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Original Article

Evaluation of Bone Metabolic Markers in Breast Cancer with Bone Metastasis

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Purpose: In the present study, four bone metabolic markers were examined to clarify them meaning and clinical value in the detection of bone metastasis (BM) from breast cancer.

Methods: we examined serum carboxyterminal telopeptide of type I collagen (ICTP), tartrate resistant acid phosphatase (TRACP), total alkaline phosphatase (ALP) and urinary type I collagen cross-linked N-telopeptides (NTx) as potential markers. These bone markers were evaluated simultaneously in 156 breast cancer patients; 114 patients without metastasis (group A), 23 patients with BM (group B) and 19 patients with metastasis at sites other than bone (group C).

Results: The mean values of ICTP and TRACP in group B were significantly greater than those in group A. Group B consisted of the patients with varying degrees of BM and variation in their treatments. The patients in group B were divided into BM (+) and BM (++) according to hot spots in bone scan. ICTP and TRACP were elevated in BM (++) patients compared to BM (+) patients ($p<0.05$). The values of ICTP and TRACP of the twelve patients without treatment in group B were significantly higher than those in group A. In the treated patients of group B, the mean values of ICTP and TRACP were lower in responders and cases of stable disease than those with progression. NTx and ALP were inferior to ICTP and TRACP for clinical evaluation of BM.

Conclusions: We confirmed that ICTP and TRACP might be useful markers for screening and monitoring BM in breast cancer.

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Key words: Breast cancer, Bone metastasis, Bone metabolic marker, Tartrate resistant acid phosphatase, Carboxyterminal telopeptide of type I collagen

Breast cancers frequently have bone metastasis (BM) that is difficult to find and treat. BM is a common clinical problem, affecting 70% of women with breast cancer[1]. We are under pressure to detect early BM and to monitor the response to treatment. Until recently, we did not have valid methods for detecting and monitoring BM except clinical symptoms and radiological findings. Bone scintigraphy is commonly performed in the staging and postoperative monitoring of breast cancer. It is characterized by remarkable sensitivity and the ability to provide a whole skeletal examination which can detect suspicious bone metastatic sites on a single procedure. Nevertheless, its low specificity is often misleading in the management of these patients. Moreover, it cannot be used easily and repeatedly in routine follow up because of exposure to radiation. For other radiological modalities such as X-ray, CT and MRI, it is also by no means easy to settle the matter in terms of convenience, economy and effectiveness.

On the other hand, the new biochemical markers of bone metabolism have been developed and applied to various bone diseases. The recent development of sensitive biochemical markers to determine changes in bone metabolism has provided new insights into the mechanisms of progression of BM. We need to change our strategy of only relying on radiological modalities for the detection and monitoring of BM.

Although there are many reports about the diagnostic value of bone markers for BM, the clinical value of such markers is still controversial. Therefore, in the present study, we examined serum carboxyterminal telopeptide of type I collagen (ICTP), tartrate resistant acid phosphatase

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Abbreviations:
BM, Bone metastasis, ICTP, Carboxyterminal telopeptide of type I collagen; TRACP, Tartrate resistant acid phosphatase; ALP, Total alkaline phosphatase; NTx, Urinary type I collagen cross-linked N-telopeptides; FRBM, First recurrence in the form of bone metastasis

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(TRACP), total alkaline phosphatase (ALP) and urinary type I collagen cross-linked N-telopeptides (NTx) as potential markers. These bone markers were evaluated simultaneously for breast cancer patients with and without BM and appropriate markers were determined for the detection of metastatic bone tumor from breast cancer.

Patients and Methods

Subjects

We studied 156 women (median age 55 years, range 30-87 years) with breast cancer followed up at the department of Surgery, Ashikaga Red Cross Hospital. They had a first treatment for primary breast cancer during 1988 and 1997, which consisted in almost all cases of a radical mastectomy or breast conserving surgery. Patients with severe hepatic (except liver metastasis) and bone metabolic diseases were excluded. No patient had received bisphosphonate therapy before, or during this study.

All were referred for bone scintigraphy with Tc-99m hydroxy methylene diphosphonate for screening, then X-ray examination was done for suspicious lesions to diagnose BM, and if necessary, other diagnostic means, such as MRI and CT scan, were used to confirm the presence of BM. Furthermore, the patients were carefully followed. Among all the patients, 23 were confirmed to have BM.

Blood and urine samples from all patients were collected between July 1997 and March 1999. We measured ICTP (ng/ml), TRACP (IU/l), ALP (IU/l), urinary NTx (nM BCE/mmol Cr) and other routine laboratory tests. Follow-up measurements were performed at various points after surgery.

Assay

The blood samples were centrifuged immediately after blood was drawn for 10 minutes, at 3000 × g after the minimum time required for complete coagulation. The serum was collected and stored at -70℃ until the analysis. Urine samples were also stored under the same condition. All assays were completed within a few days after sampling. TRACP activity was determined by an improved spectrophotometric assay according to the method of Osawa *et al.*[2] in an automated analyzer (OLYMPUS AU600, Tokyo, Japan). Briefly, the substrate used was 2,6-dichloro-4-acetylphenilphosphate (Nitto Boseki Co., Ltd., Tokyo, Japan). Reagent 1 consisted of citrate buffer (0.1 mol/L sodium citrate-HCl, pH 5.4 bovine serum albumin, which gives a final concentration of 3.8 g/L in assay mixture) containing 26 mmol/L L (+)-tartaric acid; reagent 2 contained the substrate (6.0 mmol/L 2,6-dichloro-4-acetylphenilphosphate in sodium citrate-HCl, pH 3.0). A 20 μl aliquot of serum sample mixed with 400 μl of the buffer solution and then 100 μl of the substrate solution was added to start the reaction. We monitored the reaction in rate mode from 140 to 240 s at 340 nm and calculated the enzyme activity from ΔA/min.

Serum ICTP levels were measured by a radioimmunoassy (RIA) based on the two-antibody method, with a commercial ICTP-RIA kit (Orion Diagnostic, ESOP, Finland, provided by Chugai Diagnostics Science Co., Ltd., Tokyo Japan)[3]. ALP was assayed by a method proposed by the German Society for Clinical Chemistry using a commercially available kit (International Reagents Co., Ltd., Kobe, Japan). NTx was measured directly in urine by ELISA using a specific monoclonal antibody to NTx[4] (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan), according to the modified method of Eyre[5].

Statistics

All values were expressed as the mean ± SD. Means were compared by t-test, one-way analysis of variance (ANOVA) followed by Scheffe's F test and Kruskal-Wallis test, and p values below 0.05 were considered significant.

Results

We divided the patients according to the presence of BM; group A (n=114) included patients without metastasis, group B (n=23) patients with BM (and other metastasis), group C (n=19) patients with metastasis at sites other than bone. Patient characteristics are shown in Table 1. There were no significant differences in mean age, menopausal status, hormone receptor status, or histological type of breast cancer among the groups.

Table 2 shows the correlation between bone markers and the presence of BM. The mean values of ICTP and TRACP in patients with BM were significantly higher than those without metastatic disease ($p<0.01$). Similarly, they were significantly higher in patients with BM as compared with those with metastasis other than BM ($p<0.05$). Thus, they were specifically elevated for BM. ALP and NTx did not differ in any groups.

Table 3 shows the characteristics of patients

Table 1. Patient Characteristics

	All patients (n=156)	group A (n=114)	group B (n=23)	group C (n=19)	*P* (A-C)
Age (yr) at sampling					
Medean	55	54	64	57	NS
Range	30-87	30-87	38-87	34-81	
Menopausal status					
Pre	64 (41)	50 (44)	7 (30)	7 (37)	
Post	71 (46)	46 (40)	14 (64)	11 (58)	NS
Unknown	21 (13)	18 (16)	2 (9)	1 (5)	
Mean T (cm) (n=143)	3.0	2.7	3.4	3.7	NS
SD	1.8	1.5	2.6	2.2	
Stage					
0-I	42 (27)	35 (31)	5 (22)	2 (11)	
II	87 (56)	66 (58)	10 (43)	11 (58)	*P*<0.01
III	17 (11)	13 (11)	2 (9)	2 (11)	
IV	10 (6)	0 (6)	6 (26)	4 (21)	
Estrogen receptor					
Positive	99 (64)	73 (64)	17 (74)	9 (47)	
Negative	38 (24)	31 (27)	3 (13)	4 (21)	NS
Unknown	19 (12)	10 (9)	3 (13)	6 (32)	
Progesterone receptor					
Positive	82 (53)	65 (57)	12 (52)	5 (26)	
Negative	54 (35)	38 (33)	8 (35)	8 (42)	NS
Unknown	20 (13)	11 (10)	3 (13)	6 (32)	
Histology					
Papillotubular carcinoma	57 (37)	45 (39)	7 (30)	5 (26)	
Solid-tubular carcinoma	52 (33)	34 (30)	10 (43)	8 (42)	NS
Scirrhous carcinoma	29 (19)	24 (21)	2 (9)	3 (16)	
Other/Unknown	18 (11)	11 (10)	4 (17)	3 (16)	
Nodal status					
n0	84 (54)	70 (61)	7 (30)	7 (37)	
n1α	32 (20)	19 (17)	7 (30)	6 (32)	
n1β	12 (8)	7 (6)	3 (13)	2 (11)	*P*<0.05
n2	8 (5)	7 (6)	1 (4)	0 (0)	
Unknown	20 (13)	11 (10)	5 (22)	4 (21)	

group A: patients without metastasis
group B: patients with BM
group C: patients with metastasis at sites other than bone
Numbers in parentheses are percentages

with BM (group B). Group B patients were classified in two subgroups according to the number of hot spots on the bone scan. We defined the degree of BM : BM (+) (n=15) for patients with one to three hot spots, BM (++) (n=8) for patients with four or more hot spots. We estimated that the total area of hot spots in BM (+) was within three times as much as one thoracic vertebra.

Fig 1 shows the correlation between bone markers and the degree of BM. The mean values of ICTP and TRACP in not only BM (++) but also BM (+) were significantly elevated compared to BM (-) (= group A), and those in BM (++) were significantly higher than those in BM (+). Those markers were clearly related to the degree of BM. In NTx, there was a significant difference between BM (-) and in BM (++), but there was no difference between BM (-) and in BM (+).

Some of the metastatic cases have had systemic treatment before inclusion in this study. First recurrence in the form of BM (FRBM) (n=12) in group B included patients with no treatment for BM and with new appearance of BM while receiving treatment for metastasis to other sites. At the time of sampling for the assay, the patients who were receiving treatment for BM (n=11) were divided into two subgroups according to their response to treatment, which was evaluated based on the crite-

Table 2. Correlation Between Bone Markers and Presence of BM

	group A (n=114)	group B (n=23)	group C (n=19)
Age at sampling (yr.)	56.7±11.8	63.2±13.1	55.9±13.9
ICTP (ng/ml)	3.5± 1.2*	5.4± 2.2*#	4.1± 1.9#
TRACP (IU/l)	7.3± 1.4*	9.4± 1.9*#	8.0± 1.6#
ALP (IU/l)	139.7±43.3	156.7±59.9	135.5±54.2
NTx/Cr (nM BCE/mmol Cr)	45.3±24.7	59.7±34.2	42.5±22.6

*$p<0.01$, #$p<0.05$, other NS
group A: patients without metastasis
group B: patients with BM
group C: patients with metastasis at sites other than bone

Table 3. Characteristics of the Patients with BM (group B)

Pt. No.	Age (yr) at sampling	Degree of BM	Sites of BM	Other metastatic sites	Treatment at sampling
1	79	+	Th	–	END
2*	87	+	Th	–	–
3	64	++	multiple	–	END
4	44	+	Th	–	CHE+END
5	54	+	Th	–	CHE+END
6*	54	+	Th	–	CHE+END
7*	47	+	Sternum	–	–
8*	60	+	L	–	CHE+END
9*	76	++	multiple	–	–
10*	67	++	C, Sternum	LYM	–
11	68	++	multiple	PUL, LYM	CHE
12	67	+	Sternum	PUL, SKI	CHE+END
13*	48	++	multiple	LYM	–
14	78	+	Sternum	LYM	CHE+END
15	61	+	L	LYM	CHE
16	56	++	multiple	PUL, LYM	END
17	71	++	L, Sternum	–	CHE+END
18	69	++	multiple	SKI	–
19*	77	+	Sternum	–	–
20*	51	+	Sternum	–	CHE
21*	57	+	Pelvic, Skull	–	–
22*	81	+	Skull	PUL	–
23*	38	+	Skull	PUL, HEP	CHE+END

*First recurrence in the form of BM (FRBM) n=12
C: Cervical spine, Th: Thoracic spine, L: Lumbar spine, LYM: Lymph node, PUL: Pulmonary, SKI: Skin, HEP: Hepatic, CHE: Chemotherapy, END: Endocrine therapy

ria established by the Japanese Breast Cancer Society[6]. Five patients had radiological evidence of progressive disease (PD), and six patients were in the responder-stable group (NC-CR) including in no change (NC), partial response (PR) and complete response (CR).

The correlation between bone markers and the response to treatment of BM is shown in Fig 2. There was a significant difference between PD and NC-CR in ICTP and TRACP ($p<0.05$). There were no significant differences of any markers in terms of mean value between group A and NC-CR. In PD, the mean values, except ALP, were significantly higher than those in group A ($p<0.01$, not shown in Fig 2). The mean values of ICTP and TRACP of the twelve patients in FRBM were significantly higher than those in group A (ICTP $p<0.05$, TRACP $p<0.01$).

Discussion

In this study, we mainly tested bone resorption

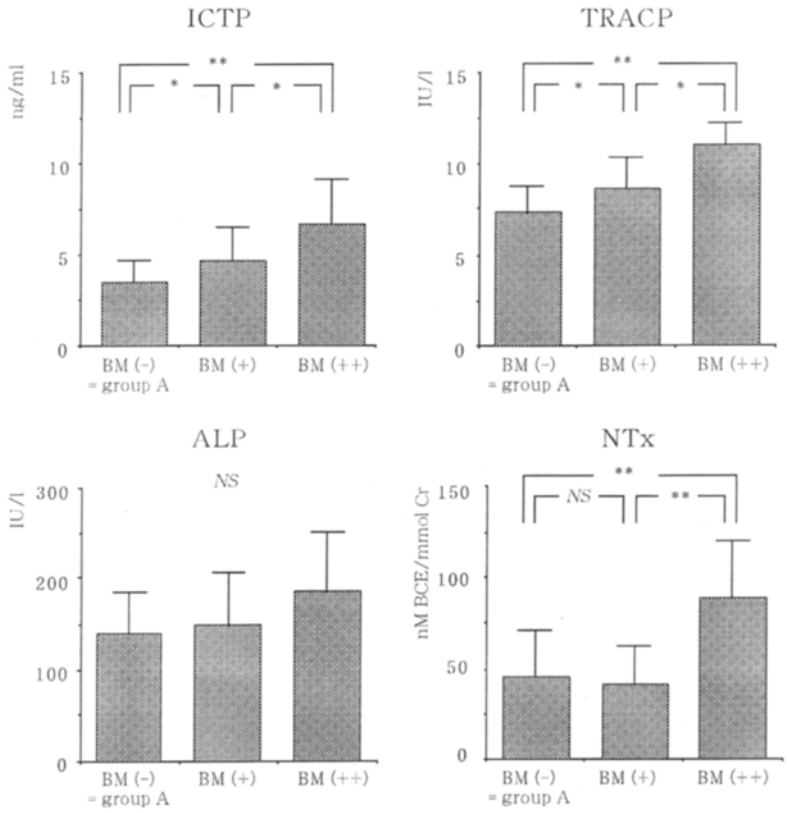

Fig 1. Correlation between bone markers and the degree of BM.
BM (−): n=114, BM (+): n=15, BM (++): n=8, $*p<0.05$, $**p<0.01$

markers: serum TRACP, ICTP and urinary NTx. Serum TRACP is a unique bone resorption marker that reflects osteoclast function. Regarding its sensitivity to tartaric acid, total acid phosphatase can be divided into a tartrate-sensitive fraction and a tartrate-resistant fraction. The former contains prostatic acid phosphatase, a diagnostic marker of prostatic cancer[7], the latter is TRACP, which is localized exclusively in osteclasts[8,9]. In the initial stage of BM, cancer cells involving bone marrow are concerned with producing osteoclast activating factors. Activated osteoclasts absorb bone and secrete TRACP during bone resorption[10] and bone matrix itself is rich in osteoclast activating factors. Thus bone resorption is increased by factors released by breast cancer cells such as PTHrP, TGF-α, or prostaglandins, which may stimulate osteoclast activity either locally or via the bloodstream[11], and serum TRACP is increased.

Serum TRACP activity has been reported to proportionally increase in patients with increased bone resorption[12-15]. Therefore, TRACP could be a useful marker for BM. However, recently TRACP has not been discussed as a clinical bone metabolic marker, because the standards differed among investigators and the colorimetric assay of TRACP, which is widely used, is inferior in precision and reproducibility compared to the results using various substrates. Though there are other methods to measure TRACP using ELISA[16] and also detection of the 5b isoenzyme of ACP[17], these are too complicated and too expensive to use in ordinary practice. Accordingly, we adopted the new measurement method of TRACP with an improved spectrophotometric assay using an automated analyzer, which is more stable than previous equipment and is easy and economical to use[2]. Degradation of extracellular collagen occurs during bone resorption, and the various cross-linking components are released either in peptide-free or in peptide-bound form. ICTP, released through type I collagen degradation, includes those cross-links[18] and is found in an immunochemically intact form in blood[19]. NTx are N-telopeptide cross-links derived from bone type I collagen as a result of bone resorption and are excreted in the urine. Thus the bone markers measured in this study represented different metabolic points in the bone resorption process.

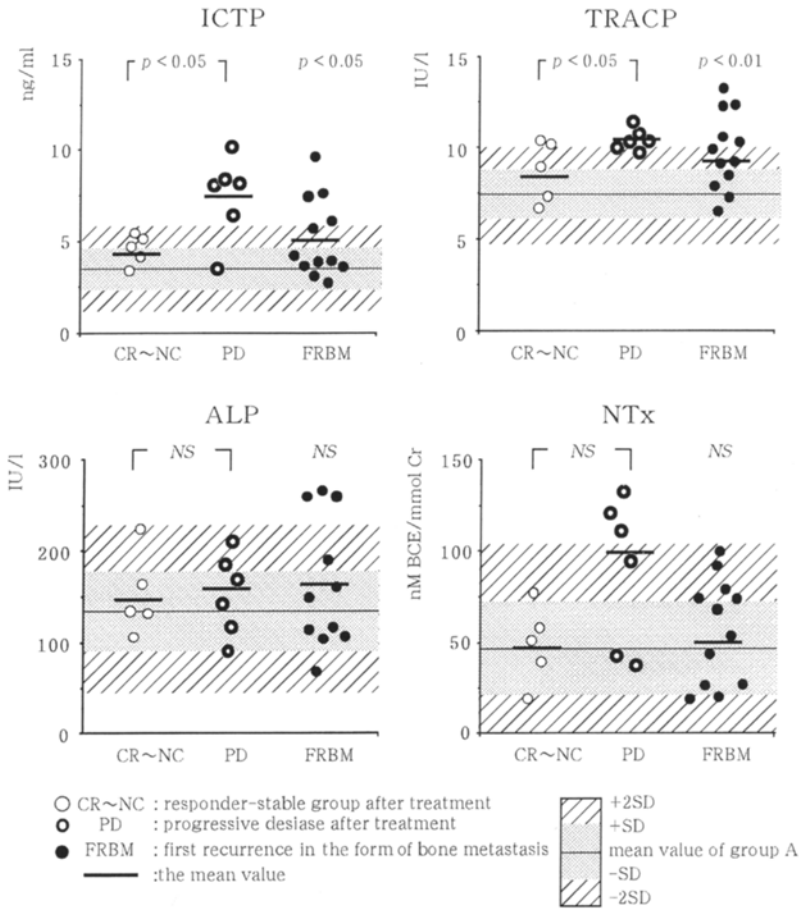

Fig 2. Correlation between bone markers and response to treatment of BM. The *p* value of FRBM was compared to group A.

As an initial approach, we measured four bone metabolic markers in breast cancer patients and compared the results in cases with and without BM. We showed that ICTP and TRACP were specifically elevated in the BM group. The poor sensitivity of NTx makes this parameter an unsuitable marker for detecting BM. While group B included patients with a variety of treatments and histories, the mean values of ICTP and TRACP in this group were significantly higher than those in group A or C. Fig 1 shows that concentrations of ICTP and TRACP significantly increased in parallel with the degree of BM, on the other hand NTx was not elevated with only positivity of BM. ICTP and TRACP reflected the semi-quantitative analysis of bone scintigraphy better. Moreover, in FRBM cases (Fig 2), serum ICTP and TRACP were significantly higher than those without BM. It is important to note that in most cases of FRBM there was little variation in TRACP. The mean value of TRACP increased although the patients with FRBM included treatment with metastasis to sites other than bone. It seems that the osteolytic response is differs between TRACP and other markers. In patients treated for BM, the mean values of ICTP and TRACP in the PD group were significantly higher than those in the NC-CR group. Thus measuring ICTP and TRACP could distinguish PD from NC-CR patients. It follows from this that ICTP and TRACP helped the detect and monitor BM in breast cancer.

We have recently shown that measurement of TRACP, urinary pyridinoline and deoxypyridinoline are reliable methods to evaluate the degree of bone resorption in patients with BM[20]. Koizumi *et al.*[21] concluded that ICTP seemed to be the best of the seven markers his group studied. Similarly, Shimozuma *et al.*[22] showed that serum ICTP appears to be the most indicative marker of bone metastasis from breast cancer among six bone turnover markers. ICTP was reported to allow clinicians to monitor patients' response to chemotherapy[23]. On the

contrary, Demers *et al.*[24] maintained that NTx measurement was the most predictive biochemical marker for the presence of bone metastases.

There is no previous paper evaluating ICTP and TRACP simultaneously for breast cancer patients. Our conclusion is that measurements of serum ICTP and TRACP concentration were clinically useful for screening and monitoring breast cancer patients with BM. For accurate detection of BM, various investigations may be needed. Our results were derived from small numbers, and longer follow-up and repeated measurement of markers are needed to determine what are the false positive and occult bony metastasis rates. The value of each biochemical marker reflects the total of change in whole bone and cannot be used for the localization of BM. Bone scintigraphy will still play a major role in skeletal localization[25]. However, bone markers can be measured repeatedly, less-invasively and conveniently to monitoring BM in breast cancer patients. They are likely to improve the clinical assessment of BM.

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