ANTICANCER RESEARCH *25*: 4639-4646 (2005)

# Evaluation of Tartrate-resistant Acid Phosphatase (TRACP) 5b as Bone Resorption Marker in Irradiated Bone Metastases

STEPHAN MOSE[1,5], CHRISTIAN MENZEL[2], ANDREAS A. KURTH[3], KIRSTIN OBERT[1], ULLA RAMM[1], KLAUS EBERLEIN[1], HEINZ-D. BOETTCHER[1] and UWE PICHLMEIER[4]

[1]*Department of Radiooncology,* [2]*Department of Nuclear Medicine and* [3]*Department of Orthopedic Surgery, Stiftung Friedrichsheim, Johann Wolfgang Goethe - University Hospital Frankfurt Main, Theodor-Stern Kai 7, D-60590 Frankfurt/Main;*
[4]*Department of Medical Biometry and Epidemiology, University of Hamburg, Martinistrasse 52, 20246 Hamburg;*
[5]*Department of Radiotherapy and Radiooncology, Schwarzwald-Baar Klinikum Villingen-Schwenningen, Vöhrenbacher Straße 23, D-78048 Villingen-Schwenningen, Germany*

**Abstract.** *Background: The aim of our study was to evaluate if the determination of the active isoform 5b of tartrate-resistant acid phosphatase (TRACP 5b) provides the possibility to monitor the effect of local radiotherapy in bone metastases and if TRACP 5b will predict further osseous progression. Materials and Methods: In 48 breast cancer patients with bone metastases, patients' characteristics, diagnostic imaging and laboratory investigation, tumor- and therapy-related parameters were registered at the beginning and the end of radiotherapy, as well as 6 and 12 weeks afterwards. TRACP 5b activity was measured using a solid phase immunofixed enzyme activity assay with the monoclonal antibody O1A. Results: During follow-up, progression in another part of the skeleton was diagnosed in 31 patients (65%). There was a significant decrease of TRACP 5b in patients without progression in non-irradiated regions, whereas in progressive disease, TRACP 5b levels remained stable with a slightly increasing tendency ($p<0.007$). In patients with ≤3 metastases, all TRACP 5b values were significantly lower than the values of those with >3 metastases ($p=0.01$). Conclusion: In patients without further osseous progression, TRACP 5b is able to monitor the effectiveness of local radiotherapy. The estimation of sensitivity and specificity based on each TRACP 5b value demonstrates that the ability to discriminate between those patients with or without osseous progression increases with time.*

*Correspondence to:* Priv. Doz. Dr. med. Stephan Mose, Schwarzwald-Baar Klinikum Villingen-Schwenningen, Department of Radiotherapy and Radiooncology, Vöhrenbacher Straße 23, D-78048 Villingen-Schwenningen, Germany. Tel: 0049-7721-93-3401, Fax: 0049-7721-93-3409, e-mail: stephan.mose@sbk-vs.de

*Key Words:* TRACP 5b, radiotherapy, bone metastases, breast cancer.

In addition to routine clinical investigation, evaluation of pain scores and the use of X-ray imaging and/or bone scintigraphy (1), the evaluation and monitoring of local radiotherapy in the treatment of bone metastases, as well as the disease activity, may be improved by biochemical markers of bone formation and/or bone resorption. However, neither in diagnosis nor in monitoring systemic or local therapies of osseous metastases, are bone markers – with the exception of the alkaline phosphatase (AP) with its known restrictions – routinely used. This is based on the lack of bone-related specificity [*e.g.* AP], cross-reactivity with bone and liver [*e.g.* bone-specific AP (BAP)], their circadian rhythm [*e.g.* (deoxy)pyridinoline crosslinks (CPD, PYD), osteocalcin (OC), bone sialoprotein (BSP)], the occurrence of some markers in other tissues [*e.g.* procollagen peptides (PICP, PINP)] and – in contrast to serum C-terminal telopeptide cross-linked collagen (ICTP) which demonstrates better results – on the higher analytical and biological variations in urinary-based assays (*e.g.* ICTP, INTP) (2-5).

Recently, the active isoform 5b of tartrate-resistant acid phosphatase (TRACP 5b) has been considered a specific serum marker of osteoclast activity and a useful marker of bone resorption (6-13). Whereas several enzymatic assays (2, 14, 15) as well as immunoassays utilizing antibodies specific for TRACP (16, 17) lack specificity because of the ubiquitous occurrence of acid phosphatases, Halleen and co-workers established an immunoassay using the monoclonal antibody O1A which, in contrast to TRACP 5a derived from macrophages and dentritic cells (10, 12, 18), specifically recognizes the osteoclastic isoform 5b in both human bone and human serum (6, 19). In clinical data, there was a low biological variation over 24 hours. Furthermore, there was neither a dependence on liver or

kidney function, nor on feeding (20, 21). Similiar data were published by Nakanishi *et al.* (7), who comparably established a kinetic assay by taking advantage of the heparin-induced inhibition of TRACP 5a in contrast to 5b activity.

Because of these promising pre-clinical and clinical data, the aim of our study was to evaluate if the determination of TRACP 5b generally provides the possibility of monitoring the effect of local radiotherapy in bone metastases and if TRACP 5b may have an additional and predictive value regarding further osseous progress.

## Materials and Methods

Among 112 patients with osseous, non-surgically treated metastases of a solid tumor, registered between 09/2001 and 08/2003, 77 patients were considered eligible. Because of prognosis ≤3 months (n=15), decline or lack of compliance (n=5), lack of fluency in German (n=5), soft tissue metastases with osseous infiltration (n=7), unexpected death (n=2), or bone fracture during radiotherapy (n=1), 35 of these 112 patients were excluded. With regard to the homogeneity of the cohort, only female breast cancer patients (mean 60.4±12.9 years) with osseous metastases (n=48) were included in this study. In accordance with the Declaration of Helsinki, all patients provided written informed consent.

The patients' characteristics, diagnostic imaging and laboratory investigation (X-ray imaging, isotope bone scan, alkaline phosphatase, TRACP 5b), tumor- and therapy-related parameters were registered at the beginning ($T_1$) and at the end of radiotherapy ($T_2$) as well as 6 ($T_3$) and 12 weeks ($T_4$) afterwards. Pain was evaluated due to a visual analogue scale (0=no pain, 10=maximum pain). Progression of osseous disease was defined using the patients' clinical presentation as well as the imaging of irradiated and non-irradiated bone regions: i. increased use of analgesic medication due to WHO-categories and/or increased pain; ii. recalcification in irradiated bones (X-ray) and increase of the number and/or the extent of (non-)irradiated osseous metastases (X-ray, bone scan at $T_1$, $T_3$ and/or $T_4$). Although after 6 and 12 weeks of radiotherapy 8 and 4 patients had died, respectively, in all patients complete information regarding further osseous progress was available.

Serum samples obtained were separated from blood cells within 2 hours, frozen, and stored at –80˚C. TRACP 5b activity was measured using the TRACP 5b-immunoassay as described by Halleen *et al.* (9). In brief, this commercially available method (medac GmbH, Wedel, Germany) is a solid phase immunofixed enzyme activity assay for the determination of osteoclast-derived TRACP 5b in human serum samples using a highly characterized, specific monoclonal antibody O1A. Microtiter wells are coated with these anti-TRACP antibodies. Bound TRACP 5b activity is determined at a pH where TRACP 5a is inactive and TRACP 5b is highly active, shown with a chromogenic substrate (p-nitrophenylphosphate). After termination of this reaction, the absorbance is read in a microtiter plate reader and the color intensity is directly proportional to the activity of TRACP 5b present in the sample.

The frequencies and percentages of all variables were recorded. Continuous data were summarized by median, quartiles, mean, standard deviation, minimum and maximum. Comparisons of ordinal and continuous variables were performed with Wilcoxon-Mann-Whitney tests. The overall value of TRACP 5b was assessed through receiver operating characteristic (ROC) curves, constructed by varying the cut-point used to determine which TRACP 5b value will be considered abnormal and then plotting the resulting sensitivities against the corresponding specificities. As a measure of accuracy, the area under the curves (AUC) was calculated. For a nonparametric comparison of AUCs on the same individuals evaluated at different visits, the method of DeLong *et al.* (22) was applied, which takes into account the correlated nature of the data. All *p*-values presented are two-tailed.

Table I. *Patients' characteristics, tumor- and therapy-related parameters of 48 breast cancer patients with bone metastases before initiation of radiotherapy (SD=standard deviation).*

| Patients' characteristics | Mean value±SD | Range |
|---|---|---|
| Age (years) | 60.4±12.9 | 33-86 |
| Karnofsky-Index (%) | 80 | 40-100 |
| **Osseous metastases** | **Patients (n)** | **%** |
| Osteolytic | 42 | 87.5 |
| Mixed | 6 | 12.5 |
| Solitary lesion | 5 | 10.4 |
| ≤3 metastases | 7 | 14.6 |
| >3 metastases | 36 | 75.0 |
| **Simultaneous systemic treatment** | **Patients (n)** | **%** |
| Endocrine therapy | 33 | 68.8 |
| Chemotherapy | 5 | 10.4 |
| Bisphosphonates | 45 | 93.8 |
| **Analgesic drugs** | **Patients (n)** | **%** |
| No analgesic drugs | 9 | 18.8 |
| WHO I | 16 | 33.3 |
| WHO II | 7 | 14.6 |
| WHO III | 16 | 33.3 |

## Results

At the time of admittance to our department, bone metastases had been diagnosed 33.9 months (median value 7.9 months, range 0-192 months) before. As seen in Table I, most of the patients suffered from osteolytic and multiple metastases. In most women, any kind of continuous systemic treatment (chemo- or endocrine therapy, bisphosphonates) was given; this therapy started at least 2½ months before radiotherapy was indicated. Most of the women (81.2%) were given an analgesic medication; co-analgesics were applied in 5 patients (10.4%) (Table I). Irradiation was indicated in case of pain (60.4%), impending bone fracture

| Visit | Osseous progression | N | Mean | SD | Min | Median | Max | *P* |
|---|---|---|---|---|---|---|---|---|
| Baseline ($T_1$) | no | 17 | 5.55 | 2.48 | 1.8 | 5.1 | 9.4 | 0.1258 |
| | yes | 31 | 8.80 | 6.39 | 3.0 | 6.0 | 25.4 | |
| End of RT ($T_2$) | no | 17 | 4.48 | 1.94 | 1.7 | 3.8 | 8.1 | 0.0068 |
| | yes | 31 | 8.93 | 7.53 | 2.5 | 6.8 | 38.8 | |
| 6 weeks after RT ($T_3$) | no | 14 | 3.96 | 1.99 | 1.9 | 3.0 | 8.4 | 0.0023 |
| | yes | 22 | 7.63 | 5.00 | 3.0 | 5.6 | 21.8 | |
| 12 weeks after RT ($T_4$) | no | 11 | 3.14 | 1.57 | 1.7 | 3.0 | 7.5 | 0.0004 |
| | yes | 21 | 8.42 | 5.20 | 2.3 | 6.4 | 21.6 | |
| Last examination/ Progression ($T_P$) | no | 17 | 4.15 | 2.12 | 1.7 | 3.1 | 8.1 | 0.0003 |
| | yes | 31 | 9.54 | 7.52 | 2.3 | 6.9 | 38.8 | |

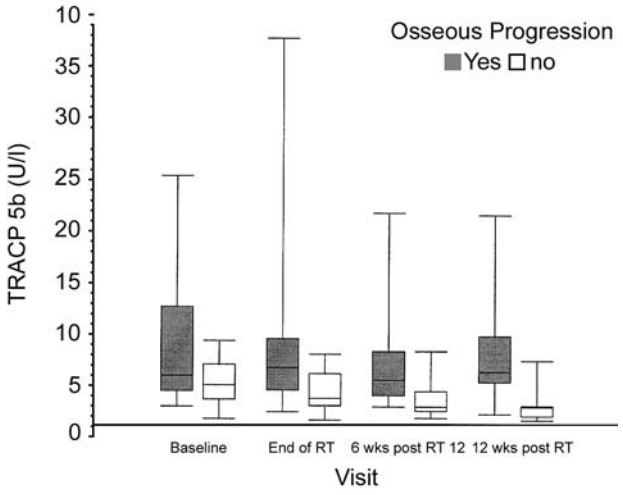



Figure 1. *Descriptive statistics and Box-Whisker diagrams of TRACP 5b (U/l) in breast cancer patients with bone metastases during and after the course of radiotherapy (RT) stratified by later observed osseous progression in non-irradiated regions (SD = standard deviation).*

(10.4%) or both (29.2%). Radiotherapy was administered with a 6 or 25 MV linear accelerator (2-3 Gy/day, 5x/week, total dose 30-40 Gy, median 36 Gy).

As demonstrated by the visual analogue scales, pain was reduced by radiotherapy (median value at $T_1$: 5, $T_2$: 2.5, $T_3$: 1, $T_4$: 1). Furthermore, at the beginning of therapy 18.8% (n=9/48) of patients did not take any analgesic medication, whereas 12 weeks after irradiation there was no use of analgesics in 52.6% (n=20/38). We observed a reduction of analgesic drugs in all WHO-categories (WHO 1: 33.3% to 13.1%, WHO II: 14.6% to 7.9%, WHO III: 33.3% to 26.3%).

Twelve weeks after radiotherapy, X-ray imaging of the irradiated region showed a recalcification in 85.3% of patients (n=29/34). After radiotherapy, in 31 patients (64.6%) progression in another part of the skeleton was diagnosed ($T_3$: n=10, $T_4$: n=21), whereas in 17 patients (35.4%) bone scans did not show new metastases. Stratifying by the number of osseous metastases, a progression was found in 4 out of 12 patients with ≤3 lesions and in 27 out of 36 patients with >3 metastases.

Observing all TRACP 5b values, the median values did not show any systematic changes over time ($T_1$: 5.9 U/l; $T_2$: 6.1 U/l; $T_3$: 4.5 U/l, $T_4$: 5.4 U/l). Stratifying statistical analysis by later observed osseous progression in non-irradiated regions, a significant decrease of TRACP 5b in those patients without new diagnosed bone metastases (median value at $T_1$ and $T_4$: 5.1 U/l to 3.0 U/l) was found, whereas in progressive disease TRACP 5b activity remained stable with a slightly increasing tendency (median value at $T_1$ and $T_4$: 6.0 U/l to 6.4 U/l) (Figure 1). Furthermore, after the end of radiotherapy, TRACP 5b values in patients without later progression were significantly lower than those in the other women. At the date of progression or last evaluation in the 17 patients without osseous progression,

the median TRACP 5b value was 3.1 (range 1.7–8.1), whereas in those 31 patients with progression the median was 6.9 (range 2.3–38.8) ($p=0.0003$) (Figure 1). Addressing the assumption that TRACP 5b activity may be influenced by osseous metastatic burden (23), our data demonstrated that, in patients with ≤3 metastases, TRACP 5b values at $T_2$ and $T_P$ were significantly lower than values in those with >3 metastases ($p=0.01$) (Table II).

Using the ROC-analysis, the AUCs at the time of the last examination or progression ($T_P$) was significantly larger than the AUC at baseline ($T_1$) ($p=0.049$) and showed a trend to be larger than the AUC at the end of irradiation ($T_2$) ($p=0.095$). Furthermore, the baseline AUC significantly differed from the AUC measured at the end of treatment ($p=0.006$) (Figure 2). If only those patients who were alive during the complete follow-up after radiotherapy were included in the analysis, no further information was obtained (AUC at $T_1$: 0.65, $T_2$: 0.76, $T_P$: 0.85). Sensitivities and specificities at selected TRACP 5b values demonstrated that the ability to discriminate between patients with or without osseous progression increases over time (Figure 2).

Table II. *Comparison of TRACP 5b values in breast cancer patients with bone metastases (≤3 versus >3 metastases) at baseline, end of radiotherapy (RT) and at time of progression/last examination.*

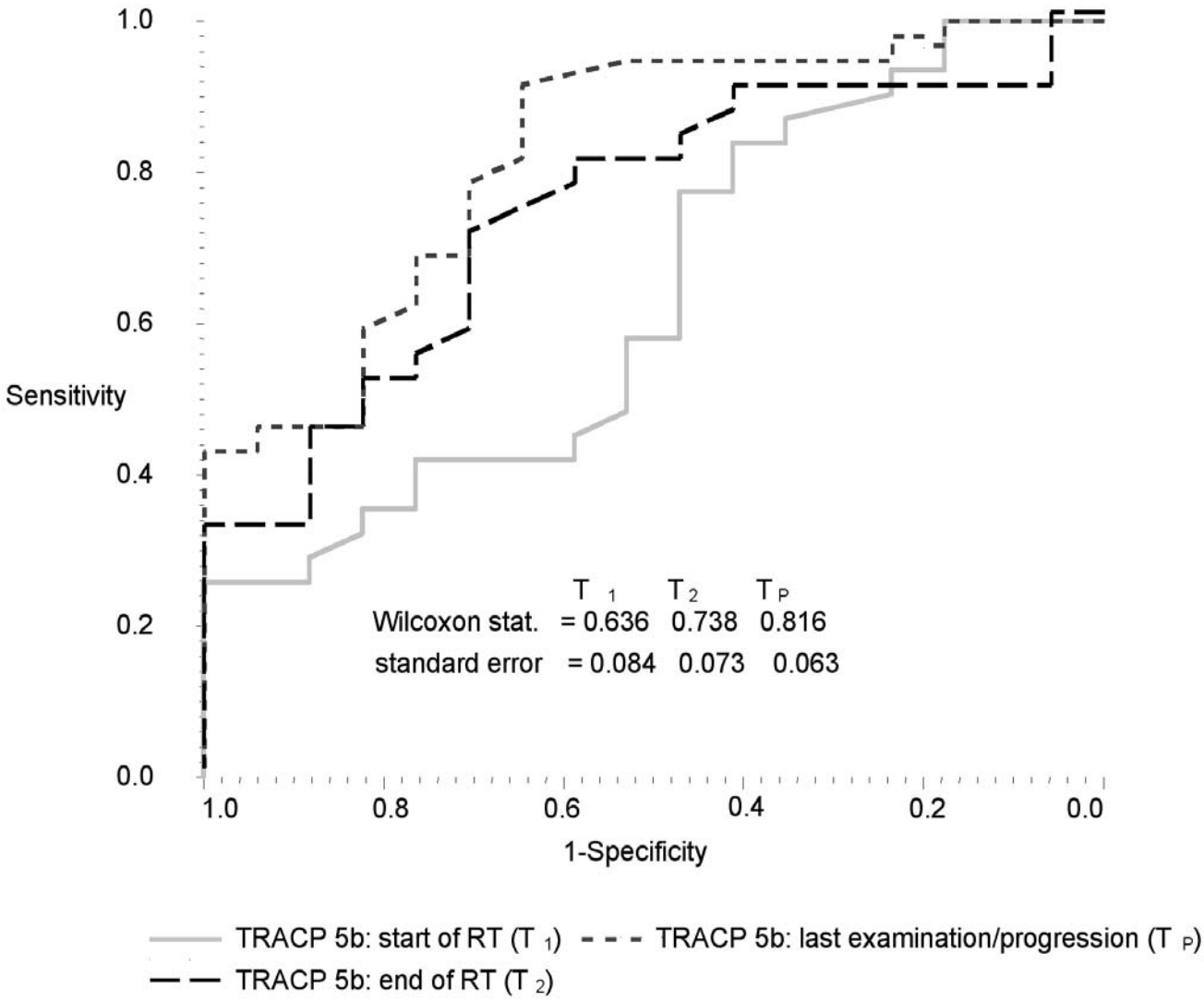
| | ≤3 metastases (n=12) | >3 metastases (n=36) | |
|---|---|---|---|
| $T_1$ (Baseline) | | | |
| Median | 4.4 | 6.4 | $p=0.08$ |
| Range | 1.8 – 7.5 | 2.2 – 25.4 | |
| $T_2$ (End of RT) | | | |
| Median | 3.75 | 6.75 | $p=0.01$ |
| Range | 1.7 – 9.3 | 2.5 – 38.8 | |
| $T_P$ | | | |
| Median | 3.9 | 6.35 | $p=0.01$ |
| Range | 1.7 – 9.3 | 2.0 – 38.8 | |

## Discussion

Although X-ray imaging regarding recalcification and pain evaluation scales together with the recording of analgesic drugs appear to allow for the measurement of pain reduction and treatment response in a reasonable manner, an objective evaluation of the effect of irradiation in treating bone metastases is difficult (1, 2, 24, 26). Furthermore, taking into consideration the osseous progression in non-irradiated regions during clinical follow-up and the monitoring of the ongoing systemic treatment by established, albeit criticized, diagnostic imaging (26), the evaluation of treatment as well as the disease activity may be improved by the use of bone markers (2, 4, 15).

While in healthy pre- and postmenopausal women, as well as in women with osteoporosis, TRACP 5b activity is similar to that of other bone markers (OC, BAP, PICP, PINP, ICTP, INTP) (6, 9, 11), TRACP – measured with an enzymatic assay – in breast cancer patients with newly diagnosed osseous metastases initially failed to demonstrate an efficiency compared to ICTP and BAP (2). Using a spectrophotometric assay, Wada *et al.* (15) already favored TRACP because of its easy, fast and economical method of measurement, although other bone markers (AP, DPD, PYD) yielded comparable results. In an early clinical study using one of the newly established immunoassays, 80% of breast cancer patients with bone metastases were found to have elevated TRACP 5b values compared to healthy pre- and postmenopausal women, resulting in a higher sensitivity than observed for other bone markers (BSP, ICTP, INTP) (9). Other authors examined healthy women and breast cancer patients at primary diagnosis without signs of osseous metastases and did not find any difference in bone marker activity, whereas a significant difference was observed in patients in whom bone metastases were newly diagnosed regarding TRACP 5b (Table III) (23, 25, 27) and BAP, ICTP and INTP (23, 25).

Furthermore, based on experimental results (8), bone markers may be appropriate for monitoring the treatment of bone metastases. Clinical data obtained in patients with multiple myeloma (28), as well as in breast cancer patients with osseous metastases (27), demonstrated increasing TRACP 5b values, in spite of further bisphosphonate treatment when progress of bone metastases occurred compared to stable disease. Interestingly, in our study we found comparable results regarding TRACP 5b values: in patients responding to local irradiation without further progression in non-irradiated regions, values decreased during the course of follow-up, while – independent of local response – TRACP 5b levels remained stable or increased again when there was further osseous progress. Based on the ROC-analysis, we can cautiously conclude that, despite local response to radiotherapy in patients with TRACP 5b values >3.8 U/l, further progression is probable. In this context, the possible influence of simultaneously given systemic therapy on TRACP 5b values should be discussed. Although there was no control group without radiotherapy, it seems probable that these therapies had no influence on TRACP 5b activity, since they were initiated at least 2½ months before irradiation was indicated. Furthermore, our baseline values (median 5.9 U/l) were comparable to literature data reported in breast cancer patients with osseous metastases before any treatment (5.1-6.1 U/l) (Table III) (9, 23, 27).




| TRACP 5b (U/l) | | $T_1$ | $T_2$ | $T_P$ |
|---|---|---|---|---|
| 3.8 | Sensitivity (%) | 84 | 81 | 90 |
| | Specificity (%) | 35 | 58 | 65 |
| 4.1 | Sensitivity (%) | 84 | 77 | 77 |
| | Specificity (%) | 41 | 59 | 71 |
| 4.5 | Sensitivity (%) | 77 | 77 | 77 |
| | Specificity (%) | 47 | 71 | 71 |
| 5.5 | Sensitivity (%) | 55 | 61 | 68 |
| | Specificity (%) | 53 | 70 | 71 |
| 6.8 | Sensitivity (%) | 45 | 48 | 48 |
| | Specificity (%) | 59 | 82 | 82 |

Figure 2. *ROC curves and associated area under the curves (AUC) for TRACP 5b values at baseline (T1), end of radiotherapy (T2), as well as at the time of the last examination or progression of disease (TP). For selected cut-points of TRACP 5b, estimates of sensitivity and specificity were extracted from ROCs.*

Table III. *Comparison of literature data demonstrating TRACP 5b values in healthy women and breast cancer patients without/with osseous metastases.*

| | Premenopausal women | Postmenopausal women | Healthy women | Breast cancer patients without osseous metastases | Breast cancer with osseous metastases before treatment | Reference patients |
|---|---|---|---|---|---|---|
| n | 60 | 40 | - | - | 20 | Halleen (9)[a] |
| TRACP 5b U/l | 2.15±0.83 | 3.21±1.05 | | | 6.1±4.75 | |
| n | - | - | 53 | 56 | 16 | Capeller (27)[a] |
| TRACP 5b U/l | | | 3.37±0.9 | 3.72±1.2 | 5.16±2.0 | |
| n | 41 | 132 | - | 161 | 42 | Koizumi (25)[a] |
| TRACP 5b U/l | 2.92±0.99 | 3.48±1.06 | | 3.08±0.79 | 4.79±1.60 <6 lesions<br>6.89±2.38 6-20 lesions<br>10.76±6.30 >20 lesions | |
| n | - | - | 60 | 30 | 30 | Chao (23)[b] |
| TRACP 5b U/l | | | 2.83±1.1 | 2.93±0.64 | 5.42±2.5 | |

[a]Enzyme immunoassay using the monoclonal antibody O1A
[b]Immunoassay using the monoclonal antibody 14G6

Another interesting issue is the assumption that the level of TRACP 5b activity may depend on the extent and/or the number of metastases (23). Koizumi *et al.* (25) observed relevant differences between TRACP 5b values regarding the number of metastases (<6, 6-20, >20 lesions) before any treatment (Table III). Comparable results were obtained in patients with different extents of myeloma bone disease (28). In our baseline data, we did not find a relevant difference between low and high metastatic tumor load (≤3 *vs.* >3 lesions); however, a significance was seen during therapy and follow-up, respectively. Based on these results, it is obviously justified to state that TRACP 5b increases in relation to the metastatic burden.

In conclusion, our data indicate the TRACP 5b levels can be used to monitor the clinical effect of local radiotherapy in patients without further osseous progression. Furthermore, TRACP 5b independently enabled us to discriminate between those patients with or without osseous progression during follow-up which may have a predictive value – independent of local therapy – for ongoing systemic treatment. Regardless of the necessary comparison with other relevant bone markers considering the different pathways of bone formation and resorption, and regardless of the criticisms of the new analytical methods in TRACP 5b evaluation (13), our data cautiously support the clinical data regarding TRACP 5b and the therapy and follow-up of bone metastases. Therefore, in a prospective controlled clinical trial, further effort should be focused on the evaluation of TRACP 5b levels compared to other bone markers to determine if TRACP 5b provides an improvement in prediction of osseous progression and in monitoring local or systemic treatment of bone metastases.

## References


1 Koswig S, Buchali A, Boehmer D, Schlenger L and Budach V: Palliative radiotherapy of bone metastases. A retrospective analysis of 176 patients. Strahlenther Onkol *175*: 509-514, 1999.

2 Plebani M, Bernardi D, Zaninotto M, De Paoli M, Secchiero S and Sciacovelli L: New and traditional serum markers of bone metabolism in the detection of skeletal metatases. Clin Biochem *29*: 67-72, 1996

3 Seibel MJ and Woitge HW: Biochemical markers of bone metabolism – Update 1999. Clin Lab *45*: 237-256, 1999

4 Seregni E, Martinetti A, Ferrari L and Bombardieri E: Clinical utility of biochemical marker of bone remodelling in patients with bone metastases of solid tumors. Q J Nucl Med *45*: 7-17, 2001.

5 Mose S, Menzel C, Kurth AA, Obert K, Breidert I, Borowsky K and Boettcher HD: Tartrate-resistant acid phosphatase 5b as serum marker of bone metabolism in cancer patients. Anticancer Res *23*: 2783-2788, 2003

6 Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ and Väänänen HK: Tartrate-resistant acid phosphatase 5b: a novel serum marker of bone resorption. J Bone Miner Res *15*: 1337-1345, 2000.

7 Nakanishi M, Yoh K, Miura T, Ohasi T, Rai SK and Uchida K: Development of a kinetic assay for band 5b tartrate-resistant acid phosphatase activity in serum. Clin Chem *46*: 469-473, 2000.

8 Alatalo SL, Halleen JM, Hentunen TA, Mönkkönen J and Väänänen HK: Rapid screening method for osteoclast differentiation *in vitro* that measures tartrate-resistant acid phosphatase 5b activity secreted into culture medium. Clin Chem *46*: 1751-1754, 2000.

9 Halleen JM, Alatalo SL, Janckila AJ, Woitge HW, Seibel M and Väänänen HK: Serum tartrate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. Clin Chem *47*: 597-600, 2001.

10 Janckila AJ, Takahashi K, Sun SZ and Yam LT: Tartrate-resistant acid phosphatase isoform 5b as serum marker for osteoclastic activity. Clin Chem *47*: 74-80, 2001.

11 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.
12 Janckila AJ, Neustadt DH, Nakasato YR, Halleen JM, Hentunen T and Yam LT: Serum tartrate-resistant acid phosphatase isoforms in rheumatoid arthritis. Clinica Chimica Acta *320*: 49-58, 2002.
13 Igarashi Y, Lee MY and Matsuzaki S: Acid phosphatases as markers of bone metabolism. J Chromatography B *781*: 345-358, 2002.
14 Scarnecchia L, Minisola S, Pacitti MT, Carnevale V, Romagnoli E, Rosso R and Mazzuoli GF: Clinical usefulness of serum tartrate-resistant acid phosphatase activity determination to evaluate bone turnover. Scand J Clin Lab Invest *51*: 517-524, 1991.
15 Wada N, Ishii S, Ikeda T, Enomoto K and Kitajima M: Serum tartrate-resistant acid phosphatase as a potential marker of bone metastases from breast cancer. Anticancer Res *19*: 4515-4522, 1999.
16 Halleen JM, Hentunen TA, Hellman J and Väänänen HK: Human bone tartrate-resistant acid phosphatase: purification and development of an immunoassay. J Bone Miner Res *11*: 1444-1452, 1996.
17 Halleen JM, Hentunen TA, Karp M, Käkönen SM, Petterson K and Väänänen HK: Characterization of serum tartrate-resistant acid phosphatase and development of a direct two-site immunoassay. J Bone Miner Res *13*: 683-687, 1998.
18 Janckila AJ, Nakasato YR, Neustadt DN and Yam LT: Disease-specific expression of tartrate-resistant acid phosphatase isoforms. J Bone Miner Res *18*: 1916-1919, 2003.
19 Halleen JM, Karp M, Viloma S, Laakson P, Hellman J, Käkönen SM, Stehan JJ, Holmes SD, Väänänen HK and Petterson K: Two-site immunoassays for osteoclastic tartrate-resistant acid phosphatase based on characterization of six monoclonal antibodies. J Bone Miner Res *14*: 464-469, 1999.
20 Halleen JM: Tartrate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. Anticancer Res *23*: 1027-1030, 2003.
21 Hannon RA, Clowes JA, Eagleton AC, Al Hadari AA, Eastell R and Blumsohn A: Clinical performance of immunoreactive tartrate-resistant acid phosphatase isoforms 5b as a marker of bone resorption. Bone *34*: 187-194, 2004.
22 DeLong ER, DeLong DM and Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics *44*: 837-845, 1988.
23 Chao TY, Ho CL, Lee SH, Chen MMJ, Janckila AJ and Yam LT: Tartrate-resistant acid phosphatase 5b as a serum marker of bone metastasis in breast cancer patients. J Biomed Sci *11*: 511-516, 2004.
24 Hoskin PJ: Radiotherapy in the management of bone metastases. *In:* Bone Metastases – Diagnosis and Treatment. Rubens RD, Fogelman I (eds.). London, Springer Verlag, pp. 171-185, 1991.
25 Koizumi M, Takahashi S1991 Ogata E: Comparison of serum bone resorption markers in the diagnosis of skeletal metastasis. Anticancer Res *23*: 4095-4100, 2003.
26 Rankin S: Radiology. *In:* Bone Metastases – Diagnosis and Treatment. Rubens RD, Fogelman I (eds.). London, Springer Verlag, pp. 63-81, 1991.
27 Capeller B, Caffier H, Sütterlin MW and Dietl J: Evaluation of tartrate-resistant acid phosphatase (TRAP) 5b as serum marker of bone metastases in human breast cancer. Anticancer Res *23*: 1011-1016, 2003.
28 Terpos E, De La Fuente J, Szydlo R, Hatjiharissi E, Viniou N, Meletis J, Yataganas X, Goldman JM and Rahemtulla A: Tartrate-resistant acid phosphatase isoform 5b: a novel serum marker of monitoring bone disease in multiple myeloma. Int J Cancer *106*: 455-457, 2003.

*Received May 24, 2005*
*Accepted July 22, 2005*