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SHORT REPORT

VEGF: A Surrogate Marker for Peripheral Vascular Disease

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Abstract This study aims to evaluate the value of VEGF as a surrogate marker for peripheral vascular disease (PVD). Prior to treatment, serum VEGF levels were evaluated by enzyme-linked immunosorbent assay (ELISA) in 293 PVD patients. Risk factors and clinical parameters of PVD were documented. Twenty-six age-matched healthy volunteers served as controls. Serum VEGF values strongly correlated with Fontaine stages ($p < 0.006$, stage IV vs. controls). High VEGF values prior to treatment were associated with poor outcome. Serum VEGF appears to indicate the severity of PVD and might serve as a surrogate indicator of disease severity.

Introduction

Vascular endothelial growth factor (VEGF) is expressed under hypoxic conditions and leads to angiogenesis and arteriogenesis. Matsui et al.[1] showed in a smaller series that serum VEGF values in PVD patients were elevated. Here, we examined serum VEGF levels prospectively in a large number of PVD patients and determined whether VEGF might serve as a surrogate marker of PVD.

Report

Study design and patients

The study included 293 patients with a history of peripheral vascular disease (PVD), who visited the 'Vascular Center', University of Regensburg, between August 2004 and July 2005 receiving 350 treatments (e.g., surgery, intervention, PGE_1 and exercise). The study population consisted of 187 men and 106 women with a median age of 67.8 years

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(117 smokers, 238 hypertensive patients, 122 diabetics and 197 patients received medication due to lipid disorders). None of the patients had known cancer.

Twenty-six age-matched healthy volunteers (long-term follow-up patients after minor hand surgery) served as controls, including five smokers. All patients gave written informed consent to participate in this study as approved by the local ethics committee.

All patients received a physical examination, ankle to brachial index, a standardised treadmill test and either a magnetic resonance (MR) angiography or an angiogram.

Venous fasting blood samples of non-traumatic venipunctures were obtained. If traumatic punctures occurred, an indwelling intravenous catheter was inserted and blood was drawn 6 h later. Full blood count, low-density lipid (LDL) and high-density lipid (HDL) cholesterol, triglyceride, creatinine, VEGF and other analytes were determined. In a subset of 10 patients, measurements were also performed from the contralateral cubital vein and 24 h after the first measurement.

Measurement of serum VEGF

Serum VEGF determination was performed because activated platelets are a major source of VEGF.[2] Blood was collected in plain vacutainer tubes with no additives.[2] Samples were processed at room temperature 30–60 min after collection centrifuged and serum stored at −80 °C. VEGF concentrations were determined using an enzyme-linked immunosorbent assay (ELISA; Quantikine human VEGF Immunoassay, R&D Systems). VEGF concentrations (pg ml^{-1}) were determined by constructing a standard curve using recombinant VEGF.

Results

Correlation of baseline VEGF values and Fontaine stages (Fig. 1)

Baseline VEGF values showed strong correlation with the Fontaine stage in PVD patients. VEGF values in Fontaine IV patients were almost doubled than in healthy controls. Baseline VEGF values of the 12 patients who required a major amputation were significantly higher than in PVD patients without amputation (580 ± 17 pg ml^{-1} vs. 1216 ± 125 pg ml^{-1}).

Influence of risk factors or co-morbidities

Smoking significantly increased VEGF levels in healthy volunteers. However, regression analysis revealed that only hypercholesterolaemia showed a significant inverse correlation to VEGF levels ($p < 0.032$). Diabetes, hypertension or smoking showed no correlation to VEGF values in PVD patients ($p = 0.339$, $p = 0.861$, $p = 0.295$, respectively).

Discussion

VEGF is an angiogenic factor that plays an important role in the development of arteriosclerosis[3] and is induced in ischaemic tissue via HIF1α. Although exogenous application of VEGF based on the concept of 'therapeutic angiogenesis' has already been performed in cardiovascular disease and PVD, little is known about VEGF expression at different stages of PVD. We aimed to close this gap by examining whether VEGF might serve as a surrogate or prognostic marker of PVD.

Our study presenting serum VEGF levels in 293 patients confirms the observation of smaller series that serum VEGF levels are elevated in PVD.[1] Furthermore, we showed a correlation of serum VEGF with the clinical Fontaine stages (Fig. 1). This finding contrasts to a report from Porcu et al.[4] in smaller series of 36 patients, which showed no VEGF increase with Fontaine classes.

Although there are several ways to evaluate PVD severity, it is sometimes difficult to differentiate in patients with diabetes between Fontaine stage III PVD and other diseases such as neuropathy or spinal canal stenosis. In such situations, serum VEGF might serve as a new surrogate marker. New parameters are needed to determine PVD severity in a growing number of older patients and those with multiple co-morbidities. We hypothesise that VEGF might be such a parameter and further studies with VEGF in combination with other parameters are warranted.

We observed no correlation between VEGF values and confounding risk factors of PVD including smoking, hypertension and diabetes. This contrasts with the other reports[3] showing higher VEGF levels in these patients. However, as the Fontaine stages of these patients were not clearly stated, the elevated VEGF values might also reflect greater PVD severity. However, we noticed in accordance with Kimura et al.,[3] a significant inverse correlation between hypercholesterolaemia and VEGF. Unfortunately, the underlying effects of these observations cannot be determined from our study since we did not examine healthy controls with these risk factors.

In summary, serum VEGF is a parameter that can be determined easily and reliably in PVD. Serum VEGF values

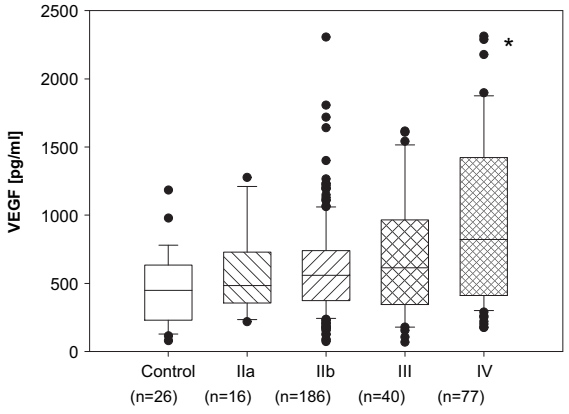

Figure 1 Serum VEGF levels in PVD patients with different Fontaine stages prior to treatment. Results are shown as median, 10th, 25th, 75th and 90th percentiles. *$p = 0.006$ Fontaine stage IV versus control (Kruskal-Wallis ANOVA on Ranks followed by Dunns method, Sigma Stat 2.03, Jandel Scientific).

show a strong correlation with the clinical Fontaine stages describing the severity of PVD and may serve as a surrogate indicator of disease severity.

Conflict of Interest

M.S. received fees from Gore, Cook and Datascope, Medtronic and Otsuka.

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