Are Basic Fibroblast Growth Factor and Vascular Endothelial Growth Factor Prognostic Indicators in Pediatric Patients with Malignant Solid Tumors?

Marie-Dominique Tabone,1 Judith Landman-Parker, Brigitte Arcil, Marie-Claude Coudert, Iliana Gerota, Marc Benbunan, Guy Leverger, and Christine Dosquet


ABSTRACT

Angiogenesis plays an important role in the growth, progression, and metastasis of solid tumors. Among angiogenic factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) appear to be useful markers in adults with cancer. The aim of this pilot study was to determine the levels of VEGF in serum and bFGF in serum and urine of children with solid tumor at diagnosis (as measured by ELISA), and to investigate whether these parameters provide prognostic information. Forty consecutive patients with different types of cancer were prospectively included in this study. Median values of all studied angiogenic factors were higher in patients than in controls (n = 40), and the differences were statistically significant for bFGF in serum and urine: 10 versus 3 pg/ml (P = 0.0004) and 6406 versus 0 pg/g of creatinine (P < 0.0001), respectively. Among patients, median serum values of bFGF and VEGF were higher in children with metastatic disease (n = 14) than in those with localized disease (n = 26). The difference was statistically significant for serum bFGF: 17.5 versus 6 pg/ml (P = 0.02). Serum angiogenic factor levels correlated with outcome. The estimated event-free survival at 3 years was 79% for patients with normal bFGF values (n = 13) versus 42% (n = 26; P = 0.02) for those with high levels, and 71% in case of normal VEGF values (n = 20) versus 38% (n = 19; P = 0.04) for those with high levels. No benefit of normal urinary bFGF values was observed. Our results provide a rationale for exploring the clinical interest of bFGF and VEGF measurements in body fluids of a larger group of children with cancer.

INTRODUCTION

A number of observations show that angiogenesis plays an important role in the growth, progression, and metastasis of solid tumors (1). Many studies conducted in adults with various types of cancer have reported a relationship between intratumor microvessel density and tumor aggressiveness (2, 3). The switch of a tumor to angiogenic phenotype is believed to involve a change in the local equilibrium between angiogenic inducers and inhibitors (4). Concerning angiogenic factors, certain patterns of expression of bFGF2 and VEGF have been described in a wide variety of animal and human tumors (4). Elevated levels of bFGF and VEGF have been detected in the urine and/or serum of a substantial number of patients with malignancies (2, 5, 6), and several studies have shown the value of these laboratory parameters as prognostic indicators (5, 7).

There is an extensive body of data on angiogenic factors in adults with cancer. However, the types and distribution of the malignancies that occur in the pediatric age group differ markedly from those that occur in adults (8), and studies in children are limited in number. The adverse prognostic value of high tumor vascularity has been demonstrated in children with neuroblastoma (9). Tumor bFGF expression in high-grade gliomas (10), as well as high bFGF levels in cerebrospinal fluid of children with brain tumors (11), have been associated with adverse outcome. Increased levels of urinary bFGF have been found in pediatric patients with acute lymphoblastic leukemia (12). In patients with Wilms’ tumor, preoperative levels of urine bFGF correlated with staging (13). However, knowledge concerning the profile of angiogenic factors in biological fluids of children with malignant tumor still needs to be improved, and the potential utility of such knowledge in the management of these children needs to be tested.

Therefore, the aim of this study was to determine the levels of VEGF in serum and bFGF in serum and urine of children with untreated solid tumors and to investigate whether these parameters provide prognostic information.

PATIENTS AND METHODS

Patients. This prospective pilot study was conducted between May 1996 and January 1999 according to the principles of the Declaration of Helsinki and the rules of our institution. During this period, consecutive patients admitted to our pediatric oncology center for diagnosis and treatment of a solid tumor were enrolled with parental oral informed consent. Children

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2 The abbreviations used are: bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; EFS, event-free survival.
with hematological malignancy and/or documented infection were excluded from the study.

**Controls.** Normal healthy children undergoing free medical check-ups in a well-child care center were recruited as controls to determine normal values of angiogenic factors. Children with a history of infectious disease during the month preceding blood and urine sampling were also excluded. Specimens from 20 males and 20 females, 3 months to 13 years of age (median, 2 years) were assayed.

**Blood and Urine Collection.** Samples from patients and controls were collected into dry sterile tubes. Serum and urine were immediately frozen (−80°C) in aliquots until assay. For patients, samples were collected before treatment. When a surgical biopsy was indicated, samples were collected just before or at least 6 days after the biopsy.

**Assay of Angiogenic Factors.** The levels of bFGF (urine and serum) and VEGF (serum) were assayed as described previously (14) with sandwich enzyme immunoassay methods (Quantikine; R & D Systems, Minneapolis, MN) using capture monoclonal and horseradish peroxidase-conjugated polyclonal antibodies that were specific for each factor. Standard curves were constructed using serial dilutions of recombinant human bFGF or VEGF (165-amino acid form). The minimum detectable concentrations were estimated to be 1 pg/ml for bFGF and 9 pg/ml for VEGF. The intra- and interassay variations were all <10%. Each sample was tested in duplicate. The bFGF concentrations in urine were expressed in pg of bFGF/g of creatinine.

**Statistical Analysis.** Results are presented as the median and range. The differences in angiogenic factor distribution between groups were analyzed using the nonparametric Mann-Whitney rank-sum test. Correlation coefficients between the different parameters were calculated using the nonparametric Spearman rank test. For survival analysis, the stopping date was November 1, 1999. Survival curves were plotted according to the Kaplan-Meier method. These curves were compared among subgroups of patients using the log-rank test. Cutoff levels of angiogenic factors were defined as the higher value of the 95% confidence interval in patients with metastases at diagnosis. Above the cutoff levels, patients were classified as having high angiogenic factors; below or at the cutoff levels, they were classified as having normal angiogenic factors. Events were defined as progression of disease under treatment or recurrence after a first complete remission. Follow-up periods were defined as the interval between diagnosis and last contact, defined as the date of death or date of last visit.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Localized disease, n (n = 26)</th>
<th>Disseminated disease and site of metastases, n (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td>5</td>
<td>BM* and bone, (n = 3)</td>
</tr>
<tr>
<td>(n = 12)</td>
<td></td>
<td>BM (n = 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone (n = 1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone and liver (n = 1)</td>
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<tr>
<td></td>
<td></td>
<td>Distant lymph node (n = 1)</td>
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<tr>
<td></td>
<td></td>
<td>Lung (n = 2)</td>
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<tr>
<td>Nephroblastoma</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>(n = 10)</td>
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<td></td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>4</td>
<td>Lung (n = 3)</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>(n = 5)</td>
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<tr>
<td>Rhabdomyosarcoma</td>
<td>1</td>
<td>BM, bone, and lung (n = 1)</td>
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<tr>
<td>(n = 2)</td>
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<td></td>
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<tr>
<td>Hepatoblastoma</td>
<td>1</td>
<td>Lung (n = 1)</td>
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<td>(n = 2)</td>
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<td></td>
</tr>
<tr>
<td>Undifferentiated carcinoma</td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td>(n = 2)</td>
<td></td>
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</tr>
</tbody>
</table>

* BM, bone marrow.

**RESULTS**

**Patient Characteristics.** Forty patients, 21 males and 19 females, 1 month to 17 years of age (median, 3 years and 3 months) were studied. Diagnoses and initial disease status are detailed in Table 1. N-myc oncogene amplification was investigated in all cases of neuroblastoma. More than 10 copies were observed in two patients with metastatic disease. Among patients with nephroblastoma free of hematogenous metastasis, both kidneys were involved in two cases. The rhabdomyosarcoma histological subtype was embryonal in the patient with localized disease and alveolar in the patient with metastases at diagnosis.

**Angiogenic Factor Values.** Concentrations of bFGF (serum and urine) and VEGF (serum) in patients and controls are presented in Table 2. Of the 40 patients included in this study, 39 had blood samples taken for serum assays and 29 had sufficient urine samples taken for the bFGF assay. In controls, no correlation was found between either age and serum angiogenic factor values ($r' = -0.02$; $P = 0.90$; $n = 31$ for VEGF; $r' = -0.16$; $P = 0.45$; $n = 22$ for bFGF) or between age and urinary levels of bFGF ($r' = 0.11$; $P = 0.50$; $n = 40$). Median values of all studied angiogenic factors were higher in patients than in controls. For bFGF, the differences were statistically significant for both serum (Fig. 1A) and urine (Fig. 1B). For VEGF, the difference between patients and controls did not reach statistical significance.

The cutoff value for serum bFGF was 4 pg/ml; 26 of 39 patients had high serum bFGF concentrations. For VEGF, the
The cutoff value was 402 pg/ml; high levels were found in 19 of 39 patients. At the cutoff value of 1116 pg/g of creatinine for urinary bFGF, 23 of 29 patients had high concentrations.

In children with solid tumors, no correlation was found either between serum and urinary levels of bFGF ($r = 0.26$; $P = 0.18$; $n = 28$) or between serum bFGF and serum VEGF levels ($r = 0.26$; $P = 0.11$; $n = 39$).

Among patients, median serum values for bFGF and VEGF were higher in children with metastatic disease than in those with localized disease (Table 3). The difference was statistically significant for serum bFGF (Fig. 2). For urinary bFGF, differences between metastatic and local disease were not significant.

The angiogenic factor values in patients with localized bone tumors (osteosarcoma or Ewing sarcoma), neuroblastoma, or nephroblastoma are detailed in Table 4. High serum concentrations of VEGF were observed in 22, 20, and 57% of cases, respectively. For bFGF, the proportions of patients with elevated serum concentrations were 44, 80, and 29% among those with bone tumor, neuroblastoma, and nephroblastoma, respectively. Urinary bFGF values were over the cutoff value in 50% of patients with localized bone tumor, in all cases of localized neuroblastoma, and in 83% of patients with nephroblastoma. The number of patients was too small in each subgroup to allow statistical analysis.

**Follow-up and Survival Analysis of Patients.** Median follow-up for the whole group of patients was 22 months (range, 3–42 months). At the time of survival analysis, 27 patients were alive, with a median follow-up of 27 months (range, 8–42 months), and 13 patients had died 3–34 months after diagnosis. Median survival time for these patients was 11 months. Estimated overall survival and EFS of the whole group of patients were 52% (95% CI 44%–60%) and 45% (95% CI 37%–53%), respectively, at 36 months.

Serum bFGF levels correlated with outcome. The estimated EFS of patients with normal serum bFGF levels was 79% (95% CI 69%–89%) at 3 years, whereas it was 42% (95% CI 24%–59%) for the subgroup of patients with high serum bFGF levels ($P = 0.02$; Fig. 3A). High VEGF concentrations appeared to be an adverse prognostic factor (Fig. 3B), with a 3-year EFS of 71% (95% CI 62%–80%) among patients with normal VEGF values and of 38% (95% CI 24%–52%) among patients with high VEGF values ($P = 0.04$). Normal urinary bFGF values had no beneficial effect on EFS (50% (95% CI 35%–65%) versus 68% (95% CI 56%–80%); $P = 0.67$).

**DISCUSSION**

Since Folkman’s hypothesis (15) in 1971 that angiogenesis plays a crucial role in the development of malignant tumors, the correlation of tumor microvessel density with cancer aggressiveness has been demonstrated over a wide spectrum of adult malignant diseases (2, 3). Substantial progress has been made in the methods available for evaluating tumor angiogenesis. Microvessel counting in cancer tissues after immunohistochemical staining remains the reference method, but this technique is difficult to standardize (3). Many researchers have found an association between tumor microvessel density and disease aggressiveness.
and the degree of VEGF expression by immunohistochemical methods (16–19). Similarly, the relationship between quantification of specific angiogenic factor mRNAs and tumor angiogenesis (20) or tumor aggressiveness (21) has also been documented in various cancers. Angiogenic factors have been assayed in cytosolic extracts of tumors, and the prognostic interest of VEGF in primary breast carcinoma has been demonstrated (22). Tumor angiogenesis can also be indirectly assessed in vivo by angiogenic factor measurements in body fluids (2). A sensitive method for bFGF detection was first described in 1991 (23). Subsequently, the clinical value of bFGF measurements in urine was demonstrated in patients with various types of cancer (5), as well as the clinical value of bFGF levels in serum of patients with renal cell carcinoma (14, 24) or breast cancer (25). Elevated serum VEGF levels have been found in patients with various types of tumors (6, 26–28). In patients with breast cancer, serum VEGF levels correlate with microvessel density and VEGF expression in tumor tissues (6). Furthermore, serum VEGF appears to be a useful marker for monitoring the clinical course after surgery for breast (6) and ovarian cancers (29). In our study, blood VEGF was similarly assayed in serum. Because platelets contain VEGF (30), some have concluded that plasma is more suitable than serum for blood VEGF measurements (31). In fact, platelets as well as leukocytes contain VEGF (30), with high VEGF levels being found in both cell types in patients with cancer (32). VEGF in the serum of patients with malignancy originates from the tumor and from blood cells and reflects the degree of mitogenicity of serum on endothelial cells, suggesting that platelets and leukocytes may scavenge biologically active VEGF (7). For these reasons, the measurement of VEGF in the serum of patients with malignancy appears more appropriate than its measurement in plasma (7, 32).

Carcinomas are rare in children, in contrast to adults. Solid malignant tumors that occur in children are more often embryonal tumors and sarcomas (8). In general, the mechanisms of oncogenesis appear to differ from those in adults, with the environmental aspect being less important in children. Furthermore, pediatric tumors usually are characterized by a rapid doubling time and a better response to chemotherapy. The usefulness of angiogenic factor assays therefore needs to be validated in pediatric oncology, as it has been in adult oncology. In children with solid tumors before treatment, we detected increased VEGF and bFGF levels in serum, as well as increased bFGF levels in urine. These results are in agreement with those in adult patients (5, 6, 33). We also found that children with metastatic disease at the time of diagnosis had higher circulating levels of bFGF and VEGF than children with localized cancer, as reported previously in adults (14, 34), although the difference was not statistically significant for VEGF. In pediatric patients with Wilms’ tumor, Lin et al. (13) found that patients with stage III, stage IV, or bilateral tumors had significantly higher urinary bFGF levels than controls. In contrast, bFGF levels in the urine of patients with stage I or II were similar to bFGF levels in controls. In our study, no significant difference was found between urinary bFGF levels in patients with localized tumors or in those with hematogenous metastases at diagnosis.

In agreement with Lin et al. (13), we found no correlation between bFGF level in serum and the corresponding urinary level. The reason for this lack of correlation could be attributable in part to the complex pattern of clearance of bFGF from serum, which involves, after i.v. injection in animals, deposition of bFGF within solid organs, preferentially the kidney, liver, spleen, and arterial wall (35).

The most clinically relevant observation in our study was the adverse prognostic value of high pretreatment levels of serum VEGF and bFGF. Our results in children confirm those of previous studies in which univariate analysis showed the prognostic value of serum VEGF (7, 28) and bFGF (14) in adults with cancer. In contrast to Nguyen et al. (5), we did

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**Table 3** Angiogenic factor concentrations according to disease extension

<table>
<thead>
<tr>
<th>Patients with metastatic disease</th>
<th>Patients with localized disease</th>
<th>P (Mann-Whitney test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum VEGF (pg/ml)</td>
<td>525 (125–1,147)</td>
<td>323 (41–1,296)</td>
</tr>
<tr>
<td>(n=14)</td>
<td>(n=25)</td>
<td></td>
</tr>
<tr>
<td>Serum bFGF (pg/ml)</td>
<td>17.5 (4–40)</td>
<td>6 (1–71)</td>
</tr>
<tr>
<td>(n=14)</td>
<td>(n=25)</td>
<td></td>
</tr>
<tr>
<td>Urinary bFGF (pg/g of creatinine)</td>
<td>4,949 (0–27,215)</td>
<td>6,492 (0–260,000)</td>
</tr>
<tr>
<td>(n=9)</td>
<td>(n=20)</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent median; numbers in parentheses represent range.

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**Fig. 2** Individual bFGF serum values in children with metastatic or localized tumor. Data points are the means of two determinations; bars, median values. The Mann-Whitney rank-sum test was used to assess significance.
not find that high levels of urinary bFGF influenced outcome. Our results were obtained from patients suffering from various types of solid tumors admitted to a pediatric oncology center whose recruitment policy explains the lack of inclusion of children with brain tumors, who are managed in other centers. The limited number of patients in this pilot study must be borne in mind when interpreting statistical results, especially in the case of urinary bFGF. However, even in a study involving a larger number of patients with nephroblastoma, the disease-free survival of patients with normal preoperative urinary bFGF levels was not statistically different from that of patients with elevated preoperative levels (13).

The finding of a correlation between serum bFGF and VEGF and outcome in our cohort of children provides a rationale for exploring this issue in a larger prospective study. This could permit multivariate analysis of the prognostic importance of angiogenic factor levels in body fluids of children with tumors, taking into account the initial extent of the disease. Furthermore, serial measurements of VEGF and bFGF during therapy and follow-up could be evaluated for their ability to predict disease progression or early metastases. The monitoring of angiogenic factors in body fluids is easy to perform in clinical practice. In the treatment of life-threatening hemangiomas in infancy, urinary levels of bFGF have been very valuable in initiating IFN-α-2a therapy and in adjusting dosage of the drug (2). The effectiveness of other antiangiogenic therapies, alone or combined with chemotherapy, has been demonstrated in a variety of experimental solid tumor models, including murine and rat osteosarcoma (36, 37), neuroblastoma (38, 39), and rhabdomyosarcoma (40, 41). The monitoring of angiogenic factors might also be useful for assessing the antiangiogenic activity of angiogenesis inhibitors in human (42). Although there have been great improvements in the survival of children with cancer in recent decades, new prognostic indicators are still needed in some cases, as well as new therapeutic approaches in patients whose tumors are refractory to conventional chemotherapy. Our results suggest that antiangiogenic therapy could be considered in such cases.

**REFERENCES**


**Table 4** Angiogenic factor concentrations in patients with localized bone tumors (osteosarcoma and Ewing sarcoma), neuroblastoma, or nephroblastoma

<table>
<thead>
<tr>
<th>Bone tumor</th>
<th>Neuroblastoma</th>
<th>Nephroblastoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum VEGF (pg/ml)</td>
<td>304 (41–630)</td>
<td>165 (42–1,293)</td>
</tr>
<tr>
<td>Serum bFGF (pg/ml)</td>
<td>4 (1–14)</td>
<td>15 (2–22)</td>
</tr>
<tr>
<td>Urinary bFGF (pg/g of creatinine)</td>
<td>826 (0–20,885)</td>
<td>10,444 (3,783–19,111)</td>
</tr>
</tbody>
</table>

*Values represent median; numbers in parentheses represent range.

*Proportion of patients with high angiogenic factor values among tested patients.

Fig. 3 EFS of children with solid tumors according to serum bFGF (A) and serum VEGF (B) levels. The log-rank test was used to assess significance. One of the 13 patients with normal bFGF and 13 of the 26 patients with high bFGF had metastases at diagnosis, as well as 4 of the 20 patients with normal VEGF and 10 of the 19 patients with high VEGF.


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