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The role of choline (Cho) in the diagnostics and differentiation of brain tumours with HMRS technique

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Summary

Background: The aim of the research was a comprehensive analysis of Cho concentration and Cho/Cr, NAA/Cho, NAA/Cho+Cr ratios for the purposes of the diagnostics and differentiation of brain tumours (the type of the pathological lesion in patients with brain tumours) with the use of HMRS technique.

Material/Methods: The HMRS examinations were performed with the use of the MRI Signa Excite 1.5T system, in PRESS technique (TR=1500 ms, TE=35 ms) and involved 100 patients with brain tumours (age range: 18 to 81 yrs, mean age 50.61). Spectra were taken from three different locations: tumour centre, the tumour edge and contralateral unchanged cerebral tissue.

All patients underwent surgery followed by histopathological analysis, on the basis of which two groups were separated (benign tumours, malignant tumours –50 cases each).

Additionally, 30 healthy volunteers in the age of 20 to 79 years (mean age 40.8) were examined.

Results: The comparison of the examined patients with the control group revealed significantly higher Cho concentrations in patients with brain tumours.

The analysis of Cho concentration was also performed with consideration of the age factor (under and over 60 years of age). Significantly lower mean Cho concentrations were discovered in a group of patients under 60 years of age.

The analysis of Cho concentrations and Cho/Cr ratios reveled statistical significance for two factors: voxel location factor and the type of the pathological lesion. The average of Cho concentration and Cho/Cr ratios were higher in the group of patients with malignant tumours. The highest Cho concentrations and Cho/Cr ratios were observed in the tumour centre.

The relative NAA/Cho and NAA/Cho+Cr ratios were statistically significant when taking into consideration the voxel location factor only.

The results received from contralateral normal cerebral tissue (the internal model) were compared with control group (the external model). Mean values of Cho concentration were slightly (insignificantly) increased in the internal model, especially in the group of patients with malignant brain tumour.

Conclusions: Cho is an important metabolite, useful in the diagnostics of brain tumours and differentiation of the type of brain tumour.

Key words: proton magnetic resonance spectroscopy (1-HMRS) • choline • brain • tumours

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Background

Population studies carried out in the last decades of the XXth century reveal an increased incidence of CNS tumours.

In Poland, there are 10–12 CNS tumours diagnosed per 100 000 persons-years, which constitutes 2% of all neoplasms.

Over 50% of the primary intracranial neoplasms are of neuroepithelial origin. These cancers are much more frequent

in men. Other most frequent type of cancers are the meningiomas (about 25% of all primary tumours), 2 times more frequent in women. Acoustic neurinomas constitute 5–8%, metastatic cancers from other organs 5–10% (25% of all cancers metastased to the brain) and adenomas of the pituitary gland – benign tumours 8–10% of all primary tumours. The incidence of malignant gliomas and meningiomas increases with age [1].

The clinical picture of the brain tumours is very heterogenous and depends on the type of tumour and its location. The basic methods applied in tumour diagnostics as well as intra- and post-therapeutical follow-up examinations are currently: the computed tomography and magnetic resonance tomography [2].

These techniques are useful in lesion detection, in determining its location, size, and vascularisation. However, some cases are connected with a problem of differentiation between the neoplastic lesions and the focal lesions of some other type, e.g. ischaemic focus or focus of post-radiation necrosis. In such instances, the method supplementing the diagnostics imaging is so called MRS, i.e. magnetic resonance spectroscopy, because it is a well-known fact that every pathology causes specific metabolic changes before appearing on imaging studies [2].

MRS, magnetic resonance spectroscopy, as a completely non-invasive method, allows for the evaluation of the chemical composition of a given tissue sample VOI (volume of interest).

The analysis involves the identification of the spectral lines appearing in pathologies, the evaluation of concentrations of selected metabolites and calculation of their mutual relative proportions. And thus, the MRS examination *in vivo* allows for the detection of pathologies in a very early stage, facilitates the differential diagnosis of the morphological lesions, enables the evaluation of the course of the pathological process, and finally, enables the monitoring of the treatment process. Due to the fact that this diagnostic method is not invasive and allows for the evaluation of the metabolic state of the whole system, the MRS examination performed *in vivo* is called a 'non-invasive biochemical biopsy' [3].

In the MRS technique, the key role is played by proton MR spectroscopy (^{1}HMRS) due to the highest concentration of hydrogen in tissues and the strongest signal induced by protons in the chosen area [2].

Spectroscopy is currently performed routinely in the evaluation of the type of lesion, malignancy grade of the brain tumour, its response to the treatment course, and detection of possible recurrences [4–8].

At the present level of technological advancement, it is possible to identify only a few metabolites in the ^{1}HMRS spectrum of the brain. The source of the strongest resonance signals are: N-acetylaspartate (NAA), choline (cho) and creatinine (Cr), together with phosphocreatinine (PCr). Other metabolites, the evaluation of which may be significant in brain pathologies are: lipids (Lip), lactic acid

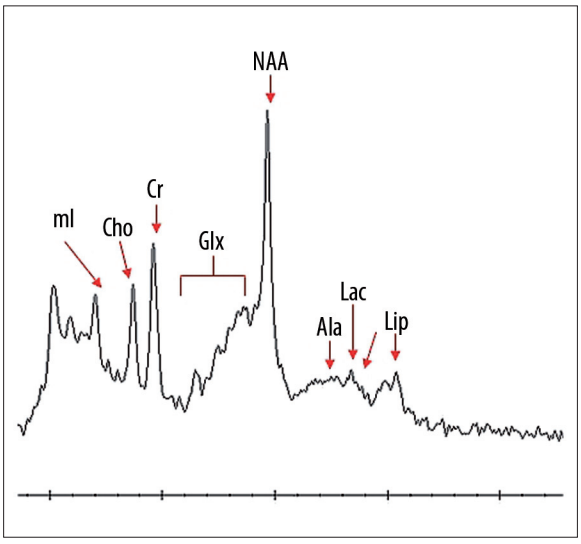

Figure 1. HMRS spectrum – healthy individual.

(Lac), glutamine and glutamic acid (Glx), and alanine (Ala) – Figure 1 [2].

One of the most important metabolites creating the HMRS spectrum is choline, an organic chemical compound – (2-Hydroxyethyl)trimethylammonium hydroxide. It constitutes some of the phospholipids (an important compound of the cell membrane), lecithine and sphingomyelin. Sphingomyelin is a component of the *myelin* sheaths of the nerves, and lecithine protects them.

In the metabolic processes, choline is the precursor of acetylcholine (neuromediator synthesised in cholinergic neurons) and phosphatidylcholine, and a product of sphingomyelin disintegration. Choline can be found in human body in a free form, or as a part of water-soluble and emulsified compounds (plasmalogen, glycerophosphocholine, phosphorylcholine, cytidinediphosphohcholine, acetylcholine), as well as compounds soluble in fats (phosphatidylcholine and sphingomyelin)

In the HMRS spectrum of the brain, choline is a source of a strong signal and is visible as a single peak – 3.21 in the ppm scale – for which responsible are the protons of the trimethylamonines N-$(CH_3)_3$ present in choline-containing compounds, in cytozole, including glicerophosphocholine, phosphocholine and choline. The influence of individual components on the level of the peak (its area) is currently not well known, and thus the change of its value may follow both from the change in mutual proportions of the above mentioned compounds, as well as from changes in their total concentration (combined). Phosphatidylcholine and sphingomyelin, i.e. compounds including choline and present in the cell membrane, do not have (most probably) any influence on peak generation, due to a limited mobility of these particles within the cell membrane.

To put it simply, choline could be described as an indicator of myeline disintegration and cell proliferation. This is reflected by the metabolic cycle of the active cells and cell membrane metabolism [9,10].

Table 1. The summary of analysed benign and malignant tumours after tumour resection and histopathological analysis.

Benign tumours n=50	Glioma, n=25	Astrocytoma diffusum, n= 3
		Astrocytoma fibrillare diffusum II, n=1
		Astrocytoma pilocyticum I, n= 2
		Oligodendroglioma II, n= 2
	Meningioma (I and II), n=19	
	Schwannoma, n= 6	
Malignant tumours n=50	Glioma (2 II/III, 12 III, 5 III/IV, 17 IV), n=36	Astrocytoma II/III, n=2
		Astrocytoma malignum (12 III grade, 3 III/IV), n=15
		Astrocytoma malignum/glioblastoma multiformae III/IV, n=2
		Glioblastoma multiformae IV, n=16
		Gliosarcoma IV, n=1
	Cancer, n=9	
	Adenocarcinoma, n=4	
	Chordoma, n=1	

Pathological lesions resulting in the change of the level and area of the choline peak can be observed as relatively large fluctuations of the height and half-width of the peak.

The analysis of choline concentration (area below the peak) is used for further calculations, i.e. proportions of concentrations. The most frequently analysed ratios are: Cho/Cr and Cho/NAA.

According to many authors, the role of Cho concentration assessment is crucial in the differentiation of the CNS focal lesions, especially when evaluating their malignancy and the degree of malignancy. Most of the papers on malignant brain tumours reveal a substantial increase in Cho concentration [4–8] but in some of the articles it was pointed out that this increase may be insignificant or even reduced [11,12]. That is why, the aim of this work was to analyse, on the basis of own material, the tendencies connected with Cho peak revealed in HMRS spectrum within brain tumours, and to differentiate the type of the lesion and to define the best method of voxel localisation as well as the method of analysis of data obtained during acquisition.

Material and Methods

Patients and control group

The MR and HMRS examinations were performed in the group of 100 patients in the age ranging from 17 to 81 years (50.61±16.34 – mean ± standard deviation). The study group included:

- 52 women in the age of 17–78 (49.88±17.24 – mean ± standard deviation),
- 48 men in the age of 17–81 years (51.40±15.45 – mean ± standard deviation).

All patients were diagnosed with tumour in a previously performed MRI. In order to define the type of lesion before the elective surgery, the whole group was subjected to the HMRS examination. On the basis of the histopathological examination after the surgical procedure, we classified the patients into two groups: with benign and malignant tumours.

The group with benign tumours involved 50 patients in the age of 17–77 years (46.04±17.65 – mean ± standard deviation). The group with malignant tumours involved 50 patients in the age of 26–81 years (55.18±13.60 – mean ± standard deviation). The data are shown in Table 1.

The control group composed of 30 healthy individuals in the age of 20–79 years (40.8±18.3 – mean ± standard deviation). The study group included:

- 18 women in the age of 20–70 years (36.39±16.13 – mean ± standard deviation),
- 12 men in the age of 23–79 (47.42±19.36 – mean ± standard deviation).

All patients from the control group were diagnosed as free of intellectual disturbances, craniocerebral injuries, drugs and alcohol addictions, poisonings, as well as history of neurological and psychiatric treatment. Also in this case we performed the imaging study first, followed by the HMRS.

HMRS examination technique

The HMRS examination was performed with the use of the MR Signa Excite (GEMS) system, and a superconducting magnet with magnetic field induction of 1.5T.

We applied the transmitting-receiving coil used for standard head examinations.

Patients

The HMRS study protocol included:

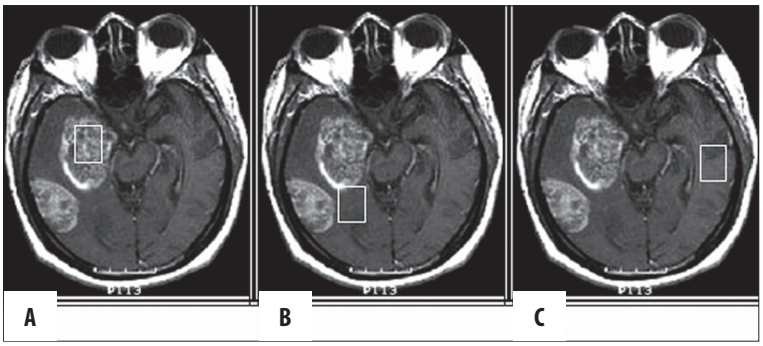

Figure 2. The voxel location: (**A**) the tumour centre, (**B**) the tumour border, (**C**) contralateral unchanged cerebral tissue.

1. 3D localiser,
2. Localiser in SE sequence, T2-weighted in the axial section,
3. PRESS sequence – tumour centre,
4. PRESS sequence – tumour border,
5. PRESS sequence – contralateral, normal tissue.

The volume of interest was selected on the basis of images obtained in the SE T2 sequence. From among 10-20 slices obtained in the localisation study, we selected those with the best image of the tumour. In each patient, the measurements were performed in three different locations. The first measurement was done by placing the voxel in the centre of the pathological lesion (Figure 2A). We tried to visualise the solid mass and to avoid necrotic and osseous tissue, and the fluid-filled spaces.

The second measurement concerned the area at the borderline of the lesion and the surrounding normal (unchanged) tissue (Figure 2B). The third measurement was performed within the normal, contralateral brain tissue (Figure 2C).

Voxel size, depending on tumour size and tumour location, ranged from 1.58 cm^3 to 14.96 cm^3 in all locations.

Control group

The HMRS studies of the control group were performed according to the following protocol:
1. 3D localiser
2. FSE T2 in axial section (localiser for spectroscopic examination),
3. SE T1 in axial section,
4. SE PD in axial section,
5. FLAIR in axial section,
1. 3-plane localiser, 19 s,
2. PRESS sequence – right frontal lobe.

Every person from the control group underwent measurements in the region of the right frontal lobe.

The voxel was placed with the use of similar standards as in patients with brain tumour (we avoided the osseous tissue and the spaces filled with fluid or air).

Voxel size ranged from 1.54 cm^3 to 8.27 cm^3.

All HMRS studies were performed in the SVS technique (single voxel spectroscopy). We used the PRESS sequence (Point Resolved Spectroscopy Sequence). Three parameters were used:
TR =1500 ms,
TE =35 ms,
Number of acquisitions: 64 or 128,
Sequence time: 2.14 min or 3.43 min.

The number of acquisitions and, appropriately, the time of sequence depended on the voxel size. Larger voxels required a lower number of acquisitions, which shortened the sequence time. In case of small voxels, we increased the number of acquisition and thus increased the sequence time (guided by the rule 'the smaller the voxel, the weaker the signal-to-noise ratio').

Before every PRESS sequence, there was an automated prescanning performed. It consisted of 'shimming' and water suppression. During shimming, all heterogeneities of the stable magnetic field were regulated. The results of shimming were presented as the full width at half maximum and amounted to 2–7 Hz.

During an automated process of water suppression, we suppressed the signal of fluids present in the volume of interest. The level of water suppression amounted in most cases to 98 or 99%. A few cases failed to obtain the optimal results of the shimming process (FWHM values exceeding 7 Hz or water suppression below 98%) and it was necessary to perform shimming manually.

Data analysis

The analysis of the obtained spectra used an original software: SAGE (GEMS). We measured the areas below the selected peaks, proportional to the concentration of particular metabolites.

For the purposes of this work we selected some data on choline (Cho):
- Choline – Cho 3.22 ppm $(-CH_3)_3$.

In order to evaluate Cho/Cr, NAA/Cho+Cr ratios in the HMRS spectrum, we analysed other metabolites as well:
- N-acetylaspartate – **NAA** 2.02 ppm $(-CH_3)$,
- Creatinine, phosphocreatinie – CR+PCr 3.02 ppm $(-CH_3)$.

The analysis involved:
- Evaluation of the total Cho concentration (measurement of areas below the selected peaks),

Table 2. The summary of mean Cho concentrations in analysed locations among patients with brain tumours and healthy volunteers with consideration of standard deviation and standard errors for 95% confidence interval.

Location	Mean	Standard deviation	Standard error	Confidence interval	
				–95%	95%
Voxel 1	0.0253	0.0192	0.0019	0.0214	0.0290
Voxel 2	0.0183	0.0140	0.0015	0.0160	0.0218
Voxel CG	0.0144	0.0049	0.0009	0.0125	0.0162

Table 3. The summary of statistical significance differences between mean Cho concentrations in analysed locations among patients with brain tumours and healthy volunteers, $p<0.05$.

Location	SS $\times 10^{-4}$	Degrees of freedom	MS $\times 10^{-4}$	F	p
Voxel 1	27.09	1	27.09	9.32	0.0028
Voxel 2	4.67	1	4.67	3.03	0.0841

SS – sum of squares; MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

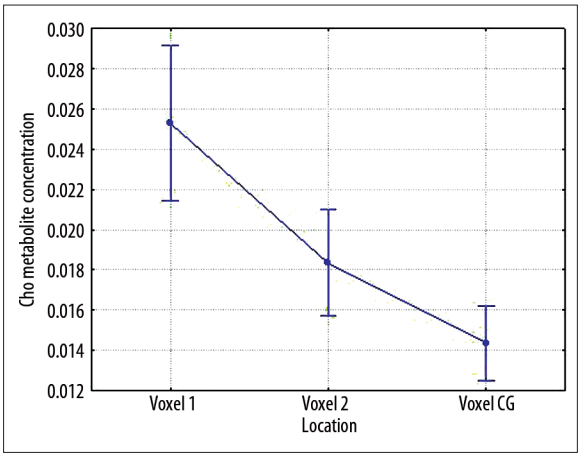

Figure 3. Mean Cho concentrations in analysed locations among patients with brain tumours and healthy individuals.

- Relative concentration ratios of choline and creatinine (Cho/Cr), and of other metabolites to choline (we based on the reports of the world literature): NAA/Cho, NAA/Cho+Cr.

The database of the baseline results was created in Excel. That program was also used for data processing. The statistical analysis was carried out with the use of the STATISTICA software and the application of one-way analysis of variance.

Results

The comparative analysis of mean Cho concentrations, on the basis of the measurements carried out in the control and the study group of patients with tumour (regardless of tumour type – benign/malignant) in two locations: in the lesion centre and at the border of the lesion

In order to find out whether Cho is a significant metabolite in the analysis of ^{1}HMRS spectra of the brain, we performed the analysis comparing mean Cho concentrations in patients with tumour and in persons from the control group. In case of patients with tumour, we analysed every measurement in the centre of the lesion (voxel 1) and at the border of the lesion (voxel 2). In the control group, the metabolites were analysed in a selected area of the right frontal lobe.

Data of patients from both groups were compared:
- Voxel 1 with a control group voxel (CG voxel),
- Voxel 2 with a control group voxel (CG voxel).

One-way analysis of variance was applied.

1. Analysis of Cho concentration for the whole study group

Mean Cho concentrations measured in the group of patients with tumour were compared with analogous values obtained in the control group. Higher mean Cho concentrations were found in the group of patients with brain tumour. The highest mean values were revealed in the measurements taken in the lesion centre, lower in the location of voxel 2, and the lowest in the locations of the control group (Figure 3, Table 2).

Statistically significant differences were found in:
- the comparisons of mean Cho concentrations in voxel 1 with a voxel of the control group, $p=0.0028$.

No statistically significant differences were found in:
- the comparisons of mean Cho concentrations in voxel 2 with a voxel of the control group, $p=0.0841$.

The results of the measurements concerning the significance of the differences in mean Cho concentrations were listed in Table 3.

When analysing the statistically obtained results, the authors revealed that significantly higher Cho concentrations are found within voxels located in the lesion

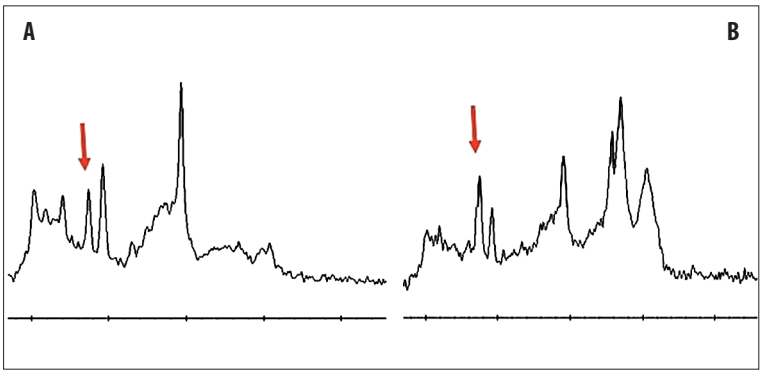

Figure 4. The examples of HMRS spectra: (**A**) healthy volunteer, (**B**) patient with brain tumour.

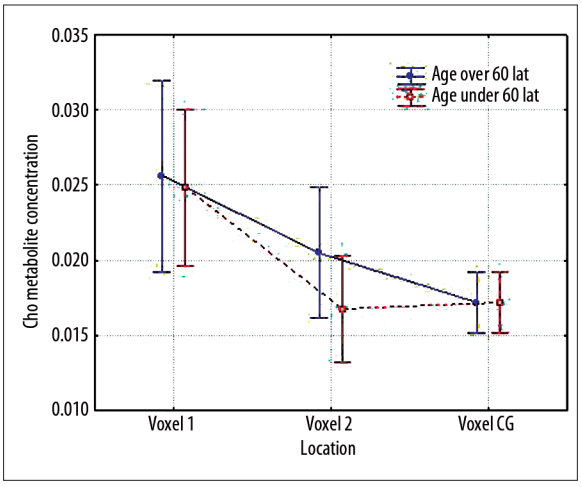

Figure 5. Mean Cho concentrations in analysed locations among patients with brain tumours and healthy individuals, with consideration of age distribution (under and over 60 years of age).

centre, as compared to voxel locations in the control group ($p<0.05$).

However, no significant differences in the mean Cho concentration were revealed between location on the lesion border and locations in the control group ($p>0.05$). In this case, we revealed only a tendency for higher mean Cho concentrations on the lesion border.

We concluded that Cho concentration is an important parameter in HMRS spectrum evaluation in patients with brain tumour.

Some examples of spectra in a healthy individual and in a patient with brain tumour were shown in Figure 4.

2. The analysis of Cho concentrations for the whole group, with respect to age distribution

We analysed the results of examinations carried out in 100 patients in the age ranging from 17 to 81 years, divided into two age groups:

- 66 patients in the age of up to 60 years (from 17 to 59, 41.71±12.35 – mean ± standard deviation),
- 34 patients in the age of over 60 (from 61 to 81, 67, 88±5.90 – mean ± standard deviation).

Table 4. The group under 60 years of age – the summary of mean Cho concentrations in analysed locations among patients with brain tumours and healthy volunteers with consideration of standard deviation and standard errors for 95% confidence interval.

Location	Men	Standard deviation	Standard error	Confidence interval	
				–95%	95%
Voxel 1	0.0256	0.0190	0.0030	0.0204	0.0326
Voxel 2	0.0205	0.0164	0.0027	0.0154	0.0263
Voxel CG	0.0117	0.0048	0.0012	0.0094	0.0145

Table 5. The group over 60 years of age – the summary of mean Cho concentrations in analysed locations among patients with brain tumours and healthy volunteers with consideration of standard deviation and standard errors for 95% confidence interval.

Location	Mean	Standard deviation	Standard error	Confidence interval	
				–95%	95%
Voxel 1	0.0249	0.0195	0.0025	0.0193	0.0293
Voxel 2	0.0168	0.0120	0.016	0.0143	0.0209
Voxel CG	0.0172	0.0035	0.0009	0.0151	0.0192

Table 6. The group under 60 years of age – the summary of statistical significance differences between mean Cho concentrations in analysed locations among patients with brain tumours and healthy volunteers, $p<0.05$.

Location	SS $\times 10^{-4}$	Degrees of freedom	MS $\times 10^{-4}$	F	p
Voxel 1	24.36	1	24.36	9.14	0.0038
Voxel 2	8.90	1	8.90	4.51	0.0385

SS – sum of squares; MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

Table 7. The group over 60 years of age – the summary of statistical significance differences between mean Cho concentrations in analysed locations of patients with brain tumours and healthy volunteers, $p<0.05$.

Location	SS $\times 10^{-4}$	Degrees of freedom	MS $\times 10^{-4}$	F	p
Voxel 1	5.82	1	5.82	1.86	0.1764
Voxel 2	0.02	1	0.02	0.02	0.8914

SS – sum of squares; MS – mean squarel F – Fisher and Snedocor test results; p – level of significance.

For comparisons, the results obtained in 30 healthy individuals in the age ranging from 20 to 79 (40.8±18.3 – mean ± standard deviation) were used:
- 16 patients in the age of up to 60 years (from 20 to 59, 33.19±11.79 – mean ± standard deviation),
- 14 patients in the age of over 60 years (from 61 to 79, 64.79±5.10 – mean ± standard deviation).

We compared mean Cho concentrations in patients with brain tumour, with mean Cho concentrations revealed in the control group. The analysis was performed in two age groups: under and over 60 years.

Group under 60 y.o.a.

In comparison to the control group, the mean Cho concentrations in patients with brain tumour were higher. The highest value was found in the tumour centre (Figure 5, Table 4).

Group over 60 y.o.a.

In comparison to the control group, the mean Cho concentrations in patients with brain tumour were significantly higher in the measurements within the lesion centre. A significantly lower mean concentration was found in the location of voxel 2 (Figure 5, Table 5).

In the group of patients under 60 y.o.a., statistically significant differences were found in:
- the comparisons of mean Cho concentrations in voxel 1 with a voxel of the control group, $p=0.0038$,
- the comparisons of mean Cho concentrations in voxel 2 with a voxel of the control group, $p=0.0385$,

In the group of patients over 60 y.o.a., no statistically significant differences were found in:
- the comparisons of mean Cho concentrations in voxel 1 with a voxel of the control group, $p=0.8914$,
- the comparisons of mean Cho concentrations in voxel 2 with a voxel of the control group, $p=0.1764$.

The results of measurements concerning significant differences between mean Cho concentrations in both groups were presented in Tables 6 and 7.

When analysing the statistically obtained results for the whole study group divided into age groups, the authors revealed that significantly higher Cho concentrations are found within voxels located both in the lesion centre and on its border, as compared to voxel locations in the control group ($p<0.05$). This concerns only the group of patients under 60 years of age.

In the group of patients over 60 years old, the mean Cho concentrations did not reach the level of statistical significance ($p > 0.05$). However, in this group, we revealed a tendency for higher mean Cho concentrations among patients with brain tumour, as compared to the control group.

I. The comparison of mean Cho concentrations in the group of patients with malignant and benign tumours

By applying the one-way analysis of variance, we evaluated different Cho concentrations. We took into consideration: lesion type (benign and malignant), voxel location (voxel 1 – in the lesion centre, voxel 2 – lesion border, voxel 3 – normal, unchanged tissue) and interactions of these two factors. The results were presented in Figure 6 and Table 8.

Mean Cho concentrations differ, depending on the type of the detected lesion. Higher mean concentrations can be found in the group of patients with malignant lesion.

In both groups of patients, the mean Cho concentrations differ, depending on voxel location.

Higher mean concentrations can be revealed in the lesion centre, lower on the border of the lesion. In locations within a normal tissue (voxel 3), the differences in mean Cho concentrations found between both groups are not significant.

Statistically significant differences were found in:
- the comparisons of groups of patients with benign and malignant lesions, $p=0.0016$,

Table 8. The summary of mean Cho concentrations in analysed locations of patients with benign and malignant brain tumours, considering standard deviation and standard errors for 95% confidence interval.

Location	Lesion type	Mean	Standard deviation	Standard error	Confidence interval	
					–95%	95%
Voxel 1	Total	0.0252	0.0191	0.0020	0.0212	0.0292
	Benign	0.0197	0.0125	0.0018	0.0159	0.0234
	Malignant	0.0309	0.0229	0.0035	0.0239	0.0379
Voxel 2	Total	0.0183	0.0132	0.0014	0.0155	0.0210
	Benign	0.0144	0.0083	0.0012	0.0119	0.0168
	Malignant	0.0223	0.0160	0.0024	0.0174	0.0272
Voxel 3	Total	0.0154	0.0075	0.0008	0.0138	0.0169
	Benign	0.0154	0.0079	0.0012	0.0130	0.0177
	Malignant	0.0153	0.0071	0.0011	0.0132	0.0175

Table 9. Evaluation of the significance of differences between mean Cho concentrations in the analysed locations, in the group of patients with benign and malignant brain tumour, $p<0.05$.

	SS	Degrees of freedom	MS	F	p
Absolute term	0.1043	1	0.1043	399.74	0.0000
Type of lesion	0.0028	1	0.0028	10.58	0.0016
Error	0.0230	88	0.0003		
Location	0.0047	2	0.0023	15.90	0.0000
Location* type of lesion	0.0015	2	0.0008	5.13	0.0068
Error	0.0258	176	0.0001		

SS – sum of squaresl MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

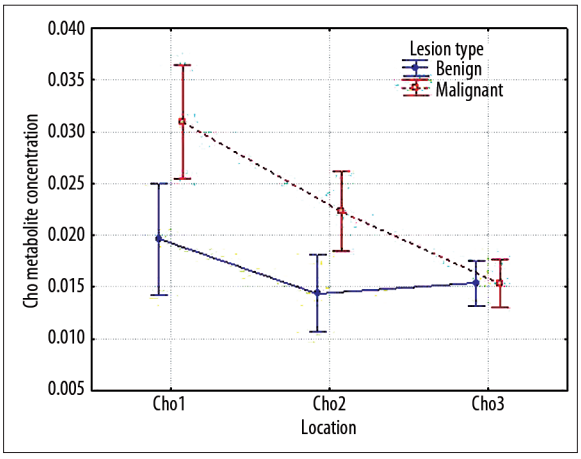

Figure 6. Mean Cho concentrations in analysed locations among patients with benign and malignant brain tumours.

- the comparisons of voxel from the lesion centre and from its border, p=0.0000,
- interactions between lesion type and voxel location, p=0.0068.

Results of calculations were presented in Table 9.

The comparison of mean Cho concentrations shows a high level of statistical significance ($p<0.05$) both for the lesion type (higher mean values for the group of patients with malignant tumours) and voxel location (the highest mean values in the centre of the lesion), as well as for interactions of both these factors.

On that basis we came to the conclusion that Cho concentration is an important parameter in the evaluation of HMRS spectrum in patients with brain tumour, depending on the lesion type (benign/malignant tumour).

Examples of spectra in patients with benign and malignant tumours were shown in Figure 7.

II. The comparison of mean Cho/Cr ratios in the group of patients with malignant and benign tumours

We evaluated differences in metabolite ratios by applying one-way analysis of variance. The type of lesion (benign, malignant), voxel location (lesion centre – voxel 1, lesion border – voxel 2, voxel 3 – normal tissue) and interactions of these two factors were taken into consideration.

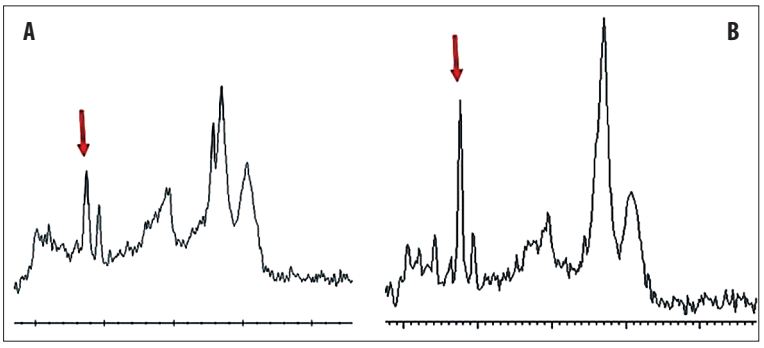

Figure 7. The examples of HMRS spectra:
(**A**) patient with benign brain tumour,
(**B**) patient with malignant brain tumour.

Table 10. The summary of mean Cho/Cr ratios in analysed locations among patients with benign and malignant brain tumours considering standard deviation and standard errors for 95% confidence interval.

Location	Type of lesion	Mean	Standard deviation	Standard error	Confidence interval	
					–95%	95%
Voxel 1	Total	3.9963	5.4378	0.5732	2.8573	5.1352
	Benign	2.9661	4.0359	0.5951	1.7676	4.1646
	Malignant	5.0733	6.4680	0.9751	3.1069	7.0397
Voxel 2	Total	1.7400	1.9427	0.2048	1.3331	2.1469
	Benign	1.1897	0.3438	0.0507	1.0876	1.2918
	Malignant	2.3153	2.6506	0.3996	1.5095	3.1212
Voxel 3	Total	1.0484	0.4163	0.0439	0.9612	1.1356
	Benign	1.0507	0.4677	0.0689	0.9118	1.1896
	Malignant	1.0461	0.3601	0.0543	0.9366	1.1556

Table 11. Evaluation of the significance of differences between mean Cho/Cr values in the analysed locations, in the group of patients with benign and malignant brain tumour, $p<0.05$.

	SS	Degrees of freedom	MS	F	p
Absolute term	1394.9	1	1394.9	105.89	0.0000
Type of lesion	78.1	1	78.1	5.93	0.0169
Error	1159.2	88	13.2		
Location	433.7	2	216.9	22.51	0.0000
Location *type of lesion	50.2	2	25.1	2.61	0.0766
Error	1695.5	176	9.6		

SS – sum of squares; MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

1. Cho/Cr ratio analysis

Mean Cho/Cr values differ, depending on the lesion type (Figure 8, Table 10).

Higher mean values can be found in the group of patients with malignant tumour.

Mean Cho/Cr values also differ, depending on location. Higher mean values can be found in lesion centre, lower on the border of the lesion. Within the normal, contralateral tissue (voxel 3), the differences in Cho/Cr found between both groups are insignificant.

Statistically significant differences were found in:
- the comparisons of voxel from the lesion centre and from its border, $p=0.0000$,
- the comparisons of groups of patients with benign and malignant lesions, $p=0.169$.

No statistically significant differences were found in:
- interactions between lesion type and voxel location, $p=0.0766$.

Table 12. The summary of mean NAA/Cho values in the analysed locations in the group of patients with brain tumour of benign and malignant type, with consideration of standard deviation and standard errors for 95% confidence interval.

Location	Type of lesion	Mean	Standard deviation	Standard error	Confidence interval	
					–95%	95%
Voxel 1	Total	1.1080	0.9313	0.0982	0.9130	1.3031
	Benign	0.9014	0.6612	0.0975	0.7051	1.0978
	Malignant	1.3240	1.1155	0.1682	0.9849	1.6632
Voxel 2	Total	1.6041	1.6966	0.1788	1.2487	1.9594
	Benign	1.8071	2.2511	0.3319	1.1386	2.4756
	Malignant	1.3918	0.7512	0.1132	1.1634	1.6202
Voxel 3	Total	1.9398	0.8093	0.0853	1.7703	2.1093
	Benign	2.0634	0.9945	0.1466	1.7680	2.3587
	Malignant	1.8106	0.5359	0.0808	1.6477	1.9736

Table 13. Evaluation of the significance of differences between mean NAA/Cho values in the analysed locations, in the group of patients with benign and malignant brain tumour, $p<0.05$.

	SS	Degrees of freedom	MS	F	p
Absolute term	648.1	1	648.1	454.58	0.0000
Type of lesion	0.5	1	0.5	0.32	0.5750
Error	125.5	88	1.4		
Location	30.9	2	15.4	10.58	0.0000
Location* type of lesion	8.9	2	4.4	3.04	0.0503
Error	256.9	176	1.5		

SS – sum of squares; MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

Results of calculations were presented in Table 11.

The comparison of mean Cho/Cr values showed a high level of statistical significance ($p<0.05$) both for the lesion type (higher mean values for the group of patients with malignant tumour) and voxel location (the highest mean values in the centre of the tumour).

No statistical significance ($p>0.05$) of mean Cho/Cr values was found for the interaction of both these factors.

It was concluded that the Cho/Cr ratio is an important parameter in HMRS spectrum evaluation in patients with brain tumours, depending on the lesion type (benign/malignant tumour).

3. NAA/Cho analysis

Mean NAA/Cho ratio values differ depending on the type of lesion and location, which was shown in Figure 9, Table 12.

In malignant lesions, as compared to the group of benign tumours, the mean values are lower; the only exception is lesion centre in which the mean NAA/Cho value is higher.

Mean NAA/Cho values differ depending on location as well. The lowest mean values are found in tumour centre (especially in the group of patients with benign tumour), higher on the border of the lesion, and the highest within the normal, healthy tissue.

Statistically significant differences were found in:
- the comparisons of voxel from the lesion centre and from its border, $p=0.0000$.

No statistically significant differences were found in:
- the comparisons of groups of patients with benign and malignant lesions, $p=0.5750$,
- interactions between lesion type and voxel location, $p=0.0503$.

Results of calculations were presented in Table 13.

The statistical analysis of mean NAA/Cho values showed a high level of statistical significance ($p<0.05$) for voxel location only (the lowest mean values in the centre of the lesion).

No statistical significance ($p>0.05$) of mean NAA/Cho values was found for lesion type and the interaction between lesion type and voxel location.

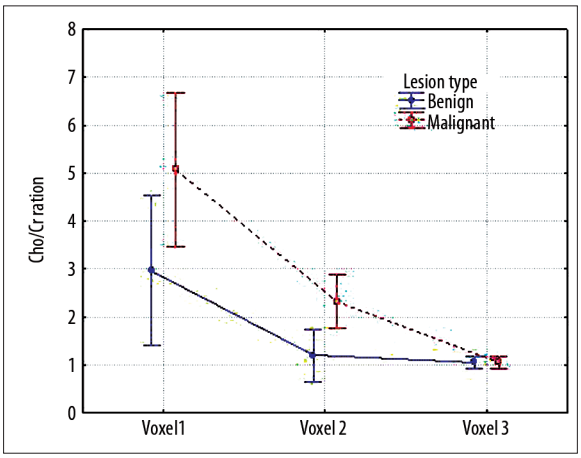

Figure 8. Mean Cho/Cr ratios in analysed locations among patients with benign and malignant brain tumours.

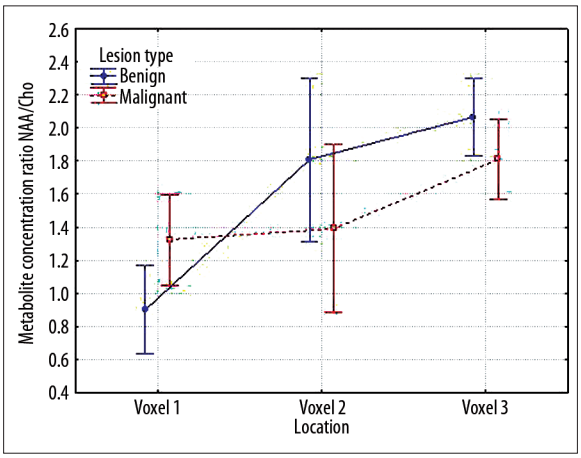

Figure 9. Mean NAA/Cho ratios in analysed locations in patients with benign and malignant brain tumours.

Table 14. The summary of mean NAA/Cho+Cr values in the analysed locations in the group of patients with brain tumour of benign and malignant type, with consideration of standard deviation and standard errors for 95% confidence interval.

Location	Type of lesion	Mean	Standard deviation	Standard error	Confidence interval	
					–95%	95%
Voxel 1	Total	0.6387	0.4608	0.0488	0.5416	0.7357
	Benign	0.6933	0.4360	0.0643	0.5638	0.8228
	Malignant	0.5802	0.4842	0.0738	0.4312	0.7292
Voxel 2	Total	0.7958	0.3328	0.0353	0.7257	0.8659
	Benign	0.8064	0.2750	0.0405	0.7247	0.8881
	Malignant	0.7844	0.3883	0.0592	0.6649	0.9039
Voxel 3	Total	1.0484	0.4163	0.0439	0.9612	1.1356
	Benign	1.0507	0.4677	0.0690	0.9118	1.1896
	Malignant	1.0461	0.3601	0.0543	0.9366	1.1556

On lesion border and within the contralateral unchanged tissue, we revealed a tendency for higher mean NAA/Cho values in the group of patients with benign tumour, as compared to the group of patients with malignant tumours (no statistical significance).

4. NAA/Cho+Cr analysis

Mean NAA/Cho+Cr values differ depending on the type of lesion. The data were shown in Figure 10, Table 14.

Higher mean values are found in the group of patients with benign lesion.

Mean NAA/Cho+Cr values differ depending on location as well. Lower mean values are found in lesion centre, higher on the border of the lesion, and the highest within the normal, healthy tissue.

Statistically significant differences were found in:
- the comparisons of voxel from the lesion centre and from its border, p=0.0000.

No statistically significant differences were found in:
- the comparisons of groups of patients with benign and malignant lesions, p=0.0615,
- interactions between lesion type and voxel location, p=0.6525.

Results of calculations were presented in Table 15.

The statistical analysis of mean NAA/Cho+Cr values showed a high level of statistical significance ($p<0.05$) for voxel location only (the lowest mean values in the centre of the lesion).

No statistical significance ($p>0.05$) of mean NAA/Cho+Cr values was found for lesion type and the interaction of lesion type and voxel location.

In all locations we revealed a tendency for higher mean NAA/Cho+Cr values in the group of patients with benign tumour, as compared to the group of patients with malignant tumour (no statistical significance).

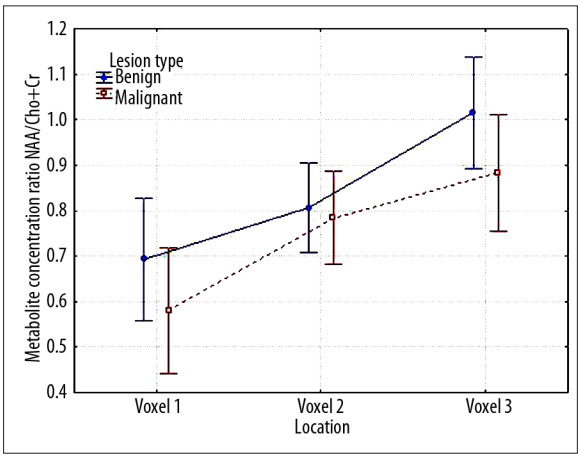

Figure 10. Mean NAA/Cho+Cr ratios in analysed locations in patients with benign and malignant brain tumours.

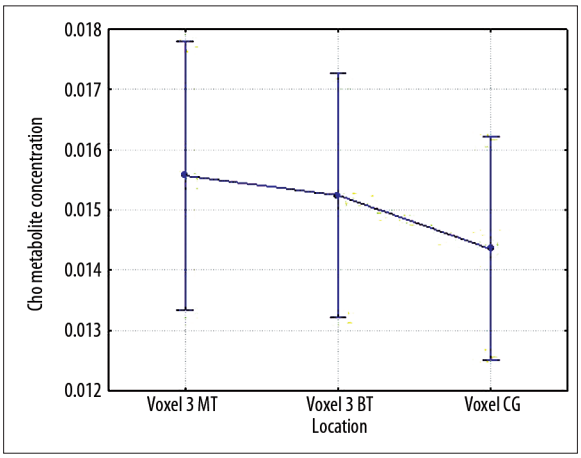

Figure 11. Mean Cho concentrations in the location studies (normal, unchanged tissue – voxel 3 MT and BT), in the group of patients with brain tumour of benign and malignant type, as well as in the control group (voxel CG).

Table 15. Evaluation of the significance of differences between mean NAA/Cho+Cr values in the analysed locations, in the group of patients with benign and malignant brain tumour, $p<0.05$.

	SS	Degrees of freedom	MS	F	p
Absolute term	168.1	1	168.1	1146.56	0.0000
Type of lesion	0.5	1	0.5	3.59	0.0615
Error	12.8	87	0.1		
Location	4.3	2	2.2	12.20	0.0000
location* type of lesion	0.2	2	0.1	0.43	0.6525
Error	31.0	174	0.2		

SS – sum of squares; MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

Table 16. The summary of mean Cho concentrations in the location studies (normal, unchanged tissue – voxel 3 MT and BT) in the group of patients with brain tumour of benign and malignant type, as well as in the control group (voxel CG), with consideration of standard deviation and standard errors for 95% confidence interval.

Location	Mean	Standard deviation	Standard error	Confidence interval	
				–95%	95%
Voxel 1 MT	0.0156	0.0078	0.0011	0.0133	0.0178
Voxel 3 BT	0.0152	0.0071	0.0010	0.0132	0.0173
Voxel CG	0.0144	0.0049	0.0009	0.0125	0.0162

Table 17. Evaluation of the significance of differences between mean Cho concentrations in the analysed locations, in the group of patients with benign and malignant brain tumour (normal, unchanged tissue – voxel 3 MT and BT) and in the control group (voxel CG), $p<0.05$.

Metabolite	SS $\times 10^{-4}$	Degrees of freedom	MS $\times 10^{-4}$	F	P
Cho	0.28	2	0.14	0.29	0.7476

SS – sum of squares; MS – mean square; F – Fisher and Snedocor test results; p – level of significance.

III. The comparison of the external model (control group) with the internal model (healthy part of the brain in patients with benign and malignant tumours), on the basis of mean Cho concentrations

We compared the mean concentrations of some selected metabolites, measured in:
- the normal, unchanged part of brain of the patients with benign tumour (BT) – voxel 3 BT,
- the normal, unchanged part of brain of the patients with malignant tumour (MT) – voxel 3 MT,
- the right frontal lobe in individuals from the control group.

One-way analysis of variance was used.

The data obtained in the patients with tumour were compared with the data of the control group:
- voxel 3 BT with voxel 3 MT,
- voxel 3 BT with voxel from the control group,
- voxel 3 MT with voxel from the control group.

Mean Cho concentrations differ slightly, depending on the location. The data were shown in Figure 11 and Table 16. The comparable mean Cho concentrations are found in location within the unchanged, normal tissue in the patients with tumour (slightly increased in the group of patients with malignant tumour). They are higher in comparison to the control group (voxel CG).

No significant differences were found in the comparison of mean Cho concentration in selected locations, $p=0.7476$

Results of calculations were presented in Table 17.

The obtained results of comparisons of mean Cho concentrations in the contralateral, normal tissue with external control group showed some slight and insignificant statistical differences.

We revealed a tendency of higher mean Cho concentrations in the normal, unchanged tissue in patients with tumour than in the external control group.

Discussion

By analysing the so far published study results concerning patients with brain tumours, we may say that one of the basic, evaluated metabolites is choline.

In our work, the baseline for the evaluation of the degree of Cho concentration change is the comparative analysis of results of patients with brain tumour and of the external control group. Higher Cho concentrations were revealed in the measurements in patients with brain tumour than in the control group. The highest concentrations were found in the centre of lesion, slightly lower on the border of the tumour, and the lowest in the control group. The discussed differences are statistically significant. The obtained results are in this case in agreement with the majority of results presented in the collected articles, underscoring the increased Cho concentrations in patients with brain tumours in comparison to the control group [6,9,13,14].

Additionally, the mean Cho concentrations in patients with brain tumour were compared with an analogous mean value measured in the control group. The analysis was carried out in two age groups: under and over 60 years of age. We reviewed articles published in the world literature, concerning differences in metabolism depending on the age of the patient and describing the use of HMRS techniques in the examination of the physiological aging process. Those articles revealed differences in metabolite concentration and in metabolite ratios [10,15–17]. The statistically significant increase of Cho concentration in the group of older patients, in comparison to the group of younger individuals, as well as Cho/Cr increase with age was shown in the work by Pfefferbaum et al. [15].

According to own results, both in the older (over 60 years of age) and in the younger age group (under 60 years of age), the mean Cho concentrations in patients with brain tumour were higher than in the control group. These differences are statistically significant in the group of individuals under 60 years of age.

In the group over 60 years of age we revealed only a tendency (no statistical significance) of higher mean Cho concentrations in patients with brain tumour. The tendency was particularly well marked when comparing the results from lesion centre and from voxel of the control group. In comparison to that location, the mean concentrations measured on lesion border and in the control group were lower.

The key issue of this work was the evaluation of differences in Cho concentration. The evaluation considered the lesion type (benign or malignant), voxel location (voxel 1 – lesion centre, voxel 2 – lesion border, voxel 3 – normal, unchanged tissue) and interactions of these two factors. By evaluating the interactions between the voxel location and the type of lesion in the group of patients with brain tumour, it was possible to answer the question whether the differences between the patient groups (benign/malignant tumour) are stable for every voxel location.

The results of own studies confirm the results presented in the collected literature [4,6,7,9,13,14,18]. Authors of the aforementioned works revealed the increased mean Cho concentrations in patients with malignant tumour. The above mentioned articles do not involve a comprehensive analysis of the results obtained in the HMRS examinations, including the information on the influence of voxel location and lesion type on Cho concentration.

The results of the study revealed statistical significance of voxel location (lesion centre, lesion border, contralateral unchanged tissue), lesion type (benign/malignant tumour) and interactions between those two factors. Depending on the lesion type, mean Cho concentrations differ significantly. Higher mean Cho concentrations can be found in the group of patients with malignant lesion.

Mean Cho concentrations differ depending on the location as well. Higher mean values are found in the lesion centre, lower on the lesion border, the lowest in the healthy tissue.

The obtained results indicate that Cho concentration is an important parameter in the evaluation of HMRS spectrum of patients with brain tumour, depending on the type and location of the lesion.

Data on metabolite concentrations are frequently not sufficient because the area below the peak does not reflect the value of absolute concentration of the metabolite and is only proportionate to it. Measurements of absolute concentrations are time-consuming and complicated. This leads to the situation in which most authors use measurement techniques based on concentration values close to the absolute concentrations of metabolites and present their study results in the form of concentration ratios.

The most frequently presented ratio is Cho/Cr, as creatinine is believed to be a referential metabolite, the concentration of which remains relatively stable. This ratio allows for an objective evaluation of the spectrum.

Assuming that the concentration of creatinine does not change substantially with voxel location, this will be mainly choline (in the numerator) that will influence the Cho/Cr ratio. The analysis of choline concentration in different locations, presented in our work, revealed higher concentrations of that metabolite in patients with malignant brain tumour, especially in its centre. Thus, we may expect that the Cho/Cr ratio will be higher in those patients as well.

The results of the published studies show an increased Cho/Cr ratio within tumours, as compared to the unchanged, normal tissue. These works reveal a changing value of that indicator, depending on the tumour type. They also show a higher Cho/Cr ratio in patients with malignant tumours [5–8,18–20].

The analysis of own results confirms the results presented in the above mentioned articles. We revealed both an increased choline concentration in patients with malignant lesions and a significantly increased Cho/Cr ratio in that group of patients, as compared to the group of patients with benign lesions. Statistical significance was also revealed during the analysis of the location factor. The highest values of the Cho/Cr ratios were found in the lesion centre; the lowest in the normal, healthy tissue.

An indicator frequently mentioned in the literature is the NAA/Cho ratio, as the concentrations of both these metabolites change substantially in patients with brain tumours. Considering the fact that NAA concentration decreases in patients with brain tumours, while the Cho concentration increases, we should expect lower mean NAA/Cho values in the tumour centre than on the border of the lesion or in the unchanged, normal tissue,

Articles 4, 5, 6, 13, 21 presented results showing NAA/Cho ratio decrease in patients with brain tumour, as compared to the normal, unchanged tissue, and a possibility of lesion differentiation on the basis of the NAA/Cho values (lower values in patients with a malignant tumour).

According to the results of our work, the statistical significance was obtained by the factor of voxel location. The lowest NAA/Cho values could be found in the tumour centre, as compared to the normal, unchanged tissue. No significance was revealed in the NAA/Cho analysis regarding lesion type. In this case we revealed only a tendency of lower NAA/Cho values in patients with a malignant tumour.

The paper by Bulakbasi et al. [4] presents the analysis of the NAA/Cho+Cr indicator. It was shown that, similarly to the NAA/Cho concentration ratio, this indicator is statistically significant in malignant and benign tumour differentiation.

Own results confirmed (similarly to the above mentioned work) lower NAA/Cho+Cr values in the group of malignant tumours than in the group of benign tumours. However, no statistical significance was found. Statistically significant was tumour location only. The highest values of this indicator were obtained within the normal, unchanged tissue, and the lowest within the lesion centre.

Taking into consideration the results obtained for that indicator, we may conclude that the analyses of that ratio do not improve the differentiation of the tumour type, especially in the situation of NAA/Cho ratio analysis.

Additionally, we performed a comparative analysis of Cho concentrations in the contralateral areas, in the group of patients with a malignant and benign tumour (voxel 3 – BT and MT), compared with an external control group (voxel CG). On the basis of the obtained results we concluded that the Cho concentrations in the measurements from voxels located in the unchanged tissue of the patients with a lesion are insignificantly higher than the values for the external control group (no statistical significance). When comparing study results obtained within the healthy tissue of patients with a tumour (voxel 3 MT and BT), we revealed a slightly higher (statistically not significant) mean Cho concentration in patients with benign tumour than in the group of malignant tumours.

Kumar et al. [6] were the first to compare the results of examinations performed within the normal unchanged tissue of patients with brain tumour and the results obtained in the external control group. Those authors revealed also that the differences in Cho concentrations between the external and the internal model are minor and statistically not significant.

Taking into consideration the obtained results and literature reports, we may conclude that an additional measurement within the unchanged tissue may seem unnecessary, especially in the situation when we are in possession of the results of the control group (external model). This is particularly important for the patients in severe conditions, for whom every long-lasting MRI and HMRS examination may be arduous. On the other hand, the lack of the external model can be replaced with an additional measurement within the contralateral tissue.

In the studied material, patients with a tumour producing inhomogenous signal intensification (e.g. malignant astrocytomas of the III and IV grade in the WHO scale – 36 cases) were constituting a large group. Considering the heterogeneity and size of such tumours, it is necessary to perform a few measurements from the lesion area, to diagnose the

regions of necrosis and of high cell activity [13,22]. This work used only those spectra that revealed the metabolic cycle of the active cells and membrane metabolism (Cho peak clearly dominates over other peaks and is considerably higher than in the control group). Regions with necrosis, showing carbohydrate catabolism, producing spectra with a clearly visible peak of lactate or reduced peaks of other metabolites were not evaluated. When taking into account the heterogeneity of the anaplastic astrocytomas and GBM, it is possible to avoid mistakes connected with a right interpretation of the spectra. Acquisition within the necrotic region and the region of high proliferation may falsify the results. Spectra obtained in such a way may even indicate Cho decrease, as compared to the control group. This explains the fact of paradoxally contradictory results of some studies [11,12]. Unambiguous results are guaranteed by a detailed selection of the volume of interest and by author's choice of those voxels only, in which the spectra indicate a high cellular activity.

Conclusions

1. The performed HMRS examinations and the analysis of the results revealed a significant role of choline in the diagnostics of patients with brain tumour.

2. Cho concentration is a significant factor differentiating patients with brain tumour (higher mean values) from the control group (especially patients under 60 years of age).

3. Mean Cho/Cr, NAA/Cho values are an important factor differentiating the lesion type (benign/malignant) in patients with brain tumour.

4. For the measurements performed in the centre and border of the lesion, the results of the control group (external model) or of the normal, unchanged tissue (contralateral) of patients with brain tumour (internal model) may be used as the reference values.

References:

1. Didkowska J, Wojciechowska U, Tarkowski W et al: Nowotwory złośliwe w Polsce w 2000 roku. Krajowy Rejestr Nowotworów Centrum Onkologii, Warszawa, 2003
2. Brandao LA, Dominigues RC, Cecil KM: MR spectroscopy of the brain. Lippincott Williams&Wilkins, Philadelphia, 2003
3. Young IR: Methods in biomedical magnetic resonance imaging and spectroscopy. Willey, New York 2000
4. Bulakbasi N, Kocaoglu M, Ors F et al: Combination of single-voxel proton MR spectroscopy and apparent diffusion coefficient calculation in the evaluation of common brain tumors. Am J Neuroradiol, 2003; 24(2): 225–33
5. Law M, Yang S, Wang H et al: Glioma grading: sensitivity, specificity, and predictive values of perfusion MR imaging and proton MR spectroscopic imaging compared with conventional MR imaging. Am J Neuroradiol, 2003; 24: 1989–98
6. Kumar A, Kaushik S, Tripathi RP et al: Role of *in vivo* proton MR spectroscopy in the evaluation of adult brain lesions: Our preliminary experience. Neurology India, 2003; 51(4): 474–78
7. Majos C, Alonso J, Aguilera C et al: Adult primitive neuroectodermal tumor: proton MR spectroscopic findings with possible application for differential diagnosis. Radiology, 2002; 225(2): 556–66
8. Walecki J, Jurkiewicz E: Diagnostyka obrazowa nowotworów ośrodkowego układu nerwowego. Polski Przegląd Neurologiczny, 2007; (3): 155–71
9. Soares DP, Law M: Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. Clinical Radiology, 2009; 64: 12–21
10. Urbanik A: Ocena procesu starzenia się mózgu metodą protonowej spektroskopii rezonansu magnetycznego. Wyd. Dęby Rogalińskie, Kraków, 2002
11. Londoñoa A, Castilloa M, Armaoa D et al: Unusual MR Spectroscopic Imaging Pattern of an Astrocytoma: Lack of Elevated Choline and High myo-Inositol and Glycine Levels. Am J Neuroradiol, 2003; (24): 942–45
12. Saraf-Lavia E, Bowena BC, Pattanya P et al: Proton MR Spectroscopy of Gliomatosis Cerebri: Case Report of Elevated Myoinositol with Normal Choline Levels. Am J Neuroradiol, 2003; 24: 946–51
13. Hourani R, Brant LJ, Rizk T et al: Can proton MR spectroscopic and perfusion imaging differentiate between neoplastic and nonneoplastic brain lesions in adults? Am J Neuroradiol, 2008; (29): 366–72
14. Majos C, Aguilera C, Alonso J et al: Proton MR Spectroscopy Improves Discrimination between Tumor and Pseudotumoral Lesion in Solid Brain Masse. Am J Neuroradiol, 2009; 30(3): 544–51
15. Pfefferbaum A, Adalsteinsson E, Spielman D et al: *In vivo* spectroscopic quantification of the N-acetyl moiety, creatine, and choline from large volumes of brain gray and white matter: effects of normal aging. MRM, 1999; 41: 276–84
16. Lundbom N, Barnett A, Bonavita S et al: MR image segmentation and tissue metabolite contrast in 1H spectroscopy imaging of normal and aging brain. MRM, 1999; (41): 841–45
17. Ross B, Michaelis T et al: Clinical applications of magnetic resonance spectroscopy. Magnetic Resonance Quarterly, 1994; 10: 191–247
18. Mukherji SK: Clinical Applications of Magnetic Resonance Spectroscopy. Wiley-Liss, 1998
19. Fayed N, Modrego PJ: The contribution of magnetic resonance spectroscopy and echoplanar perfusion-weighted MRI in the initial assessment of brain tumours. J Neurooncol, 2005; 72(3): 261–65
20. Majós C, Julià-Sapé M, Alonso J et al: Brain Tumor Classification by Proton MR comparison of diagnosis accuracy at short and long TE. Am J Neuroradiol, 2004; 25: 1696–704
21. Walecki J, Grieb P, Chojnacka E et al: Spektroskopia protonowa MR *in vivo* guzów wewnątrzczaszkowych. Doniesienie wstępne. Pol Przegl Radiolog, 1998; 63: 225–32
22. Walecki J: Neuroradiologia. Upowszechnienie Nauki – Oswiata „Un-O", Warszawa, 2000