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ORIGINAL ARTICLE

Tumor size and karyometric variables in brain astrocytoma

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Summary

Purpose: *To show any possible correlation of some karyometric variables with tumor size in patients with brain astrocytoma, in order to confirm karyometry as an objective histological method.*

Patients and methods: *The study included 63 patients of different ages and both genders with brain astrocytoma histologically confirmed on the surgically removed material. In all patients maximal tumor excision was done, and all were postoperatively treated according to different therapeutic protocols. Tumor size (preoperative CT scan) was correlated with the duration of survival and the values of some karyometric tumor variables: area, density, maximal axis, mean axis, minimal axis, circumference, roundness, integrated optical density (IOD) and number of nuclei.*

Results: *Patients were separated into 3 groups according to the average tumor diameter. There were 34 cases of medium-sized tumors, 12 of small and 17 of large-sized tumors, and their respective survival was 83, 97 and 24 weeks. Patients with large tumors had statistically shorter survival compared to those with medium and small tumors (log-rank test, $p=0.0122$). Seven out of 9 examined karyometric variables were significantly related ($p<0.05$) to the tumor size: area, maximal axis, mean axis, minimal axis, circumference, roundness and IOD.*

Conclusion: *Patients with larger tumors have shorter survival. The results of our morphometric analysis of the tumor cell nuclei, after correlation with CT findings, revealed that nuclear pleomorphism and larger nuclear size are associated with larger brain astrocytomas.*

Key words: brain astrocytoma, karyometry, survival, tumor size

Introduction

The clinical presentation of patients with brain astrocytoma can be associated with tumor site, patient personality and, above all, with biological tumor characteristics. Growth rate, infiltrative nature and perifocal edema are directly associated with biological tumor behavior, i.e. its malignant nature, in case of brain astrocytoma described by the current nomenclature adopted by the World Health Organization (WHO). However, the original WHO classification does not separate astrocytomas clearly in a prognostically valid way. Histological elements, disregarded in the WHO classification, are also important for survival. Classifications based on a limited number of histological characteristics are not satisfactory, so classification techniques are needed which must involve as many histological parameters as possible in order to be reliable [1].

Tumor cells have been intensely analysed with precise and quantitative morphological methods in order to accurately determine biological tumor features and their malignant potential and, therefore, patient survival. Karyometric analyses have been thus developed, which involve analysis of various nuclear parameters [2]. The number of parameters analysed, both direct and indirect, is around 50 [3]. These analyses can be performed in two (asterologic or planimetric method) or three dimensions (stereologic method). The method of karyometric analysis of volume-dependent nuclear

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volume based on the intercept length was introduced in 1988 [4] and has been a commonly performed method at various institutes of pathology.

Nuclear architecture determines the biochemistry of tumor cells, and is determined by the above mentioned karyometric variables. Biochemistry of an astrocytoma cell defines tumor growth rate i.e. its size, and is the consequence of a complex gene expression in each of the phases of tumor growth.

Our aim was to assess any possible correlation between the tumor size on preoperative brain CT scan with survival and a number of karyometric variables in patients with brain astrocytoma.

Patients and methods

At the Institute of Pathology in Nis, karyometric analysis most commonly involves the following variables:

1. Nuclear roundness: the value is 1 for a perfect circle, and the value diminishes as the roundness of the nucleus diminishes. Nuclear roundness is the quotient of the multiplied area 1 and 4π with the square of the circumference [3].
2. Nuclear area: it is defined as the number of pixels in the digitalized image of the nucleus.
3. Density of nuclear chromatin: it reflects the mitotic potential of malignant cells [3]. Preparing for mitosis, the genetic material is increased within the nucleus and density is increased.
4. Nuclear diameter (large, small and medium nuclear axis): raising the equivalent diameter to the 3rd degree and multiplying it by π/6 we can obtain the volume of the equivalent sphere, taken as nuclear volume.
5. Perimeter: it is directly proportional to nuclear diameter, i.e. nuclear roundness.
6. Number of nuclei: it is an important component in cellularity determination. Hypercellularity is the basic histopathological characteristic of a malignancy.
7. Integrated optical density (IOD): it is a novel morphometric parameter which can be determined only with computerized television systems [3]. It is an indirect variable based on the area which determines the nuclear content of DNA.

This study included 63 patients surgically treated from 16 April 1995 to 31 December 2001 at the Clinic of Neurosurgery in Nis. The patients were postoperatively monitored till 1 March 2003.

The data taken from patient medical records included dates of primary and subsequent operations for relapse, histological grade of the primary tumors and of the relapses and preoperative brain CT findings.

Patients were divided into 3 subgroups according to tumor diameter on preoperative brain CT. The first group consisted of small tumors (average diameter < 25 mm); medium-sized tumors (average diameter 25-50 mm) comprised the second group; and the third group was composed of large (>50 mm) lesions. Average tumor diameter ($D_{1\text{-}3\ max}$) was defined as the quotient of the sum of largest tumor dimensions (a, b, c) in all 3 planes divided by 3.

$$\overline{D}_{1\text{-}3\ max}=(a+b+c)/3$$

Maximal tumor excision was done in all patients and postoperative by they were treated according to different therapeutic protocols currently in use at the Clinic of Oncology, Clinical Centre Nis.

The date of death was recorded based on the available medical documentation from the Clinics of Neurosurgery and Oncology, but most commonly by contacting the relatives of the deceased.

The processing of the histological material included 63 brain astrocytomas and was performed at the Institute of Pathologic Anatomy, Clinical Centre Nis. Each sample was taken in the form of 4-8 slices. Fixation of the material was performed for 24 h in 10% formaldehyde. Slices 4 μm thick were hematoxylin & eosin (H&E)-stained for histological tumor diagnosis.

Morphometric analysis of the material involved digital processing of several photographs taken from different locations from each slide. The assessment was done on light microscope Olympus BX-50, Tokyo, Japan, with ×40 magnification, and 100 nuclei were measured for each patient. The microscope was connected to the analog video camera SONY DXC-107P in order to scan microscopic image. Video signal was further processed with appropriate software for digital image analysis.

Statistical analysis

For statistical analysis, Pearson's correlation and log-rank test were used. P-values lower than 0.05 were considered as statistically significant.

Results

The first group with 12 (19.05%) patients, with $D_{1\text{-}3\ max} < 25$ mm, had average survival of 83 weeks (range 34-133). The second group with 34 (53.97%) patients, with $50\ mm > D_{1\text{-}3\ max} > 25$ mm, had average survival of 97 weeks (range 57-137), and the third one, with 17 (26.98%) cases and $D_{1\text{-}3\ max} < 50$ mm, had average survival of 24 weeks (range 11-37; Table 1).

At the completion of statistical processing, we had 25.00% of censored patients with small lesions,

around 8.82% of those with medium-sized tumors, and around 5.88% of those with large disease (Table 2).

The survival difference was statistically significant (Figure 1) and was related to tumor size as shown in CT findings. Those with large disease had significantly shorter survival compared to those with medium-sized and small tumors (log-rank, p=0.0122).

Tumor size distribution in relation to histological grade was as follows (Table 3): both cases of grade I astrocytomas were small lesions (100%), grade II astrocytomas were small-sized in 6 cases (50%) and medium-sized in another 6 (50%); anaplastic astrocytomas (AA/grade III) were small-sized in 1 case (12.5%), medium in 4 cases (50%) and large-sized in 3 cases (37.5%); glioblastoma multiforme (GBM/grade IV) were small-sized in 3 cases (7.3%), medium-sized in 24 cases (58.5%), and large-sized in 14 cases (34.2%).

The results of statistical analysis demonstrated that 7 out of 9 investigated karyometric variables were significantly associated with medium tumor diameter values. There was statistically significant correlation between the surface, big, small and medium axes, circumference, roundness, IOD, and tumor size ($p<0.05$; Table 4).

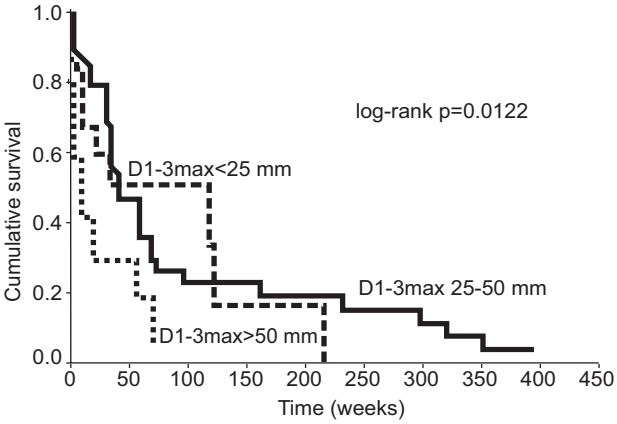

Figure 1. Survival of the patients surgically treated for brain astrocytoma in relation with the preoperative CT size of the tumors.

Table 1. Survival of patients surgically treated for brain astrocytoma in relation with preoperative CT tumor size

$D_{1\text{-}3\ max\ (mm)}$	*Patients n*	*Average survival (weeks)*	*95% CI*
< 25	12	83.42	33.61-133.22
25-50	34	96.64	56.52-136.75
> 50*	17	23.76	10.76-36.77

*p=0.0122, 95% CI: 95% confidence interval

Discussion

Tumor development is the consequence of transformation of astrocytic cells into tumor cells via complex gene changes [5].

Brain tumor growth requires adherence of tumor cells to adjacent structures, degradation of the surrounding matrix, migration of tumor cells, angiogenesis and tumor cells proliferation [6].

Tumor angiogenesis induces activation of adjacent endothelial cells which dissolve the surrounding extracellular matrix, migrate towards the tumor, proliferate and form new vascular network, supplying the tumor with nutrients and oxygen and removing metabolic products.

Degradation of extracellular matrix induces depolymerization of hyaluronic acid, which facilitates tumor invasion, and thus induced oligosaccharides lead to further migration and proliferation of endothelial cells [7].

Brain astrocytoma angiogenesis is induced by

Table 2. Relationship of the deceased and censored patients surgically treated for brain astrocytoma in relation with preoperative CT tumor size

$D_{1\text{-}3\ max\ (mm)}$	*Patients n*	*Deceased n*	*Censored n*	*% Censored*
< 25	12	9	3	25.00
25-50	34	31	3	8.82
> 50	17	16	1	5.88
Total	63	56	7	11.11

Table 3. Distribution of medium tumor diameter in relation with histological tumor grade

Grade	$D_{1\text{-}3\ max}$ *<25 mm n (%)*	$D_{1\text{-}3\ max}$ *25-50 mm n (%)*	$D_{1\text{-}3\ max}$ *>50 mm n (%)*	*Total n (%)*
I	2 (100)	–	–	2 (100)
II	6 (50)	6 (50)	–	12 (100)
III	1 (12.5)	4 (50)	3 (37.5)	8 (100)
IV	3 (7.3)	24 (58.5)	14 (34.2)	41 (100)
Total	12 (19.0)	34 (27)	17 (54.0)	63 (100)

Table 4. Coefficients of correlation (R) between tumor size and karyometric variables

Karyometric variables	*Area*	*Density*	*Max axis*	*Min axis*	*Mean axis*	*Circumference*	*Roundness*	*IOD*	*Number of nuclei*
$D_{1\text{-}3\ max}$	**0.44**	0.23	**0.50**	**0.42**	**0.47**	**0.50**	**0.27**	**0.59**	0.00

Bold numbers denote statistical significance ($p<0.05$) with the severity of peritumoral edema.
IOD: integrated optical density

gene expression coding for angiogenetic growth factors in tumor cells, such as vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF). Such an expression is the consequence of disturbed function of tumor suppressor gene p53 and VHL [8]. The principles of interaction of VEGF and receptors VEGF1 and VEGF2 are the basis of paracrine control of angiogenesis and endothelial proliferation. This paracrine control is normally switched on in an embryonic brain, switched off in an adult brain and re-activated during tumor progression [9]. Increased levels of tyrosine kinase receptors VEGF1 and VEGF2 were found in the endothelium of grade II, III and IV astrocytomas, as well as in the endothelium of tissues immediately adjacent to the tumor [10].

In all brain astrocytomas there exists a zone of infiltration. In most pilocytic astrocytomas this is a narrow, regular infiltration zone around the tumor, while in high grade astrocytomas this is a wide zone around the tumor. In high grade astrocytomas this infiltration may involve more than one lobe and sometimes invades the contralateral brain hemisphere, where spikes of tumor cells along the white matter pathways may be identified microscopically [11]. These spikes are not visible preoperatively on CT and MRI scans, intraoperatively macroscopically, or postoperatively on traditional H&E staining. Only most up-to-date immunohistochemical staining (Ki-67, MIB-1) and biochemical methods (increased VEGF far away from the tumor involved area of the brain) can shed light on the three-dimensional infiltration of high grade astrocytomas [6,12].

However, in routine neurosurgical practice, the field of interest during resection is the tumor volume visualized on CT or MRI, i.e. a narrow zone up to 1.5 cm away from the tumor, also known as the zone of "pseudo-cleavage", i.e. the edema zone through which tumor resection is most commonly done.

Astrocytoma volume is directly proportional to tumor growth rate, which in GBM is 0.0013 cm^3/day, doubling the tumor volume every 60 days. After contrast medium administration brain CT shows the regions of the brain with higher concentration of tumor cells (e.g. 8000 cells per mm^3), while portions peripheral to these regions with tumor cell concentration reduced up to 80 times (e.g. 100 cells per mm^3) represent healthy brain tissue, i.e. the tissue involved with edema [13]. This wide diffusion of tumor cells into a "healthy" tissue is the essence of limited success in the surgical treatment of high grade astrocytomas.

The significance of tumor volume for the survival of astrocytoma patients was clearly identified by Swanson et al. [13], who defined the survival as an interval needed for volume A tumor (on the first CT) to reach volume B (tumor volume on autopsy). In a mathematical model which included tumor growth rate and diffuse coefficient, the survival of GBM patients was 158 days for tumors originating in the white matter and 256 days for tumors originating in the gray matter.

In addition to the Swanson et al. study, some other authors also studied the association of tumor volume and survival. Wood et al. [14] demonstrated in a large group of 510 patients with malignant gliomas that tumor volume is of great prognostic significance independent of other known prognostic variables. Other authors, too, found statistically significant correlation of preoperative volume of astrocytomas on contrast-enhanced CT and survival [15,16]. Scerrati and colleagues [17] in their paper described 3 groups of low grade astrocytomas, where maximal tumor diameters on preoperative CT were up to 3 cm, 3-5 cm, and over 5 cm. Patients with tumors >5 cm had significantly shorter survival compared with the other two groups ($p=0.0193$). In the study of Wurschmidt et al. [18] the group of patients with largest astrocytoma diameter > 5 cm had also statistically shorter survival ($p=0.04$). In our study too, the group of patients with preoperative tumor diameters >5 cm lived significantly shorter than other two groups (with tumors <2.5 cm and 2.5-5.0 cm; $p=0.0122$).

The distribution of average tumor diameter in relation with histological grade in our study roughly demonstrated that with rising astrocytoma grade the average tumor diameter rises as well.

The results of our morphometric analyses demonstrated that most of the studied karyometric variables correlated with tumor volume.

Total absence of correlation was noted only for the number of nuclei in the visual field. This is understandable since it is known that for any brain tumor the increased extracellular matrix (the main component of which is water) is a most important factor for tumor volume and not its cellularity. In the study of Bruehlmeier et al. [19], after appropriate measurement with 76 Br-bromide and PET, the authors concluded that the extracellular matrix is more abundant within a tumor compared to the peritumoral space involved with edema or distant brain portions uninvolved with edema.

From the results of karyometric analyses taken as a whole, it can be concluded that tumor volume is directly proportional to the size of nuclei (of more irregular shape). Larger nuclei suggest intense synthetic processes, while irregular shape suggests increased nuclear membrane surface and consequent increase of the number of nuclear pores. Among other things, the transport through these pores of informational RNA to the ribosomes takes place where proteins necessary for tumor growth and development are synthesized.

Among these substances included are proteolytic enzymes necessary for tumor invasion of healthy brain tissue, as consequence of degradation of the extracellular matrix elements (laminin, type IV collagen, fibronectin and vitronectin, etc.). Moreover, the process of moving of astrocytic cells across the elements of extracellular matrix is associated with changes in numerous receptors, including homotypic cellular (receptors integrins and a tumor suppressor molecule called DCC which is deleted in colon cancer), then hyaluronate receptors (CD44, RHAMM, i BEHAB) and matrix protein receptors important for tumor development (SPARC). The nuclei of tumorous astrocytes are the site of genetic material for the transcription of information necessary for the synthesis of growth factors and their receptors, such as PDGF-AA, PDGF-BB, PDGFR α, PDRGF β, TGFα, EGFR, i bFGF [11]. Karyometric measurements can provide an insight into the intensity of autocrine regulation of tumor growth.

Conclusion

Patients with larger brain astrocytoma have shorter survival. Correlating karyometric variables with brain CT findings, it was found that larger nuclei, higher degrees of nuclear wrinkling and increased IOD are associated with larger tumors and shorter survival.

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