European Review for Medical and Pharmacological Sciences 2019; 23: 5277-5284

Study on diagnostic value of P1NP and β-CTX in bone metastasis of patients with breast cancer and the correlation between them

C.-T. ZUO[1], D.-C. YIN[1], H.-X. FAN[2], M. LIN[3], Z. MENG[1], G.-W. XIN[1], Y.-C. ZHANG[1], L. CHENG[3]

[1]Department of General Surgery, The Third People's Hospital of Qingdao, Qingdao, P.R. China
[2]Department of General Surgery, Hiser Medical Center of Qingdao, Qingdao, P.R. China
[3]Department of Orthopedics, The Third People's Hospital of Qingdao, Qingdao, P.R. China

Abstract. – OBJECTIVE: This study aimed to investigate the diagnostic value of the total amino-terminal propeptide of type 1 procollagen (P1NP) and C-terminal telopeptide of β-I collagen (β-CTX) in bone metastasis of patients with breast cancer and the correlation between them.

PATIENTS AND METHODS: The medical records of 73 patients were retrospectively analyzed. These patients with breast cancer were treated in Oncology, General Surgery, and Orthopedic Departments in The Third People's Hospital of Qingdao from March 2014 to April 2017, including 40 patients with bone metastasis (bone metastasis group) and 33 patients with no bone metastasis (non-bone metastasis group). Other 40 healthy people who received physical examination in the same period were selected as the control group. The expression of P1NP and β-CTX in plasma were detected by the Enzyme-linked immunosorbent assay, and the correlation between them was analyzed.

RESULTS: There were significant differences in P1NP and β-CTX concentrations among the three groups ($p<0.05$). The concentrations of P1NP in the control group and the non-bone metastasis group were significantly lower than that in the bone metastasis group ($p<0.05$); the concentrations of β-CTX in the control group and the non-bone metastasis group were significantly lower than that in the bone metastasis group ($p<0.05$). P1NP: AUC=0.852, sensitivity: 72.5%, specificity: 93.9%, CUT OFF=66.44. β-CTX: AUC=0.883, sensitivity: 85.0%, specificity: 84.8%, CUT OFF=69.8. Joint detection: AUC=0.952, sensitivity: 84.8%, specificity: 99.5%, CUT OFF=99.5. The results of the concentrations of P1NP and β-CTX in the bone metastasis group detected by the Pearson correlation analysis showed that their concentrations were positively correlated in the bone metastasis group (r=0.764, $p<0.05$).

CONCLUSIONS: P1NP and β-CTX in plasma have a high diagnostic value for bone metastasis of breast cancer and have important significance in the diagnosis of bone metastasis and disease monitoring.

Key Words:
P1NP, β-CTX, Breast cancer, Bone metastasis, Diagnostic value, Correlation.

Introduction

The statistics show that breast cancer is the most common malignant tumor in women and its incidence accounts for 32% of female malignant tumors, ranking first. It is one of the main causes of female cancer death[1,2]. There are more than 1.3 million new breast cancer patients in the world every year, and the mortality rate is increasing year by year[3]. According to reports, there were 234,000 breast cancer patients in the United States in 2015, and more than 40,000 people die from breast cancer every year[4]. China is a country with a high incidence of breast cancer, and it has been reported that breast cancer is associated with family history, obesity, uncontrolled oral contraceptives, and mental pressure[5].

Metastasis refers to the bone injury caused by a malignant tumor outside the bone tissue metastasized by blood. Breast cancer patients are most likely to have bone metastasis. The data showed that more than 70% of breast cancer patients have bone metastasis, and breast cancer is most likely to spread to the bone[6,7]. Once the patient has bone metastasis, he will suffer a

Corresponding Author: Lei Cheng, MD; e-mail: c382qn@163.com

variety of complications, including pathological fracture, spinal cord compression, bone pain, and other symptoms, and in severe cases the paralysis of the limbs will be caused, which is a serious threat to the quality of life[8]. The total amino-terminal propeptide of type 1 procollagen (P1NP) is an important specific marker of bone formation. A study has shown that the abnormal expression of P1NP is associated with the development of cancer. In particular, the expression of P1NP was significantly increased when bone metastasis occurred[9]. The c-terminal telopeptide of β-I collagen (β-CTX) is an important marker of bone resorption, which is mainly produced by type I collagen degradation. According to Hu et al[10], β-CTX can be easily degraded under normal conditions and is less common in blood. When the patient's β-CTX inter-day Coefficient of Variance (inter-day CV) exceeds 10%, the probability of bone metastasis is significantly increased[11]. P1NP and β-CTX are valuable in the diagnosis of bone metastasis in breast cancer[11]. But, whether there is the same effect in patients with breast cancer has not been shown in the literature. The diagnosis contributes to the control of cancer in advance and improves the survival rate. Therefore, by detecting P1NP and β-CTX in the plasma of patients with breast cancer, we explored both values in bone metastasis of breast cancer patients and provided a reference for clinical practice.

Patients and Methods

Information and Methods

The medical records of 73 breast cancer patients diagnosed by pathological examination and treated in Oncology, General Surgery, and Orthopedic Departments in The Third People's Hospital of Qingdao from March 2014 to April 2017 were retrospectively analyzed. The patients were divided into bone metastasis group and non-bone metastasis group according to the existence of metastasis, and normal volunteers were collected as the control group in this study. All 40 cases in the bone metastasis group were female, with an age range of 30-56 years and an average age of (50.5±4.9) years, and the number of bone metastasis was 1.33±0.57. All 33 patients in the non-bone metastasis group were female, with an age range of 45-55 years and an average age of (49.9±5.1) years. All 40 cases in the control group were female, with the age range of 40-57 years and an average age of (52.1±4.5) years. TNM staging was conducted according to the ACJJ stage 8. The diagnostic criteria of bone metastasis were: (1) screening diagnostic method of bone metastasis, bone radionuclide scanning; (2) imaging diagnostic methods of bone metastasis; (3) bone biopsy and pathological diagnosis if necessary. This study was approved by the Medical Ethics Committee of The Third People's Hospital of Qingdao, and the informed consent was signed by both the family and the patient.

Inclusion and Exclusion Criteria

The inclusion criteria were: over 18 years old; with breast cancer; female; no kinship between groups; no congenital heart disease and other malignant tumors; complete clinical data. The informed consent was signed by both the family and the patient.

The exclusion criteria were: not cooperate with the treatment; with cognitive dysfunction, depression, anxiety; with language communication problems; with important basic functional defects; admission treatment of breast cancer for recurrence.

Major Reagents

P1NP kit was purchased from Azelasi Biotechnology Co., Ltd (Linyi, China). (EK-2612); β-CTX kit was purchased from Yiji Industrial Co., Ltd (BN65500121; Shanghai, China).

ELISA Detection

5 ml fasting venous blood was collected in the early morning and placed in EDTA anticoagulant tube standing for 30 minutes, and then centrifuged at 1000 rpm for 15 min. The plasma was collected for the follow-up experiment. 50 μl of different concentrations of the standard solution was added into blank micropore; 50 μl of distilled water and 50 μl of antibody were added into blank control pore; 40 μl of sample was added to other micropores, and then, 10 μl of biotin-labeled antibody was added, respectively. The plate was blocked for 30 min at 37°C. During the washing of the plate, the washing fluid was guaranteed to be full and not overflowing, and discarded 30 seconds after standing; then, the plate was dried. The above steps were repeated for 5 times. 50 μl of enzyme-labeled solution was added into each pore, and the plate was blocked for 60 min at 37°C again; then, the plate was washed again for 5 times. For the last time, we used a water absorbent paper to pat dry thoroughly. The plate was blocked with 100 μl/pore horseradish peroxidase (HRS) for 15 min exposed to dark at 37°C. 100

μl/pore chromogenic substrate TMB was added and incubated for 20 min at room temperature without light. At last, 50 μl/pore stop solution was added, the detection was conducted by the Enzymatic marker in 15 min, and the maximum absorption wavelength was recorded at 450 nm. Three groups of repeat holes were set up, and the experiment was repeated 3 times.

Statistical Analysis

In this study, SPSS 20.0 software package (IBM Corp., Armonk, NY, USA) was used for the statistical analysis of the experimental data, while GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) was used to draw figures. The enumeration data were expressed as rate (%) and analyzed using the Chi-square test. The measurement data was represented by mean±standard deviation (Mean±SD). The measurement data between the two groups that conformed to normal distribution were analyzed by the *t*-test (expressed in *t*). The One-way ANOVA was used for comparisons among multiple groups, and the LSD-*t* test was used as a post-hoc test. The Pearson analysis was used for the correlation analysis of P1NP and β-CTX. The receiver operating curve (ROC) and the area under the curve (AUC) were used to evaluate the efficacy parameters of plasma P1NP and β-CTX in the diagnosis of the bone metastasis of breast cancer. There was a statistical difference when $p<0.05$.

Results

Data Analysis of Three Groups of Patients

By comparing the clinical data of patients in the three groups, it was found that there was no difference in the clinical data among the three groups ($p>0.05$). In addition, by comparing the pathological data of bone metastasis group and non-bone metastasis group, it was found that there was no difference in tumor size, differentiation degree, TNM staging, ER, PR, and Her-2 between the two groups ($p>0.05$), but there was a difference in Ki-67 ($p<0.05$), suggesting that the data were comparable (Table I).

Expression of P1NP and β-CTX in the Three Groups

The detection of P1NP and β-CTX in the three groups by ELISA showed that there were significant differences in P1NP and β-CTX concentrations between the bone metastasis group, the non-bone metastasis group, and the control group ($p<0.05$). The concentrations of P1NP in the control group and the non-bone metastasis group were significantly lower than that in the bone metastasis group ($t_{\text{control group}}=7.221$, $p_{\text{control group}}<0.001$; $t_{\text{non-bone metastasis group}}=6.995$, $p_{\text{non-bone metastasis group}}<0.001$), and there was no difference in the concentration of P1NP between the non-bone metastasis group and the control group ($t=0.130$, $p=0.897$). The concentrations of β-CTX in the control group and the non-bone metastasis group were significantly lower than that in the bone metastasis group ($t_{\text{control group}}=8.523$, $p_{\text{control group}}<0.001$; $t_{\text{non-bone metastasis group}}=6.546$, $p_{\text{non-bone metastasis group}}<0.001$), and there was no significant difference in the concentration of β-CTX between the non-bone metastasis group and the bone metastasis group ($t=1.558$, $p=0.122$) (Table II, Figure 1).

Correlation Analysis of P1NP and β-CTX in Patients of the Bone Metastasis Group

The Pearson correlation analysis of P1NP and β-CTX concentrations in the bone metastasis group showed that their concentrations were positively correlated in the bone metastasis group ($r=0.764$, $p<0.05$) (Figure 2).

Evaluation of Energy Efficiency of P1NPand β-CTX in the Diagnosis of Bone Metastasis in Breast Cancer

By drawing P1NPX, β-CTX, and combined diagnostic ROC curves, it was found that in P1NP, AUC=0.852, the sensitivity was 72.5%, the specificity was 93.9%, and CUT OFF=66.44. In β-CTX, AUC=0.883, the sensitivity was 85.0%, the specificity was 84.8%, and CUT OFF=69.8. In joint detection: AUC=0.952, the sensitivity was 84.8%, the specificity was 99.5%, and CUT OFF=99.5. The results showed that joint detection was better than single detection (Figure 3).

Discussion

Breast cancer is the most common malignant tumor in women. Statistics from IARC (International Agency for Research) show that more than 14 million patients were diagnosed with cancer in 2012, of which more than 12% were diagnosed with breast cancer, and more than 500,000 died from breast cancer or its complications every year[12,13]. The incidence of

Table I. Data analysis of three groups of patients [n (%)].

Groups	Bone metastasis group (n=40)	Non-bone metastasis group (n=33)	Control group (n=40)	$F/X^2/t$	*p*
Age					
>50	25 (62.50)	20 (60.61)	23 (57.50)	0.212	0.899
≤50	15 (37.50)	13 (39.39)	17 (42.50)		
BMI (kg/m^2)	21.32±1.62	21.58±1.43	21.68±1.58	0.569	0.568
Hypertension history				0.499	0.779
Yes	20 (50.00)	17 (51.52)	23 (57.50)		
No	20 (50.00)	16 (48.48)	17 (42.50)		
Diabetes history				0.499	0.779
Yes	15 (37.50)	13 (39.39)	18 (45.00)		
No	25 (62.50)	20 (60.61)	22 (55.00)		
Nationality				0.591	0.744
Han nationality	35 (87.50)	30 (90.91)	37 (92.50)		
Minority nationality	5 (12.50)	3 (9.09)	3 (7.50)		
Amenorrhea				0.553	0.759
Yes	22 (55.00)	18 (54.55)	19 (47.50)		
No	18 (45.00)	15 (45.45)	21 (52.50)		
TNM staging				0.256	0.613
Stage III	10 (25.00)	10 (30.30)			
Stage IV	30 (75.00)	23 (69.70)			
Tumor size				0.613	0.434
>20 mm	30 (75.00)	22 (66.67)			
≤20 mm	10 (25.00)	11 (33.33)			
Differentiation degree				0.178	0.673
Well differentiated	38 (95.00)	32 (96.97)			
Poorly differentiated	2 (5.00)	1 (3.03)			
ER					
positive	32 (80.00)	24 (72.73)		0.535	0.464
negative	8 (20.00)	9 (27.27)			
PR					
positive	29 (72.50)	20 (60.61)		1.159	0.282
negative	11 (27.50)	13 (39.39)			
Her-2					
positive	15 (37.50)	13 (39.39)		0.027	0.869
negative	25 (62.50)	20 (60.61)			
Ki-67 cell number ≥50%	26 (65.00)	12 (36.36)		5.941	0.015
Ki-67 cell number <50%	14 (35.00)	21 (63.64)			

breast cancer in China is relatively low compared with the average incidence in the world. However, the incidence and mortality of breast cancer in recent 20 years were found to be on the rise. The IARC predicts that the number of new breast cancer patients in China may exceed 230,000/year by 2030[14,15]. Breast cancer is a kind of multifactorial and systemic comprehensive

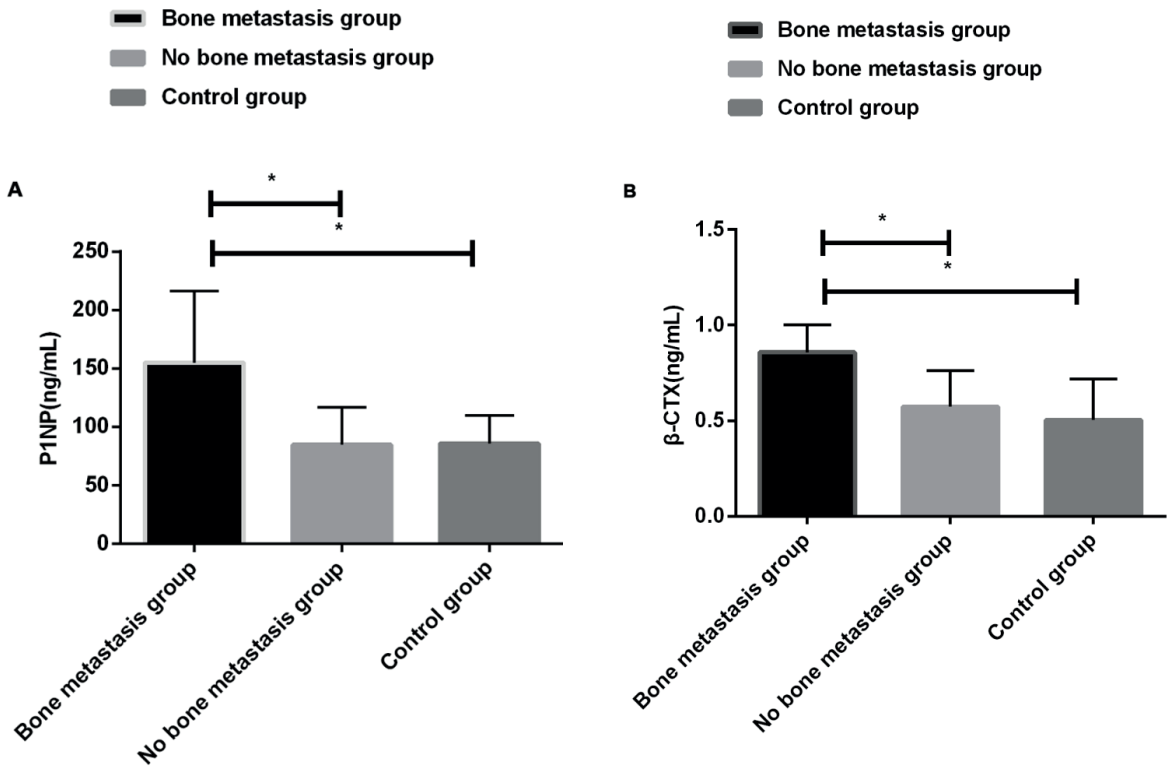

Figure 1. Expression of P1NP and β-CTX in three groups. ***A,*** Detection of P1NP by ELISA: there were significant differences in P1NP concentration between the bone metastasis group, the non-bone metastasis group, and the control group (p<0.05). The concentrations of P1NP in the control group and the non-bone metastasis group were significantly lower than that in the bone metastasis group (p<0.05), and there was no difference in the concentration of P1NP between the non-bone metastasis group and the control group (p>0.05). ***B,*** Detection of P1NP by ELISA: there were significant differences in β-CTX concentration between the bone metastasis group, the non-bone metastasis group, and the control group (p<0.05). The concentrations of β-CTX in the control group and the non-bone metastasis group were significantly lower than that in the bone metastasis group (p<0.05), and there was no significant difference in the concentration of β-CTX between the non-bone metastasis group and the bone metastasis group (p>0.05). *p<0.05.

malignant tumor, and it is believed that the pathogenesis of breast cancer is related to family genetic history, environment, and other factors[16].

Bone metastasis is more common in malignant tumors. Research[17,18] shows that more than 80% of breast cancer patients have bone metastasis, and more than 23% of early breast cancer patients (stage I) have bone metastasis. It is difficult to detect bone metastasis in patients with early breast cancer. Currently, the diagnosis mainly depends on the imaging methods, but there are some limitations in the different imaging methods. For example, it is difficult to find bone trabecular lesions in X-ray diagnosis when bone metastasis lesions are less than 10 mm. Compared with X-ray, ECT (single photon emission CT) can detect bone injury in advance, but its specificity is low, and the false positive rate is too high so it cannot be used as the diagnostic standard of bone

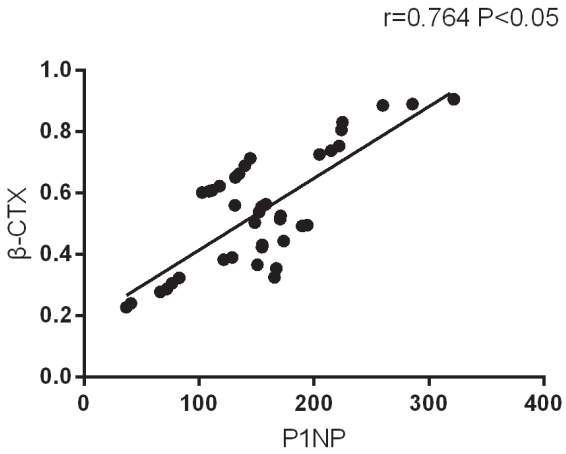

Figure 2. Correlation analysis of P1NP and β-CTX in patients of bone metastasis group. P1NP and β-CTX concentrations were positively correlated in the bone metastasis group (r=0.764, p<0.05).

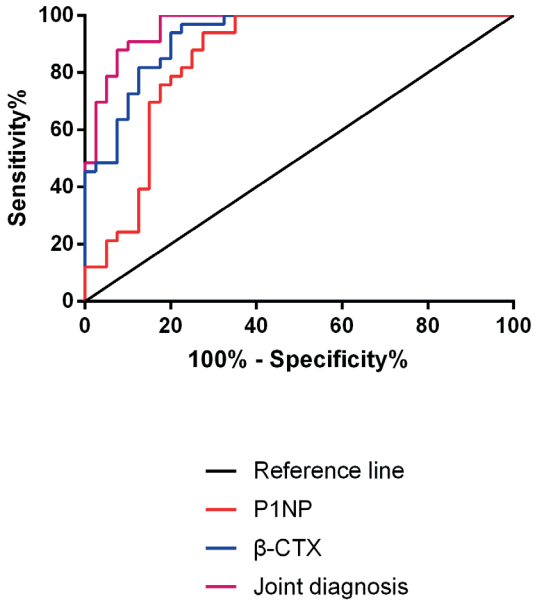

Figure 3. The ROC curve of P1NP and β-CTX in the diagnosis of bone metastasis in breast cancer. In P1NP, AUC=0.852, the sensitivity was 72.5%, the specificity was 93.9%, and CUT OFF=66.44; in β-CTX, AUC=0.883, the sensitivity was 85.0%, the specificity was 84.8%, and CUT OFF=69.8; in joint detection: AUC=0.952, the sensitivity was 84.8%, the specificity was 99.5%, and CUT OFF=99.5.

metastasis. CT has great damage to the human body because of its radioactivity; while, even if PET/CT performed well in the diagnosis of bone metastasis, it was not affordable for ordinary families[19-21].

Bone metabolism indicator is more convenient and less harmful to patients than imaging in the diagnosis of bone metastasis. It has been reported that bone metabolism can be used as a marker for the diagnosis of bone metastasis in cancer[22]. However, the diagnostic specificity of the conventional indicator (Ca, P, PTH) is low, due to the interference of other factors[23]. Type I collagen, the most abundant collagen in the human body, can form a huge acidophilic fiber called collagenous fiber. It is expressed in cicatricial tissue, inner periosteum of the myofibril, and organic part of bone[24]. P1NP is an important index to reflect the synthesis of type I collagen. A study has shown that P1NP is a specific marker of type I collagen deposition and can well reflect the bone formation rate and the activity of osteocytes[25]. While β-CTX, as a type I collagen decomposition product, is an important indicator of bone resorption. According to Bilezikian et al[26], the differential expression of CTX in tumor patients with bone metastasis is a good reflection of bone resorption. Therefore, by detecting the expression of P1NP and β-CTX in plasma of patients with early breast cancer, this study explored the feasibility of P1NP and β-CTX in the diagnosis of bone metastasis of early breast cancer in order to provide the basis for clinical diagnosis.

In this study, by detecting the bone metastasis of early breast cancer patients in The Third People's Hospital of Qingdao, it was found that the concentration of P1NP in the bone metastasis group was significantly higher than that in the control group and the non-bone metastasis group, and there was no difference in the concentration of P1NP between the non-bone metastasis group and the control group. Lumachi et al[27] found that the high expression of P1NP in the plasma of patients with bone metastasis of early breast cancer was significantly higher than that in the control group, which indicated that the high expression of P1NP in patients with bone metastasis could be used as a diagnostic criterion of bone metastasis. By detecting the β-CTX in plasma of patients with bone metastasis of early breast cancer, it was found that the expression of β-CTX in plasma of early bone metastasis was significantly higher than that in the non-bone metastasis group and the control group, and there was no difference in the expression between the non-bone metastasis group and the control group. Pollmann et al[28] showed that the P1NP and β-CTX levels in the plasma of breast cancer patients with bone metastasis were significantly higher than those without bone metastasis, which were consistent with our results. These results suggest that P1NP and β-CTX are valuable in the diagnosis of early breast cancer patients with bone metastasis. At the same time, the correlation between the two markers was also analyzed, and the results showed that there was a positive correlation between the expression of P1NP and β-CTX in the plasma of patients with bone metastasis of early breast cancer. Pan et al[29] also showed a positive correlation between the expression of P1NP and β-CTX in plasma of patients with bone metastasis of lung cancer, which suggests that P1NP and β-CTX are also associated with other cancers. At the end of the study, by drawing the ROC curve for P1NP and β-CTX, it was found that in P1NP, AUC=0.852, the sensitivity was 72.5%, and the specificity was 93.9%; in β-CTX, AUC=0.883, the sensitivity was 85.0%, and the specificity was 84.8%; in joint detection, AUC=0.952, the sensitivity was 84.8%, and the specificity was

99.5%. The results showed that the joint detection of the expressions of P1NP and β-CTX is more helpful for the diagnosis of bone metastasis in early breast cancer than single detection. With the exploration of bone metastasis in patients with lung cancer by joint detection of P1NP and β-CTX expression, Wang et al[30] showed that the result was AUC=0.833, which was similar to our results. This also shows that the joint detection of P1NP and β-CTX is equally effective in detecting bone metastasis of other cancers.

However, there are still some limitations in this study. We did not count the survival of the patient. It is not clear whether these two indicators can be used as prognostic indicators of bone metastasis. Whether the small amount of specimen makes a difference in the result, needs to be further verified. Therefore, to verify the results of the study, the sample size will be increased, and the follow-up of the patients will be followed in future studies.

Conclusions

We showed that the expression of P1NP and β-CTX in plasma has certain diagnostic value for bone metastasis of early breast cancer and is worthy of clinical promotion.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE, Gelmon KE, Giordano SH, Hudis CA, Solky AJ, Stearns V, Winer EP, Griggs JJ. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline Update on Ovarian Suppression. J Clin Oncol 2016; 34: 1689-701.
2) Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015; 386: 1341-1352.
3) Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet 2015; 386: 1353-1361.
4) Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, Thürlimann B, Senn HJ, Panel Members. Tailoring therapies--improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015; 26: 1533-1546.
5) DeSantis C, Ma J, Bryan L, Jemal A. Breast cancer statistics, 2013. CA Cancer J Clin 2014; 64: 52-62.
6) Gareen IF, Hillner BE, Hanna L, Makineni R, Duan F, Shields AF, Subramaniam RM, Siegel BA. Hospice admission and survival after 18F-fluoride PET performed for evaluation of osseous metastatic disease in the national oncologic PET registry. J Nucl Med 2018; 59: 427-433.
7) Hillner BE, Hanna L, Makineni R, Duan F, Shields AF, Subramaniam RM, Gareen I, Siegel BA. Intended versus inferred treatment after 18F-fluoride PET performed for evaluation of osseous metastatic disease in the national oncologic PET registry. J Nucl Med 2018; 59: 421-426.
8) Davila D, Antoniou A, Chaudhry MA. Evaluation of osseous metastasis in bone scintigraphy. Semin Nucl Med 2015; 45: 3-15.
9) Fisher A, Srikusalanukul W, Fisher L, Smith PN. Lower serum P1NP/βCTX ratio and hypoalbuminemia are independently associated with osteoporotic nonvertebral fractures in older adults. Clin Interv Aging 2017; 12: 1131-1140.
10) Hu T, Yang Q, Xu J, Zhang Z, He N, Du Y. Role of β-isomerized C-terminal telopeptides (β-CTx) and total procollagen type 1 amino-terminal propeptide (tP1NP) as osteosarcoma biomarkers. Int J Clin Exp Med 2015; 8: 890-896.
11) Song B, Li X, Zhou Q, Yang X, Jiang Y, Wang A. Application of bone turnover markers PICP and β-CTx in the diagnosis and treatment of breast cancer with bone metastases. Clin Lab 2018; 64: 11-16.
12) Finn RS, Crown JP, Lang I, Boer K, Bondarenko IM, Kulyk SO, Ettl J, Patel R, Pinter T, Schmidt M, Shparyk Y, Thummala AR, Voytko NL, Fowst C, Huang X, Kim ST, Randolph S, Slamon DJ. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16: 25-35.
13) D'Angelo A, Rinaldi P, Belli P, D'Amico R, Carlino G, Grippo C, Giuliani M, Orlandi A, Infante A, Manfredi R. Usefulness of automated breast volume scanner (ABVS) for monitoring tumor response to neoadjuvant treatment in breast cancer patients: preliminary results. Eur Rev Med Pharmacol Sci 2019; 23: 225-231.
14) Blum JL, Robert N, Andersen J, Favret A, Ward P, Osborne C, Pippen J. Use of biomarkers to guide decisions on adjuvant systemic therapy in early-stage invasive breast cancer. J Clin Oncol 2018; 36: 428-429.
15) Swain SM, Baselga J, Kim SB, Ro J, Semiglazov V, Campone M, Ciruelos E, Ferrero JM, Schneeweiss A,

Heeson S, Clark E, Ross G, Benyunes MC, Cortés J, CLEOPATRA Study Group. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 2015; 372: 724-734.

16) Nagata T, Shimada Y, Sekine S, Hori R, Matsui K, Okumura T, Sawada S, Fukuoka J, Tsukada K. Prognostic significance of NANOG and KLF4 for breast cancer. Breast Cancer 2014; 21: 96-101.

17) Epstein-Peterson ZD, Sullivan A, Krishnan M, Chen JT, Ferrone M, Ready J, Baldini EH, Balboni T. Postoperative radiation therapy for osseous metastasis: outcomes and predictors of local failure. Pract Radiat Oncol 2015; 5: e531-e536.

18) Sampath SC, Sampath SC, Mosci C, Lutz AM, Willmann JK, Mittra ES, Gambhir SS, Iagaru A. Detection of osseous metastasis by 18F-NaF/18F-FDG PET/CT versus CT alone. Clin Nucl Med 2015; 40: e173-177.

19) Segrelles JD, Medina R, Blanquer I, Marti-Bonmati L. Increasing the efficiency on producing radiology reports for breast cancer diagnosis by means of structured reports. A comparative study. Methods Inf Med 2017; 56: 248-260.

20) Mittal S, Kaur H, Gautam N, Mantha AK. Biosensors for breast cancer diagnosis: a review of bioreceptors, biotransducers and signal amplification strategies. Biosens Bioelectron 2017; 88: 217-231.

21) Passarelli MN, Newcomb PA, Hampton JM, Trentham-Dietz A, Titus LJ, Egan KM, Baron JA, Willett WC. Cigarette smoking before and after breast cancer diagnosis: mortality from breast cancer and smoking-related diseases. J Clin Oncol 2016; 34: 1315-1322.

22) Hu T, Yang Q, Xu J, Zhang Z, He N, Du Y. Role of β-isomerized C-terminal telopeptides (beta-CTx) and total procollagen type 1 amino-terminal propeptide (tP1NP) as osteosarcoma biomarkers. Int J Clin Exp Med 2015; 8: 890-896.

23) 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: 131-137.

24) Ponticos M, Papaioannou I, Xu S, Holmes AM, Khan K, Denton CP, Bou-Gharios G, Abraham DJ. Failed degradation of JunB contributes to overproduction of type I collagen and development of dermal fibrosis in patients with systemic sclerosis. Arthritis Rheumatol 2015; 67: 243-253.

25) Hansen NU, Karsdal MA, Brockbank S, Cruwys S, Rønnow S, Leeming DJ. Tissue turnover of collagen type I, III and elastin is elevated in the PCLS model of IPF and can be restored back to vehicle levels using a phosphodiesterase inhibitor. Respir Res 2016; 17: 76.

26) Bilezikian JP, Watts NB, Usiskin K, Polidori D, Fung A, Sullivan D, Rosenthal N. Evaluation of bone mineral density and bone biomarkers in patients with type 2 diabetes treated with canagliflozin. J Clin Endocrinol Metab 2016; 101: 44-51.

27) Lumachi F, Basso SM, Camozzi V, Tozzoli R, Spaziante R, Ermani M. Bone turnover markers in women with early stage breast cancer who developed bone metastases. A prospective study with multivariate logistic regression analysis of accuracy. Clin Chim Acta 2016; 460: 227-230.

28) Hansen NU, Karsdal MA, Brockbank S, Cruwys S, Rønnow S, Leeming DJ. Tissue turnover of collagen type I, III and elastin is elevated in the PCLS model of IPF and can be restored back to vehicle levels using a phosphodiesterase inhibitor. Respir Res 2016; 17: 76.

29) Lumachi F, Santeufemia DA, Del Conte A, Mazza F, Tozzoli R, Chiara GB, Basso SM. Carboxy-terminal telopeptide (CTX) and amino-terminal propeptide (PINP) of type I collagen as markers of bone metastases in patients with non-small cell lung cancer. Anticancer Res 2013; 33: 2593-2596.

30) Jung K, Lein M, Ringsdorf M, Roigas J, Schnorr D, Loening SA, Staack A. Diagnostic and prognostic validity of serum bone turnover markers in metastatic renal cell carcinoma. J Urol 2006; 176: 1326-1331.