CURRENT ONCOLOGY
A Canadian Cancer Research Journal

ORIGINAL ARTICLE

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

Curr Oncol. 2017 June;24(3):e180-e184 www.current-oncology.com

INTRODUCTION

Lung cancer is currently one of the leading causes of cancer-related mortality for both men and women worldwide[1,2]. It is known to be responsible for more than 1,000,000 deaths annually all over the world[3]. 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[4]. Detection of biomarkers in BALF could therefore serve as an important method for lung cancer diagnosis[5–13].

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[4].

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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[14].

Bronchoalveolar Lavage

The BALF samples were collected and analyzed as described in detail in our earlier studies[4–6]. 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 noncancer control group. The lung cancer group included 22 cases of squamous cell carcinoma, 17 of adenocarcinoma, and 5 of small-cell lung cancer.

TABLE I Characteristics of the study patients

Characteristic	Patient group	
	Lung cancer	Benign condition
Patients (*n*)	44	35
Mean[a] age (years)	63.8±1.5	51.9±2.3
Mean[a] body mass index (kg/m^2)	22.6±0.6	23.1±0.7
Sex (*n*)		
Men	37	20
Women	7	15
Smoking status (*n*)		
Smokers	31	15
Mean[a] pack–years	32.1±5.5	15.6±5.3
Nonsmokers	13	20
Histologic type (*n*)		
Squamous cell carcinoma	22	
Adenocarcinoma	17	
Small-cell lung cancer	5	

[a] With standard error.

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), but the difference did not reach statistical significance ($p = 0.6692$). 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)].

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.

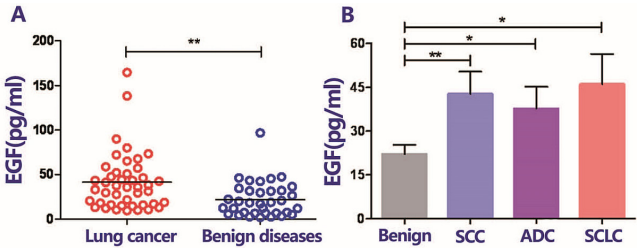

FIGURE 1 Levels of epidermal growth factor (EGF) in bronchoalveolar lavage fluid. (A) Levels of EGF were significantly higher in patients with lung cancer than in patients with benign conditions (**$p < 0.01$ by t-test; the horizontal lines mark mean values). (B) Similarly, subgroup analysis by tumour histology in lung cancer patients showed statistical differences in EGF levels (**$p < 0.01$ and *$p < 0.05$ by t-test). SCC = squamous cell carcinoma; ADC = adenocarcinoma; SCLC = small-cell lung cancer.

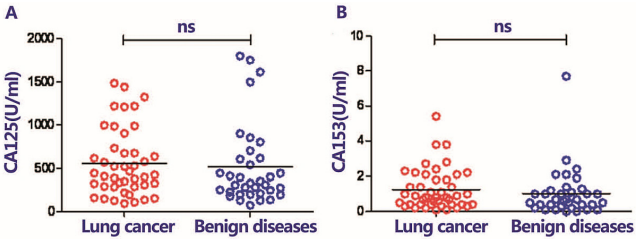

FIGURE 2 Levels of cancer antigen 125 (CA125) and 15-3 (CA15-3) in bronchoalveolar lavage fluid. (A) Levels of CA125 were not significantly higher in patients with lung cancer than in patients with benign conditions [p = nonsignificant (ns) by t-test]. (B) Levels of CA15-3 were not significantly different in patients with lung cancer than in patients with benign conditions (p = ns by t-test). Horizontal lines mark mean values.

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[15]. 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[16,17]. In a recent report, Ying *et al.*[18] undertook

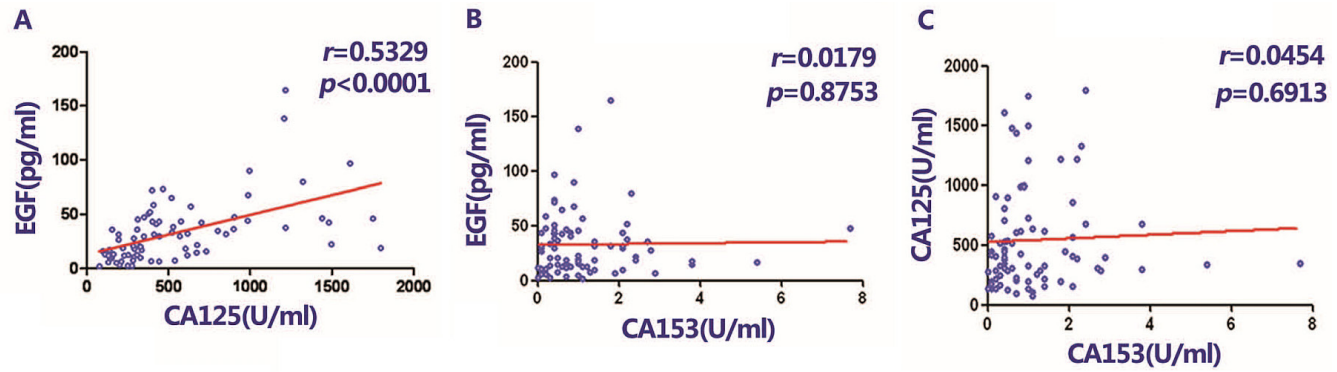

FIGURE 3 Correlations of epidermal growth factor (EGF), cancer antigen 125 (CA125), and cancer antigen 15-3 (CA15-3) in bronchoalveolar lavage fluid. (A) A significant statistical correlation was observed between EGF and CA125. (B,C) No significant statistical correlation was found between EGF and CA15-3 or between CA125 and CA15-3.

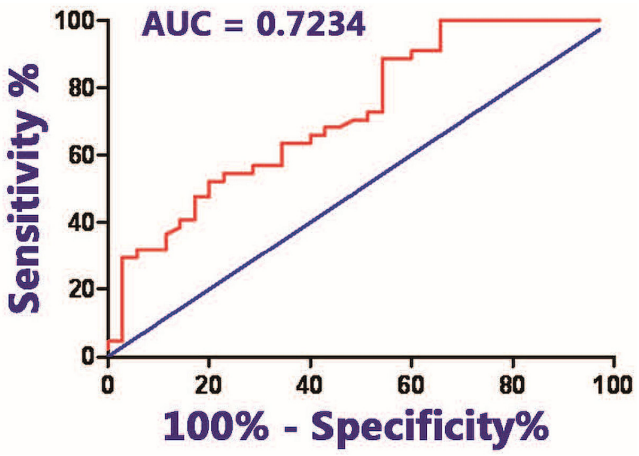

FIGURE 4 A receiver operating characteristic curve was plotted to evaluate the threshold value of epidermal growth factor (EGF) in differentiating lung cancer from benign conditions. At a cut-off value of 27.22 pg/mL, EGF reached a sensitivity of 63.6% [95% confidence interval (CI): 47.8% to 77.6%] and 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

Cut-off value (pg/mL)	Sensitivity		Specificity		Likelihood ratio
	(%)	95% CI	(%)	95% CI	
8.56	100	92.0 to 100.0	34.3	19.1 to 52.2	1.52
12.18	90.9	78.3 to 97.5	40	23.9 to 57.9	1.52
12.79	88.6	75.4 to 96.2	45.7	28.8 to 63.4	1.63
18.86	70.5	54.8 to 83.2	51.4	34.0 to 68.2	1.45
27.22	63.6	47.8 to 77.6	65.7	47.8 to 80.9	1.86
35.25	52.3	36.7 to 67.5	80	63.1 to 91.6	2.61
46.15	31.8	18.6 to 47.6	94.3	80.8 to 99.3	5.57
47.35	29.6	16.8 to 45.2	97.1	85.1 to 99.9	10.34

CI = confidence interval.

a large-scale retrospective analysis of the prognostic value of serum CA125 in 645 lung cancer patients. They found that CA125 could be used as an independent predictive marker for prognosis in patients with lung cancer. In our study, we demonstrated a significant correlation between EGF and CA125 in BALF. However, concentrations of CA125 or CA15-3 in BALF were not elevated in our lung cancer patients. That observation suggests that measurement of CA125 or CA15-3 in BALF has poor diagnostic value in lung cancer.

In recent years, several studies have investigated biomarkers in BALF of lung cancer. Emad *et al.*[7] demonstrated that the mean level of lactate dehydrogenase in BALF was significantly higher in patients with malignant pulmonary nodules than it was in patients with benign nodules ($p < 0.001$). In another study, Ohta and colleagues[11] evaluated the expression of vascular endothelial growth factor in 41 patients with lung cancer and 7 patients with noncancerous diseases. They observed that concentrations of vascular endothelial growth factor in BALF were significantly greater in lung cancer patients than in patients with noncancerous diseases ($p = 0.047$). Moreover, the BALF of patients with lung cancer, compared with the BALF of healthy subjects, was found to contain a higher level of transforming growth factor β1[13]. However, neither study performed a ROC analysis to calculate the sensitivity and specificity of the biomarkers of interest in predicting lung cancer, to define a possible threshold value.

Our study showed that the levels of EGF in BALF were significantly higher in patients with lung cancer than in patients with benign diseases. In addition, we found that detection of EGF in BALF reached a sensitivity of 63.6% and a specificity of 65.7% for lung cancer. To the best of our knowledge, our study is the first in which levels of EGF in BALF were measured in patients both with lung cancer and with benign diseases. The difficulty of discriminating lung cancer from non-neoplastic respiratory diseases is a common and challenging clinical problem. In addition to the traditional tissue-based analysis, our study provides an evident possibility for an evaluation of EGF levels in BALF. The findings from our study indicate that measurement of EGF in BALF might be helpful in distinguishing malignancy from benign disease in pulmonary medicine.

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 in-clinic benefit.

ACKNOWLEDGMENTS

This work was supported by the Medical Science and Technology Plan Projects of Ningbo (no. 2016A03, C. Cao) and the Science and Technology Project of Ningbo (no. 2016C51019, S. Sun).

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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REFERENCES

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 2010;60:277–300.
2. Cao C, Zhang Y, Wang R, *et al.* Excision repair cross complementation group 1 polymorphisms and lung cancer risk: a meta-analysis. *Chin Med J (Engl)* 2011;124:2203–8.
3. Sihoe AD, Yim AP. Lung cancer staging. *J Surg Res* 2004;117:92–106.
4. Cao C, Sun SF, Lv D, Chen ZB, Ding QL, Deng ZC. Utility of VEGF and sVEGFR-1 in bronchoalveolar lavage fluid for differential diagnosis of primary lung cancer. *Asian Pac J Cancer Prev* 2013;14:2443–6.

5. Chen Z, Xu Z, Sun S, *et al.* TGF-β1, IL-6, and TNF-α in bronchoalveolar lavage fluid: useful markers for lung cancer? *Sci Rep* 2014;4:5595.
6. Cao C, Chen ZB, Sun SF, Yu YM, Ding QL, Deng ZC. Evaluation of VEGF-C and tumor markers in bronchoalveolar lavage fluid for lung cancer diagnosis. *Sci Rep* 2013;3:3473.
7. Emad A, Emad V. The value of BAL fluid LDH level in differentiating benign from malignant solitary pulmonary nodules. *J Cancer Res Clin Oncol* 2008;134:489–93.
8. Charalabopoulos K, Karakosta A, Bablekos G, *et al.* CEA levels in serum and BAL in patients suffering from lung cancer: correlation with individuals presenting benign lung lesions and healthy volunteers. *Med Oncol* 2007;24:219–25.
9. Cremades MJ, Menéndez R, Rubio V, Sanchis J. Fibronectin in bronchoalveolar lavage fluid in lung cancer: tumor or inflammatory marker? *Respiration* 1998;65:178–82.
10. Bugdayci G, Kaplan T, Sezer S, *et al.* Matrix metalloproteinase-9 in broncho-alveolar lavage fluid of patients with non–small cell lung cancer. *Exp Oncol* 2006;28:169–71.
11. Ohta Y, Ohta N, Tamura M, *et al.* Vascular endothelial growth factor expression in airways of patients with lung cancer: a possible diagnostic tool of responsive angiogenic status on the host side. *Chest* 2002;121:1624–7.
12. Cremades M, Menéndez R, Pastor A, Llopis R, Aznar J. Diagnostic value of cytokeratin fragment 19 (CYFRA 21-1) in bronchoalveolar lavage fluid in lung cancer. *Respir Med* 1998;92:766–71.
13. Domagała-Kulawik J, Hoser G, Safianowska A, Grubek-Jaworska H, Chazan R. Elevated TGF-beta1 concentration in bronchoalveolar lavage fluid from patients with primary lung cancer. *Arch Immunol Ther Exp (Warsz)* 2006;54:143–7.
14. Kozower BD, Larner JM, Detterbeck FC, Jones DR. Special treatment issues in non-small cell lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* 2013;143:e369S–99S.
15. Lemos-Gonzalez Y, Rodríguez-Berrocal FJ, Cordero OJ, Gómez C, Páez de la Cadena M. Alteration of the serum levels of the epidermal growth factor receptor and its ligands in patients with non-small cell lung cancer and head and neck carcinoma. *Br J Cancer* 2007;96:1569–78.
16. Ghosh I, Bhattacharjee D, Das AK, Chakrabarti G, Dasgupta A, Dey SK. Diagnostic role of tumour markers CEA, CA15-3, CA19-9 and CA125 in lung cancer. *Indian J Clin Biochem* 2013;28:24–9.
17. Dai H, Liu J, Liang L, *et al.* Increased lung cancer risk in patients with interstitial lung disease and elevated CEA and CA125 serum tumour markers. *Respirology* 2014;19:707–13.
18. Ying L, Wu J, Zhang D, *et al.* Preoperative serum CA125 is an independent predictor for prognosis in operable patients with non–small cell lung cancer. *Neoplasma* 2014;62:602–9.