Serum TRACP 5b and ICTP as Markers of Bone Metastases in Breast Cancer

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Abstract. *Background: The purpose of this cross-sectional study was to evaluate the value of serum tartrate-resistant acid phosphatase 5b (TRACP 5b) and carboxyterminal telopeptide of type I collagen (ICTP) separately and in combination as markers of bone metastases compared to total alkaline phosphatase (tALP) in breast cancer. Materials and Methods: Two groups of patients were studied, one with verified bone metastases (N=46) and one without bone metastases (N=141). Bone marker levels were correlated with the presence or absence of bone metastases. Results: Serum TRACP 5b concentrations exhibited the largest area under the receiver-operating characteristics (ROC) curve (AUC=0.845), followed by ICTP (0.818) and tALP (0.814) when all patients were included in the analysis. With the combination of TRACP 5b and ICTP, the AUC increased to 0.881. In multivariate regression analysis, all three markers were significant predictors of bone metastases. Conclusion: Serum TRACP 5b, ICTP and tALP exhibited equal performances in the detection of bone metastases. The combination of TRACP with ICTP did not significantly improve the detection of bone metastases over tALP.*

Bone metastases (BM) are a frequent complication of advanced breast cancer. When growing in the bone micro-environment, breast cancer cells secrete osteoclast-activating cytokines and hormones, which induce bone resorption at the site of bone metastases (1). The resulting bone destruction causes significant morbidity such as bone pain, pathological fractures, impaired mobility, hypercalcemia and spinal cord compression (2). Reliable detection of metastatic bone disease is crucial for primary staging because it influences the therapeutic decision. Recent reports postulate improvements in the survival time of certain subpopulations of breast cancer patients, even with BM, when treated early with bisphosphonates (3). Therefore, the diagnosis of BM should be made as early as possible. In addition, tools are needed for monitoring the response to therapy of BM.

Human serum contains two forms of tartrate-resistant acid phosphatase (TRACP), namely TRACP 5a derived from macrophages and TRACP 5b derived from osteoclasts (4, 5). TRACP 5b is a promising new marker of bone resorption (6, 7). The TRACP 5b-specific immunoassay used in this study detects only active TRACP 5b molecules that have been liberated from osteoclasts into the circulation recently. Thus, with this assay, bone resorption can be measured specifically at the time of sample collection. This assay has been shown to have low biological and analytical variabilities (6, 7). There are some advantages of TRACP 5b compared to other bone markers. TRACP 5b activity does not show marked dependence on food intake or diurnal rhythm (8). In addition, TRACP 5b activity is not affected by liver or kidney function (7, 8), which is an important issue in oncology considering patients with, *e.g.,* additional liver metastases. The limitation is on the long-term storage temperature, which must be –70˚C or lower (6). Serum TRACP 5b levels are affected by changes in both pathological and physiological bone turnover. Increased TRACP 5b concentrations have been detected in conditions with increased bone resorption, such as osteoporosis, Paget's disease, renal bone disease, multiple myeloma and breast and prostate cancer patients with BM (9-14). Serum TRACP

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5b is also decreased during antiresorptive therapy (15-18), and can be used to predict future bone fracture risk (19).

Carboxyterminal telopeptide of type I collagen (ICTP) is a cross-linked product of collagen I degradation generated by matrix metalloproteinases. Increased concentrations of ICTP have been shown to be closely correlated with increased pathological bone resorption in clinical conditions, such as rheumatoid arthritis and cancer BM, but to be rather insensitive to changes in physiological bone collagen turnover (20, 21).

Theoretically, combining TRACP 5b and ICTP should increase the reliability of the markers to detect bone metastases. TRACP 5b is specifically derived from osteoclasts, but its serum levels are affected by changes in both pathological and physiological bone turnover. After breast cancer has been diagnosed, observing elevated TRACP 5b levels during follow-up most probably indicates bone metastases, but it can also indicate elevated physiological bone turnover, especially in patients who additionally have postmenopausal osteoporosis. ICTP, in turn, is specific for pathological collagen degradation, but it is not bone-specific. Thus, the combined elevation of TRACP 5b and ICTP should, in theory, indicate nothing else but BM.

The combination of serum TRACP 5b and ICTP was hypothesized to improve the diagnosis of BM in breast cancer. The aim of the present cross-sectional study was therefore: a) to assess the value of TRACP 5b and ICTP in the detection of BM in breast cancer and b) to assess whether these markers could increase the specificity and sensitivity of BM detection, as compared with serum total alkaline phosphatase (tALP), the routinely clinically used marker of BM in breast cancer.

Table I. *Patient characteristics, comparison of breast cancer patients without (BM–) and with (BM+) bone metastases.*

	BM– (N=141) N (%)	BM+ (N=46) N (%)	*p*-value
Postmenopausal N (%)	110 (78%)	43 (93%)	0.0088**
Previous therapy			
No previous therapy	65 (46%)	3 (7%)	<0.0001**
Chemotherapy	43 (30%)	25 (54%)	0.004**
Irradiation	53 (38%)	29 (63%)	0.003**
Endocrine	31 (22%)	28 (61%)	<0.0001**
Present systemic therapy			
Chemotherapy	16 (11%)	8 (17%)	0.288**
Endocrine therapy (excluding aromatase inhibitor)	30 (21%)	26 (56%)	<0.0001**
Aromatase inhibitor	4 (3%)	11 (24%)	<0.0001****
Bisphosphonates	3 (2%)	16 (35%)	<0.0001****
Soft tissue metastases (Other than bone)			
No metastases	123 (87%)	0 (0%)	
Local progression only	5 (4%)	0 (0%)	
Metastases to parenchymal structures	13 (9%)	31 (67%)	
Mean age (range)	58 (31-87)	61 (38-89)	0.091*
Time from diagnosis in days (Median, range)	149 (20-10045)	1967 (30-6542)	<0.0001***

Statistical method: **t*-test, **Chi-square, ***Wilcoxon rank sum, ****Fisher exact test.

Materials and Methods

Serum samples were collected from consecutive breast cancer patients who had a histologically confirmed diagnosis attending the Department of Oncology in Turku University Hospital, Turku, Finland, and were stored at –70˚C. The clinical data were collected from the patients' files, including information on primary diagnosis, current status, treatment of breast cancer and use of all medications and treatments that could affect bone metabolism. The presence of BM was verified by reviewing skeletal scintigrams and X-rays with the clinical follow-up data.

Serum tALP was determined using a kit manufactured by Roche Diagnostics GmbH (Mannheim, Germany). The measurements were performed with Hitachi 917 equipment (Hitachi Ltd., Tokyo, Japan). Serum ICTP was measured by a commercially available competitive radioimmunoassay (Orion Diagnostica, Espoo, Finland). Tartrate-resistant acid phosphatase 5b (TRACP 5b) activity was measured using an in-house immunoassay (6) that is also available commercially (BoneTRAP® assay, SBA-Sciences, Oulu, Finland). The inter-assay CV of the TRACP 5b assay is 7.7%.

All patients provided informed consent and the study was approved by the joint ethical committee of Turku University Hospital and the University of Turku.

Statistical methods. The Chi-square and Fisher exact tests were used in comparison of categorical patient characteristics and the *t*-test or Wilcoxon rank sum test were used to compare numeric variables. Logistic regression was used to analyze the association between BM with several bone markers. The following analyses were performed for serum concentrations of TRACP 5b, ICTP and tALP: a) univariate analyses with all patients included, b) univariate analysis without patients who received bisphosphonates and/or aromatase inhibitors, c) multivariate analysis with all patients and d) multivariate analysis without patients who received bisphosphonates and/or aromatase inhibitors. In all models, standard deviation of the explanatory variable was used as a unit to calculate the odds ratios (OR) so that the ORs were comparable between the variables and models. The main criterion for assessing model discriminative ability was the non-parametric estimate of the area under (AUC), the receiver-operating characteristics (ROC) curve (22) and the sensitivity and specificity. When comparing areas under ROC curves, methods described by Hanley and McNeil (23) were used. A *p*-value of less than 0.05 was considered statistically significant. Statistical computations were performed using the SAS System for Windows version 8.2.

Table II. *Mean levels (±SD) of TRACP 5b, ICTP and tALP in breast cancer patients without (BM–) and with (BM+) bone metastases.*

	BM– Mean±SD (N)[1]	BM+ Mean±SD (N)[1]	BM– Mean±SD (N)[2]	BM+ Mean±SD (N)[2]	BM– Mean±SD (N)[3]	BM+ Mean±SD (N)[3]
TRACP5b (U/l)	3.2±1.2 (141)	6.2±3.0(46)	3.2±1.2 (134)	5.4±2.7(23)	3.1±1.3(7)	6.9±3.2 (23)
ICTP (U/ml)	4.3±1.7(133)	9.9±7.0(46)	4.3±1.7(129)	8.2±4.3(23)	4.2±1.7(4)	11.6±8.7(23)
tALP (U/ml)	150.2±47.4(121)	327.2±257.4(46)	151.2±46.5(114)	264.2±122.0(23)	135.0±61.6 (7)	390.2±335.2(23)

[1]all patients included, [2]excluding patients treated with bisphosphonates/aromatase inhibitors, [3]only patients treated with bisphosphonates/aromatase inhibitors.

Table III. *Association of TRACP 5b, ICTP and tALP with bone metastases in logistic regression analysis.*

		All patients			Without patients on bisphosphonates and/or aromatase inhibitor drugs		
	Predictor	OR[1] (95% CI)		*p*-value	OR[2] (95% CI)		*p*-value
Univariate analysis	TRACP 5b	6.5	(3.6-13.6)	<0.001	3.6	(2.1-7.0)	<0.001
	ICTP	16.7	(6.4-54.0)	<0.001	4.5	(2.5-9.2)	<0.001
	tALP	8.1	(4.1-18.8)	<0.001	3.2	(2.1-5.4)	<0.001
Multivariate analysis	TRACP 5b	2.9	(1.4-6.6)	0.007	1.6	(0.8-3.6)	0.194
	ICTP	5.3	(1.5-21.4)	0.012	2.6	(1.3-6.0)	0.013
	tALP	2.9	(1.3-7.2)	0.012	1.8	(1.1-3.2)	0.026

[1]Corresponds to an increase of one SD (TRACP 5b SD=2.24, tALP SD=161, ICTP SD=4.54).
[2]Corresponds to an increase of one SD (TRACP 5b SD=1.71, tALP SD=77.5, ICTP SD=2.65).

Results

Patient characteristics and information regarding previous and present cancer therapies of the two groups studied, without BM (BM–) and with radiologically confirmed BM (BM+), are provided in Table I. The mean age of the patients was 60 years (range 31-89). The mean (±SD) serum concentrations of each marker in the BM– and BM+ groups are shown in Table II.

When the serum concentrations of all studied markers were analyzed with univariate logistic regression analysis, all three markers (TRACP 5b, ICTP, tALP) exhibited a statistically significant association with the presence of BM, even when patients treated with bishosphonates and/or aromatase inhibitors were excluded (Table III).

Analysis of the odds ratios indicated that an increase of the magnitude of one SD in serum TRACP 5b concentration corresponded to a 6.5-fold higher risk of BM. An increase of the magnitude of one SD in serum ICTP and tALP concentrations corresponded to a 8.1-fold and 16.7-fold times higher risk of BM, respectively. The distribution of tALP values was remarkably skewed with a few very high values increasing the standard deviation. Because of this, the corresponding OR was very high. When the patients on bisphosphonates and/or on aromatase inhibitors were excluded, the corresponding ORs were 3.62 for TRACP 5b, 3.19 for ICTP and 4.52 for tALP. In this group, no outlining values for tALP were included (Table III).

When the serum concentrations of the various markers were analyzed by comparing their AUCs, serum TRACP 5b exhibited the largest AUC (0.845), followed by ICTP (0.818) and tALP (0.814) when all patients were included in the analysis (Figure 1a). These differences were not, however, statistically significant between TRACP 5b and tALP ($p=0.26$), ICTP and tALP ($p=0.53$) or TRACP 5b and ICTP ($p=0.53$).

When the analysis was made without patients using bisphosphonates and/or aromatase inhibitors, the comparison of AUC statistics was as follows: TRACP 5b 0.798, ICTP 0.764 and tALP 0.797 (Figure 1b). There were no statistically significant differences between TRACP 5b and tALP ($p=0.49$), ICTP and tALP ($p=0.31$) or TRACP 5b and ICTP ($p=0.31$).

In the multivariate regression analysis, all three markers remained statistically significant predictors of BM when all patients were included. However, when patients with bisphosphonates and/or aromatase inhibitors were excluded, TRACP 5b did not remain a significant predictor for BM (Table III, multivariate analysis).

In the multivariate analysis with the three markers combined (TRACP 5b, ICTP and tALP) AUC was 0.896, as shown in Figure 1c. When patients with bishosphonates and/or aromatase inhibitors were excluded, the AUC was

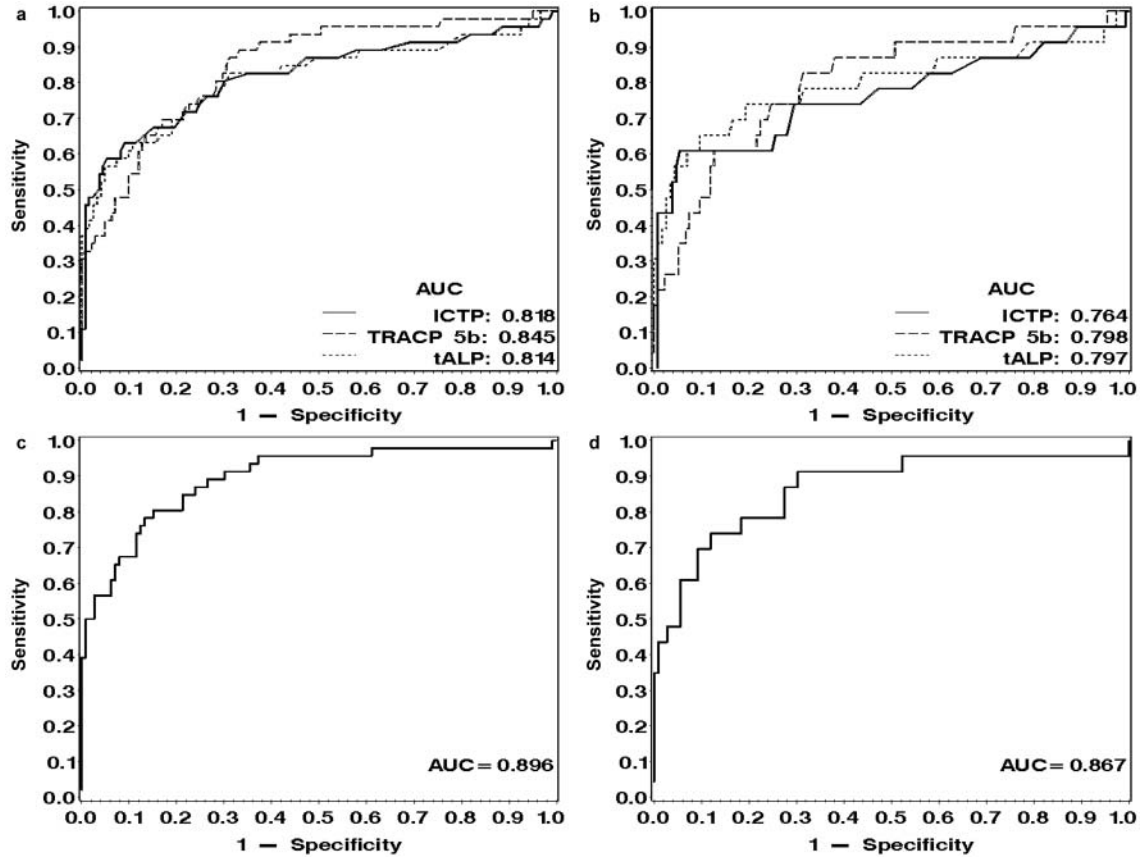

Figure 1. *ROC curves for TRACP 5b, ICTP and tALP. a) Univariate analysis for all patients, b) multivariate analysis for all patients, c) univariate analysis without patients who received bisphosphonates and/or aromatase inhibitors and d) multivariate analysis without patients who received bisphosphonate and/or aromatase inhibitors. AUC=Area under the ROC curve.*

0.867 as shown in Figure 1d. The combination seemed to slightly improve the detection power for BM.

Since TRACP 5b and ICTP are indicators of different metabolic processes, these two were combined and tested against tALP. A comparison of the combination TRACP 5b and ICTP (AUC=0.881) against tALP (AUC=0.814) all patients included (p=0.09) is shown in Figure 2a. The corresponding comparison of TRACP 5b and ICTP (AUC=0.835) *vs.* tALP (AUC=0.797) is shown in Figure 2b, without patients treated with aromatase inhibitors and/or bisphosphonates (p=0.28).

The sensitivity and specificity were estimated by finding the lowest cut-off values for each marker, with a sensitivity of minimum 85% as a cut-off and the best possible value for specificity. In this setting, the cut-off value was 3.65 for TRACP 5b, with a sensitivity of 87% and specificity of 69%. For ICTP, the cut-off was 4.2, with a sensitivity of 87% and specificity of 53%. For tALP, the cut-off was 145, with a sensitivity of 87% and specificity of 50%. When the patients using bisphosphonates and/or aromatase inhibitors were excluded, the corresponding cut-off values and percentages were: TRACP 5b cut-off 3.3, sensitivity 87%, specificity 62%; ICTP cut-off 3.6, sensitivity 87%, specificity 31%; and tALP cut-off 135, sensitivity 87%, specificity 40%.

When all patients were included and when the cut-point criteria were fulfilled with both TRACP 5b (>3.65) and ICTP (>4.2), the sensitivity was 78.3% and the specificity 82%. When the patients using aromatase inhibitors and/or bisphosphonates were excluded, the corresponding percentages were 83% and 68%.

Discussion

BM are frequent complications in breast cancer. Their diagnosis usually relies on radiology and bone scintigraphy, which may be limited by low sensitivity and specificity. Furthermore, due to the flare phenomenon, bone scans need to be interpreted with caution within 6-9 months after a change in therapy (24). As a consequence, this effect may delay the gathering of critical information on treatment efficacy. It is obvious that better tools for the early diagnosis and monitoring of breast cancer BM are needed.

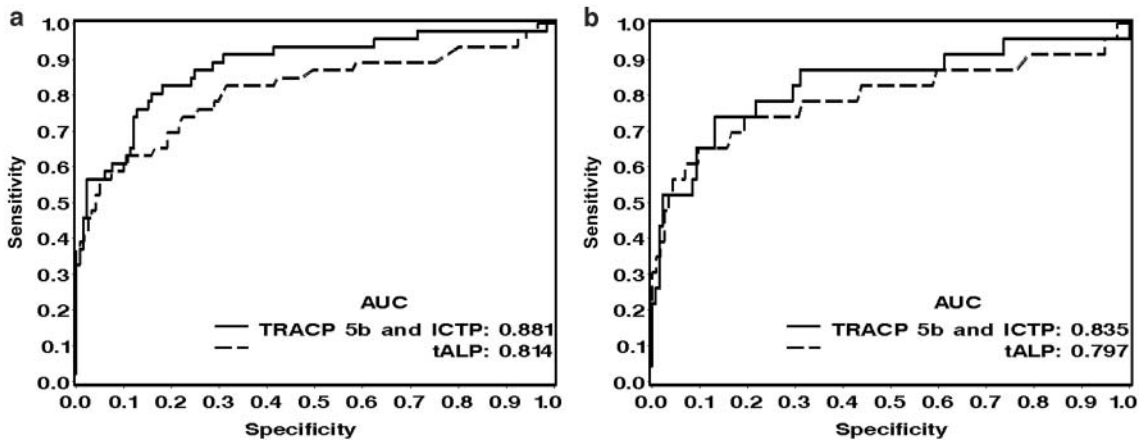

Figure 2. *ROC curves for the combination of TRACP 5b and ICTP and for tALP including (a) and excluding (b) patients with aromatase and/or bisphosphonate treatment. AUC=Area under the ROC curve.*

The current, widespread clinical practice is to measure serum tALP, an indicator of osteoblast activity, also in conditions such as breast cancer, where increased osteoclast activity and osteolysis dominate. The paradox in using serum tALP measurements for the diagnosis or follow-up of osteolytic bone metastases is even further pronounced by the fact that increased bone resorption at the site of developing BM is not, unlike in healthy bone, coupled to increased bone formation (25). Therefore, serum markers of bone resorption might be more sensitive than tALP in the diagnosis of breast cancer BM.

The findings of the present study indicated that the bone resorption markers TRACP 5b and ICTP were equally sensitive and specific in the detection of BM in breast cancer patients compared with tALP. The fact that the tested serum markers of bone resorption did not outperform tALP may be because the patients in this study had already been treated. Typically, when osteolytic lesions respond to treatment, the physiological coupling between bone resorption and formation is partly restored and serum concentrations of bone formation mirror the events of bone resorption again (25). Our results suggested that further studies are needed to characterize the value of serum markers of bone resorption in the early diagnosis of bone metastases, in untreated patients.

We further set out to test the hypothesis that, by combining TRACP 5b, ICTP and tALP, the sensitivity and specificity of detecting BM in breast cancer patients could be increased, compared to when the single markers were used alone. This is because the use of composite markers, consisting of two or more markers of a given biological phenomenon or disease, *e.g.,* breast cancer, may result in a better diagnostic performance than the use of any of the markers alone (26). Of the various marker combinations tested in this study, the AUC value was higher for the combination of TRACP 5b and ICTP than for any of the markers alone, but the difference did not reach statistical significance, possibly due to the small sample size. The observed increased AUC value may, however, suggest that, by combining serum measurements of TRACP 5b and ICTP, the bone metastatic diagnostic value of these markers might be increased.

Treatment with bisphosphonates and/or aromatase inhibitors decreases bone resorption and bone marker levels and thus it is important to obtain pre-treatment marker values for each individual when using bone markers for treatment monitoring. Unfortunately, we were not able to obtain pre-treatment values for our study subjects. Our patients with BM who received treatment with bisphosphonates and/or aromatase inhibitors had higher post-treatment TRACP 5b, ICTP and tALP levels than the observed values of those patients who did not receive such treatment. Because bone marker levels decrease during such treatment, the pre-treatment values of these patients must have been substantially higher than the observed post-treatment values. This is reasonable because treatment was started in patients with the most severe disease. Thus, excluding the patients who received treatment in this study means excluding patients with most severe metastatic bone disease and the highest bone marker values. Therefore, the observed decrease in the diagnostic value of TRACP 5b, ICTP and tALP after excluding these patients was reasonable and expected.

In conclusion, the present findings indicated that serum TRACP 5b and ICTP were at least equally sensitive and specific markers of BM in breast cancer patients as tALP. Our results encourage further study of TRACP 5b and ICTP in the detection of breast cancer BM and their combined and separate roles in predicting skeletal changes during the treatment of breast cancer.

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