

## Vascular endothelial growth factor and soluble intercellular adhesion molecule-1 in lung adenocarcinoma with malignant pleural effusion: Correlations with patient survival and pleural effusion control

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The mechanisms by which vascular endothelial growth factor (VEGF) and soluble intercellular adhesion molecule-1 (sICAM-1) contribute to lung cancer growth have not been fully elucidated. This study aimed to assess the role of VEGF and sICAM-1 in control of pleural effusions (PE) and survival in patients with primary human lung adenocarcinoma.

Using enzyme-linked immunoadsorbent assay, the concentrations of VEGF and sICAM-1 were measured in pleural effusions and serum from a total of 79 lung adenocarcinoma patients with malignant pleural effusions (MPE) and 24 patients with tuberculosis. Data were correlated with the efficacy of MPE control and survival.

Compared to patients with tuberculosis, the levels of VEGF and sICAM-1 in both PE and serum were significantly higher in patients with lung adenocarcinoma. Statistically significant correlation was observed between PE VEGF levels and MPE control. PE VEGF $\geq$ 2760 pg/ml was used as a cut-off point for failure to MPE control (odds ratio=7.06; 95% confidence interval (CI), 2.40-20.78;  $P < 0.001$ ). The median progression-free survival (PFS) from response assessment was 3 months. In a multivariate analysis, PE VEGF (hazard ratio [HR], 1.16; 95% CI, 1.02-1.32), serum sICAM-1 (HR, 1.90; 95% CI, 1.17-3.07) were confirmed as independent prognostic factors for PFS.

The levels of VEGF in PE can be used to predict the therapeutic efficacy in the control of MPE and this, together with serum level of sICAM-1 is potential survival factors in lung adenocarcinoma patients with MPE.

*Key words: vascular endothelial growth factor, soluble intercellular adhesion molecule-1, malignant pleural effusion, lung adenocarcinoma, survival*

Malignant pleural effusion (MPE) represents a common (500 new cases/million population/yr) clinical presentation predominantly found in patients with lung adenocarcinoma [1, 2]. The presence of MPE is an important sign of recurrent or advanced malignancies and has short life expectancy [3, 4]. In spite of its frequent occurrence in clinical situation, limited studies have been reported on the management of MPE and patients in general have little improvement from available therapies. Thus, understanding of the molecular mechanisms of MPE pathophysiology is important and may provide more safer and effective therapeutic options.

Study of the molecular mechanisms of MPE have found that new blood vessel formation (angiogenesis) and increased permeability of existing and newly formed blood vessels are important in the development of MPE [5]. The vascular en-

dothelial growth factor (VEGF) pathway is critical in tumor angiogenesis and becomes an important therapeutic target. The VEGF family, by binding to their cognate tyrosine kinase receptors, stimulates endothelial cell proliferation and migration, increases permeability of existing blood vessels in the tumor microenvironment [6]. Several lines of evidence suggest that expression of VEGF in peripheral lung tumors is important in the development of MPE [7, 8].

Elevated serum or plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) have been reported in patients with several human malignancies [9, 10] and the presence of sICAM-1 correlated with tumor progression and metastasis [11-13]. In non-small cell lung cancer patients, increased level of sICAM-1 was correlated with the patient short survival and poor response to chemotherapy [14]. One

of the underlying mechanisms is that sICAM-1 can bind to circulating cytotoxic lymphocytes, thereby helping tumors to escape immune recognition. In addition, sICAM-1 may act as an angiogenic factor to promote angiogenesis and stimulate tumor cell growth [15, 16]. Nevertheless, the role of sICAM-1 in the development of MPE has not been fully studied. Because the poor survival of lung adenocarcinoma patients with MPE, we thereby examined the role of sICAM-1 in this malignancies.

Treatment decisions in routine practice depended mainly on awareness of main prognostic determinants. For MPE patients, evacuation of the pleural fluid, prevention of re-accumulation and prolong survival are the main goals of management [17-19]. In the current study, we focused on these two important factors to determine the possible role in the clinical outcomes of patient survival and MPE control.

## Patients and methods

**Patients.** The PE and serum samples from two different groups of patients were collected. The first group was com-

prised of tuberculosis patients with PE (n=24). The diagnosis of TB was based on a positive culture from the PE or a pleural biopsy. The second group were patients with MPE-associated lung adenocarcinoma (n=79). Patients with previously untreated lung adenocarcinoma with MPE were enrolled prospectively into this study at two institutions (Nanjing Chest Hospital and Jinling Hospital, Nanjing, China) between January 2009 and July 2010. To be eligible for MPE-associated lung adenocarcinoma, patients were verified by cytological analysis or a pleural biopsy; an Eastern Cooperative Oncology Group performance status (PS) from 0 to 2; normal hepatic, renal, and hematologic function and no concomitant serious comorbidities were found.

Eligible patients were initially performed therapeutic thoracentesis. Chemical pleurodesis was attempted after completed expansion of the lung followed by pleural fluid evacuation with no evidence of trapped lung. The pleurodesing agent was bleomycin. At the same time, at least two cycles of cisplatin and gemcitabine combination chemotherapy was repeated every 3 weeks.

The treatment response of malignant effusion was evaluated according to the following criteria: (1) complete response, no fluid reaccumulation and patients were free of symptoms for at least 4 weeks as determined by chest radiography, ultrasonography and/or CT scan and clinical evaluation; (2) partial response, recurrence of effusion to less than 50% of the original effusion volume, patients were asymptomatic and no need for thoracentesis for symptom relief within 4 weeks after treatment; (3) failure to response, recurrence of effusion greater than 50% of the original volume, patients were symptomatic and need for thoracentesis to relieve symptoms within 4 weeks of treatment. Objective response of malignant effusion included complete response and partial response [21].

The study protocol was approved by Jinling Hospital Medical Ethical Committee. All patients provided written informed consent before study-related procedures were performed.

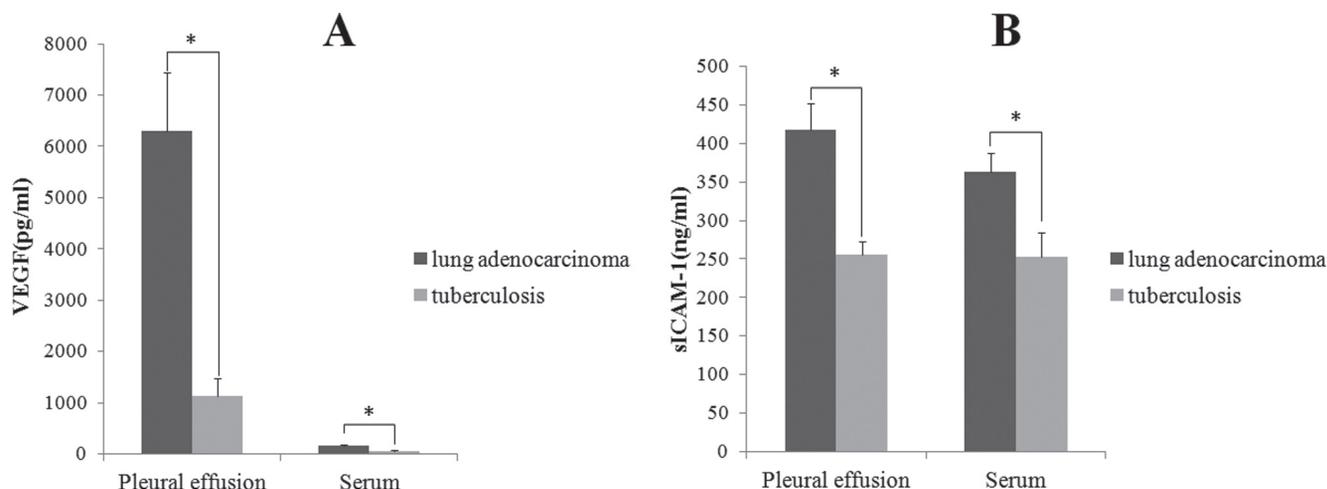
**Methods.** Specimens of peripheral blood and effusions were collected before pleurodesis and chemotherapy. Effusions and sera were obtained in sterile tubes and centrifuged immediately at 4°C. Cell-free supernatants were collected and aliquots were stored frozen at -70°C until use. Effusions and sera VEGF and sICAM-1 levels were measured with the commercially available VEGF and sICAM-1 ELISA kits obtained from R&D Systems (Minneapolis, USA) according to the manufacturer's instructions.

**Statistical methods.** Statistical analyses were performed using by SPSS 13 for Windows. Differences in the values among the groups were determined by analysis of variance (ANOVA) procedure or nonparametric Mann-Whitney U-test and correlation analysis of different covariates was used with the Spearman rank-order correlation. In order to find the cut-off value and to evaluate and compare the performance of factors in predicting response to therapy, receiver operating characteristic (ROC) curves and Youden Index were constructed. The Kaplan-Meier method was used to

**Table 1. Lung adenocarcinoma patients Characteristics.**

Variable	No.of patients	%
Characteristic of lung adenocarcinoma patients	79	
Gender		
Men	48	61
Women	31	39
age, y		
<65	34	43
≥65	45	56
Smoking history		
never smoker	33	42
former smoker	24	30
smoker	22	28
Stage		
M1a	55	70
M1b	24	30
ECOG PS		
0	37	47
1	33	42
2	9	11
Characteristic of tuberculosis patients	24	
Gender		
Men	19	79
Women	5	21
age, y		
<65	20	83
≥65	4	17
Smoking history		
never smoker	7	29
former smoker	12	50
smoker	5	21

ECOG PS: Eastern Cooperative Oncology Group performance status



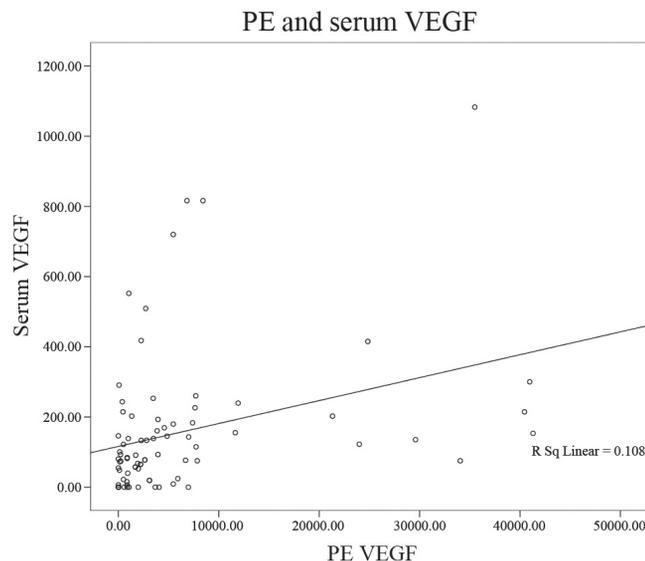
**Figure 1.** The levels of VEGF (A) and sICAM-1 (B) for 79 lung adenocarcinoma and 24 tuberculosis patients in serum and pleural effusion. (A) The levels of VEGF in both PE and serum were significantly higher in lung adenocarcinoma patients than that in patients with tuberculosis (for PE:  $6299.28 \pm 1141.33$  and  $1131.94 \pm 354.38$  pg/ml,  $p < 0.001$ ; for serum:  $157.34 \pm 22.64$  pg/ml and  $64.42 \pm 13.59$  pg/ml,  $p = 0.018$ ). (B) the levels of sICAM-1 in both PE and serum were significantly higher in lung adenocarcinoma patients than that in patients with tuberculosis patients (for PE:  $418.38 \pm 34.46$  and  $255.06 \pm 18.19$  ng/ml,  $p = 0.016$ ; for serum:  $363.88 \pm 23.11$  ng/ml and  $252.39 \pm 32.47$  ng/ml,  $p = 0.013$ ). \* indicates: difference of mean values between lung adenocarcinoma and tuberculosis groups by nonparametric Mann-Whitney U-test ( $P < 0.05$ ). VEGF: vascular endothelial growth factor; sICAM-1: soluble intercellular adhesion molecule-1; PE: pleural effusions.

estimate survival and progression-free survival (PFS) distributions. Log-rank tests were used for univariate comparisons of survival and PFS end points. Cox proportional hazards regression model was used in the multivariate analysis to assess the prognostic potential of covariates adjusted for the possible confounding effects of all other factors. Progression-free survival was defined as time from diagnosis to documented progression or death, censored at the date of the last documented disease evaluation for patients without a PFS event reported. A value of  $p < 0.05$  was considered significant.

**Results**

**Measurement of factor levels in PE and serum.** The main characteristics of patients were summarized in Table 1. For both PE and serum, there were significant differences of VEGF and sICAM-1 levels between the overall mean of lung adenocarcinoma and tuberculosis (Figure 1). The levels of VEGF in both PE and serum were significantly higher in lung adenocarcinoma patients than that in patients with tuberculosis (for PE:  $6299.28 \pm 1141.33$  and  $1131.94 \pm 354.38$  pg/ml,  $p < 0.001$ ; for serum:  $157.34 \pm 22.64$  pg/ml and  $64.42 \pm 13.59$  pg/ml,  $p = 0.018$ ). Similarly, the levels of sICAM-1 in both PE and serum were significantly higher in lung adenocarcinoma patients than that in patients with tuberculosis patients (for PE:  $418.38 \pm 34.46$  and  $255.06 \pm 18.19$  ng/ml,  $p = 0.016$ ; for serum:  $363.88 \pm 23.11$  ng/ml and  $252.39 \pm 32.47$  ng/ml,  $p = 0.013$ )

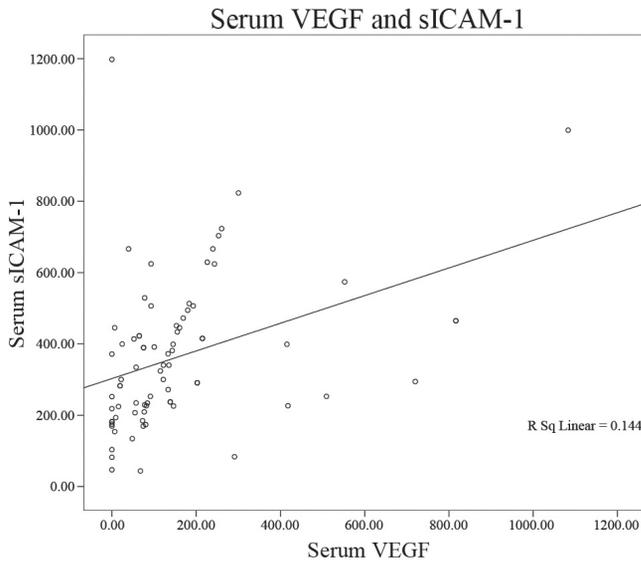
We found that no correlations were observed between PE VEGF or sICAM-1 levels and gender, ages, smoking, stages and



**Figure 2.** Correlation between VEGF in PE and serum for lung adenocarcinoma patients. When performing a Spearman rank-order correlation, we found the statistically significant correlation between VEGF values in PE and serum ( $R = 0.499$ ,  $p < 0.001$ ). VEGF: vascular endothelial growth factor; PE: pleural effusions.

PS. Similarly, there was no association between serum VEGF or sICAM-1 levels and these clinical characteristics.

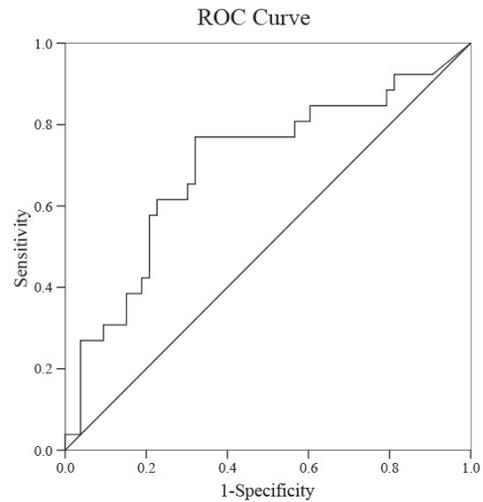
We found also the following statistically significant correlations: VEGF values in PE and serum ( $R = 0.499$ ,  $p < 0.001$ ) (Figure 2) and the VEGF and sICAM-1 values in serum



**Figure 3.** sICAM-1 correlated with VEGF in serum for lung adenocarcinoma patients. When performing a Spearman rank-order correlation, we found the statistically significant correlation between the VEGF and sICAM-1 values in serum ( $R=0.512$ ,  $p<0.001$ ). VEGF: vascular endothelial growth factor; sICAM-1: soluble intercellular adhesion molecule-1.

( $R=0.512$ ,  $p<0.001$ ) (Figure 3). However, no association was observed between sICAM-1 values in PE and serum ( $p=0.453$ ) and the VEGF and sICAM-1 values in PE ( $p=0.753$ ).

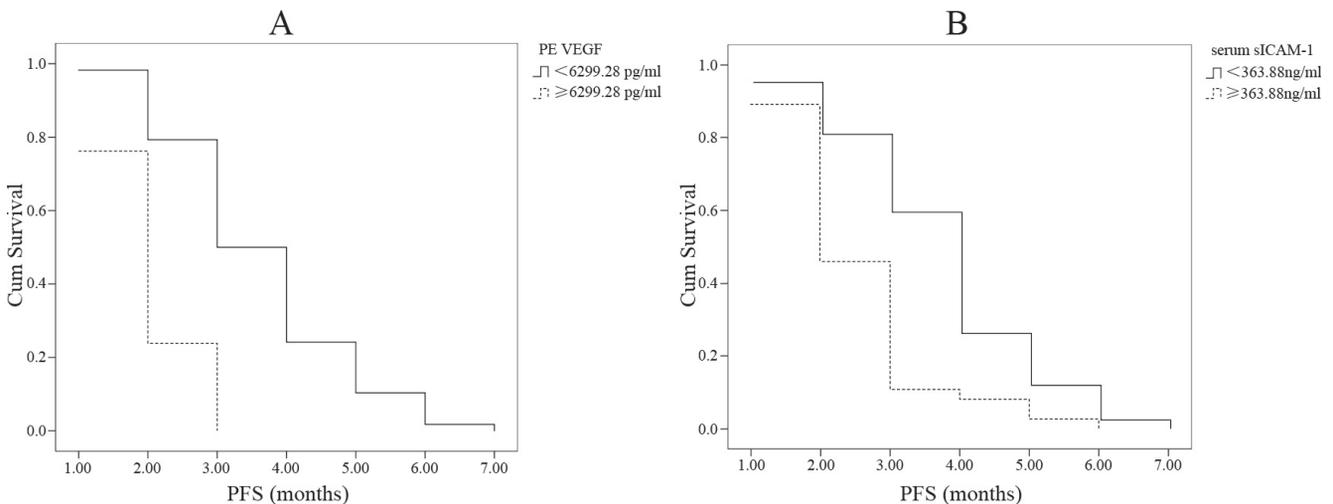
**Association between PE VEGF level and MPE control.** For MPE control, postero-anterior chest radiograph or ultrasonography was obtained as the baseline study and compared in every 3 weeks. Patients were divided into objective response



**Figure 4.** Receiver-operating characteristic curve analysis to assess the value of VEGF in malignant pleural effusions that predicted an Objective response to pleural effusions control. The area under the ROC curve was 0.70 (95% CI, 0.57-0.83).

(67%) and failure groups (33%). VEGF level in MPE was the only one significant factor for effusion control when comparing with other demographic and laboratory data (age, gender, smoking, stage, PS, serum VEGF, PE and serum sICAM-1) ( $P=0.005$ ).

A receiver operating characteristic (ROC) curve analysis was carried out to assess whether PE levels of VEGF could predict an OR to MPE control (Figure 4). The area under the ROC curve was 0.70 (95% CI (confidence interval), 0.57-0.83). The best efficacy was observed at 2760 pg/ml, with a sensitivity



**Figure 5.** Kaplan-Meier survival curve of PE VEGF (A), serum sICAM-1 (B). (A) Patients with a PE VEGF level  $<6299.28$  pg/ml survived longer than those who had PE VEGF level  $\geq 6299.28$  pg/ml (3.64 months vs. 2.00 months,  $P<0.001$ ). (B) Patients who had serum sICAM-1  $<363.88$  ng/ml (the mean value of serum sICAM-1) survived longer than patients who had serum sICAM-1  $\geq 363.88$  ng/ml (3.76 months vs. 2.57 months,  $P<0.001$ ).

of 77% and a specificity of 68% (positive predictive value, 54%; negative predictive value, 86%). Based on the ROC curve and Youden index analysis, MPE VEGF $\geq$ 2760 pg/ml was used as a poor indicator for effusion control as this was the value with the best efficiency in terms of sensitivity and specificity. Logistic analysis confirmed the correlation between PE VEGF and OR to MPE control (odds ratio=7.06; 95% confidence interval, 2.40-20.78; P<0.001).

**Survival analysis according to factor levels.** For the 79 lung adenocarcinoma patients with MPE, the median PFS from the landmark date was 3 months. In the univariate analysis, a statistically significant prognostic impact on PFS from the landmark date for PS and disease stage, PE VEGF, serum sICAM-1 were observed. Patients with a PE VEGF level<6299.28 pg/ml (the mean value of MPE VEGF) survived longer than those who had PE VEGF level $\geq$ 6299.28 pg/ml (3.64 months vs. 2.00 months, P<0.001). Patients who had serum sICAM-1<363.88 ng/ml (the mean value of serum sICAM-1) survived longer than patients who had serum sICAM-1 $\geq$ 363.88 ng/ml (3.76 months vs. 2.57 months, P<0.001). The landmark survival curves for PE VEGF (A), serum sICAM-1 (B) were shown in Figure 5.

When a Cox multivariate regression model was applied, a statistically significant prognostic impact on PFS from the landmark date for PE VEGF and serum sICAM-1 were observed (Table 2). We found that independently prognostic roles of PE VEGF [hazard ratio (HR), 1.16; 95% CI, 1.02-1.32], serum sICAM-1 (HR, 1.90; 95% CI, 1.17-3.07) were confirmed. No correlations were observed in patient survival and the serum VEGF or sICAM-1 levels.

**Discussion**

We examined VEGF and sICAM-1 in patients with tuberculosis and MPE-associated lung adenocarcinoma and showed that the levels in PE and serums were significantly higher in patients with lung adenocarcinoma than in patients

with tuberculosis. Based on our data, we found that there are good linear correlations between VEGF level in PE and in serum, and between VEGF and sICAM-1 values in serum. The results from this study support the hypothesis that PE VEGF $\geq$ 2760 pg/ml could be used as a poor indicator for MPE control. More importantly, for MPE-associated lung cancer patients, PE VEGF and serum sICAM-1 levels were considered as strong and independent predictors of PFS therapy in our multivariate analysis.

Several lines of studies agreed with our results demonstrating that VEGF levels are higher in malignant effusions from various cancers (including lung carcinomas, breast and mesotheliomas [22]) than that in benign diseases [23-26]. These results demonstrated that VEGF expression was associated with MPE. Reliable animal experiments [7] showed that the production of MPE required tumor cells to invade the pleura and to express high levels of VEGF. They found that when lung cancer cells were injected into mice, the level of VEGF expression directly correlated with the amount of MPE. Besides, the vascular permeability and pleural fluid formation induced by cancer cells can be significantly reduced when the cells are transfected with antisense VEGF<sub>165</sub> gene. Furthermore, other reports also demonstrated that administration of VEGF antagonists significantly reduced MPE formation in mice, all of which indicated the essential role of VEGF in MPE formation [8, 27]. However, VEGF values in PE has limited efficacy as a diagnostic tool as values between malignant and benign effusions could be overlapped [23, 24].

Current treatment options of MPE such as indwelling pleural catheters or chemical-induced pleural fibrosis, aimed to drain or eliminate the pleural space and had little benefit for a significant number of patients [28, 29]. For patients with MPE, understanding the treatment efficacy and the prognostic determinants are critical to adjust treatment options. Our data suggested that PE VEGF might be a reliable marker to assess MPE control outcome and to refine prognosis of PFS for lung adenocarcinoma patients with MPE.

**Table 2. Survival of patients according to several variables.**

Characteristic	p	HR	95% CI for HR	
			Lower	Upper
Gender	0.18	0.65	0.34	1.22
Age	0.09	0.61	0.35	1.07
Stage	0.75	0.90	0.47	1.72
Smoking	0.46	1.27	0.68	2.40
ECOG PS	0.21	1.31	0.86	2.00
MPE control	0.68	0.88	0.48	1.62
PE VEGF	0.02	1.16	1.02	1.32
Serum VEGF	0.97	1.00	0.85	1.17
PE sICAM-1	0.53	0.92	0.69	1.21
Serum sICAM-1	0.01	1.90	1.17	3.07

ECOG PS: Eastern Cooperative Oncology Group performance status; sICAM-1: soluble intercellular adhesion molecule-1; VEGF: vascular endothelial growth factor. p<0.05 was considered significant.

On the other hand, we found significantly elevated serum levels of VEGF in patients with MPE-associated lung adenocarcinoma compared with tuberculosis. Although the prognostic role of serum VEGF level has been supported by a strong rationale but several other studies published data [14, 30] including ours did not confirm this. Of course, there are some limitations in our study. Studies found that both white blood cell and platelet counts correlated with serum VEGF [31, 32]. Serum VEGF level differed depending upon the counts of platelet and white blood cells. There is some uncertainty regarding serum VEGF levels with MPE treatment efficacy and the survival significance of a high serum VEGF level. Therefore, more and large multicenter randomized studies including serum VEGF levels of patients with lung cancer are needed to further resolve this uncertainty and to increase our knowledge and experiences in separating patients.

In this study, we demonstrated that serum sICAM-1 was an independent prognostic indicator for PFS. This result is in concordance with a randomized study [14], which evaluated the combination of chemotherapy and bevacizumab and showed that serum baseline level of sICAM-1 was prognostic for survival and predictive of response to chemotherapy with or without bevacizumab. The release of sICAM-1 is modulated by several cytokines and various growth factors. One in vitro study [33] using cultured endothelial cells showed that sICAM-1 levels reflected ICAM-1 expression on these cells and endothelial cells were an important source. Although the physiological role of sICAM-1 was not completely understood, previous reports hypothesized that sICAM-1 might perform dual functions that were essential for tumor growth by inducing angiogenesis [15, 16] and might facilitate escape of tumor cells from immune surveillance [9]. Therefore, patients with better PFS may have lower circulation sICAM-1 levels. However, no correlations were observed in MPE treatment efficacy and serum or MPE sICAM-1. The mechanism of this phenomenon is still unclear, more in-depth studies are needed to explore the nature of this phenomenon.

In summary, our study indicates that measurement of PE VEGF as a surrogate marker prior to chemical pleurodesis can be used to assess therapeutic efficacy and predict prognosis in lung adenocarcinoma patients with MPE. Also, serum sICAM-1 is a significant negative PFS marker of patients with MPE-associated lung adenocarcinoma. We realize that the cohort size of patients in this study is small and this limits the power of multivariate analyses. Nevertheless, our data warrants validation and refinement in larger scale prospective trials in the future.

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