# Circulating Serum Vascular Endothelial Growth Factor is Not a Prognostic Factor of Non-small Cell Lung Cancer

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**Introduction:** High circulating serum vascular endothelial growth factor (VEGF) levels might reflect enhanced angiogenesis in patients suffering from non-small cell lung cancer (NSCLC). This study aimed at determining the prognostic significance of circulating VEGF as a prognostic factor in NSCLC.

**Methods:** Four hundred fifty-one histologically or cytologically proven and previously untreated NSCLC patients have been studied. Median follow-up was 13 years and 9 months. Eleven clinical and biologic variables were recorded. The levels of circulating VEGF were measured in the serum by quantitative immunoassay. Patients have had received conventional treatment (without anti-VEGF therapy) according to the international guidelines. All statistical tests were two-sided.

**Results:** Receiver operating characteristic curves (area under the ROC curve: 0.66 ± 0.05) showed that circulating VEGF serum level did not demonstrate a high sensitivity–specificity relationship, and therefore, demonstrated a low ability to differentiate NSCLC from benign lung diseases. A 600 pg/mL level of circulating VEGF serum was considered as threshold with 40.8% of NSCLC patients presenting with a high level. The circulating VEGF distribution differed significantly according to disease stage, nodal status, and performance status (PS), with the highest levels observed in metastatic stage, positive mediastinal nodal status, and poor PS. In univariate survival analysis, patients with a high pretreatment circulating VEGF serum level proved to have a shorter overall survival when compared with patients presenting with a circulating VEGF serum level ≤600 pg/mL. However, in the Cox proportional hazard model, this variable was not included in the panel of independent determinants of a poor outcome that was as follows: advanced or metastatic diseases according to the 6th edition of the staging system, PS ≥2, nodal status $N_{2-3}$, metastatic disease, neuron-specific enolase >12.5 ng/mL, CYFRA 21-1 >3.6 ng/mL.

**Conclusion:** The prognostic information given by a high circulating VEGF serum level is not an independent determinant of survival owing to a high relationship with main prognostic variables such as PS, stage of the disease, and nodal status. This finding does not preclude a putative prognostic impact of in situ detection of VEGF and VEGF receptors in tumor specimen.

**Key Words:** Circulating vascular endothelial growth factor, Angiogenesis, Non-small cell lung cancer, Prognosis.

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Treatment decisions for non-small cell lung cancer (NSCLC) in routine practice mainly depended on awareness of main prognostic determinants.[1–4] For patients accrued in clinical trials, knowledge of prognostic determinants is also critical to stratify randomization according to pertinent factors and to adjust statistical survival impact of treatment.[5] In the Eastern Cooperative Oncology Group trial 1594 randomized trial of chemotherapy in metastatic NSCLC, patients presenting with performance status (PS) greater than 1 experienced an excessively high rate of toxicity,[6] and female patients proved to have a better prognosis than male patients.[5] Hitherto, the most widely accepted negative prognostic determinants of NSCLC are metastatic disease stage, positive nodal status, poor PS,[1,2] weight loss[3] and, although inconstancy, male gender.[5] Nonsquamous histologies have been variously reported as positive prognostic factors but whether or not this feature is independent of or correlated with patient gender (adenocarcinoma having been more frequently observed in women) is still debatable.[7] We previously published that the prognostic information given by a high serum CYFRA 21-1 level is independent from other well-known variables such as PS and disease stage, and is perennial throughout extended follow-up period. A high neuron-specific enolase (NSE) level also prognosticates a poor outcome probably by reflecting tumor heterogeneity and underestimated neuroendocrine differentiation.

NSCLC clinically behaves aggressively with a rapid growth and metastatic spread. As both features are thought to be angiogenic-dependent processes, therapy targeting angiogenesis has been considered as a potential new approach. Bevacizumab is a recombinant humanized monoclonal antibody that binds vascular endothelial growth factor (VEGF).

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When given in combination with standard platinum-based doublet regimens, bevacizumab has been shown to prolong survival of previously untreated advanced NSCLC when compared with chemotherapy alone.[8,9] The recent approval of bevacizumab in combination with first-line therapy for a selected subgroup of NSCLC stimulated further clinical researches of other angiogenesis targeted therapies. In this setting, different proteins involved in the angiogenesis could be detected in the serum of patients suffering from various malignancies, including lung cancer.[10] Therefore, circulating VEGF serum level might be regarded as one of putative markers, and could help the appraisal of angiogenesis.

Hitherto, the prognostication of patient outcome using angiogenesis serum marker remains a matter of controversy. To accurately determine whether or not circulating VEGF serum level adds prognostic information, the herein study simultaneous appraised pretreatment level of circulating VEGF serum and other known putative survival determinants in a large NSCLC population followed-up during a long period of time.

## PATIENTS AND METHODS

### Patients

Four hundred fifty-one consecutive patients referred to the Montpellier—Nîmes University Hospital between February 1990 and June 1998 were prospectively entered in a databank informing a sera bank (Table 1). Eligibility criteria consisted of histologically or cytologically proven and previously untreated NSCLC. Consequently, patients suffering from small cell lung cancer and patients admitted for adjuvant treatment (after surgery), second-line therapy or palliative care after anticancer treatment failure were not eligible. Histologic subclassification was done according to the WHO classification.[11] PS was estimated according to the Eastern Cooperative Oncology Group[12] and the percentage of weight loss during the previous 4 months was recorded. Staging was performed by exhaustive procedures according to the 6th edition of the Union Internationale Contre le Cancer tumor node metastases classification.[13] The following investigations were performed: clinical examination, standard chest radiograph, computed tomography (CT) scan of chest and upper abdomen, fiber-optic bronchoscopy, liver sonography, and bone scanning. Mediastinoscopy was used to establish nodal status in NSCLC patients with nonmetastatic disease and evidence of mediastinal lymph node enlargement on chest CT-scan. Brain CT scan was done on clinical indication until 1992 and systematically performed thereafter.

### Controls

The serum markers were measured in 49 consecutive patients with nonmalignant pulmonary diseases. Patients in this benign chronic lung disease group were affected by diseases resulting from chronic obstructive pulmonary disease with or without infectious complications, postinfectious bronchiectiasis, silicosis, and severe asthma. Most of the patients in this group suffered from tobacco consumption.

**TABLE 1.** Patients' Demography and Disease Characteristics

| Variables | No. of Patients (%) |
|---|---|
| Total | 451 |
| Age (yr), median ± SD | 61 ± 10 |
| Gender | |
| Male | 409 (91) |
| Female | 42 (9) |
| ECOG performance status | |
| <2 | 237 (52.9) |
| ≥2 | 211 (47.1) |
| Stage grouping (mountain) | |
| I | 41 (9) |
| II | 4 (0.9) |
| IIIa | 56 (12.4) |
| IIIb | 146 (32.3) |
| IV | 204 (45.2) |
| Histology | |
| Squamous cell | 250 (55.4) |
| Adenocarcinoma | 132 (29.3) |
| Large cell carcinoma | 69 (15.3) |
| Weight loss (%) | |
| ≤5% | 307 (71.2) |
| <5% | 124 (28.8) |
| Blood leukocyte count | |
| ≤10,000/μL | 242 (54.4) |
| >10,000/μL | 203 (45.6) |
| Serum NSE level | |
| ≤12.5 ng/mL | 335 (74.6) |
| >12.5 ng/mL | 114 (25.4) |
| Serum CYFRA 21-1 level | |
| ≤3.6 ng/mL | 227 (50.3) |
| <3.6 ng/mL | 224 (49.6) |
| Serum VEGF level | |
| ≤600 pg/mL | 267 (59.2) |
| >600 pg/mL | 184 (40.8) |
| Serum albumin level | |
| ≤32 g/L | 66 (15.1) |
| >32 g/L | 370 (84.9) |

ECOG, Eastern Cooperative Oncology Group; NSE, neuron-specific enolase; VEGF, vascular endothelial growth factor.

### Treatment

A medical panel composed of thoracic surgeons, chest physicians, radiologists, radiotherapists, and medical oncologists discussed the case of each patient to design a treatment program to be submitted for patient's approval. Particular attention was paid to the agreement between each individual proposal and the international guidelines.

NSCLC patients with stage I or II disease underwent surgery in an attempt at complete resection. Patients suffering from pathologically demonstrated N2 disease received cisplatin-based neoadjuvant chemotherapy followed by surgery whenever possible. Other patients with PS ≤2 and distant metastases (stage IV) or gross mediastinal involvement (stage IIIb and unresectable stage IIIa) were treated by a cisplatin-based chemotherapy. Radiotherapy was applied in locally

advanced stages according to a concurrent chemoradiotherapy schedule.[14] As adjuvant to anticancer therapy, best supportive care, including palliative radiation therapy when needed, was proposed to patients according to their symptoms and impact of disease on quality of life. Treatment was decided upon according to clinical and routine biologic findings and without knowledge of the circulating VEGF, CYFRA 21-1 or NSE levels. Disease stage, PS, and comorbidities were obviously taken into account in therapeutic choice. Hence, treatment was not considered as a prognostic variable in this study.

## Biochemical Measurements

A blood sample was taken from each patient at presentation, the serum was separated and stored at −180°C until tested.

Serum NSE was measured by ELSA NSE™, a solid phase two-site immunoradiometric assay (Cis Biointernational, Gif/Yvette, France). Two monoclonal antibodies were prepared against sterically remote antigenic sites on the NSE molecule, the first one specific for the NSE is coated in the ELSA solid phase, and the second radiolabeled with 125iodine is used as a tracer. The NSE molecules present in the standards or the samples to be tested were sandwiched between the two antibodies. After the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer was easily removed by a washing step. The radioactivity bound to the ELSA is proportional to the concentration of NSE present in the sample. The calculated concentration of NSE was expressed in ng/mL.

CYFRA 21-1™ (Centocor Diagnostics, Malvern, PA and Cis Biointernational) is a solid phase immunoradiometric assay based on the two-site sandwich method. In this method the cytokeratin 19 is recognized by two mouse MoAb, KS 19-1 and BM 19-21, directed against two different epitopes of a fragment of cytokeratin subunit 19, which is referred to as serum CYFRA 21-1. MoAb KS 19-1 coated polystyrene spheres were incubated with 200 $\mu$L of patient serum, control serum, or standard curve (composed of the following concentrations of cytokeratin 19: 0, 3, 8, 25, and 50 ng/mL) for 20 hours between 2 and 8°C. Afterward, the solid phase was washed with distilled water and then incubated with 0.85 $\mu$CI/mL of 125iodine labeled BM 19-21 for 3 hours between 2 and 8°C. Finally, the solid phase was washed again with distilled water to cancel the nonfixed labeled reagents. Radioactivity was counted in a well-type gamma counter (Autogamma; Packard Instrument Company, Chicago, IL) and expressed in cpm. The calculated concentration of cytokeratin 19 was expressed in ng/mL.

The upper limit of normal values for leukocytes was 10,000/$\mu$L. The lower limits of normal values were 32 g/L and 135 mmol/L for albumin and serum sodium, respectively.

Serum VEGF level was measured using the Calbiochem Human enzyme-linked immunosorbent assay kit (QIA51, Merck Biosciences, Darmstadt, Germany). This sandwich immunoassay uses a monoclonal antibody immobilized onto the surface of the plastic wells to capture VEGF present in sera and a polyclonal antibody tracer, labeled using horseradish peroxidase. The kit measured both the isoforms, $VEGF_{165}$ and $VEGF_{121}$, and allows its use to quantify natural human VEGF protein in sera. The sample to be assayed (100 $\mu$L of patients' samples and standards) are pipetted into the wells and any human VEGF present binds to the capture antibody. After incubation at room temperature for 2 hours, wells were washed 3 times and incubated with 200 $\mu$L of the VEGF conjugate for 2 hours. Each well was washed as before, and then incubated for 25 minutes with 50 $\mu$L of substrate solution ($H_2O_2$ + tetramethylbenzidine). The reaction was stopped with 50 $\mu$L of $H_2SO_4$ 2*N* and absorbance was measured using a spectrophotometer plate reader at dual wavelengths of 450/540 nm. The standard curve was composed of the following concentrations of Sf21-expressed recombinant human VEGF 165: 31.2, 62.5, 125, 250, 500, and 1000 pg/mL, and concentrations of unknowns were determined by interpolation from the standard curve. The sensitivity of the test is 9.0 pg/mL. The levels of circulating VEGF were measured blindly without any clinical information given.

## Statistics

Receiver operating characteristic (ROC) curves were constructed using both patient and control subject serum marker levels in an attempt to establish a sensitivity–specificity relationship. Areas under the ROC curves were calculated.[15] A comparison between the areas under the ROC curves (AUC-ROC) was made using the Z statistic (two-tailed test). Version 1.0 of AccuROC for Windows 95 software was used.[16]

The serum tumor marker was not distributed normally; thus, to analyze the distribution of tumor markers in subsets of patients, results were expressed as median, and variation was expressed as interquartile range (IR). Nonparametric statistical analyses were used: differences between two independent groups were determined by means of the Mann-Whitney *U* test with the Bonferroni correction for multiple comparisons; differences between more than two groups were determined by means of Kruskal–Wallis one-way analysis of variance.

Survival was defined from the date of sampling to the date of death of any cause. Survival data were updated in February 2008 and 12 patients (2.6%) were lost to follow-up. Median follow-up was 13 years and 9 months. Probability of survival was estimated by the Kaplan–Meier method.[17] Single variable survival analyses were done by means of Wilcoxon and log-rank tests and multivariate regression was done with Cox's model.[18] Cox's model analysis was written after a Boolean coding of all variables which reach a 0.15 *p* level using the results of univariate analysis. For each variable, the proportional hazard assumption was tested graphically: survival was analyzed using the SAS software package.

# RESULTS

## Marker Distribution According to Pretreatment Variables

ROC curve for circulating VEGF serum was constructed with specificity calculated using the results of titration in the nonmalignant lung disease group, whereas sensi-

 

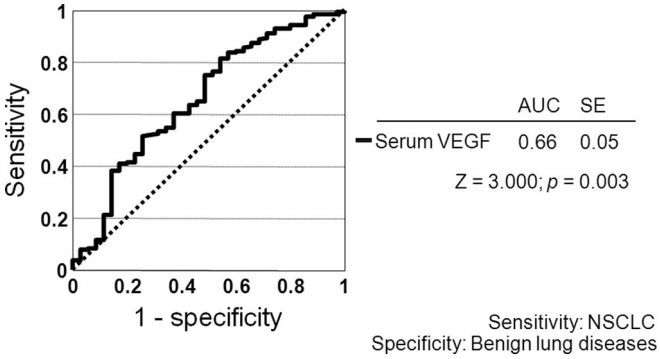


**FIGURE 1.** Receiver operating characteristic constructed using the sensitivity–specificity relationship circulating vascular endothelial growth factor (VEGF) serum to discriminate non-small cell lung cancer (NSCLC) patients and patients with a nonmalignant pulmonary disease. AUC, area under the curve; SE, standard error.

tivity was measured in the whole NSCLC population (Figure 1). Areas under the ROC curves (AUC-ROC) were 0.66 ± 0.05; Z statistics: 3.0; $p = 0.002$ when compared with the no-discrimination line. These results suggested that circulating VEGF serum level did not demonstrate a clear sensitivity–specificity relationship when its ability to differentiate NSCLC from benign lung diseases was tested. By using this ROC curve, a 600 pg/mL level of circulating VEGF serum was considered as threshold with 40.8% of NSCLC patients presenting with a high level. In the whole NSCLC population, median (IR) was 518 pg/mL (309 to 870); and circulating VEGF serum levels ranged from 6 to 2456 pg/mL.

The circulating VEGF serum level varied significantly according to stage grouping in NSCLC inasmuch as the highest level was observed in stage IV disease and the lowest in stage I–II (Kruskal–Wallis test: 11.2; $p = 0.01$; Figure 2). Similarly, patients presenting with a mediastinal lymph node involvement (Kruskal–Wallis test: 11.1; $p = 0.01$; Figure 3) or a poor PS (Kruskal–Wallis test: 26.6; $p < 0.0001$; Figure 4) have higher median and IR circulating VEGF serum when compared with patients presenting with opposite features.

## Survival Analysis

Survival was analyzed in the whole patient population. Patients lost to follow-up were considered for the real time of participation to the study and right censored afterward. Univariate analyses were shown in Table 2. Patients with a high pretreatment circulating VEGF serum level proved to have a shorter overall survival when compared with patients presenting with a circulating VEGF serum level ≤600 pg/mL (median survival in months [95% CI]: 6.4 [5.3–8.7] and 10.6 [8.3–13.2] respectively, log-rank: $p = 0.0002$; Figure 5). However, in the Cox proportional hazard model, this variable was not included in panel of independent determinants of a poor outcome that were as follows: Mountain stage grouping (advanced or metastatic diseases according to the 6th edition of the staging system), PS 2 or 3, nodal status $N_{2-3}$, metastatic disease, serum NSE >12.5 ng/mL, and serum CYFRA 21-1 >3.6 ng/mL (Table 3).

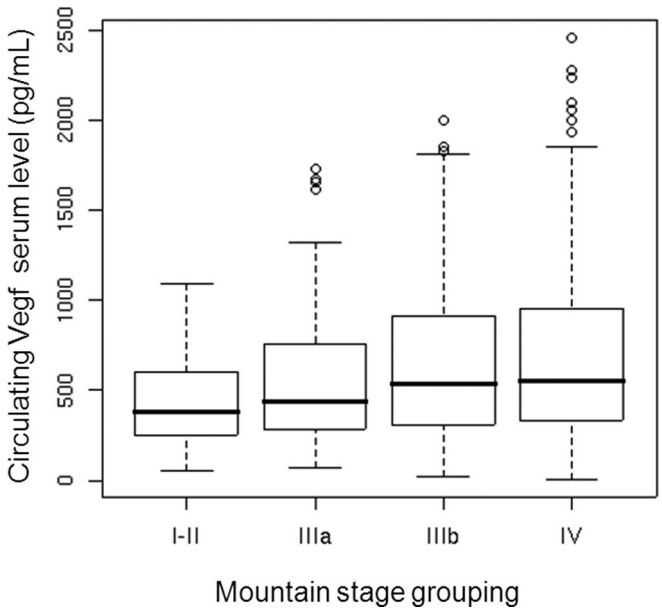


**FIGURE 2.** Circulating vascular endothelial growth factor (VEGF) serum distribution according to Mountain stage grouping in non-small cell lung cancer (6th edition of the staging system). Horizontal bar, median value; columns, interquartile range; vertical bar, 95% confidence interval. Kruskal–Wallis test: 11.2; $p = 0.01$.

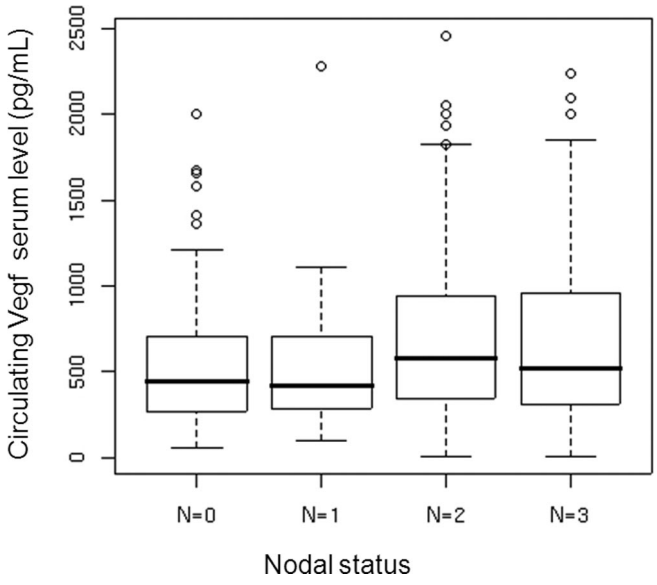


**FIGURE 3.** Circulating vascular endothelial growth factor (VEGF) serum distribution according to the nodal status in non-small cell lung cancer. Horizontal bar, median value; columns, interquartile range; vertical bar, 95% confidence interval. Kruskal–Wallis test: 11.1; $p = 0.01$.

## DISCUSSION

In this study, we investigated whether or not pretreatment circulating VEGF serum level, as one of the angiogenesis markers, is a prognostic factor of NSCLC patient sur-

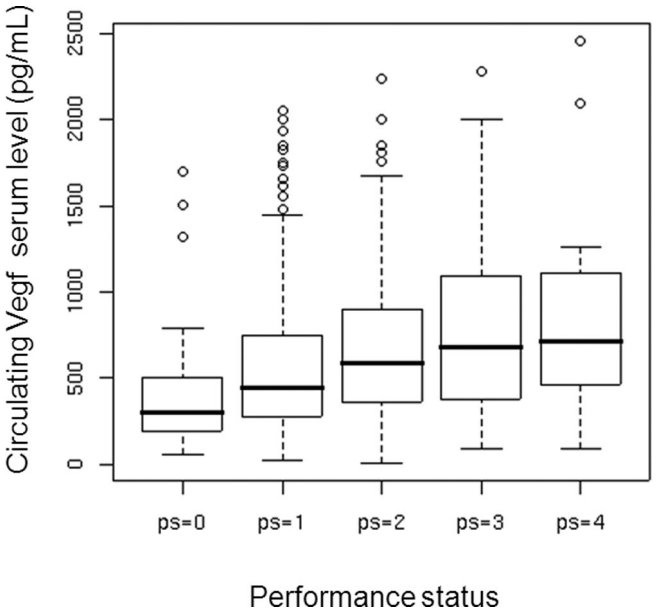


**FIGURE 4.** Circulating vascular endothelial growth factor (VEGF) serum distribution according to performance status in non-small cell lung cancer. Horizontal bar, median value; columns, interquartile range; vertical bar, 95% confidence interval. Kruskal–Wallis test: 26.6; $p < 0.0001$.

vival. Although patients presenting with a high circulating VEGF serum level proved to have a shorter survival than patients with circulating VEGF lower than its threshold value, this feature does not independently determine survival in multivariate analysis. Possible interpretation of this negative result could be based on high relationship between circulating VEGF serum and main prognostic variables such as PS, stage of the disease and nodal status.

Angiogenesis is recognized as an important feature of tumor progression in many human malignancies,[19] including NSCLC. Although antiangiogenic agents aim at limiting stromal expansion rather than modifying tumor cell proliferation, they are considered as indirect ways in achieving tumor expansion arrest. Interestingly, the antitumor effect of antiangiogenic therapy is considered not to be affected by tumor resistance such as multidrug resistance that limits conventional anticancer therapies.

Different clues suggesting the importance of angiogenesis in promoting tumor growth have been observed in NSCLC. Some are in situ markers of angiogenesis and other are circulating serum markers.

In the former group, a high microvessel density has been shown to indicate a poor prognosis.[20] This feature obviously seems as the final result of angiogenesis. Therefore, it is not surprising that most of the literature on this subject concluded in the same way regarding a poorer prognostic outcome for patients presenting with a high count of microvessel density.[20,21] Further explorations of in situ markers have also suggested that high immunohistochemical staining using either anti-VEGF antibodies or antiplatelet-derived endothelial cell growth factor correlates with high microvessel density and poor patient outcome.[21] Although more debatable, the negative prognostic significance of high staining for matrix metalloproteinases (MMP) have been suggested, particularly, MMP-9[22] and MMP-2.[23,24] MMP-2 expression, as analyzed by protein-staining status or by in situ hybridization, proved to correlate with overall survival, but reached

**TABLE 2.** Univariate Analysis in NSCLC Patients

| Variable and Levels | Median Survival (95% CI, mo) | *p* | |
|---|---|---|---|
| | | Wilcoxon | Log-Rank |
| Sex | | | |
| Female | 13.8 (8.1–18.2) | 0.42 | 0.41 |
| Male | 8.2 (6.9–9.7) | | |
| Age[a] | | | |
| >70 yr | 7.2 (5.4–10.7) | 0.29 | 0.38 |
| ≤70 yr | 8.9 (7.3–10.6) | | |
| Performance status | | | |
| 0–1 | 14.5 (11.8–17.3) | $<10^{-4}$ | $<10^{-4}$ |
| >1 | 5.2 (4.2–6) | | |
| Tumor status | | | |
| $T_{1-2}$ | 14.5 (9.6–20.8) | $3 \times 10^{-4}$ | $<10^{-4}$ |
| $T_{3-4}$ | 7.6 (6.5–9.1) | | |
| Nodal status | | | |
| $N_{0-1}$ | 18.4 (13.6–24.8) | $<10^{-4}$ | $<10^{-4}$ |
| $N_{2-3}$ | 6.9 (6.1–8.3) | | |
| Metastases | | | |
| M0 | 13.6 (10.7–15.8) | $<10^{-4}$ | $<10^{-4}$ |
| M1 | 5.4 (4.7–6.2) | | |
| Stage grouping[a] | | | |
| I–II | 81 (32.4–NR) | $<10^{-4}$ | $<10^{-4}$ |
| IIIa | 15.8 (8.9–27.6) | | |
| IIIb–IV | 6.7 (5.8–8.3) | | |
| Histology | | | |
| SQC | 8.9 (6.9–10.8) | 0.95 | 0.88 |
| Non-SQC | 8.2 (6.5–10.6) | | |
| Weight lost | | | |
| ≤5% | 9.6 (7.8–10.9) | 0.012 | 0.125 |
| >5% | 6.5 (4.7–9.1) | | |
| Circulating VEGF serum | | | |
| ≤600 pg/mL | 10.6 (8.3–13.2) | $<10^{-4}$ | 0.0002 |
| >600 pg/mL | 6.4 (5.3–8.7) | | |
| Serum albumin | | | |
| ≥32 g/L | 9.7 (7.8–11) | $<10^{-4}$ | $<10^{-4}$ |
| <32 g/L | 3.8 (2–5.4) | | |
| Leukocytes | | | |
| ≤10,000/mL | 12.4 (10.6–14.5) | $<10^{-4}$ | $<10^{-4}$ |
| >10,000/mL | 5.4 (4.3–6.6) | | |
| Neuron specific enolase | | | |
| ≤12.5 ng/mL | 10 (8.3–11.8) | $<10^{-4}$ | $<10^{-4}$ |
| >12.5 ng/mL | 5.3 (3.7–7.6) | | |
| CYFRA 21-1 | | | |
| ≤3.6 ng/mL | 14.5 (10.8–16.3) | $<10^{-4}$ | $<10^{-4}$ |
| >3.6 ng/mL | 5.9 (5–6.9) | | |

[a] Sixth edition of the staging system.

SQC, squamous cell carcinoma; NR, not reached; 95% CI, 95% confidence interval; VEGF, vascular endothelial growth factor; NSCLC, non-small cell lung cancer.

 

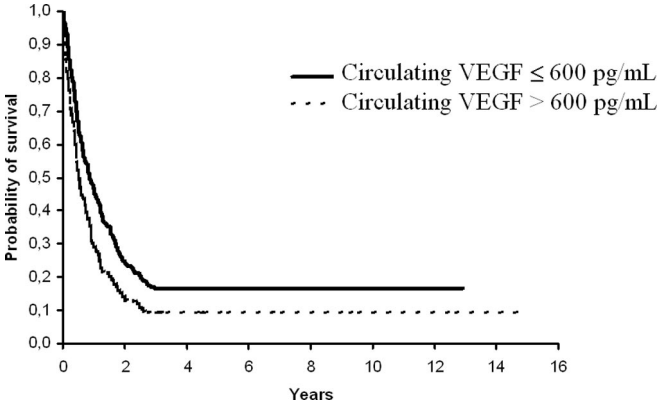


**FIGURE 5.** Probability of survival of non-small cell lung cancer patients with normal and elevated pretreatment circulating vascular endothelial growth factor (VEGF) serum level; Kaplan–Meier curves were constructed taking into account the whole population survival. Dotted line, circulating VEGF serum >600 pg/mL; dark line, circulating VEGF serum ≤600 pg/mL. Median survival in months [95% CI]: 6.4 [5.3–8.7] and 10.6 [8.3–13.2], respectively, log-rank: $p = 0.0002$.

**TABLE 3.** Estimated Hazard Ratio for Significant Variables

| Variables | Hazard Ratio (95% CI) | *p* |
|---|---|---|
| Mountain stage IIIa–IV | 2.23 (1.33–3.73) | 0.0023 |
| NSE >12.5 ng/mL | 1.61 (1.27–2.05) | <0.0001 |
| CYFRA 21-1 >3.6 ng/mL | 1.50 (1.20–1.86) | 0.0003 |
| Nodal status $N_{2-3}$ | 1.64 (1.24–2.17) | 0.0005 |
| Metastatic disease | 1.58 (1.25–2.00) | 0.0001 |
| Performance status 2–4 | 1.77 (1.43–2.19) | <0.0001 |

CI, confidence interval; NSE, neuron-specific enolase.

significance in the univariate analysis only, whereas it was not a prognostic determinant of lung cancer in multivariate analysis.[24] Conversely, low expression of tissue inhibitors of matrix metalloproteinase resulting in a tissue inhibitors of matrix metalloproteinase–MMP imbalance has been considered as a putative target for antiangiogenic therapy.[22] Destruction of the extracellular matrix is a necessary prerequisite for vessel invasion toward metastasic process. Although MMPs play a key role in the initial step of angiogenesis, MMP inhibitors failed to demonstrate a survival benefit in cancer. This observation might have resulted from a partial or incomplete angiogenesis process blockade. Finally, adhesion molecules, such as integrin $\alpha v\beta 3$ which is over expressed in lung cancer, are considered as important step in tumor growth and angiogenesis and have been recently explored as targets in NSCLC therapy.[25]

Circulating angiogenic factor are detectable in the serum of patients suffering from different human malignancies. The most evaluated markers are VEGF, basic fibroblast growth factor, platelet-derived growth factor; transforming growth factor, interleukin-8; hepatocyte growth factor, platelet-derived endothelial cell growth factor, and angiogenin.[19,26] In some malignancies such as gastrointestinal cancer[27] or melanoma,[28] high serum levels of VEGF, interleukin-8, and basic fibroblast growth factor have been identified as prognostic factors. In a population of 125 patients affected by various stage of melanoma and who underwent different combinations of cytokines and cytotoxic therapies, both overall and progression free survivals were independently determined by the three aforementioned circulating angiogenic factors.[28]

In lung cancer, recent literature regarding circulating angiogenic agents produced conflicting results.[10,29–33] It has been suggested that circulating VEGF serum level could be regarded as a surrogate for evaluating tumor-related angiogenesis. Some studies have pointed out a possible correlation between a high serum VEGF level and a poor prognosis in NSCLC patients.[31,33] Although the prognosis significance of circulating VEGF serum level has been supported by a strong rationale and several concordant studies, other studies, including the herein reported, did not. One can consider that the positive studies have had evaluated serum level in small patient populations with sometimes undefined time of serum sampling. In a study stating that circulating VEGF serum is a prognostic determinant of NSCLC, a careful analysis of the results indicates that this parameter shared its prognostic information with other well-known prognostic factors such as disease stage and PS.[31] In multivariate analysis, only the latter well-known prognostic factors were independent prognostic determinants, whereas circulating VEGF serum level was not retained into the Cox model, a similar observation to the one done in our study.

Strengths of our study are (i) a levelheaded number of accrued patients allowing a good reliability of survival analyses of 11 clinical and biologic variables in multivariate analysis; (ii) a long-term follow-up and low rate of patients lost to follow-up. These features authorize confidence in the survival analyses.

There are also some limitations. Although patients have been prospectively accrued in this study, the original purpose of this sera bank (strictly stored at −180°C until tested) was to evaluate the prognostic meaning of a high serum CYFRA 21-1 level. This immunoradiometric assay referred to as the detection of a perfectly identified cytokeratin 19 fragment (epitope sequences lying within the sequences 311–335 and 346–367).[34] The prognostic significance of CYFRA 21-1 has been demonstrated by our research program,[35] and thereafter, confirmed in a meta-analysis.[4] In a previously published study, we demonstrated that the prognostic information given by a high serum CYFRA 21-1 level is independent from other well-known variables such as PS and disease stage, and is perennial throughout extended follow-up period. A high NSE level also prognosticates a poor outcome probably by reflecting tumor heterogeneity and underestimated neuroendocrine differentiation.[36] However, in the nineties, the case for thrombocytopenia, as a putative prognostic variable, was not suspected and the databank was not developed for angiogenic factor analysis purpose. It is now recognized that both platelet count and circulating VEGF serum might be correlated.[30] Because we are not able to give data regarding the former parameter there is part of uncertainty regarding the survival significance of a high circulation VEFG level. Another lim-

itation in the herein study was the long period accrual time and the early start of the study (1990). Nevertheless, the use of cisplatin-based chemotherapy for patients suffering from locally advanced or metastatic disease was early recognized in our institution insofar as we actively participated in the study by Le Chevalier, the first one demonstrating the benefit of third-generation drug plus cisplatin chemotherapy when compared with second-generation drug combination or single-drug chemotherapies.[37] A similar commitment in research and application of concurrent chemoradiotherapy has lead to the combined modality treatment of unresectable stage III NSCLC patients in our study. Therefore, one can consider that our patient population received first-line treatment in a manner that has anticipated the main current guidelines regarding (i) use of cisplatin plus third-generation drug doublets in metastatic patients and (ii) multimodality treatments favoring concurrent chemoradiotherapy in stage IIIb.

Interpretation of negative result from our study is shared in common with other studies analyzing circulating angiogenic factors in lung cancer patients. (i) We investigated one angiogenic agent (VEGF) and tried to correlate its level with prognosis, but none of the patient received antiangiogenic therapy. However, in the randomized study evaluating combination of chemotherapy and bevacizumab,[8] VEGF levels were predictive of response to antiangiogenic therapy but not survival.[38] Serum VEGF could vary in patients receiving bevacizumab in other malignancies suggesting that circulating VEGF might be useful in predicting and monitoring tumor response to anticancer therapies.[26] Therefore, the conclusion of our study does not preclude a possible role of circulating VEGF serum as a surrogate marker for angiogenesis in patient who undergo antiangiogenesis therapy. (ii) It is tempting to define serum markers of angiogenesis, and similar attempts are made for other signaling pathways that promote NSCLC progression such as epidermal growth factor receptor activation. However, complex pathways are rarely reflected by a simple circulating marker. In this setting, we previously demonstrated that neither HER-2 nor EGFR extracellular domains specific levels were associated with a particular prognosis of NSCLC patients.[39] A similar phenomenon might have occurred in the case of circulating VEGF serum that seems a weaker prognostic indicator than its in situ counterpart or its in situ receptors detections.[40–43] (iii) Finally, taking into account the high number of endogenous factors promoting endothelial cells differentiation and proliferation toward tumor angiogenesis, considering a sole factor as a putative surrogate for such a complex process might sound as a naive concept.[19,44]

In the herein study a high circulating VEGF serum did not independently determine prognosis of NSCLC: patients with such a high circulating angiogenic marker proved to have a poorer overall survival than patients with the opposite feature owing to the fact that serum VEGF is strongly correlated with disease stage. This finding could be considered as an additional clue that angiogenesis process partly induces tumor progression and, consequently, contributes to a negative impact on NSCLC prognosis. However, nodal status, stage of the disease and PS that are clearly correlated with a high circulating VEGF serum remain the most important prognostic variables suggesting that circulating VEGF level is only one of the numerous endogenous factors that promote angiogenesis.

## REFERENCES


1. Paesmans M, Sculier JP, Libert P, et al. Prognostic factors for survival in advanced non-small-cell lung cancer: univariate and multivariate analyses including recursive partitioning and amalgamation algorithms in 1052 patients. The European Lung Cancer Working Party. *J Clin Oncol* 1995;13:1221–1230.
2. Charloux A, Hedelin G, Dietemann A, et al. Prognostic value of histology in patients with non-small cell lung cancer. *Lung Cancer* 1997;17:123–134.
3. Ross PJ, Ashley S, Norton A, et al. Do patients with weight loss have a worse outcome when undergoing chemotherapy for lung cancers? *Br J Cancer* 2004;90:1905–1911.
4. Pujol JL, Molinier O, Ebert W, et al. CYFRA 21-1 is a prognostic determinant in non-small-cell lung cancer: results of a meta-analysis in 2063 patients. *Br J Cancer* 2004;90:2097–2105.
5. Wakelee HA, Wang W, Schiller JH, et al. Survival differences by sex for patients with advanced non-small cell lung cancer on Eastern Cooperative Oncology Group trial 1594. *J Thorac Oncol* 2006;1:441–446.
6. Sweeney CJ, Zhu J, Sandler AB, et al. Outcome of patients with a performance status of 2 in Eastern Cooperative Oncology Group Study E1594: a phase II trial in patients with metastatic non-small cell lung carcinoma. *Cancer* 2001;92:2639–2647.
7. Thun MJ, Lally CA, Flannery JT, Calle EE, Flanders WD, Heath CW Jr. Cigarette smoking and changes in the histopathology of lung cancer. *J Natl Cancer Inst* 1997;89:1580–1586.
8. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2007;356:1373–1374.
9. Manegold C, von Pawel J, Zatloukal P, et al. Randomised, double-blind multicentre phase III study of bevacizumab in combination with cisplatin and gemcitabine in chemotherapy-naïve patients with advanced or recurrent non-squamous non-small cell lung cancer (NSCLC): BO17704. *J Clin Oncol* 2007 ASCO Annual Meeting Proceedings Part I. Vol 25, No. 18S:LA7514.
10. Laack E, Scheffler A, Burkholder I, et al. 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* 2005;50:51–58.
11. Travis WD, Colby TV, Corrin B, Shimosato Y, Brambilla E, in collaboration with L. H. Sobin and pathologists from 14 countries. Histological Typing of Lung and Pleural Tumors, 3rd ed. Berlin: Springer, 1999.
12. Zubrod C, Schneiderman M, Frei EJ. Appraisal of methods for the study of chemotherapy of cancer in man: comparative therapeutic trial of nitrogen mustard and triethylene thiophosphoramide. *J Chron Dis* 1960;11:7–33.
13. Sobin LH, Wittekind C. Lung and pleural tumours. In Cancer IUA (Ed.), TNM Classification of Malignant Tumours, 6th Ed. New York: Wiley & Sons, 2002. Pp. 99–103.
14. Rowell NP, O'Rourke NP. Concurrent chemoradiotherapy in non-small cell lung cancer. *Cochrane Database Syst Rev* 2004:CD002140.
15. Hanley JA, McNeil BJ. The meaning and use of area below the receiver operating characteristic curves. *Radiology* 1982;143:29–36.
16. Vida S. A computer program for non-parametric receiver operating characteristic analysis. *Comput Methods Programs Biomed* 1993;40:95–101.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–481.
18. Cox JD, Yesner RA. Causes of treatment failure and death in carcinoma of the lung. *Yale J Biol Med* 1981;54:201–207.
19. Scappaticci FA. Mechanisms and future directions for angiogenesis-based cancer therapies. *J Clin Oncol* 2002;20:3906–3927.
20. Kadota K, Huang CL, Liu D, et al. The clinical significance of lymphangiogenesis and angiogenesis in non-small cell lung cancer patients. *Eur J Cancer* 2008;44:1057–1067.
21. O'Byrne KJ, Koukourakis MI, Giatromanolaki A, et al. Vascular endo-


 

thelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small-cell lung cancer. *Br J Cancer* 2000;82:1427–1432.
22. Mino N, Takenaka K, Sonobe M, et al. Expression of tissue inhibitor of metalloproteinase-3 (TIMP-3) and its prognostic significance in resected non-small cell lung cancer. *J Surg Oncol* 2007;95:250–257.
23. Leinonen T, Pirinen R, Böhm J, Johansson R, Kosma VM. Increased expression of matrix metalloproteinase-2 (MMP-2) predicts tumour recurrence and unfavourable outcome in non-small cell lung cancer. *Histol Histopathol* 2008;23:693–700.
24. Yamamura T, Nakanishi K, Hiroi S. Expression of membrane-type-1-matrix metalloproteinase and metalloproteinase-2 in non-small cell lung carcinomas. *Lung Cancer* 2002;35:249–255.
25. Albert JM, Cao C, Geng L, Leavitt L, Hallahan DE, Lu B. Integrin alpha v beta 3 antagonist Cilengitide enhances efficacy of radiotherapy in endothelial cell and non-small-cell lung cancer models. *Int J Radiat Oncol Biol Phys* 2006;65:1536–1543.
26. Poon RT, Fan ST, Wong J. Clinical implications of circulating angiogenic factors in cancer patients. *J Clin Oncol* 2001;19:1207–1225.
27. Hyodo I, Doi T, Endo H, et al. Clinical significance of plasma vascular endothelial growth factor in gastrointestinal cancer. *Eur J Cancer* 1998;34:2041–2045.
28. Ugurel S, Rappl G, Tilgen W, Reinhold U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. *J Clin Oncol* 2001;19:577–583.
29. Ustuner Z, Saip P, Yasasever V, et al. Prognostic and predictive value of vascular endothelial growth factor and its soluble receptors, VEGFR-1 and VEGFR-2 levels in the sera of small cell lung cancer patients. *Med Oncol* 2008; (Epub ahead of print).
30. Choi JH, Kim HC, Lim HY, et al. Vascular endothelial growth factor in the serum of patients with non-small cell lung cancer: correlation with platelet and leukocyte counts. *Lung Cancer* 2001;33:171–179.
31. Laack E, Köhler A, Kugler C, et al. Pretreatment serum levels of matrix metalloproteinase-9 and vascular endothelial growth factor in non-small-cell lung cancer. *Ann Oncol* 2002;13:1550–1557.
32. Katsabeki-Katsafli A, Kerenidi T, Kostikas K, et al. Serum vascular endothelial growth factor is related to systemic oxidative stress in patients with lung cancer. *Lung Cancer* 2008;60:271–276.
33. Dudek AZ, Mahaseth H. Circulating angiogenic cytokines in patients with advanced non-small cell lung cancer: correlation with treatment response and survival. *Cancer Invest* 2005;23:193–200.
34. Bodenmüller H, Ofenloch-Hähnle B, Lane EB, Dessauer A, Böttger V, Donié F. Lung cancer-associated keratin 19 fragments: development and biochemical characterisation of the new serum assay Enzymun-Test CYFRA 21-1. *Int J Biol Markers* 1994;9:75–81.
35. Pujol JL, Grenier J, Daurès JP, Daver A, Pujol H, Michel FB. Serum fragment of cytokeratin subunit 19 measured by CYFRA 21-1 immunoradiometric assay as a marker of lung cancer. *Cancer Res* 1993;53:61–66.
36. Pujol JL, Boher JM, Grenier J, Quantin X. Cyfra 21-1, neuron specific enolase and prognosis of non-small cell lung cancer: prospective study in 621 patients. *Lung Cancer* 2001;33:89–94.
37. Le Chevalier T, Pujol JL, Douillard Y, et al. A three-arm trial of vinorelbine (Navelbine) plus cisplatin, vindesine plus cisplatin, and single agent vinorelbine in the treatment of non-small cell lung cancer: an expanded analysis. *Semin Oncol* 1994;21:28–34.
38. Dowlati A, Gray R, Sandler AB, Schiller JH, Johnson DH. Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab—an Eastern Cooperative Oncology Group Study. *Clin Cancer Res* 2008;14:1407–1412.
39. Jacot W, Pujol JL, Boher JM, Lamy JM. Serum EGF-receptor and HER-2 extracellular domains and prognosis of non-small-cell lung cancer. *Br J Cancer* 2004;91:430–433.
40. Niklińska W, Burzykowski T, Chyczewski L, Nikliński J. Expression of vascular endothelial growth factor (VEGF) in non-small cell lung cancer (NSCLC): association with p53 gene mutation and prognosis. *Lung Cancer* 2001;34(Suppl 2):S59–S64.
41. Liao M, Wang H, Lin Z, Feng J, Zhu D. Vascular endothelial growth factor and other biological predictors related to the postoperative survival rate on non-small cell lung cancer. *Lung Cancer* 2001;33:125–132.
42. Baillie R, Carlile J, Pendleton N, Schor AM. Prognostic value of vascularity and vascular endothelial growth factor expression in non-small cell lung cancer. *J Clin Pathol* 2001;54:116–120.
43. Arinaga M, Noguchi T, Takeno S, Chujo M, Miura T, Uchida Y. Clinical significance of vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 in patients with non-small cell lung carcinoma. *Cancer* 2003;97:457–464.
44. Herbst RS, Onn A, Sandler A. Angiogenesis and lung cancer: prognostic and therapeutic implications. *J Clin Oncol* 2005;23:3243–3256.