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Prognostic Value of Serum VEGF in Melanoma Patients: a Pilot Study

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Abstract. *Background: Vascular endothelial growth factor (VEGF) is involved in angiogenesis. We investigated the association of VEGF serum levels (pre-treatment and follow-up) with outcome in patients with melanoma. Patients and Methods: Serum levels of VEGF in melanoma patients at diagnosis and during follow-up were analysed with enzyme-linked immunoassays. Patients were followed up with physical examination and ultrasound scans of the liver every three months and thorax X-ray annually. The VEGF serum level was evaluated six-monthly. Results: From February 1996 to February 2000, 33 patients were enrolled. Ninety-two serum blood samples were collected. Patients had a median age of 60 years (range 32-82). Twenty patients were males, 13 females. One patient presented with stage IA disease, 2 with stage IB, 11 with stage IIA, 4 with stage IIB, 8 with stage III and 5 with stage IV. Two patients were affected by uveal melanoma. The melanomas were predominantly located at the extremities or trunk (26/33). The median serum level of VEGF at diagnosis was 249 ng/ml (minimum: 9 ng/ml, maximum: 1215 ng/ml). The median survival of all 33 patients was 45.1 months. The median time-to-progression was 36.7 months. Patients with lower or higher serum VEGF values showed no statistically significant differences in survival. In contrast, high serum VEGF values were associated with shorter disease-free survival as compared with lower values (median DFS: 25 vs 60 months, p=0.048 at log-rank test). Conclusion: Our results suggest that serum VEGF could be of prognostic value in melanoma.*

Vascular endothelial growth factor (VEGF) is the founding member of a family of closely related cytokines that exert critical functions in vasculogenesis. It belongs to the platelet-derived growth factor (PDGF) superfamily of growth factors. VEGF is as a tumor-secreted protein that potently increases vascular permeability and promotes the formation of new blood vessels. VEGF stimulates endothelial cells to migrate and divide. Members of the VEGF family include VEGF-A, B, C, D, E and placenta growth factor (PlGF) (1).

VEGF is overexpressed by the vast majority of solid human cancers (2). VEGF is overexpressed not only by invasive cancer cells, but also by at least some premalignant lesions (*e.g.*, precursor lesions of breast, cervix and colon cancers); furthermore, the expression levels increase in parallel with malignant progression. There is also good evidence that oncogenes and tumor suppressor genes promote tumor growth, in part by modulating the angiogenic response induced by VEGF (3,4).

On binding to its receptors, VEGF initiates a cascade of signaling events that begins with autophosphorylation of both receptor tyrosine kinases, followed by activation of numerous downstream proteins including phospholipase C , PI3-K, GAP, the Ras GTPase-activating protein, MAPK and others. It seems that VEGFR-2 is responsible for mediating microvascular permeability, for the rapid early increase in $[Ca^{2+}]_i$, and for subsequent endothelial cell proliferation and migration. The signaling steps mediated through VEGFR-1 have been less well characterized. VEGFR-1 may have an independent role in stimulating cell motility and may also dampen certain signaling pathways and biological effects (*e.g.*, cell proliferation) mediated by VEGFR-2 (5).

Interestingly, the amount of VEGF expressed by cancer cells has been found to correlate with poor prognosis in many types of tumors, including carcinomas of the breast, kidney, colon, brain, ovary, cervix, thyroid, bladder, esophagus and prostate, and in osteoid and soft tissue sarcomas and pediatric tumors (6).

There is contradictory evidence about the prognostic value of the serum level of VEGF in melanoma. The present pilot study was conducted in order to explore a possible association between basal or follow-up VEGF values and outcomes in patients affected by malignant melanoma.

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Table I. *Characteristics of 33 patients.*

		basal sVEGF values	
	No.	lower (<300pg/ml)	higher (>300pg/ml)
Age			
median (range)	60 (32-82)		
	<60	8	9
	>60	10	6
Sex			
Males	20	10	10
Females	13	8	5
Stage			
IA	1	1	-
IB	2	2	-
IIA	11	7	4
IIB	4	3	1
III	8	2	6
IV	5	3	2
uveal	2	-	2
Breslow (referred to the primary lesion)			
Unknown	5	4	1
<1 mm	3	2	1
>1 mm and < 2 mm	8	4	4
>2 mm and < 4 mm	10	5	5
> 4 mm	7	3	4
Location of primary melanoma			
extremities	13	6	7
eyes	2	-	2
trunk	13	7	6
head	5	5	-

Patients and Methods

Patients with histologically confirmed malignant melanoma were enrolled in this prospective investigation. Patients with stage I/II disease were treated only by surgery. Patients with stage III disease received adjuvant interferon-based therapy after surgery. Patients with stage IV disease were treated with multidisciplinary approaches (surgery, radiotherapy, chemotherapy, immunotherapy). Pretreatment evaluation included a complete history and physical examination with a computed tomography (CT) of the chest, sonography or CT of the upper abdomen, radionuclide bone scan, complete blood cell count and serum chemistry analysis.

Twenty millilitres of venous blood were taken at diagnosis and at six-month intervals. Samples were subsequently centrifuged at 2100 g for 10 minutes at 4˚C. The supernatants were transferred into microtubes and stored at -70˚C until use.

Serum samples were analysed for VEGF with Human VEGF Immunoassay Quantikine™ (R&D System, Inc., Minneapolis, MN, USA). This assay employs a quantitative sandwich enzyme immunoassay technique. The cut-off of serum VEGF level was derived from literature review (<300 ng/ml: low, >300 ng/ml: high). Blood samples were not collected after progression. The minimum detectable concentration of VEGF is <9.0 pg/ml. Each serum sample was determined twice.

The TTP (time-to-progression) was measured from the diagnosis until progression. The OS (overall survival) was measured from the diagnosis until death resulting from any cause. Patients who were lost at follow-up or who died without documentation of disease progression were considered to have had tumor progression at the time of death, unless there was sufficient documented evidence to conclude that progression did not occur before death. The distribution of TTP and survival time was estimated using the Kaplan-Meier method. Differences between groups in terms of time-to-event outcomes were studied with the log-rank test. P values <0.05 were considered statistically significant. All statistical tests were performed using MedCalc software.

Results

Patient and tumor characteristics. From February 1996 to February 2000, 33 patients were enrolled into this study. The patient and tumor characteristics are shown in Table I. Patients had a median age of 60 years (range 32-82). Twenty patients were males, 13 females. One patient presented with stage IA disease, 2 with stage IB, 11 with stage IIA, 4 with stage IIB, 8 with stage III and 5 with stage IV. Two patients were affected by uveal melanoma. Fifty-one per cent of patients had a tumor Breslow index >2mm (17/33). The Breslow index was unknown for 5 advanced patients. The melanomas were predominantly located at the extremities and trunk (26/33). Differences between basal sVEGF values (lower *vs* higher) and patient and tumor characteristics are shown in Table I. The median serum level of VEGF at diagnosis was 249 ng/ml (minimum: 9 ng/ml, maximum: 1215 ng/ml). The difference with healthy control (provided by R&D Systems) was not significant (data not shown).

Correlation between serum levels VEGF and outcome. With a median follow-up of 58 months, 31 patients progressed and 16 patients died. The median survival of all 33 patients was 45.1 months (range: 19.9-96.5 months). The median TTP was 36.7 months (range: 16.7-91.2 months). Ninety-two serum blood samples were collected. Each sample was classified as high or low for VEGF values as described in the Methods section and was determined at six-monthly intervals. VEGF values of each sample (low *vs* high) were associated to events (progression or death) at each visit. For survival analysis, only serum levels of VEGF at diagnosis were considered.

Patients with lower or higher serum VEGF values showed no statistically significant differences in survival although a trend was observed (data not shown). By contrast, high serum VEGF values were associated with shorter disease-

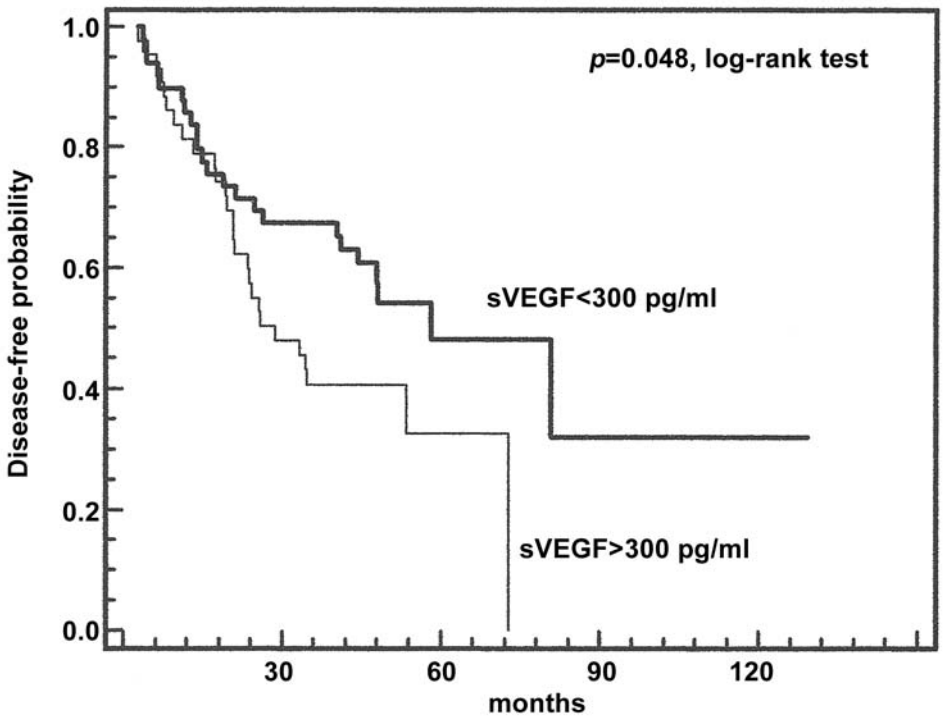

Figure 1. *Kaplan-Meier estimate of time-to-progression by VEGF values.*

free survival as compared with lower values (median DFS: 25 *vs* 60 months, $p=0.048$ at log-rank test) (Figure 1).

Discussion

The present pilot study was planned with an explorative aim, considering that there were few data on the prognostic value of serum VEGF. Surprisingly, we found that the serum level of VEGF >300 pg/ml was able to predict shorter DFS in melanoma patients. However, we did not find any association between serum VEGF values and OS (although a trend was observed). Obviously, due to the small sample of our series, such results need to be confirmed in larger series.

VEGF is expressed in primary and metastatic melanomas while the benign counterpart is negative. In fact, some authors have proposed that assessment of VEGF expression might aid in the differential diagnosis between dysplastic nevi and melanomas (7). Furthermore, VEGF receptors are involved in promoting neovascularization and glomeruloid microvascular proliferations, focal proliferative buddings of endothelial cells resembling a renal glomerulus, in melanoma, breast, endometrial and prostate cancer. These phenomena are associated with an impaired prognosis (8). VEGF is involved in molecular progression of malignant melanoma (9,10) Again, it has recently been demonstrated that exposure of primary cutaneous melanoma cells to dacarbazine resulted in the up-regulation of interleukin-8 (IL-8) and VEGF increasing tumor growth and metastatic behavior *in vivo* (11).

Recent data showed that VEGF-C expression may be important in regional lymphatic disease in melanoma (12). A recent study demonstrated that VEGF-C expression is correlated with localization of melanoma metastases in the lymph nodes (13). However, a study on 202 melanoma specimens demonstrated VEGF expression by IHC in 42 (20.8%) samples, but multivariate analysis performed using Cox proportional hazards method did not show a statistically significant survival difference between the VEGF-positive and -negative groups ($p=0.25$) (14). Another study failed to find any prognostic power for serum VEGF in 96 patients with primary or metastatic melanoma (15). Ugurel *et al.* showed that serum levels of VEGF in 125 melanoma patients was an independent predictor of both disease-free progression and survival (16).

The present pilot study suggests that serum VEGF could promote melanoma progression and it is consistent with the hypothesis that the higher the production of VEGF, the higher the chances for cancer to establish, to grow and to become clinically evident. Obviously, these results need to be confirmed in larger series than the present one. Based on this hypothesis, the perspective of using VEGF as a

target for therapeutic approach is intriguing. VEGF could contribute to the failure of immune protection and to the promotion of genes involved in tumor invasion and metastasis and, thus, worsen patient outcome.

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