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ORIGINAL ARTICLE

Application of Bone Turnover Markers PICP and β-CTx in the Diagnosis and Treatment of Breast Cancer with Bone Metastases

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SUMMARY

Background: **This study is to investigate the application of the bone turnover markers type I procollagen carboxy-terminal propeptide (PICP) and β-isomerized forms of type I collagen breakdown products (β-CTx) in the diagnosis and treatment of breast cancer with bone metastases.**
Methods: **A total of 162 breast cancer patients were included in this study. There were 70 cases with bone metastasis (BM group) and 92 cases without bone metastasis (non-bone metastasis, NBM group). The levels of the bone turnover markers PICP and β-CTx were measured using Electro-Chemiluminescence Immunoassay to compare the difference between BM and NBM group, before and after treatments in the NBM group, and to analyse the relationship with therapeutic effects.**
Results: **The BM group had higher PICP and β-CTx levels than the NBM group and also higher in the non-luminal type group than the luminal type group, the differences were all statistically significant. However, no statistically significant differences were found among the pN0, pN1, pN2, and pN3 subgroups of the NBM group. Among the 70 cases of BM patient after 3 months of treatment, there were 48 cases that showed clinical benefits, with significantly reduced PICP and β-CTx levels ($p = 0.02$, $p = 0.00$, respectively), but 22 cases showed disease progression with elevated PICP and β-CTx levels ($p = 0.01$, $p = 0.04$, respectively).**
Conclusions: **The bone turnover markers PICP and β-CTx have crucial value in the diagnosis and treatment efficacy evaluation for women of breast cancer with bone metastases.**
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INTRODUCTION

Breast cancer is a common malignant cancer in females worldwide which develops from breast cells. In recent compiled data, the incidence of breast cancer has ranked first among all types of cancer in females, and the death rate due to breast cancer has also ranked first among women aged 20 to 59 years [1]. It often develops distant metastases. Bone metastasis is a common type of distant metastasis in breast cancer with an incidence of 65 - 75% [2]. Once the diagnosis of bone metastasis in breast cancer is confirmed, the median survival time is

2 years and the prognosis is poor [3]. Bone metastases in breast cancer also greatly affect the quality of life, and any form of bone metastasis can cause serious complications such as pain, fractures, hypercalcaemia, and spinal nerve compression symptoms [4].

Therefore, recent clinical research has focused on the early diagnosis of breast cancer bone metastasis, efficacy monitoring of breast cancer patients with bone metastases during treatment and the early determination of whether patients can benefit from treatment. Type I procollagen carboxy-terminal propeptide (PICP) is a biochemical marker of bone formation, and β-isomerized forms of type I collagen breakdown products (β-CTx) is a biochemical marker of bone resorption. Bone metastasis in breast cancer is a mixed bone metastasis and mainly consists of osteolytic bone metastasis. These two markers together can be used for the early diagnosis of breast cancer bone metastases and to evaluate the efficacy of the treatment of breast cancer bone metastases, employing the full clinical value of bone turnover markers [5]. The present study investigated the value of bone turnover markers in the diagnosis and treatment of breast cancer, and the results are reported as follows.

MATERIALS AND METHODS

Subjects and grouping

During the period of July 2011 to July 2015, we enrolled 162 breast cancer cases that were confirmed by histopathology. The patients were all female, aged 41 - 73 years, with a median age of 57 years. Among them, 70 cases had bone metastasis. The diagnostic process of bone metastasis was as follows: electroconvulsive therapy (ECT) was used in the initial screening, and positive cases then received X-ray, computed tomography (CT), magnetic resonance imaging (MRI) or other imaging examinations to confirm. There were 92 cases without bone metastasis, which were further divided according to different pathological conditions: 1) based on the axillary lymph node metastasis conditions, the non-bone metastasis group was divided into the pN0 (19 cases), pN1 (27 cases), pN2 (29 cases), and pN3 (17 cases) subgroups; and 2) based on the hormone receptor conditions, the non-bone metastasis group was divided into the luminal type (42 cases) and non-luminal type (50 cases) subgroups. Axillary lymph node staging was conducted according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging manual [6]. Based on the conditions after axillary lymph node dissection, the pN0 group had no histologically confirmed regional lymph node metastasis; the pN1 group had micro-metastases, or metastases to 1 - 3 axillary lymph nodes, and/or the absence of clinical findings, but internal mammary lymph node metastasis was discovered by sentinel lymph node biopsy; the pN2 group had metastases in 4 - 9 axillary lymph nodes, or clinical findings of internal mammary lymph node metastasis without axillary lymph node metastasis; and the pN3 group had metastases in 10 or more axillary lymph nodes, subclavian lymph node metastasis, clinical findings of internal mammary lymph node metastasis with one or more axillary lymph node metastases, three or more axillary lymph node metastases, associated with internal mammary lymph node metastasis confirmed by sentinel lymph node biopsy in the absence of clinical findings, or ipsilateral supraclavicular lymph node metastasis. According to the 2011 St. Gallen consensus, breast cancer can be divided into two groups based on different molecular typing [7]. The luminal type is ER and (or) PR positive and HER-2 negative or positive. The non-luminal type is HER-2 positive and ER and PR negative or triple negative.

Inclusion criteria

Breast cancer was confirmed by histopathology, the patients had an expected survival > 3 months and the patients had normal blood examination results, liver and kidney functions and electrocardiograms. All of the patients signed informed consent forms.

Exclusion criteria

Patients with a traumatic bone fracture 1 year before enrollment; patients with rheumatic arthritis, osteoarthritis, and bone metabolism diseases; and patients who had received bisphosphonates, hormone or calcium agent therapy one month before screening or who had received radiotherapy within a month of the screening were excluded.

Research content

1) We measured the levels of the bone turnover markers PICP and β-CTx in all of the enrolled patients and compared these levels between the bone metastasis group and non-bone metastasis group and among different lymph node staging subgroups and different molecular typing subgroups of the non-bone metastasis groups.

2) For breast cancer patients with bone metastases, we measured the pre-treatment initial peripheral blood levels of the bone turnover markers PICP and β-CTx. According to the clinical conditions of the patients, we conducted radiotherapy, chemotherapy or endocrine therapy together with bisphosphonate bone protection treatment, with bisphosphonate therapy performed once per month for a continuous treatment of 2 years. Three months after treatment, we began to retest for the bone turnover markers and compared the levels with the initial values to assess changes in bone turnover markers before and after treatment. Bone ECT examination was conducted again after 3 months of treatment.

3) Follow-up was performed by telephone or email, and the last follow-up was October 2015. The evaluation of breast cancer bone metastasis treatment efficacy was performed according to the 2000 response evaluation criteria in solid tumours (RECIST). Increases in the degree of bone pain and clinical symptoms or the

appearance of new lesions in the radiographic examination were all considered to represent disease progression. We also measured the bone turnover marker levels when the patients showed progression in the clinical assessment. Patients with alleviated bone pain and clinical symptoms or remission or stable conditions in the imaging examination were included in the clinical benefit group.

Statistical methods

The statistical analysis was conducted using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The data were tested for a normal distribution and were expressed $\bar{x} \pm s$. A paired *t*-test was used to compare the mean levels before and after treatment, and a multi-group *t*-test and analysis of variance were used to compare the means between groups. $p < 0.05$ was considered statistically significant.

RESULTS

All of the patients had good compliance, and there were no lost cases.

We compared the patients in the bone metastases group with those in the non-bone metastases group. The PICP β-CTx levels were higher in the bone metastasis group than in the non-bone metastasis group. The PICP comparison between the two groups yielded $t = 3.063$ and $p = 0.013$, indicating that the difference was statistically significant. The β-CTx comparison yielded $t = 2.660$ and $p = 0.047$, indicating that the difference was statistically significant. The details are listed in Table 1. The comparison of PICP levels among the pN0, pN1, pN2, and pN3 subgroups without bone metastasis yielded $F = 0.282$ and $p = 0.838$, indicating that the differences were not statistically significant. The β-CTx comparison yielded $F = 1.300$ and $p = 0.288$, indicating that the differences were not statistically significant. The details are listed in Table 2.

The comparison of the PICP levels between the luminal and non-luminal type subgroups of the non-bone metastasis group yielded $t = 2.080$ and $p = 0.044$, indicating that the difference was statistically significant, and the β-CTx comparison yielded $t = 1.974$ and $p = 0.048$, indicating that the difference was statistically significant. The bone turnover marker levels were higher in the non-luminal type group than in the luminal type group. The details are listed in Table 3.

Patients in the non-bone metastasis group were tested for bone turnover markers once every three months during follow-up. We found that 17 cases showed abnormal elevations of bone turnover markers, of which PICP was increased by an average of 2.3-fold and β-CTx was increased by an average of 1.9-fold. However, there were no symptoms of bone pain or other bone-related events. Further investigation using bone scanning and other imaging examinations revealed that 13 of these 17 patients had bone metastasis, 1 had pulmonary metastasis, 1 had chest wall recurrence, and 2 had liver metastasis.

There were 48 breast cancer patients with bone metastases in the clinical benefit group. The initial PICP level was (127.58 ± 34.70) ng/mL and that of β-CTx was 0.543 ± 0.255 ng/mL, whereas the levels after 3 months of treatment were 38.308 ± 26.451 ng/mL for PICP and 0.212 ± 0.112 ng/mL for β-CTx. A comparison of the PICP levels before and after treatment yielded $t = 2.694$ and $p = 0.016$, indicating statistically significant differences, and a comparison of the β-CTx levels before and after treatment yielded $t = 3.878$ and $p = 0.001$, indicating statistically significant differences. The details are listed in Table 4.

There were 22 breast cancer patients with bone metastases in the clinical progression group. The initial PICP level was 34.074 ± 17.436 ng/mL and that of β-CTx was 0.166 ± 0.086 ng/mL, whereas the levels after 3 months of treatment were 63.476 ± 19.175 ng/mL for PICP and 0.377 ± 0.138 ng/mL for β-CTx. A comparison of the PICP levels before and after treatment yielded $t = -4.949$ and $p = 0.008$, indicating statistically significant differences, and a comparison of the β-CTx levels before and after treatment yielded $t = -3.719$ and $p = 0.042$, indicating statistically significant differences. The details are listed in Table 4.

The patients underwent bone ECT again at 3 months after treatment. In the clinical benefit group, 37 patients showed stable imaging results but had decreased bone turnover marker levels, and the bone turnover markers indicated that the clinical treatment was effective before the clinical imaging reached this conclusion.

DISCUSSION

Imaging is currently the main evaluation tool used for the diagnosis of bone metastases in breast cancer. However, it has the disadvantages of radiation risk, a long cycle, and an inability to be evaluated for early efficacy. Compared to conventional imaging diagnostic methods, the measurement of bone turnover markers has the following advantages: it is non-invasive, highly specific, simple, fast, capable of reflecting minor bone changes, and evaluating the bone metastasis treatment efficacy in early stages and displays increasing superiority over other methods [8].

Bone metastases in breast cancer are mixed bone metastases that mainly consist of osteolytic metastases and bone formation and resorption co-exist when bone metastases occur. Therefore, combining biochemical indicators reflecting either bone resorption or formation for the diagnosis and monitoring of breast cancer bone metastasis can fully utilize the clinical value of bone turnover markers [9]. In this study, we used bone turnover markers with good sensitivity and specificity, PICP and β-CTx [10]. During the maturation process, type I procollagen forms the fragment of PICP. The PICP level in the serum reflects the ability of osteoblasts to synthesize

Table 1. Comparison of PICP and β-CTx levels in BM and NBM groups (ng/mL, $\bar{x} \pm s$).

Groups	Cases	PICP level	β-CTx level
BM	70	106.33 ± 45.32	0.59 ± 0.35
NBM	92	58.52 ± 40.47	0.32 ± 0.35
t		3.06	2.66
p		0.01	0.04

BM - bone metastasis, NBM - non-bone metastasis, PICP - Type I procollagen carboxy-terminal propeptide, β-CTx - β-isomerized forms of type I collagen breakdown products.

Table 2. Comparison of PICP and β-CTx levels in different groups of NBM patients (ng/mL, $\bar{x} \pm s$).

Groups	n	PICP	β-CTx
pN0	19	47.96 ± 34.34	0.32 ± 0.20
pN1	27	62.27 ± 50.87	0.55 ± 0.44
pN2	29	58.29 ± 40.20	0.59 ± 0.37
pN3	17	63.83 ± 36.04	0.56 ± 0.28
F		0.28	1.30
p		0.84	0.29

Table 3. Comparison of PICP and β-CTx levels in different molecular types (ng/mL, $\bar{x} \pm s$).

Groups	n	PICP	β-CTx
No-luminal type	42	71.45 ± 23.71	0.62 ± 0.37
Luminal type	50	47.20 ± 18.39	0.43 ± 0.31
t		2.08	1.98
p		0.04	0.048

Table 4. Comparison of PICP and β-CTx levels before and after treatment (ng/mL, $\bar{x} \pm s$).

Groups		PICP level	p	β-CTx level	p
Clinical benefit group (48 cases)	Pre-treatment	127.58 ± 34.70	0.02	0.54 ± 0.26	0.00
	After treatment	38.31 ± 26.45		0.21 ± 0.11	
Clinical progression group (22 cases)	Pre-treatment	34.07 ± 17.44	0.01	0.17 ± 0.09	0.04
	After treatment	63.48 ± 19.18		0.38 ± 0.14	

collagen and is a specific marker for the monitoring of osteoblast activity and bone formation. The continuous monitoring of PICP in individuals is helpful for the diagnosis and efficacy evaluation of breast cancer bone metastasis [11]. β-CTx is a biochemical indicator of bone resorption; it can reflect osteolytic changes, and its degree of increase is consistent with that of osteoclast activity.

This study found that the PICP and β-CTx levels were higher in the bone metastasis group than in the non-bone metastasis group, and the difference was statistically significant, consistent with previous reports from other countries [12]. Therefore, measuring the serum PICP and β-CTx levels can significantly improve the diagnostic efficiency of bone metastases in breast cancer, and PICP and β-CTx can act as important serological markers for the diagnosis of breast cancer bone metastasis in conjunction with imaging. For breast cancer patients with bone metastases, the clinical benefit groups showed significantly reduced PICP and β-CTx levels during treatment compared to pre-treatment levels, and the differences were statistically significant ($p < 0.05$). By contrast, the PICP and β-CTx levels of the clinical progression group were significantly increased, and the differences were statistically significant ($p < 0.05$) compared to the pre-treatment levels. The results and findings of this study are similar to those reported by researchers abroad [13], suggesting that comprehensive assessment using the two types of bone turnover markers, a bone formation marker and a bone resorption marker, could objectively reflect the changes in bone metastasis during the treatment of the two groups of patients. Changes in bone turnover markers are closely related to clinical benefits or clinical progression and can facilitate treatment efficacy assessment to provide a basis for further clinical treatment. Voorzanger et al. [9] found that bone turnover markers had a high sensitivity and specificity in clinical application, and monitoring the changes in bone turnover markers in breast cancer bone metastasis patients receiving bisphosphonate treatment could accurately evaluate the treatment efficacy and prognosis.

This study also found that 37 cases in the clinical benefit group showed ameliorated clinical symptoms. Although bone scan results had not yet revealed manifestations of clinical remission, bone turnover markers had decreased significantly at this stage. Therefore, bone turnover markers can detect therapeutic effects significantly earlier than routine radiological monitoring and can provide an objective basis for the future treatment plan. Consequently, a delay in radiographic assessment alone would not lead to a delay of treatment plans, ensuring the smooth progress of clinical diagnosis and treatment.

Bone turnover markers can reflect the microscopic changes of bone structure and can detect therapeutic effects significantly earlier than conventional imaging examinations. We reasoned that we could utilize bone turnover markers in follow-up examinations of patients with early breast cancer, thereby conducting an early diagnosis of bone metastases in breast cancer and evaluating the risk factors for breast cancer bone metastasis; thus, bone turnover markers could play a greater role in clinical practices. Smith et al. [14] summarize the high-risk factors for breast cancer bone metastasis, including the clinical stage and the number of axillary lymph node metastases. Therefore, one focus of the present study was to investigate whether it is possible to conduct early auxiliary diagnosis of breast cancer bone metastases by monitoring bone turnover markers in high-risk patients during breast cancer follow-up, thus enabling the diagnosis and subsequent treatment of bone metastasis at the earliest possible stage. In the present study, during the follow-up of breast cancer patients without bone metastases, we found that 17 cases had abnormal increases in bone turnover markers, of which PICP was increased by 2.3-fold and β-CTx was increased by 1.9-fold. The increases in bone turnover markers occurred when there were no symptoms of bone pain or other bone-related events. Further investigation using bone scanning and other imaging examinations revealed that 13 out of these 17 patients had bone metastasis. Therefore, measuring bone turnover markers can timely reflect disease conditions in clinical practices, detect bone metastases earlier than conventional imaging, provide an objective basis for planning further treatment, avoid delay in the clinical observation of disease conditions, and have important clinical value.

Based on the above findings, we attempted to explore the differences in baseline bone turnover marker levels in early breast cancer patients without bone metastases under different clinical pathological conditions to provide evidence for a follow-up evaluation of high-risk patients for breast cancer bone metastases. Koopman et al. [15] reported that when the numbers of axillary lymph node metastases were no less than 4, the prevalence of the first bone metastasis was 12.2% within 2 years after surgery and 26.8% within 10 years after surgery. Dent et al. [16] also suggested that the presence of axillary lymph node metastasis indicated an increased possibility of bone metastases, and the incidence of bone metastasis in breast cancer was elevated with increased numbers of axillary lymph node metastases. It is possible that as lymph node metastases increased, the baseline levels of bone turnover markers might also increase. We found that the levels of bone turnover indicators were significantly higher in patients with axillary lymph node metastasis (i.e., N+ group) than in those without lymph node metastasis (N0 group), but there were no statistically significant differences among the N1, N2, and N3 groups, which might be caused by the small sample sizes of the subgroups. In addition, further long-term follow-up studies should be designed to confirm the results. In terms of immunohistochemical pathology, ER-positive tumors have better differentiation and lower probabilities of visceral metastases [17]. In this study, the patients were divided into the luminal type and non-luminal type subgroups in accordance with breast cancer molecular typing, and the bone turnover marker levels were higher in the non-luminal type group than in the luminal type group, with statistically significant differences between the two groups. However, for breast cancers of different molecular typing, the correlations between baseline bone turnover marker levels and the occurrence of bone metastasis and the differences in the probability of bone meta-

stases between the two groups of patients still require verification by long-term follow-up, and there is yet no conclusion.

CONCLUSION

In summary, compared to conventional imaging methods, bone turnover markers have the advantages of being simple, economic, highly specific, quantitative, and capable of reflecting clinical condition changes in a timely manner. Bone turnover markers can be used as important auxiliary indicators in addition to conventional imaging examinations and have important clinical significance during the diagnosis, treatment, and follow-up of patients.

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Declaration of Interest:
All authors declare that there were not any conflicts of interest.

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