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ORIGINAL ARTICLE

Peritumoral edema and karyometric variables in astrocytoma of the brain

A. Kostic[1], D. Mihailovic[2], S. Veselinovic[3], G. Tasic[4], D. Radulovic[4], I. Stefanovic[1]

[1]Clinic of Neurosurgery, [2]Institute of Pathologic Anatomy, [3]Clinic of Oncology, [4]Institute of Neurosurgery, Clinical Centre Nis, Nis, Serbia

Summary

Purpose: *The aim of this study was to evaluate karyometry as a quantitative and objective histological method by showing correlation of some karyometric variables with the severity of peritumoral edema in patients with brain astrocytoma.*

Patients and methods: *63 patients of different ages and both genders were enrolled. The patients were diagnosed with astrocytoma of the brain, histologically confirmed on the surgically removed material. Maximal tumor excision was performed in all patients, who were postoperatively treated according to current oncologic therapeutic protocols. The intensity of perifocal edema (preoperative CT scan) was correlated to the duration of survival and the values of 9 karyometric tumor variables: area, density, maximal axis, mean axis, circumference, roundness, integrated optical density and number of nuclei.*

Results: *There were 17 cases with small perifocal edema, 19 with medium-sized and 27 with large perifocal edema, and their respective survival was around 149, 62 and 48 weeks. Those with small edema had statistically significant prolonged survival compared to those with medium and large perifocal edema (log-rank test, $p=0.045$). Six out of 9 karyometric variables examined were significantly related ($p<0.05$) to the intensity of peritumoral edema: long and mean axis, circumference, roundness, integrated optical density and number of nuclei.*

Conclusion: *Patients with larger peritumoral edema have shorter survival. Correlation of karyometric variables with CT findings revealed that higher degrees of tumor cellularity and nuclear wrinkling with increased integrated optical density is associated with larger peritumoral edema.*

Key words: brain astrocytoma, karyometry, peritumoral edema, survival

Introduction

Histological analysis has an element of subjectivity, especially in the estimation of the malignant potential of precancerous lesions and histological and nuclear grade of certain malignant lesions. This is the reason why the objective morphometric methods, especially caryometry, gain in significance. A precise and reliable diagnosis of any tumor has not only academic and ethical significance, but also great economic value, evident mainly in early diagnosis and timely, relatively cheap treatment, and avoidance of over-diagnosis, leading to reduction of redundant and expensive therapeutic procedures.

In order to analyze the shape and size of tumor cell nuclei, various approaches have been applied by several investigators [1,2] and the number of analyzed parameters, direct and indirect, is about 50 [3]. We most commonly determine the size of nuclei and nucleoli (karyometric analysis) in two dimensions (asterologic and planimetry methods) or as a volume (stereologic method). During the last years, at the Institute of Pathology in Nis, karyometric analysis of the volume-dependent nuclear volume is being performed, based on the intercept length. The method was introduced by Gundersen et al. in 1988 [2], and at the Institute of Pathology by Kutlesic and Mihailovic in 1990, with over 800 karyometric analyses performed through to 1997 [3].

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Author and address for correspondence:

Aleksandar Kostic, MD
Ul.Branka Krsmanovica 15/16
18000 Nis
Serbia
Tel: +381 18 571618
Fax: +381 18 512048
E-mail: aleko@bankerinter.net

Commonly examined karyometric variables are:

Shape of tumor cell nuclei

Determination of the nuclear roundness can predict the biologic course of various carcinomas with variable reliability. The roundness is "1" for full circle, and with greater deviation from full circle the roundness value is lower. The nuclear roundness is a quotient of the multiplied area l and 4π with the square of the circumference [3].

Nuclear area

Nuclear area is defined as the number of pixels on the digitalized nuclear image. Nuclear surface, together with nuclear roundness, quantitatively defines the level of nuclear pleomorphism (nuclear atypia) [4], and together with the number of nuclei in the visual field it defines cellularity. We then speak of nuclear area percentage (NAP) or nuclear area fraction (NAF), and their values are expressed in the percentages of the visual field. Since the nuclear area represents a fundamental element of cellularity as well as pleomorphism, it is a karyometric parameter with which we may expect association with higher degrees of malignancy of a tumor, frequently connected with survival.

Density of chromatin within the nucleus

Preparation of a cell for division leads to an internuclear increase of genetic material and increase of density. Density represents the mitotic potential of a malignant cell [3].

Nuclear diameter

This is a karyometric parameter related to the major, minor and mean nuclear axis – quantitative indicators of nuclear size. Larger nuclei characterize the S-phase, when doubling of DNA takes place.

Raising the equivalent of nuclear surface diameter to the cube and multiplying with π/6, the volume of the equivalent sphere, taken as the nuclear volume, is obtained.

Nuclear perimeter

This is a karyometric parameter directly correlated with nuclear diameter i.e. with nuclear roundness.

Number of nuclei

This parameter is an essential component to establish cellularity, the basic histopathologic feature of a malignancy.

Integrated optical density

This is an indirect variable based on the area, determining in fact the nuclear DNA content. It is a new morphometric parameter which can be determined solely aided by digital video systems [3].

Karyometric measurements help obtain quantitative indicators of nuclear architecture which in turn determine the biochemistry of a tumor cell, and biochemistry dictates the elements of a clinical picture, where peritumoral edema has a significant role. Peritumoral edema is an integral part of tumor mass-effect and it is a direct cause of the final and fatal tumor effect.

The aim of our study was to investigate the possible correlation between the severity of peritumoral edema, as assessed by karyometric variables and preoperative CT of the brain, and survival of surgically treated patients with brain astrocytoma.

Patients and methods

The study enrolled 63 patients surgically managed at the Clinic of Neurosurgery in Nis from May 1995 to December 2001. Their postoperative status was monitored until March 2003.

The data collected from the patients' records included date of first operation and operation at relapse, histological grades of the primary tumor and relapse, and contrast-enhanced brain CT findings before treatment initiation.

The studied patients were divided into 3 groups according to the severity of the peritumoral edema visualized on preoperative brain CT. Large edema in this study was the one with maximal diameter > 2 times longer than maximal tumor diameter; intermediate edema was the one with maximal diameter < 2 and > 1.5 times longer than maximal tumor diameter; and small edema was the one with maximal diameter <1.5 times longer than maximal tumor diameter.

In all of the cases maximal surgical tumor reduction was performed, together with postoperative treatment in accordance with current oncologic therapeutic protocols.

Information on postoperative treatment was obtained from the patients' records at the Clinic of Oncology, Clinical Centre Nis.

The date of death was noted based on the available medical documentation at the Clinic of Neurosurgery and Clinic of Oncology, but most commonly based on the contact with the patients' relatives.

Histological material analysis involved the samples of 63 brain astrocytomas and took place at the Institute of Pathologic Anatomy, Clinical Centre Nis. Each sample was processed in the form of 4-8 sections. Fixation of surgical material was performed during 24 h with 10% formaldehyde solution. Sections 4 μm thick were stained with hematoxylin & eosin.

Morphometric analysis of the histological material involved digital processing of several shots at a time, taken from different locations for each section plate. The investigation was done with light microscope Olympus BX-50 (Tokyo, Japan) with magnification x40 and in each patient 100 nuclei were measured. The microscope was connected to an analogic Sony DXC-107P video camera to scan microscopic images. The video signal was further processed with the software for digital image processing.

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 27 (42.9%) patients, with large peritumoral edema, had median survival of 46 weeks (range 23-73). The second group with 19 (30.2%) patients, with medium-sized edema, survived for a median of 62 weeks (range 23-101), and the third, with 17 (28.9%) patients, with small peritumoral edema, survived for a median of 149 weeks (range 76-221) (Table 1).

At the end of statistical processing, we had around 7.41% of the censored patients from the large edema group, around 5.26% of the censored from the medium-sized edema group, and 23.53% of those with small peritumoral edema (Table 2).

Table 1. Survival (in weeks) of the patients surgically treated for brain astrocytoma according to the degree of peritumoral edema

Edema	*Number of patients*	*Average survival (weeks)*	*95 % CI*[§]
Large	27	47.96	22.69-73.23
Medium	19	62.00	22.76-101.24
Small*	17	148.68	76.04-221.33

*p=0.045, [§]95% confidence interval

Table 2. Relationship of the censored and deceased patients surgically treated for brain astrocytoma according to the degree of peritumoral edema

Edema	*Patients, n*	*Deceased*	*Censored*	*% censored*
Large	27	25	2	7.41
Medium	19	18	1	5.26
Small	17	13	4	23.53
Total	63	56	7	11.11

The difference in survival was statistically significant (Figure 1) in relation to peritumoral edema in CT findings. The group of patients with small edema lived significantly longer compared to the groups with medium or large edema (log-rank, p=0.045).

The distribution of severity of peritumoral edema according to histological grade was as follows (Table 3): small peritumoral edema occurred in both cases (100%) of pilocytic astrocytoma (PA), in 9 cases (75%) of grade II astrocytoma, in one case (12.5%) of anaplastic astrocytoma (AA) and in 5 cases (12.2%) of glioblastoma multiforme (GBM). Medium-sized peritumoral edema was found in 2 cases (16.7%) of grade II astrocytoma, in 3 cases (37.5%) of AA and in 14 cases (34.1%) of GBM. Large peritumoral edema was found in 1 case (8.3%) of grade II astrocytoma, in 4 cases (50%) of AA and in 22 cases (53.7%) of GBM.

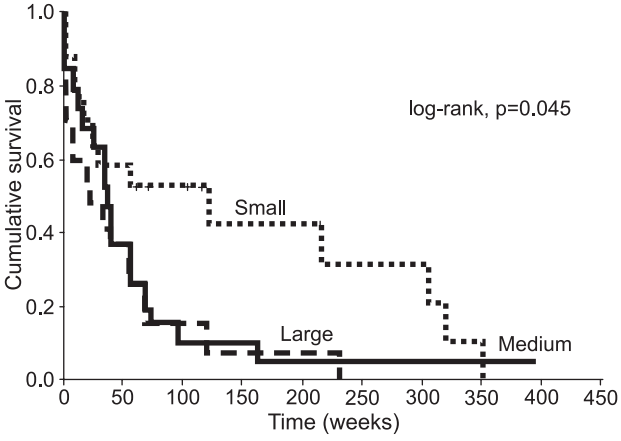

Figure 1. Survival of patients treated for brain astrocytoma in relation to preoperative CT assessment of peritumoral edema.

Table 3. The distribution of severity of peritumoral edema, according to histological grade of the tumor

Grade	*Large edema n (%)*	*Medium edema n (%)*	*Small edema n (%)*	*Total n (%)*
I	–	–	2 (100)	2 (100)
II	1 (8.3)	2 (16.7)	9 (75)	12 (100)
III	4 (50)	3 (37.5)	1 (12.5)	8 (100)
IV	22 (53.7)	14 (34.1)	5 (12.2)	41 (100)
Total	27 (42.9)	19 (31.1)	17 (26)	63 (100)

The results of statistical processing demonstrated that 6 out of 9 tested karyometric variables were significantly related to the severity of peritumoral edema. These were long and mean axes, circumference, roundness, integrated optical density and number of nuclei (Table 4). The correlations were significant at the level of $p<0.05$.

Discussion

The pathophysiology of brain edema is a multifactorial phenomenon. The basis of tumor-related edema is of vasogenic origin, where firm endothelial bonds making the blood-brain barrier are broken. In this type of edema, serum proteins are "squeezed" through the widened "tight bonds" (zonulae occludentes) from the blood vessels, so the extracellular space is expanded. Thus, the distorted blood-brain barrier makes the application of contrast media justified in various diagnostic methods for visualization of brain tumors. Generally, perifocal vasogenic edema does not affect cellular function, but due to increased tissue pressure, it does affect the regular brain blood flow. High local pressure increases the resistance to venous drainage and leads to congestion and venous dilatation. The concentrations of occludine [5] and claudine [6], which are the building components of the firm endothelial bonds, are lowered in the microcirculation of high-grade brain astrocytomas. This enables exudation of intravascular proteins into the extracellular space of the brain parenchyma. Once reaching the extracellular space, the edema quickly spreads along the white matter pathways. Brain tumors release vasoactive substances and components which destruct the endothelium (arachidonic acid, excitatory neurotransmitters, eicosanides, bradychinines, histamines and free radicals). In highly malignant astrocytomas an increase of arachidonic acid (and its metabolites) is found, even 40-fold higher compared to normal brain tissue. Arachidonic acid is the most important precursor of eicosanides in humans. Eicosanides, such as tromboxan (TX) A2 and leukotriene (LT) C4, are known to directly correlate with the severity of peritumoral edema as seen on CT scans [7].

Some new studies point out the significance of overexpression of aquaporine 1 [8,9] and 4 [3,8], the proteins of the water channels on the microvasculature in brain astrocytomas, which is essential for an alternative way of brain edema generation. The mechanism involves increased flow of water through the channels, with blood-brain barrier preserved, preventing transudation of plasma macromolecules [10].

In the region of peritumoral edema, tumor proteolytic agents induce tissue degradation, which is a precondition for tumor cell invasion [11]. On the other hand, intercapillary distance is increased in the region of peritumoral edema, which, if double than normal, significantly reduces pO2 and leads to hypoxia [12]. Due to hypoxia, the production of energy in the tissues is based upon the transfer to glycolysis, where the glycolytic pathway leads to lactic acid formation, with production of only 2 ATP molecules, in contrast to 32 molecules obtained by oxidative phosphorylation in a normal brain. Such depleted energy stores lead to inhibition of ion transport systems and accumulation of Na^+ and Cl^- ions in the cells, which induces absorption of water in the cell, its enlargement, and finally death.

A statistically significant correlation of peritumoral edema severity and survival in brain astrocytoma was also found in the studies by Levin et al. [13] and Yamada et al. [14].

In the study by Steinhoff et al. [15], perifocal edema was found in 88% of the cases of GBM with mainly medium-sized and large edemas, while in the study by Fernandez et al. [16] almost all investigated PA (80 in total), cerebral or cerebellar, had perifocal edema. In our study all GBM cases had an accompanying perifocal edema, with 87.8% having medium-sized or large edemas, and in both PA cases perifocal edema was scarce.

The results of our karyometric tests indicate that large, synthetically active tumor nuclei, with increased area and irregular shape are the location of intense iRNA transcription to synthesize the substances influencing the concentration of occludin, claudin 3, aqua-

Table 4. Coefficients of correlation (R) between the severity of peritumoral edema and karyometric variables

	Karyometric variables								
	Area	*Density*	*Max axis*	*Min axis*	*Mean axis*	*Circumference*	*Roundness*	*IOD**	*Number of nuclei*
Severity of peritumoral edema	0.24	0.03	**0.35**	0.18	**0.27**	**0.31**	**0.36**	**0.30**	**0.33**

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

porin 1 and 4, and other substances important for the development of peritumoral edema.

Karyometric measurements may produce quantitative elements of the biologic potential of a tumor. Altered measurements (surface and diameter) of shape, circumference, density, integrated optical density and number of nuclei in glial cells, are the consequence of altered normal or activation of new gene expressions and consequent biochemical processes. Nuclear architecture reflects the metabolic activity of tumor cells, and biochemistry of the cell is the immediate cause of terminal, fatal effects of brain tumors and target of the current oncologic therapeutic protocols.

Conclusion

Patients with larger peritumoral edema have shorter survival. Correlating karyometric variables with brain CT findings, it was found that higher degrees of tumor cellularity and nuclear wrinkling with increased integrated optical density are associated with larger peritumoral edema and shorter survival.

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