Peritumoral angiogenesis around primary and metastatic brain neoplasms. Morphometric analysis

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In an interface between brain tumour and surrounding tissue there occur simultaneously two very important phenomena. On the one hand there is a proliferation of peritumoral vessels penetrating into the neoplasm in which they make alike tumoral vessels. On the other hand, neoplastic cells penetrate from the tumour into the vicinity along peritumoral vessels. To determine the influence of the histological type of different brain tumours, their malignancy degree as well as location in the central nervous system on peritumoral vessels morphological appearance, the detailed morphometric analysis was carried out. The morphological examination and computerised morphometric analysis were conducted on 166 primary and metastatic CNS neoplasms taken during routine neurosurgical procedure. It turned out that the peritumoral angiogenesis depends predominantly on the malignancy of brain tumours. This angiogenesis may be modified by local environmental factors — it is more evident within the white matter than in the cerebral cortex. One of the important factors may be reactive peritumoral astrogliosis. There is no specific CNS region predisposed to the development of peritumoral angiogenesis.

key words: brain tumours, morphometric analysis, peritumoral angiogenesis

INTRODUCTION

It is generally accepted that angiogenesis plays a very important role in tumour growth. Angiogenesis means the sprouting of vessels from preexisting vasculature [19]. In an interface between brain tumour and surrounding tissue there occur simultaneously two very important phenomena. On the one hand there is a proliferation of peritumoral vessels penetrating into the neoplasm [6]. On the other hand, neoplastic cells penetrate from the tumour into the vicinity along peritumoral vessels [7]. These processes allow the neoplasm to invade new areas. Hence, the term angiogenesis is more adequate from the point of view of neoplasm pathogenesis than the term vasculogenesis — the appearance of new vessels during embryonic brain development [4, 8, 20].

The interest in the angiogenesis within the peritumoral tissue has been known for many years. Mossakowski [16], Kunicki and Stefanicka-Wiechowa [12] described vascular structures within and around gliomas. Sosiński [25] did the same with reference to different brain tumours. Kroh and Stoltenberg-Didinger [11] observed more intensive neovascularisation at the edge of experimentally induced spinal cord gliomas. Attention is mainly focused on a microcirculation around the brain neoplasm [27, 28]. Recently, it has been emphasised that peritumoral capillaries can be involved rather in the resolution of oedema than its formation [2]. The question whether the morphological and functional characteristics of peritumoral vessels depend entirely on factors released from neoplastic cells, irrespec-
tive of brain tumour location, or are regulated by peritumoral environment, including anatomical properties of normal tissues sticking to the neoplasm, is still open.

A morphometric study was done of the vascular neoformation and measurement of haemodynamics within the brain tumours [5, 14, 27]. Peritumoral capillaries were investigated morphometrically by Bertosi et al. [2] but the findings were based on analysis of several anaplastic astrocytomas. To determine the influence of the histological type of different brain tumours, their malignancy degree as well as location in the central nervous system (CNS) on peritumoral vessels morphological appearance, the detailed morphometric analysis was carried out.

**MATERIAL AND METHODS**

The morphological examination and computerised morphometric analysis were conducted on 166 CNS neoplasms taken during routine neurosurgical procedure. The tumours were divided into 6 groups: group I — 32 astrocytomas I and II, group II — 40 glioblastoma multiforme, group III — 23 ependymomas, group IV — 16 oligodendrogliomas, group V — 12 medulloblastomas and group VI — 43 metastases. The age of patients ranged from 17 to 78 years. In all cases, besides the neoplasm, a small amount of surrounding nervous tissue was also visible. There were no cases with intentional expansion of operated area. The specimens, including control group, were fixed in formalin for 18–22 hours. Paraffin-embedded specimens were stained with haematoxylin and eosin, and according to Weigert. The sections were also immunostained for GFAP and factor VIII-related antigens.

In order to analyse CNS vessels, they were first scanned from light microscope by video camera to frame grabber and then to computer imaging system of morphological analysis. The number of vessels, their calibre and vascular wall thickness were examined in white matter, cerebral and cerebellar cortex, and in grey matter of basal ganglia. The calibre and vascular wall thickness were determined using DISTANCE procedure. The DISTANCE means the length given in micrometres. This feature was measured clicking on the beginning and ending points of the vector (i.e. evaluated object). Because the measurement values should appear in real units (micrometres) a calibration was needed. Sampling density was 0.48 pixels/μm 100x magnification. This meant that the length of vector on the monitor screen had corresponded with the real thickness of the vascular wall and with vascular calibre in micrometres. 10 measurements of the DISTANCE were done within the thinnest region of the wall and the smallest calibre of each evaluated vessel. The number of vessels was evaluated within the scanned image, corresponding with real region of 0.0048 mm² in area.

The control measurements of the number of vessels, vascular wall thickness and calibre of vessels were taken within the appropriate regions of the brain. The brains were taken from 15 individuals, aged 38–59 years, who had died of non-neurological disorders: myocardial infarction, renal insufficiency, cirrhosis of liver, gastric or rectal cancer.

Specimens were scanned (3–6 images per case) under a light microscope (Axioskop, Zeiss, Germany) with a Plan-Neofluar 100x (1.3 oil) objective and a two-tube colour TV camera (JVC TK 1070E) illuminated by a 100W halogen bulb. The images were analysed with the KONTRON KS-100 v.2.0 imaging system (license No. 0100176).

Statistical differences between groups were checked using Wilcoxon, Kruskal-Wallis and U Mann-Whitney tests. The level of significance was taken as p < 0.01 and alpha = 0.05.

**RESULTS**

The mean number of vessels in the peritumoral areas of neoplasms located in the different CNS regions, with respect to white matter, to cortex and to grey matter of basal ganglia is presented in Figure 1. The mean number of vessels was significantly higher in the peritumoral white matter of all types of neoplasms compared to control group (p < 0.001). Especially this concerned glioblastoma multiforme and metastatic tumours. The mean number of cortical vessels was significantly higher in the peritumoral areas of cerebellar astrocytomas I and II, supratentorial ependymomas and metastases in comparison with the controls (p < 0.05).

Exclusively astrocytomas I and II located in the cerebellum and supratentorial ependymomas were surrounded by denser vascular network than tumours of the same histological type, located in other regions (p < 0.5). In the peritumoral regions of glioblastomas, oligodendrogliomas and metastatic tumours, the mean number of vessels, both in white matter and in cortex, was similar in all investigated CNS regions.

The mean calibre and wall thickness of vessels in peritumoral white matter of different CNS regions is presented in Figure 2. The vessels were significantly larger in peritumoral white matter of glioblastoma multiforme, ependymomas, medulloblastomas, and predominantly, metastases (p < 0.001). In glioblastomas and metastases the vessels were also significantly thicker (p < 0.01) in comparison with control group. The vascular parameters only slightly differed from each other in the same type of the neoplasm located in different CNS regions.
Within the peritumoral cortex, the mean vascular calibre and wall thickness were significantly higher in metastases ($p < 0.001$). In other tumours, with the exception of astrocytomas I and II, the morphometric parameters were slightly increased (Fig. 3).

In 4.6% of metastatic tumours vascular glomeruloid structures were observed in peritumoral areas (Fig. 4), in many vessels blood circulation seemed to be preserved (Fig. 5A). In the majority of vessels, endothelial cells demonstrated distinct immunoreaction against factor VIII re-
Control group

<table>
<thead>
<tr>
<th>Caliber of vessels in white matter</th>
<th>Frontal lobe</th>
<th>Parietal lobe</th>
<th>Temporal lobe</th>
<th>Basal ganglia</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>13.39</td>
<td>12.52</td>
<td>10.23</td>
<td>10.87</td>
<td>10.87</td>
</tr>
<tr>
<td>SD ±</td>
<td>6.15</td>
<td>5.21</td>
<td>6.26</td>
<td>4.62</td>
<td>4.62</td>
</tr>
<tr>
<td>In cortex</td>
<td>Mean = 7.43</td>
<td>Mean = 7.95</td>
<td>Mean = 6.31</td>
<td>Mean = 7.21</td>
<td>Mean = 7.31</td>
</tr>
<tr>
<td>SD ±</td>
<td>3.37</td>
<td>3.4</td>
<td>2.24</td>
<td>2.58</td>
<td>3.41</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vascular wall thickness in white matter</th>
<th>Frontal lobe</th>
<th>Parietal lobe</th>
<th>Temporal lobe</th>
<th>Basal ganglia</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2.56</td>
<td>2.89</td>
<td>2.58</td>
<td>2.89</td>
<td>2.89</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.67</td>
<td>0.91</td>
<td>0.64</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>In cortex</td>
<td>Mean = 1.64</td>
<td>Mean = 1.89</td>
<td>Mean = 1.84</td>
<td>Mean = 1.76</td>
<td>Mean = 2.16</td>
</tr>
<tr>
<td>SD ±</td>
<td>0.44</td>
<td>0.46</td>
<td>0.52</td>
<td>0.55</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Statistical significance between investigated tumors and control group was presented on page 5.

**Figure 2.** The mean caliber  and wall thickness  of vessels in peritumoral white matter in the neoplasms located in different CNS regions.
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Glioblastomas demonstrated vascular glomeruli in the peritumoral white matter (25% of cases) (Fig. 6), and less frequently, in cortex (8.3%). The morphometric parameters of the majority of vessels in peritumoral cerebral cortex of glioblastomas did not differ from control group, and the interface between the tumour and peritumoral cerebral cortex was sharp. Around the majority of tumours, especially of a malignant nature, surrounded by evident vascular network, distinct glial reaction was found (Figs. 4, 6, 7). In the vicinity of glioblastomas, cerebral cortex demonstrated minimal vascularisation and gliosis was not large in comparison with the peritumoral white matter and with the peritumoral cortex in the vicinity of metastases, where rich reactive gliosis appeared. In general, the peritumoral angiogenesis was the greater where the glial reaction was more evident.

**DISCUSSION**

Theoretically, a few environmental factors could determine the peritumoral angiogenesis in the CNS. On the one hand there are structural and functional differences between the vessels of grey matter, especially of cerebral and cerebellar cortex, and white matter [3, 13, 21, 29]. In general, within the grey formations the vascular network is denser while vessels are smaller. On the other hand, the histological type of tumour and its malignancy could influence the angiogenesis [26]. We have decided to analyse the relationship between the factors mentioned and intensity, as well as the morphological appearance of peritumoral angiogenesis.

Our investigations revealed that angiogenesis was significantly more intensive in the peritumoral white matter, in spite of lesser vascular network compared to grey matter. The number of vessels of white matter

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**Figure 3.** The mean caliber and wall thickness of vessels in peritumoral cerebral cortex in the neoplasms located in different CNS regions.
turned out to be significantly greater predominantly in the vicinity of malignant tumours. The evident increase in the number of cortical vessels was observed only in the neighbourhood of metastases. It is hard to explain the relatively small reactivity of vessels within the cortex adjacent to glioblastomas. Small neoformation of vessels was combined with poor penetration of tumour into the cortical structures. The invasion took place mainly by means of perineuronal satelitosis.

In the peritumoral white matter of malignant neoplasms and supratentorial ependymomas, the vascular network consisted of pathologically large vessels with thickened wall. In the peritumoral cortex, vessels were evidently changed only in metastases. In the cortex located in the vicinity of other tumours, vascular parameters were almost unchanged.

Based on the foregoing observations we may presume that the malignancy of the tumour plays the most important role in the appearance of vessels surrounding CNS neoplasms. White matter seems to be susceptible to the impact of the neighbourhood of neoplasm. The differences between normal angioarchitec-

Figure 4. Vascular glomeruloid structures and evident glia reaction in the peritumoral area of metastatic neoplasm, HE × 400.

Figure 5A, B. Distinct immunoreaction against factor VIII related antigen in the endothelial cells of thick-walled peritumoral vessels with preserved blood circulation, × 400.

Figure 6. Vascular glomeruli and reactive gliosis in the peritumoral white matter of glioblastoma multiforme, HE × 400.

Figure 7. The evident peritumoral glia reaction and vascular neoformation around oligodendrogioma, HE × 400.
ture connected with individual CNS regions seems not to play such a crucial part, because within the same CNS region the number of peritumoral vessels and their appearance differed, depending on the histological type of the neoplasm and its malignancy. The process of tissue shrinkage during a preparation of histological specimens was also not of great importance in our cases, because the preparation was similar in both investigated and control group. The vessels were usually pathological, irrespective of the age of patients. We have regarded this as a result of the influence of neoplasms. It seems that the impact of the patients' age on the morphological appearance of peritumoral vessels is minimal, and covered by effect of the neoplasm; furthermore, it was not age-related vascular pathology.

The reactive gliosis around the tumour may play a role in the development of peritumoral angiogenesis. Zhang and Olsson [30] believe that activated glial cells can produce biological factors, which may alter the structural and functional properties of the peritumoral tissues. In general, our cases have demonstrated that the greater the angiogenesis, the more intensive the peritumoral gliosis. Vascular endothelial growth factor (VEGF), a specific mitogen for endothelial cells [19, 24], is released by astroglial and microglial cells [18, 19]. This may explain the relationship between peritumoral angiogenesis and gliosis. In the cortex adjacent to glioblastomas, gliosis was relatively not large in comparison with the peritumoral white matter and with the peritumoral cortex in the vicinity of metastases, which demonstrated much more evident reactive gliosis. Schiffer et al. [23] have also reported that vessel density in the cortex infiltrated by malignant gliomas increased in some cases only.

Vascular glomeruloid structures are found within the glioblastomas and brain metastases, regardless of the histological type of the latter [10, 25]. We also observed numerous glomeruli structures around the glioblastomas and metastases in both white matter and in cortex. Schiffer et al. [23] postulated that vascular glomeruli develop when the cortex is completely invaded by the neoplasm. Meanwhile, we also found them within the peritumoral tissue insignificantly or not infiltrated by malignant glioma cells. They appeared even though the peritumoral network was not dense. Stewart et al. [26] also stressed that despite the development of glomeruli structures, the vessel density in peritumoral cortex of malignant gliomas remained very low. Morphological appearance of glomeruloid structures was similar in the peritumoral tissue and within the tumour. This phenomenon and the presence of vascular glomeruloid structures exclusively around the tumours, in which they are commonly present, also support the predominant influence of tumoral component on the peritumoral vascular angiogenesis. This influence seems to be direct and indirect. Stewart et al. [26] observed that peritumoral vessels not immediately invested by neoplastic cells demonstrated abnormalities similar to vessels involved by the tumour. They suggest that direct contact of vessels with the tumour is not required and that this inductive influence is exerted over a distance.

The above-mentioned data indicate that both environmental factors connected with the peritumoral areas and the tumour itself simultaneously stimulate neoinformation of vessels in the tissue adjacent to the neoplasms, however the impact of neoplasm seems to be much greater or even crucial. With some exceptions, we did not find evident differences in the morphological appearance of peritumoral vessels, which could depend on the location of the tumour in the specific CNS region. Arosarena et al. [1] pointed to the influence of peritumoral environment on endothelial differentiation within glial tumours, but Molnar et al. [15] did not observe host-site influence on angiogenesis. The evident peritumoral vascularisation around the malignant neoplasms might favour the spreading of tumours into the surrounding nervous tissue along the vessels in the tumour-brain interface [5]. The angiogenesis results in an exponential tumour growth [9, 17, 19].

CONCLUSIONS

1. The peritumoral angiogenesis depends predominantly on the malignancy of brain tumour.
2. The environmental effect on peritumoral angiogenesis is greater in white matter. One of the important factors may be reactive peritumoral astrogliosis.
3. There is no specific CNS region predisposed to the development of peritumoral angiogenesis.

REFERENCES


