Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
295
JNMU
Journal of Nanjing Medical University, 2008, 22(5):295–298
www.elsevier.com/locate/jnmu
Research Paper

The evaluation of Tracp5b as a marker for monitoring treatment results of bone metastasis in breast cancer patients

Xiaoyun Huang, Yan Si, Jia Zhao, Qiang Ding*

Department of Breast Surgery, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Received 4 June, 2008

Abstract

Objective: To evaluate the sensitivity of serum tartrate-resistant acid phosphatase 5b(Tracp5b) activity in monitoring bisphosphonate treatment results of bone metastasis in breast cancer(BC) patients. **Methods:**The serum activities of Tracp5b, CEA, CA153 were measured in 58 BC patients, including 26 without bone metastasis, 32 with bone metastasis. The serum activities of Tracp5b, CEA, CA153 were also measured in 19 patients with bone metastasis after 3 months of bisphosphonate treatment. Eighteen healthy women with age from 34 to 70 served as control. **Results**:Serum Tracp5b was significantly elevated in patients with bone metastasis compared with that in all any other groups($P < 0.05$). The sensitivity of Tracp5b was 78.13% and the specificity was 86.36%. The sensitivity of CA153 was 37.50% and the specificity was 77.27%. The sensitivity of CEA was 21.88% and the specificity was 84.09%. The serum activity of Tracp5b decreased significantly($P < 0.05$) after 3 months of bisphosphonate treatment, while the levels of CA153 and CEA were unchanged. **Conclusion:**Serum Tracp5b activity is a useful diagnostic marker for bone metastasis in BC patients and can be used to evaluate the treatment results of bisphosphonate.

Key words: breast neoplasm; bone metastasis; tartrate-resistant acid phosphatase 5b; bisphosphonate; carcinoembryonic antigen; carbohydrate antigen 153

INTRODUCTION

In breast cancer(BC), patients with bone metastasis (BM) may have a prolonged clinical course. Pain and pathologic fracture are the major complications of bone metastasis and can significantly debilitate the life quality of patients[1]. Because of the introduction of bisphosphonates, pain management of bone metastasis in breast cancer patients has been improved[2]. Although the markers CA 153 and CEA are in clinical use to monitor the stage IV breast cancer, a sensitive diagnostic marker to detect bone metastasis and specifically monitor bisphosphonate treatment effects on bone metabolism is still lacking[3].

Bone metastasis is believed to be associated with the disruption of the normal coupling between osteoblasts and osteoclasts[4]. In most cases, the osteoclasts are preferentially activated and resulted in bone destruction[5]. Pathologic examination of a metastatic bony lesion typically reveals the presence of breast cancer cells accompanied by increased numbers of osteoclasts/osteoblasts in the vicinity[6]. The activated osteoclasts may erode bone heavily; however, the efficiency of new bone formation by osteoblasts does not keep pace, resulting in net bone loss.

The diagnosis of bone metastasis in breast cancer patients starts from the appearance of clinical symptoms. It is then confirmed by roentgenography and/or by bone scintigraphy[6]. Recently, tartrate-resistant acid phosphatase 5b(Tracp5b) has been recognized as a marker of osteoclasts[7]. There are two isoforms of type 5 Tracp in human serum, 5a and 5b[8], and the difference between them is the presence of sialic acid in 5a but not in 5b. Purified human osteoclastic Tracp is type 5b, which is a sensitive and specific marker for bone metastasis in breast cancer patients[9]. This study aims to evaluate the

*Corresponding author.
E-mail address: dqnj2000@yahoo.com.cn

sensitivity of serum Tracp5b activity in monitoring treatment results of bone metastasis in breast cancer patients compared with that of CEA and CA153.

MATERIALS AND METHODS

Patients

Fifty-eight breast cancer patients(26 without bone metastasis, 32 with newly diagnosed bone metastasis) in the Department of Breast Surgery in the First Affiliated Hospital of Nanjing Medical University between November 2005 and February 2007 were studied after informed consent had been obtained. Patients ranged in age from 28 to 75 years. Bone metastasis was diagnosed from clinical symptoms, radiological features, and whole-body bone scintigraphy. All breast cancer patients received chemotherapy, hormone therapy after modify radical mastectomy, and 19 patients with bone metastasis received bisphosphonate therapy. These patients were followed up by physical check-up regularly, including tumor markers detection, sonography, computerized tomography, and bone scintigraphy as clinically indicated. Eighteen healthy women with ages from 34 to 70 years were recruited as a control group after informed consent had been obtained.

Collection of serum

Serum of each patient was collected after they entered this study. For normal healthy women, sera were collected once when they entered the study. For bone metastasis patients, sera were collected twice before and after 3 months bisphosphonate therapy. Venous blood was drawn, and clotted at room temperature for 30 minutes, then was stored for no more than 4 hours at 4℃ before centrifugation to collect serum. All sera were stored at −80℃ for no more than 1 month and thawed at room temperature immediately before TRACP5b activity was measured.

Serum Tracp5b activity assay

TRACP5b activity was measured by enzyme linked immunosorbent serologic assay(ELISA). The kit was bought from Immunodiagnostic Systems Limited(IDS Co. England) this assay uses a TRACP-specific antibody (14G6) to immobilize serum TRACP. The bound TRACP5b activity is subsequently estimated using 4-nitrophenyl phosphate as substrate at pH 6.1. At this pH, the activity of serum TRACP5a is minimized whereas that of TRACP5b remains high. The activity of the TRACP5b in healthy women was less than 4.15 U/L (the range was from 1.81 to 3.37 U/L in pre-menopause Asian women and was from 2.34 to 4.04 U/L in post-menopausal women, provided by the assay producer).

CEA and CA153 activity assay

Serum CEA and CA153 activities are indicators of breast tumor and were measured by Electrochemiluminescence Immunoassay with Elecsys 2010 provided by Hitachi/Roche Company. The normal range of the CEA activity in healthy women was less than 4.3 ng/ml(provided by assay producer). The normal range of the CA153 serum level in healthy women was less than 25 U/ml(also provided by assay producer).

Statistical analysis

All data are expressed as $\bar{x} \pm s$ and performed with ANOVA test using SPSS13.0 to assess the independent groups and treatment effects in each group, respectively. A *P*-value less than 0.05 was considered to be statistically significant. The change percentage of the three markers was calculated according to the following formula: [(posttreatment data-pretreatment data)/pretreatment data] × 100%.

RESULTS

Serum Tracp5b activity, CA153 and CEA levels in breast cancer patients with or without bone metastasis and healthy women

The means of serum Tracp5b activity, CA153 and CEA levels in 32 breast cancer patients with bone metastasis were 5.03 U/L, 39.45 U/ml and 5.44 ng/ml, respectively. The means of serum Tracp5b activity, CA153 and CEA levels in 26 breast cancer patients without bone metastasis were 3.29 U/L, 18.25 U/ml and 2.79 ng/ml, respectively. In the health control group, the means were 2.48 U/L, 12.53 U/ml and 1.12 ng/ml, respectively. As shown in ***Table 1***, the mean values of all these three markers are significantly higher in breast cancer patients with bone metastasis than those in healthy women($P < 0.0001$). The Tracp5b activity in bone metastasis group is significantly higher compared with that in the group without bone metastasis($P < 0.05$). The serum level of CEA or CA153 is not correlated with bone metastasis($P = 0.084$ & 0.196 respectively).

Table 1 **The levels of serum Tracp5b, CA153 and CEA in healthy women, breast cancer(BC) patients with or without bone metastases(BM)** ($\bar{x} \pm s$)

group	*n*	Tracp5b(U/L)	CA153(U/ml)	CEA(ng/ml)
health	18	2.48 ± 0.84*	12.53 ± 7.25*	1.12 ± 0.81*
BC(without BM)	26	3.29 ± 0.89*	20.30 ± 8.32*	2.79 ± 1.59*
BC(with BM)	32	5.03 ± 0.35	38.86 ± 31.63	5.44 ± 5.51

Compared with BC(withBM), *$P < 0.05$.

Sensitivity and specificity of serum Tracp5b activity compared with those of CA153 and CEA as a marker for bone metastasis in BC

The serum TRACP5b activity to identify bone metastasis in BC had a sensitivity of 78.13% and a specificity of 86.36%. The sensitivity and specificity of CEA were 21.88% and 84.09%, respectively. The sensitivity and specificity of CA153 were 37.50% and 77.27%, respectively. As shown in ***Table 2***, the serum Tracp5b activity was more sensitive to bone metastasis than the level of CEA or CA153(P= 0.001 and 0.002, respectively). But the specificity of Tracp5b versus that of CEA and CA153 had no significant difference(P = 0.476 and 0.995, respectively). There were also no differences between CEA and CA153 in the sensitivity and specificity as a marker to monitor bone metastasis of BC(P= 0.602 and 0.389,respectively).

Table 2 **The sensitivity and specificity of serum Tracp5b, CA153 and CEA in diagnosing bone metastases in breast cancer patients**

(%)

Variable	Sensitivity(%)	Specificity(%)
Tracp5b	78.13(25/32)	86.36(38/44)
CA-153	40.63(13/32)*	77.27(34/44)
CEA	21.88(7/32)*	84.09(37/44)

Compared with Tracp 5b, $^{*}P < 0.05$.

Treatment response of serum Tracp5b activity, CEA and CA153 levels in breast cancer patients with bone metastasis before and after treatment

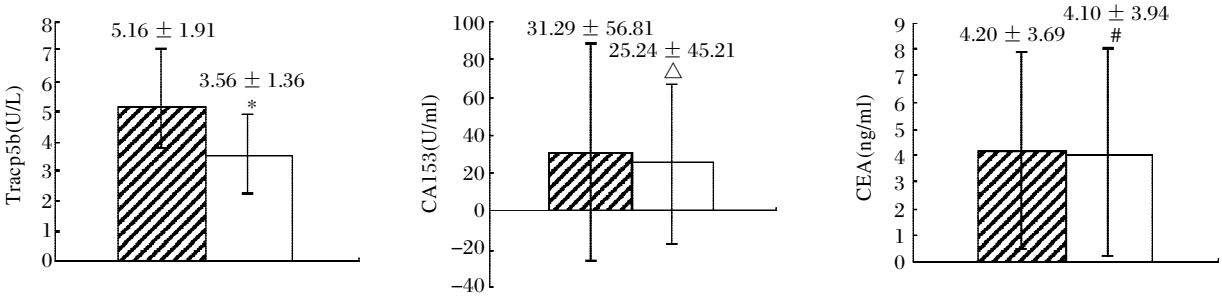
Nineteen patients were evaluate for bisphosphonate treatment response during the study period. The Tracp5b activity, CEA and CA153 levels of 19 paired serum samples, before and after treatment, were tested. Among the three biomarkers, Tracp5b was highly significant decreased after treatment(P = 0.033, ***Fig. 1***). The mean change percentage of TRACP5b activity was -31.01%. However, there was no significant decrease in CEA and CA153 levels after treatment(P = 0.94 and 0.74 respectively, ***Fig. 1***). The mean changes percentage of CEA and CA153 levels were -2.38% and -19.33%, respectively.

DISCUSSION

Bone metastasis is an important issue in breast cancer patients. At postmortem examination, the incidence of bone metastasis in breast cancer patients can be as high as 70%[1]. In bone metastasis it is believed that the osteoclasts are preferentially activated and result in bone destruction[10].

The most important measures for the diagnosis and follow-up of bone metastasis in breast cancer patients are plain radiography, bone scintigraphy, computerized tomography, and magnetic resonance[11]. However, each image measure has its own limitations, and some of them are ineffective-cost. Moreover, the fear of radiation related injury has limited the frequent uses of repeated image studies. Some biochemical markers for diagnosis and follow-up of bone metastasis are useful because the markers are more sensitive to systemic events than local events[12]. Furthermore, markers respond more rapidly to treatment and can discriminate between healing lesions and progressive lesions[13].

CA153 is a normal product of breast cells, while in cancer it is frequently over-expressed. CA153 is recommended for monitoring the course and response to therapy of breast cancer. The elevation of CA153 in breast cancer is found in over 30% of patients with stage IV disease[14]. The best use of this marker for breast cancer patients remains controversial. It is not recommended monitoring unless the marker was high at the time of initial treatment. CEA is the most useful marker to monitor the treatment of cancer especially in gastrointestinal(GI) and, in particular, colorectal cancer. CEA is also found helpful in monitoring patients with cancers of the rectum, lung, breast[15], liver, pancreas, stomach and ovary. Not all cancers produce CEA. Like many other tumor markers, CEA and CA153 lack specificity and sensitivity to monitor bone metastasis in BC patients.



Compared with the post-treatment, $^{*}P$= 0.033; $^{\triangle}P$= 0.743; $^{\#}P$= 0.941.

Fig. 1 The comparison of serum Tracp5b of pre-and post-treatment with bisphosphonate in bone metastases patients

Tracp5b is normally secreted by osteoclasts during bone resorption[8,16]. It is usually as a marker of osteoclast's activity and number. The activity of Tracp5b can be specifically measured in serum by immunoassays and has been proposed as a marker of bone resorption[17]. It has been also reported that serum Tracp5b may not be affected by food intake or renal or hepatic diseases. In addition, the clinical variation of Tracp5b is very low[9]. Serum Tracp5b activity may be a useful marker in the detection and follow-up of breast cancer patients with bone metastasis[18,19]. In this study, we have shown that serum Tracp5b activity are significantly higher in breast cancer patients with bone metastasis than that in normal subjects. Therefore, it is plausible to use Tracp5b to diagnose and monitor bone metastasis in breast cancer patients. Serum Tracp5b activity decreased after effective treatment with antiresorptive bisphosphonates and increased again after such treatment failed[20]. Therefore, serum Tracp5b activity has been proposed as a useful diagnostic marker to monitor BM in BC. Further analysis revealed that Tracp5b activity was the most sensitive marker in monitoring treatment response of bone metastasis in breast cancer patients.

The limitation of this study is the relatively fewer number of patients who were available for treatment response. Furthermore, there is no standard criterion for evaluation of the treatment response on bone metastasis in breast cancer patients. We chose the time of 3 months after bisphosphonate therapy as the only point to compare the treatment results. Therefore, we do not have enough data to make a correlation between the changes of Tracp5b activities and the treatment results.

Our results suggest using Tracp5b to follow up breast cancer patients with bone metastasis after treatment. Tracp5b is perhaps more sensitive than any other markers for this purpose, as shown in this study, but further study with more patients is needed to confirm our current findings.

References

[1] Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer* 1987;55:61-6.

[2] Kohno N. Treatment of breast cancer with bone metastasis: bisphosphonate treatment current and future. *Int J Clin Oncol* 2008; 13:18-23.

[3] Halleen JM, Ylipahkala H, Alatalo SL. Serum tartrate-resistant acid phosphatase 5b, but not 5a, correlates with other markers of bone turnover and bone mineral density. *Calcif Tissue Int* 2002;71:20-5.

[4] Akhtari M, Mansuri J, Newman KA, Guise TM, Seth P. Biology of breast cancer bone metastasis. *Cancer Biol Ther* 2008;7:3-9.

[5] Wada N, Fujisaki M, Ishii S, Ikeda T, Kitajima M. Evaluation of bone metabolic markers in breast cancer with bone metastasis. *Breast Cancer* 2001;8:131-7.

[6] Peyruchaud O. Mechanisms of bone metastasis formation. *J Soc Biol* 2007;201:229-36.

[7] Capeller B, Caffier H, Sütterlin MW, Dietl J. Evaluation of tartrate resistant acid phosphatase(TRAP) 5b as serum marker of bone metastases in human breast cancer. *Anticancer Res* 2003;23:1011-5.

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

[9] Miyazaki S, Igarashi M, Nagata A, Tominaga Y, Onodera K, Komoda T. Development of immunoassays for type-5 tartrate-resistant acid phosphatase in human serum. *Clin Chim Acta* 2003; 329:109-15.

[10] Chung YC, Ku CH, Chao TY, Yu JC, Chen MM, Lee SH. Tartrate-resistant acid phosphatase 5b activity is a useful bone marker for monitoring bone metastases in breast cancer patients after treatment. *Cancer Epidemiol Biomarkers Prev* 2006;15:424-8.

[11] Koizumi M, Takahashi S, Ogata E. Comparison of serum bone resorption markers in the diagnosis of skeletal metastasis. *Anticancer Res* 2003;23:4095-9.

[12] Rosenbrock H, Seifert-Klauss V, Kaspar S, Busch R, Luppa PB. Changes of biochemical bone markers during the menopausal transition. *Clin Chem Lab Med* 2002;40:143-51.

[13] Terpos E, de la Fuente J, Szydlo R, Hatjiharissi E, Viniou N, Meletis J, *et al.* Tartrate-resistant acid phosphatase isoform 5b: a novel serum marker for monitoring bone disease in multiple myeloma. *Int J Cancer* 2003;106:455-7.

[14] Duffy MJ. CA 153 and related mucins as circulating markers in breast cancer. *Ann Clin Biochem* 1999;36:579-86.

[15] Halleen JM. Tartrate-resistant acid phosphatase 5B is a specific and sensitive marker of bone resorption. *Anticancer Res* 2003;23:1027-9.

[16] Vaananen HK, Zhao H, Mulari M, Halleen JM. The cell biology of osteoclast function. *J Cell Sci* 2000;113:377-81.

[17] Hannon RA, Clowes JA, Eagleton AC, Al Hadari A, Eastell R, Blumsohn A. Clinical performance of immunoreactive tartrate-resistant acid phosphatase isoform 5b as a marker of bone resorption. *Bone* 2004;34:187-94.

[18] Chao TY, Ho CL, Lee SH, Chen MM, Janckila A, Yam LT. Tartrate-resistant acid phosphatase 5b as a serum marker of bone metastasis in breast cancer patients. *J Biomed Sci* 2004;11:511-6.

[19] Martinetti A, Seregni E, Ripamonti C, Ferrari L, De Conno F, Miceli R, *et al.* Serum levels of tartrate-resistant acid phosphatase-5b in breast cancer patients treated with pamidronate. *Int J Biol Markers* 2002;17:253-8.

[20] Capeller B, Caffier H, Sutterlin MW, Dietl J. Evaluation of tartrate-resistant acid phosphatase (TRAP) 5b as serum marker of bone metastases in human breast cancer. *Anticancer Res* 2003;23:1011-5.