Levels of Vascular Endothelial Growth Factor in Serum and Pleural Fluid Are Independent Predictors of Survival in Advanced Non-small Cell Lung Cancer: Results of a Prospective Study

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Abstract. *Aim: To further evaluate the prognostic significance of pre-treatment serum and pleural fluid levels of vascular endothelial growth factor VEGF in patients with non-small cell lung cancer (NSCLC) presenting with malignant pleural effusion (MPE). Patients and Methods: Forty consecutive newly-diagnosed patients with NSCLC with MPE at presentation but without distant metastases were prospectively enrolled. The prognostic value of serum and pleural fluid VEGF levels for overall survival (OS) and progression-free survival (PFS) was assessed by Cox regression analysis. Results: The median serum VEGF level was significantly higher in patients compared to healthy controls ($p<0.001$). Serum VEGF higher than 375 pg/ml, pleural fluid VEGF greater than the median value and the presence of progressive disease were all significantly associated with reduced OS and PFS, both in univariate and multivariate analyses. Conclusion: The results of our study suggest that increased pre-treatment serum and pleural fluid levels of VEGF may be independent predictors of a worse survival in patients with advanced-stage NSCLC.*

Non-small cell lung cancer (NSCLC) is an aggressive malignancy, typically characterized by rapid growth, tendency for early metastatic spread and resistance to conventional chemotherapy. The majority of cases present with locally advanced or distant metastatic disease at the time of diagnosis, precluding curative surgical resection of the primary tumor (1). Despite recent advances in treatment of advanced NSCLC, including the advent of patient-tailored targeted-therapies, the overall prognosis of these patients remains poor, with a median survival of less than 12 months in most series (2).

Angiogenesis is defined as the growth of new blood vessels stemming from pre-existing vasculature, and is a dynamic process controlled by a fine balance between proangiogenic and antiangiogenic factors (2). As shown by experimental and clinical data, angiogenesis plays a critical role not only in physiological events (such as embryonal development and wound healing), but also in the development, growth and metastasis of a variety of solid tumors, including NSCLC (3-5). Vascular endothelial growth factor (VEGF) is among the key proangiogenic signaling proteins involved in regulation of endothelial cell proliferation and migration, vascular permeability and stromal degradation, thereby enhancing the formation of new blood vessels, penetration of tumor cells through vessel walls and their metastatic dissemination (6-10). In recent years, immunohistochemical evaluation of VEGF expression in tumor tissue (11-13), as well as quantitative measurement of VEGF levels in the plasma, serum or other body fluids of patients with NSCLC (3, 6, 14-18), have attracted considerable research interest as potential indicators of treatment response and overall prognosis. Likewise, from a therapeutic standpoint, VEGF-targeted agents, such as the monoclonal antibody to VEGF bevacizumab, are increasingly used in combination with chemotherapy for treatment of advanced NSCLC (19, 20).

Most, but not all, previous studies have suggested that higher pre-treatment serum VEGF levels may be associated with reduced survival in patients with NSCLC (2, 3, 14, 16, 21), in accordance with the results of similar studies on other solid tumor types (22-24). However, the independent prognostic value of this biomarker remains poorly-established

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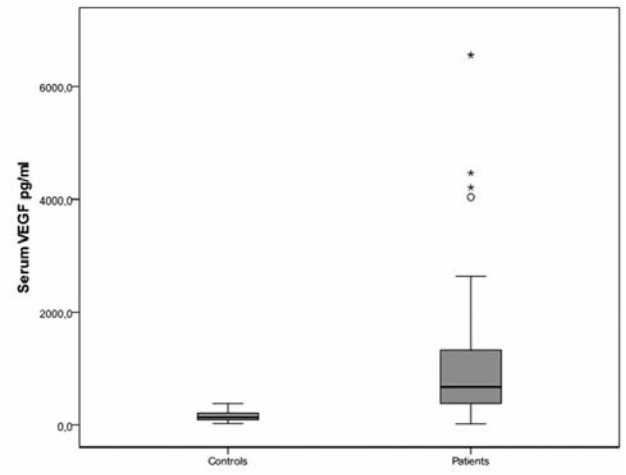

Figure 1. *Box plots for serum vascular endothelial growth factor levels in patients and controls. Top and bottom of box represent 75th and 25th percentile, respectively; middle bar in box represents median value; top and bottom lines extending from box represent maximum and minimum observed values, respectively.*

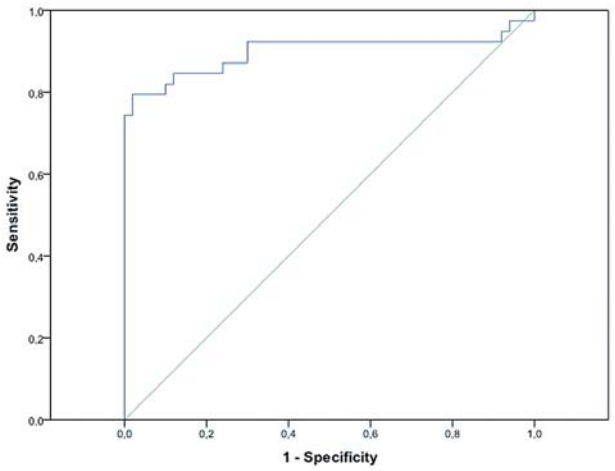

Figure 2. *Receiver operating characteristic (ROC) curve of serum vascular endothelial growth factor for the discrimination between patients and controls.*

and largely controversial, while little is known about the potential clinical relevance and prognostic significance of VEGF levels in pleural fluid.

The primary objective of the present study was to prospectively evaluate the potential prognostic value of pre-treatment VEGF levels in serum and pleural fluid from patients with NSCLC presenting with malignant pleural effusion (MPE). We also aimed to investigate the diagnostic utility of serum VEGF levels in discriminating between patients with NSCLC and healthy individuals.

Patients and Methods

Study population. We prospectively studied 40 consecutive patients newly diagnosed with NSCLC who were referred to the Oncology Unit GPP of Sotiria General Hospital, Athens, Greece, between September 2009 and September 2013. Fifty age- and sex-matched healthy controls were also included in the study. The study protocol was approved by the Institutional Ethics Committee (approval number: 2230) and written informed consent was obtained from all patients and controls. Inclusion criteria were defined as follows: histologically- or cytologically-confirmed diagnosis of NSCLC, age >18 years, presence of MPE at diagnosis, absence of distant metastases and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of less than 2. Patients who had been previously treated for NSCLC or patients with other concomitant malignancy were excluded from the study. Tumors were classified using the World Health Organization (WHO) histological classification (25). Staging was carried out according to the seventh edition of the American Joint Committee of Cancer (AJCC) TNM classification system (26). Standard staging procedures were used, including a complete history and physical examination, blood tests, chest x-ray, computed tomography (CT) of the chest and abdomen and CT or magnetic resonance imaging of the brain; bone involvement was documented by plain radiography or bone scintigraphy.

All patients were treated with platinum-based chemotherapy regimens without bevacizumab, with or without palliative radiotherapy, as determined by the attending physician according to the needs of individual patients. Follow-up evaluations (clinical examination, CT scan and routine laboratory investigations) were carried out at 3-month intervals. Response to chemotherapy was assessed using Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1) (27), and was classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). Progression-free survival (PFS) was calculated from the start date of the first chemotherapy cycle to the time of first documentation of PD or until death by any cause. Overall survival (OS) was defined as the time from diagnosis to the date of death by any cause.

Collection of samples and measurement of VEGF levels. Blood samples and pleural fluid specimens were collected from all cases prior to the initiation of any therapeutic intervention. Briefly, peripheral venous blood samples were collected from patients and controls after overnight fasting and were allowed to coagulate at room temperature for 30 min. Serum was separated by centrifugation at 2000×g for 10 minu. Pleural fluid specimens were collected by thoracentesis and centrifuged at 2000×g for 10 min. Serum and pleural fluid supernatants were stored at −70˚C until tested. Albumin, glucose and lactate dehydrogenase (LDH) concentrations were also measured in the serum and pleural fluid specimens using standard laboratory procedures.

Serum and pleural fluid VEGF levels were determined using a commercially available enzyme-linked immunoassay (ELISA) kit (VEGF-A Human ELISA kit; BioVendor Laboratory Medicine

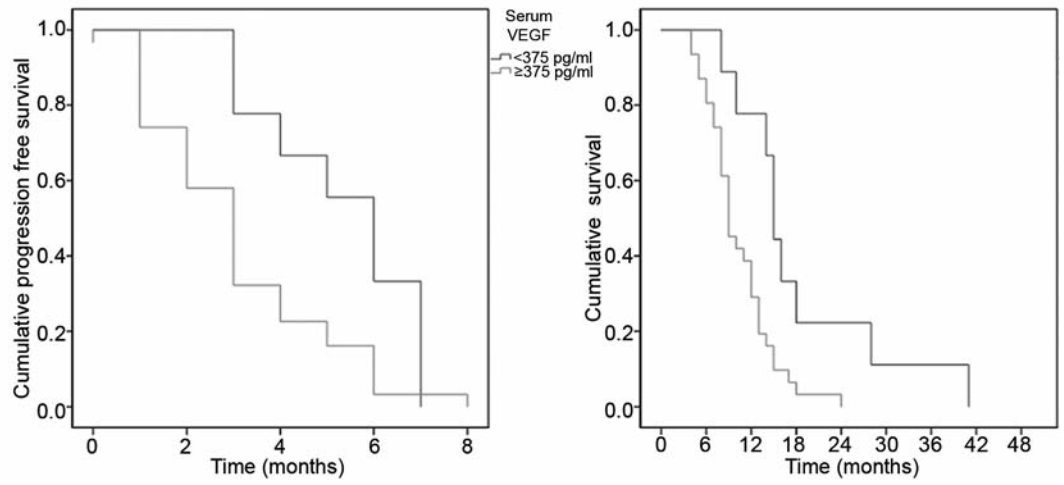

Figure 3. *Kaplan-Meier progression-free survival and overall survival estimates according to serum vascular endothelial growth factor level.*

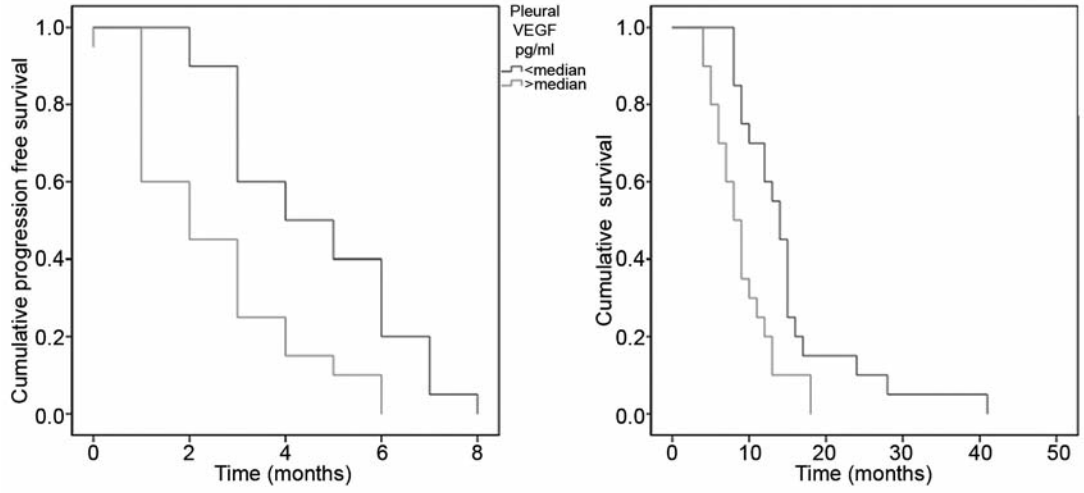

Figure 4. *Kaplan-Meier progression-free survival and overall survival estimates according to pleural fluid vascular endothelial growth factor level.*

Inc., Brno, Czech Republic), according to the manufacturer's instructions. This is a sandwich immunoassay using biotin-labeled antibody for the detection of the soluble isoforms of human VEGF (VEGF 165 and 121). A standard curve was created using measurements at the following VEGF concentrations: 15.6, 31.3, 62.5, 125.0, 500.0 and 1000 pg/ml, while in-between values were evaluated using the curve. The sensitivity (lower limit of detection) of this method was 7.5 pg/ml. All assays were run in duplicate.

Statistical analysis. Quantitative variables are expressed as mean values with standard deviation (SD), or as median values and interquartile range (IQR). Qualitative variables are expressed as absolute and relative frequencies. The diagnostic accuracy of serum VEGF levels in discriminating between patients with NSCLC and healthy individuals was assessed using receiver operating characteristic (ROC) curves. The overall performance of the ROC analysis was quantified by computing the area under the curve (AUC). An area of 1 indicates perfect performance, while a value of 0.5 indicates a performance that was not different from that expected by chance. Sensitivity and specificity were calculated for different cut-off values, and the optimal cut-off values were determined using ROC analysis.

Serum and pleural fluid VEGF levels were correlated with demographic and clinicopathological variables (gender, age, smoking history and pack-years, PS and weight loss, histological type of tumor, serum and pleural fluid levels of albumin and glucose, pleural fluid LDH levels, LDH ratio of pleural fluid/serum, and treatment response). For the comparisons of proportions, chi-square tests were used. Mann-Whitney tests were used for the comparison of serum and pleural VEGF levels

Table I. *Baseline demographics and clinicopathological characteristics of patients.*

Sex N (%)	
Men	29 (72.5)
Women	11 (27.5)
Mean age (SD), years	65 (8.5)
Age, years, N (%)	
≤65	20 (50.0)
>65	20 (50.0)
Smoking, N (%)	
No	5 (12.5)
Yes	35 (87.5)
Mean pack-years (SD)	65.9 (31.6)
Histological type, N (%)	
Adenocarcinoma	21 (52.5)
Squamous cell carcinoma	5 (12.5)
Not otherwise specified	14 (35)
Performance status, N (%)	
0	12 (30.0)
1	28 (70.0)
Weight loss >10%, N (%)	
Yes	5 (12.5)
No	35 (87.5)
Mean LDH (SD), U/l	381.4(173.2)
LDH ratio*>2 , N (%)	
No	27 (67.5)
Yes	13 (32.5)
Mean (SD) albumin in pleural fluid, g/dl	4.2 (0.7)
Mean (SD) albumin in serum, g/dl	7 (0.9)
Mean (SD) glucose in pleural fluid, mg/dl	127.4 (30.1)
Mean (SD) glucose in serum, mg/dl	113.3 (22.1)
Treatment response, N (%)	
Compete response	0 (0.0)
Partial response	9 (22.5)
Stable disease	16 (40.0)
Progressive disease	15 (37.5)
Median (IQR) VEGF in pleural fluid, pg/ml	2449.3(1293.9-5694.9)
Median (IQR) VEGF in serum, pg/ml	697.7 (377.5-1339.5)

LDH: Lactate dehydrogenase ; SD: standard deviation ; VEGF: vascular endothelial growth factor; *LDH ratio pleural fluid/serum.

between two groups and Kruskal-Wallis tests for their comparison between three groups. Spearman's correlation coefficient was used to explore the association of serum and pleural VEGF with other continuous variables.

Life-table analyses were used to calculate the cumulative survival rate (standard errors) for specific time intervals. The prognostic value of each variable for OS and PFS was first assessed by univariate Cox regression analysis. Variables that showed significant association with the outcome were included in the multivariate Cox proportional-hazard model in a stepwise method. The assumption of proportional hazards was evaluated by testing for interaction with a continuous time variable. Kaplan-Meier survival estimates were graphed over the follow-up period. All reported *p*-values are two-tailed. Statistical significance was set at $p<0.05$ and analyses were conducted using SPSS statistical software (version 19.0; SPSS Inc., Chicago, IL, USA).

Results

Demographics and clinicopathological characteristics. A total of 40 patients with a mean age of 55.5 years (SD=8.2 years), 29 men and 11 women, participated in the study. Serum VEGF levels were also measured in 50 controls with similar age and sex distribution ($p=0.517$ and $p=0.795$, respectively). The control group had a mean age of 54.3 years (SD=9.1 years) and comprised of 35 men and 15 women. Baseline demographics and clinicopathological characteristics of patients are presented in Table I. The majority of patients (87.5%) had a positive smoking history; with a mean (SD) of 65.9 (31.6) pack/years. The predominant histological type was adenocarcinoma (52.5%). PS was 0 and 1 in 70% and 30% of patients, respectively. Weight loss more than 10% was noted in 12.5% of patients. Nine patients (22.5%) had PR, while 16 (40%) and 15 (37.5%) patients had SD and PD, respectively. The mean duration of follow-up was 12.1 months (SD=6.9) with a median of 10.5 months (IQR: 8 to 15 months). All patients died during the follow-up period.

Serum and pleural fluid VEGF levels. The median serum VEGF levels were 138.2 (IQR=92.2-218.3) pg/ml in the control group and 697.7 (IQR=377.5-1339.5) pg/ml in the patient group ($p<0.001$) (Figure 1). ROC curve analysis (Figure 2) showed that the optimal cut-off of serum VEGF for the discrimination between patients and controls was 375 (pg/ml), with sensitivity equal to 76.9% and specificity equal to 98.0%. The AUC was 0.90 [95% confidence interval (CI)=0.92-0.98], which significantly differs from 0.5 ($p<0.001$). Only one of the controls (2%) had a serum VEGF level of more than 375 (pg/ml) and the corresponding proportion was 76.9% for patients. The median pleural VEGF levels were 2449.3 (IQR=1293.9-5694.9) pg/ml. Serum VEGF levels were highly correlated with pleural VEGF levels (r=0.68, $p<0.001$).

The association of pleural and serum VEGF levels with the remaining clinicopathological parameters is shown in Table II. Serum VEGF levels were negatively associated with age and positively associated with LDH and serum glucose level. Pleural VEGF levels were positively associated with pack-years and pleural LDH level. The VEGF level in pleural fluid was significantly increased in adenocarcinomas compared to other histological types.

Survival analysis. Table III presents the results of univariate Cox regression analyses for PFS and OS in association with all study parameters. Increased pack-years were associated with increased hazard for disease progression and worse OS. Patients with squamous cell carcinoma had almost three-times greater hazard [hazard ratio (HR)=3.07, 95% CI=1.02-9.21] for progression compared to patients having

Table II. *Association of pleural fluid and serum vascular endothelial growth factor levels with demographic and clinicopathological variables.*

	VEGF in pleural fluid (pg/ml)		VEGF in serum (pg/ml)	
	Median (IQR)	*p*-Value	Median (IQR)	*p*-Value
Sex				
Men	2457.3 (1883.0-4174.7)	0.617*	766.9 (456.0-1344.7)	0.220*
Women	1295.9 (948.0-13558.2)		384.9 (200.5-1324.0)	
Age (years), r (*p*-value)	–0.14 (0.387)		–0.35 (0.029)	
Age (years)				
≤65	2342.5 (1613.0-5055.6)	0.935*	896.9 (546.3-1460.9)	0.083*
>65	2578.7 (1217.0-6019.2)		456.8 (320.6-900.8)	
Smoking				
No	2700.1 (2529.6-2831.4)	0.668*	384.9 (291.2-767.5)	0.500*
Yes	2243.8 (1259.0-6585.1)		713.8 (411.1-1344.7)	
Pack-years, r (*p*-value)	0.37 (0.027)		0.26 (0.129)	
Histological type				
Adenocarcinoma	2831.4 (2154.0-7145.6)	0.023**	914.2 (411.1-1505.1)	0.089**
Squamous cell carcinoma	2737.5 (2457.3-3782.0)		623.0 (598.8-678.3)	
Not otherwise specified	1589.5 (693.2-2239.5)		435.7 (197.6-1305.2)	
Performance status				
0	2160.0 (1293.9-8425.3)	0.883*	648.4 (208.1-1447.3)	0.616*
1	2493.4 (1317.9-4814.0)		697.7 (432.4-1196.6)	
Weight loss >10%				
Yes	1883.0 (1376.8-2831.4)	0.790*	456.0 (411.1-914.2)	0.854*
No	2457.3 (1291.8-5936.5)		713.8 (375.0-1344.7)	
LDH U/l, r (*p*-value)	0.32 (0.049)		0.35 (0.025)	
LDH ratio*>2				
No	2243.8 (1291.8-5453.3)	0.444*	766.9 (350.0-1344.7)	0.965*
Yes	2831.4 (1376.8-6585.1)		678.3 (411.1-1087.9)	
Albumin in pleural fluid, r (*p*-value)	0.10 (0.548)		0.15 (0.381)	
Albumin in serum, r (*p*-value)	0.10 (0.593)		0.05 (0.796)	
Glucoseinpleural fluid, r (*p*-value)	–0.29 (0.082)		–0.14 (0.406)	
Glucose in serum, r (*p*-value)	0.03 (0.863)		0.43 (0.013)	
Treatment response				
Partial response	2239.5 (883.2-2598.4)	0.115**	766.9 (291.2-1416.6)	0.339**
Stable disease	2046.5 (1217.0-4617.6)		638.5 (296.8-930.5)	
Progressive disease	2737.5 (1987.1-13558.2)		767.5 (449.5-2632.7)	

*Mann-Whitney test; **Kruskal-Wallis test; r: Spearman correlation coefficient.

NSCLC not otherwise specified. Furthermore, patients with SD or PD had greater hazard for progression and worse survival in comparison to those that had PR. For every 300-unit increase in serum VEGF level, the hazard for progression increased by 10%, while that for death increased by 19%. Those with serum VEGF of more than 375 pg/ml had 2.19-times greater hazard for progression and 2.93-times greater hazard for death. Patients with pleural fluid VEGF greater than the median value had 2.55-times greater hazard for progression and 2.46-times greater hazard for death.

Kaplan-Meier PFS and OS estimates for patients with serum VEGF greater than 375 pg/ml are presented in Figure 3. The aforementioned survival estimates for pleural fluid VEGF levels greater than the median value are presented in Figure 4. For patients with serum VEGF of more than 375 pg/ml, the cumulative survival rate for the first 6 months was 87% (SE=6%), for 12 months 44% (SE=9%) and for 18 months 17% (SE=4%); for patients with serum VEGF less than 375 pg/ml, the cumulative survival rate for 6 months was 100% (SE=0%), for 12 months 78% (SE=14%) and for 18 months 43% (SE=16%).

When multiple Cox regression analysis was conducted in a stepwise method for PFS, it was found that serum VEGF more than 375 pg/ml (HR=2.41, 95% CI=1.10-5.22, p=0.028), pleural fluid VEGF greater than the median (HR=2.58, 95% CI=1.30-5.13, p=0.007), as well as presence of PD (HR=5.93, 95% CI=2.55-13.76, p<0.001) were independent predictors of poorer PFS. Multiple Cox regression analysis further revealed that serum VEGF more than 375 (pg/ml) (HR=2.92, 95% CI=1.24-6.84, p=0.013)

Table III. *Results of univariate Cox regression analyses for progression-free and overall survival.*

	Progression		Survival	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Sex				
Men	1.00*		1.00	
Women	0.96 (0.47-1.97)	0.911	0.87 (0.42-1.8)	0.711
Age (years)	1.01 (0.97-1.05)	0.670	1 (0.96-1.04)	0.942
Age (years)				
≤65	1.00		1.00	
>65	1.29 (0.69-2.44)	0.424	1.15 (0.61-2.16)	0.660
Smoking				
No	1.00		1.00	
Yes	0.88 (0.34-2.28)	0.795	0.99 (0.38-2.55)	0.985
Pack-years	1.01 (1-1.02)	0.040	1.01 (1-1.02)	0.044
Histological type				
NOS	1.00		1.00	
Adenocarcinoma	1.77 (0.87-3.63)	0.117	1.97 (0.93-4.19)	0.079
Squamous cell	3.07 (1.02-9.21)	0.046	2.86 (0.93-8.75)	0.066
Performance status				
0	1.00		1.00	
1	1.53 (0.76-3.08)	0.235	1.41 (0.71-2.82)	0.328
Weight loss >10%				
Yes	1.00		1.00	
No	0.93 (0.36-2.4)	0.878	1.26 (0.49-3.25)	0.636
LDH U/ l (per 100 unit increase)	1.20 (0.98-1.46)	0.073	1.05 (0.86-1.26)	0.650
LDH ratio*>2				
No	1.00		1.00	
Yes	1.19 (0.60-2.35)	0.625	0.80 (0.40-1.62)	0.537
Albumin in pleural fluid, g/dl	0.90 (0.57-1.44)	0.674	0.94 (0.59-1.50)	0.791
Albumin in serum, g/dl	0.76 (0.46-1.23)	0.261	0.99 (0.60-1.62)	0.953
Glucose in pleural fluid, mg/dl	1.00 (0.99-1.01)	0.700	1.00 (0.98-1.01)	0.558
Glucose in serum, mg/dl	1.00 (0.99-1.02)	0.928	1.00 (0.98-1.02)	0.798
Treatment response				
Progressive disease	1.00		1.00	
Partial response	0.13 (0.05-0.36)	<0.001	0.05 (0.01-0.16)	<0.001
Stable disease	0.23 (0.10-0.52)	0.001	0.10 (0.03-0.30)	<0.001
VEGF in pleural fluid >median	2.55 (1.30-5.00)	0.006	2.46(1.27-4.79)	0.008
VEGF in serum >375 pg/ml	2.19 (1.01-4.76)	0.048	2.93 (1.26-6.80)	0.012

and pleural fluid VEGF greater than the median (HR=2.27, 95% CI=1.15-4.47, p=0.017) were independent predictors of poor OS. Additionally, treatment response was another factor independently associated with OS, and patients with PD had 12.03 times greater hazard for death (HR=12.03, 95% CI=4.01-36.06, p<0.001).

Discussion

VEGF is a potent angiogenic regulator with a crucial role in the initiation and progression of solid malignancies (3, 6, 28). Accumulating evidence suggests that circulating levels of this factor in patients with NSCLC may, at least partly, reflect the extent of tumor-related angiogenesis and thus serve as a marker of disease aggressiveness and progression (12-14). Moreover, it is also being increasingly recognized that serum or plasma VEGF measurement by ELISA is a reliable technique, with significant practical and safety advantages over VEGF immunostaining performed on tumor biopsy specimens, especially among patients with metastatic or inoperable disease (6, 21, 29, 30).

Increased concentrations of serum VEGF have been consistently observed in patients with NSCLC compared to healthy controls (18, 31, 32), and are occasionally associated with other adverse clinicopathological prognostic variables, including advanced disease stage, positive nodal status and poor PS (18, 29). In line with these previous observations, serum VEGF levels were significantly higher in our patient population compared to their sex- and age-matched controls; furthermore, a cut-off

point of 375g/ml yielded high sensitivity and specificity (76.9% and 98.0%, respectively) in our cohort, thus further supporting the notion that this biomarker may be useful in discriminating between patients with NSCLC and healthy individuals.

Several authors have suggested a possible association between increased circulating VEGF levels and a worse prognosis in both the early and metastatic NSCLC settings (3, 14, 16, 31). Shimanuki *et al*. (14), Dudek and Mahaseth (33), Crohns *et al*. (34), as well as Brattstrom *et al*. (35), reported that increased pre-treatment levels of serum VEGF correlate with reduced OS, while Hanrahan *et al*. (16) found that high baseline serum or plasma VEGF may be predictive of PFS advantage in patients with advanced-stage disease only. In another study of patients with advanced-stage NSCLC, a significant association between increased pre-treatment levels of serum VEGF and a worse OS was observed, both in univariate and multivariate analyses (3). Additionally, a recent meta-analysis, pooling data from 17 studies and including 2,366 patients with lung cancer, similarly concluded that high levels of circulating (plasma or serum) VEGF may predict poor OS (21). In the present series of patients with advanced-stage NSCLC, increased serum VEGF levels were associated with both reduced OS and PFS, independently of other clinicopathological prognostic parameters, thus largely concurring with the aforementioned results.

In contrast to these findings, other researchers have reported that measurement of circulating VEGF levels may not correlate with survival or provide independent prognostic information, in addition to well-established prognostic parameters such as disease stage or PS (6, 17, 18, 29), and that the complex pathway of angiogenesis may not be accurately reflected by a single endogenous factor (18). According to the results of a prospective ECOG study (36), baseline serum VEGF levels correlate with disease-free survival but not OS. Chakra *et al*. (18) found that high serum VEGF levels were associated with a poorer OS in univariate but not in multivariate analysis. Therefore, this finding was attributed to a strong correlation between serum VEGF and disease stage and the authors concluded that this biomarker may not independently predict prognosis of NSCLC.

All these data taken together suggest that the clinical utility of serum VEGF as a prognostic biomarker in NSCLC remains controversial. Potential causes of this significant discrepancy among studies may include the following: small sample size of some series; varying distribution of clinicopathological prognostic parameteres (such as disease stage, PS and tumor histology) between study cohorts; variable length of follow-up; use of different assay procedures/antibodies for the detection of VEGF; and use of different cut-off points to discriminate between low and high VEGF subgroups. With regards to the latter, it should be pointed out that many previous authors have employed mean or median values as cut-off points, instead of optimal cut-offs as determined by ROC curve analysis. Additional large-scale prospective studies adopting standardized immunoassay protocols and cut-offs are therefore warranted, so as to more accurately determine the prognostic significance of circulating VEGF (21, 35).

Recent studies have demonstrated that VEGF levels in MPE caused by a variety of malignancies (including NSCLC) may be higher than in non-malignant pleural effusions, as well as in exudates as compared to transudates (37-40). Furthermore, VEGF levels seem to be present at significantly higher levels in pleural fluid than in serum or plasma, indicating that VEGF production from mesothelial cells-and possibly also from tumor cells-within the pleural cavity may be disproportionate to its systemic production (35, 41, 42). Consistent with these prior findings, the median serum and pleural VEGF levels in our patient population were 697.7 pg/ml and 2449.3 pg/ml, respectively, thus further supporting the idea that VEGF may accumulate in the pleural space of patients with MPE.

In addition to previous reports on the prognostic value of serum VEGF, there is also certain limited evidence suggesting that VEGF levels in pleural fluid may also provide clinically relevant prognostic information in NSCLC (41, 42). Hsu *et al*. (42), in a study of patients with NSCLC-associated MPE, reported that the level of VEGF in pleural fluid may correlate with the amount of effusion and serum VEGF level, as well as with reduced overall survival, albeit not as an independent predictor. Hooper *et al*. (41), similarly reported a statistically significant association between increased VEGF levels in pleural fluid and shorter OS in the NSCLC subgroup of patients included in their study, while failing to observe any correlation between survival and pleural fluid VEGF among patients with epithelioid mesothelioma. In the present series, VEGF levels in pleural fluid were significantly and independently associated with shorter OS and PFS, while a statistically significant correlation was also observed between serum and pleural fluid VEGF levels. To the best of our knowledge, this is the first study to report that VEGF in pleural fluid may represent an independent prognostic biomarker in NSCLC.

Our reported results should nevertheless be evaluated in light of certain limitations. The number of patients included in the present study was relatively small, but still sufficient for statistical analysis. We also failed to include a control group of patients with benign pleural effusions, which prevented us from comparing VEGF levels in pleural fluid between patients with NSCLC and patients with inflammatory or other benign conditions. Another limitation of our study is the fact that platelet and leucocyte counts were not taken into account; therefore, the potential correlation between serum VEGF levels and peripheral

blood cells-which may release, as previously hypothesized, angiogenic factors including VEGF-was not investigated. On the other hand, the main strengths of our study include its prospective design, consecutive patient enrolment, and homogeneity of the study population, as well as long and complete follow-up of all participants.

In conclusion, we herein demonstrated that increased VEGF levels in serum and pleural fluid may each be independently associated with worse PFS and OS among patients with NSCLC presenting with MPE. Future prospective studies are required to confirm these findings in larger patient cohorts and to further explore the potential value of these candidate biomarkers in predicting not only survival, but also response to targeted anti-angiogenic therapies.

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