Pretreatment serum levels of matrix metalloproteinase-9 and vascular endothelial growth factor in non-small-cell lung cancer

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Background: Matrix metalloproteinase (MMP)-9 and vascular endothelial growth factor (VEGF) are two proteins involved in angiogenesis. In the present study we investigated the association of pretreatment MMP-9 and VEGF serum levels with clinicopathological parameters and outcome in patients with non-small-cell lung cancer (NSCLC).

Patients and methods: From February 1998 to October 1999, pretreatment serum levels of MMP-9 and VEGF were analysed in 118 patients with enzyme-linked immunoassays. At diagnosis 50 patients (42%) were staged as early disease (I/II), 27 patients (23%) as locally advanced (IIIA/IIIB), and 41 patients (35%) had metastatic disease (IV). In 72 of the 118 patients tumours were resected and 46 patients received combination chemotherapy with gemcitabine and vinorelbine.

Results: The median survival of all 118 patients was 602 days. The 72 patients who had undergone surgery had a median survival of 972 days and the 46 patients who were treated with chemotherapy had a median survival of 298 days ($P<0.001$). Resected patients with stage I/II disease and an MMP-9 serum level $\leq$1293 ng/ml or a VEGF serum level $\leq$630 pg/ml had a significantly longer survival (median survival longer than 1218 days) than patients with higher serum levels (median survival 421 days) ($P=0.001$ for MMP-9; $P=0.04$ for VEGF). No significant difference in survival was observed in patients with resected stage III disease. Besides tumour stage, Karnofsky performance status and gender, the pretreatment serum level of MMP-9 was identified as an independent prognostic factor in a multivariate Cox regression analysis.

Conclusions: Future studies may support our hypothesis that the pretreatment serum level of MMP-9 is a new powerful prognostic marker and can help to stratify NSCLC patients with stage I/II disease into low- and high-risk groups.

Key words: angiogenic factors, matrix metalloproteinase-9, non-small-cell lung cancer, prognostic factors, vascular endothelial growth factor

Introduction

In Western Europe and in the United States, lung cancer is the most frequent cause of death among malignant diseases. More than one million new cases of lung cancer occur worldwide every year [1]. Non-small-cell lung cancer (NSCLC) accounts for more than 75% of pulmonary carcinomas. Up to now surgical resection has been the therapy of choice in early stages of the disease. However, half of the patients relapse after complete resection indicating that NSCLC is a disseminated disease from the beginning in a substantial proportion of patients. Currently the best predictor of outcome for patients with NSCLC is the TNM classification [2, 3]. However, it is known that the prognosis of patients within each stage of disease may vary considerably. The aim of the current research was to find new prognostic factors to assess individual risk profiles.

Angiogenesis represents the formation of new blood vessels from existing vasculature. Neovascularisation is a requirement for solid tumour growth beyond 1–2 mm in diameter [4, 5]. The angiogenic process is a balance between stimulatory and inhibitory factors. The pro-angiogenic stimuli may be released by the tumour, stromal and inflammatory cells, by the extracellular matrix, or by the endothelial cells themselves. Tumour cells secrete or induce the release of growth factors
which stimulate migration and proliferation of endothelial cells. Furthermore these factors may be involved in capillary morphogenesis or release of proteolytic enzymes [6–8].

Matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) are two of the most potent factors involved in angiogenesis. The MMPs belong to a family of zinc-dependent neutral endopeptidases. Under physiological conditions they are capable of degrading extracellular matrix and basement membrane components. Increased MMP activity has been implicated in tumour invasion and the formation of metastases. Although 18 members of the MMP family have been described [9], the gelatinases MMP-9, formerly known as gelatinase B or 92 kDa type IV collagenase, and MMP-2 have been most consistently detected in malignant tissues and are associated with tumour aggressiveness and metastatic potential [10–12].

VEGF is the most potent and specific growth factor (e.g. proliferation and migration) for endothelial cells. High levels of expression of VEGF are found in many solid tumour types including colon [13], breast [14], gastric [15] and lung [16] cancer. It has been reported that overexpression of VEGF in tumour tissue represents an independent prognostic factor in patients with breast [17], gastric [18] and squamous-cell lung [19] cancer. Four isoforms of VEGF (VEGF121, VEGF165, VEGF189, VEGF206) exist in human tissues [20]. VEGF189 and VEGF206 are cell-associated and have strong affinity to cell-surface proteoglycans [20] whereas VEGF121 and VEGF165 are secretory forms.

The prognostic value of pretreatment serum levels of MMP-9 and VEGF in patients with NSCLC is still unclear.

The aim of the present study was to analyse the association of pretreatment serum levels of MMP-9 and VEGF with clinicopathological parameters and outcome, and to evaluate their prognostic relevance in patients with NSCLC.

**Patients and methods**

Patients with cytologically or histologically confirmed NSCLC were enrolled in this prospective investigation. Patients with stage I/II disease were treated only by surgery. If resection was complete no adjuvant radiotherapy was applied. Patients with operable locally advanced disease, stage IIIA, received radiotherapy after surgery. Patients with inoperable locally advanced or metastatic disease were part of a phase II study and were treated with a combination chemotherapy containing gemcitabine and vinorelbine [21]. Patients received gemcitabine 1000 mg/m² as a 30-min infusion followed 1 h later by vinorelbine 30 mg/m² as a 15-min infusion on days 1, 8 and 15 every 4 weeks.

Pretreatment evaluation included a complete history and physical examination with evaluation of the Karnofsky performance status score, chest X-rays in postero–anterior and lateral views, a computed tomography (CT) scan of the chest, sonography or CT of the upper abdomen, radionuclide bone scan, fiberoptic bronchoscopy with bronchoaspirate and/or brushing and/or bronchial biopsy, complete blood cell count, and serum chemistry analysis. Brain CT was only performed if clinically indicated. Patients who had signs of metastasis in mediastinal lymph nodes in the CT scan of the chest received a mediastinoscopy before surgery. All patients gave informed consent before entering this study.

Twenty millilitres of venous blood were taken before surgery or chemotherapy from each non-fasting patient, and was subsequently centrifuged at 2100 g for 7 min at 4°C. The supernatant was transferred into microtubes and stored at −70°C until use.

Serum samples were analysed for MMP-9 and VEGF with Human MMP-9 (total) Immunoassay Quantikine™ and Human VEGF Immunoassay Quantikine™ (R&D Systems, Inc., Minneapolis, MN, USA). The principle of these assays employs a quantitative sandwich enzyme immunoassay technique. The minimum detectable concentration of MMP-9 is ≤0.156 ng/ml and of VEGF≤9.0 pg/ml. Each serum sample was determined twice. The mean intrabatch coefficients of variation range between 1.9% and 2.9% for the MMP-9 immunoassay and between 4.5% and 6.7% for the VEGF immunoassay. MMP-9 and VEGF concentrations were calculated using Delta SOFT 3 computer software (Bio Metallics, Inc., Princeton, NJ, USA).

**Statistical analysis**

The median follow up of all patients was 945 days (minimum 154 days, maximum 1218 days). Dates of diagnosis, relapse and death were recorded. From these data, survival curves were prepared according to the Kaplan–Meier method for time to death. Survival curves were compared with a log-rank test. The serum concentrations of MMP-9 and VEGF in our study population were compared with data from healthy controls (37 persons) provided by R&D Systems with a two-tailed Student’s t-test. The Kruskal–Wallis test was used to compare serum levels of MMP-9 or VEGF with tumour stage.

All factors were investigated for their prognostic value in a univariate analysis using the Cox regression model. Variables with sufficient statistical prognostic power (P < 0.1) were investigated, then subjected to a multivariate analysis and to a model selection approach in order to determine a final parsimonious prognostic model for patient survival based on this data set. Forward and backward variable selection and likelihood-ratio statistic were calculated with SPSS software (Version 10.0; SPSS, Inc., Chicago, IL, USA). The outcome of the Cox regression was described quantitatively by the statistical estimate of the regression parameter β and its standard error, its risk ratio exp (β), the 95% confidence interval, and the respective R value obtained from the Wald test statistic. MMP-9 and VEGF were categorised in quartiles (MMP-9: ≤806 ng/ml, 807 to ≤1293 ng/ml, 1294 to ≤1912 ng/ml, >1912 ng/ml; VEGF: ≤417 pg/ml, 418 to ≤630 pg/ml, 631 to ≤1205 pg/ml, >1205 pg/ml) for survival and Cox regression analysis.

All statistical tests were performed using SPSS software.

**Results**

**Patient characteristics**

From February 1998 to October 1999, 118 patients were prospectively enrolled into this study. Patient characteristics are provided in Table 1. The majority of patients were men (75%). Patients had a median age of 63 years (range 39–86), and a median Karnofsky performance status of 90%. The predominant histological type was adenocarcinoma. Fifty patients (42%) were staged as early disease (stage I and II), 27 (23%) as locally advanced disease (stage IIIA and IIIB) and 41 patients (35%) had metastatic disease (stage IV) at the time
of diagnosis. Seventy-two of the 118 patients underwent surgery, of whom 22 patients with locally advanced disease received radiotherapy after tumour resection. Forty-six patients with inoperable locally advanced or metastatic disease received a combination chemotherapy with gemcitabine and vinorelbine. Up to now a relapse has occurred in 26 of the 72 (36%) resected patients.

The median serum levels of MMP-9 and VEGF of all 118 patients were 1293 ng/ml [MMP-9: maximum 3055 ng/ml, minimum 156 ng/ml; mean 1389 ± 730 ng/ml (standard deviation, SD)] and 633 pg/ml (VEGF: maximum 6241 pg/ml, minimum 69 pg/ml; mean 917 ± 909 pg/ml), respectively. In comparison with healthy controls (mean serum level of MMP-9: 436 ng/ml, mean serum level of VEGF: 220 pg/ml) provided by R&D Systems the serum levels of MMP-9 and VEGF were significantly elevated in our patient population [t-test for MMP-9: mean difference 953 ng/ml (95% confidence interval 820–1086, P < 0.0005); t-test for VEGF: mean difference 697 pg/ml (95% confidence interval 531–862, P < 0.0005)]. More than 80% of our patients had MMP-9 and VEGF serum levels above the cutoff of the mean serum level plus 2 SDs of a healthy control group.

Serum levels of MMP-9/VEGF and patient characteristics

A significant relationship between serum levels of MMP-9 and tumour stage was observed (P = 0.01). Patients with early disease had a median serum level of 910 ng/ml (range 156–3055), those with locally advanced disease 1340 ng/ml (range 259–2892), and those with metastatic disease 1796 ng/ml (range 161–2897) (Figure 1). The relationship between serum levels of VEGF and tumour stage was also highly significant (P < 0.0005). The median serum levels in patients with stage I/II disease was 506 pg/ml (range 69–2087), with stage IIIA/B disease 630 pg/ml (range 80–2708), and with stage IV disease 890 pg/ml (range 315–6241) (Figure 2).

No relationship was observed between serum levels of MMP-9 as well as VEGF and the different histological types (adenocarcinoma, squamous-cell carcinoma, large-cell carcinoma).

Correlation between serum levels of MMP-9/VEGF and outcome

The median survival of all 118 patients was 602 days [range 29–1218+ (‘+’ indicates that the patient with the longest
follow up is still alive). The 72 patients who had undergone surgery had a median survival of 972 days (range 35–1218+), whereas the 46 patients who were treated with chemotherapy had a median survival of 298 days (range 29–989) ($P < 0.00005$) (Figure 3).

The median survival of patients with stage I/II disease was longer than 1218 days (range 35–1218+), for patients with stage IIIA/B disease 587 days (range 42–1189+), and for patients with stage IV disease 301 days (range 29–989) ($P < 0.00005$) (Figure 4).

A significant correlation between serum concentration of MMP-9 and survival was observed for the 72 patients who had undergone surgery. Comparing the survival curves of the serum level quartiles of MMP-9 we found an overall significant difference ($P = 0.01$), whereas no significant difference existed for the 46 patients who were treated with chemotherapy ($P = 0.11$).

Patients with resected disease and an MMP-9 serum level of 1293 ng/ml or lower (i.e. cutoff level between the second and third quartile) had a significantly longer survival [median survival longer than 1218 days (range 42–1218+)] than those with a serum level of higher than 1293 ng/ml [median survival 540 days (range 35–1168+)] ($P = 0.002$).

Subanalysis of resected patients with stage I/II and stage IIIA/B disease has demonstrated that only patients with stage I/II disease and an MMP-9 serum level of 1293 ng/ml or lower had a significantly longer survival [median survival longer than 1218 days (range 46–1218+)] than those with a serum level of higher than 1293 ng/ml [median survival 421 days (range 35–1168+)] ($P = 0.001$) (Figure 5), whereas no significant difference was found in patients with resected stage IIIA/B disease ($P = 0.49$) nor in patients who were treated with chemotherapy ($P = 0.06$).

Comparing the VEGF serum level quartiles with regard to survival of the 72 resected patients ($P = 0.28$) and the 46 patients who were treated with chemotherapy ($P = 0.10$) no significant difference was observed.

An analysis including all resected patients has also demonstrated no significant difference in survival for patients with a VEGF serum level of 630 pg/ml or lower (cutoff level between the second and third quartile) versus higher than 630 pg/ml ($P = 0.20$). On the other hand, subanalysis showed a significant survival benefit for patients with stage I/II disease and a VEGF serum level of 630 pg/ml or lower [median survival longer than 1218 days (range 103–1218+) versus 421 days (range 35–1125+)] ($P = 0.04$) (Figure 6). No significant difference in survival was observed for patients

Figure 3. Kaplan–Meier survival curves of 72 patients who had undergone surgery and of 46 patients who were treated with chemotherapy.

Figure 4. Kaplan–Meier survival curves of 118 non-small-cell lung cancer patients according to tumour stage.

Figure 5. Kaplan–Meier survival curves of 50 patients with stage I/II disease stratified according to pretreatment matrix metalloproteinase-9 (MMP-9) serum levels.

Figure 6. Kaplan–Meier survival curves of 50 patients with stage I/II disease stratified according to pretreatment vascular endothelial growth factor (VEGF) serum levels.
with resected stage IIIA/B disease ($P = 0.38$). In contrast, patients treated with chemotherapy who had a VEGF serum level of 630 pg/ml or lower did survive significantly longer than those with serum levels higher than 630 pg/ml [median survival 630 days (range 29–989) versus 290 days (range 46–587)] ($P = 0.02$).

In a univariate Cox regression analysis tumour stage, pretreatment serum levels of MMP-9 and VEGF, platelet count, haemoglobin concentration, lactate dehydrogenase concentration, the histological type, and the Karnofsky performance status had prognostic significance in survival of all NSCLC patients ($n = 118$) (Table 2). Using a multivariate analysis ($P$ limit $\leq 0.1$), only tumour stage, Karnofsky performance status, gender and the pretreatment serum level of MMP-9 were identified as independent prognostic factors, whereas histological type, age, platelet count, haemoglobin concentration, lactate dehydrogenase concentration and VEGF serum level had no impact on survival in this patient population (Table 3).

### Discussion

The aim of the present investigation was to analyse the association between pretreatment serum levels of MMP-9 and VEGF and clinicopathological parameters and outcome in patients with NSCLC.

We observed a significant elevation of serum levels of MMP-9 and VEGF in our patient population in comparison with healthy controls. Similar results were reported for

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**Table 2. Results of the univariate Cox regression analysis for clinical factors and serum parameters ($n = 118$)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
<th>$n$</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>–</td>
<td>118</td>
<td>1.02</td>
<td>0.99–1.04</td>
<td>0.24</td>
</tr>
<tr>
<td>Histological type</td>
<td>Squamous-cell carcinoma</td>
<td>41</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Adenocarcinoma</td>
<td>46</td>
<td>1.00</td>
<td>0.55–1.81</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Large-cell carcinoma</td>
<td>31</td>
<td>2.09</td>
<td>1.15–3.79</td>
<td>0.02</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>89</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>29</td>
<td>0.62</td>
<td>0.34–1.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Tumour stage</td>
<td>I/II</td>
<td>50</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IIIA/B</td>
<td>27</td>
<td>1.88</td>
<td>0.97–3.65</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>41</td>
<td>4.02</td>
<td>2.19–7.36</td>
<td>0.0005</td>
</tr>
<tr>
<td>Karnofsky performance status</td>
<td>≤70</td>
<td>14</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>41</td>
<td>0.64</td>
<td>0.32–1.29</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>50</td>
<td>0.29</td>
<td>0.14–0.60</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>13</td>
<td>0.24</td>
<td>0.24–0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>MMP-9</td>
<td>≤806 ng/ml</td>
<td>29</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>807 to ≤1293 ng/ml</td>
<td>30</td>
<td>1.78</td>
<td>0.81–3.92</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>1294 to ≤1912 ng/ml</td>
<td>30</td>
<td>3.06</td>
<td>1.43–6.55</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>&gt;1912 ng/ml</td>
<td>29</td>
<td>4.82</td>
<td>2.20–10.55</td>
<td>0.0005</td>
</tr>
<tr>
<td>VEGF</td>
<td>≤417 pg/ml</td>
<td>29</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>418 to ≤630 pg/ml</td>
<td>30</td>
<td>1.36</td>
<td>0.62–2.96</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>631 to ≤1205 pg/ml</td>
<td>29</td>
<td>2.14</td>
<td>1.02–4.51</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>&gt;1205 pg/ml</td>
<td>30</td>
<td>3.63</td>
<td>1.72–7.64</td>
<td>0.001</td>
</tr>
<tr>
<td>Platelet count</td>
<td>≤400/nl</td>
<td>93</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>&gt;400/nl</td>
<td>25</td>
<td>2.12</td>
<td>1.23–3.67</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemoglobin ($n = 117$)</td>
<td>9 to &lt;12 g/dl</td>
<td>28</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>12 to &lt;15 g/dl</td>
<td>68</td>
<td>0.62</td>
<td>0.36–1.07</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>≥15 g/dl</td>
<td>21</td>
<td>0.35</td>
<td>0.15–0.83</td>
<td>0.02</td>
</tr>
<tr>
<td>Lactate dehydrogenase ($n = 112$)</td>
<td>≤200 U/l</td>
<td>83</td>
<td>1$^a$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>&gt;200 U/l</td>
<td>29</td>
<td>2.59</td>
<td>1.50–4.46</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$^a$Reference group in univariate analysis.

CI, confidence interval; MMP-9, matrix metalloproteinase-9; VEGF, vascular endothelial growth factor.
patients with various types of cancer, including lung cancer [22–32].

In our investigation we found a significant positive correlation between serum levels of both MMP-9 and VEGF and tumour stage. So far, only one group [33] has described a significant elevation of serum VEGF in a small patient population with advanced NSCLC in comparison with those with early disease; no significant difference was detected between locally advanced and metastatic disease. Furthermore a positive correlation between VEGF serum level and size of the primary tumour has been reported [34]. In contrast, other authors did not find a significant correlation between VEGF serum level and tumour stage or the presence of distant metastases in 70 patients with small-cell or non-small-cell lung cancer [25].

To the best of our knowledge, our findings are the first report of a significant association between MMP-9 serum level and tumour stage in NSCLC. So far, two groups could not identify a relationship between MMP-9 serum or plasma levels and tumour stage in NSCLC in small patient populations [29, 30].

So far, no association has been reported between serum levels of MMP-9 or VEGF and the different histological types of NSCLC (squamous-cell carcinoma, adenocarcinoma, large-cell carcinoma) [25, 29, 33], supporting our findings.

We observed a significant correlation between serum levels of MMP-9 as well as VEGF and survival in resected patients with stage I/II disease. Patients with an MMP-9 serum level of 1293 ng/ml or lower or a VEGF serum level of 630 pg/ml or lower had a significantly longer survival than patients with higher serum concentrations. No significant difference was found in patients with stage IIIA/B disease, whereas a significant correlation between VEGF serum level and survival was observed in the patients with metastatic disease who were treated with chemotherapy.

In the present study, tumour stage, histological type, serum level of MMP-9 as well as VEGF, Karnofsky performance status, haemoglobin concentration, lactate dehydrogenase concentration and platelet count had prognostic significance in the univariate Cox regression analysis. However, in a multivariate analysis only tumour stage, the Karnofsky performance status, gender and pretreatment MMP-9 serum level were independent prognostic factors.

The prognostic impact of MMP-9 and VEGF serum or plasma levels still remains unclear. In most published studies, serum VEGF has no prognostic influence on survival [24, 25]. The prognostic role of VEGF expression in tumour tissue is discussed controversially by several study groups [35–41]. In an immunohistochemical study of 69 stage I/II NSCLC patients no prognostic influence of VEGF expression has been found [36]. Only in the context of microvessel density did VEGF expression yield prognostic information in 81 operable NSCLC patients [37]. In contrast, VEGF expression was found to be a significant prognostic factor in a univariate analysis in 223 patients with operable NSCLC [35]. Other study groups identified VEGF expression in tumour tissue as an independent prognostic factor in NSCLC [38–40].

To the best of our knowledge, this is the first report identifying MMP-9 serum level as an independent prognostic factor in NSCLC in a multivariate analysis. A formerly described significant correlation between MMP-9 plasma level and 1-year survival in 79 NSCLC patients by Ylisirnio et al. [42] could not be confirmed in a multivariate analysis. The same authors could not show prognostic significance for

### Table 3

Final results of the forward and backward selection of the multivariate Cox regression analysis (P limit ≤0.1) of pretreatment features. Clinical factors and serum parameters were included in this model (n = 118)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (baseline male)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Female</td>
<td>-0.74</td>
<td>0.33</td>
<td>0.48</td>
<td>0.25–0.90</td>
<td>0.02</td>
</tr>
<tr>
<td>Tumour stage (baseline stage I/II)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IIIA/B</td>
<td>0.50</td>
<td>0.36</td>
<td>1.65</td>
<td>0.82–3.35</td>
<td>0.16</td>
</tr>
<tr>
<td>IV</td>
<td>1.35</td>
<td>0.36</td>
<td>3.85</td>
<td>1.90–7.78</td>
<td>0.0005</td>
</tr>
<tr>
<td>Karnofsky PS (baseline ≤70)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>80</td>
<td>1.60</td>
<td>0.67</td>
<td>4.95</td>
<td>1.33–18.46</td>
<td>0.02</td>
</tr>
<tr>
<td>90</td>
<td>1.26</td>
<td>0.63</td>
<td>3.53</td>
<td>1.03–12.10</td>
<td>0.04</td>
</tr>
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<td>100</td>
<td>0.65</td>
<td>0.63</td>
<td>1.91</td>
<td>0.56–6.57</td>
<td>0.30</td>
</tr>
<tr>
<td>MMP-9 (baseline &lt;807 ng/ml)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>807 to 1293 ng/ml</td>
<td>0.13</td>
<td>0.43</td>
<td>1.14</td>
<td>0.49–2.65</td>
<td>0.77</td>
</tr>
<tr>
<td>1294 to ≤1912 ng/ml</td>
<td>0.98</td>
<td>0.41</td>
<td>2.68</td>
<td>0.73–7.86</td>
<td>0.02</td>
</tr>
<tr>
<td>&gt;1912 ng/ml</td>
<td>1.12</td>
<td>0.43</td>
<td>3.06</td>
<td>1.49–14.23</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CI, confidence interval; MMP-9, matrix metalloproteinase-9; PS, performance status.
serum MMP-9 in a univariate analysis in another comparable patient population [43].

Similar to VEGF, contradictory results exist concerning the prognostic impact of MMP-9 expression in lung cancer tissue detected by immunohistochemistry methods. In one investigation the expression of MMP-9 in tumour tissue did not correlate with survival in 90 patients with stage I–IV adenocarcinomas of the lung [44], whereas a significant association was observed in 79 patients with adenocarcinomas staged as T1 disease [45]. In an additional further study with 169 NSCLC patients with stage I–IIIA the expression of MMP-9 in tumour tissue was identified as an independent prognostic factor [46]. In this investigation a significant proportion of tumours co-expressed MMP-9 and epidermal growth factor receptor (EGFR). The co-expression of both markers was significantly associated with a poor prognosis. The authors discussed that the EGFR signalling pathway may play an important role in the invasive behaviour of NSCLC via specific up-regulation of MMP-9 [46].

In conclusion, our results demonstrate a correlation between tumour stage and pretreatment serum levels of both MMP-9 and VEGF. However, in our patient population only the pretreatment serum level of MMP-9 was identified as an independent prognostic factor and had a higher prognostic relevance than those of VEGF. Future studies should be conducted and may support our hypothesis that the pretreatment MMP-9 serum level is a new powerful prognostic marker and may help to assess an individual risk profile for NSCLC patients with stage I/II disease—together with classical prognostic markers like TNM stage and performance status—in order to stratify these patients into low- and high-risk groups. Based on the data of this investigation, we plan a large study where patients with stage I/II disease are stratified for adjuvant chemotherapy according to their serum levels of MMP-9 before surgery. Furthermore, 200 patients with metastatic disease have currently been enrolled in an ongoing study to investigate the prognostic value of pretreatment MMP-9 and VEGF serum levels in patients who received palliative chemotherapy.

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References


