

RESEARCH ARTICLE

Markers of Bone Metastases in Breast and Lung Cancers

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Abstract

Aim and Background: The aim of the present study was to evaluate correlations between serum osteocalcin, osteoprotegerin and NTX (Cross-linked N-telopeptides of Type I Collagen) and urinary NTX in breast and lung cancer patients with bone metastases. These four markers are considered to have important roles in bone formation, resorption and metastases. **Methods:** Four markers were determined in the sera of 60 breast cancer and 21 lung cancer patients and healthy controls (n=30). Serum levels were studied using ELISA and EIA. **Results:** The median levels of serum osteoprotegerin (p<0.001) and osteocalcin (p=0.003) were higher in patients. Significant correlations were observed between the serum NTX-osteocalcin (r=0.431; p<0.001), serum NTX-osteoprotegerin (r=0.42; p=0.003) and serum NTX - urine NTX (r=0.255; p=0.022). **Conclusion:** We conclude that osteocalcin, osteoprotegerin and NTX are independent diagnostic tools. Due to the ease of urine collection, urine NTX may be applied routinely to allow early detection of bone metastases and indicate progression of the disease.

Keywords: Bone metastases - cancer - tumor marker

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Introduction

Metastatic spread of cancer to bone is a prevalent extension of many malignancies. Over one-fourth of all cancer patients demonstrate some degree of metastatic disease to bone. Patients with breast and prostate cancer are particularly at risk for metastatic bone disease: >75% of these patients may ultimately have bone metastases of their primary malignancy (Roodman, 2012).

Oncologists have traditionally relied on bone scan and (or) bone survey to detect disease dissemination. These methods, although quite specific, are relatively insensitive in detecting early metastatic lesions to bone. The recent development of relatively sensitive and specific biochemical markers of bone turnover (Kamiya et al., 2012) has generated interest in the potential use of these markers for the early detection of bone metastases, and their use in assessing the efficacy and response to antiresorptive medications lended assistance for treating patients with metastatic bone disease (Johansen et al., 2007; Jung et al., 2011).

In healthy bone, the osteoblast-expressed proteins bind the receptor activator of NF-kappa B ligand (RANKL), and osteoprotegerin (OPG) regulates bone remodeling. RANKL (also known as OPGL, ODF, and TRANCE), a cell membrane-bound tumor necrosis factor superfamily member, was shown to bind its receptor, RANK, which is expressed on osteoclast precursors (Lacey et al., 1998; Yasuda et al., 1998) and stimulates osteoclast formation and activation. In vitro, OPG blocks osteoclastogenesis

in a dose-dependent manner. OPG binding to RANKL on osteoblast/stromal cells, blocks the RANKL-RANK ligand interaction between osteoblast/stromal cells and osteoclast precursors. This has the effect of inhibiting the differentiation of the osteoclast precursor into a mature osteoclast (Simonet et al., 1997).

Osteocalcin is a small protein, unique to bone and tooth dentine, synthesized in osteoblasts and, once secreted, binds strongly to hydroxyapatite. The small fraction of the newly-synthesized protein, which fails to bind to hydroxyapatite spills over into the circulation (Lee and Tung, 2011).

Human bone is continuously remodeled through a coupled process of bone resorption by osteoclasts followed by bone formation by osteoblasts. This process is necessary for normal development and bone growth as well as skeletal integrity. Measurement of specific degradation products of bone matrix allows analysis of the rate of bone remodeling. Approximately 90% of the bone organic matrix is made of type 1 collagen. This helical protein is stabilized by cross-links at its N- terminal and C-terminal ends and forms the basic fabric of bone tissue (Calvo et al., 1996).

The degradation products of type 1 collagen, NTX and CTX are released into the circulation and urine during bone resorption. Chung et al. demonstrated high urine levels and Tamiya et al. found high serum levels of NTX in lung cancer patients. (Chung et al., 2005; Tamiya et al., 2011).

We planned to determine the serum concentrations of

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the bone markers including osteocalcin, osteoprotegerin, serum and urine concentrations of NTX in breast and lung cancer patients. These markers were examined “statistically” to determine which marker or markers were the best predictors of the presence of bone metastases.

Materials and Methods

Totally 81 patients who had histologically confirmed breast (n=60; 60 women) and lung cancer (n=21; 16 men, 5 women) associated with scintigraphic evidence and radiographic confirmation (plain X ray and/or magnetic resonance) of bone metastases were included in the study consecutively admitted to the Istanbul University, Oncology Institute during one-year period, January 2011 to December 2011. Serum and urine samples were obtained on first admission before any type of treatment was given. Staging was performed on a pathological basis according to the American Joint Committee on Cancer (AJCC). The median age of patients was 55 (30-74) years for breast cancer patients and 58 (42-79) years for lung cancer patients.

Normal healthy subjects (n=30; 10 men, 20 women) with median age of 53 (28-69) years were included as the control group. Controls were blood donors undergoing regular physical and laboratory examinations. Our study on human materials has been approved by the relevant institutional committee (Local Ethics Committee, Number: 1393). Written informed consent was obtained from all patients included in the study. The protocol was consistent with the Declaration of Helsinki (2000).

Serum sample and spot urine specimen collection were obtained from each patient in the morning. Blood samples were obtained by venipuncture and clotted at room temperature. Sera and urine were collected following centrifugation and frozen immediately at -20°C until analysis. For all patients, routine chemical assays,

including blood and urine creatinine was determined on a routine automated clinical chemistry analyzer (Rayto, RT-1904C Chemistry Analyzer, Atlanta GA, USA).

Solid phase- enzyme linked immunosorbent assay (ELISA) was used to determine the serum values of osteocalcin as ng/mL (Quidel Corporation Headquarters, San Diego, CA, USA) and osteoprotegerin as U/L (BioVendor Lab. Med.Inc. Czech Republic). We determined the concentration of NTX in serum and urine by EIA (Osteomark; Ostex International, Seattle, WA) as nmol BCE/L (BCE: Bone Collagen Equivalents). The detection limit of the NTX assay was 25 nmol BCE/L and reference range for NTX was 0.8-1.2 mg/dl creatinine.

SPSS software (version 16; SPSS, Chicago, IL) was used for statistical analysis. The Mann-Whitney U test was used to evaluate differences between patients with bone metastases and normal controls. Spearman’s correlation test was used to correlate different serum parameters.

A two-tailed p value <0.05 was considered statistically significant. The report design was adopted from the standarts for reporting diagnostic accuracy (STARD) group [Bossuyt et al., 2004]. This article was reviewed by a biostatistician from I.U.Oncology Institute, Preventive Oncology, Biostatistics and Epidemiology Department.

Results

The mean and median values, standard deviations, and ranges of serum osteoprotegerin and osteocalcin in patients with bone metastases and control group are shown in Table 1. The median serum osteoprotegerin (p<0.001) and osteocalcin (p=0.003) levels were significantly different in patients compared with healthy controls (Table 1). The graphs for the significances between the groups are shown in figures (Figure 1-3).

The median values of NTX were 15.6 (1-47.5) nmol BCE/L in sera and 465 (29-2300) nmol BCE/L in

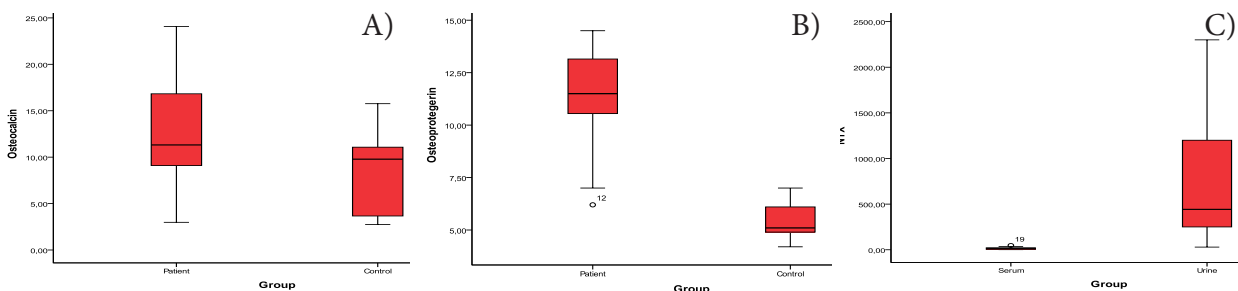


Figure 1. Median Serum in the Groups. A) Osteocalcin Values in the Groups, B) Osteoprotegerin Values and C) Median Serum and Urine NTX Values.

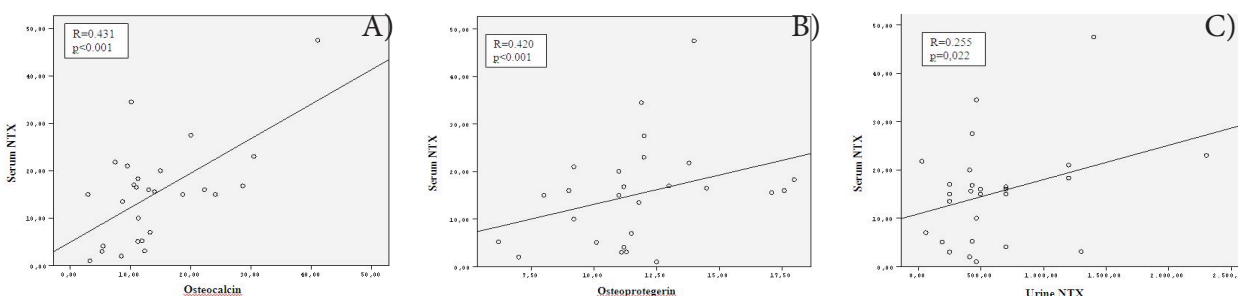


Figure 2. The Correlation. A) Serum NTX and Osteocalcin Levels, B) Serum NTX and Osteoprotegerin Levels C) Serum and Urine NTX

Table 1: The Levels of Tests According to the Groups (x±sd: mean±standard deviation; m: median)

	Osteoprotegerin (U/L) x±sd; m (min-max)	Osteocalcin (ng/mL) x±sd; m (min-max)	Serum Ntx (nmol BCE/L) x±sd; m (min-max)	Urine Ntx (nmol BCE/L) x±sd; m (min-max)
Patient (n=81)	11.71±2.84; 11.3 (6.2-18)	14.13±8.7; 11.32 (2.98-41.05)	16.5±9.6; 15.6 (1-47.5)	478±287; 465 (29-2300)
Control (n=30)	5.42±0.81; 5.1 (4.2-7)	9.08±4.44; 9.78 (2.74-15.76)		
p	<0.001	=0.003		

urine. The NTX excretion of sera and urine exhibited a significant difference between the measurements (15.6 vs 465 nmol BCE/L, respectively).

The cut-off levels (x±2sd) were for osteoprotegerin (6.1-17.4) U/L, for osteocalcin (0-31.5) ng/mL, for serum NTX (0-35.7) nmol BCE/L and for urine NTX (0-1052) nmol BCE/L.

We observed significant correlations between serum NTX-osteocalcin (r=0.431; p<0.001), serum NTX-osteoprotegerin (r=0.42; p=0.003) and serum NTX-urine NTX (r=0.255; p=0.022). These results are shown in Figures (Figure 4-6).

Discussion

Skeletal homeostasis is maintained by balanced processes of osteolysis and osteogenesis. Several factors across the cancer process (e.g., adjuvant therapies, bone metastases) can disturb this balance. Circulating levels of specific biochemical markers released during bone turnover may provide insight into the risk of bone metastasis. In addition, bone turnover marker levels and alterations might reflect tumor-bone interactions and response to the treatment in patients with bone metastases (Lipton et al., 2011). The use of these markers in oncology includes monitoring of anticancer treatment in patients with malignant disease metastatic to the bones (therapeutic monitoring), predicting the risk of bone relapse in patients with a first diagnosis of potentially curative, early-stage malignant tumors (prognostic use), and making an early diagnosis of malignant bone disease in patients with a known malignant tumor to start early bone-targeted treatment and avoid skeletal-related events (diagnostic use) (Joerger and Huober, 2012). Biochemical markers of bone turnover can be used to assess effectiveness of treatment in the patient with metabolic bone disease (Meier and Kraenzlin, 2012).

In 60 breast carcinoma patients, Kamby et al. evaluated the utility of serum osteocalcin levels to detect the presence of bone metastases. They found that the level of osteocalcin was high 50 % of patients (Kamby et al., 1993). Salem et al. evaluated statistically high osteocalcin levels in the study with 47 breast cancer patients and they found that osteocalcin levels in patients with metastatic lesions were higher than the levels in non-metastatic patients (Salem et al., 2007).

We determined high osteocalcin and osteoprotegerin levels all in patients as like as the literature. In our study, there were statistical significances between the patients and control group for osteocalcin (p=0.003) and osteoprotegerin (p<0.001). The NTX excretion of patients who underwent sera and urine collection for exhibited a significant difference between the measurement (15.6 vs 465 nmol BCE/L, respectively). Demers et al. suggested

that urine N-telopeptide measurements had the highest correlation and the most significant association with the probability of bone metastases [Demers et al., 1995]. Joerger M and Huober J have also claimed the same, they revealed that OPG shows low sensitivity for bone metastases in patients with breast or lung cancer, whereas urinary NTX has higher sensitivity for the diagnosis of bone metastases [Joerger and Huober, 2012].

Significant correlations were observed between serum NTX - osteocalcin (r=0.431; p<0.001), serum NTX - osteoprotegerin (r=0.42; p=0.003) and serum NTX - urine NTX (r=0.255; p=0.022) in this study. These "correlation data" demonstrate that different bone markers are reflecting different aspects of bone metabolism and diagnostic properties. Despite several studies on these markers in the literature, this is the first study investigating correlation of osteoprotegerin, osteocalcin, NTX in serum and NTX in urine together.

We concluded that osteocalcin, osteoprotegerin and NTX are independent diagnostic tools together and useful markers in monitoring patients with skeletal metastases. Due to the ease of urine collection, urine NTX may be applied in the routine to indicate early detection of bone metastases and progression of the disease.

Acknowledgements

This study has been approved by the Local Ethics Committee, Number: 1393.

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