



Relationship Between IL-18 and Bone Metastasis in Female Breast Cancer

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OBJECTIVE

Breast cancer is a metastatic disease that frequently (in approximately 70% of cases) affects the skeletal system. The aim of the present study was to compare serum IL-18 levels in breast cancer patients with and without bone metastases with healthy controls.

METHODS

Included were a total of 154 female breast cancer patients with bone metastases (n=53; Group 2) and without bone metastases (n=51; Group 1), as well as 50 healthy control subjects (Group 3). Serum IL-18 levels were compared among the groups.

RESULTS

Mean serum IL-18 levels were significantly different between Groups 1 and 3 ($p<0.001$), Groups 2 and 3 ($p<0.001$), and Groups 2 and 1 ($p=0.020$). In receiver operating characteristic (ROC) curve analysis performed between Groups 1 and 2, sensitivity of serum IL-18 levels in patients with bone metastases was nearly 26 percent.

CONCLUSION

Lower rates of IL-18 sensitivity were detected in breast cancer patients with bone metastasis.

Keywords: IL-18; bone metastasis; breast cancer.

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Introduction

Breast cancer is the most frequently occurring type of cancer in women, and the most common cause of cancer-related mortality after lung cancer. One of every 9 women has a risk of developing invasive breast cancer during her lifetime.[1] Nearly 25% of breast cancer metastases initially involve bone, and bone metastases are observed in nearly 70% of patients with metastatic breast cancer. Bone metastases are important etiological

factors for morbidity and mortality. With the presence of bone metastases secondary to breast cancer, pain, hypercalcemia, fractures, and nerve compression are frequently observed.[2]

Tumor biology should be better understood, and mechanisms of the metastatic process should be more comprehensively investigated in an effort to decrease development of bone metastasis in breast cancer, emergence of related complications, and mortality rates.

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Table 1. Distribution of number and ages of female study participants according to group

Group	Number of volunteers n=154	Mean±SD age of volunteers
1	51	52.65±9.54
2	53	55.04±13.45
3	50	53.12±12.61

The aim of the present study was to compare serum IL-8 levels in breast cancer patients with and without bone metastases and healthy individuals. In consideration of potentially emergent outcomes, degree of sensitivity of serum IL-18 levels in the detection of bone metastases in breast cancer patients and optimal treatment strategies are discussed.

Materials and Methods

Sampling method

A total of 104 female patients with breast cancer who presented to the Outpatient Clinic of the Mersin University Faculty of Medicine Department of Medical Oncology between March 2011 and March 2012 were included. Patients aged over 18 years with histopathologically confirmed breast cancer were divided into Groups 1 and 2. Fifty-one patients, including some in remission and some without bone metastases who regularly attended follow-up visits, were selected for Group 1. Fifty-three patients, included in Group 2, had bone metastases. Number and location of bone metastases were not taken into consideration. Patients without bone metastases, but with metastases in different organs were not included. Fifty healthy women who had consulted the hospital within the aforementioned time interval were included as the control group, Group 3. Controls were women aged over 18 years with no known chronic or autoimmune disease, or active infection.

From the volunteers, 5-mL fresh blood samples were drawn into flat-bottom test tubes, and centrifuged at 4000 rpm for 10 minutes at ambient temperature to separate sera. Serum samples were conserved in Eppendorf tubes at -25°C until analysis. The samples to be analyzed were pipetted out from Eppendorf tubes at -25°C and left to thaw at room temperature before analyses were performed. Human IL-18 was analyzed with a DSX Automated Microelisa Processing System (Dynex Technologies Inc., Chantilly, VA, USA), using human IL-18 enzyme-linked immunosorbent assay (ELISA) kit (MBL Ltd. code no: 7620) and sandwich ELISA method. Reference range was 36.1—257.8 pg/mL.

Statistical analysis

Categorical variables were expressed as numbers and percentages, while continuous variables were expressed as mean±SD. One-way analysis of variance was used for intergroup comparison of IL-18 levels and patient ages. If intergroup differences were found, post-hoc analysis with Bonferroni correction was performed. Receiver operating characteristic (ROC) curve analysis was used to obtain cutoff values for IL-18. Level of significance of statistical analyses was accepted as $p < 0.05$.

Results

Mean ages among the groups were similar (Table 1).

Distribution of cancer stages of Group 1 patients were: stage I, 24%; stage II, 49%; stage III, 27%. All patients had metastatic disease, and were included in stage IV. Regarding histopathological diagnosis, invasive ductal carcinoma was the most common diagnosis in Groups 1 and 2 (83%). Invasive lobular carcinoma (10%), invasive papillary carcinoma (6%), and medullary carcinoma (1%) were also observed.

Mean serum IL-18 level of Group 2 (1528.38±1081.28 pg/mL) was higher than that of Group 1 (1146.16±495.09 pg/mL). Mean serum IL-18 levels of Groups 1 and 2 were higher than that of Group 3 (480.33±234.62 pg/mL; Table 3)

A statistically significant difference was found among serum IL-18 levels ($p < 0.001$). Mean serum IL-18 levels of Groups 3 and 2 were statistically significantly different ($p < 0.001$). Mean serum IL-18 levels of Groups 3 and 1 were statistically significantly different ($p < 0.001$), as were those of Groups 1 and 2 ($p = 0.020$).

Table 2. Comparison of hormone receptor and HER2 positivities in Groups 1 and 2

	Group 1	Group 2
Estrogen receptor positivity	62%	79%
Progesterone receptor positivity	56%	67%
HER 2 positivity	35%	24%
Triple negative	13%	8%
Triple positive	17%	15%

Table 3. IL-18 values (mean±SD) among the groups

	Group 1 Mean±SD (n=51)	Group 2 Mean±SD (n=53)	Group 3 Mean±SD (n=50)
IL-18 level	1146.16±495.09 (pg/mL)	1528.38±1081.28 (pg/mL)	480.33±234.62 (pg/mL)

As a result of ROC curve analysis, cutoff value of IL-18 in breast cancer patients with and without bone metastasis was 1773.63 pg/mL. Diagnostic value of IL-18 level in the detection of bone metastasis when used as a marker is shown in Table 4.

Area under the curve (AUC) value for serum IL-18 levels in Groups 1 and 2 was 0.576 (p=0.1782). An AUC value of less than 0.60 signifies that this test will not be statistically useful when discriminating between breast cancer patients with and without bone metastases.

Discussion

Prognostic parameters are still a subject of debate. In clinical practice, most important prognostic and predictive information regarding breast cancer is obtained from histopathological evaluation (incorporating size of tumor, extent of axillary lymph node involvement, presence of lymphatic and vascular invasion, histological grading of the tumor, and estrogen/progesterone status).[3] Breast cancer patients are most commonly lost due to distant metastases, and bone metastases develop in approximately 70% of these cases.[4] The determination of prognostic factors is of the utmost importance, so that the disease may be detected at an early stage, potential metastases may be evaluated, existing metastases may be optimally monitored, and convenient treatment strategies may be determined. The current lack of auxiliary markers to aid in the detection of bone metastases in breast cancer has lead researchers to conduct new studies.

Increased levels of serum IL-8 in some cancer patients has been reported, and it has been determined that disease progression in patients with increased serum IL-18 levels is more fatal. Takubo et al. demonstrated that non-Hodgkin's lymphoma patients with serum IL-18

levels over 2000 pg/mL were at higher risks.[5] Kawabata et al. reported that gastric cancer patients with higher serum IL-18 levels had shorter survival times.[6] These results suggest that serum IL-18 levels may be used as a prognostic factor in some cancer patients. However, while IL-18 has been associated with some cancer types, the mechanisms of cancer pathogenesis and antitumor activity have yet to be made clear. Still, IL-18 may presumably be an important marker in the progression of breast cancer,[6] as serum IL-18 levels in breast cancer patients who developed bone metastases were found to be significantly higher in several studies.[7,8]

In light of these results, the usefulness of serum IL-18 as a marker of the potential development of bone metastases in breast cancer patients was presently investigated.

Serum IL-18 levels were compared among breast cancer patients with and without bone metastases and healthy women. Breast cancer patients with other organ metastases were not included. In a similar study conducted by Soheir et al. in 2005, no significant difference in serum IL-18 levels was found between breast cancer patients with bone or other organ metastases.[8] However, in the present study, serum IL-18 levels of all breast cancer patients (with and without metastases) were found to be higher than those of the healthy controls (Table 3). Serum IL-18 levels in the group with bone metastases were found to be significantly higher (p=0.020). In a similar study conducted by Günel et al. in 2002, serum IL-18 levels in the metastatic and non-metastatic groups were found to be higher than those of the healthy group, while serum IL-18 levels of the group with bone metastases were higher than those of the nonmetastatic group.[7] However, in several studies, some performed by Günel et al., breast cancer patients with metastases in other organs constituted a

Table 4. Diagnostic value of serum IL-18 level

Cytokine	Cutoff value (pg/mL)	Area under curve	Sensitivity (%)	Specificity (%)	p
IL-18	1773.63	0.576	26.4	96.1	0.17

separate study group. Still, serum IL-18 levels of breast cancer patients with bone metastases were statistically significantly higher, compared to those of breast cancer patients with other organ metastases.[7,9] Following the important discovery that primary osteoblasts can also secrete IL-18 receptor components, many authors have suggested that osteoblasts are target cells in IL-18 response and that IL-18 exerts a physiological effect on osteogenic cells.[10]

IL-18 exerts its effects on osteoblasts, not via production of INF- γ , but rather via production of granulocyte macrophage colony-stimulating factor (GM-CSF).[11] Increased GM-CSF also induces dose-related increases in the proliferation rate of human osteoblasts.[12] IL-18 can inhibit osteoclastogenesis by increasing osteoprotegerin in stromal or osteoblastic cells.[10] In addition to its physiological interactions with osteogenic cells, IL-18 also plays a role in pathological processes. In the development of bone metastasis in breast cancer patients, as an autocrine defense mechanism, the organism releases IL-18 from osteoclasts and osteoblasts.[11] This phenomenon can be thought to justify the detection of higher IL-18 levels in the presence of bone metastasis. However, it should be investigated further in comprehensive studies.

As evidenced by theoretical experiments, IL-18 can inhibit development of osteolytic metastases of human lung cancer or human breast cancer cells.[13,14] In addition, initial treatment with recombinant IL-18 or IL-18 binding protein reportedly decreased the number of distant metastases in mice cancer models.[15]

As mean serum IL-18 values of patients with bone metastases were significantly higher than those without metastases ($p=0.020$) in the present study, it can be said that IL-18 may be useful in the detection of bone metastases. The cutoff value of serum IL-18 for the groups with and without bone metastases was 1773.63 pg/mL, and keeping in mind that patients with serum IL-18 levels above this cutoff value had bone metastases, nearly 27% of this group of patients exceeded the cutoff value. However, other patients with serum IL-18 values below the cutoff had conclusively confirmed bone metastases. Therefore, serum IL-18 may not be a sensitive marker in the monitorization of breast cancer patients with bone metastases. At the same time, the lower sensitivity of IL-18 in the present study may be related to bisphosphonates used by breast cancer patients with bone metastases.

Bisphosphonates, which effect bony structures via pathways similar to those involved in the mechanism of IL-18, also exert an impact on the secretion of RANKL,

an osteoclast differentiation factor.[16] RANKL secreted from osteoblastic precursor cells binds to RANK receptors released from osteoclasts and osteoclast precursors.[17] The activity of RANKL is blocked by OPS, which prevents bone resorption and acts as an antagonist receptor of RANKL.[18] With this mechanism, bisphosphonates prevent osteoclastic bone resorption, as is done by IL-18. It has been demonstrated in experimental studies using mice that bisphosphonates decrease skeletal metastases and tumor burden of bone.[19,20]

Considering these results, it can be concluded that serum IL-18 levels will decrease in response to decreased tumor burden in the bone, and that bone metastases will regress in patients using bisphosphonates. This phenomenon, which narrows the gap between IL-8 levels in groups with and without bone metastases, may explain the decreased sensitivity of this test. The authors believe that further studies will yield more accurate outcomes and conclude the debate surrounding this issue.

Disclosure Statement

The authors declare no conflicts of interest.

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