Diagnostic value of epidermal growth factor, cancer antigen 125, and cancer antigen 15-3 in bronchoalveolar lavage fluid of lung cancer

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ABSTRACT

Aim In the present study, we assessed the diagnostic value of epidermal growth factor (EGF) and cancer antigens 125 (CA125) and 15-3 (CA15-3) in bronchoalveolar lavage fluid (BALF) of lung cancer from 79 enrolled patients with suspected lung cancer.

Methods All patients underwent fibrescopic examination, during which BALF samples were collected. Levels of EGF, CA125, and CA15-3 were determined in BALF using commercially available test kits.

Results The results showed that levels of EGF in BALF were significantly higher in patients with lung cancer than in patients with benign diseases (p < 0.01); no significant differences for CA125 (p = 0.67) or CA15-3 (p = 0.43) in BALF were observed between the lung cancer patients and the non-cancer control subjects. With a cut-off value of 27.22 pg/mL, EGF showed a sensitivity of 63.6% and a specificity of 65.7% in predicting the malignant nature of pulmonary disease.

Conclusions The study findings suggest that levels of EGF are significantly increased in BALF from patients with lung cancer than in BALF from patients with benign disease. Detection of the level of EGF in BALF is proposed as a noninvasive test to identify patients at high risk for lung cancer.

Key Words Lung cancer, bronchoalveolar lavage fluid, epidermal growth factor, cancer antigen 125, cancer antigen 15-3

INTRODUCTION

Lung cancer is currently one of the leading causes of cancer-related mortality for both men and women worldwide. It is known to be responsible for more than 1,000,000 deaths annually all over the world. The high mortality from lung cancer occurs mainly because more than half of all patients present with metastasis at the time of diagnosis.

It has been reported that 5-year survival in lung cancer is generally lower than 15%. Although detection of lung cancer at an earlier stage could potentially increase the survival rate, seeking a diagnosis method with high sensitivity and specificity is still a difficult and challenging clinical problem. One promising approach is the identification and detection of lung cancer–specific biomarkers at an early stage. In our previous reports, we found that levels of cancer-specific cytokines rose much earlier and presented at higher concentrations in bronchoalveolar lavage fluid (BALF) than in peripheral blood. Detection of biomarkers in BALF could therefore serve as an important method for lung cancer diagnosis.

Currently, fibre-optic bronchoscopy is regularly performed when patients are suspected of having lung cancer, during which bronchial washing is traditionally used. Given that some cytokines are produced directly by the tumour or by non-tumour cells in response to the presence of tumour cells, elevation of those cytokines can be detected earlier than radiographic abnormalities can.

* These authors contributed equally to the present work.
Investigating specific molecular markers in airways is an important adjunct to routine bronchoscopy for lung cancer diagnosis. In the present study, we prospectively assessed the expression of epidermal growth factor (EGF) and cancer antigens 125 (CA125) and 15-3 (CA15-3) in airways by comparing levels in benign lung diseases and in lung cancer.

METHODS

Patients
The study included 79 patients admitted to the Affiliated Hospital of Medical College, Ningbo University, Ningbo, P.R.C., with suspected lung cancer. Approval for the study was obtained from the Institutional Review Board for Human Studies of the Affiliated Hospital of Medical College, Ningbo University, and informed consent was obtained from all participants. All patients had histologically confirmed disease; those who had already received preoperative chemotherapy or radiotherapy were excluded. The experiments were performed in accordance with the evidence-based clinical practice guidelines published by the American College of Chest Physicians.

Bronchoalveolar Lavage
The BALF samples were collected and analyzed as described in detail in our earlier studies. In brief, the bronchus on the disease side was washed with 37°C physiologic saline, and the fluid was gently withdrawn into a siliconized container placed in iced water. The chilled lavage fluid was passed through a nylon filter to remove mucus and then centrifuged at 3000 rpm for 10 minutes. The cell pellets were separated from their supernatant and stored at –80°C.

Measurements of EGF, CA125, and CA15-3
The EGF levels were measured using Quantikine sandwich ELISA kits (R&D Systems, Minneapolis, MN, U.S.A.), and all assays were conducted according to the manufacturer’s guidelines. Levels of CA125 and CA15-3 were determined using commercially available electrochemiluminescence immunoassay kits (Beckman Coulter, Brea, CA, U.S.A.).

Statistical Analysis
Data are presented as means with standard error. Comparisons between groups used the Student t-test. The relationships between the various markers were determined using Pearson correlations. Receiver operating characteristic (ROC) curves were constructed as plots of the percentage of true positives (sensitivity) against the percentage of false positives (100 specificity) to determine the area under each curve. A probability value of less than 0.05 was considered statistically significant. The analysis was conducted using the SPSS (version 13.0: SPSS, Chicago, IL, U.S.A.) and GraphPad Prism (version 5.0: GraphPad Software, San Diego, CA, U.S.A.) software applications.

RESULTS

Patient Characteristics
Table I summarizes the basic demographic characteristics for the 79 patients (44 with lung cancer, 35 with noncancerous diseases). Mean age of the lung cancer patients was 63.8 ± 1.5 years (standard error), and 37 (84.1%) were men. Mean age of the patients with benign diseases (20 men, 15 women) was 51.9 ± 2.3 years. The percentage of smokers was 70.1% in the lung cancer group and 42.9% in the noncancerous disease control group. The lung cancer group included 22 cases of squamous cell carcinoma, 17 of adenocarcinoma, and 5 of small-cell lung cancer.

EGF in BALF
Levels of EGF were significantly higher in patients with lung cancer than in patients with benign diseases [41.2 ± 4.9 pg/mL vs. 22.1 ± 3.3 pg/mL, p = 0.0027, Figure 1(A)]. When categorized by tumour histology, differences between the patients with various lung cancer histologies and the patients with benign diseases were found to be statistically significant [squamous cell carcinoma: 42.8 ± 7.6 pg/mL, p = 0.0065; adenocarcinoma 37.8 ± 7.5 pg/mL, p = 0.0292; small-cell lung cancer: 46.1 ± 10.3 pg/mL, p = 0.0155; Figure 1(B)].

CA125 and CA15-3 in BALF
As Figure 2(A) shows, the level of CA125 was slightly higher in the group of lung cancer patients than in the group of patients with noncancerous diseases [558.5 ± 58.0 U/mL vs. 517.4 ± 79.1 U/mL, p = 0.5329, Figure 2(B)]. In addition, the levels of CA15-3 in BALF were not significantly different between the lung cancer patients and the patients with benign diseases [1.3 ± 0.2 U/mL vs. 1.0 ± 0.2 U/mL, p = 0.428, Figure 2(B)].

Correlations of EGF, CA125, and CA15-3 in BALF
The correlation between EGF and CA125 in BALF [r = 0.5329, p = 0.0001, Figure 3(A)] was significant. However, the level of CA15-3 in BALF showed no correlation with the level of EGF [r = 0.0179, p = 0.8753, Figure 3(B)] or of CA125 [r = 0.0454, p = 0.6913, Figure 3(C)].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient group</th>
<th>Lung cancer</th>
<th>Benign condition</th>
</tr>
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<tbody>
<tr>
<td>Patients (n)</td>
<td>44</td>
<td>35</td>
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</tr>
<tr>
<td>Mean± age (years)</td>
<td>63.8±1.5</td>
<td>51.9±2.3</td>
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<tr>
<td>Mean± body mass index (kg/m²)</td>
<td>22.6±0.6</td>
<td>23.1±0.7</td>
<td></td>
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<tr>
<td>Sex (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>37</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>7</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Smoking status (n)</td>
<td></td>
<td></td>
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<tr>
<td>Smokers</td>
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<td>15</td>
<td></td>
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<tr>
<td>Mean± pack–years</td>
<td>32.1±5.5</td>
<td>15.6±5.3</td>
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<tr>
<td>Nonsmokers</td>
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<td>20</td>
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<tr>
<td>Histologic type (n)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>22</td>
<td></td>
<td></td>
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<tr>
<td>Adenocarcinoma</td>
<td>17</td>
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<tr>
<td>Small-cell lung cancer</td>
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</table>

a With standard error.
Diagnostic Performance of EGF in BALF of Lung Cancer

A ROC analysis was performed to calculate the sensitivity and specificity of EGF in predicting lung cancer, with the aim of determining a possible threshold value. As Figure 4 shows, the area under the ROC was 0.7234. Various cut-off values of EGF, with their associated sensitivities and specificities, were proposed (Table II). The optimal threshold for discrimination of cancerous from noncancerous disease was 27.22 pg/mL. Using a cut-off value of 27.22 pg/mL, EGF reached a sensitivity of 63.6% (95% confidence interval: 47.8% to 77.6%) and a specificity of 65.7% (95% confidence interval: 47.8% to 80.9%) in the differential diagnosis of lung cancer.

DISCUSSION

In the present prospective study, we assessed the diagnostic value of the expression of EGF, CA125, and CA15-3 in BALF of lung cancer. All 79 patients enrolled in the study underwent fibrescopic examination, during which BALF samples were collected. Levels of EGF, CA125, and CA15-3 in BALF were determined using commercially available test kits. The study findings suggest that EGF levels in BALF are significantly higher in patients with lung cancer than in patients with benign diseases; however, CA125 and CA15-3 levels in BALF are not significantly different between the two groups.

The mean level of EGF in BALF of the non-cancer group was 22.1 ± 3.3 pg/mL—a level much lower than was observed in patients with lung cancer (41.2 ± 4.9 pg/mL). When the patients with lung cancer were categorized by tumour histology, levels of EGF in squamous cell carcinoma, adenocarcinoma, and small-cell lung cancer were 42.8 ± 7.6 pg/mL, 37.8 ± 7.5 pg/mL, and 46.1 ± 10.3 pg/mL respectively. The differences in the EGF levels in BALF were statistically significant for each histologic type of lung cancer and for benign disease.

Higher EGF concentrations were found in malignancy in our study, in agreement with earlier data regarding EGF in serum from lung cancer patients. The diagnostic value of EGF in BALF was further evaluated by ROC analysis. The area under the ROC curve was 0.7234. The optimal threshold for discriminating cancerous from noncancerous lesions was 27.22 pg/mL. Using a cut-off value of 27.22 pg/mL, EGF reached a sensitivity of 63.6% and a specificity of 65.7% in the differential diagnosis of lung cancer.

In clinical practice, CA125 and CA15-3 are the tumour markers most widely used for the differential diagnosis of lung cancer. In a recent report, Ying et al. undertook
FIGURE 4 A receiver operating characteristic curve was plotted to evaluate the threshold value of epidermal growth factor (EGF) in differentiating lung cancer from benign conditions. At a cut-off value of 27.22 pg/mL, EGF reached a sensitivity of 63.6% (95% confidence interval (CI): 47.8% to 77.6%) and a specificity of 65.7% (95% CI: 47.8% to 80.9%). AUC = area under the curve.

TABLE II Diagnostic performance of epidermal growth factor in bronchoalveolar lavage fluid from patients with lung cancer

<table>
<thead>
<tr>
<th>Cut-off value (pg/mL)</th>
<th>Sensitivity (%) 95% CI</th>
<th>Specificity (%) 95% CI</th>
<th>Likelihood ratio</th>
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<tr>
<td>8.56</td>
<td>100 92.0 to 100.0</td>
<td>34.3 19.1 to 52.2</td>
<td>1.52</td>
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<tr>
<td>12.18</td>
<td>90.9 78.3 to 97.5</td>
<td>40 23.9 to 57.9</td>
<td>1.52</td>
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<tr>
<td>12.79</td>
<td>88.6 75.4 to 96.2</td>
<td>45.7 28.8 to 63.4</td>
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<tr>
<td>18.86</td>
<td>70.5 54.8 to 83.2</td>
<td>51.4 34.0 to 68.2</td>
<td>1.45</td>
</tr>
<tr>
<td>27.22</td>
<td>63.6 47.8 to 77.6</td>
<td>65.7 47.8 to 80.9</td>
<td>1.86</td>
</tr>
<tr>
<td>35.25</td>
<td>52.3 36.7 to 67.5</td>
<td>80 63.1 to 91.6</td>
<td>2.61</td>
</tr>
<tr>
<td>46.15</td>
<td>31.4 18.6 to 47.6</td>
<td>94.3 80.8 to 99.3</td>
<td>5.57</td>
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<td>47.35</td>
<td>29.6 16.8 to 45.2</td>
<td>97.1 85.1 to 99.9</td>
<td>10.34</td>
</tr>
</tbody>
</table>

CI = confidence interval.

CONCLUSIONS

Levels of EGF were significantly higher in BALF from lung cancer patients than in BALF from patients with benign diseases. Detecting EGF in BALF might be helpful for the differential diagnosis of lung cancer. Future studies are wanted to validate our results and elucidate their clinical benefit.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood Current Oncology’s policy on disclosing conflicts of interest, and we declare that we have none.

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