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ICTP in Bone Metastases of Lung Cancer

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A B S T R A C T

Bone metastases often appear in advanced stages of lung cancer. They are the result of modulation of bone metabolism by tumor cells that migrated into bone microenvironment and degraded bone organic matrix. Measurement of C-terminal telopeptide of type I collagen (ICTP) in the serum of subjects with lung cancer with and without bone metastases and healthy population is the way to explore bone resorption. In 343 subjects included in this research ICTP level was significantly higher in the bone metastasis than other two groups. The existence of pathologic fracture significantly increased ICTP level. ICTP showed sensitivity of 66.0% in bone metastases at 95.0% specificity in lung cancer stages IA and IB. ICTP is a good diagnostic marker in detection of bone metastasis of lung cancer. Its level can distinguish lung cancer with and without bone involvement and can be used as an addition to standard techniques used in diagnostics of bone metastasis.

Key words: *bone matrix, lung neoplasms, osteoclasts, osteolysis*

Introduction

Lung cancer frequently develops bone metastases in advanced stages of disease. At initial diagnosis 16% of non-small cell lung cancer (NSCLC) and 38% of small cell lung cancer (SCLC) patients present with bone metastasis[1]. At the time of death, the majority of patients have bone metastases[2].

Lung cancer metastases to bone are predominantly osteolytic. Osteolysis results from the increased number and activity of osteoclasts in bone remodeling process.

Once caught in the bone marrow vasculature, tumor cells adhere to endothelial cells, and extravasate to bone microenvironment[3]. The attachment to bone matrix is facilitated by bone sialoprotein (BSP), often overexpressed in lung cancer cells[4–7]. It is assumed that tumor cells bind to BSP through the integrin $\alpha_v\beta_3$ expressed on their surface whilst glutamate-rich regions of BSP attach to crystals of hydroxyapatite[8].

In the bone microenvironment tumor cells secrete parathyroid hormone-related peptide (PTHrP) which shares 70% homology with parathyroid hormone (PTH) and has similar biologic function binding to PTH/PTHrP receptors[9–12]. PTHrP stimulates the production of receptor activator of nuclear factor-κB ligand (RANKL) on the surface of stromal cells and osteoblasts. RANKL then binds to receptor activator of nuclear factor-κB (RANK) on osteoclast progenitors. Activation of transcription factors AP1 and NF-κB in osteoclast progenitors leads to their differentiation[13].

Simultaneously, PTHrP binds to PTH/PTHrP receptors in kidneys and stimulates the tubular resorption of calcium what causes hypercalcaemia which is one of the typical traits of bone metastasis[14].

Degradation of bone matrix which is rich with growth factors causes their release into the bone microenvironment. The release of TGF-β subsequently induces the signal transduction of Smad proteins[15–17]. This process stimulates the proliferation of tumor cells and their production of PTHrP which closes the »vicious circle« of the osteolytic bone metastasis.

In optimal pH from 4,7 to 6,8 cathepsin K from osteoclasts can cleave collagen fibers on several sites on triple helix, but primarily on telopeptide region generating fragments from 70 to 80 kDa[18–20]. Its concentration in osteoclasts is the highest relative to cathepsins S, L and B[21].

Osteoclasts strongly express matrix-metalloproteinase 9 (MMP-9) which is beside MMP-2 an enzyme that has

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an ability to cleave denaturated collagen type I between residues 775 and 776 thus generating N and C-terminal fragments in optimally neutral pH[19,22–24]. The positive correlation between serum levels of MMP-9 and ICTP has been proven by Ylisirniö et al.[25].

Normally, the pH value in subosteoclastic resorption zone ranges from 3.5 and 4.5, but it is hypothesized that the buffer activity of dissolved salts from the bone (Ca^{2+} and PO_4^{3-}) shifts the pH value towards the neutral[18,20].

The C-terminal telopeptide of type I collagen (ICTP) is found only in C-terminal part of the mature collagen type I in which the crosslinks between fibrils have been formed. Since the 90% of bone organic matrix is composed of collagen type I that makes ICTP good potential marker of bone resorption process.

Increased ICTP levels can be found in bone metastases of breast, prostate, lung cancer and other malignancies[26,27]. ICTP levels can discriminate lung cancer with and without bone metastasis[26]. Also, ICTP level reflects the extent of skeletal involvement and the associated bone pain[26,27]. It has been proven to be a good prognostic marker in lung cancer elevated level implying higher risk of death than those below cut-off value[25].

The aim of this study is to investigate the level of ICTP in lung cancer with proven bone metastases, lung cancer in stage IA or IB prior to surgery and phenotypic healthy individuals, and to test the differences in ICTP level between studied groups.

The clinical application of ICTP depends on population characteristics, patient medical history, sample size

TABLE 1
CHARACTERISTICS OF THE STUDY POPULATION

		Patients with bone metastasis	Patients in stage IA/IB	Phenotypic healthy individuals
Number		144	99	100
Gender	Male	101	74	31
	Female	43	25	69
Age		64.0±10.2	62.8±9.9	46.6±12.4
Histological type of lung cancer	Adenocarcinoma	60	27	–
	Squamous cell carcinoma	38	46	–
	Small cell lung cancer	12	2	–
	Adenosquamous or other mixed types of non-small cell lung cancer	10	3	
	Large cell carcinoma	3	7	–
	Carcinoids	1	11	
	Other (other mixed types, non-differentiated)	20	3	–
Stage	IA	–	16	–
	IB		83	
Pathologic fracture	Present	19	–	–
	Not present	125	–	–
Months after initial diagnosis of lung cancer		0–84.0±13.0	–	–
Number of affected metastatic sites	1	104	–	–
	2	17	–	–
	3	12	–	–
	4	7	–	–
	5 and more	4	–	–
Location of bone metastasis	Rib	69	–	–
	Vertebra	64	–	–
	Pelvis	20	–	–
	Femur	18	–	–
	Scapula	14	–	–
	Humerus	10	–	–
	Calvarial bones	8	–	–
	Sternum	5	–	–
	Clavicle	4	–	–
	Other	15		

included in research, sampling time in relation to other diagnostic invasive procedures, and clinically defined control groups that are expected to have a different dynamics of ICTP level than the healthy population.

Subject selection

Samples from 144 lung cancer patients with bone metastasis were obtained at the time of the first evidence of osteolytic lesions proven by scinthigraphy and x-ray scans, before treatment of bone metastases by irradiation and biphosphonates.

Second group is consisted of 99 lung cancer patients who underwent surgical treatment (lobectomy or pneumonectomy). Samples were collected at the time of diagnosis prior to surgery, and patient selection has been made according to the pathologic diagnosis made after surgical treatment, so only the patients in stage IA or IB were selected in order to be positive there was no disease spreading outside the thoracic wall.

Third group is composed of 100 healthy individuals who had no visible symptoms of any disease nor had knowledge about any disease or disorder.

Marker assay

Blood samples were collected by standard venipuncture into sterile tubes containing clot activator. After centrifugation sera were stored at –20°C until analysis.

ICTP measurement was done using commercially available immunoassay Uniq ICTP EIA (Orion Diagnostica, Finland), according to the manufacturer's instructions. ICTP concentration is given in μg/L.

Statistical analysis

Differences between groups were tested by Mann-Whitney U-test or Kruskal-Wallis ANOVA on Statistica 7.1 software package (Statsoft, USA). ROC curves were calculated using Analyse-it 1.73 (Analyse-it Software, UK).

P-value below 0.05 was considered significant.

Results

The characteristics of 343 subjects enrolled in the study are presented in Table 1.

Highest median ICTP levels are seen in lung cancer patients with bone metastasis (Table 2).

Comparison between groups revealed significant differences between the bone metastasis population and other two groups (Figure 1). Also, significant differences

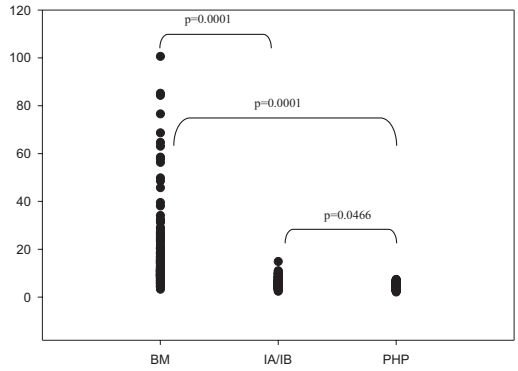

Fig. 1. Comparison of ICTP levels between groups. BM=patients with bone metastasis, IA/IB=patients in stage IA or IB, PHP= phenotypic healthy population.

have been found between the population of patients in stage IA and IB and phenotypic healthy individuals, but not to the extent as in comparison with bone metastasis patients.

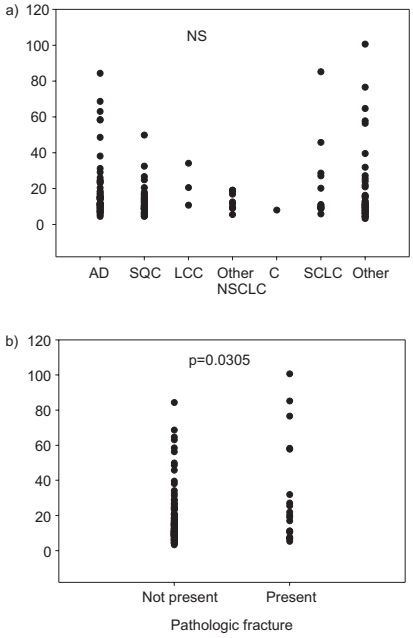

Fig. 2. a) Comparison in ICTP level among various histological types of lung cancer with bone metastasis; b) Differences in ICTP level between patients without and with pathologic fracture. NS=non-significant, AD=adenocarcinoma, SQC=squamous cell carcinoma, LCC=large cell carcinoma, Other NSCLC= adenosquamous and other mixed types of non-small cell lung cancer, C=carcinoid, SCLC=small cell lung cancer.

TABLE 2
SERUM LEVEL OF ICTP IN THREE GROUPS OF STUDIED POPULATION

	Patients with bone metastasis	Patients in stage IA/IB	Healthy individuals
Median ICTP (μg/L)	12.0	5.8	4.3
Inter-quartile range	9.1–20.5	4.7–7.2	3.2–5.5

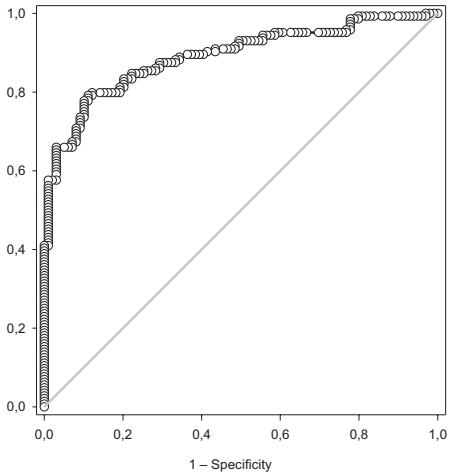

Fig. 3. ROC curve for discrimination of bone metastasis from stage IA/IB lung cancer. AUC=0.891, 95% CI of AUC=0.851 to 0.931, p<0.0001; ◊=ICTP.

The comparison of ICTP levels according to the histological type of lung cancer in bone metastasis group did not reveal any significant correlation (Figure 2a). On the contrary, Mann-Whitney U-test showed significant differences in ICTP level between subjects with and without pathologic fracture as a result of bone metastasis (Figure 2b). Obtained median ICTP value in patients without the fracture is 11.7 μg/L and with pathologic fracture 20.1 μg/L.

Furthermore, ROC curve is constructed using two datasets; truly positive=patients with proven bone metastasis, and truly negative=lung cancer stage IA or IB (Figure 3).

The cut-off value of 9.9 μg/L is selected from the ROC curve which corresponds to 66.0% of sensitivity and 95.0% of specificity. That gives positive predictive value of 95.0% and negative predictive value of 65.7%.

Discussion

The results obtained in this research show significantly higher ICTP values in lung cancer with bone metastasis than in lung cancer in stages IA and IB and phenotypic healthy population. This confirms the mechanism of osteolyis in lung cancer bone metastasis resulting predominantly from the increased osteoclast maturation and activation and subsequent bone organic matrix degradation[13,25]. The median ICTP value in patients in stage IA and IB is closer to the one measured in healthy population suggesting the absence of increased bone resorption in the early stages of lung cancer.

All three groups compared together showed significant differences between lung cancer with bone metastases and other two groups. Such differences have been previously found in breast, lung and prostate cancer with and without bone metastasis[26,28–30]. The ability of ICTP to discriminate between lung cancer with and without bone metastasis suggests its use as a potential marker of bone collagen degradation in the advanced stages of lung cancer where there is suspicion about bone involvement based on symptoms and available imaging techniques.

The difference in ICTP levels between lung cancer of stage IA and IB and phenotypic healthy population can be explained by the destruction of extracellular matrix surrounding primary lung tumor. Although not as high as the ones obtained by the comparison with the bone metastasis group these differences suggest a certain level of degradation of collagen fibers in the epithelial tissue and stroma built from the helical units of collagens I and III[31]. These results have been previously described in lung cancer[25].

The histological type of lung cancer with bone metastasis did not significantly influence ICTP levels as the mechanism of osteoclast activation being common to all predominantly osteolytic bone metastasis.

The existence of pathologic fracture influenced significantly the ICTP level being higher than in bone metastasis without fracture. These results confirm that the increase in serum level of ICTP is mainly a consequence of tumor involvement of the bone organic matrix that is more advanced in patients who have pathologic fractures.

ROC curve revealed very good AUC and ratio of sensitivity and specificity. The high sensitivity in bone metastasis makes ICTP good diagnostic marker as an additional tool to standard diagnostic techniques. ICTP can be primarily used in monitoring of lung cancer patients with the suspicion, increased risk or already present bone metastasis because its level reflects bone resorption resulting from modulation of bone metabolism by tumor cells.

Conclusion

This research showed that ICTP is good diagnostic tool that can detect bone metastasis of lung cancer with high sensitivity. Also, it can be used to distinguish lung cancer with and without bone metastasis. These results suggest its application as a complement to standard diagnostic techniques in diagnosis and monitoring of the advanced lung cancer with high risk of bone involvement or already present bone metastasis.

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ICTP KOD KOŠTANIH METASTAZA RAKA PLUĆA

S A Ž E T A K

Koštane metastaze često su prisutne u uznapredovanim stadijima raka pluća. One su rezultat modulacije koštanog metabolizma od strane tumorskih stanica koje su migrirale u koštanu mikrookolinu i degradirale koštani organski matriks. Mjerenje C-terminalnog telopeptida kolagena tipa I (ICTP) u serumu ispitanika sa i bez koštanih metastaza, te zdravoj populaciji, način je na koji je moguće ispitati koštanu resorpciju. Kod 343 ispitanika obuhvaćenih istraživanjem razina ICTP-a bila je značajno viša kod koštanih metastaza, nego kod preostale dvije grupe. Postojanje patološke frakture značajno je utjecalo na povećanje razine ICTP-a. ICTP je pokazao senzitivnost od 66,0% kod koštanih metastaza koja odgovara 95,0%-tnoj specifičnosti kod raka pluća stadija IA i IB. ICTP je dobar dijagnostički biljeg za detekciju koštanih metastaza raka pluća. Njegovom razinom moguće je razlikovati rak pluća sa i bez zahvaćenosti kostiju, te se može koristiti kao dodatak standardnim tehnikama dijagnostike koštanih metastaza.