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Research Paper

Diagnostic and prognostic validity of serum bone turnover markers in bone metastatic non-small cell lung cancer patients

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1. Background

The incidence of bone metastases (BM) in advanced non-small-cell lung cancer (NSCLC) patients is estimated to range from 30% to 40% [1,2]. The presence of BM often results in pathologic remodeling of the affected bone compartment, making affected bones vulnerable to skeletal related events (SREs). SREs include pathologic fractures, spinal cord compression, requirement for radiation, surgery to bone and hypercalcemia, all reducing quality of life and worsening prognosis [3]. BM is a poor prognostic survival factor [4]. Therefore, early diagnosis and adequate treatment of BM is critically important issues of the clinical management of NSCLC patients.

To detect BM in NSCLC patients, bone scintigraphy combined with plain radiographs, computerized tomography (CT) and magnetic resonance imaging (MRI) is recommended. But routine radiography only gives definite diagnosis when the bone is already substantially damaged by the tumor. Although scintigraphy is more sensitive, its specificity is not satisfactory due to pseudo-positive values caused by inflammation and traumatic fracture. Any abnormal scintigraphic findings should always be verified by radiographic ones [5]. Bone scintigraphy is also a more expensive, invasive, time-consuming, and exposes cancer patients to irradiation, limiting its use for monitoring purposes.

Since BM impairs the balance between bone formation and bone resorption, altered bone remodeling activities can be assessed directly by measuring the components of affected bone cells or indirectly by analyzing metabolic products released from the bone matrix by changed rates of bone formation or resorption. Numerous new analytical tools for bone turnover markers (BTMs) have improved the diagnosis of BM. These BTMs have been recommended as helpful tools for assessing BM [6]. Collectively, there seem to be a diversity of findings depending on cancer type and the type of BTMs used. Previous researches had explored the applications of BTMs in NSCLC patients [7–19], nevertheless, as to the optimal markers and their proper application in BM screening, there hasn't been a consistent agreement, which greatly hampered the BTMs usage in clinical practice.

Therefore, we (1) measured serum markers of bone formation and bone resorption as noninvasive analytes of bone turnover in NSCLC patients with or without BM, (2) assessed the diagnostic accuracy of these BTMs as potential indicators of BM in NSCLC patients, combined diagnostic effectiveness of BTMs and (3) evaluated with univariate and multivariate analysis of the usefulness of BTMs to make a prognosis in NSCLC patients with BM. We selected bone-specific alkaline phosphatase (BALP), N-terminal midfragment of osteocalcin (N-MID) and aminoterminal propeptide of type I collagen (PINP) as bone formation markers, β-cross-linked carboxyterminal telopeptide of type I collagen (β-CTx) as bone resorption markers.

2. Materials and methods

2.1. Patients and samples

2.1.1. Patients

Our retrospective study included 414 newly diagnosed NSCLC patients that were investigated and treated in the department of internal oncology of the Sixth People's Hospital, Shanghai Jiao Tong University between January 2010 and December 2013. All

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participants signed approved written consents; the study was done in accordance with the Helsinki Declaration II and Standards of Good Clinical Practice. The Local Ethical Committee has approved the study protocol.

The study consisted of three groups: Group A included 193 NSCLC patients without BM at diagnosis, Group B included 221 NSCLC patients with BM at diagnosis, and Group control included 179 healthy volunteers. The diagnosis of all NSCLC patients was confirmed by histological or cytological examination of specimens taken from bronchoscopy or by CT-guided fine needle biopsy. Cancer stage was assigned according to the TNM system. All patients underwent bone scanning using a radionuclide (Technetium-99m) scintigraphy together with plain radiographs, CT and/or MRI to verify and quantify the presence of BM. In special cases, affected bone lesions by CT-guided fine needle biopsy were used to diagnose BM.

2.1.2. Patients' evaluation

Baseline evaluation included clinical assessment, bone survey, evaluation for extraskeletal disease and serum BTMs determination.

Clinical evaluation included assessment of performance status according to the World Health Organization (WHO) criteria. SREs at diagnosis were recorded, patients were followed up for survival every 3 months and SREs in follow-up were also recorded.

Bone survey included bone scintigraphy and plain radiological, as well as CT or MRI when necessary. Patients were initially classified according to the type and bulk of BM, based on the findings of the bone survey. BM type was characterised as lytic, blastic or mixed. The bulk of BM concerned the number of sites involved and was graded as previously proposed by Soloway [20]. Briefly, Soloway 0 refers to patients without BM; Soloway 1 refers to patients with <6 BM; Soloway 2 refers to patients with <20 BM; Soloway 3 refers to patients with >20 but less than a "super scan"; Soloway 4 refers to patients with "super scan" that is defined by a $>75\%$ involvement of the ribs, vertebrae, and pelvic bones.

2.1.3. Samples

Blood samples were collected in plastic tubes between 07:30 and 09:00 a.m., stored in ice and centrifuged at 2000*g* for 15 min, at 4 °C, within 2 h from venipuncture. Blood samples were collected before the administration of any anticancer treatment after initial diagnosis.

Bone Formation Markers. BALP was determined by the Tandem-MP Ostase Immunoenzymetric Assay (Beckman Colter, Fullerton, CA), which specifically quantifies BALP with low immunoreactivity for the liver/kidney isoforms [21]. N-MID (N-MID-Osteocalcin Assay, Roche) and PINP (Total PINP-Assay, Roche) were measured on the Elecsys 2010 analyzer (Roche). The PINP assay is a new electrochemiluminescent assay that detects both tri- and monomeric PINP forms.

Bone Resorption Markers. β-CTX was determined by the β-CrossLaps Assay (Roche) on the Elecsys 2010 analyzer [22].

2.2. Statistical analysis

Statistical calculations were performed with SPSS® 13 for Windows™ and GraphPad® Prism® 4.03. All results are expressed as mean $\pm$ SD. We used the nonparametric Kruskal–Wallis ANOVA with Dunn's post test, the Mann–Whitney *U* test, Spearman's rank correlation coefficients and the Kolmogorov–Smirnov distribution fitting procedure. Diagnostic accuracy was evaluated by Receiver-operating characteristic (ROC) curve analysis. For the combined diagnostic effectiveness of BTMs, the probability was fitted by logistic regression model and then analysed by ROC curves. The Kaplan–Meier product limit method was used to determine survival probability in subgroups. Univariate and multivariate analysis of risk factors predicting NSCLC specific death was performed using the Cox proportional hazards regression model. Differences and associations were considered statistically significant if $p<0.05$.

3. Results

3.1. Demographics characteristics

221 patients suffered from clinically manifest BM and 33.5% had more than seven BM lesions. In group A, 1 or more metastases in the lung, liver, and other sites (excluding brain) were present in 49.1% patients, while in group B the number is 44.6%. The majority of the patients in both groups received chemotherapy during the study (55.4% in the group A and 80.5% in the group B), and 22.8% in the group A and 29.0% in the group B received target therapy. There were no age or sex difference among the subgroups. For further clinicopathologic data see Table 1 and 2.

3.2. Serum BTMs in the study groups

Fig. 1 shows the scatter plots and medians of all BTMs among the subgroups. Since all markers showed a Gaussian distribution using the Kolmogorov–Smirnov test, we calculated the parametric upper 95% reference limits. Briefly, ANOVA analysis showed bone formation BALP, PINP, N-MID and bone resorption β-CTx values were higher in BM patients than in the control group ($p<0.05$) and in patients without BM ($p<0.05$), but no difference was found between the control group and the group without BM ($p>0.05$). For further data see Fig. 1.

3.3. BTMs as diagnostic Indicators of BM

3.3.1. ROC analyses between NSCLC patients with or without BM

ROC analyses were performed to characterize the diagnostic usefulness of the BTMs, which is to differentiate NSCLC patients with or without BM (Fig. 2 and Table 3). Both bone formation and resorption markers were helpful in this respect. ROC curves were drawn according to the markers based on true-postive ratio

Table 1
Demographic and Clinicopathologic Characteristics of the Study Groups.

Characteristics	Control Group	Group A	Group B
Sex			
Female	84	89	95
Male	95	104	126
Age	57 ± 11	55 ± 9	59 ± 10
Tumor stage:			
T1	–	23	–
T2	–	37	–
T3	–	47	–
T4	–	86	221
Pathologic type			
Adenocarcinoma	–	91	120
Squamous cell carcinoma	–	28	29
Adenosquamous carcinoma	–	10	8
Poor differentiated carcinoma	–	50	53
Alveolar cell carcinoma	–	6	5
Large cell carcinoma	–	8	6
Therapy			
Operation	–	88	2
Chemotherapy	–	107	178
Target therapy	–	44	64
General condition			
ECOG:0-1	179	159	182
ECOG:2	0	34	39