Circulating Vascular Endothelial Growth Factor (VEGF) Levels in Advanced Stage Cancer Patients Compared to Normal Controls and Diabetes Mellitus Patients with Critical Ischemia

Yoka H. Kusumanto, Coby Meijer, Wendy Dam, Nanno H. Mulder and Geke A.P. Hospers

Dept. of Medical Oncology, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands.

Abstract: Anti-angiogenic therapy is emerging as a valuable tool in the treatment of patients with cancer. As VEGF is a central target in anti-angiogenic therapy, its levels in the circulation might be relevant in selecting tumor types or patients likely to respond to this treatment. Additional VEGF has been recognized as a key factor in the pathogenesis of diabetic retinopathy. Recently anti-angiogenic therapy has been advocated in this situation.

We measured VEGF levels in whole blood in 42 patients with high grade (n = 26) and low grade (n = 16) end stage cancer, and in 28 healthy controls and 37 patients with diabetes related vascular disease. Only 2/26 patients in the group of high grade cancer had significantly elevated VEGF levels, 1/16 in the low grade group and 1/28 in the healthy control group. In contrast, in 10/37 diabetic patients the mean VEGF levels were significantly elevated compared to the other groups. The mean level in these diabetic patients was significantly elevated compared to the other groups.

These data indicate the limitation of the use of circulating VEGF levels as a potential selection criterion for anti-angiogenic therapy in cancer patients and suggest further studies into its application in the management of diabetic complications.

Keywords: VEGF level, cancer, diabetes mellitus, Critical Limb Ischemia

Introduction

Anti-angiogenic therapy is emerging as an important strategy in the treatment of cancer (Ferrara, 2005). Following extensive in vitro and preclinical testing over many decades the therapeutic implications of tumor angiogenesis are finally having an impact in the clinic (Folkman, 1971). Up till now it is impossible to predict activity of anti-angiogenic therapy for particular tumor types or individual patients. However the favorable results of VEGF antibodies suggest that circulating levels of VEGF might give an indication of the potential of this treatment in tumors of different grades of malignancy or even in individual patients. A profile of elevated VEGF levels in the more aggressive cancers compared to slow growing tumors and in those tumor types known to respond to VEGF antibody therapy, would support further studies on VEGF levels as predictive markers.

Increased circulating VEGF levels have also been observed in patients with diabetes mellitus (Chiarelli et al. 2000; Valabhji et al. 2001). A variety of factors, implicated in the development of diabetic complications, have been shown to upregulate VEGF expression in vitro, including high glucose concentrations and advanced glycation end-products (Williams, 1997; Okamoto et al. 2002). Vascular proliferation is known to play a role in the development of diabetic retinopathy. It is therefore not surprising that developments in anti-angiogenic therapy in cancer have been closely followed in the field of ophthalmology. Preliminary evidence suggests that this treatment form either with bevacizumab or with its derivative ranibizumab is highly effective (Rosenfeld, 2005a; Rosenfeld, 2005b; Miller, 2005; Puliafito, 2005).

To further study the prevalence of elevated circulating VEGF levels in cancer patients and in diabetics we measured, in a population of patients who were referred with advanced stage cancer, the incidence of increased VEGF levels and compared them to values in the normal population and in a group of

Correspondence: G.A.P. Hospers, M.D., Ph.D., Dept. of Medical Oncology, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
Tel: +31 50 361 2821/1847; Fax: +31 50 361 4862; Email: g.a.p.hospers@int.umcg.nl

non-cancer diabetic patients known to have severe ischemic vascular disease.

Patients and Methods

Forty-two patients who had incurable metastatic cancer and referred for palliative therapy were studied (median: 55 (range 19–75) years). These patients were separated in two groups, one with slow growing differentiated neuro-endocrine carcinoid tumors, comprising 16 patients, and a second group of aggressively progressive solid tumors of diverse origin, comprising 26 patients (Table 1). The 6 "others" in Table 1 are extragonadal germ cell tumor, metastatic insulinoom, myxoid solitary fibrous tumor, neuroendocrine pancreas tumor (no hormone producing), lung (non small cell) and testicular cancer.

Thirty seven diabetic patients (median: 71 (range 40–84) years of age, diabetic duration: median 16 years (range 0.5–55) HbA1c median: 7.6 (range 5.8–12.2)) with end stage vascular disease of the limbs were included. In the diabetic patient group the following investigations were performed: demographic characteristics such as age and diabetic duration and body mass index but also clinical assessment of edema were included. Concentrations of hemoglobin A1c (HbA1c), fasting glucose, cholesterol and triglycerides, C-reactive protein (CRP), creatinin, and albumin excretion ratio (two overnight urine collections) were measured. Standard laboratory assays were used. Fundus photographs of the retina were performed and graded as follows: no retinopathy, background retinopathy, pre-proliferative and proliferative diabetic retinopathy. Ankle and toe pressures were measured according to conventional procedures using an 8 MHz Parkes Doppler. Ankle to brachial index (ABI) and Toe to brachial index (TBI) were calculated as the quotient of absolute ankle and toe pressures to the simultaneously measured brachial pressure, respectively.

As a control population 28 healthy (median: 29 (18–54) years) volunteers were studied and randomly recruited from the medical and laboratory personal. Diabetic patients with evidence of systemic complications or systemic disease otherwise were excluded i.e. 1) proliferative eye disease, 2) history of malignancy or severe co-morbidity. The study was approved by the local Human Investigations Committee.

Table 1. Distribution of elevated VEGF levels.

Subjects	n	Elevated VEGF level
Controls	28	1
Carcinoid patients	16	1
Aggressive solid tumor patients	26	2
• colon cancer	8	0
• breast cancer	4	0
• renal cancer	3	0
• melanoma	5	0
• others	6	2
Diabetes mellitus	37	10*

Number of patients with elevated VEGF levels (i.e. >1200 pg/ml; 95% confidence interval in healthy controls: 157.7–1200.0 pg/ml).
*: *P* = 0.015 by Mantel Haenzl chi square test.

VEGF measurements

Venous blood was collected in sterile tubes containing CTAD (sodium citrate, theophylline, adenosine, dipyridamole, Becton Dickinson Vacutainer systems, France, Europe). Blood samples were diluted with two volumes of PBS (phosphate buffered saline) and subsequently lysed by freezing and thawing twice. Aliquots were stored at –80 °C.

VEGF levels were determined in duplicate using the Quantikine human VEGF enzyme-linked immunosorbent assay (ELISA) (R & D systems Inc. Minneapolis, MN). The minimum detection level was 9.0 pg/ml in whole blood as quoted by the manufacturer.

Statistics

The Kolmogorov-Smirnov test was used to confirm the assumption of normal distribution of VEGF samples in the healthy control group. The number of patients with elevated levels was compared between the groups using the mantel haenzl sqi square test. Mean VEGF levels were compared using the two sided student T test (unpaired). Statistical significance was set at $p < 0.05$.

Results and Discussion

Circulating VEGF mainly reflects VEGF derived from peripheral blood cells, including platelets and leucocytes. Therefore, we used whole blood for the measurement of VEGF, which contains all cell compartments, as was recommended previously (Salven et al. 1999).

In the present study we found a 95% confidence interval of values in our control population (n = 28) between 157.71 pg/ml and 1200.03 pg/ml. Individual VEGF levels above the upper limit of 1200 pg/ml were considered to be elevated. Accordingly, in three out of 42 patients with advanced stage cancer VEGF levels were elevated, one patient with carcinoid cancer, one with an aggressive no hormone producing neuro-endocrine tumor and one with lung cancer, compared to one out of 28 cases in the control group and 10 out of 37 cases in the patients with diabetes (p = 0.015, Table 1).

In the cancer patients there was a tendency toward higher levels in patients with aggressive solid tumors compared to controls although this did not reach significance (p = 0.08, Table 2). There was no difference of occurrence of high levels of whole blood VEGF levels between aggressive and the more differentiated and slower growing carcinoid tumors. Also VEGF levels in the patients with colonic cancer, a tumor type that is accepted as an indication for angiogenic therapy did not exceed normal levels. As angiogenesis is necessary for tumor growth and the metastatic process, many attempts at quantifying this process have been made. Direct measurements include determination of micro vessel density in the tumor (Blann et al. 2002; Blann et al. 2001; Cascinu et al. 2000). However this method although effective in these studies in colorectal cancer requires tumor samples, limiting its applicability in the clinic as does immunohistochemistry on tumor cells or vessels. Indirect measurements include the various pro- or anti angiogenic factors in blood. Interestingly Tien et al. (Tien et al. 2006) found peripherically determined venous VEGF levels to be not inferior to levels downstream in the venous blood of gastro-intestinal tumors. Moreover VEGF levels correlated the most closely to patients clinico pathological characteristics.

A considerable number of studies have linked blood VEGF levels to tumor stage and prognosis in patients with cancer. Reports usually indicate that cancer patients tend to have higher levels of VEGF than controls, and that levels correlate with adverse prognostic factors. Subgroup analysis, for example comparing long-and short-term survivors, was suggestive for the existence of a relation of malignancy grade with VEGF levels. This effect was striking in an early study in lung cancer (Ohta et al. 1996). The same correlations were found in liver cancer, breast cancer and colon cancer (Torimura et al. 1998; Toi et al. 1995; Eppenberger et al. 1998; Gasparini et al. 1997; Takahashi et al. 1995; Cascinu et al. 2000; Ishigami et al. 1998). Yet, the question whether a relation can be found between VEGF levels and tumor stage has been answered equivocally in various studies. In renal cell cancer such a correlation was absent, but in planocellular esophagus cancer it was striking (Edgren et al. 2001; Wallner et al. 2001). The same was found in cervical cancer and differentiated thyroid cancer (Bachtiary et al.; Tuttle et al. 2002).

Usually it is assumed that tumor angiogenesis under the influence of elevated VEGF levels is the biological phenomenon involved. However the endpoint of that process, micro vessel density, was not always related to VEGF levels, even when these levels had been found to have predictive clinical relevance (Yudoh et al. 2001). In the latter study from Yudoh et al. in sarcoma patients, local relapse, metastatic progression and short survival were predicted by high tissue levels of VEGF but not with micro vessel density. In another study in bone sarcoma patients however, serum levels of VEGF were elevated in contrast to tissue levels in Ewing sarcoma (Holzer et al. 2001). On the other hand an apparent relation between tumor burden and circulating VEGF levels was shown by the rapid decrease of elevated levels after surgery for such different tumors as esophageal cancer and childhood Wilms tumor (McDonnell et al. 2001; Blann et al. 2001). Yet, in view of the rarity of

Table 2. VEGF whole blood levels.

	Controls	All cancers	Carcinoids tumors	Aggressive solid mellitus	Diabetes
VEGF levels (pg/ml)	491.7 ± 275.5	592.6 ± 351.8	525.6 ± 101.9 n.s.	634.1 ± 311.9	928.9 ± 443.2
P-value		n.s.	n.s.	n.s.*	0.0001**

Mean VEGF levels of patient groups are compared to the healthy control group by two-sided student T-test. *: *P* = 0.08, for trend. **: P < 0.05, considered statistically significant.

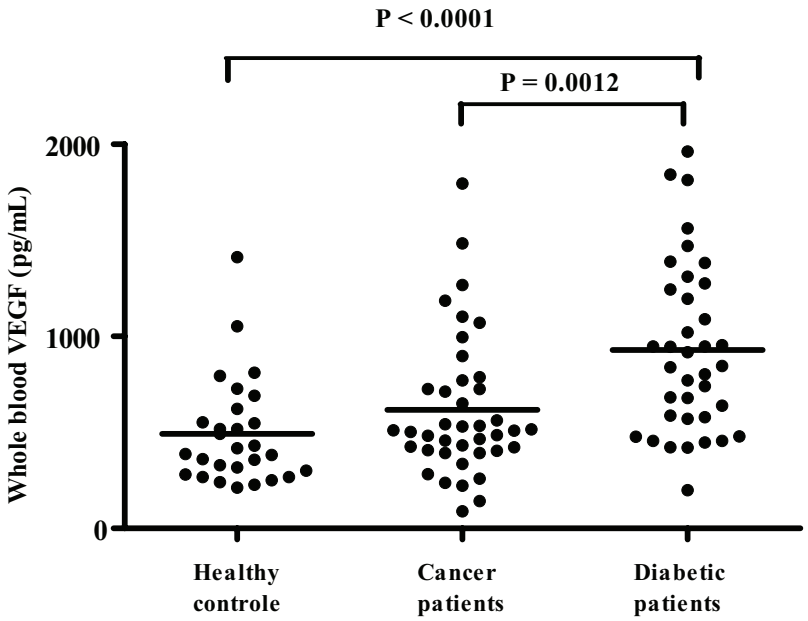

Figure 1. Mean VEGF levels. P < 0.05 is considered significant (unpaired Student T test).

increased VEGF levels found in our cancer patients it is doubtful that VEGF levels will become an important guideline in the treatment with anti-angiogenic drugs on an individual or tumor type oriented basis.

As tissue anoxia is considered to be a major stimulus for VEGF production vascular insufficiency could also in non-malignant disease lead to increased VEGF levels (Maulik et al. 2000). For this purpose we studied a group of patients with end stage diabetic vascular insufficiency and found significantly elevated VEGF levels when mean VEGF levels ($p = 0.001$, Table 2 and Fig. 1) as well as numbers of patients with elevated levels were compared. Although there was a trend for higher VEGF levels with duration of diabetes this did not reach significance ($p = 0.08$, data not shown). In addition we found no relation with other risk factors for diabetic complications such as, HbA1c, and albumin excretion ratio (AER), but also the degree of ankle edema, renal function, lipids, retinopathy and ABI/TBI were not related to circulating VEGF levels (data not shown). The role of VEGF in the development of diabetic vascular complications has become an increasingly intense studied subject in view of the rising prevalence of diabetes. Perhaps the strongest case for VEGF as a growth factor in diabetic vascular disease is proliferative diabetic retinopathy (Clermont et al. 1997; Malecaze et al. 1994). The results of studies on the relation with circulating VEGF levels were however not unequivocal with positive correlations found in proliferative retinopathy (Sydorova and Lee 2005; Chaturvedi, 2000). Our findings confirm that VEGF levels can be elevated in diabetes and that high levels are indeed common with end stage vascular disease as opposed to diabetics with healthy vessels (Chiarelli et al. 2000; Valabhji et al. 2001; Blann et al. 2002). The common occurrence of elevated VEGF levels in diabetic patients with vascular disease suggest that anti-angiogenic treatment directed at this growth factor is logical. This was already found to be successful in the treatment of diabetic retinopathy with ranibizumab, a derivative of bevacizumab. Interestingly, early clinical studies in the treatment of chronic ischemic limb disease with VEGF as a therapeutic agent, thus increasing endogenous VEGF levels, showed beneficial effects without causing severe adverse effects, even in diabetic patients (Baumgartner et al. 1998; Shyu et al. 2003).

In conclusion, our study indicates that the potential of the use of circulating VEGF levels as a selection criterion for anti-angiogenic therapy in cancer patients is limited. Significantly elevated VEGF levels in end stage solid tumor patients are rare (2/26), such levels can be found also in healthy controls (1/28) and and most strikingly, in diabetic patients with ischemic vascular disease (10/37). Future studies should reveal the biologic relevance

and hence the diagnostic and therapeutic implications for the treatment of vascular complications in diabetic patients.

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