

VEGF in Patients with Non-small Cell Lung Cancer during Combination Chemotherapy of Carboplatin and Paclitaxel

MASATO SHINGYOJI, SOICHIRO ANDO, HIROKI NISHIMURA, TAKAHIRO NAKAJIMA,
AKI ISHIKAWA, MEIJI ITAKURA, TOSHIHIKO IIZASA and HIDEKI KIMURA

Department of Thoracic Disease, Chiba Cancer Center, Chiba, Japan

Abstract. *Background:* Vascular endothelial growth factor (VEGF) is a potent angiogenic factor related to tumor growth and metastasis. However, little is known about the clinical significance of circulating VEGF in cancer patients. *Patients and Methods:* Eighteen patients with non-small cell lung cancer received chemotherapy using carboplatin and paclitaxel. Plasma levels of VEGF were analyzed at baseline and after 2 cycles of chemotherapy. *Results:* Partial remission was observed in 3 patients (16.7%), stable disease in 10 patients (55.6%) and progressive disease in 5 patients (27.8%). Patients with partial remission or stable disease had significantly lower levels of plasma VEGF than did patients with progressive disease, both at baseline ($p=0.0341$) and after 2 cycles of chemotherapy ($p=0.0265$). There were no significant changes of plasma VEGF during chemotherapy. *Conclusion:* Pretreatment plasma levels of VEGF are a useful marker for predicting disease control by chemotherapy.

Tumor angiogenesis, the formation of new blood vessels, is an important biological feature of tumor growth and metastasis, and vascular endothelial growth factor (VEGF) is one of the most potent angiogenic factors. VEGF plays a role in enhancing endothelial cell proliferation and survival, increasing migration and invasion of endothelial cells, increasing the permeability of existing vessels, formation of a lattice network for endothelial cell migration, and enhancing chemotaxis and homing of bone marrow-derived vascular precursor cells. In addition to its proangiogenic effects, VEGF has several important functions that are independent of vascular processes, including autocrine effects on tumor cell functions (survival, migration, and

invasion), immune suppression and homing of bone marrow progenitors to prepare an organ for subsequent metastasis (1). With regard to tumor development, VEGF may be an important target in cancer therapy. It has been shown that the use of bevacizumab, a monoclonal antibody targeting VEGF, adds a survival benefit to chemotherapy with carboplatin and paclitaxel (2). Moreover, VEGF was reported to be a marker that correlates with the number of circulating endothelial cells and mobilizing endothelial precursors that may involve tumor angiogenesis (3, 4). Thus, circulating levels of angiogenic factors could theoretically reflect the overall angiogenic activity of the tumor.

The ability to measure angiogenic factors in peripheral blood has certain potential advantages compared with the evaluation of angiogenic activity in tumor tissue. Sampling can be performed non-invasively and repeatedly, and precise quantification is possible, in contrast to the semiquantification achieved with immunostaining. Although elevated levels of circulating angiogenic factors have been found in various types of cancer, little is known about the clinical significance of these factors. This study examined the correlation between circulating VEGF and clinical outcomes such as response to chemotherapy and survival, for patients with advanced non-small cell lung cancer (NSCLC).

Patients and Methods

Patients. The study population consisted of 18 patients with NSCLC. None of the patients had received prior chemotherapy or radiotherapy. Seventeen patients had advanced stage NSCLC and one experienced recurrence after lobectomy. In order to be eligible for first-line combination chemotherapy, patients were required to have the following: cytologically or histologically proven NSCLC; measureable unresectable stage III-IV disease or recurrence after surgery; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; normal hepatic, renal and hematological function, and no concomitant or serious comorbidities. Stage classification was based on the UICC-TNM classification. Written informed consent was obtained from each patient prior to the start of the study.

Treatment plan. All patients received 6 mg/ml/min carboplatin area under the curve (AUC) and 200 mg/m² paclitaxel on day 1 every 3 or 4 weeks. Paclitaxel was given as a 2-h infusion, followed by a

Correspondence to: Masato Shingyoji, Department of Thoracic Disease, Chiba Cancer Center, 666-2, Nitona-cho, Chuo-ku, Chiba, 260-8717 Japan. Tel: +81 432645431, Fax: +81 432659515, e-mail: mshingyoji@chiba-cc.jp

Key Words: Plasma, VEGF, non-small cell lung cancer, chemotherapy, paclitaxel.

1-h infusion of carboplatin. The chemotherapy regimen was repeated for a maximum of 6 cycles unless there was evidence of disease progression or intolerance to the study treatment.

Treatment assessments. Before entering the study, patients underwent a medical history evaluation and physical examination, and tumor measurements of lesions were assessed by imaging techniques such as computed tomography (CT) of the chest and abdomen, magnetic resonance imaging (MRI) of the brain, and bone scintigraphy. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors guidelines (5). Radiological assessments were performed by CT scans of the chest and abdomen after every 2 cycles of chemotherapy.

Samples. Peripheral blood samples were obtained from the patients before chemotherapy and after 2 cycles of chemotherapy (day 21 to 28) just prior to the third cycle. For plasma collection, blood samples were drawn into a vacutainer containing sodium ethylenediaminetetraacetic acid and immediately centrifuged at 3,000 rpm. Plasma samples were collected and stored at -20°C until VEGF measurements were taken.

Measurement of plasma VEGF. Plasma samples were analyzed for VEGF using the Quantikine Human VEGF Immunoassay (R&D Systems Inc., Minneapolis, MN). The assay employs a quantitative sandwich enzyme immunoassay technique. The minimum detectable concentration of VEGF was 15.6 pg/ml. The method was prepared to recognize both VEGF-121 and VEGF-165, which represent the most biologically active molecular forms of total circulating VEGF.

Statistical analysis. Experimentally determined values for VEGF were compared with clinical factors on median values of plasma VEGF, and statistical significance was determined using the Mann-Whitney *U*-test. Spearman's rank correlation coefficient was calculated to analyze the relationship between plasma VEGF and serum carcinoembryonic antigen (CEA) levels. The effect of chemotherapy on plasma VEGF levels was assessed using the Wilcoxon signed-rank test to compare baseline levels to those after 2 cycles of chemotherapy. Patients were also divided into 2 cohorts on the basis of whether their VEGF levels were greater than the median value. Log-rank tests were then performed to compare progression-free and overall survival. Statistical analyses were performed using the statistical packages Stat View for Windows version (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics. The patient characteristics are listed in Table I. Of the 18 patients, 14 were males (78%), and the median age was 59 years (range, 45-72). Thirteen patients (72.2%) had adenocarcinoma and 11 patients (61%) had stage IV disease.

Plasma VEGF levels and patient characteristics. The mean±standard error of the mean (SEM) for plasma VEGF levels at baseline was 88.4±18.5 pg/ml. No association was observed between the patients' sex, age, histological type, ECOG performance status, disease stage (Table II) or serum CEA levels and VEGF levels at baseline (Spearman's rank correlation coefficient, -0.172, / *P*-value=0.5001).

Table I. Patient characteristics.

	Number (n=18)
Gender	
Male	14
Female	4
Age (years)	
<60	10
≥60	8
Histology	
Adenocarcinoma	13
Squamous cell carcinoma	4
Adenosquamous cell carcinoma	1
ECOG Performance status	
0	7
1	11
Stage	
IIIA	3
IIIB	3
IV	11
Recurrence after surgery	1
Serum CEA (ng/ml)	
≤5	8
>5	10
Response to 1st-line chemotherapy	
PR	3
SD	10
PD	5

ECOG, Eastern Cooperative Oncology Group; CEA, carcinoembryonic antigen; PR partial remission; SD, stable disease; PD, progressive disease.

Plasma VEGF levels and response to chemotherapy. Partial remission (PR) was observed in 3 patients (16.7%), stable disease (SD) in 10 patients (55.6%) and progressive disease (PD) in 5 patients (27.8%) (Table I). Patients with PR or SD had significantly lower levels of plasma VEGF than did patients with PD, both at baseline (Table II) and after 2 cycles of chemotherapy (*p*=0.0265, Figure 1). There was no statistically significant difference between the patients' plasma VEGF levels before treatment and after 2 cycles of chemotherapy for any of the patients (*p*=0.9811), for the group of patients with PR or SD (Figure 1), or for the group of patients with PD (Figure 1). After 2 cycles of chemotherapy, the mean plasma VEGF level for all the patients was 68.0±12.7, the mean for the PR group was 48.5±6.9, and the mean for the SD group was 118.8±34.7 pg/ml.

Plasma VEGF levels and survival. There was disease progression in all 18 patients and 14 patients died. The median progression-free survival was 5.5 months (range 1.4-13.9) and median overall survival was 26 months (range 4.9-38.6). There was no significant difference in overall survival (log-rank test, *p*=0.7099 calculated using plasma VEGF levels at baseline; *p*=0.3258 calculated using plasma levels after 2

Table II. Baseline plasma VEGF levels and clinical factors.

	Number (n=18)	Baseline VEGF (pg/ml; Mean±SEM)	P-value
Gender			
Male	14	93.3±21.7	0.6710
Female	4	71.5±38.0	
Age (years)			
<60	10	104.2±86.7	0.2863
≥60	8	68.7±23.7	
Histology			
Adenocarcinoma	13	77.9±21.3	0.4969
Squamous cell carcinoma	4	117.7±49.0	
ECOG Performance status			
0	7	94.8±29.6	0.8209
1	11	84.4±24.7	
Stage			
IIIA/IIIB	6	63.4±25.0	0.2278
IV	11	108.7±25.6	
Response			
PR/SD	13	70.1±21.2	0.0341
PD	5	136.2±30.4	

VEGF, Vascular endothelial growth factor; SEM, standard error of the mean; ECOG, Eastern Cooperative Oncology Group; PR, partial remission; SD, stable disease; PD, progressive disease.

cycles of chemotherapy) or progression-free survival (log-rank test, $p=0.3981$ calculated using plasma VEGF levels at baseline; $p=0.0789$ calculated using plasma levels after 2 cycles of chemotherapy) between patients whose plasma VEGF levels were above or below the mean of each time point. The mean level of plasma VEGF at baseline was 88.4 pg/ml and after 2 cycles of chemotherapy was 68.0 pg/ml.

Discussion

Six different mRNA splice forms of *VEGF* (VEGF-121, -145, -165, -183, -189, -206) have been discovered. The shorter isoforms (VEGF-165, VEGF-145, and VEGF-121) are secreted peptides that may act as diffusible agents, whereas the longer isoforms remain cell associated. Most tumor cell types produce several VEGF isoforms simultaneously, but VEGF-121 and VEGF-165 are the predominant variants (6). There have been several reports studying the relationship between clinical outcome and circulating VEGF (VEGF-121 and VEGF-165) levels in patients with advanced NSCLC. However, most of these reports measured VEGF levels in serum, not in plasma. Hyodo *et al.* reported on the stability of VEGF levels in plasma, in contrast to its instability in serum. The levels of serum VEGF in drawn blood samples were also found to increase during clot formation, and this increase may be caused by the release from platelets (7). Therefore, in this study we measured VEGF levels in plasma.

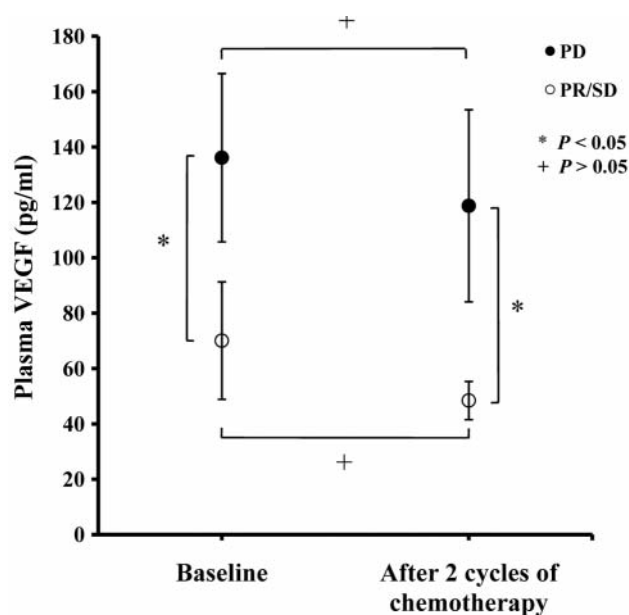


Figure 1. Plasma levels of VEGF (mean±standard error) at baseline and after 2 cycles of chemotherapy in patients with non-progressive disease (PR/SD) and in patients with progressive disease (PD). Both at baseline and after 2 cycles of chemotherapy, plasma VEGF levels in patients with PD were significantly higher than in those with non-PD. There was no statistically significant difference between pretreatment levels of plasma VEGF and the levels after 2 cycles of chemotherapy in either group.

In our study, there was a significant correlation between disease control and plasma VEGF levels, both at baseline and after 2 cycles of chemotherapy. Several reports have examined the correlation between the effectiveness of chemotherapy and circulating VEGF levels in patients with advanced NSCLC. Two reports showed that serum VEGF levels at baseline were significantly correlated with response to chemotherapy in NSCLC patients (8, 9). Three other studies found no correlation (10-12). All 5 of the studies looked at VEGF levels in serum and treated patients using cisplatin-based combination chemotherapy rather than taxanes. Our results suggested that NSCLC patients with high levels of plasma VEGF tended to be resistant to chemotherapy. It was observed that overexpression of VEGF in tumor cells was induced by hypoxia, inactivation of tumor suppressor genes, and activated oncogenes that are part of the ras/MAP-kinase signal transduction pathway (6). One explanation might be that high VEGF levels are related to hypoxia and vascular permeability in tumors, which might lead to insensitivity to anticancer drugs and difficulty of delivery and diffusion of the drugs to the tumor (13, 14).

Laack *et al.* (9) showed that serum VEGF levels at baseline predicted overall survival, and Lissoni *et al.* (8) showed that normalization of serum VEGF levels by chemotherapy was

related to good prognosis in patients with PR or SD. Our study did not show plasma VEGF to be significantly associated with survival. Likewise, Sandler *et al.* did not find a relationship between survival and plasma VEGF at baseline, although it was shown that when bevacizumab, a monoclonal antibody targeting VEGF, was added to combination chemotherapy using carboplatin and paclitaxel, there was proven survival effectiveness (2). The discrepancy between the results of our study and previous studies in terms of response and survival might be explained by differences in the study populations analyzed in terms of histology, chemotherapy regimens, including second-line or later therapy, sample treatment, and sample size. In our study, patients with adenocarcinoma were dominant and gefitinib was administered to 4 patients. In 3 out of the 4 patients, gefitinib was very effective and likely had the most impact on survival.

A few studies have reported on changes in circulating VEGF levels in NSCLC patients during chemotherapy. Two reports showed no significant changes in serum VEGF during treatment (10, 11). However, Lissoni *et al.* showed significant changes in serum VEGF during chemotherapy with cisplatin and etoposide among patients with PR or SD as compared with PD (8). No significant changes were found in plasma VEGF levels between baseline and 2 cycles of chemotherapy with carboplatin and paclitaxel in the present study. Based on these inconsistent results, further studies are needed to clarify the clinical significance of circulating VEGF. In previous studies, cisplatin-based combination chemotherapy was administered without the use of taxanes, contrary to the use of carboplatin and paclitaxel in our study. There have been some reports that have shown a relationship between paclitaxel and angiogenesis, and the effects of paclitaxel on endothelial cells and tumor angiogenesis have been discussed (4, 14-16). Furstenberger *et al.* reported an increase of circulating endothelial progenitor cells and serum VEGF levels in breast cancer patients after 2 cycles of anthracycline and/or taxane-based neoadjuvant chemotherapy (4). In addition, another report showed that plasma VEGF significantly correlated with the number of circulating endothelial cells in breast cancer and lymphoma patients, which supports the belief that plasma VEGF may reflect the number of circulating endothelial cells related to tumor angiogenesis (3). We hypothesized that plasma VEGF levels would change with the use of paclitaxel for NSCLC patients, but our study did not show an increase of plasma VEGF levels after 2 cycles of taxane-based chemotherapy. Shaked *et al.* showed that circulating endothelial progenitor cells increased 4 hours after cancer patients received paclitaxel-based therapy, but without a significant change of plasma VEGF levels. Our negative results may be due to the fact that the time points for sampling and the chemotherapy regimen might have

been inappropriate, or that the influence on endothelial cells by paclitaxel might not occur in NSCLC, or that circulating VEGF might not be a good surrogate marker for angiogenesis as Shaked *et al.* showed (14).

Conclusion

Pretreatment plasma levels of VEGF appear to be a useful marker for predicting disease control by chemotherapy, although no definitive conclusions could be drawn on the basis of this study. In view of the behavior of VEGF, which is different from that of conventional tumor markers, levels of circulating VEGF could be a biomarker for angiogenesis, and may improve the clinical management of cancer patients. Further studies involving a larger number of patients, appropriate sampling time points, and different chemotherapy regimens are necessary to further elucidate and confirm this.

References

- 1 Ellis LM and Hicklin DJ: VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 8: 579-591, 2008.
- 2 Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilienbaum R and Johnson DH: Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 355: 2542-2550, 2006.
- 3 Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G and Bertolini F: Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. *Blood* 97: 3658-3661, 2001.
- 4 Furstenberger G, von Moos R, Lucas R, Thurlimann B, Senn HJ, Hamacher J and Boneberg EM: Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. *Br J Cancer* 94: 524-531, 2006.
- 5 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 92: 205-216, 2000.
- 6 Neufeld G, Cohen T, Gengrinovitch S and Poltorak Z: Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 13: 9-22, 1999.
- 7 Hyodo I, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K and Kotani Y: Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. *Eur J Cancer* 34: 2041-2045, 1998.
- 8 Lissoni P, Rovelli F, Malugani F, Brivio F, Fumagalli L and Gardani GS: Changes in circulating VEGF levels in relation to clinical response during chemotherapy for metastatic cancer. *Int J Biol Markers* 18: 152-155, 2003.
- 9 Laack E, Scheffler A, Burkholder I, Boeters I, Andritzky B, Schuch G, Gorn M, Vohwinkel G, Edler L, Fiedler W and Hossfeld DK: Pretreatment vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) serum levels in patients with metastatic non-small cell lung cancer (NSCLC). *Lung Cancer* 50: 51-58, 2005.

- 10 Tas F, Duranyildiz D, Oguz H, Camlica H, Yasasever V and Topuz E: Serum vascular endothelial growth factor (VEGF) and bcl-2 levels in advanced stage non-small cell lung cancer. *Cancer Invest* 24: 576-580, 2006.
- 11 Mihaylova Zh, Ludovini V, Gregorg V, Floriani I, Pistola L, Toffaneti F, Ferraldeschi M, Spreafico A, Ceresoli GL, Bellet M, Darwish S, Tonato M and Raynov J: Serum level changes of matrix metalloproteinases 2 and 9, vascular endothelial growth factor and epidermal growth factor receptor during platinum-based chemotherapy in advanced non-small cell lung cancer patients. *J BUON* 12: 105-111, 2007.
- 12 Yazar A, Soydinc H, Ertan E, Yasasever V and Tas F: The role of serum vascular endothelial growth factor and matrix metalloproteinase-9 in predicting response to chemotherapy in patients with advanced non-small cell lung cancer. *South Med J* 101: 327-328, 2008.
- 13 Bertout JA, Patel SA and Simon MC: The impact of O₂ availability on human cancer. *Nat Rev Cancer* 8: 967-975, 2008.
- 14 Shaked Y, Henke E, Roodhart JM, Mancuso P, Langenberg MH, Colleoni M, Daenen LG, Man S, Xu P, Emmenegger U, Tang T, Zhu Z, Witte L, Strieter RM, Bertolini F, Voest EE, Benezra R and Kerbekl RS: Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* 14: 263-273, 2008.
- 15 Belotti D, Vergani V, Drudis T, Borsotti P, Pitelli MR, Viale G, Giavazzi R and Taraboletti G: The microtubule-affecting drug paclitaxel has antiangiogenic activity. *Clin Cancer Res* 2: 1843-1849, 1996.
- 16 Lennernas B, Albertsson P, Lennernas H and Norrby K: Chemotherapy and antiangiogenesis-drug-specific, dose-related effects. *Acta Oncol* 42: 294-303, 2003.

Received February 11, 2009

Revised April 27, 2009

Accepted May 6, 2009