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Bone turnover markers in women with early stage breast cancer who developed bone metastases. A prospective study with multivariate logistic regression analysis of accuracy☆

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ABSTRACT

Background: The skeleton is the most common site of metastasis for breast cancer and the periodic measurement of circulating bone turnover markers (BTMs) can be useful. The aim of this study was to prospectively evaluate the diagnostic accuracy of a panel of BTMs in the early detection of bone metastases (BMs).
Methods: We reviewed the medical records of 297 postmenopausal women with early stage luminal-type invasive ductal carcinoma (IDC). Twenty-six patients who developed isolated BMs during follow-up and 24 randomly selected controls were studied. The two groups were matched according to age, final disease staging, and follow-up. All patients underwent periodic measurement of total and bone-specific (BSAP) alkaline phosphatase, CTX, ICTP, osteocalcin, NTX, PINP, and TRACP5b.
Results: Only BSAP, CTX, PINP, and TRACP5b were significantly ($p < 0.05$) associated with the group, and the logistic regression analysis excluded CTX from the model. The AUC (ROC curve) for TRACP5b alone, which was the most accurate marker, and for the combination of BSAP + PINP + TRACP5b was 0.784 (95% CI: 0.651–0.916) and 0.889 (95% CI: 0.798–0.981), respectively.
Conclusion: According to our results, the measurement of these three markers together should be performed in all postmenopausal patients with luminal-type IDC, when an early diagnosis of BMs is required.

1. Introduction

The skeleton is the most common site of metastasis for breast cancer (BC) and can also be the only location of the metastatic disease, especially in patients with advanced or aggressive tumors, including those with triple-negative (TN) BC [1]. It has been shown that the cumulative incidence of bone metastases (BMs) among women with BC may reach 22% and that adverse skeletal-related events (SREs) occur in nearly one-half of such patients [2,3]. Younger age, high (>2) histologic grade (G) and disease stage (T > 2, ≥4 positive nodes) increase the risk of BMs occurrence, whereas the presence of multiple metastases and the TN phenotype significantly affect the mortality rate ratio regardless of initial staging and treatment [2,4,5]. Delayed diagnosis seriously affects the quality of life of patients because it is associated with several adverse SREs and disabilities. It also leads to a poor prognosis and the reduction of both disease-free survival and overall survival [4,5]. Even if the measurement of bone turnover markers (BTMs) is not routinely recommended in patients with cancer, it can be useful in suggesting the need to anticipate imaging studies, such as radionuclide bone scanning, ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET), or MRI, for localizing BMs [6–8].

The aim of this study was to prospectively evaluate the diagnostic accuracy of a panel of eight BTMs in the early detection of BMs in patients with BC.

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2. Materials and methods

2.1. Study population

With the aim of having homogeneous study groups, we reviewed the medical records of 297 postmenopausal women with early stage (T1-2, N0, M0), luminal-type (estrogen receptor-positive, HER2-negative), G1-2 invasive ductal carcinoma (IDC) treated with breast conserving surgery. All of the patients received adjuvant endocrine therapy and external radiation therapy, which was optionally associated with personalized chemotherapy, according to the postsurgical staging. Patients with histories of other malignancy, concomitant acute or chronic bone diseases requiring bisphosphonate therapy or calcium and vitamin D supplementation, renal or hepatic failure, as well as those with non-luminal type, node-positive (N+), non IDC and high grade (G3) BC, were excluded from the study. After giving their informed consent, all patients underwent periodic (every 4–5 months) measurement of the following BTMs: (i) bone formation markers, including total alkaline phosphatase (TAP), bone-specific alkaline phosphatase (BSAP), osteocalcin (OC), and amino-terminal propeptide of type I collagen (PINP), and (ii) bone resorption markers, including isoform 5b of the osteoclast enzyme tartrate-resistant acid phosphatase (TRACP5b), carboxy-terminal (CTX) and amino-terminal (NTX) cross-linked telopeptides of type 1 collagen, and ICTP (or CTX-MMP), another carboxy-terminal telopeptide cleaved from type I collagen by matrix metalloproteinase (MMP) during bone resorption [9]. CEA and CA 15-3 serum levels were also routinely measured.

Twenty-six patients (median age 64 years, range 50–74 years) developed isolated BM during follow-up (median 41 months, range 22–50 months) and were considered cases. The controls were 24 randomly selected patients in whom the presence of BMs was excluded using ^{18}F-FDG PET. The groups were matched according to age, final disease staging, and follow-up. A recent (≤20 days) BTMs assay was available in both cases and controls.

2.2. Serum markers assay

As reported in Table 1, the eight BTMs were measured using automated spectrophotometric methods (TAP, BSAP, TRACP5b), automated sandwich chemiluminescence immunoassay (OC, PINP, CTX), manual competitive inhibition enzyme-linked immunosorbent assay (NTX), and manual competitive radioimmunoassay (ICTP). Table 1 also reports the limits of detection (LoD) and quantitation (LoQ) of each BTM, and the relative cut-off limits referred to the previously obtained data from laboratory archival information [12,14,15].

2.3. Statistical analysis

The results of BTMs assay were dichotomized and reported in the database as above (positive) or below (negative) the optimal cut-off. Student's *t*-test was used to compare continuous variables as mean ± standard deviation (SD). The chi-square (χ^2) test or the Fisher exact probability test was used for inter-group analysis of dichotomized data. The parameters found to be significant in univariate analysis were assessed for the multivariate analysis using forward stepwise logistic regression model, to evaluate what variables were independent. The parametric statistical Wald test was also used. Odds ratio (OR) estimates and the relative 95% confidence intervals (CI) were calculated. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were also obtained. The receiver operating characteristic (ROC) curve and the area under the curve (AUC) as its summary index were used to evaluate the diagnostic performance of the prediction model. The level of significance was defined as p-value <0.05. The software Statistica (StatSoft, Tulsa, OK, USA, version 2012) was used for statistical calculation.

3. Results

Table 2 reports the values of each BTM (mean ± SD) in both groups. CEA and CA 15-3 serum levels at the time of BM onset did not

Table 1
Assay methods for the detection of the bone turnover markers considered in the study, their analytical performances express as limits of detection (LoD) and quantitation (LoQ), and each relative cut-off considered in the study.

Marker (unit of measure)	Method	Assay technique	Equipment	Manufacturer	LoD	LoQ	Cut-off
TAP (U/L)	Automated spectrophotometric assay [10]	Bowers and McComb procedure using of para-nitro-phenyl-phosphate	Dimension Vista 1500	Siemens Healthcare, Newark, NJ, USA	5	10	150
BSAP (μg/L)	Automated spectrophotometric immunoassay [11]	Magnetic microparticles labeled with streptavidin, a biotin labeled BSAP-specific monoclonal antibody and an enzyme substrate	iSYS	Immunodiagnostic Systems, Bolton, UK	0.4	1.0	25
Osteocalcin (μg/L)	Automated CLIA [12]	Anti-OC monoclonal antibody labeled with 4-aminobutyl-N-etyl-isoluminol and magnetic microbeads coated with anti-fluorescein isothiocyanate	Maglumi 2000 Plus	SNIBE, Shenzen, China	0.13	0.25	45
PINP (μg/L)	Automated CLIA	Anti-PINP biotinilated monoclonal antibody labeled with acridinium ester derivative and magnetic particles coated with streptavidin	iSYS	Immunodiagnostic Systems, Bolton, UK	1	1.5	65
TRACP5b (U/L)	Automated spectrophotometric immunoassay	Magnetic microparticles labeled with streptavidin, a biotin labeled anti-TRACP5b monoclonal antibody and an enzyme substrate	iSYS	Immunodiagnostic Systems, Bolton, UK	0.6	0.9	5
CTX (μg/L)	Automated CLIA	Two highly specific monoclonal antibodies directed against a specific amino-acid sequence of CTX, streptavidin coated magnetic particles and acridinium ester derivative as a tracer	iSYS	Immunodiagnostic Systems, Bolton, UK	0.023	0.033	0.83
NTX (nmol BCE/L)	Manual ELISA [13]	A NTX epitope adsorbed onto microplate wells and a horseradish labeled monoclonal antibody	Alisei	Alere, Scarborough, ME, USA	3.2	4.0	30
ICTP (μg/L)	Manual RIA	Competitive RIA method using ^{125}I-labelled ICTP as tracer molecule and a polyclonal rabbit antiserum as detection reagent	Wallac Wizard	Orion Diagnostika, Espoo, Finland	0.6	1.0	6

TAP = total alkaline phosphatase, BSAP = bone-specific alkaline phosphatase, PINP = amino-terminal propeptide of type I collagen, TRACP5b = tartrate-resistant acid phosphatase isoform 5b, CTX = carboxy-terminal cross-linked telopeptide of type 1 collagen, NTX = amino-terminal cross-linked telopeptide of type 1 collagen, BCE = bone collagen equivalent, ICTP (CTX-MMP) = carboxy-terminal telopeptide cleaved from type I collagen by matrix metalloproteinase (MMP), CLIA = chemiluminescence immunoassay, ELISA = enzyme-linked immunosorbent assay, RIA = radioimmunoassay.