

## Morphological analysis of vascular density in ependymomas

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### Abstract

*Ependymomas generally show slow growth rate and are associated with a long clinical history. In some cases however the biology of these tumours is considered to be unpredictable on the basis of histologic criteria. Density of microvessels was shown to serve in various malignant neoplasms as a prognostic factor that correlates with increased risk of metastasis and overall free survival.*

*Some data suggest that density of blood vessels may be of prognostic value also in patients with neuroepithelial tumours. The aim of this study was to determinate whether that observation can be applied to ependymomas. The materials included 51 ependymomas G2 and G3 according to the WHO classification. Vasculature was visualized immunohistochemically in paraffin-embedded sections of tumour samples with CD31 and FVIII antibody. Density of blood vessels was calculated using a computed image analyzing system. The data were statistically evaluated.*

*The density of blood vessels in anaplastic (WHO G3) ependymomas was shown to be significantly higher than that in WHO G2 type of the tumour, while there was no statistical difference between subtypes of WHO G2 ependymomas. The results suggest a connection between density of vasculature and the degree of histological malignancy in gliomas of ependymal derivation.*

**Key words:** tumour vascularity, microvessel density, ependymoma, CD31, FVIII-von Willebrand factor.

### Introduction

Ependymomas are the rarest subtype of gliomas, comprising 3-9% of these tumours. Most ependymomas show slow growth rate and relatively long natural history [18]. Histopathological features describing tumour properties are however not independently associated with prognosis of survival [5,21]. According to Schiffer et al. (1991) the number of mitoses is an important prognostic factor in cases of supratentorially situated ependymomas, whereas endothelial proliferations and necrotic foci are less useful

in predicting patient survival than in astrocytic and oligodendroglial tumours [21].

Some data suggest however that malignant transformation in astrocytomas, oligodendrogliomas and ependymomas depends, among other things, on proliferation of blood vessels [3].

Formation of new blood vessels is known to occur in a variety of physiologic and pathologic processes, and it is also known that proliferation of solid neoplasms is dependent upon angiogenesis [4,7,8,10,24,25].

Quantity and density of the microvessels have been shown to be an important prognostic indica-

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tor of biologic aggressiveness, risk of metastases and survival in many types of human neoplasms [2,15,22,23,26,27].

The aim of this paper was to present the results of calculation of microvessel density in some histologic subtypes of WHO grade 2 ependymomas and in anaplastic, WHO grade 3 ones, and its correlations with basic clinical data of patients.

## Material and methods

The investigation was performed on postoperative material from 51 cases of ependymoma. The group consisted of 26 males and 25 females aged from 3 months to 68 years (mean age was 34 years); 26 patients were younger than 16, while the remainder were older than 21.

Cerebellar location of tumour was the most common (26 cases), followed by supratentorial (17) and intraspinal (8).

The histopathological diagnosis of ependymoma was based on the criteria of the WHO 2000 Classification of Tumours of the Nervous System (Kleihues, Cavenee, 2000) [11].

The investigated cohort consisted of 13 tumours presenting typical for ependymoma WHO grade II, histopathological features (called by us "classic" subtype) (10 males and 3 females); 29 ones were characterized by dense cellularity (cellular subtype) (19 females and 10 males), an additional 6 showed mixed "classic" and cellular picture (5 males and 1 female), and 3 fulfilled morphological criteria of anaplastic variant (2 females and 1 male).

Eight "classic" ependymomas were situated in the cerebellum, 3 in cerebral lobes and 1 in the spinal cord. Cellular tumours were located as follows: 13 subtentorial, 13 supratentorial and 3 intraspinal; 4 ependymomas of mixed microscopic architecture were located in the cerebellum and an additional 2 in the spinal cord. Anaplastic variant was found in 3 different places (1 supratentorial, 1 subtentorial and 1 intraspinal).

Age of the patients ranged from 4 to 66 years in "classic ependymomas"; 1 to 67 years in tumours of cellular type, 3 months to 48 years in mixed type and 2 to 68 years in anaplastic ependymomas.

The formalin-fixed, paraffin-embedded biopsy specimens of the tumours were cut into slides 4 µm thick and stained with H-E. Slices with the highest vascularity were selected for immunohistochemical

procedures with monoclonal mouse anti-human antibody against endothelial cells – CD31, clone JC70A (DAKO), dilution 1:40; and monoclonal antibody against FVIII-von Willebrand factor, clone F8/86 (DAKO) dilution 1:50. Diaminobenzidine was used as chromogen. The proper and control reaction attached to each of the reagents.

Immunohistochemically stained slides of tumour tissue were microscopically analyzed under 100× magnification in order to find areas of the highest neovascularisation (hot spots). Then the number of blood vessels from 10 hot spots was counted using a computed image analyzing system (Jenaval microscope, Panasonic camera, software Multiscan) under 400× magnification.

All calculated data were collected in a database for statistical evaluation. The median, maximal and minimal values and the standard deviation were estimated. Non-parametric tests were used to compare the groups (U-Mann-Whitney median test, Kruskal-Wallis's ANOVA). Correlations were calculated according to the formulae set for non-parametric data by R. Pearson.

## Results

The vasculature of all examined tumours was well developed, but distribution of blood vessels was heterogeneous. There were vascular-rich areas, especially in the periphery of tumours and also almost avascular regions. The small vessels were thin walled and padded with a single layer of endothelial cells. The cells showed positive membranous reaction with CD31 antibody and cytoplasmic granular pattern with antibody anti-FVIII, von Willebrand factor. Vascularity was particularly robust in anaplastic ependymomas, although microvascular glomeruloid proliferations, the histopathological hallmark of glioblastoma, were not observed.

Values of vascular density measured on slices immunostained with CD 31 antibody ranged, for the whole investigated group of tumours, from 18 to 62 vessels/mm<sup>3</sup> (mean 34.4 vessels/mm<sup>3</sup>). In cellular subtype of ependymomas it was 18 to 59 (mean 31.7); in "classic" subtype, 19 to 50 (mean 32.4); in mixed ones, 28 to 50 (mean 39.1) and in anaplastic ependymomas, 55 to 62 (mean 59). The results are summarized in Table I.

Similar results were obtained when measured on slices stained with antibody against FVIII-von Wille-

brand factor. Values of vascular density for the cohort ranged between 15 and 75 vessels/mm<sup>3</sup> (mean value, 35.1). In cellular ependymomas it was 15-55 (mean 31.4); "classic" subtype, 23-51 (mean 34.1); mixed, 26-49 (mean 37.8), and 63-75 (mean 68.6) in anaplastic ependymomas. The results are summarized in Table II.

The difference of mean value of vascular network density between anaplastic (WHO grade 3) and the whole group of WHO grade 2 ependymomas is statistically significant ( $p < 0.0001$ ). There was no statistical difference however of the parameter between examined subtypes of WHO grade 2 ependymomas.

No statistically significant relations were found between density of blood vessels within the tumour and its location or between the density of vasculature and age and gender of the patients.

## Discussion

Vascularity of neoplastic tumours reflects to a high degree the ability to induce formation of new blood vessels that is called angiogenesis. Microvessel density does not distinguish however newly formed blood vessels from native ones and does not correlate with the degree of endothelial cell proliferation [13].

In our study we have chosen two antibodies labelling cells of blood vessel wall, antibody against FVIII related antigen which stains mainly cells of

large blood vessels [16], and antibody against CD31 labelling endothelial cells of small vessels. Results of immunostaining of the latter antibody are, according to Parums et al., 1990 [17] more consistent than that of monoclonal and polyclonal antibodies to factor VIII related antigen. The results of our study do not show a statistically significant difference between density of microvessels measured in slices stained with anti-F VIII and anti-CD31 antibodies; however, the value of blood vessels stained with the latter was slightly higher.

Angiogenesis is known to be an important factor influencing growth of solid neoplasms. It has been shown on animal models that tumours are not able to expand beyond 2-3 mm<sup>3</sup> without proliferations of newly formed microvessels which penetrate neoplastic tissue [3].

High vascular density in most types of tumours is an unfavourable prognostic indicator mainly because it is related to increased risk of dissemination and relapses survival free of metastases [2,15,22,23,26,27].

Tumours of the brain only rarely metastasize outside the central nervous system, and that is why the question arises whether they are really angiogenesis-dependent tumours [28].

The role of vascular density as a prognostic factor in gliomas is indeed controversial. Some authors [1,13,14] have shown that the higher the density of blood vessels in a tumour's tissue, the shorter the survival, while others [29] did not find a significant

**Table I.** Results of CD 31 immunoexpression in the examined ependymomas

Microscopic type	No.	Mean	St. deviation	St. error	Minimum	Maximum
cellular	29	31.7589	10.9989	2.0424	18.0000	59.0000
classical	13	32.4615	9.2612	2.5686	19.0000	50.0000
mixed	6	39.1667	8.0104	3.2702	28.0000	50.0000
anaplastic	3	<b>59.0000</b>	3.6056	2.0817	55.0000	62.0000
total	51	34.4118	11.8037	1.6528	18.0000	62.0000

**Table II.** Results of vWf immunoexpression in the examined ependymomas

Microscopic type	No.	Mean	St. deviation	St. error	Minimum	Maximum
cellular	29	31.4828	10.1405	1.8830	15.0000	55.0000
classical	13	34.1538	9.2362	2.5617	23.0000	51.0000
mixed	6	37.8333	8.9312	3.6462	26.0000	49.0000
anaplastic	3	<b>68.6667</b>	6.0277	3.4801	63.0000	75.0000
total	51	35.0980	12.7988	1.7922	15.0000	75.0000

difference of microvascular density between astrocytoma grades II and III, suggesting that these tumours “use” preexisting vasculature. It was shown moreover, on autopsy material of glioblastomas, that mean vascular density was higher within the tumour tissue than in the surroundings; however, in approximately 50% of examined fields there was no difference in comparison with non-infiltrated white matter of the brain [28].

Some authors [9,13] postulate a correlation between patients’ age and microvascular density in astroglial tumours. According to these data it is worth stressing that in our series of ependymomas, a correlation between microvessel density of tumour tissue and age of the patients was not revealed.

Prognostic factors in ependymomas based on histologic criteria are not satisfactorily established yet, especially in children. Some authors [18,19] have proved the prognostic value of such factors as number of mitoses, endothelial hyperplasia, necrosis, high hypoxia score, intracranial location of the neoplasm and age of patients lower than 4 years. It was shown moreover that proliferation of endothelial cells and presence of necrotic foci are less important in predicting survival than in other types of glial tumours [19]. It is suggested also that, for patients with intracranial ependymoma, the mitotic and the MIB-1 indices are the only features that have independent prognostic significance [12].

Our investigations show that density of blood vessels in ependymomas grade 2 according WHO classification is lower than in anaplastic (WHO grade 3) subtype of the tumour. This is in agreement with the results given by Gilhuis and coworkers [6].

We are aware, however, that the number of anaplastic ependymomas in our material (3 instead of at least 5 cases) does not completely fulfil the rules of statistical analysis.

On the other hand we did not find significant differences of vascular density between subtypes within the group of ependymomas of WHO grade 2. It is worth stressing that density of vascular network is, even in cellular ependymomas (whose characteristic feature is high cellularity), similar to other morphological subtypes of this tumour group.

In summary, our observations demonstrate that angiogenesis by means of measure of microvessel density probably plays a role as one of the factors determining the biological behaviour of ependymal gliomas.

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