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Evaluation of serum and pleural levels of endostatin and vascular epithelial growth factor in lung cancer patients with pleural effusion

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ABSTRACT

Objective: To evaluate the diagnostic value of endostatin (ES), vascular endothelial growth factor (VEGF) and carcinoembryonic antigen (CEA) in both serum and pleural effusion of lung cancer patients. **Methods:** Levels of ES, VEGF and CEA in 52 malignant pleural effusion due to lung cancer and 50 patients with non-malignant disease were measured by using sandwich enzyme-linked immunosorbent assay and microparticle enzyme immunoassay. **Results:** The ES, VEGF and CEA levels in pleural effusion and serum, and their ratio(F/S) were higher in lung cancer group than that in benign group, and the differences were statistically significant($P<0.05$). The diagnostic efficiency of ES+VEGF for lung cancer was superior to either single detection. The diagnostic efficiency of ES+VEGF+CEA was superior to either ES+VEGF or ES+CEA. **Conclusions:** The results suggest that ES, VEGF and CEA might be useful in the differentiation between benign and malignant pleural effusion due to lung cancer. In comparison with either single determination of concentration in serum or pleural fluid, the combined detection of two or three markers is of important clinical significance in the diagnosis of lung cancer.

1. Introduction

The majority of neoplasms can cause pleural effusion during their progression. In adults, about 38% to 52% patients of pleural effusion were malignant. The main reason of malignant pleural effusion (MPE) is lung cancer, with an incidence of 24% to 42%[1]. The development of MPE is usually a negative prognostic symptom, and is associated with poor quality of life. Therefore, the differentiation between malignant and benign pleural effusion is closely related to its treatment and prognosis.

In 1997, O'Reilly and his colleagues discovered endostatin (ES), which is an important angiogenesis inhibitor and plays an important role in tumor development, invasion and metastasis[2]. Several previous studies have shown that endostatin expression levels have a close relationship with malignant tumors' progress and prognosis[3]. Vascular endothelial growth factor (VEGF), a potent growth factor for endothelial cells, is thought to be the key mediator in forming MPE. A large number of studies have shown that tumor blood vessel growth depends on the balance of various positive and negative regulatory factors[4-6], of which VEGF is the most universal tumor angiogenic factor, whereas ES is the strongest inhibitor of tumor angiogenesis, both playing an important role in growth and metastasis of lung cancer.

To date, many previous studies have investigated the role of ES and VEGF in tumor samples and serum in the diagnosis for patients with lung cancer, but the levels of ES and VEGF in pleural effusion have not been fully evaluated. In this study, we used enzyme-linked immunosorbent assay (ELISA) to detect the levels of VEGF and ES in the blood and pleural fluid of patients with benign or malignant pleural effusion, our objective was to assess the diagnostic value of ES and VEGF in both serum and pleural effusion of lung cancer patients.

2. Materials and methods

2.1. Population studied

Between October 2008 and February 2011, the serum

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and pleural effusion were available from 102 patients. The pleural effusion was collected thoracoscopically. Diagnosis of lung cancer was histologically or cytologically proven in 52 cases (35 men and 17 women, aged 28-76 years). In all the patients, cancer cells were found in pleural effusion by cytological evaluation. These patients comprised 38 with adenocarcinomas, 7 with squamous cell carcinomas, and 7 with small cell carcinoma. Patients with benign disease comprised 39 with tuberculous pleurisies, 7 with pleuropneumonias and 4 with empyema (33 men and 17 women, 19-78 years of age). Tuberculous pleurisy was diagnosed with a pleural biopsy specimen showing typical epitheloid cell granuloma, and/or clinical and laboratory data suggestive of tuberculosis and response to specifc antituberculous therapy. A pleural effusion was considered to be pleuropneumonic when there was an acute febrile illness with purulent sputum, and pulmonary infltration in the absence of malignancy or other disease causing pleurisy. Empyema was diagnosed with macroscopic pus, microorganisms on pleural fuid Gram stain or culture in patients with pleural effusion associated with a lung infection. They were under an IRB approved protocol and we received written informed consent from each patient.

2.2. Collection of biological material

Percutaneous thoracic puncture was used to collect the pleural fluid. Blood samples were simultaneously obtained. Drawn blood was allowed to coagulate at RT for 15 min and centrifuged for 15 min at 3 000 rp/min to obtain the serum. Pleural effusions were centrifuged in the same conditions. Cell-free supernatants were collected, and aliquots were stored at -70 ℃ until they were analyzed.

2.3. Determination of tumor marker levels

The concentrations of ES (Market; Saint Louis, US), VEGF (Biological Engineering; Saint Louis, US) in pleural effusion and sera were determined using the ELISA kit according to the manufacturer's instructions with modifcations for the pleural effusions and sera. Measurements were taken and standard curve fttings were done using a precision microplate reader (Sunrise-Remote; Tecan, Austrian). Samples in which the levels were outside the linear part of the curve were diluted and reanalyzed. The method for the quantitative measurement of CEA (Abbott Laboratories; Chicago, US) was based on microparticle enzyme immunoassay technology.

2.4. Statistical analysis

We used commercial SPSS 13.0 software for statistical analysis. Measurement data and numeration data were compared with t test and χ^2 test, respectively. Diagnostic accuracy of tumor markers was compared by analyzing receiver operating characteristic (ROC). Results from patients with malignant pleural effusions were used to select cut-off values for sensitivity and specifcity for all markers. P less than 0.05 was considered to be statistically signifcant.

3. Results

As shown in Table 1, patients with lung cancer presented higher serum and pleural effusion levels of ES, VEGF, and CEA than those with benign pleural effusion, the differences were statistically significant ($P<0.05$). The F/S ratio was also significantly higher in lung cancer group than that in benign group for ES and VEGF ($P<0.05$) as well as CEA ($P<0.01$).

Table 2 shows the diagnostic sensitivity, specifcity, positive predictive value, negative predictive value, and accuracy of ES, VEGF and CEA for diagnosing malignant pleural effusion due to lung cancer. Cut-off points were determined by the maximum sum of sensitivity and specifcity. We used cut-off points of 163.57 ng/mL, 211 ng/L, and 8 ng/mL in pleural effusion ES, VEGF and CEA, respectively; cut-off points of 98.96 ng/mL, 125 ng/L, and 8 ng/mL in serum ES, VEGF and CEA, respectively. As shown in Table 2, the diagnostic efficiency of each marker was slightly higher in pleural effusion than in serum; the diagnostic efficiency of VEGF alone was higher than ES alone but lower than CEA alone. The combined detection of two of the three markers in pleural effusion or serum was superior to that of any single detection. The concomitant determination of serum and pleural levels of the three markers was of the superior diagnostic efficiency (Figure 1).

Table 1
Levels of ES, VEGF and CEA in fluid and serum and fluid/serum(F/S) ratio in benign and malignant pleural effusions.

Marker		Benign (n=50)	Lung cancer group (n=52)	P value
ES (ng/mL)	Fluid	138.4±98.3	205.3±115.4	<0.01
	Serum	97.6±11.2	160.5±21.7	<0.01
	F/S	1.1±0.6	1.4±0.8	<0.05
VEGF (ng/L)	Fluid	122.4±85.1	355.8±131.5	<0.01
	Serum	52.6±48.5	135.5±119.7	<0.01
	F/S	2.1±0.9	2.5±1.1	<0.05
CEA (ng/mL)	Fluid	2.3±1.5	208.0±70.5	<0.001
	Serum	1.5±0.3	52.4±15.6	<0.01
	F/S	2.1±0.2	8.4±1.6	<0.01

ES=endostatin; VEGF=vascular endothelial growth factor; CEA=carcinoembryonic antigen; F/S=fluid/serum.

Table 2

Measures of diagnostic accuracy for ES, VEGF and CEA assays in fluid and serum in lung cancer-associated malignant pleural effusions.

Marker		Sensitivity(%)	Specificity(%)	Accuracy(%)	Positive predictive value (%)	Negative predictive value (%)
ES	Fluid (>163.57 ng/mL)	69.3	71.5	70.2	72.8	70.3
	Serum (> 98.96 ng/mL)	62.4	74.1	68.6	66.5	71.1
VEGF	Fluid (> 211 ng/L)	76.6	73.8	74.5	85.7	65.5
	Serum (>125 ng/L)	75.3	72.6	73.3	83.1	64.0
CEA	Fluid (>8 ng/mL)	78.2	91.2	85.5	90.9	80.6
	Serum (>8 for ng/mL)	64.8	100	77.5	100	72.0
ES+VEGF	Fluid	90.5	70.0	81.0	83.5	85.8
	Serum	90.1	69.7	80.2	83.2	85.7
ES+CEA	Fluid	99.6	69.4	88.6	85.4	98.6
	Serum	99.0	67.9	85.2	83.2	95.8
ES+VEGF+CEA	Fluid	100.0	73.2	87.3	83.1	100.0
	Serum	100.0	72.5	88.6	84.5	100.0

ES=endostatin; VEGF=vascular endothelial growth factor; CEA=carcinoembryonic antigen; F/S=fluid/serum.

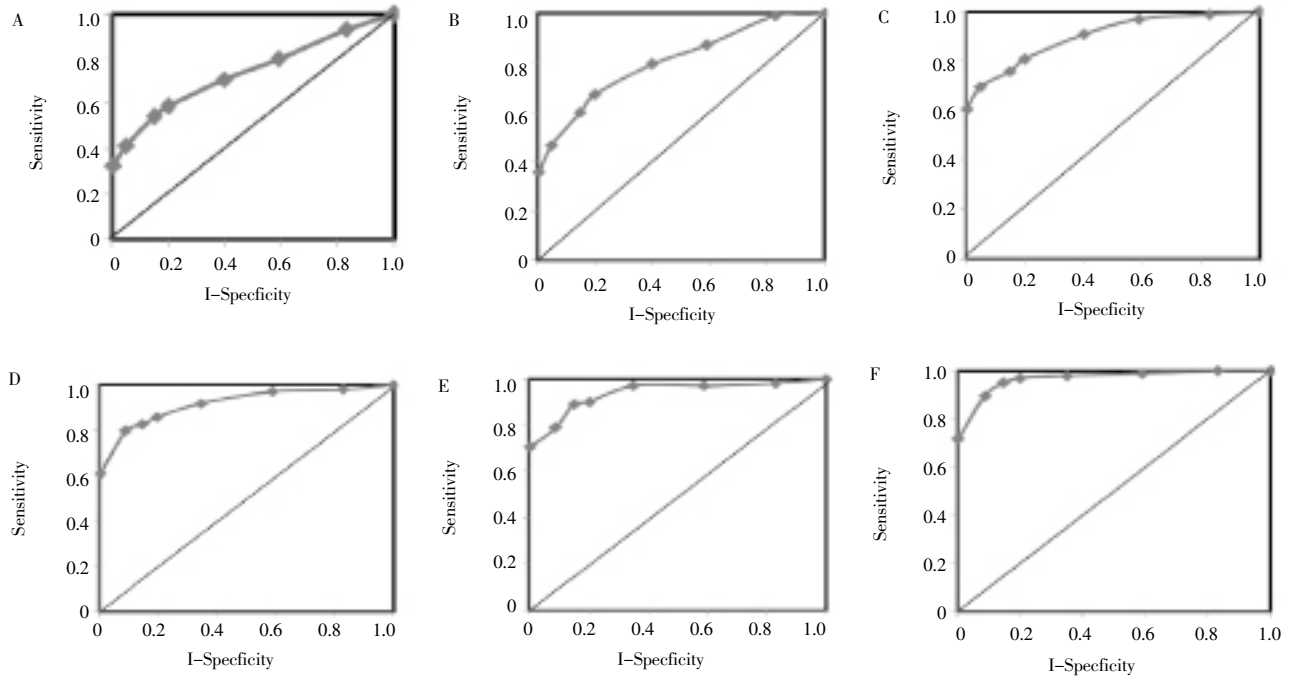

Figure 1. ROC curve in pleural effusion.

ES ROC curve (A), VEGF ROC curve (B), CEA ROC curve (C), ES+VEGF ROC curve (D), ES+CEA ROC curve (E), ES+VEGF+CEA ROC curve (F).

4. Discussion

The etiological diagnosis of pleural effusion is frequently a problem in clinical practice, especially in terms of -the differentiation between malignant and benign pleural effusion, due to the significant difference in the treatment and prognosis involved. Thoracentesis with cytopathologic study of the pleural fluid, the principal diagnostic method, presents great variation in its sensitivity (40%-60%), which is increased by up to 7% when a pleural biopsy is also performed[7,8]. Although surgical procedures (thoracoscopy and thoracotomy) present better diagnostic sensitivity for malignant cases (90%)[9], they are expensive and are not available at all medical centers. Recently, with the development of molecular biology in cancer, tumor markers were widely used in the diagnosis of pleural effusion. We investigated VEGF and ES in the blood and pleural fluid in patients with benign diseases or MPE due to lung cancer.

ES is a potent anti-angiogenic factor derived from the C-terminal region of collagen XVIII and is implicated in the regulation of physiological and pathological angiogenesis[10]. Some studies[11-13] have shown that ES can activate tyrosine kinase and induce endothelial cells to form various signaling complexes, thereby inducing tumor vascular endothelial cell into the process of apoptosis and inhibiting microvascular generation. In the process of tumor formation, new blood vessels provide nutrients for tumor cells, which make endothelial cells proliferate faster; as more tumor blood vessels grow, the tumor volume increases faster. In this scenario, the ES can effectively inhibit angiogenesis and tumor growth[14].

VEGF, as a vascular endothelial cell specific growth factor, has a role in promoting vascular endothelial cell mitosis.

In most solid tumor growth and metastasis processes, VEGF plays an important role in pleural metastasis and malignant pleural effusion[15,16]. VEGF significantly increases the permeability of the blood vessels, for which it is so-called vascular permeability factor. It has been shown that VEGF can induce angiogenesis also by stimulating endothelial cell mitosis and can activate matrix metalloproteinase by combining with specific receptors on the surface of endothelial cells, leading to endothelial cell proliferation; because new blood vessels are necessary conditions in tumor growth, invasion and metastasis, the VEGF-stimulated formation of plasma protein extravasation and cell matrix lead to the tumor formation, during which VEGF also releases growth factors to promote endothelial cell proliferation, migration, thereby promoting the proliferation of tumor blood vessels[17].

In recent years, VEGF and ES have been investigated for their diagnostic values in lung cancer. There have been some research about ES and VEGF in tumor samples and serum[6,11,18,19], but their levels in pleural effusion have not been fully evaluated. In this study, we detected VEGF and ES levels in serum and pleural effusion by ELISA for 52 lung cancer patients and 50 patients with benign diseases, and we compared the diagnostic efficiencies of VEGF and ES with CEA (the tumor marker which currently provides the best diagnostic accuracy[20]). The results show that in lung cancer group, the ES, VEGF, CEA levels in pleural effusion and serum, and their ratio(F/S) were significantly higher than that in benign group. The diagnostic efficiency of ES+VEGF for lung cancer was superior to that of any single detection. The combined detection of all three markers further improves the diagnostic sensitivity and accuracy.

Our findings demonstrate that determining serum and pleural fluid levels of the tumor markers ES, VEGF and CEA is useful in differentiating between benign and malignant pleural effusion due to lung cancer. In comparison with either single determination of concentration in serum or pleural fluid, the combined detection of two or three markers was of important clinical significance in the diagnosis of lung cancer. In clinical practice, when cytopathologic study of the pleural fluid is negative, the concomitant detection of serum and pleural levels of ES, VEGF and CEA may help clinicians decide whether to obtain a cytological/histological specimen by invasive means to investigate a possible diagnosis of malignancy.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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