Carboxy-terminal Telopeptide (CTX) and Amino-terminal Propeptide (PINP) of Type I Collagen as Markers of Bone Metastases in Patients with Non-small Cell Lung Cancer*

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Abstract. *The early diagnosis of non-small cell lung carcinoma (NSCLC) is difficult, and 30-40% of patients with NSCLC develop bone metastases (BMs) during the course of their disease. Because the delayed demonstration of skeletal involvement may seriously affect survival, there is a need for early diagnosis of BMs. Unfortunately, the sensitivity of common serum tumor markers is low and they are used mainly for monitoring the efficacy of therapy and detection of recurrence. The aim of this study was to evaluate the utility of a panel of serum biomarkers in patients with NSCLC and BMs. Sixteen patients (11 males, 5 females; median age=64 years, range 54-68 years) with NSCLC and BMs (cases), and 18 age- and stage-matched patients without BMs (controls) underwent measurement of serum carboxy-terminal telopeptide of type I collagen (CTX), tartrate-resistant acid phosphatase isoform type 5b (TRAP5b) and amino-terminal propeptide of type I collagen (PINP), carcinoembryonic antigen (CEA) and fragments of cytokeratin 19 (CYFRA 21-1. CTX (443.7±945.1 vs. 402.7±28.4 pg/ml, p=0.003) and PINP (75.9±11.4 vs. 64.1±7.5 μg/l, p=0.001) were significantly higher in patients with BMs, while the mean value of the other markers did not differ (p=NS) between cases and controls. The sensitivity, specificity and accuracy were 73.3%, 86.7% and 79.4% for CTX; 55.5%, 62.5% and 58.8% for CEA; 65.0%, 78.6% and 70.6% for CYFRA; 30.4%, 76.2% and 67.6% for TRAP5b; and 72.2%, 81.2% and 76.5% for PINP, respectively. The area under the receiver operating characteristic curve (AUC) for CTX was 0.68. In conclusion, CTX and PINP measurement can be useful in monitoring patients with NSCLC during follow-up, with the aim of detecting BMs early.*

Non-small cell lung carcinoma (NSCLC) is the most common type of lung cancer and represents the major cause of cancer death worldwide (1). Overall, the 5-year survival rate of patients with NSCLC is poor, and barely exceeds 10% (2). The early diagnosis of NSCLC is difficult. The sensitivity of common serum tumor markers, such as carcinoembryonic antigen (CEA) and fragments of cytokeratin 19 (CYFRA 21-1), is low and they are used mainly for monitoring the efficacy of therapy and detection of recurrence (3).

Unfortunately, 30-40% of patients with NSCLC develop bone metastases (BMs) during the course of their disease. Pain is the most common symptom, but up to 20-25% of patients are asymptomatic (4). Both symptomatic and asymptomatic patients are potential candidates for systemic chemotherapy, together with an additional treatment strategy using bisphosphonates (BP) or denosumab, the first fully human monoclonal antibody to receptor activator of nuclear factor-κB (RANK) ligand (5-7). Because the delayed demonstration of skeletal involvement may seriously affect survival, there is a need for early diagnosis of BMs, which are usually osteolytic and distributed mainly in the spine, pelvis and ribs (4). Several bone biomarkers have been proposed, such as carboxy-terminal telopeptide of type I collagen (CTX) and tartrate-resistant acid phosphatase isoform type 5b (TRAP5b), which are markers of bone resorption generated by different mechanisms, and amino-terminal propeptide of type I collagen (PINP).

The aim of this study was to evaluate the utility of a panel of serum biomarkers in patients with NSCLC and BMs.

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Table I. *Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of serum markers in patients with non-small cell lung carcinoma and bone metastases.*

Marker	TP	FP	TN	FN	Sensitivity	Specificity	PPV	NPV	Accuracy
CTX	14	2	13	5	73.7	86.7	87.5	72.2	79.4
CEA	10	6	10	8	55.5	62.5	62.5	55.5	58.8
CYFRA 21-1	13	3	11	7	65.0	78.6	81.2	61.1	70.6
TRAP5b	7	9	16	2	30.4	76.2	43.7	88.9	67.6
PINP	13	3	13	5	72.2	81.2	81.2	72.2	76.5

CTX, Carboxy-terminal telopeptide of type I collagen; CEA, carcinoembryonic antigen; CYFRA 21-1, cytokeratin fragment 21-1; TRAP5b, tartrate-resistant acid phosphatase isoform type 5b; PINP, amino-terminal propeptide of type I collagen; TP, true-positive; FP, false-positive; TN, true-negative; FN, false-negative. PPV, Positive predictive value; NPV, negative predictive value.

Patients and Methods

Sixteen patients (11 males, five females; median age=64 years, range 54-68 years) with NSCLC and BMs (cases), and 18 age- and stage-matched patients without BMs (controls) underwent measurement of serum CTX, TRAP5b, PINP, CEA, and CYFRA 2. CTX was measured by an automated immunometric assay, TRAP5b and CEA by two-site quantitative enzyme-linked immunosorbent sandwich assay (ELISA), PINP by radioimmunoassay (RIA), and CYFRA 21-1 by immunochemiluminescent assay. BMs were revealed by positron-emission tomography (PET) using the glucose analog tracer ^{18}F-2-deoxy-fluoro-D-glucose (FDG) scanning or ^{99m}Tc-methyldiphosphonate (MDP) bone scintigraphy, and confirmed by bone X-ray, fine-needle aspiration biopsy or core biopsy. The obtained cut-off values (at 95% specificity) were 400 pg/ml, 5 U/l, 4.9 ng/ml, 65 μg/l, and 2.7 ng/ml for CTX, TRAP5b, CEA, PINP and CYFRA 21-1, respectively.

The data are expressed as means±standard deviation (SD). Sensitivity was defined as true-positives (TP)/TP+false-negatives (FN); specificity as true-negatives (TN)/TN+false-positives (FP); positive predictive value (PPV) as TP/(TP+FP); negative predictive value (NPV) as TN/(TN+FN); and accuracy as (TN+TP)/total patients. Odds ratio (OR) and the associated 95% confidence intervals (CI) were estimated for patients with high *versus* low levels of each marker (8, 9). The coefficient of variation of test samples at different dilutions was used to determine the interassay precision, as previously reported (9).

The receiver operating characteristic (ROC) curve to test sensitivity *versus* FP rate (1– specificity) for the more reliable marker was drawn and the area under the curve (AUC) was obtained. Student's *t*-test and Fisher's exact probability test were used to compare results. The significance level was set at $p<0.01$.

Results

CTX (443.7±945.1 *vs*. 402.7±28.4 pg/ml, $p=0.003$) and PINP (75.9±11.4 *vs*. 64.1±7.5 μg/l, $p=0.001$) were significantly higher in patients with BMs, while the mean value of the other markers did not differ (p=NS) between cases and controls.

The sensitivity, specificity, PPV, NPV and accuracy of CTX, TRAP5b, PINP, CEA, and CYFRA 21-1 are reported in Table I. The relative ORs for accuracy were 18.20 (95% CI 2.99-110.7, $p<0.0001$), 11.26 (95% CI 2.21-57.20, $p=0.002$), 6.81 (95% CI 1.41-32.8, $p=0.012$), 6.22 (95% CI 1.06-36.5, $p=0.038$) and 2.08 (95% CI 0.53-8.23, $p=0.29$) for CTX, PINP, CYFRA 21-1, TRAPb5 and CEA, respectively. The AUC for CTX was 0.68, and Figure 1 shows the relative ROC curve.

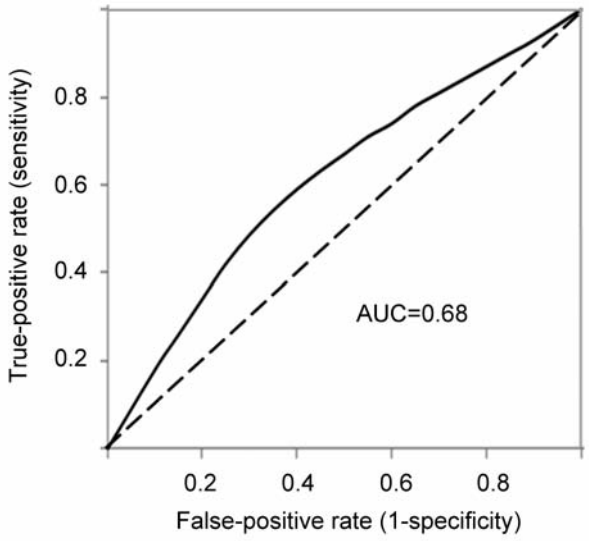

Figure 1. *Receiver operator characteristic (ROC) curve and area under the curve (AUC) for analysis of carboxy-terminal telopeptide of type I collagen (CTX) in detecting bone metastases from non-small cell lung carcinoma.*

Discussion

It has been observed that elevated levels of serum CEA and CYFRA 21-1were associated with a worse outcome in patients with NSCLC (10, 11). CEA is one of the most widely used tumor markers, especially for patients with colorectal cancer, and in the detection of liver metastasis,

while CYFRA 21-1 was originally a marker of advanced urothelial carcinoma of the bladder (12, 13). They are the most important markers in NSCLC and both have been shown to be of prognostic value (14). NSCLC accounts for 85-90% of cases of lung cancer and bone is one of the most common distant sites of metastasis in NSCLC and BMs may lead to several skeletal-related events (15, 16).

Diagnosis of BMs in patients with NSCLC usually relies on symptoms (*i.e.* pain) or image studies, such as ^{18}F-FDG PET/computed tomography (CT) and ^{99m}Tc-MDP scintigraphy (17, 18). However, bone turnover markers, such as serum CTX, PINP and TRAP5b, have also been shown to be useful during follow-up and BP therapy (19, 20). More than 90% of organic bone matrix consists of type I collagen and PINP is a bone formation marker and one of the two pro-peptides of type I procollagen, reflecting the rate of synthesis of type I collagen (21). CTX is a marker of osteoclast activity used to assess the level of bone resorption and indicates bone metabolic activity in several diseases, including rheumatoid arthritis and osteoarthritis (22, 23). TRAP5b is another marker of bone resorption that is secreted into the circulation exclusively by osteoclasts and rapidly inactivated by loss of iron (24). It is elevated both in patients with alterations of bone mineral density (BMD) and osteoporosis, and in those with BMs, especially from breast cancer (25, 26).

Several other markers of bone turnover may act as indicators of BMs in NSCLC, including bone-specific alkaline phosphatase (BAP), osteocalcin, pyridinoline cross-linked carboxy-terminal telopeptides of type I collagen (ICTP) and *N*-terminal cross-linked telopeptide of type I collagen (NTx). It has been found that NTx may increase in patients with extensive bone metastatic burden, while TRAP5b increases in patients with a small bone metastatic burden (27).

Our results suggest that serum CTX and PINP are the most useful markers of BMs, having an accuracy ranging from 76.5% to 79.4%, with a specificity of more than 80%. In an another study, ICTP had better sensitivity and accuracy than CTX, and it has also been observed that serum NTx levels are significantly related to the presence of BMs in patients with NSCLC (28, 29). In a previous study we found that both NTx and BAP are specific markers of bone remodelling, but their usefulness is limited in early diagnosis of metastatic disease (30).

Conclusion

Biochemical markers of bone turnover are useful in monitoring treatment response in patients with cancer and in diagnosing metastatic bone disease, and can be used to determine the time-to-tumor progression (31, 32). Unfortunately, their sensitivity and specificity ranges widely, according to the stage and type of cancer, as well as pathological characteristics and number of BMs.

Our preliminary results suggest that serum CTX and PINP measurement could be useful in monitoring patients with NSCLC during follow-up, with the aim of detecting BMs early.

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