



## PLASMA EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN PATIENTS WITH BRAIN TUMORS

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### ABSTRACT

**OBJECTIVE:** Angiogenesis and angiogenic growth factors are of basic importance in the development and progression of brain tumours. The purpose of the study was to evaluate the plasma levels of two angiogenic factors Vascular Endothelial Growth Factor (VEGF) and Basic Fibroblast Growth Factor (bFGF) in patients with brain tumors and potential possibilities to use them with predictive value.

**MATERIAL – METHOD:** In order to determine amount of VEGF and bFGF in plasma were examined 35 patients, (glioblastoma multiforme, GBM, n=7; astrocytoma, n=7; meningioma, n=11; and healthy control group, n=10) were analysed. For determination of plasma concentrations of angiogenic factor, highly specific enzyme-linked immunosorbent assays (ELISAs) were used. The data underwent regression and correlation analysis for estimation of the eventual interrelations.

**RESULTS:** Median levels of bFGF in glioblastoma patients were higher compared with those with low grade gliomas, meningiomas or healthy patients. Highest levels of VEGF concentrations were detected in plasma derived from patients suffering from LGG. Plasma expression of these angiogenic factors shows dynamic changes pre- and post-operative, but cannot be explained only with tumor resection, grade or type of the tumor, and probably is result of physiological regeneration of the operative wound. No correlation between the survival time of the patients and the plasma levels of the tested growth factors was obtained.

**CONCLUSIONS:** Expression of VEGF and bFGF in plasma are not reliable marker.

The detectability of the angiogenic factors VEGF and bFGF in the plasma of patients suffering from various types of brain tumours is described. The plasma detectability of the individual angiopoietic factors seems to depend at least partly on the tumour type as well as on tumour progression. This might be of prognostic and therapeutic relevance.

**Key words:** VEGF, bFGF, GBM, meningioma, astrocytoma, plasma

Angiogenesis plays a crucial role in tumour growth, particularly in GBM.

The degree of tumor angiogenesis seems to be a significant predictor of tumor progression, recurrence, and metastatic spread in a variety of malignant tumors [1, 2].

There are many growth factors and cytokines involved in the complex mechanism of angiogenesis.

Of the all known angiogenic factors, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are the most common in malignant tumors. [3, 4]

Astrocytic tumors represent the majority of primary intra-axial brain tumors in adults, and immunohistochemical studies have shown high concentrations of bFGF [5] and VEGF [6] in these tumors.

Vascular endothelial growth factor (VEGF) is a secreted dimer with characteristic kinase domain receptor binding sites [7, 8].

Endothelial expression of VEGF was detected in all angiogenesis stages, namely survival, proliferation, migration and permeability, underlining the central role of this growth factor in new vessel formation [9].

Up-regulation of VEGF transcription in tumour cells due to hypoxia [10, 11] represents a stimulus for angiogenesis. Notably, in gliomas, expression levels of VEGF and its receptor highly correlate with their malignancy grade [12].

Fibroblast growth factor bFGF is the first discovered angiogenic growth factor. [13]

They stimulate fibroblast as well as endothelial cell growth and are therefore of vital importance in the process of angiogenesis, [14] and also play a significant part in at least three of the four phases of wound healing: inflamma-

tion, repair and regeneration [14, 15, 16]. Further important functions of FGFs include tumour development and progression.

FGF-2 or basic FGF is one of the most potent mitogens and chemotactic factors of the vascular endothelial cell. Recently, it has been demonstrated that basic FGF and VEGF have synergistic effects on angiogenesis in vivo [17].

## PATIENTS AND METHODS

**Study population.** The study participants were patients at the Department of Neurosurgery, Medical University of Varna, between July 2012 and December 2013. All blood samples were taken from forearm veins prior to the resection of intracranial tumours or any other treatment modalities. A control postoperative blood samples were taken in early postoperative period- 7-th day after surgery. A written informed consent was received from each patient prior to vein puncture. The study was approved by the local Ethics Committee. The diagnosis for each group was made by histological investigation of surgically removed tumour tissue by experienced pathologists.

**Sample collection.** Peripheral blood samples were obtained on the day of surgery. The blood samples were collected into 7 ml vacutainer EDTA tubes and were immediately cooled on ice. Blood was centrifuged (3000 rpm for 10 min), and supernatant serum was separated and stored at -70° C until an enzyme-linked immunosorbent assay for the angiogenic factors was performed.

**ELISA assays.** These analyses were performed with

commercially available ELISA kits (Quantikine® Human VEGF and Quantikine® Human bFGF Immunoassay, all from R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Briefly, standards were prepared using the provided recombinant proteins. One hundred  $\mu$ l of assay diluent followed by 50  $\mu$ l of either standard or plasma sample were added to each well and incubated for 2 h on a constant shaker at room temperature. Subsequently, wells were washed 3 to 4 times with washing buffer (R&D Systems) using the ELX Auto strip Washer and 200  $\mu$ l of conjugate were added to each well. After an additional incubation period of 2 h under constant shaking at room temperature, which was followed by the described washing procedure, 200  $\mu$ l of substrate solution was applied to each well and samples were incubated for 30 min under light protection. The reaction was stopped by addition of stop solution (50  $\mu$ l) and the ELISA plate was read at 450 nm within 30 min using Powerwave ELISA reader. The limit of quantification was specific for each assay and given as 3.0 pg/ml for bFGF and 5.0 pg/ml for VEGF.

**Patient characteristics.** In this study, blood samples were collected from a total of 35 patients suffering from malignant or benign brain tumours undergoing a neurosurgical investigation and tumour-directed intervention. The study participants suffered from one of the following brain tumours: meningioma (n=11); GBM WHO Grade IV (n=7); astrocytoma WHO I-II (n=7). Control group (n=10), healthy volunteers, with no anamnestic or clinical data for chronic disease or inflammation. For detailed patient characteristics, see Table 1.

**Table 1.** Demographic data of brain tumour patients separated into four groups.

	GBM	Astrocytoma	Meningeoma	Healthy
Number of patients	7	7	11	10
Gender, n (%)				
Male	3 (43%)	3 (43%)	2 (18%)	5 (50%)
Female	4 (57%)	4 (57%)	9 (82%)	5 (50%)
Age, years (mean-S.D.)	60 (9,2)	56,7(20,3)	54	
Died	7	7	0	0
Survival (days) (mean-S.D)	106(97,97)	294( 655)		

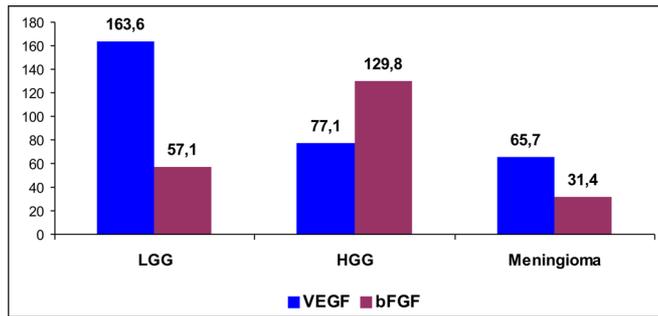
### GBM- Glioblastoma multiforme

**Statistical analysis.** Statistical analyses were performed with SPSS Version 11.0. Comparisons between groups were calculated using unpaired Student's t-test. The differences were considered as significant when  $p \leq 0.05$ . Parameters are indicated as grouped mean values  $\pm$  S.D. The association between the 2 angiogenesis factors were evaluated with Pearson's correlation coefficient. The same test was used for performing a correlation analysis between the survival rates of brain tumour patients in different groups.

## RESULTS

**Plasma concentrations of bFGF and VEGF in the study population.** In a first step, the bFGF and VEGF concentration in peripheral blood samples was measured (Figure 1). Although they were detectable in all plasma samples, a strong interindividual variability was observed, which depended at least partly on the tumour type. Interestingly, the mean VEGF concentration in the GBM group ( $77,1 \pm 16,0$  pg/ml)

**Fig. 1.** Preoperative plasma concentrations of bFGF and VEGF



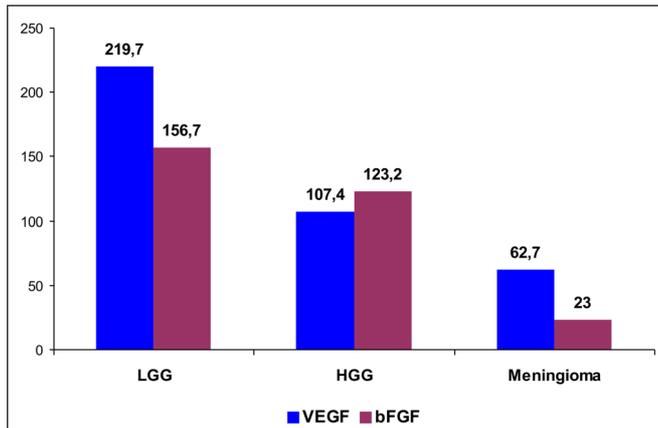
Was much lower than that observed in patients with LGG ( $163,6 \pm 226,2$  pg/ml) and correspond to those in meningioma group ( $66 \pm 29,0$  pg/ml).

The concentration of bFGF did not parallel those measured for VEGF (Fig. 1). The bFGF plasma concentrations of the HGG group ( $130 \pm 135,0$  pg/ml) were significantly higher than those of patients suffering from LGG ( $57,1 \pm 81$  pg/ml,  $p < 0.????????$ ) and meningioma group ( $31,4 \pm 15,0$  pg/ml).

However, no statistically significant difference was detected between the plasma VEGF levels of the GBM and the meningioma groups.

Comparative analysis of the values of VEGF and bFGF before and after surgery demonstrated statistically significant increase of the factors in patients with LGG ( $p < 0.05$ ). (Fig. 2). VEGF increases approximately 1.5 times, while bFGF is approximately triplicate his value.

**Fig. 2.** Postoperative plasma concentrations of bFGF and VEGF



In HGG group significant difference was observed only in VEGF values ( $p < 0.05$ ). bFGF values did not show statistically difference, although the decreasing of the values was observed.

In patients with Meningiomas was not found statistical difference in preoperative and postoperative data of VEGF and bFGF, despite decreases in the values of both factors.

*Correlation of VEGF and bFGF plasma levels with patient survival.* The expression levels of distinct angio-

poinetin factors have been shown to depend at least partial on the tumour malignancy grade. Therefore, the plasma levels of VEGF and bFGF were correlated with the patients' survival time. The individual survival data were obtained from personal conversation with the family of each patient. Data were available for all of the patients with HGG and LGG, all of them had died at the time of this study. The mean survival periods of the GBM patients and of the patients suffering from LGG were  $106,29 \pm 97,97$  days and  $293,6 \pm 655,1$  days, respectively. The survival data were compared with the plasma levels of the individual growth factors using Pearson's correlation coefficient. However, none of the 2 growth factors was found to correlate with the survival of the patients.

## DISCUSSION

Similar to other tumour entities, angiopoiesis is of central importance in brain tumour growth. The role of angiopoietin factors in the development of brain tumours was extensively studied with respect to mRNA and protein at the tissue level however, the data is limited about their plasma detectability in this type of tumor patients. This paper describes considerable plasma values of the angiopoietic factors VEGF and bFGF in samples derived from meningioma patients as well as in the plasma of patients suffering from HGG and LGG. Previously, VEGF was found to be highly detectable in the plasma derived from various kinds of primary solid tumours [19-20]. Earlier studies performed on brain tumours including GBM and astrocytoma demonstrated high tissue expression levels of bFGF and VEGF [4, 18]. As reported earlier [3, 4, 6, 13], VEGF and bFGF are synergistically involved in the formation of new blood vessels. Thus, a correlation of the plasma concentrations of VEGF and bFGF in all patients with brain tumors was expected. However, the data did not show any significant correlation. This might be due to high interindividual differences of VEGF concentrations within the same group in the study population. Interestingly, the VEGF concentration was significantly higher in patients with Astrocytoma when compared with patients suffering from GBM. This may be a result of a limited permeability of the blood-brain barrier as well as rapid intracerebral degradation at the site of secretion might additionally contribute to the relatively low VEGF concentrations in the plasma derived from GBM patients. No significant difference was found between the individual tumor.

A correlation of VEGF and bFGF expression levels was demonstrated in distinct tumors, which led to the hypothesis that bFGF represents one of the key mediators in angiogenesis. Therefore, the absence of a significant correlation between the plasma levels of VEGF and bFGF in our study population was surprising.

Our data demonstrate a lack of association between serum levels of angiogenic factors to tumor grade. A possible explanation for this observation is that direct secretion of the angiogenic factors by the tumor into the subarachnoid space, with the blood-brain barrier serving as a filter, is the basis for the significant gradient between CSF and serum levels of these factors. This assumption is sup-

ported by the observation that CSF levels of angiogenic factors of patients with systemic malignancies are low, despite elevated levels of angiogenic factors in the serum [21].

Dynamic changes that we observed pre- and post-operatively show statistical difference, but cannot be explained only with the brain surgery and tumor resection and reduction. Probably the operative wound, no matter how small she is, and subsequent natural healing, interfere and influence results. Further studies are required to firmly establish the role of regenerative process in VEGF and bFGF plasma dynamics.

Relations between angiogenic factor levels and patient survival period did not have statistical significance.

Malignant brain tumours have a poor prognosis, which is in part due to delayed diagnosis but also due to their aggressive growth potential with limited therapeutic options. Plasminogen activator inhibitor (PAI-1), glial fibrillary acidic protein, low molecular weight caldesman, cathepsin D and others were suggested to represent potential plasma markers for brain tumour diagnosis and surveillance. With respect to the importance of vascular growth factors in tumour biology with potential implication some new therapeutic options, these data on plasma levels of angiopoietin factors might form the basis to extend the diagnostic armamentarium in neuro-oncology. In this context, the

recently reported success of VEGF-targeted therapies in GBM treatment seems of specific interest.

Although no data are available for the potential application of this treatment modality for low grade gliomas, the high plasma levels of angiopoietic factors found in our patients with LGG suggests the applicability of VEGF blockade-based therapeutic regimes for this selected patient group. The success of therapeutic strategies involving the inhibition of growth factor pathways strongly depends on the identification of susceptible patients. Therefore, the identification of plasma-detectable vascular growth factors in the course of brain tumours might be not only of diagnostic, but also of prognostic and therapeutic relevance.

#### CONCLUSION:

The detectability of the angiogenic factors VEGF and bFGF in the plasma of patients suffering from various types of brain tumours is described. The plasma detectability of the individual angiopoietic factors seems to depend at least partly on the tumour type as well as on tumour progression. This might be of prognostic and therapeutic relevance.

Dynamic changes in values of VEGF and FGF cannot be associated with tumour type and progression and probably due to physiological angiogenesis.

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