Is there any correlation between levels of serum osteopontin, CEA, and FDG uptake in lung cancer patients with bone metastasis?


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ABSTRACT

Objective: In this study, an evaluation was made of the relationship between the serum levels of carcinoembryonic antigen (CEA), osteopontin (OPN), and the semi-quantitative parameters of 18-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) in lung cancer patients with bone metastasis.

Material and methods: The evaluation included 42 non-small cell lung cancer (NSCLC) and 31 small cell lung cancer (SCLC) patients who were referred to our institution for staging by 18F-FDG PET/CT. The biochemical parameters measured included CEA and OPN serum levels.

Results: Serum levels of OPN in NSCLC patients with and without bone metastasis were 21.20 ± 4.97 ng/ml and 13.33 ± 4.53 ng/ml, respectively (p < 0.05). In SCLC patients with and without bone metastasis serum OPN levels were 23.95 ± 4.78 ng/ml and 17.30 ± 3.09 ng/ml, respectively (p < 0.05). Serum levels of CEA in NSCLC patients with and without bone metastasis were 33.79 ± 6.49 ng/ml and 11.74 ± 2.96 ng/ml, respectively (p < 0.05). In SCLC patients with and without bone metastasis serum levels of CEA were 28.93 ± 4.59 ng/ml and 13.88 ± 4.47 ng/ml, respectively (p < 0.05). There were no correlations between primary tumor SUV max, and serum levels of CEA and OPN.

Conclusions: Bone metastasis can be detected in patients with lung cancer by measuring CEA and OPN levels. Increased levels of CEA and OPN levels may be considered an early warning sign in patients needing accurate imaging, as they are at higher risk of bone metastasis.

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¿Existe correlación entre los niveles de osteopontina en suero sanguíneo, CEA y captación de 18F-FDG en pacientes con metástasis óseas por cáncer de pulmón?

RESUMEN

Objetivo: Evaluar la relación entre los niveles de antígeno carcinoembrionario (CEA), osteopontina (OPN) y los valores semicuantitativos (SUV) de la PET/TC con 18F-FDG en pacientes con metástasis óseas por cáncer de pulmón.

Material y método: Se incluyeron 40 pacientes con cáncer de pulmón de células no pequeñas (NSCLC) y 31 pacientes con cáncer de pulmón de células pequeñas (SCLC) referidos a nuestro centro para la realización de un estudio PET/TC con 18F-FDG de estadificación. Se analizaron los niveles sanguíneos de OPN y CEA.

Resultados: Los niveles de OPN en pacientes con NSCLC con y sin metástasis óseas fueron de 21.20 ± 4.97 ng/ml y 13.33 ± 4.53 ng/ml, respectivamente (p < 0.05). En pacientes con SCLC con y sin metástasis óseas fueron de 23.95 ± 4.78 ng/ml y 17.30 ± 3.09 ng/ml, respectivamente (p < 0.05). Los niveles sanguíneos de CEA en pacientes de NSCLC con y sin metástasis óseas fueron de 33.79 ± 6.49 ng/ml y 11.74 ± 2.96 ng/ml, respectivamente (p < 0.05). En pacientes con SCLC con y sin metástasis óseas fueron de 28.93 ± 4.59 ng/ml y 13.88 ± 4.47 ng/ml, respectivamente (p < 0.05). No hubo correlación entre el SUV máximo del tumor primario, los niveles OPN ni de CEA.

Conclusiones: La metástasis ósea puede ser detectada en pacientes con cáncer de pulmón con la determinación de los niveles de OPN y CEA. Los niveles incrementados de CEA y OPN pueden ser considerados como una señal de advertencia temprana en pacientes que necesitan imágenes precisas, porque ellos están en mayor riesgo de metástasis en el hueso.

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**Introduction**

Lung cancer is the leading cause of cancer deaths in the world. It is divided into two major types: non-small cell lung cancer (NSCLC) (85%) and the prognostically poorer small cell lung cancer (SCLC) (15%). The skeleton is the most common site for distant metastasis. Clinical presentation of bone metastasis includes severe bone pain, limitation of movement, hypercalcemia and pathologic fractures. Early diagnosis and aggressive treatment of these complications play important roles in improving the patient's quality of life, and accurate staging of patients with lung cancer is critical for appropriate treatment.

In recent years, positron-emission tomography/computed tomography (PET/CT), using the glucose analog 18-fluoro-2-deoxyglucose (18F-FDG), has been indicated to provide a more accurate diagnostic approach than the conventional methods. PET/CT has proven to be a very effective imaging modality for the bone metastases of lung cancer. PET/CT has also been recognized for particular value in primary tumor staging and it allows differentiation of tumor tissue from post-obstructive atelectasis. The molecular mechanisms leading to the development of bone metastasis in lung cancer are still unclear. One of the best known tumor markers in the management of lung cancer is the carcinoembryonic antigen (CEA), and higher CEA concentrations were found in advanced stages. Osteopontin (OPN) mediates biological processes, such as adhesion, migration, invasion, proteolysis, enhanced cell survival, and angiogenesis, and several studies have indicated a correlation between high OPN expression and poor patient outcomes in lung cancer. Additionally, it is known that there is a relationship between the biochemical markers and existence of bone metastases. Reliable blood tests for the early detection and monitoring of progression are a “holy grail” in cancer diagnostics. To the best of our knowledge, this is the first study to evaluate bone metastasis in lung cancer using 18F-FDG PET/CT imaging functional data and its correlation with CEA and OPN. The purpose of this study was to directly compare the results of semi-quantitative analyses by 18F-FDG PET/CT with biochemical parameters including CEA and OPN.

**Material and methods**

**Patients**

Over 10 months, 42 NSCLC patients (34 males and 8 females) and 31 SCLC patients (26 males and 5 females), referred to our institution for 18F-FDG PET/CT scanning for staging were included. The diagnoses of the lung cancer patients were confirmed by histological or cytological examinations of specimens taken from bronchoscopy, or by computerized tomography-guided fine needle biopsies. At the time of 18F-FDG PET/CT, pathologic TNM staging was evaluated according to the criteria of American Joint Committee on Cancer (AJCC). Among the NSCLC patients, 15 cases were in stage I/II, 27 cases were in stage III/IV. Among the SCLC patients 14 cases were in stage I/II and 17 cases were in stage III/IV. The control group consisted of 20 healthy volunteers (11 males and 9 females). Approval was received from the local ethics committee, and each patient signed an informed consent form. Patients with chronic kidney disease, osteoporosis, connective tissue disease, degenerative bone disease, traumatic fracture and history of current medication use affecting bone metabolism were not included in the study. Patients were excluded if they had received chemotherapy, radiotherapy or surgery, a history of extrapulmonary cancer or pregnancy. Histological diagnosis of bronchioloalveolar cell carcinoma subtype were also excluded from the study. All patients were imaged using 18F-FDG PET/CT.

**18F-FDG PET/CT imaging**

The patients fasted overnight, for at least 12 h. PET/CT whole body imaging was performed after an intravenous injection of approximately 12 mCi (444 MBq) 18F-FDG; Patients with a fasting blood glucose level above 120 mg/dl were excluded. After one hour waiting period in a quiet room, the patient was imaged using an integrated PET/CT camera, which consisted of a 16-slice CT gantry, integrated with an SLO-based fullring PET scanner (Siemens Biograph 16, Siemens, Knoxville, TN, USA). The CT was performed with 120–200 mAs adjusted to the patient's body weight at a 140 kV and from the base of the skull to the proximal thighs. For attenuation correction and image fusion, the PET images were reconstructed by using an iterative algorithm (ordered-subset expectation maximization: two iterations, eight subsets). The reconstructed PET, CT, and fused images were displayed by commercially available software (e-soft/VSIM, Siemens Medical Solutions) in axial, coronal, and sagittal planes. Maximum intensity projection (MIP) PET images and integrated and co-registered PET/CT images were visually evaluated by two experienced nuclear medicine physicians. The SUVmax was determined by drawing region of interest (ROI) around the primary tumor on the transaxial slices, and calculated according to the following formula: measured activity concentration [Bq/ml] × body weight [kg]/injected activity [Bq].

**Biochemical assessment**

The fasting blood samples taken on the day of the PET/CT scan were centrifuged, and the serum samples were divided into portions and kept at −80°C until analyses. The biochemical parameters in the serum: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), Ca, albumin, and total protein were determined using a Beckman Coulter AU 5800 auto analyzer and Beckman reagents within five days of the PET/CT scan for all patients. The serum concentration of CEA was performed at the Clinical Biochemistry Laboratory of our institution using the Beckman Coulter UniCel Dxl 800 with Beckman reagents. The OPN serum levels were measured using a Platinum ELISA kit (eBioscience, San Diego, CA, USA).

**Bone metastasis evaluation**

All patients were evaluated for bone metastasis by 18F-FDG PET/CT. 18F-FDG PET/CT results were read by 2 specialists and classified into (1) normal/benign, (2) positive for bone metastases, or (3) equivocal (the image could not be confidently categorized into one of the former two subgroups, requiring additional imaging procedures). When the interpretation differed among specialists the following criteria were used for confirmation. (1) Progression of bone lesion on the follow-up PET/CT; (2) confirmed bone metastasis by simple radiography, bone scintigraphy or magnetic resonance imaging (MRI); (3) concordance between positive initial findings on PET/CT and symptoms and (4) histopathological confirmation.

**Statistical analysis**

Results were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS version 19.0) software. Differences among the groups were obtained using the Duncan’s multiple range test option. For the correlation between primary tumor SUVmax, CEA and, OPN levels. Pearson’s correlation coefficients were computed. p < 0.05 were considered.
Table 1

<table>
<thead>
<tr>
<th>Site of metastasis</th>
<th>Number of cases (n) [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>33 (39.8)</td>
</tr>
<tr>
<td>Vertebral</td>
<td>25 (75.8)</td>
</tr>
<tr>
<td>Rib</td>
<td>18 (54.5)</td>
</tr>
<tr>
<td>Femur</td>
<td>12 (36.4)</td>
</tr>
<tr>
<td>Pelvis</td>
<td>16 (48.5)</td>
</tr>
<tr>
<td>Arm–shoulder</td>
<td>8 (24.2)</td>
</tr>
<tr>
<td>Cranial</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>Lung</td>
<td>6 (4.2)</td>
</tr>
<tr>
<td>Liver</td>
<td>7 (9.6)</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>5 (6.8)</td>
</tr>
<tr>
<td>Brain</td>
<td>1 (1.4)</td>
</tr>
</tbody>
</table>

Multiple locations of metastasis can be seen in the same patient, percentages of bone metastases are calculated accepting bone metastases as 100%.

Statistically significant. For all parameters, mean ± standard deviation values are indicated for each group separately.

Results

Seventy-three lung cancer patients, mean age 62.98 ± 10.17 years, were included in the study. The mean age of the patients was 64.4 ± 11.60 years in those with bone metastasis, and 61.68 ± 8.61 years in those without bone metastasis. It was determined that 42 patients (60%) had NSCLC, whereas 31 (40%) had SCLC and lung cancer showing neuroendocrine differentiation. Twelve of the cases of NSCLC were identified as squamous cell, while 15 cases were adenocarcinoma. In 15 NSCLC cases, type determinations could not be made.

The distribution of the cases according to metastases is shown in Table 1. Bone was the most common metastatic area in our group, with six (18.2%) of the cases having single-bone metastasis, and 27 (81.8%) having multiple bone metastases. The most common metastasis area was the vertebral bones (75.8%).

All lung cancers showed mild to marked uptake of FDG in the primary tumor site on the $^{18}$F-FDG PET/CT scan. We found that in NSCLC patients with and without bone metastasis the mean SUVmax were 8.94 (range 6.83–15.72) and 8.62 (range 5.25–13.87), respectively. In SCLC patients with and without bone metastasis the mean SUVmax were 7.74 (range 2.75–12.91) and 5.24 (range 5.01–10.91), respectively. SUVmax values with and without bone metastasis were not statistically significant in lung cancer patients ($p > 0.05$) (Fig. 1).

NSCLC and SCLC patients with or without bone metastasis had increased levels of OPN compared to controls ($p < 0.05$). The mean OPN levels in NSCLC patients with and without bone metastasis were 21.20 ± 4.97 ng/ml and 13.33 ± 4.53 ng/ml respectively ($p < 0.05$). Also the mean OPN levels in SCLC patients with and without bone metastasis were 23.95 ± 4.78 ng/ml and 17.30 ± 3.09 ng/ml respectively ($p < 0.05$).

The mean CEA levels in NSCLC patients with and without bone metastasis were 33.79 ± 6.49 ng/ml and 11.74 ± 2.96 ng/ml respectively ($p < 0.05$). The mean CEA levels in SCLC patients with and without bone metastasis were 28.93 ± 4.59 ng/ml and 13.88 ± 4.47 ng/ml respectively ($p < 0.05$). In patients with bone metastasis, compared to patients without metastasis, the serum CEA and OPN levels were found to be significantly higher ($p < 0.05$) (Figs. 2 and 3). There were no correlation between primary tumor SUVmax, serum CEA and OPN levels ($p > 0.05$).

The relationship between certain biochemical and bone metabolic markers and the existence of bone metastasis is given in Table 2. There were no correlations between the routine clinical serum marker levels in patients with bone metastasis, compared to patients without metastasis ($p > 0.05$ for all). In this study, the sensitivities of the routine biomarkers were found to be lower in evaluating the metastasis to bone at an early stage. Studies involving more patients are required in order to recommend their usage in bone metastasis.

Discussion

Lung cancer represents one of the most common and aggressive human malignancies, and reducing the mortality of lung cancer remains an important public health goal. In patients with lung cancer, the early diagnosis of bone metastasis is crucial. The identification of sensitive serum biomarkers would allow earlier and more efficient treatment, and improve the prognosis in these patients.

Contrast enhanced CT remains the mainstay for staging of lung cancer. However, PET has particular value in nodal staging of lung cancer and also in determining the presence of distant metastatic disease. In a study by Gould et al., the sensitivity of PET/CT for metastasis was 85% and the specificity was 95% as compared with a
Table 2
Comparison of various biochemical measurements in patients with and without bone metastases.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control group (n = 20)</th>
<th>NSCLC patients without BM (n = 22)</th>
<th>NSCLC with BM (n = 20)</th>
<th>SCLC patients without BM (n = 16)</th>
<th>SCLC patients with BM (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>10.56 ± 2.78</td>
<td>20.52 ± 7.77</td>
<td>21.62 ± 9.40</td>
<td>17.75 ± 7.29</td>
<td>22.13 ± 8.21</td>
</tr>
<tr>
<td>GGT</td>
<td>14.35 ± 3.38</td>
<td>82.18 ± 28.29</td>
<td>80.33 ± 45.27</td>
<td>37.25 ± 18.99</td>
<td>64.33 ± 40.52</td>
</tr>
<tr>
<td>ALP</td>
<td>79.42 ± 8.19</td>
<td>47.59 ± 26.58</td>
<td>84.21 ± 38.45</td>
<td>88.12 ± 21.84</td>
<td>114.61 ± 47.52</td>
</tr>
<tr>
<td>Ca</td>
<td>9.20 ± 0.48</td>
<td>9.42 ± 0.80</td>
<td>9.61 ± 0.48</td>
<td>9.24 ± 0.31</td>
<td>9.13 ± 0.55</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.81 ± 0.21</td>
<td>3.72 ± 0.55</td>
<td>3.74 ± 0.07</td>
<td>3.88 ± 0.45</td>
<td>3.42 ± 0.53</td>
</tr>
<tr>
<td>Total protein</td>
<td>6.87 ± 0.18</td>
<td>6.90 ± 0.77</td>
<td>7.40 ± 0.98</td>
<td>6.74 ± 0.48</td>
<td>5.83 ± 0.86</td>
</tr>
<tr>
<td>OPN</td>
<td>5.21 ± 3.48</td>
<td>13.33 ± 4.53</td>
<td>21.20 ± 4.97</td>
<td>17.30 ± 3.09</td>
<td>23.95 ± 4.78</td>
</tr>
<tr>
<td>CEA</td>
<td>1.57 ± 0.76</td>
<td>11.74 ± 2.96</td>
<td>33.79 ± 6.49</td>
<td>13.88 ± 4.47</td>
<td>28.93 ± 4.59</td>
</tr>
</tbody>
</table>

NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; BM, bone metastasis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; OPN, osteopontin; CEA, carcinoembryonic antigen.

Within rows, means with different letters are significantly different according to the one-way ANOVA–Duncan test (p < 0.05). Results are expressed as mean ± SD.

Tumor cells release several biomarkers, unlike their original cells, and these biomarkers play important roles in the diagnosis or follow-up in the therapy of many tumors. CEA is an oncofetal antigen produced during embryonal and fetal development. Elevated levels of serum CEA have been reported in 35–60% of NSCLC patients. When cancer metastasis emerges in bone, the cancer has metastasized hematogenously, and abnormal increases in tumor markers are so high that they are very useful for screening tests. There are only a few reports about the relationship between bone metastasis and CEA level. Shinozaki et al. reported a tumor marker for the abnormal elevation of CEA levels in cases of bone metastasis. Tsukushi et al. revealed that multiple skeletal metastases and visceral metastases were found to be correlated with the serum CEA levels; however, there was no difference between the presence of primary lesions, pathological fracture, or previous cancer history.

CEA is substantially expressed in the bone matrix, and it plays a role in cellular adhesion, migration, invasion, and proliferation by means of integrins and CD 44. The serum OPN level was reported to be higher in the group with bone metastasis in the NSCLC cases. It is indicated that OPN—expressing tumors have worse overall survival than patients with OPN-negative tumors and OPN has the potential to be used as a prognostic biomarker in NSCLC. Consistent with previous reports in our study, the serum OPN levels in both the NSCLC and SCLC groups where bone metastasis was evaluated by PET/CT were found to be significantly higher in those with bone metastasis.

There were a few limitations in our study. First, all metastatic bone lesions were not histopathologically confirmed. Second, we did not routinely perform MRI for confirmation of bone metastases in patients with negative results on 18F-FDG PET/CT and bone scan, and there might be false-negative metastatic bone lesions. Finally, all these results represent a single-center experience with a small number of patients.

In conclusion, this preliminary prospective study in a small group of lung cancer patients with bone metastasis revealed that serum CEA and OPN levels may aid to identify patients with bone metastases. Besides, 18F-FDG PET CT can identify the exact location of bone metastasis while CEA, OPN or other biomarkers only indicate presence of metastases without offering information about the metastatic sites. Therefore, bone metastasis should be meticulously explored in patients who are diagnosed with lung cancer by routine 18F-FDG PET/CT that integrates anatomical, morphological and metabolic aspects in a single examination and has the ability to perform whole-body scanning, during the period of higher CEA and OPN levels. However, we have demonstrated the potential diagnostic value of serum CEA and OPN for bone metastasis in lung cancer patients, further studies are needed to clarify the role of these biomarkers in early detection of lung cancer patients with bone metastasis.
Conflict of interests

The authors declare no conflict of interest.

References