Serum Levels of Vascular Endothelial Growth Factor Dependent on the Stage Progression of Lung Cancer*

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Study objective: In lung cancer, vascular endothelial growth factor (VEGF) is an important cytokine and is correlated with tumor vessel density, malignant pleural effusions, and coagulation-fibrinolysis factors in vitro. We investigated the correlation between serum VEGF level and stage progression in lung cancer to study the predicted value of VEGF level. We also studied whether coagulation-fibrinolysis factors and PaO$_2$ levels, which are also important factors for the prediction of the clinical course, are correlated with VEGF.

Methods: Forty-nine patients with lung cancer were investigated prospectively. VEGF levels of sera and malignant effusions, and plasma concentrations of coagulation-fibrinolysis factors were measured by enzyme-linked immunosorbent assay. We measured PaO$_2$ levels in all patients at rest.

Results: Serum levels of VEGF were increased significantly according to stage progression. Additionally, plasma concentrations of D dimer, thrombin-antithrombin complex (TAT), and tissue plasminogen activator/plasminogen activator inhibitor type I complex were elevated significantly according to stage progression. The serum VEGF level had a significant positive correlation with the TAT and D dimer levels. Serum VEGF levels had a significant negative correlation with PaO$_2$ levels. The incidence of cerebral vascular disorder was significantly higher in the patients with systemic hypoxemia than in those without ($p < 0.05$). Mean VEGF levels in malignant effusions in eight patients (five with pleural effusions, two with pericardial effusions, and one with both) were extremely high, especially in pericardial effusions ([mean ± SD] pleural effusions, 531.9 ± 285.4 pg/mL; pericardial effusion, 3,071.6 ± 81.3 pg/mL).

Conclusion: We predict that in lung cancer, VEGF production and the abnormality of the coagulation-fibrinolysis system differ depending on the stage of progression of disease. Serum VEGF levels would be affected by PaO$_2$ levels in lung cancer.

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Key words: cerebral vascular disorder; D dimer fragments; pericardial effusion; thrombin-antithrombin complex; tissue plasminogen activator/plasminogen activator inhibitor type I complex

Abbreviations: ELISA = enzyme-linked immunosorbent assay; PAI = plasminogen activator inhibitor type I; PIC = plasmin inhibitor complex; TAT = thrombin-antithrombin complex; tPA = tissue plasminogen activator; VEGF = vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is an important cytokine in cancer and is associated with increased tumor vessel density, cancer metastasis, cancer prognosis, carcinomatous pleurisy, and the coagulation-fibrinolysis system in vitro. Systemic hypoxemia induces elevations of serum VEGF levels, especially in patients with lung cancer. However, little is known about the association of VEGF levels with stage progression of lung cancer, the association of VEGF and coagulation-fibrinolysis factors in lung cancer in vivo, and VEGF levels in malignant pericardial effusion associated with lung cancer. Also, evaluation of the coagulation-fibrinolysis system and control of pericardial effusions are important in predicting the clinical course of lung cancer patients. In this study, we investigated VEGF levels in the sera and malignant effusions of 49 lung cancer patients to determine whether this parameter might be used to evaluate disease progression. We also studied whether coagulation-fi-
brinolysis factors and PaO₂ levels are correlated with VEGF in lung cancer patients.

**MATERIALS AND METHODS**

**Patients**

We prospectively investigated 49 consecutive lung cancer patients who were admitted to the Department of Respiratory Medicine (National Minami-Kyushu Hospital) from 1995 to 1999, including 39 men and 10 women whose mean (± SD) age was 62.5 ± 12.3 years. We definitely excluded the patients with clinical or conventional laboratory evidence suggestive of intravascular coagulation abnormalities. We also excluded patients with diabetes mellitus, arteriosclerosis, and those receiving anticoagulant medication. For all patients, the diagnosis of lung cancer was confirmed by the histologic examinations of biopsy and cytologic specimens taken during bronchoscopic examinations. Staging was based on the new international staging system. The staging procedure included the following: a clinical examination; standard chest radiography; CT scans of the chest, abdomen, and brain; bronchoscopy; liver ultrasonography; and bone scanning.

Adenocarcinoma was diagnosed in 28 patients, squamous cell carcinoma in 17 patients, and small cell lung cancer in 4 patients. Two patients were classified as having clinical stage I disease, 3 patients as having stage IIA disease, 13 patients as having stage IIB disease, 11 patients as having stage IIIA disease, 15 patients as having stage IIIB disease, and 5 patients as having stage IV disease. Eight patients had malignant effusions (carcinomatous pleurisy, six patients; carcinomatous pericarditis, three patients; and those without, one patient).

**Clinical Study**

We examined the PaO₂ levels (measured with the patient breathing room air at rest) in all patients. The patients whose PaO₂ levels were < 60 mm Hg were classified as having systemic hypoxemia (eight patients).

**Measurement of Coagulation-Fibrinolysis Factors and VEGF**

We measured plasma concentrations of D dimer fragments, thrombin-antithrombin complex (TAT), plasmin-α2-plasmin inhibitor complex (PIC), tissue plasminogen activator (tPA)/plasminogen activator inhibitor type I (PAI) complex, and serum VEGF in the 49 patients described above. Plasma D dimer concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody, which recognizes an antigenic determinant of D dimer, that was developed by Elms et al. Plasma TAT levels were assayed using a solid-phase ELISA kit (Enzygnost-TAT; Behringwerke AG; Frankfurt, Germany) by means of the sandwich principle and two different antibodies directed against human thrombin and antithrombin III. Plasma concentrations of PIC were assayed by commercial ELISA kits (Teijin Ltd; Tokyo, Japan), employing an antiplasminogen antibody and a peroxidase-conjugated human anti-α2-plasmin inhibitor monoclonal antibody. Plasma concentrations of tPA/PAI complex were measured by ELISA using a polyclonal antibody against PAI. VEGF concentrations in serum and malignant effusions (ie, pleural and pericardial effusions) were measured in duplicate for each sample with a commercial ELISA kit (R&D Systems; Minneapolis, MN) that recognizes the soluble isoforms VEGF₁₆₅ and VEGF₁₄₅. This assay is sensitive to 9 pg/mL (0.2 pmol) VEGF and does not cross-react with platelet-derived growth factor or other homologous cytokines. The optical density at 450 nm was measured on a plate reader (Titertek Multiskan MC; Flow Laboratories; Helsinki, Finland), and VEGF concentration was determined by linear regression from a standard curve and by computer software (Graph Pad; San Diego, CA) for analysis.

**Statistical Analysis**

All data were presented as mean ± SD. We used one-way factorial analysis of variance with a Bonferroni-Dunn test to determine the differences of VEGF levels and coagulation-fibrinolysis factors between the histologic patterns. A Spearman correlation coefficient by rank was used to measure differences in VEGF levels and coagulation-fibrinolysis factors between stages. We utilized Pearson’s correlation coefficient to evaluate the correlations between VEGF and coagulation-fibrinolysis factors or PaO₂ levels. The Mann-Whitney U test was utilized to measure the difference in VEGF levels between the patients with systemic hypoxemia and the patients without systemic hypoxemia. We used the χ² test to evaluate the incidence of cerebral vascular disorder between patients with systemic hypoxemia and those without. A p value < 0.05 was considered significant.

**Results**

The serum levels of VEGF, and the plasma concentrations of TAT, D dimer, and tPA/PAI complex were increased significantly according to the stage of progression of disease (Table 1). There were no significant differences between the histologic patterns (Table 2).

There were significant positive correlations between TAT and VEGF (r = 0.322; p < 0.05), D

| Table 1—Relationship Among VEGF, Coagulation-Fibrinolysis Factors, and the Clinical Stages of Disease* |
|-------------------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Variables                               | I         | IIA        | IIB        | IIIA       | IIIB       | IV         | p Value     |
| VEGF, pg/mL                             | 254.01 ± 184.37 | 337.46 ± 92.07 | 201.57 ± 136.75 | 288.39 ± 133.29 | 535.65 ± 200.32 | 670.61 ± 145.46 | < 0.0001 |
| TAT, ng/mL                              | 1.7 ± 0.28 | 2.57 ± 1.09 | 2.96 ± 1.20 | 2.97 ± 2.11 | 4.44 ± 1.56 | 5.56 ± 3.41 | < 0.01 |
| D dimer, ng/mL                          | 1.16 ± 0.56 | 1.43 ± 0.33 | 1.90 ± 0.29 | 105.69 ± 62.19 | 144.08 ± 118.29 | 246.71 ± 106.38 | < 0.001 |
| PIC, µg/mL                              | 1.66 ± 0.49 | 1.54 ± 1.09 | 1.58 ± 0.51 | 1.67 ± 0.25 | 1.61 ± 0.46 | 1.55 ± 0.69 | NS |
| tPA/PAI, ng/mL                          | 6.8 ± 6.22 | 7.67 ± 1.82 | 10.99 ± 3.79 | 12.04 ± 2.97 | 13.89 ± 3.21 | 15.86 ± 4.63 | < 0.001 |

*NS = not significant.
dimer and VEGF ($r = 0.42; p < 0.01$), and tPA/PAI complex and VEGF ($r = 0.365; p < 0.01$). In patients with squamous cell carcinoma, there was a stronger positive correlation between TAT and VEGF than in the whole group ($r = 0.56; p < 0.001$).

The serum VEGF levels in the patients with systemic hypoxemia were significantly higher than in those without (Fig 1). There was a significant negative correlation between VEGF and $\text{Pao}_2$ levels ($r = -0.578; p < 0.0001$) and tPA/PAI complex and $\text{Pao}_2$ levels ($r = -0.378; p < 0.01$). There was no significant correlation between hypoxemia and stage progression.

The mean VEGF level in patients with malignant pleural effusions was $531.9 \pm 285.4 \text{ pg/mL}$, and that in patients with malignant pericardial effusions was $3,071.6 \pm 81.3 \text{ pg/mL}$ (Table 3).

Three patients developed cerebral vascular disorders (two patients with transient ischemic attack, and one patient with cerebral infarction) among the patients with systemic hypoxemia; however, nobody developed cerebral vascular disorder among the patients without systemic hypoxemia. This difference was significant ($p < 0.05$).

**TABLE 2—VEGF and Coagulation-Fibrinolysis Factors Between Histologic Patterns**

<table>
<thead>
<tr>
<th>Histologic Pattern</th>
<th>Adenocarcinoma (n = 28)</th>
<th>Squamous Cell Carcinoma (n = 17)</th>
<th>Small Cell Carcinoma (n = 4)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF, pg/mL</td>
<td>$353.74 \pm 196.66$</td>
<td>$480.48 \pm 210.61$</td>
<td>$388.69 \pm 209.98$</td>
<td>NS</td>
</tr>
<tr>
<td>TAT, mg/mL</td>
<td>$3.25 \pm 2.12$</td>
<td>$4.31 \pm 1.85$</td>
<td>$2.7 \pm 1.42$</td>
<td>NS</td>
</tr>
<tr>
<td>D dimer, mg/mL</td>
<td>$225.39 \pm 103.78$</td>
<td>$229.88 \pm 115.36$</td>
<td>$147.75 \pm 44.9$</td>
<td>NS</td>
</tr>
<tr>
<td>PIC, pg/mL</td>
<td>$1.23 \pm 1.52$</td>
<td>$1.47 \pm 0.65$</td>
<td>$1.82 \pm 1.63$</td>
<td>NS</td>
</tr>
<tr>
<td>tPA/PAI, pg/mL</td>
<td>$12.92 \pm 3.87$</td>
<td>$12 \pm 3.46$</td>
<td>$9.78 \pm 7.07$</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS = not significant.

**FIGURE 1.** Comparison of serum VEGF levels between the patients with systemic hypoxemia and those without systemic hypoxemia. Serum VEGF levels in the patients with systemic hypoxemia are significantly higher than in the patients without systemic hypoxemia. ● = mean values; bars = SD in each group.

### Discussion

According to stage progression, concentrations of TAT, D dimer, and tPA/PAI complex in plasma and concentrations of VEGF in serum were significantly increased. In lung cancer patients, Gabazza et al.\(^{14}\) reported increases in levels of TAT, D dimer, and PIC in patients with advanced stages of disease (ie, stages IIIb and IV). Our results were almost the same as those of that report, except for the level of PIC, and we confirmed the activation of the coagulation-fibrinolysis system in lung cancer patients, especially those in the advanced stage of disease. However, the association of VEGF expression with bladder cancer recurrence\(^{15}\) and with lymph node metastasis in patients with non-small cell lung cancer\(^2\) has been reported. Our examination showed an increase of VEGF in patients with advanced-stage disease (ie, stages IIIb and IV) who exhibited lymph node metastasis in patients with non-small cell lung cancer\(^2\) has been reported. Our examination showed an increase of VEGF in patients with advanced-stage disease (ie, stages IIIb and IV) who exhibited lymph node metastasis or distant metastasis. Also, VEGF levels in patients with malignant pleural effusions were high, as reported by Thickett et al.\(^4\). Furthermore, as in patients with pleural effusions, we also observed an extremely high level of VEGF in patients with malignant pericardial effusions (Table 3). These findings may suggest the strong association of VEGF with lung cancer progression.

**Table 3—VEGF Levels in Malignant Effusions**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Serum VEGF, pg/mL</th>
<th>Pleural Effusion VEGF, pg/mL</th>
<th>Pericardial Effusion VEGF, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>710.5</td>
<td></td>
<td>3,138</td>
</tr>
<tr>
<td>2</td>
<td>626.8</td>
<td></td>
<td>3,096.4</td>
</tr>
<tr>
<td>3</td>
<td>360.9</td>
<td>560.1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>129.7</td>
<td>326.8</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>420.9</td>
<td>909.4</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>850.7</td>
<td>856.3</td>
<td>2,980.9</td>
</tr>
<tr>
<td>7</td>
<td>360.9</td>
<td>306.7</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>321.5</td>
<td>251.9</td>
<td>0</td>
</tr>
</tbody>
</table>
Statistical analysis showed significant positive correlations between TAT and VEGF, D dimer, and VEGF, and between tPA/PAI complex and VEGF. It has been reported that VEGF has a strong association with the coagulation-fibrinolysis system, including the induction of VEGF by thrombin in vitro.16 Our study supports this hypothesis in vivo. Interestingly, we showed that TAT and VEGF showed a stronger positive correlation in patients with squamous cell carcinoma, whose cell proliferation was said to have some associations with VEGF expression.17 Additionally, it was reported that patients with squamous cell lung cancer were likely to have coagulation-fibrinolysis system abnormalities.18 VEGF may have an association with abnormality of the coagulation-fibrinolysis system in lung cancer patients.

The serum VEGF levels and plasma concentrations of tPA/PAI complex had significant negative correlations with PaO₂ levels. Systemic hypoxemia induces VEGF expression in the lung6 and coagulation-fibrinolysis abnormalities.19 Lung cancer that develops in a central site is likely to obstruct the airway and leads to atelectasis or obstructive pneumonia. Indeed, eight patients required oxygen therapy because of systemic hypoxemia, and the incidence of cerebral vascular disorders was significantly high in these patients.

In conclusion, we reported the increased levels of VEGF and coagulation-fibrinolysis factors in patients with advanced lung cancer, the association of VEGF with the coagulation-fibrinolysis system in vitro, and the correlation of VEGF levels with systemic hypoxemia in patients with lung cancer. The measurement of serum VEGF levels may be useful to evaluate lung cancer progression. These three factors (VEGF levels, coagulation-fibrinolysis factors, and PaO₂ levels) may have intertwined associations with one another. Our study is too small to draw conclusions, but further studies addressing this point may clarify the association between these factors.

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REFERENCES
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