The level of vascular endothelial growth factor as a predictor of a poor prognosis in osteosarcoma

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We undertook a prospective study to evaluate the prognostic significance of the serum levels of vascular endothelial growth factor (VEGF) in predicting the survival of patients with osteosarcoma. The levels were measured by an enzyme-linked immunosorbent assay in 15 patients with osteosarcoma before commencing treatment. The patients were divided into two groups, with a high or a low serum VEGF level, and the incidence of metastases and overall survival rate were compared.

No significant relationship was observed between the serum VEGF levels and gender, age, the size of the tumour or the response to pre-operative chemotherapy. Patients with a serum VEGF > 1000 pg/ml had significantly worse survival than those with a level < 1000 pg/ml (p = 0.002). The serum VEGF level may be useful in predicting the prognosis for survival in patients with osteosarcoma.

Angiogenesis is essential for the growth of a tumour and its metastases. Vascular endothelial growth factor (VEGF) is the most potent stimulator of angiogenesis. Its expression has been shown to be correlated with the progression of the tumour and the prognosis in many cancers. The serum level of VEGF may be a surrogate marker of the expression of tumour VEGF. It has been shown to have prognostic significance in patients with cancer.

Angiogenesis plays an important role in the progression of osteosarcoma, and VEGF may be an important mediator of angiogenesis since there is a significant association between a high expression of VEGF in the primary tumour and a poor prognosis. A close relationship between a high serum level of VEGF and a poor prognosis has been suggested, but the lack of prospective evaluation has made its value in the prediction of the prognosis in patients with osteosarcoma uncertain. Our aim was to carry out a prospective evaluation of the prognostic significance of the serum level of VEGF before treatment in patients with osteosarcoma.

Patients and Methods
Our study had institutional approval. Between April 1998 and March 2000, 16 patients with a putative diagnosis of osteosarcoma gave informed consent to provide samples of peripheral venous blood. These were taken before biopsy and the serum was stored at -0°C until the assays were performed. One patient died from chemotherapy-related leukaemia and the remaining 15 were enrolled in the study. There were four women and 11 men with a mean age 23.6 years (9 to 60). Of the tumours, nine were located in the femur, four in the tibia, one in the humerus and one in the pelvis. Histologically, all were high grade. Paget's disease was not associated with any. The assessment before treatment included palpation of the regional lymph nodes, plain radiographs of the chest and CT of the lung and abdomen. Bone scintigraphy showed pulmonary metastases in one patient.

All the patients were managed by excision of the tumour and pre- and post-operative chemotherapy, based on a combination of high-dose methotrexate, doxorubicine, cisplatin and ifosfamide. A wide surgical margin was achieved in all patients. The response to pre-operative chemotherapy was evaluated for the surgical specimens of all patients. The histological response was graded as good ( > 90% tumour necrosis) or poor ( < 90% tumour necrosis).

The concentrations of VEGF in the serum of patients taken before biopsy were assessed by a commercially available sandwich ELISA (VEGF; IBL, Fujioka, Japan). The volume of the primary tumour was assessed on MR scans which had been taken before biopsy, and calculated by the following formula:

\[ \pi \times \text{height} \times \text{width} \times \text{depth} \]
The serum levels of VEGF were compared between groups categorised by various clinicopathological features using the Mann-Whitney U test. The curve for overall survival was drawn according to the Kaplan-Meier method and the differences were analysed by applying the log-rank test. All statistical analyses were performed using SPSS version 10.0 for Windows (SPSS Inc., Chicago, Illinois). Statistical significance was defined as \( p \leq 0.05 \).

### Results

The median serum level of VEGF was 1069.4 pg/ml (95% confidence interval (CI) 551.0 to 2075.5) which was significantly higher than that of ten healthy control subjects (median 211.5 pg/ml; 95% CI 87.5 to 335.4; \( p = 0.04 \)). No significant relationship was observed between the serum VEGF level and gender, age, the size of the tumour or the response to pre-operative chemotherapy (Table I).

All 15 patients were followed up regularly having plain radiography of the chest every six months and CT of the lung and bone scintigraphy every year. They were divided into a high and a low VEGF group by setting the median serum VEGF level as the cut-off value (1000 pg/ml) because of the small number of control subjects and the wide variation in the serum VEGF levels.

The incidence of remote metastases and the overall survival rate were compared between the two groups. During a median follow-up of 52.1 months (6 to 102), nine patients died from metastases. In the high VEGF group, eight died from pulmonary metastases and in the low VEGF group, one from liver metastases. The rate of metastases in patients with a high serum level of VEGF (8 of 8 patients; 100%) was significantly higher than that in the low serum VEGF level group (1 of 7 patients; 14.3%; \( p = 0.004 \)).

Univariate analysis was performed to investigate whether the pre-operative VEGF level was an independent factor predictive of prognosis for the patients with osteosarcoma among various other pre-operative clinical parameters (Table II). A serum VEGF level of more than 1000 pg/ml was associated with a significantly poorer survival compared with a level of 1000 pg/ml or less (\( p = 0.002 \)). A tumour larger than 200 cm\(^3\) (\( p = 0.44 \)) or a poor response to pre-operative chemotherapy (\( p = 0.34 \)) was associated with a poorer prognosis, but the difference was not statistically significant (Table II).

Figure 1 shows the cumulative overall survival curves of all 15 patients by several clinical parameters including gender, age, sensitivity to pre-operative chemotherapy, size of the tumour and the level of serum VEGF at diagnosis. When stratified into those with high and low levels using the median concentrations as cut-off values, the overall survival rates at five years were 85.7% for those with low levels and 12.5% for those with high levels.

### Discussion

We have previously shown that 63% of VEGF-positive osteosarcomas have a higher incidence of developing pulmonary metastases when compared with VEGF-negative tumours (82% vs 10%), and that patients with VEGF-positive tumours have significantly worse overall and disease-free survival rates.\(^{15}\)

Another study of 30 patients with non-metastatic osteosarcoma assessed VEGF expression using a reverse-transcriptase polymerase chain reaction on samples of tumour taken before treatment.\(^{16}\) VEGF mRNA was present in all samples, with 80% of tumours expressing the VEGF165 isoform and 20% the VEGF121 isoform. Of the patients with VEGF165-positive tumours, 83%
developed pulmonary metastases compared with 16% of those with VEGF165-negative lesions and the overall survival was significantly worse in the former. Other research has focused on the circulating VEGF levels in
patients with osteosarcoma.\textsuperscript{17-20} In one study of 27 patients, serum VEGF levels were measured in patients with bone sarcoma, including osteosarcoma, and compared with a healthy control group.\textsuperscript{17} Only patients with Ewing's sarcoma had a mean VEGF level which was significantly higher than that of healthy control subjects. A larger study evaluated the serum levels of VEGF and bFGF in 72 patients with non-metastatic bone sarcoma and in healthy subjects and patients with benign bone tumours.\textsuperscript{18} Those with bone sarcomas had higher levels of serum VEGF and bFGF than the healthy control group, but not those with benign bone tumours. Higher VEGF levels were associated with large tumours, those of a higher grade and those more locally invasive. The serum VEGF and bFGF levels did not differ among patients with osteosarcoma, Ewing's sarcoma, or chondrosarcoma.\textsuperscript{18} We have evaluated the prognostic impact of the serum levels of VEGF, bFGF and placental growth factor in a series of 16 patients with osteosarcoma.\textsuperscript{19} Those with metastatic disease at diagnosis or who developed metastases within one year had significantly higher VEGF levels than patients without early metastases. The sensitivity and specificity of the serum VEGF level above the mean for the group in identifying early metastatic disease were 100\% and 88.9\%, respectively. Another group measured the serum VEGF levels in 45 paediatric patients with solid malignant tumours including osteosarcoma, and found that the patients with high serum levels of VEGF (> 207 pg/ml) had a high potential for metastases. They concluded that the serum VEGF level could be a reliable marker for assessing paediatric malignancies with high metastatic potential.\textsuperscript{20}

In our study all eight patients with a serum VEGF level greater than 1000 pg/ml died from pulmonary metastases. This suggests a positive link between angiogenesis and the establishment of pulmonary metastases in osteosarcoma. One patient with a serum VEGF < 1000 pg/ml died from liver metastases. This is rare in osteosarcoma, but metastasis to the liver may occur through an angiogenesis-independent pathway.

In our study, as in others,\textsuperscript{12-15} the median serum level of VEGF was taken as the cut-off value, rather than the upper limit in the healthy control group or the mean ±2SD of the control group. The small number of control subjects and the wide variation in the serum levels of VEGF made it difficult to define a reliable cut-off value from that of the control group. Prospective studies which include more patients and a larger healthy control group are needed to define a reliable cut-off value for the serum VEGF level.

The findings of a correlation between high serum levels and poor prognosis may have a therapeutic implication. Active angiogenesis may play an important role in the progression of the tumour in osteosarcoma. The use of anti-angiogenic therapy in combination with systemic cytotoxic drugs may suppress tumour angiogenesis and lead to better control of the lesion.

We are aware that our hypothesis requires more detailed evaluation with a larger sample size before the ultimate significance can be determined. This limits our ability to conduct multivariate analysis. Nevertheless, we hope that our study will prompt investigators to design new studies to understand better the relationship between VEGF and the prognosis in patients with osteosarcoma. It is well known that the histological response or the size of the tumour is the most significant prognostic factor in patients with osteosarcoma. We have shown in our series that a large size of tumour or a poor response to preoperative chemotherapy was associated with a poor prognosis, but the difference was not statistically significant. The size of our group was too small to provide proper analysis of the influence of these factors and the effect of VEGF levels. However, our observations are such as to warrant fresh prospective studies designed to assess these factors.

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\textbf{References}


