

Diagnostic Significance of Measuring Vascular Endothelial Growth Factor for the Differentiation between Malignant and Tuberculous Pleural Effusion

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Malignancy and tuberculosis are common causes of lymphocytic exudative pleural effusion. However, it is occasionally difficult to differentiate malignant pleural effusion from tuberculous pleural effusion. Vascular endothelial growth factor (VEGF) is a critical cytokine in the pathogenesis of malignant pleural effusion. Endocan is a dermatan sulfate proteoglycan that is secreted by endothelial cells. Importantly, endocan mediates the vascular growth-promoting action of VEGF. The aim of this study was to evaluate the diagnostic significance of VEGF and endocan in pleural effusion. We thus measured the levels of VEGF and endocan in the pleural effusion and serum samples of patients with lung cancer ($n = 59$) and those with tuberculosis ($n = 32$) by enzyme-linked immunosorbent assay. Lung cancer included 40 cases of adenocarcinoma, 13 of squamous cell carcinoma, and 6 of small cell carcinoma. Pleural effusion VEGF levels were significantly higher in the malignant group than in the tuberculosis group ($2,091.47 \pm 1,624.80$ pg/mL vs. $1,291.05 \pm 1,100.53$ pg/mL, $P < 0.05$), whereas pleural effusion endocan levels were similar between the two groups (1.22 ± 0.74 ng/mL vs. 0.87 ± 0.53 ng/mL). The areas under the curve of VEGF and endocan were 0.73 and 0.52, respectively. Notably, the VEGF levels were similar in malignant pleural effusion, irrespective of the histological type of lung cancer. Moreover, no significant difference was found in the serum VEGF and endocan levels between patients with lung cancer and those with tuberculosis. In conclusion, high VEGF levels in pleural effusion are suggestive of malignant pleural effusion.

Keywords: endocan; malignancy; pleural effusion; tuberculosis; vascular endothelial growth factor
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Introduction

Malignancy and tuberculosis are among the more common causes of lymphocytic exudative pleural effusion (PE) (Wongtim et al. 1999). However, it is occasionally difficult to differentiate malignant PE from tuberculous PE. Usually, the first diagnostic step in the differential diagnosis of PE is to examine the pleural fluid obtained by thoracentesis. However, the sensitivity of conventional cytological examination is only 60% (Bennett and Maskell 2005). If malignancy is suspected and the pleural fluid cytology is negative, the clinical management is controversial. Invasive diagnostic procedures such as pleural biopsy or thoracoscopy are therefore needed; however, these procedures may

result in numerous complications such as pneumothorax, hemothorax, and possible cancer implantation in 4% of patients (Fiorelli et al. 2011). Given this situation, many biochemical markers have been proposed to improve the accuracy of diagnosis of malignant PE.

The vascular endothelial growth factor (VEGF) family is a family of endothelial growth factors (Zachary 2001) that possesses critical functions in angiogenesis (Senger et al. 1983), exerting a number of effects on the vascular endothelium including survival, proliferation, and differentiation (Ferrara and Henzel 1989; Ferrara et al. 2003; Hicklin and Ellis 2005). The role of VEGF in malignant PE is under investigation, with current data strongly implicating VEGF as a critical cytokine in the pathogenesis of

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malignant PE. VEGF production by intrathoracic lung cancer cells has been shown to contribute to PE production, tumor dissemination, and angiogenesis (Yano et al. 2000; Ishii et al. 2004).

Endocan, previously called endothelial-specific molecule-1, is a dermatan sulfate proteoglycan secreted by endothelial cells that has been suggested to have roles in the regulation of cell adhesion in inflammatory disorders and in tumor progression (Sarrazin et al. 2006). Recently, it was reported that endocan is expressed in tumor endothelium and that it induces tumor formation.

During tumor progression, a tumor that rapidly grows in size eventually becomes hypoxic. This activates hypoxia-inducible factor signaling, resulting in the secretion of VEGF from both tumor cells and tumor-associated stromal cells in an attempt to ensure that the tumor's oxygen requirements are met (Maurage et al. 2009). The vascular growth-promoting action of VEGF is mediated by endocan.

To the best of our knowledge, this is the first report examining the roles of VEGF and endocan in the differential diagnosis of pleural fluid of patients with tuberculous and malignant PE. In the present study, the levels of VEGF and endocan in the pleural fluid were analyzed simultaneously to evaluate their usefulness in differentiating malignant PE from PE of tuberculous origin.

Materials and Methods

Patient selection

A total of 91 patients with a definitive diagnosis of either malignant PE due to lung cancer ($n = 59$) or tuberculous PE ($n = 32$) were recruited into the study between January 2009 and July 2011 from the Department of Pulmonology, Wonkwang University Hospital. There were 61 men and 30 women, with a mean age of 63.92 ± 19.21 years (range, 17-94 years). Malignant PE caused by lung cancer was diagnosed on the basis of malignant cells detected during cytological examination of the pleural fluid and histocytologically proven by pleural biopsy. A diagnosis of tuberculous PE was based on the presence of any of the following: smear or culture positive for *Mycobacterium tuberculosis* from pleural fluid or tissue; sputum culture positive for *M. tuberculosis* in the presence of PE; and/or caseating granulomas positive for acid-fast bacilli in the pleural tissue as well as a positive response to antituberculosis treatment (Light 2010). All tuberculous PE patients were clinically free from malignant disease.

Study design

Thoracentesis was performed for diagnostic or therapeutic purposes, and pleural fluids were collected at the time of first tapping. All patients underwent a cytological examination of pleural fluids, a microbiological examination, and a routine biochemical analysis including tests for pleural protein, glucose, lactic dehydrogenase, adenosine deaminase, carcinoembryonic antigen, and cytokeratin 19 fragments (CYFRA). Blood and PE samples were collected within 24 hours of admission or on a symptomatic day before treatment, and centrifuged immediately at 4°C . Cell-free supernatants were collected as aliquots and stored at -80°C until analysis.

The serum and PE samples used in this study were provided by the Biobank of Wonkwang University Hospital, a member of the National Biobank of Korea. This study protocol was approved by the Institutional Review Board for Human Studies at the Clinical Research Center of Wonkwang University Hospital. All the recruited patients and volunteers provided written informed consent.

Measurement of VEGF and endocan in PE and serum samples

VEGF and endocan concentrations in PE and serum were measured using a sandwich enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN, USA) and an enzyme immunoassay kit (Chemicon International Inc., Temecula, CA, USA). The assays were conducted according to the manufacturer's guidelines. All samples were run in duplicate, with dilutions as appropriate, and the technicians were blinded to clinical data.

Statistical analyses

All the continuous data (e.g., age, VEGF and endocan) are presented as mean \pm standard deviation (SD). Statistical differences were based on a significance level of 0.05. All statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed using the Mann-Whitney U test for the comparison of VEGF and endocan levels between two groups. The diagnostic accuracy of VEGF and endocan in discriminating between malignant PE and tuberculous PE was compared by constructing receiver operating characteristic curves.

Results

Patient characteristics

As shown in Table 1, the study population included 32 patients with effusions defined as tuberculous PE and 59 with effusions defined as malignant PE due to lung cancer. Of the 32 patients with tuberculous PE, 22 were men (68.7%) and 10 were women (31.3%), with a mean age of 55.06 ± 22.86 years (range, 17-87 years). Among the patients with tuberculous PE, 14 were diagnosed by pleural fluid analysis, 12 demonstrated typical epithelioid cell caseous granulomas in the pleural biopsy, and the remaining 6 were diagnosed on the basis of cultured *M. tuberculosis* in the PE fluid. Of the 59 patients with malignant PE, 39 were men (66.1%) and 20 were women (33.9%), with a mean age of 68.73 ± 15.01 years (range, 31-94 years). All patients had lung cancer: there were 40 cases of adenocarcinoma, 13 of squamous cell carcinoma, and 6 of small cell carcinoma. Among the patients with malignant PE, 35 received a definitive diagnosis by pleural biopsy and 24 were diagnosed by cytology or cytospin.

There was no significant difference in pleural pH or lactic dehydrogenase and glucose levels between malignant and tuberculous PE. However, pleural protein was significantly higher in tuberculous PE ($P < 0.05$). The well-known lung cancer biomarkers carcinoembryonic antigen and CYFRA were present at significantly higher levels in malignant PE than in tuberculous PE. The adenosine deaminase level, which is important in making the diagnosis in tuberculous PE, was statistically significantly higher in tuberculous PE.

Table 1. Clinical and laboratory characteristics of patients with malignant pleural effusion (PE) and tuberculous PE.

	Malignant PE	Tuberculous PE	<i>P</i> value
Age, years (range)	68.73 ± 15.01	55.06 ± 22.86	< 0.05
Sex, male/female, n	39/20	22/10	NS
Pleural fluid			
pH	7.46 ± 0.49	7.43 ± 0.53	NS
Protein, g/dl	4.39 ± 1.00	5.18 ± 0.68	< 0.05
LDH, IU/L	954.29 ± 1420.50	905.28 ± 413.81	NS
Glucose, mg/dl	116.53 ± 51.78	109.87 ± 78.07	NS
Neutrophil, %	15.96 ± 19.66	12.40 ± 18.13	NS
Lymphocyte, %	81.53 ± 26.90	87.74 ± 18.13	NS
ADA, U/L	11.56 ± 7.18	55.79 ± 13.01	< 0.05
CEA, ng/mL	371.95 ± 795.60	2.10 ± 1.64	< 0.05
CYFRA, ng/mL	174.16 ± 282.34	44.07 ± 42.24	< 0.05

Data are presented as the medians (interquartile ranges). LDH, lactic dehydrogenase; ADA, adenosine deaminase; CEA, carcinoembryonic antigen; CYFRA, cytokeratin 19 fragments.

VEGF and endocan levels in PE

The levels of VEGF and endocan are presented in Table 2. In both malignant PE and tuberculous PE, the concentrations of pleural fluid VEGF and endocan were significantly higher than the serum levels of VEGF and endocan ($P < 0.001$). The levels of VEGF were found to be significantly higher in malignant PE than in those of tuberculous PE ($P < 0.05$). Notably, the VEGF levels were similar in malignant PE, irrespective of the histological type of lung cancer. The levels of endocan were higher in malignant PE than in those of tuberculous PE, but the difference was not statistically significant ($P = 0.059$).

Receiver operating characteristic analysis

To evaluate whether the levels of VEGF and endocan in pleural fluid could discriminate between malignant and tuberculous PE, receiver operating characteristic curves were constructed by plotting the sensitivity against 1-specificity. The receiver operating characteristic curves for carcinoembryonic antigen, CYFRA, VEGF, and endocan are shown in Fig. 1. The areas under the curve of VEGF and endocan were 0.73 and 0.52, respectively.

Discussion

The etiological diagnosis of PE is frequently a problem in clinical practice, especially in terms of the differentiation between malignant and tuberculous PE, owing to the significant differences in the treatment and prognosis involved. In the present study, the diagnostic performance of VEGF and endocan levels in PE fluid of patients with tuberculous and malignant PE was evaluated.

During the course of tumor growth and metastasis, angiogenesis is essential. VEGF is well established as a key regulator of this process. VEGF is a multifunctional cytokine that increases vascular permeability, and it is an important angiogenic and lymphogenic factor (Light and Hamm 1997; Grove and Lee 2002). VEGF plays a pivotal role in the formation of malignant PE, as it increases vascular permeability and vascular leakage of fluid (Kraft et al. 1999; Zebrowski et al. 1999). In addition, a high level of pleural VEGF has been found to be correlated with malignancy (Ishimoto et al. 2002), and thus, an increasing number of studies consider VEGF to be a marker for the diagnosis of malignant PE (Lim et al. 2000; Kishiro et al. 2002;

Table 2. Vascular endothelial growth factor (VEGF) and endocan in serum and pleural effusion (PE) fluid in differentiating malignant and tuberculous PE.

	Malignant PE	Tuberculous PE	<i>P</i> value
Serum			
VEGF, pg/mL	288.48 ± 523.07	274.03 ± 323.84	NS
Endocan, ng/mL	0.58 ± 0.33	0.51 ± 0.40	NS
Pleural fluid			
VEGF, pg/mL	2091.47 ± 1624.80	1291.05 ± 1100.53	< 0.05
Endocan, ng/mL	1.22 ± 0.74	0.87 ± 0.53	0.059

Data are presented as the medians (interquartile ranges). VEGF, Vascular endothelial growth factor.

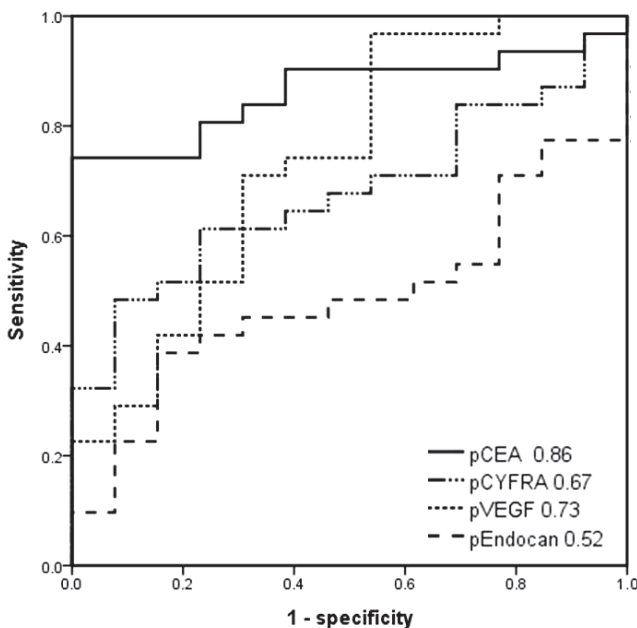


Fig. 1. Receiver operating characteristic curves for the diagnostic accuracy in discriminating malignant and tuberculous PE.

The receiver operating characteristic curve was constructed by plotting the sensitivity versus 1-specificity at different cutoff values for each of the indicated markers in pleural effusion, shown as pCEA, pCYFRA, pVEGF and pEndocan.

Hamed et al. 2004). However, conflicting results have been reported (Sack et al. 2005), and the exact role of VEGF as a diagnostic marker remains unclear. The results of meta-analysis suggest that VEGF may play a role in the diagnosis of malignant PE, while its diagnostic value is not satisfactory (Shen et al. 2012). The combination of VEGF with other markers may aid in the establishment of the diagnosis

of malignant PE.

Endocan is a secreted endothelial cell-restricted dermatan sulfate proteoglycan (Ho et al. 2003), which is preferentially expressed in lung and kidney tissues (Lassalle et al. 1996). Endocan has potential roles in the control of tumor growth and development, as well as in angiogenesis during tumor progression. In recent years, endocan has been investigated for its diagnostic and prognostic value in lung cancer. In non-small cell lung cancer patients, serum endocan values were found to be significantly elevated compared with healthy controls. High endocan values were significantly correlated with the presence of metastasis and with limited survival (Grigoriu et al. 2006). However, endocan levels in PE fluid have not been fully evaluated. Although the exact regulatory mechanism of endocan production is not well established, recent studies suggest that a number of signaling pathways and bioactive mediators are involved. The expression of endocan is upregulated by VEGF-A, VEGF-C, tumor necrosis factor- α , and transforming growth factor- β 1 (Lassalle et al. 1996; Rennel et al. 2007; Maurage et al. 2009; Delehedde et al. 2013). Because of these relations between endocan and VEGF, we compared the diagnostic efficiencies of VEGF and endocan with carcinoembryonic antigen (the tumor marker that currently provides the best diagnostic accuracy (Shi et al. 2008) and CYFRA. Our results show that in the malignant PE group, the VEGF levels in PE fluid were significantly higher than those in tuberculous PE fluid, while endocan levels showed a trend toward being higher in malignant PE ($P = 0.059$). We demonstrated in the present study that the levels of accuracy of VEGF and endocan for distinguishing malignant PE from tuberculous PE were 0.73 and 0.52, respectively. Both VEGF and endocan have low sensitivity and specificity in the diagnosis of malignant PE. To the best of our knowledge, this is the first report on the deter-

mination of VEGF and endocan pleural fluid levels in patients with malignant and tuberculous PE.

The main limitation of the present study is that it is a retrospective study with a comparatively small sample size. Furthermore, the malignant PE originated only from lung cancer. Therefore, we do not know whether the pleural fluid levels of VEGF and endocan can be used as indicators of malignant PE of other origins. In spite of the relatively high values of statistical significance obtained, further research involving a larger group of patients with malignant PE of various origins will be required to confirm the validity of our results.

In conclusion, the present study indicates that the pleural fluid levels of VEGF are significantly higher in malignant PE than in tuberculous PE, suggesting that high VEGF levels in PE are suggestive of malignant PE. These findings regarding VEGF and endocan may be important and deserve further research to clarify the role of angiogenesis in PE.

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Conflict of Interest

The authors declare no conflict of interest.

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