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Soluble Angiogenic Factors: Implications for Chronic Myeloproliferative Disorders

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The role of angiogenesis for the progressive growth and metastatic process of tumours is well established. What is not clear, though, is the clinical prognostic significance of the angiogenic factors in malignant haematological diseases. In this study, we have assessed the plasma and serum levels of two major angiogenic factors, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) in 55 patients affected by chronic myeloproliferative disorders (CMD). This series included 25 patients with essential thrombocythemia (ET), 10 patients with chronic myelocytic leukaemia (CML), 14 patients with polycythemia vera (PV), and 6 patients with primary myelofibrosis (MF), and they were compared to 20 healthy control subjects. In all patients the plasma VEGF concentration was significantly increased to the healthy control group ($P < 0.004$). The highest concentrations were found in the patients with ET (178.25 ± 125.22 pg/ml). The VEGF levels were significantly higher in CMD patients with vascular complications than those in CMD patients without complications ($P < 0.01$). The b-FGF serum levels also appeared to be significantly higher in almost all the CMD patients compared to the control group ($P < 0.07$). A significant correlation was found between the VEGF levels and the platelet count in the ET patients and the spleen index in the CML patients. VEGF level, in this study, is associated with increased risk of thrombotic complications. There is evidence of increased levels of soluble angiogenic factors in malignant haematological disorders, but their contribution to the progression of diseases is yet unclear. Am. J. Hematol. 69:159–163, 2002. © 2002 Wiley-Liss, Inc.

Key words: angiogenesis; angiogenic growth factors; chronic myeloproliferative disorders; thrombosis; extramedullary hematopoiesis

INTRODUCTION

Chronic myeloproliferative disorders (CMD) are characterized by varying degrees of extramedullary hematopoiesis (EMH) and a high incidence of thrombotic events. The mechanisms of these complications are still not clear. Our study was prompted by the recent observation of increased bone marrow (BM) microvessel density in patients with CMD [1]. The evaluation of this angiogenic activity, like solid tumours, might be a clue for a better understanding of the mechanism involved in the pathogenesis of EMH and thrombosis in these diseases.

The role of angiogenesis for the progressive growth and metastatic process of many solid tumours is well established [2–5]. Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), and their receptors seem to be the major inducers of angiogenesis. Both angiogenic factors are involved in an autocrine endothelial cell mitogenic loop. While families of FGF display their biological effects on a variety of cell types, VEGF appears to be the most selective growth factor acting on endothelial cells [6–8].

Recently, high serum levels of these molecules have been reported in various pathological conditions, including inflammation and cancer, whereas only a few data exist for hematologic neoplasms [9–17]. This is may due to the historical concept of leukaemia as a "liquid tumour". However, there is now some evidence for the concept that hematopoietic malig-

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nancies may be considered to be dependent on vascular support in the bone marrow, like solid tumours in other tissues.

Increased microvessel density has been reported in bone marrow of acute lymphoblastic leukaemia (ALL) and multiple myeloma (MM) patients [18,19]. Expression of VEGF has also been detected in non-Hodgkin's lymphoma (NHL), acute myeloid leukaemia (AML), and myelodysplastic syndromes (MDS) [20–24].

In myeloid malignancies (AML, MDS) the microvessel density seems to be correlated to disease progression [25,26].

In addition to an increased vascular density in BM, some studies have also shown elevated levels of VEGF and/or b-FGF in these conditions. However, the clinico-prognostic significance of these findings is yet unclear.

In this paper, we evaluated circulating levels of VEGF and b-FGF in a group of patients with CMD. Moreover, we compared levels of soluble angiogenic factors in two groups of CMD patients, those with and those without thrombotic complications.

MATERIALS AND METHODS

Patients

Fifty-five patients (38 female and 17 male) with a median age of 60 years were evaluated in this study. The series included 25 patients with essential thrombocythemia (ET), 10 patients with chronic myeloid leukaemia (CML), 14 patients with polycythemia vera (PV), and 6 patients with myelofibrosis (MF). The diagnosis was made according to established haematological criteria. Fourteen patients (8 with ET, 6 with PV) presented thrombotic complications occurring from 1 month prior to the initial diagnosis of CMD to the time of testing. They included complete stroke, deep venous thrombosis, and digital gangrene.

Twenty healthy control subjects, with similar sex and age distributions as the patients, were examined.

The study was performed according to the guidelines of the local Board of Ethics.

During this study, 39 patients were considered before any chemotherapy, 16 patients received platelet anti-aggregants. Patients with intercurrent infection or inflammatory process were excluded from the study.

Determination of Circulating Levels of VEGF and b-FGF

Venous blood samples were collected in two sterile tubes, an aliquot with anticoagulant (1/9 volume of 0.129 mol trisodium citrate) and a part in tubes with no anticoagulant. Serum and platelet-poor plasma samples were prepared by centrifugation at 2,000*g* for 10 min within 1 hr of collection and stored at −70°C until time of VEGF and b-FGF assays.

sVEGF (on plasma) and sb-FGF (on serum) were measured in duplicate by an enzyme-linked immunosorbent assay (ELISA) method (Quantikine, R&D system, Minneapolis, MN), according to manufacturer's instructions.

Statistical Methods

Data were expressed as average values ± standard deviation. Statistical analysis was performed by using the ANOVA one-way test and Pearson's coefficient of correlation. The significance levels was set to $P < 0.05$.

RESULTS

All CMD patients showed significantly high levels ($P < 0.004$) of sVEGF (129.42 ± 106.19 pg/ml) compared to the control patients (30.16 ± 15.08 pg/ml).

The plasma level of sVEGF was significantly higher in patients with ET (178.25 ± 125.22 pg/ml) compared to the other forms of CMD (CML 81.89 ± 41.7 pg/ml, PV 116.8 ± 100.4 pg/ml, MF 74.1 ± 27.1 pg/ml), Fig. 1. A significant difference was also observed between the CMD patients with thrombotic complications and those without thrombosis ($P < 0.01$), Fig. 2.

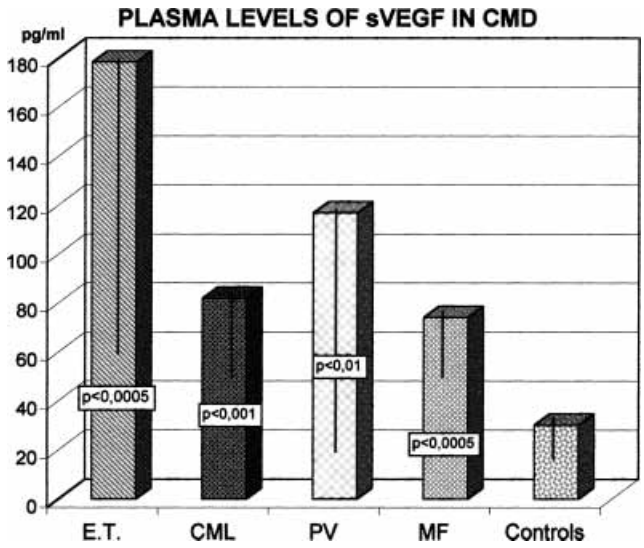

Fig. 1. Plasma levels of sVEGF in CMD.

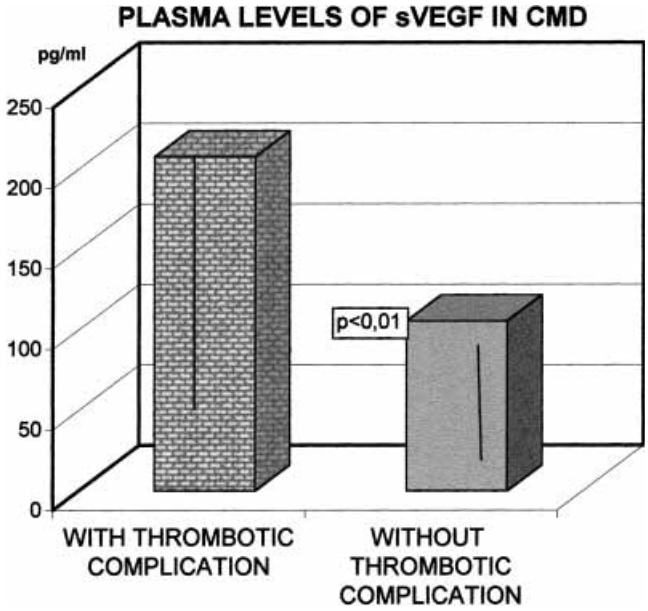

Fig. 2. Plasma levels of sVEGF in CMD patients with and without thrombotic complications.

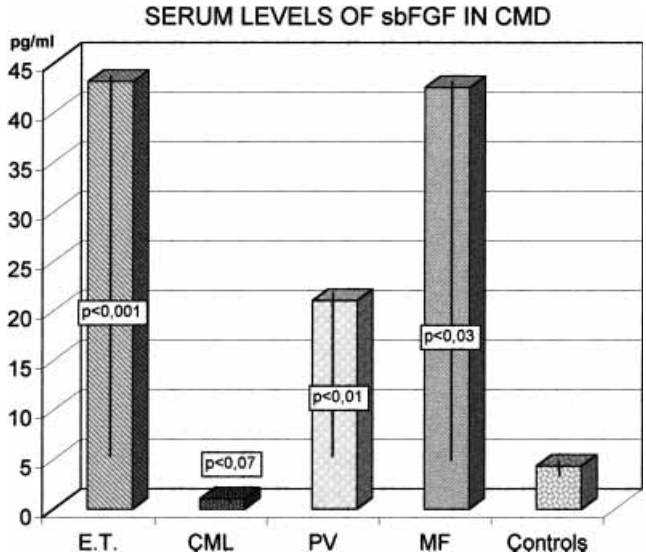

Fig. 3. Serum levels of sb-FGF in CMD.

At the same time, we found high levels of sb-FGF in almost all CMD patients compared to the control group ($P < 0.07$).

The average sb-FGF concentration was significantly higher in the patients with ET (43.1 ± 40.4 pg/ml), with MF (42.5 ± 49.79 pg/ml), with PV (21.02 ± 17.3 pg/ml), compared to the control subjects (4.35 ± 1.18 pg/ml), Fig. 3.

On the other hand, there was no significant difference between CML patients (1.02 ± 0.29 pg/ml) and the control group.

A positive correlation was found between platelet count and the sVEGF plasma levels in all ET patients ($r = 0.86$, $P < 0.0005$). A weak correlation was found with enlarged spleen (ultrasound >15 cm) in CML patients ($r = 0.94$, $P < 0.01$). However, b-FGF levels showed no correlation with platelet count.

DISCUSSION

The present investigation has demonstrated a significant increase of circulating VEGF levels in patients with CMD compared with control group. Indeed, the 80% of the patients showed 3- to 4-fold higher VEGF concentrations than the median of the control group. The highest VEGF concentrations were found in patients with ET. We also observed a significant increase of VEGF in CMD patients with thrombotic events. Moreover, there was a significant correlation between circulating VEGF and platelet count.

The presence of mRNA for VEGF has been described in platelets and megakaryocytes [27–29]. This finding has implications for processes involving platelet and endothelial cell interactions.

Previous experiments have emphasised the role of VEGF in thrombogenesis: the VEGF released by the activated platelets would seem to promote endothelial activation with a subsequent switch to a predominant prethrombotic phenotype [7,30].

This observation suggests that the increased plasma VEGF levels might be an important signalling molecule for thrombotic risk in CMD patients.

Moreover, in patients with CML the increased VEGF levels correlated with marked splenomegaly. This finding is relevant because it could confirm the hypothesis of the role of angiogenic factors in the dysregulation of hematopoiesis in CMD. Recently, Mesa and colleagues reported a substantial increase of marrow vascularity in myelofibrosis with myeloid metaplasia. In addition, they demonstrated that increased angiogenesis in MMM correlated significantly with marked splenomegaly and was found to be an independent risk factor for overall survival [31].

Both VEGF and b-FGF are endothelial cells growth factors, and b-FGF is an important inducer of stroma cell activation. Activated stroma cells, in turn, may produce other inducers of angiogenesis, such as b-FGF, IL-6, and IL-8 [32].

VEGF-stimulated endothelium may produce growth factors for myeloid and lymphoid leukaemic cells. Paracrine growth stimulation may not only be restricted to the BM microenvironment but may also take place at extramedullary sites. Under favourable

conditions, the development of EMH in haematological diseases may be initiated by this mechanism.

The serum levels of b-FGF were elevated in patients with ET, PV, and MF, while no difference was observed between CML patients and control group. Because of the limited number of samples from patients with CML that were available for the study, we were unable to understand the clinical-prognostic significance of lower b-FGF levels.

In conclusion, in our study there is evidence of increased levels of angiogenic factors in CMD patients. Anti-angiogenic therapy might be considered in patients with extramedullary hematopoiesis and/or thrombotic risk.

Follow-up studies are needed to verify the possible role of these factors as prognostic markers of diseases progression.

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