In the multivariate analysis, both a higher HG and a higher TRACP-5b level were independently associated with worse outcomes of BMFI (Table II). The mRFIs of node-positive patients were marginally different between the high-grade breast cancer and low-grade breast cancer groups (Fig. 2C). The mRFIs of node-positive patients were not significantly different between the high TRACP-5b and low TRACP-5b groups (Fig. 2D), suggesting that the effect of preoperative TRACP-5b levels on recurrence was specific to the bone. TRACP-5b was not associated with BMFI in patients with high-grade breast cancer; positive lymph node status [multivariate P=0.046; hazard ratio, 3.67 (95% confidence interval 0.94-14.31)] was the only factor independently associated with worse BMFI outcomes. These results suggest that a serum TRACP-5b level is a prognostic factor specific for bone metastasis in patients with node-positive breast cancer. The baseline characteristics of patients with node-positive breast cancer stratified with TRACP-5b demonstrated that the high TRACP-5b group includes more postmenopausal women, patients with a larger tumor size, negative ER and negative PR (Table III). Finally, prognostic value of the combination of HG and TRACP-5b was estimated. The

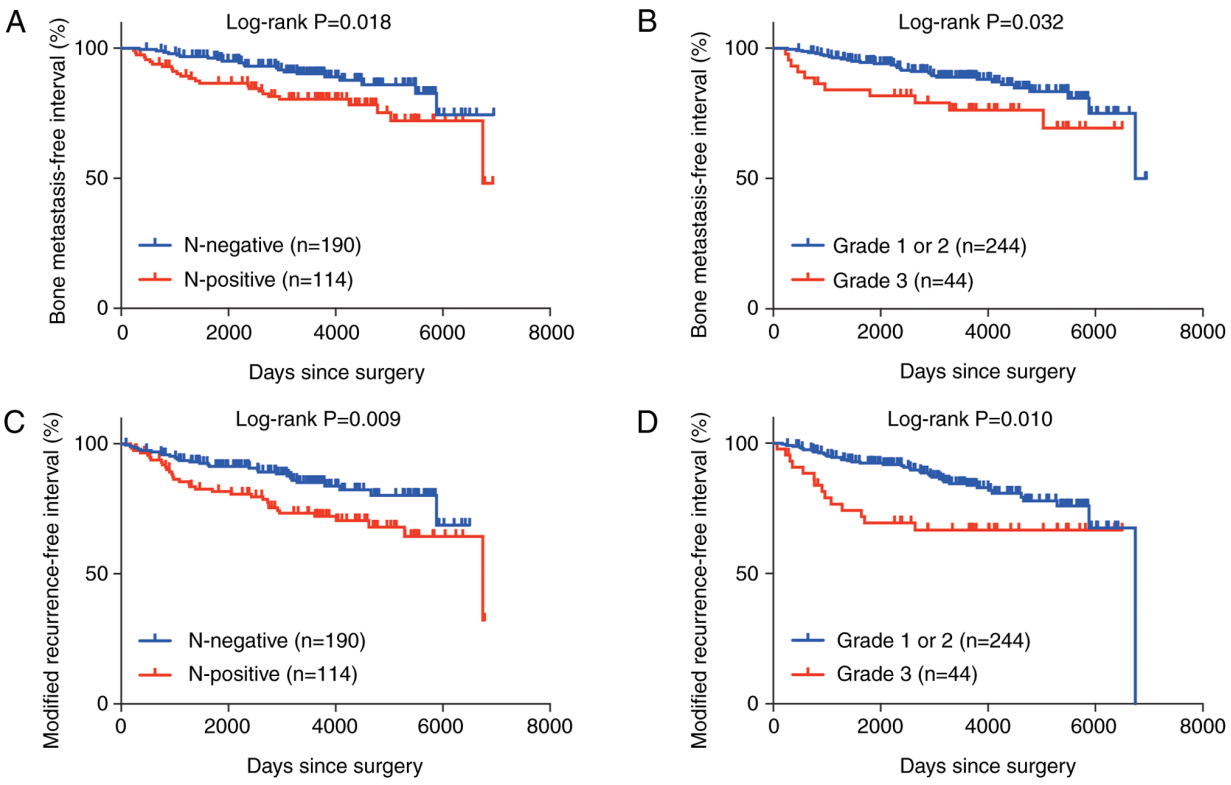

Figure 1. Kaplan-Meier curves of patients with breast cancer stratified with lymph node status or histological grade. (A) Bone metastasis-free intervals of lymph node-negative (blue line; n=190) and lymph node-positive (red line; n=114) patients. (B) Bone metastasis-free intervals of Grade 1 or 2 (blue line; n=244) and Grade 3 (red line; n=44) patients. (C) Modified recurrence-free intervals of lymph node-negative (blue line) and lymph node-positive (red line) patients. (D) Modified recurrence-free intervals of Grade 1 or 2 (blue line) and Grade 3 (red line) patients. Vertical tick indicates a censored case. N, lymph node.

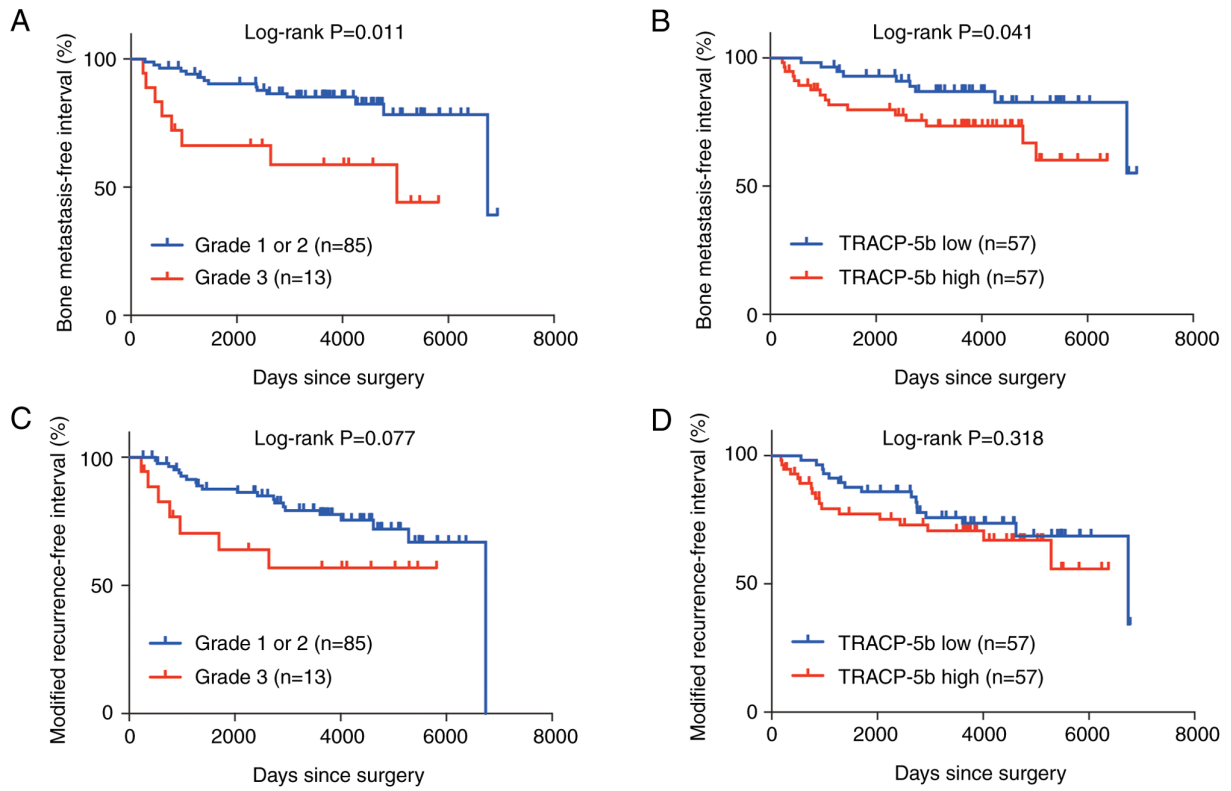

Figure 2. Kaplan-Meier curves of patients with node-positive breast cancer stratified with histological grade or TRACP-5b. (A) Bone metastasis-free intervals of Grade 1 or 2 (blue line; n=85) and Grade 3 (red line; n=13) patients. (B) Bone metastasis-free intervals of low TRACP-5b (blue line; n=57) and high TRACP-5b (red line; n=57) patients. (C) Modified recurrence-free intervals of Grade 1 or 2 (blue line) and Grade 3 (red line) patients. (D) Modified recurrence-free intervals of low TRACP-5b (blue line) and high TRACP-5b (red line) patients. Vertical tick indicates a censored case. TRACP-5b, tartrate-resistant acid phosphatase 5b.

Table II. Univariate and multivariate analyses of BMFI of patients with node-positive breast cancer.

	BMFI		
Characteristic	Univariate P-value[a]	Multivariate P-value[b]	Hazard ratio (Wald's 95% CI)
Menopausal status	0.886		
Tumor size	0.130		
ER	0.622		
PR	0.266		
HER2	0.508		
Histological type	0.773		
Histological grade	0.003	0.011	3.40 (1.42-8.15)
CEA	0.165		
CA15-3	0.452		
TRACP-5b	0.038	0.041	2.46 (1.00-6.03)

[a]Log-rank test; [b]Cox proportional hazard. BMFI, bone metastasis-free interval; CA15-3, cancer antigen 15-3; CEA, carcinoembryonic antigen; CI, confidence interval; HER2, human epithelial growth factor receptor-2; PR, progesterone receptor; TRACP-5b, tartrate-resistant acid phosphatase 5b.

Table III. Baseline characteristics of lymph node-positive patients stratified by TRACP-5b levels.

Characteristic	Low TRACP-5b, n (%)	High TRACP-5b, n (%)	P-value
Menopausal status			0.002
Premenopausal	40 (35.1)	23 (20.2)	
Postmenopausal	17 (14.9)	34 (29.8)	
Tumor size			0.024
≤2 cm	23 (20.2)	11 (9.6)	
>2 cm	34 (29.8)	46 (40.4)	
ER			0.037
Negative	7 (6.1)	17 (14.9)	
Positive	50 (43.9)	40 (35.1)	
PR			<0.001
Negative	13 (11.4)	35 (30.7)	
Positive	44 (38.6)	21 (18.4)	
Unknown	0 (0.0)	1 (0.9)	
HER2			0.082
Negative	49 (43.0)	41 (36.0)	
Positive	6 (5.3)	13 (11.4)	
Unknown	2 (1.8)	3 (2.6)	
Histological type			0.437
IDC	49 (43.0)	52 (45.6)	
Others	4 (3.5)	2 (1.8)	
Unknown	4 (3.5)	3 (2.6)	
Histological grade			0.613
Grade 1 or 2	44 (38.6)	41 (36.0)	
Grade 3	8 (7.0)	10 (8.8)	
Unknown	5 (4.4)	6 (5.3)	
CEA			1.000
Low	23 (20.2)	22 (19.3)	
High	34 (29.8)	35 (30.7)	
CA15-3			0.567
Low	25 (21.9)	21 (18.4)	
High	32 (28.1)	36 (31.6)	
Chemotherapy			0.070
No	13 (11.4)	5 (4.4)	
Yes	44 (38.6)	52 (45.6)	

CA15-3, cancer antigen 15-3; CEA, carcinoembryonic antigen; ER, estrogen receptor; HER2, human epithelial growth factor receptor-2; IDC, invasive ductal carcinoma; PR, progesterone receptor; TRACP-5b, tartrate-resistant acid phosphatase 5b.

BMFIs declined stepwise with increasing HG and increasing TRACP-5b level (Fig. 3), implying that the combination of HG and TRACP-5b offers improved predictive capability for bone metastasis in node-positive patients, as compared to utilizing either factor. No correlation was observed between bone metastasis and HG or TRACP-5b (Table SI). Accordingly, we next assessed the correlation between bone metastasis and the combination of HG and TRACP-5b. There were two ways to separate these combinations; one was to compare HG 3 or TRACP-5b high or both with HG 1/2 and TRACP-5b low, and the other was to compare HG 3 and TRACP-5b high with HG 1/2 or TRACP-5b low or both. No correlation was also observed between bone metastasis and the combination of HG and TRACP-5b in two possible ways of their separation (Table SI). The lack of correlation between them may be because such an analysis cannot take into account the time dependence of bone metastasis.

Discussion

Herein, we found that lymph node metastasis and HG are independent prognostic factors for metastasis to the bone and other organs. In patients with node-positive breast cancer, serum TRACP-5b level is associated specifically with bone metastasis. Thus, serum TRACP-5b level has a prognostic value in bone metastasis in patients with early-stage node-positive breast cancer.

The identification of risk factors for bone metastasis has been extensively pursued. In our cohort, lymph node metastasis and HG are independent risk factors for bone metastasis. Consistent with this finding, lymph node metastasis and/or HG of breast cancer have been identified as risk factors for bone recurrence (17-19). Notably, these studies and ours have included metastasis to the bone-only or bone and other site(s) as bone metastasis. Luminal subtypes have the strongest association with bone-only metastasis, whereas lymph node involvement and histological grading appears to have only a minor influence on bone-only metastasis (20). This suggests that lymph node metastasis and HG

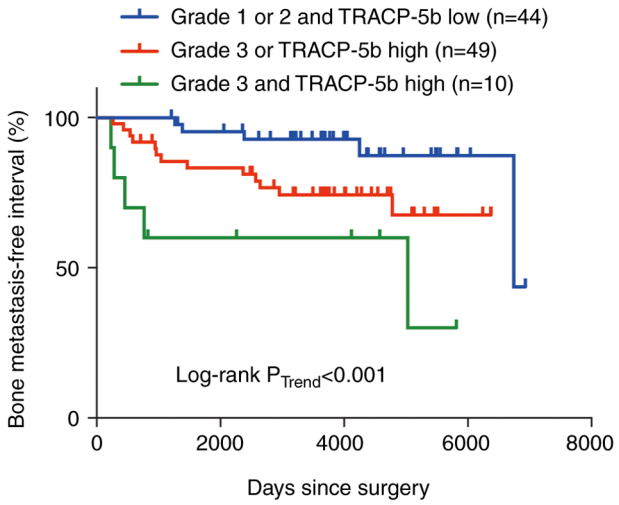

Figure 3. Kaplan-Meier curves of node-positive patients stratified with the combination of histological grade and TRACP-5b. Bone metastasis-free intervals of Grade 1 or 2 and low TRACP-5b patients (blue line; n=44), Grade 3 or high TRACP-5b patients (red line; n=49) and Grade 3 and high TRACP-5b patients (green line; n=10) are shown. The P-value of the log-rank test for trend is shown to indicate the stepwise trend between survival and the combination of increasing histological grade and elevated TRACP-5b levels. Vertical tick indicates a censored case. TRACP-5b, tartrate-resistant acid phosphatase 5b.

are not specific risk factors for bone metastasis. In line with these findings, our observations also showed that lymph node status and HG are associated not only with BMFI but also with mRFI. Although not specific to the bone, lymph node metastasis and HG remain important bone metastasis risk factors that should be considered. Applying the Fisher's exact test, a low CEA level was associated with the development of bone metastases. However, the log-rank test showed no significant difference in BMFI between the low and high CEA groups. Bone metastasis is a time-dependent event. The number of bone metastasis-negative cases may be overestimated because the current cohort included many cases with a short follow-up duration. Thus, the log-rank test results should take precedence over the 2 by 2 contingency test results.

In patients with node-positive breast cancer, we showed that serum TRACP-5b concentration is associated with BMFI, suggesting that TRACP-5b predicts bone metastasis in such patients. To date, there have been attempts to identify a bone turnover marker as a prognosticator for bone metastasis, although reports of successful identification are scarce. Type I collagen β C-terminal telopeptide (B-CTx) is released into the bloodstream during bone resorption. Hence, it is used as a bone resorption marker. In the NCIC CTG MA.14 trial, which included 621 patients with hormone receptor-positive early-stage breast cancer, patients with high serum B-CTx concentration experienced bone-only metastasis with a significantly higher incidence rate than patients with low B-CTx (hazard ratio 2.80) (21). This study did not include patients with breast cancer that metastasized to the bone and other sites. However, in clinical settings, it is not necessary to distinguish bone-only metastasis with metastasis to the bone and other sites because the therapeutic approach to bone metastasis is basically the same in both situations. In a seminal study by Fujii *et al* (4), patients with metastatic breast cancer were dichotomized in accordance with the emergence of bone metastasis; patients who experienced bone metastasis at any time point had worse overall survival than those who did not experience bone metastasis during the follow-up periods, indicating that breast cancers ever metastasizing to the bones are a subgroup distinct from breast cancers that never metastasize to the bones. Thus, this study, in which BMFI was defined irrespective of the metastatic sites other than the bones, is reasonable and even more practical to use as a prognosticator.

Previous studies have provided a rationale for TRACP-5b as a prognosticator of bone metastasis. TRACP-5b is secreted specifically from the osteoclasts, thereby reflecting precise osteoclast number (22). Osteoclasts play a vital role in the development of bone metastasis; breast cancer cells colonizing the bones become dormant in a niche for several years. Then, osteoclasts initiate a vicious cycle by remodeling the bone niche to reactivate dormant tumor cells. Proliferating breast cancer cells and osteoclasts stimulate each other to modify the bone niche, enabling breast cancer cells to form an overt metastasis (23). This mechanistic insight into the role of osteoclasts in the development of bone metastasis has been confirmed with a clinical observation; a cohort study investigating the relationship between bone metastasis and osteoporosis, in which osteoclastic resorption is increased, demonstrated that osteoporosis accelerates the progression of bone metastasis (24). These findings indicate that TRACP-5b, osteoclasts, and bone metastasis are closely related.

Our study did not restrict eligible patients to postmenopausal women with breast cancer. In postmenopausal women, the number of osteoclasts increases because the estrogen-dependent apoptosis of osteoclasts is suppressed, thereby increasing the concentration of TRACP-5b (25). However, menopausal status does not correlate with BMFI of all patients or lymph node-positive patients, suggesting that menopausal status has minimal effects on bone metastasis. In accordance with this finding, a meta-analysis has shown that the association of menopausal status with bone metastasis is not significant (26). Given the intimate association among TRACP-5b, osteoclasts, and bone metastasis, we consider that excluding premenopausal women from the analysis may lead to biased results.

This study has some limitations. First, this was a retrospective, intermediate-sized cohort study; thus, the patient background may have been biased. Nevertheless, to minimize the bias, consecutive patients who had undergone resectable breast surgery over a period were included. Some associations between factors and outcomes may not have been obvious because of the underpowered cohort size. Second, serum uric acid concentration, which may influence TRACP-5b measurement, was not considered (27). The factor could not be retrieved from medical records in a majority of the cohort. Thus, some TRACP-5b levels in patients with high uric acid may have been underestimated.

In conclusion, TRACP-5b may predict ever bone metastasis in patients with resectable node-positive breast cancer. To determine whether TRACP-5b can be a prognosticator

of bone metastasis, a prospective large-sized cohort study is warranted.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MS conceived and designed the analysis, collected the data, provided serum samples, performed the analysis and wrote the paper. YaS collected the data and performed the analysis. MS and YaS confirm the authenticity of all the raw data. KA, NM, MT, TY, YoS, TM and TT provided serum samples. KS provided serum samples and supervised the study. All authors contributed to data interpretation, and read and approved the final manuscript.

Ethics approval and consent to participate

Collection and analyses of serum samples were done in accordance with the protocol approved by the institutional review board of Osaka University (approval no. 332). This study was approved by the institutional review board of the Osaka University Hospital (approval no. 14293). Written informed consent to participate was obtained from each patient before collecting a blood sample.

Patient consent for publication

Written informed consent for publication was obtained from each patient before collecting a blood sample.

Competing interests

Nittobo Co., Ltd. funded TRACP-5b measurements for 111 out of 320 samples. MS and KS are conducting joint research unrelated to this study with Nittobo Co., Ltd. The other authors have no potential conflicts of interest.

References

1. Huang JF, Shen J, Li X, Rengan R, Silvestris N, Wang M, Derosa L, Zheng X, Belli A, Zhang XL, *et al*: Incidence of patients with bone metastases at diagnosis of solid tumors in adults: A large population-based study. Ann Transl Med 8: 482, 2020.
2. Early Breast Cancer Trialists' Collaborative Group (EBCTCG): Adjuvant bisphosphonate treatment in early breast cancer: Meta-analyses of individual patient data from randomised trials. Lancet 386: 1353-1361, 2015.
3. Gnant M, Pfeiler G, Steger GG, Egle D, Greil R, Fitzal F, Wette V, Balic M, Haslbauer F, Melbinger-Zeinitzer E, *et al*: Adjuvant denosumab in postmenopausal patients with hormone receptor-positive breast cancer (ABCSG-18): Disease-free survival results from a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 20: 339-351, 2019.
4. Fujii T, Mason J, Chen A, Kuhn P, Woodward WA, Tripathy D, Newton PK and Ueno NT: Prediction of bone metastasis in inflammatory breast cancer using a markov chain model. Oncologist 24: 1322-1330, 2019.
5. D'Oronzo S, Brown J and Coleman R: The role of biomarkers in the management of bone-homing malignancies. J Bone Oncol 9: 1-9, 2017.
6. Coleman R, Hadji P, Body JJ, Santini D, Chow E, Terpos E, Oudard S, Bruland Ø, Flamen P, Kurth A, *et al*: Bone health in cancer: ESMO clinical practice guidelines. Ann Oncol 31: 1650-1663, 2020.
7. Halleen JM, Tiitinen SL, Ylipahkala H, Fagerlund KM and Väänänen HK: Tartrate-resistant acid phosphatase 5b (TRACP 5b) as a marker of bone resorption. Clin Lab 52: 499-509, 2006.
8. Nenonen A, Cheng S, Ivaska KK, Alatalo SL, Lehtimäki T, Schmidt-Gayk H, Uusi-Rasi K, Heinonen A, Kannus P, Sievänen H, *et al*: Serum TRACP 5b is a useful marker for monitoring alendronate treatment: Comparison with other markers of bone turnover. J Bone Miner Res 20: 1804-1812, 2005.
9. Lumachi F, Basso SMM, Camozzi V, Tozzoli R, Spaziante R and Ermani M: Bone turnover markers in women with early stage breast cancer who developed bone metastases. A prospective study with multivariate logistic regression analysis of accuracy. Clin Chim Acta 460: 227-230, 2016.
10. Nishimukai A, Higuchi T, Ozawa H, Yanai A, Miyagawa Y, Murase K, Imamura M, Takatsuka Y and Miyoshi Y: Different patterns of change in bone turnover markers during treatment with bone-modifying agents for breast cancer patients with bone metastases. Breast Cancer 24: 245-253, 2017.
11. Wada N, Fujisaki M, Ishii S, Ikeda T and Kitajima M: Evaluation of bone metabolic markers in breast cancer with bone metastasis. Breast Cancer 8: 131-137, 2001.
12. Chao TY, Yu JC, Ku CH, Chen MM, Lee SH, Janckila AJ and Yam LT: Tartrate-resistant acid phosphatase 5b is a useful serum marker for extensive bone metastasis in breast cancer patients. Clin Cancer Res 11: 544-550, 2005.
13. Clowes JA, Hannon RA, Yap TS, Hoyle NR, Blumsohn A and Eastell R: Effect of feeding on bone turnover markers and its impact on biological variability of measurements. Bone 30: 886-890, 2002.
14. Yamada S, Inaba M, Kurajoh M, Shidara K, Imanishi Y, Ishimura E and Nishizawa Y: Utility of serum tartrate-resistant acid phosphatase (TRACP5b) as a bone resorption marker in patients with chronic kidney disease: Independence from renal dysfunction. Clin Endocrinol (Oxf) 69: 189-196, 2008.
15. Gourgou-Bourgade S, Cameron D, Poortmans P, Asselain B, Azria D, Cardoso F, A'Hern R, Bliss J, Bogaerts J, Bonnefoi H, *et al*: Guidelines for time-to-event end point definitions in breast cancer trials: Results of the DATECAN initiative (definition for the assessment of time-to-event endpoints in CANcer trials). Ann Oncol 26: 873-879, 2015.
16. Goldner W: Cancer-related hypercalcemia. J Oncol Pract 12: 426-432, 2016.
17. Liede A, Jerzak KJ, Hernandez RK, Wade SW, Sun P and Narod SA: The incidence of bone metastasis after early-stage breast cancer in Canada. Breast Cancer Res Treat 156: 587-595, 2016.
18. Yamashiro H, Takada M, Nakatani E, Imai S, Yamauchi A, Tsuyuki S, Matsutani Y, Sakata S, Wada Y, Okamura R, *et al*: Prevalence and risk factors of bone metastasis and skeletal related events in patients with primary breast cancer in Japan. Int J Clin Oncol 19: 852-862, 2014.
19. Huang Z, Hu C, Liu K, Yuan L, Li Y, Zhao C and Hu C: Risk factors, prognostic factors, and nomograms for bone metastasis in patients with newly diagnosed infiltrating duct carcinoma of the breast: A population-based study. BMC Cancer 20: 1145, 2020.
20. Diessner J, Wischnewsky M, Stüber T, Stein R, Krockenberger M, Häusler S, Janni W, Kreienberg R, Blettner M, Schwentner L, *et al*: Evaluation of clinical parameters influencing the development of bone metastasis in breast cancer. BMC Cancer 16: 307, 2016.
21. Lipton A, Chapman JA, Demers L, Shepherd LE, Han L, Wilson CF, Pritchard KI, Leitzel KE, Ali SM and Pollak M: Elevated bone turnover predicts for bone metastasis in postmenopausal breast cancer: Results of NCIC CTG MA.14. J Clin Oncol 29: 3605-3610, 2011.

22. Rissanen JP, Suominen MI, Peng Z and Halleen JM: Secreted tartrate-resistant acid phosphatase 5b is a marker of osteoclast number in human osteoclast cultures and the rat ovariectomy model. Calcif Tissue Int 82: 108-115, 2008.
23. Croucher PI, McDonald MM and Martin TJ: Bone metastasis: The importance of the neighbourhood. Nat Rev Cancer 16: 373-386, 2016.
24. Chen HM, Chen FP, Yang KC and Yuan SS: Association of bone metastasis with early-stage breast cancer in women with and without precancer osteoporosis according to osteoporosis therapy status. JAMA Netw Open 2: e190429, 2019.
25. Halleen JM, Ylipahkala H, Alatalo SL, Janckila AJ, Heikkinen JE, Suominen H, Cheng S and Väänänen HK: Serum tartrate-resistant acid phosphatase 5b, but not 5a, correlates with other markers of bone turnover and bone mineral density. Calcif Tissue Int 71: 20-25, 2002.
26. Liu H, Zhang X, Zhang S, Wang X and Yu S: Factors associated with bone metastasis in breast cancer: A systematic review and meta-analysis. Ann Palliat Med 10: 4435-4452, 2021.
27. Wu ZQ, Zhang Y, Xie E, Song WJ, Yang RX, Yan CJ, Zhang BF and Xu HG: High uric acid (UA) negatively affects serum tartrate-resistant acid phosphatase 5b (TRACP 5b) immunoassay. PLoS One 11: e0147554, 2016.