Evaluation of CXCL12, carbohydrate antigen CA15.3, calcium and parathyroid hormone as potential biomarkers for bone metastasis in patients with breast cancer

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Abstract. Bone is a common site for tumor cells to settle in breast cancer. It has been reported that ~70% of patients with breast cancer experience bone metastasis. C-X-C motif chemokine ligand 12 (CXCL12) is a chemokine, which plays a crucial role in bone metastasis. Currently, the diagnostic biomarkers and risk factors associated with bone metastasis are limited. Therefore, the present study aimed to investigate whether CXCL12, carbohydrate antigen 15.3 (CA15.3), a comparative biomarker, and the bone-related biomarkers, calcium (Ca^{2+}) and parathyroid hormone (PTH), could be used as diagnostic biomarkers and independent risk factors for the early detection and treatment of bone metastasis in patients with breast cancer. The present case control and cross-sectional study consisted of 25 participants who served as the control group, 25 patients newly diagnosed with breast cancer, 25 patients treated for primary breast cancer and 20 patients with breast cancer and bone metastasis. The CXCL12, CA15.3 and PTH levels were analyzed using sandwich ELISA. In addition, the Ca^{2+} levels were measured using colorimetric assay. The results revealed that the levels of CXCL12 were significantly increased. In addition, the levels of Ca^{2+} were slightly increased, whereas the PTH levels were notably decreased in patients with breast cancer experiencing bone metastasis. In addition, CXCL12 exhibited a higher diagnostic accuracy [area under the curve (AUC), 0.7940; P=0.0008] at a cut-off value of >1,392 pg/ml compared with CA15.3 (AUC, 0.6579; P=0.0756; cut-off, >21.50 U/ml). Additionally, PTH displayed a higher diagnostic accuracy (AUC, 0.7280; P=0.0092) for bone metastasis compared with Ca^{2+} (AUC, 0.6792; P=0.0427). Notably, the diagnostic accuracy was increased (AUC, 0.8040; P=0.0005) after combining CXCL12 and CA13.5. Furthermore, CXCL12, CA15.3 and PTH were identified as independent risk factors for bone metastasis. Overall, the results of the present study suggest that CXCL12, CA15.3 and PTH may be considered as promising independent risk factors, which could assist the early detection of bone metastasis and the selection of treatment for patients with breast cancer.

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Introduction

Bone is the most common site for the settlement of spreading of tumor cells in patients with breast cancer. The treatment of bone metastasis is challenging, since several complications can occur, thus affecting the lifestyle of patients (1,2). The early detection of bone metastasis could improve prevention, diagnosis and treatment. It is worth noting that screening biomarkers in blood could be a useful strategy for the early detection of bone metastasis, since this is a low-cost and non-invasive method. Previous studies have identified different biomarkers in diverse types of cancer (3,4). However, the number of studies on the identification of diagnostic and prediction biomarkers for cancer metastasis is limited.

C-X-C motif chemokine ligand 12 (CXCL12), a chemokine also known as stromal cell-derived factor-1 (SDF-1), plays a crucial role in cancer metastasis (5). Previous studies have demonstrated that CXCL12 is expressed in several types of cancer, such as esophageal squamous cell carcinoma (6), epithelial ovarian cancer (7), renal cancer (8,9), colorectal carcinoma (10) and breast cancer (11). Furthermore, another study revealed that CXCL12 was expressed in different sites of metastasis, including the liver, bones, brain and lungs (12). The content of CXCL12 has also been determined in plasma samples from patients with different types of cancer. The results demonstrated that the CXCL12 plasma levels were significantly higher in patients with esophageal cancer (13) and breast cancer (14). By contrast, it has also been reported that patients with breast cancer exhibit lower plasma CXCL12 and mRNA expression levels in the invasive tissues compared with those in the control groups (14).

The carbohydrate antigen 15.3 (CA15.3) is a glycoprotein with a molecular weight of 300-450 kDa. It is commonly used as a common tumor marker for the spread of cancer cells to lymph nodes in patients with metastatic breast cancer (15) and in monitoring adjuvant chemotherapy following surgery in patients with human epidermal growth factor receptor 2 (HER-2) negative breast cancer (16). A previous study demonstrated that CA15.3 was associated with a poor prognosis in patients with breast cancer (17). Furthermore, it was previously found that CA15.3 levels were increased in the majority of patients with metastatic breast cancer (18). However, the levels of CA15.3 have also been found to be increased in other disorders, such as chronic renal failure, colitis, and dermatological and liver diseases (19). Therefore, it was hypothesized that CA15.3 was not a specific biomarker for breast cancer.

Emerging evidence has suggested that calcium (Ca^{2+}) and parathyroid hormone (PTH) play a critical role in cancer progression by regulating cell proliferation, migration and cell death-related pathways (20-23). Bone is the most common site for Ca^{2+} storage. Therefore, Ca^{2+} hemostasis may be affected when tumor cells spread and settle into bones (24). A previous study demonstrated that elevated Ca^{2+} levels were associated with a poor prognosis of patients (25). Furthermore, in another study, increased plasma Ca^{2+} levels in patients with bladder cancer and bone metastasis displayed a high diagnostic accuracy and they were thus identified as an independent risk factor for predicting bone metastasis in these patients (26). PTH, a critical hormone, regulates the absorption of Ca^{2+} from the intestinal and its release from bones (27). In addition, PTH is involved in the development of skeletal tumors. In previous study using a preclinical model, the treatment of mice with PTH increased the number of osteoblasts and tumor colonies in bones (28). There have been several attempts to identify tumor markers for the diagnosis of different types of cancer. However, only a limited number of studies have (29-31) investigated the diagnostic accuracy of tumor markers and predicted risk factors associated with bone metastasis in patients with breast cancer.

Therefore, the present study aimed to investigate the diagnostic accuracy of CXCL12 and CA15.3, and that of the bone-related biomarkers, Ca^{2+} and PTH, and their efficiency in predicting bone metastasis in patients with breast cancer.

Patients and methods

Study design. In the present case control and cross-sectional study, a total of 25 subjects were included in the control group, 25 patients were included in group newly diagnosed with breast cancer, 25 patients were included in the treated group with primary breast cancer, and 20 patients were included in the group with bone metastasis. Bone metastasis occurred in patients with breast cancer at an average of 4 months following diagnosis. The samples from patients with primary breast cancer and those from patients with bone metastasis were selected by a physician. Bone metastasis was diagnosed by a computed tomography (CT) and positron emission tomography-CT scan. The present study was conducted between December, 2023 and May, 2024 at the Al-Amal National Hospital for Cancer Management, Baghdad, Iraq. The breast cancer subtype was identified from the pathological reports of patients. Additionally, patients with osteoporosis, liver and kidney diseases, diabetes mellitus, hypertension, and smoking and alcohol consumption history were excluded from the study. The protocol of the present study followed the Declaration of Helsinki and it was approved by College of Science, Al-Nahrain University, Baghdad, Iraq (approval no. 4225/3/2, November 15, 2023). Informed consent was obtained from all participants included in the present study.

Biomarker analysis. Peripheral blood samples were collected from participants in lithium-heparin tubes. Plasma was separated following blood centrifugation at 492 x g for 15 min at 4˚C and stored at -20˚C. The CXCL12, CA15.3 and PTH levels were measured using a sandwich ELISA assay, according to the manufacturer's instructions (Sunlong Medical™ Human CXCL12/SDF-1 ELISA kit; cat. no. EL0249Hu; Quick Step Human Carbohydrate Antigen 15-3 ELISA kit; cat. no. QS0383Hu; Quick Step Human Parathyroid Hormone ELISA kit; cat. no. QS1342Hu; all from Sunlong Biotech Co., Ltd.). The Ca^{2+} levels were determined using the Elabscience® Calcium colorimetric assay kit (cat. no. E-BC-K103M; Elabscience Biotechnology, Inc.).

Statistical analysis. All statistical analyses were performed using GraphPad Prism 8.4.3 software (Dotmatics). Data distributions were analyzed using a Kolmogorov-Smirnov test. All continuous parameters, which were not normally distributed, are expressed as the median value with minimum and maximum values. Statistically significant differences between continuous non-parametric variables were assessed using the Kruskal-Wallis test, with Dunn's multiple comparison post hoc tests among groups. Categorical variables are presented as numbers and percentages. The significant differences between categorical variables were determined using Fisher's exact and Chi-squared tests. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic accuracy of each parameter via determining the area under the curve (AUC), sensitivity and specificity values. The cut-off value of each biomarker was calculated using Youden-J index. Multiple logistic regression analyses were performed to identify the independent risk factors associated with predicting bone metastasis in patients with breast cancer. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinicopathological characteristics of the patients. The clinicopathological characteristics of the subjects in the control and breast cancer groups, including patients with newly diagnosed with primary breast cancer, treated patients with primary breast cancer and those with breast cancer with bone metastasis, are presented in Table I. The subjects in each group were age-matched (P=0.3046). In addition, no statistically significant differences were obtained between the two groups in terms of breast cancer types (P=0.2070). The majority of the patients with primary breast cancer were of luminal A type (84%), while the remaining 12 and 4% were of HER-2 and the triple-negative breast cancer (TNBC) subtype, respectively. None of the patients with primary breast cancer were diagnosed with luminal B subtype. In addition, the majority of the treated patients with primary breast cancer were of luminal A subtype (68%), while 24, 4 and 4% of these patients had the HER-2 type, TNBC and luminal B type breast cancer, respectively. In the bone metastasis group, 90 and 10% of patients were of luminal A and TNBC subtype, respectively. No patients with HER-2 and luminal B breast cancer were

Table I. Clinical characteristic and biochemical parameters of the patients with breast cancer.

Variables	Control (n=25)	Patients with primary breast cancer (n=25)	Treated patients with primary breast cancer (n=25)	Patients with bone metastasis (n=20)	P-value
Age (years), median (min-max)	48 (35-70)	45 (24-70)	50 (36-62)	51 (43-78)	0.3046
Breast cancer types, n (%)					0.2070
Luminal A		21 (84)	17 (68)	18 (90)	
Luminal B		0 (0)	1 (4)	0 (0)	
HER-2		3 (12)	6 (24)	0 (0)	
TNBC		1 (4)	1 (4)	2 (10)	
Surgery					<0.0001
Yes, n (%)		0 (0)	25 (100)	13 (65)	
No, n (%)		25 (100)	0 (0)	7 (35)	
Smoking					>0.9999
Yes, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	
No, n (%)	25 (100)	25 (100)	25 (100)	20 (100)	
Alcohol consumption					>0.9999
Yes, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	
No, n (%)	25 (100)	25 (100)	25 (100)	20 (100)	
CXCL12 (pg/ml), median (min-max)	1,296 (1137.5-637.5)	1,371 (1179.2-1687.5)	1,377 (1216.7-2470.8)	1,485 (1220.8-2579.2)	>0.9999 [a]>0.9999[b] 0.0030[c]
CA15.3 (U/ml), median (min-max)	15.74 (11.6-27.04)	14.54 (9.32-23.31)	16.89 (10.23-57.65)	21.78 (9.4-75.54)	>0.9999[a] 0.0705[b] 0.0864[c]
Ca^{2+} (mmol/l), median (min-max)	1.175 (0.831-1.247)	1.199 (0.831-1.263)	1.168 (0.851-1.297)	1.239 (0.933-1.335)	>0.9999 [a]>0.9999[b] 0.0941[c]
PTH (pg/ml), median (min-max)	43.3 (30.39-79.52)	44.27 (30.74-61.57)	43.17 (27.9-78.01)	35.09 (30.33-87.64)	>0.9999 0.6490[b] 0.0318[c]

Value are expressed as the median (min-max) or as numbers and percentages. A P-value <0.05 indicates a statistically significant difference. Fisher's exact test was used to analyze the data for surgery, smoking and alcohol consumption. The Chi-squared test was used to analyze the data for breast cancer types. The Kruskal-Wallis test with Dunn's multiple comparisons post hoc test were used to compare the CXCL12, CA15.3; Ca^{2+} and PTH levels between the primary breast cancer and the other groups. [a]P-value for comparisons between control vs. patients with primary breast cancer; [b]P-value for comparisons between patients with primary breast cancer vs. treated patients with primary breast cancer, [c]P-value for comparisons between patients with primary breast cancer vs. those with bone metastasis. TNBC, triple-negative breast cancer; CXCL12, C-X-C motif chemokine ligand 12; CA15.3, carbohydrate antigen 15.3; Ca^{2+}, calcium; PTH, parathyroid hormone.

enrolled. Furthermore, there was a statistically significant difference among the different groups in terms of the patients who underwent surgery and those who did not (P<0.0001). All patients in the treated primary breast cancer group underwent surgery, while none of the patients in the primary breast cancer group underwent surgery. Additionally, the majority of patients in the bone metastasis group (65%) also underwent surgery. None of the participants in the control and patient group had a history of smoking or alcohol abuse (Table I).

Comparison of biomarkers. Subsequently, the secretion levels of CXCL12 and CA15.3, as comparative biomarkers, and those of the bone-related factors, Ca^{2+} and PTH, were compared (Table I). Of note, no significant differences were observed in the CXCL12, CA 15.3, Ca^{2+} and PTH levels between the control and primary breast cancer groups (P>0.9999), and primary breast cancer and treated primary breast cancer groups (P>0.9999, P=0.0705, P>0.9999 and P=0.6490, respectively). These findings indicated that treatment could not affect the secretion levels of the candidate biomarkers. Notably, the CXCL12 levels were significantly increased (P=0.0030) in the primary breast cancer group compared with the bone metastasis group. The Ca^{2+} levels were slightly increased (P=0.0941), whereas those of PTH were notably decreased (P=0.0318) in the primary breast cancer group compared with the bone metastasis group. Finally, the CA15.3 levels were

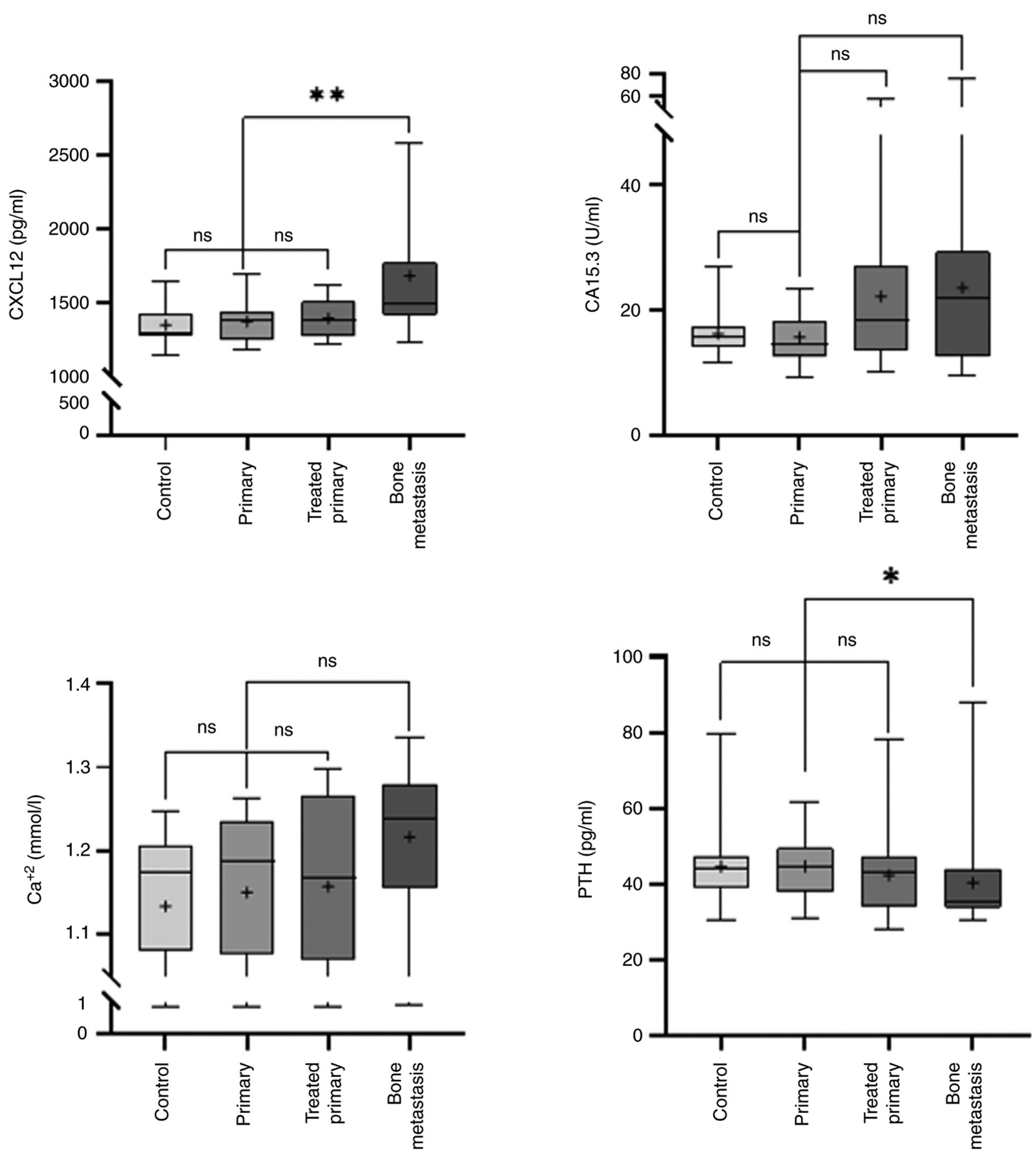

Figure 1. Comparison between the control and breast cancer groups (primary, treated primary and bone metastasis). The levels of (A) CXCL12, (B) CA15.3, (C) Ca^{2+} and PTH in the different groups are shown. The horizontal line indicates the median and the crossed line indicates the mean. *P<0.05 and **P<0.005; ns, not significant; CXCL12, C-X-C motif chemokine ligand 12; CA15.3, carbohydrate antigen 15.3; Ca^{2+}, calcium; PTH, parathyroid hormone.

slightly (P=0.0864) enhanced in the primary breast cancer group compared with the bone metastasis group (Fig. 1).

Diagnostic accuracy and AUC values of CXCL12, CA15.3, Ca^{2+} and PTH. To evaluate the diagnostic reliability of CXCL12 and CA15.3, as comparative biomarkers, and Ca^{2+} and PTH, as bone-related biomarkers in bone metastasis in patients with breast cancer compared with those with primary breast cancer, the sensitivity, specificity and cut-off values were determined using ROC analysis (Table II and Fig. 2). The results revealed that CXCL12 (AUC, 0.7940; P=0.0008; cut-off, >1,392 pg/ml) could be a potential effective biomarker for predicting bone metastasis in patients with breast cancer compared with CA15.3 (AUC, 0.6579; P=0.0756; cut-off, >21.50 U/ml). The sensitivity and specificity percentages for CXCL12 were 90 and 68%, respectively, while those for CA13.5, 52 and 88%, respectively. In terms of bone-related biomarkers, Ca^{2+} could discriminate patients with breast cancer and bone metastasis from those with primary breast cancer (AUC, 0.6792; P=0.0427; cut-off, >1.257 U/ml) with

Table II. Evaluation of CXCl12, CA15.3, Ca^{2+} and PTH as diagnostic biomarkers.

Factors	AUC	Sensitivity (%)	Specificity (%)	95% CI	Cut-off	P-value
CXCL12 (pg/ml)	0.7940	90	68	0.6552-0.9328	>1392	0.0008
CA 15.3 (U/ml)	0.6579	52	88	0.4750-0.8408	>21.50	0.0756
Ca^{2+}(mmol/l)	0.6792	45	91.6	0.5154-0.8429	>1.257	0.0427
PTH (pg/ml)	0.7280	60	88	0.5718-0.8842	<36.70	0.0092
CXCL12 + CA15.3	0.8040	90	68	0.6709-.9371	-	0.0005
CXCL12 + Ca^{2+}	0.8000	90	68	0.6650-0.9350	-	0.0006
CXCL12 + PTH	0.7800	76	75	0.6383-0.9217	-	0.0014
CXCL12 +CA15.3 +Ca^{2+} + PTH	0.7820	80	72	0.6415-0.9225	-	0.0013

A P-value <0.05 indicates a statistically significant difference. AUC, area under the curve; CI, confidence interval; CXCL12, C-X-C motif chemokine ligand 12; CA15.3, carbohydrate antigen 15.3; Ca2+, calcium; PTH, parathyroid hormone.

a sensitivity of 45% and specificity of 91.6%. However, PTH (AUC, 0.7280; P=0.0092; cut-off, <36.70 pg/ml) displayed a higher diagnostic accuracy for predicting bone metastasis, with a sensitivity of 60% and specificity of 88%, compared with Ca^{2+}, but reduced compared with CXCL12. These results indicated that CXCL12 could be the optimal biomarker for distinguishing patients with breast cancer and bone metastasis from those with primary breast cancer. Notably, the diagnostic accuracy was increased after combining CXCL12 and CA13.5 (Table II and Fig. 2E; AUC=0.8040; P=0.0005), with sensitivity and specificity rates of 90 and 68%, respectively, which were higher compared with those of CXCL12 and CA13.5 alone. Furthermore, the combination of CXCL12 + PTH and CXCL12 + CA15.3 + Ca^{2+} + PTH notably increased the diagnostic accuracy (AUC, 0.7800; P=0.0014 and AUC, 0.7820; P=0.0013), with a specificity of 75 and 72%, respectively, for discriminating patients with breast cancer and bone metastasis from those with primary breast cancer.

CXCL12, CA15.3, $Ca^{2+}$and PTH as independent risk factors of bone metastasis in patients with breast cancer. To investigate whether CXCL12, CA15.3, Ca^{2+} and PTH levels could serve as independent risk factors for predicting bone metastasis in patients with breast cancer, multiple logistic regression analysis was carried out (Table III). The analysis revealed that CXCL12 [β=0.01531; odds ratio (OR), 1.015; P=0.0261], CA15.3 (β=0.3920; OR, 1.480; P=0.0226) and PTH (β=-0.4838; OR, 0.6164; P=0.0224), but not Ca^{2+} levels (β=7.515; OR, 1.835; P=0.1932) could be independent risk factors for predicting bone metastasis.

Discussion

Bone is considered as the most common site of metastasis in patients with breast cancer. Emerging evidence has suggested that ~65-70% of patients with breast cancer will experience metastasis (32,33). Nowadays, several attempts have been made to develop novel and effective approaches for diagnosing and predicting the spread of tumor cells in distant sites. Therefore, researches have focused on the development of easy-to-apply, non-invasive and low-cost methods for the early detection of bone metastasis, thus improving treatment management. Therefore, the present study aimed to identify tumor markers in the peripheral blood of patients with breast cancer for the diagnosis and risk assessment of bone metastasis to assist in its early detection and treatment decision.

A previous study demonstrated that the mRNA expression levels of CXCL12 in breast cancer tissues and plasma content were decreased in patients with invasive breast carcinoma compared with normal breast tissues and plasma from subjects in the control group (34). On the other hand, another study found that the plasma levels of CXCL12 were significantly higher in diverse stages of breast cancer compared with the healthy group (14). However, herein, no significant changes in the CXCL12 levels were observed between patients with primary breast cancer and control subjects. This finding could be due to the inclusion of samples from different types of breast cancer, since 84% of patients were diagnosed with luminal A breast cancer, 12% with HER-2 breast cancer and 4% with TNBC. By contrast, in the study by Motyka *et al* (34), only patients with luminal A and B breast cancer were included. Additionally, in the study by Dabrowska *et al* (14), the types of breast cancer were not defined.

Of note, in the present study, the plasma levels of CXCL12 were significantly increased in patients with breast cancer and bone metastasis compared with those with primary breast cancer. In line with the present study, a previous study on patients with non-small cell lung cancer, the plasma levels of CXCL12 were notably increased in patients with bone metastasis, thus supporting the vital role of this chemokine in metastasis (35). Other research has also highlighted the key role of CXCL12 in intracellular pathways in cancer. A previous study revealed that CXCL12 upregulation following its binding to its receptor, CXCR4, stimulated different intracellular pathways, thus ultimately promoting cell proliferation, angiogenesis, survival and metastasis (5). Furthermore, in the present study, the CXCL12 content displayed a high diagnostic accuracy (AUC, 0.7940) and elevated plasma CXCL12 levels (>1,392 pg/ml) were considered as a predictive

Table III. Risk factors for bone metastasis in breast cancer.

Factor	β	OR	OR (95% CI)	P-value
CXCL12	0.01531	1.015	1.005-1.033	0.0261
CA15.3	0.3920	1.480	1.160-2.377	0.0226
Ca^{2+}	7.515	1835	0.1275-3210486883	0.1932
PTH	-0.4838	0.6164	0.3612-0.8378	0.0224

A P-value <0.05 indicates a statistically significant difference. OR, odds ratio; CI, confidence interval; CXCL12, C-X-C motif chemokine ligand 12; CA15.3, carbohydrate antigen 15.3; Ca2+, calcium; PTH, parathyroid hormone.

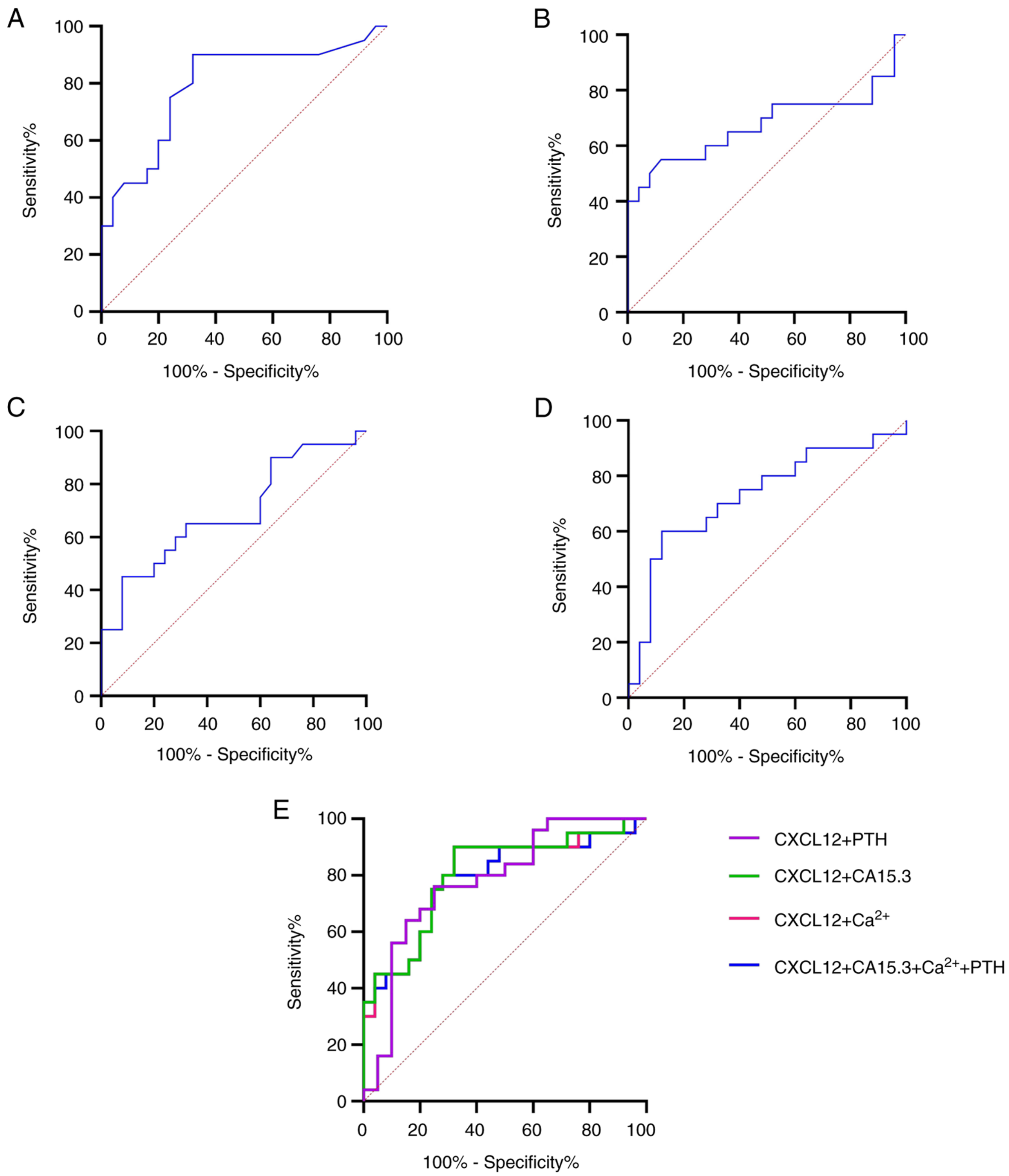

Figure 2. Receiver operative characteristic curves for the diagnosis of bone metastasis in patients with breast cancer. (A) CXCL12, (B) CA15.3, (C) Ca^{2+} (D) PTH, and (E) the combination of biomarkers. CXCL12, C-X-C motif chemokine ligand 12; CA15.3, carbohydrate antigen 15.3; Ca^{2+}, calcium; PTH, parathyroid hormone.

biomarker for the development of bone metastasis in patients with breast cancer. Additionally, CXCL12 was identified as an independent risk factor (β=0.01531; P=0.0261) for predicting the incidence of bone metastasis. To the best of our knowledge, the present study is the first to investigate CXCL12 as a diagnostic biomarker and prediction risk factor for patients with breast cancer and bone metastasis.

In the present study, the CA15.3 levels did not differ significantly compared with those in the control group. This result was not in agreement with previous studies, indicating that the levels of CA15.3 were higher in patients with breast cancer compared with healthy subjects (14,34). This finding may be due to the small sample size, which was one of the limitations of the present study. Previously, it was suggested that CA15.3 may be a good indicator for bone and liver metastasis in patients with breast cancer (36,37). In addition, the CA15.3 levels were enhanced in patients with breast cancer and bone metastasis compared with those without bone metastasis (38). The present study demonstrated that the CA15.3 levels were slightly increased in patients with bone metastasis compared with those with primary breast cancer. Furthermore, the diagnostic accuracy of CA13.5 for predicting bone metastasis was 0.6570, which was the lowest compared with other candidate biomarkers in the present study. However, the combination of CXCL12 and CA15.3 improved the diagnostic accuracy to 0.8040, which was the highest among the different combinations of biomarkers. This increase in predictive accuracy following the combination of CXCL12 and CA15.3 was also reported in previous studies investigating the accuracy of these biomarkers in predicting breast cancer (14,34). However, the combination of CXCL12 and CA15.3 in predicting bone metastasis in patients with breast cancer has not been previously investigated. Furthermore, increased CXCL12 and CA15.3 levels were associated with cancer development and spread of tumor cells to bones. In the present study, no significant differences in plasma Ca^{2+} levels were obtained between patients with primary breast cancer and the control group. However, plasma Ca^{2+} level was slightly increased in patients with bone metastasis compared with those with primary breast cancer. This finding was consistent with that reported in a previous study, demonstrating increased serum Ca^{2+} levels in patients with bladder cancer and bone metastasis compared with those without bone metastasis (26). The slight elevation of Ca^{2+} levels in the bone metastasis group could occur due to an imbalance between bone resorption and bone formation, which could in turn lead to the release of Ca^{2+} into the blood stream in bone metastasis (25). In addition, the results of the present study illustrated that Ca^{2+} alone displayed a moderate diagnostic accuracy (AUC, 0.6792; P=0.0427) with a sensitivity and specificity of 45 of 91.6%, respectively. This result was consistent with that reported in a previous study on patients with bladder cancer and bone metastasis (26). Notably, the diagnostic accuracy was increased (AUC, 0.8000, P=0.0006) after combining Ca^{2+} and CXCL12. In addition to the diagnostic accuracy, the combination of Ca^{2+} and CXCL12 also improved the sensitivity to 90%, but reduced the specificity to 68%.

A previous study found that elevated plasma PTH levels were associated with the poor prognosis of patients with advanced-stage prostate cancer experiencing bone metastasis (23). Herein, the results were inconsistent from those reported in the study by Schwartz (23), since plasma PTH levels were reduced in patients with bone metastasis compared with those with primary breast cancer. This contradiction in the results may be due to the different cancer types included in these two studies. Therefore, further studies are required in breast cancer to verify the results of the present study. Enhanced Ca^{2+} levels could mitigate the secretion levels of PTH in patients with bone metastasis, since it has been reported that increased Ca^{2+} levels can inhibit PTH secretion through a negative feedback mechanism. Of note, PTH alone displayed a satisfactory diagnostic accuracy (AUC, 0.7280; P=0.0092), which was higher compared with that recorded for CA15.3 and Ca^{2+}, but not for CXCL12. The sensitivity was increased from 60% in PTH alone to 76% in the combination of PTH with CXCL12, while specificity decreased from 88 to 75%. Nevertheless, the combination of CXCL12 + CA15.3 + Ca^{2+} + PTH displayed an improved diagnostic accuracy (AUC, 0.7820; P=0.0013), which was slightly lower than that of CXCL12 alone, with a sensitivity and specificity of 80 and 72%, respectively. The combination of CXCL12 + CA15.3 exhibited an enhanced diagnostic reliability for predicting bone metastasis in patients with breast cancer compared with other candidate biomarkers. More importantly, CXCL12, CA15.3 and PTH were identified as possible independent risk factors for predicting of bone metastasis. To the best of our knowledge, the present study was the first to assess this finding.

Although several significant findings were reported in the present study, there are some limitations that still need to be addressed. Firstly, the sample size was small in each group. Secondly, the majority of patients with breast cancer were diagnosed with the luminal A type. Thirdly, the sensitivity and specificity of the candidate biomarkers obtained were moderate. Therefore, improvements are still warranted. Finally, different tumor stages were not considered in this study. Overall, further studies with a higher number of patients with different breast cancer subtypes are required to provide more interesting results, not only in patients with breast cancer with bone metastasis, but also in those with liver metastasis. Additionally, different tumor markers, such as calcitonin and calcitriol, could be assessed to increase the sensitivity and specificity of the diagnostic reliability.

Overall, the results of the present study suggested that elevated plasma CXCL12 levels could be a novel potential diagnostic biomarker and an independent risk factor for the prediction of bone metastasis in patients with breast cancer. CA15.3, Ca^{2+} and PTH levels also exhibited significant diagnostic accuracy, but not as high as that obtained for the combination of CXCL12 and CA15.3. CXCL12, CA15.3 and PTH levels could be also considered as independent risk factors that could promote the early detection of bone metastasis in patients with breast cancer and improve the management of their treatment.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

BAA designed the concept of the study, carried out the sample collection and performed the experiments. FAR supervised the whole study, interpreted and statistically analyzed the data, and wrote and edited the manuscript. BAA and FAR confirm the authenticity of all the raw data. Both authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The protocol of the present study followed the Declaration of Helsinki and it was approved by the College of Science, Al-Nahrain University, Baghdad, Iraq (approval no. 4225/3/2, November 15, 2023). Informed consent was obtained from all participants included in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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