ORIGINAL PAPER/*ARTYKUŁ ORYGINALNY*

Magnetic resonance spectroscopy in intracranial tumours of glial origin

Spektroskopia rezonansu magnetycznego w wewnątrzczaszkowych nowotworach pochodzenia glejowego

Dariusz J. Jaskólski[1], Jan Fortuniak[1], Agata Majos[2], Witold Gajewicz[2], Wielisław Papierz[3], Paweł P. Liberski[4], Beata Sikorska[4], Ludomir Stefańczyk[2]

[1]Department of Neurosurgery and Oncology of the Central Nervous System, Medical University of Lodz, Poland
[2]Department of Radiology, Medical University of Lodz, Poland
[3]Department of Pathology, Medical University of Lodz, Poland
[4]Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Poland

Neurologia i Neurochirurgia Polska 2013; 47, 5: 438-449
DOI: 10.5114/ninp.2013.32999

Abstract

Background and purpose: To determine *in vivo* magnetic resonance spectroscopy (MRS) characteristics of intracranial glial tumours and to assess MRS reliability in glioma grading and discrimination between different histopathological types of tumours.
Material and methods: Analysis of spectra of 26 patients with glioblastomas, 6 with fibrillary astrocytomas, 4 with anaplastic astrocytomas, 2 with pilocytic astrocytoma, 3 with oligodendrogliomas, 3 with anaplastic oligodendrogliomas and 17 control spectra taken from healthy hemispheres.
Results: All tumours' metabolite ratios, except for Cho/Cr in fibrillary astrocytomas ($p = 0.06$), were statistically significantly different from the control. The tumours showed decreased Naa and Cr contents and a high Cho signal. The Lac-Lip signal was high in grade III astrocytomas and glioblastomas. Reports that Cho/Cr ratio increases with glioma's grade whereas Naa/Cr decreases were not confirmed. Anaplastic astrocytomas compared to grade II astrocytomas had a statistically significantly greater mI/Cr ratio ($p = 0.02$). In pilocytic astrocytomas the Naa/Cr value (2.58 ± 0.39) was greater, whilst the Cho/Naa ratio was lower (2.14 ± 0.64) than in the other astrocytomas. The specific feature of oligodendrogliomas was the presence of glutamate/glutamine peak

Streszczenie

Wstęp i cel pracy: Ustalenie charakterystyki spektroskopii magnetycznego rezonansu jądrowego (*magnetic resonance spectroscopy* – MRS) u chorych z nowotworami wewnątrzczaszkowymi pochodzenia glejowego oraz ocena przydatności tego badania w diagnostyce różnicowej typów histologicznych glejaków.
Materiał i metody: Przeprowadzono analizę widm MRS nowotworów u 26 chorych z glejakami wielopostaciowymi, 6 z gwiaździakami włókienkowymi, 4 z gwiaździakami anaplastycznymi, 2 z włosowatokomórkowymi, 3 ze skąpodrzewiakami, 3 ze skąpodrzewiakami anaplastycznymi oraz 17 widm kontrolnych pochodzących ze zdrowych półkul mózgu.
Wyniki: Wszystkie wskaźniki metaboliczne w przypadkach nowotworów, z wyjątkiem Cho/Cr w gwiaździakach włókienkowych ($p = 0{,}06$), różniły się znamiennie od tych w grupie kontrolnej. Nowotwory wykazywały zmniejszoną zawartość Naa i Cr oraz wysoki sygnał Cho. Sygnał Lac-Lip był wysoki w gwiaździakach III stopnia wg WHO i glejakach wielopostaciowych. Nie udało się potwierdzić doniesień, że wskaźnik Cho/Cr rośnie, a wskaźnik Naa/Cr maleje wraz ze wzrostem stopnia złośliwości glejaka. Gwiaździaki anaplastyczne wykazywały znamiennie wyższy wskaźnik mI/Cr ($p = 0{,}02$) w porównaniu z gwiaździakami II stopnia wg WHO. W gwiaździakach włosowatokomórkowych wartość Naa/Cr ($2{,}58 \pm 0{,}39$)

Correspondence address: Dariusz J. Jaskólski, Department of Neurosurgery and Oncology of the Central Nervous System, Medical University of Lodz, Barlicki Hospital, ul. Kopcińskiego 22, 90-153 Łódź, phone: +48 42 677 67 70, fax: +48 42 677 67 81, e-mail: dariusz.jaskolski@umed.lodz.pl
Received: 25.06.2012; accepted: 3.01.2013

Glx. However, this peak was absent in two out of three anaplastic oligodendrogliomas. Characteristically, the latter tumours had a high Lac-Lip signal.

Conclusions: MRS *in vivo* cannot be used as a reliable method for glioma grading. The method is useful in discrimination between WHO grade I and WHO grade II astrocytomas as well as oligodendrogliomas from other gliomas.

Key words: magnetic resonance spectroscopy, glioblastoma, astrocytoma, oligodendroglioma.

była większa, a Cho/Naa mniejsza (2,14 ± 0,64) niż w innych gwiaździakach. Skąpodrzewiaki charakteryzowała obecność szczytu glutaminianu/glutaminy (Glx), którego jednak nie obserwowano w 2 spośród 3 przypadków skąpodrzewiaków anaplastycznych. Dla tych ostatnich symptomatyczna była obecność silnego sygnału Lac-Lip.

Wnioski: Badanie MRS *in vivo* nie jest niezawodną metodą różnicującą glejaki wewnątrzczaszkowe. Wydaje się użyteczne w diagnostyce różnicowej gwiaździaków I i II stopnia wg WHO oraz w odróżnianiu skąpodrzewiaków od pozostałych glejaków.

Słowa kluczowe: MRS, glejak wielopostaciowy, gwiaździak, skąpodrzewiak.

Introduction

The progress of magnetic resonance imaging (MRI) has considerably facilitated diagnosis of brain tumours. One of its particular applications is magnetic resonance spectroscopy *in vivo* (MRS). To date, a number of reports have presented a correlation between MRS spectra and neuropathology of brain tumours [1-7]. This method proved to be helpful in *in vivo* grading of gliomas [8-11], discrimination between lymphomas and gliomas [12,13], metastases and gliomas [14], as well as neoplasms and non-neoplastic lesions [15,16]. It appears that MRS may add to the diagnostic accuracy of MRI [17], having also certain prognostic value in patients with malignant gliomas [18,19].

The aim of this study was to determine *in vivo* MRS characteristics in patients operated on at the Department of Neurosurgery, Medical University of Lodz, Poland and to compare these data with reports available in the literature, as well as to assess MRS reliability in glioma grading and discrimination between different histopathological types of tumours.

Material and methods

The study involved 89 patients with brain tumours who underwent neurosurgery at the Department of Neurosurgery of the Medical University in Lodz. There were 38 men and 51 females aged from 19 to 75. The study evaluated only patients with medium and large tumours i.e. the cases in which the investigator was able to set a voxel volume from 1 to 8 cubic centimetres. All patients gave informed consent to the study. The study protocol has been accepted by the University Bioethical Committee for Experiments on Human Subjects. The MR examinations were carried out as part of the eTumour project (LSHC-CT-2004-503094) – Web Accessible MR Decision Support System For Brain Tumour Diagnosis And Prognosis, Incorporating *In Vivo* And *Ex Vivo* Genomic And Metabolimic Data (http://cordis.europa.eu/fetch?).

The patients had an MRS examination a day prior to tumour surgery. This was performed with a Siemens Magnetom Vision scanner with magnetic flux density of 1.5T. First, T1-weighted images of a tumour were obtained in three planes (coronal, sagittal and axial) after intravenous administration of the contrast medium – gadolinium. Subsequently, the voxel was set in a solid and most strongly contrast-enhancing part of the tumour as seen in Fig. 1. The MRS examination was done by means of single voxel spectroscopy (SVS), employing

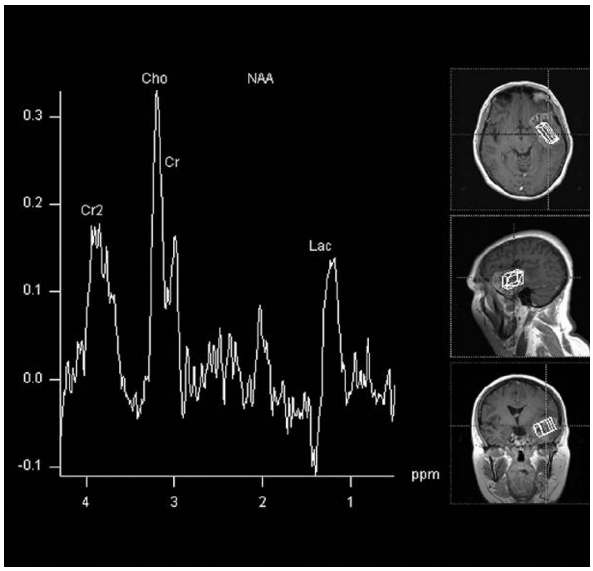

Fig. 1. The voxel placed in the solid and enhancing part of anaplastic oligodendroglioma shown in three planes (transverse, sagittal and coronal) and the characteristic spectrum of this neoplasm

Table 1. Clinical data of patients with glioblastoma

No.	Age	Sex	Tumour location
1.	64	F	Frontal
2.	54	F	Frontal
3.	63	F	Temporal
4.	20	F	Frontal
5.	38	F	Temporal
6.	60	M	Temporal
7.	48	M	Frontal
8.	63	F	Frontal
9.	59	F	Parietal
10.	64	M	Temporal
11.	53	F	Parietal
12.	68	M	Parietal
13.	70	M	Frontal
14.	50	M	Temporal
15.	53	F	Frontal
16.	64	M	Parietal
17.	45	M	Occipital
18.	54	F	Frontal
19.	48	M	Occipital
20.	64	M	Temporal
21.	54	F	Frontal
22.	49	M	Parietal
23.	66	M	Frontal
24.	26	M	Parietal
25.	56	M	Temporal
26.	55	F	Frontal

M – male; F – female

both short echo time (STEAM sequence with TE = = 30 ms or PRESS sequence with TE = 30 ms) and long echo time (PRESS TE = 136 ms or PRESS TE = 135 ms). In both instances the repetition time (TR) was 2000 ms. On average, 256 signal acquisitions were done in each sequence.

The control spectra of normal brain were obtained from the voxel placed in a healthy cerebral hemisphere in 17 patients.

All spectra were processed by means of the computer program Magnetic Resonance User Interface (MRUI, v.3) according to the protocol of the eTumour project [20]. The processing included synchronization of the spectrum phase with the reference spectrum representing only the signal of water, apodisation and removal of the signal of water employing the method of Hankel Lanczos singular values decomposition (HLSVD).

Qualitative analysis of the spectra included recognition of signals from the following metabolites: N-acetyl aspartate (Naa, chemical shift equals 2.02 ppm), choline (Cho – 3.22 ppm), creatinine (Cr – 3.02 ppm), alanine (Ala – 1.48 ppm), myo-inositol (mI – 3.56 ppm), glutamate (Glx – 2.35 ppm), lipids (Lip – 0.9 ppm and 1.3 ppm), and lactates (Lac – 1.33 ppm). The Advanced Method for Accurate, Robust and Efficient Spectral Fitting (AMARES) [21] was used for quantitative analysis. Its results were given as relative values, i.e. fractions of the signal amplitudes: Cho/Cr, Naa/Cr, Cho/Naa, mI/Cr. Contents of lactates and lipids were looked at together, as a Lac-Lip mixture [14,22], and determined by means of a semi-quantitative method: absent (–), present (+), or high (+++) [23]. Histopathological diagnosis was given in accordance with the WHO classification of brain tumours [24]. The results were compared with those obtained from the normal brain and between the examined tumours.

In the examined group there were 26 patients with glioblastomas, 17 with cerebral metastases, 14 with meningiomas, 3 with oligodendrogliomas, 3 with anaplastic oligodendrogliomas, 6 with fibrillary astrocytomas, 4 with anaplastic astrocytomas, 2 with pilocytic astrocytomas, 2 with haemangiopericytomas, 2 lymphomas, 6 schwannomas and single patients with Lhermitte-Duclos disease, choroid plexus papilloma, pituitary adenoma and, finally, chondroma. This paper deals only with tumours of glial origin. The clinical data on 26 patients with glioblastoma are given in Table 1 and on patients with other gliomas in Table 2. Mann-Whitney *U* test was used for the statistical analysis.

Results

Glioblastoma (WHO grade IV) was diagnosed in 26 patients: 14 males and 12 females aged 20 to 70 (mean age, 54 years). In the long TE sequence (Fig. 2), in 11 cases the MRS spectrum showed a high Cho signal, low peaks of Cr and Naa and a high Lac-Lip signal. In 5 other spectra, the only difference was that the Lac-Lip signals were much lower and in the other 7 there was a negative Lac signal. In 2 cases, there were low peaks of Cho, Cr and Naa together with a high Lac-Lip signal. Finally, one spectrum displayed nothing but a high Lac-Lip signal. In the short TE sequences (Fig. 3) 13 spectra had a low Cho signal and a high Lac-Lip

peak. In the other 4, apart from the aforementioned there was also a low Cr peak. In 8 cases the main finding was a high Lac-Lip signal. One spectrum had the following characteristics: high Cho, low Cr and low Lac-Lip. In 3 spectra high mI peaks were identified. Based on the spectra obtained in the long TE sequence, the following ratios were calculated: Cho/Cr = 5.65 ± 2.38, Naa/Cr = 1.65 ± 0.67 and Cho/Naa = 4.02 ± 2.09. The spectra of the short TE sequence gave two other ratios: Cho/Cr = 1.91 ± 0.72 and mI/Cr = 1.12 ± 0.33.

Anaplastic astrocytoma (WHO grade III) was found in 4 females (age 21-75, mean 52 years). In the long TE sequence (Fig. 4), 3 patients had a high Cho signal with low Cr and Naa peaks and a negative Lac band. The only difference in other spectra was a quite high Naa signal. In the short TE sequence (Fig. 5), all spectra had high Cho with low Cr and mI signals. In 3 spectra there was no Naa band. In every case there was a Lac-Lip signal which was very high in one patient.

In 6 patients fibrillary astrocytoma was diagnosed (WHO grade II). Females constituted 2/3 of this group. The age ranged from 19 to 46 years (mean, 31 years). In the long TE sequence (Fig. 6), 4 spectra displayed high Cho with low Cr and Naa signals. In the remaining cases there was also a negative Lac peak. In the short TE sequence (Fig. 7), 2/3 of the spectra had the following signals: high Cho, low Cr, low Lip-Lac and high mI. In the remainder, the Lip-Lac peak was absent. Based on the spectra obtained with the long TE sequence, the following ratios were calculated: Cho/Cr = = 4.53 ± 2.71, Naa/Cr = 1 ± 0.65, Cho/Naa = 3.32 ± 1.22. The short TE spectra gave two ratios: Cho/Cr = = 1.67 ± 0.65 and mI/Cr = 0.92 ± 0.13.

Pilocytic astrocytoma (WHO grade I) was diagnosed in 2 female patients – 35 and 39 years old. In the long TE sequence (Fig. 8) both spectra showed a high Cho and low Cr signal. In one there was a low Naa peak and a negative Lac band. Another one had no Naa band but displayed a high Lip signal. In the short TE sequence (Fig. 9) both spectra were the same, showing high Cho and lower Cr, Naa and mI with a high Lac-Lip doublet.

Oligodendroglioma (WHO grade II) was diagnos - ed in 3 patients (2 women and one man, 40-41 years). The long TE sequence spectra (Fig. 10) showed a high Cho signal in all cases (it was very high in two). Furthermore, two spectra displayed low Cr and Naa peaks. In one spectrum apart from the Cho signal there was only a negative Lac band. In the short TE sequence (Fig. 11) the following signals were observed: high Cho, low Cr, mI and Glx. One patient showed a Lip-Lac doublet.

Table 2. Clinical data of patients with gliomas of WHO grade I-III

No.	Age	Sex	Histopathology	Tumour location
1.	75	F	Anaplastic astrocytoma	Parietal
2.	21	F	Anaplastic astrocytoma	Frontal
3.	42	F	Anaplastic astrocytoma	Temporal
4.	71	F	Anaplastic astrocytoma	Temporal
5.	41	F	Oligodendroglioma	Frontal
6.	41	M	Oligodendroglioma	Frontal
7.	40	F	Oligodendroglioma	Parietal
8.	65	F	Anaplastic oligodendroglioma	Temporal
9.	63	F	Anaplastic oligodendroglioma	Parietal
10.	64	F	Anaplastic oligodendroglioma	Frontal
11.	36	F	Fibrillary astrocytoma	Frontal
12.	46	F	Fibrillary astrocytoma	Temporal
13.	26	M	Fibrillary astrocytoma	Parietal
14.	19	F	Fibrillary astrocytoma	Parietal
15.	29	M	Fibrillary astrocytoma	Temporal
16.	30	F	Fibrillary astrocytoma	Frontal
17.	35	F	Pilocytic astrocytoma	Cerebellum
18.	39	F	Pilocytic astrocytoma	Cerebellum

M – male; F – female

Anaplastic oligodendroglioma (WHO grade III) was found in 3 women (63-65 years old). All spectra obtained with the long TE sequence presented high Cho and low Cr and Naa signals (Fig. 1). Furthermore, in one patient there was a negative Lac band and in another a positive Lac-Lip signal. The short TE sequence spectra revealed only high Lac-Lip peaks.

The small number of patients restricted statistical analysis of the metabolic contents only to patients with the following tumours: glioblastoma, fibrillary astrocytoma and anaplastic astrocytoma. All ratios were statistically significantly different from the control. The only exception was the Cho/Cr ratio in fibrillary astrocytoma ($p = 0.06$). Concentration of the metabolites in glioblastoma was not significantly different when compared to anaplastic and fibrillary astrocytomas. Anaplastic astrocytomas had higher contents of mI than fibrillary astrocytomas ($p = 0.02$).

All the results are summarized in Tables 3 and 4.

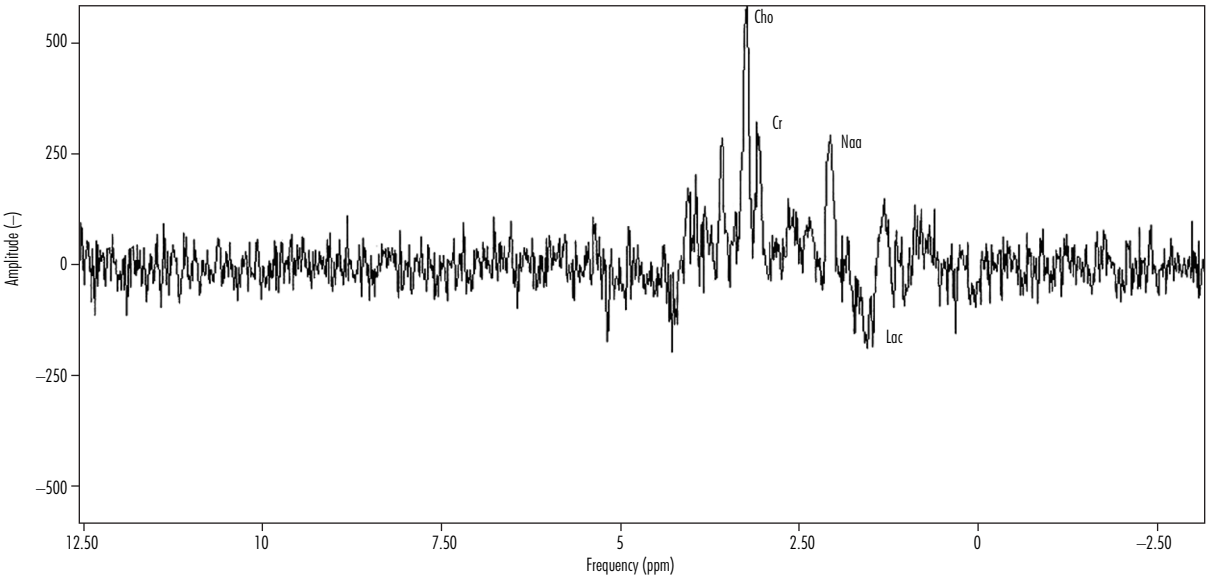

Fig. 2. Long-TE spectrum of glioblastoma

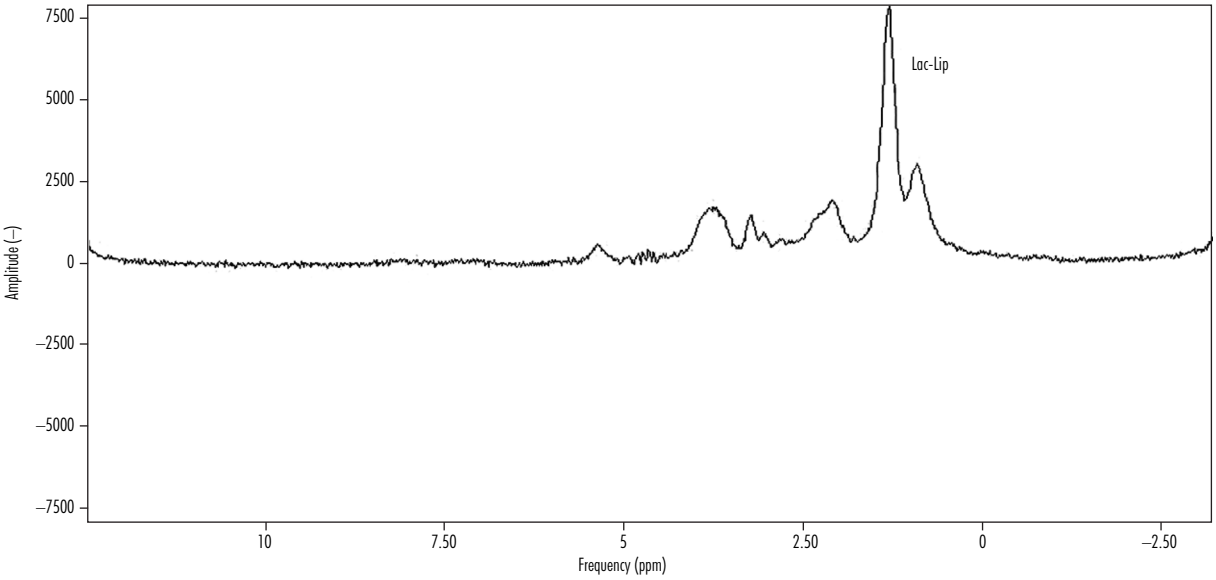

Fig. 3. Short-TE spectrum of glioblastoma

Discussion

MRS of gliomas shows decreased contents of Naa [25-28] since neurons are superseded by the neoplasm cells. For the same reason, a decreased level of Cr is observed [26,28,29]. Furthermore, astrocytomas show high Cho contents [28,30-32] due to increased synthesis of cellular membranes inside the tumour.

In our patients with diffuse gliomas those features were apparent in all types of tumours. The only exception was the Cho/Cr ratio in fibrillary astrocytomas since it was not significantly different from the one found in normal brain. This may be explained by the fact that some of these neoplasms have cellular density only slightly increased with relative preservation of the local cytoarchitecture.

Some tumours show Lac and Lip signals, which are markers of anaerobic metabolism and necrosis [33,34]. There are those who claim that high Lac-Lip concentration helps to distinguish high and low grade gliomas

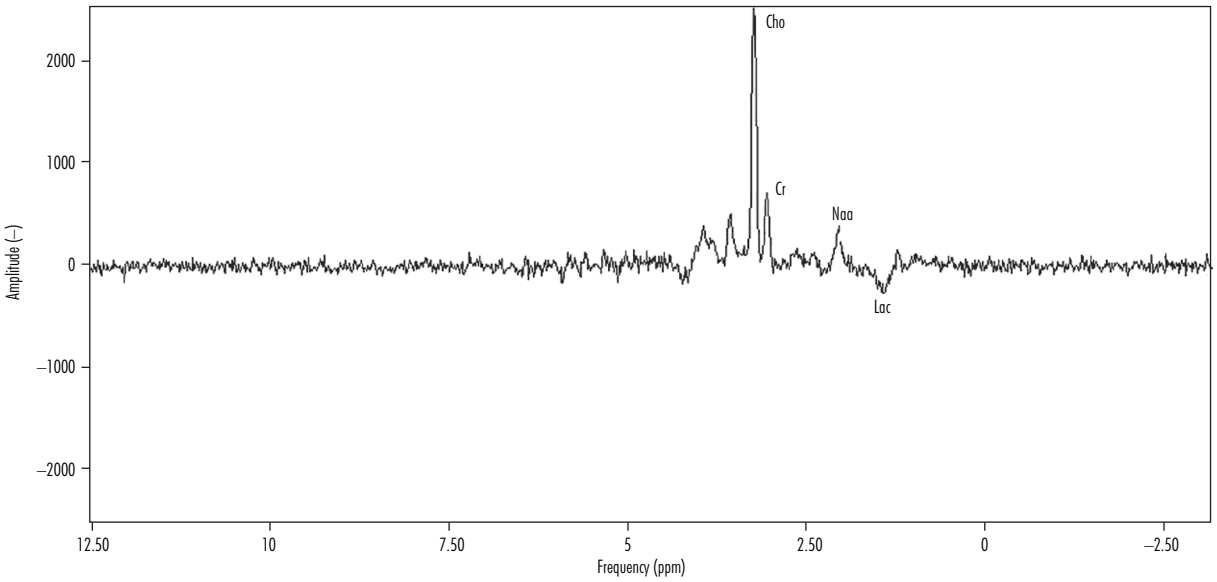

Fig. 4. Long-TE spectrum of anaplastic astrocytoma

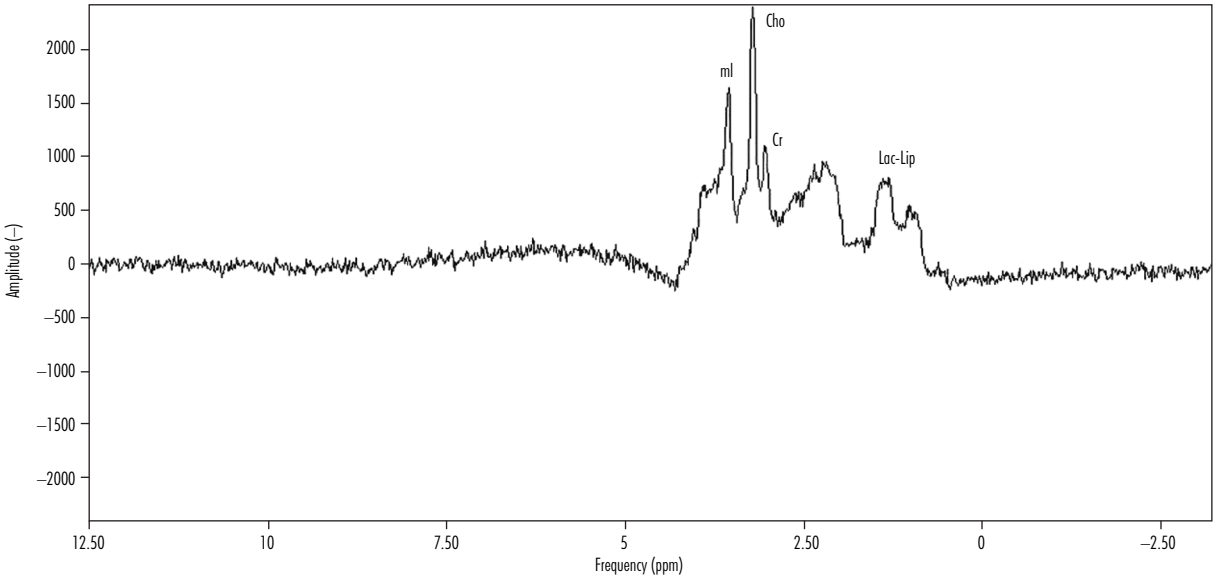

Fig. 5. Short-TE spectrum of anaplastic astrocytoma

[35-38]. One study does not support this thesis [39]. Another report showed that glioblastomas have a higher Lac-Lip concentration than anaplastic astrocytomas [6]. Interestingly enough, the main source of the Lac-Lip signal came from the lipids. Nonetheless, the authors of this study admit that absence of the Lac-Lip signal does not exclude the diagnosis of high grade glioma. In our material peak Lac-Lip was present in all instances of glioblastoma. With one exception the signal was very high (++) in the STEAM sequence. In the patients with anaplastic astrocytoma this band was always present but it was high only in one case. In contrast, fibrillary astrocytoma displayed Lac-Lip in 2/3 of the cases, none of which reached a high level. Hence, it seems that Lac-Lip signal magnitude may be of assistance in distinguishing WHO grade II from WHO II and III gliomas.

Several reports state that Cho contents measured with the Cho/Cr ratio increase with the grade of astrocytoma [35,38,40-44]. This was also proven *in vitro*

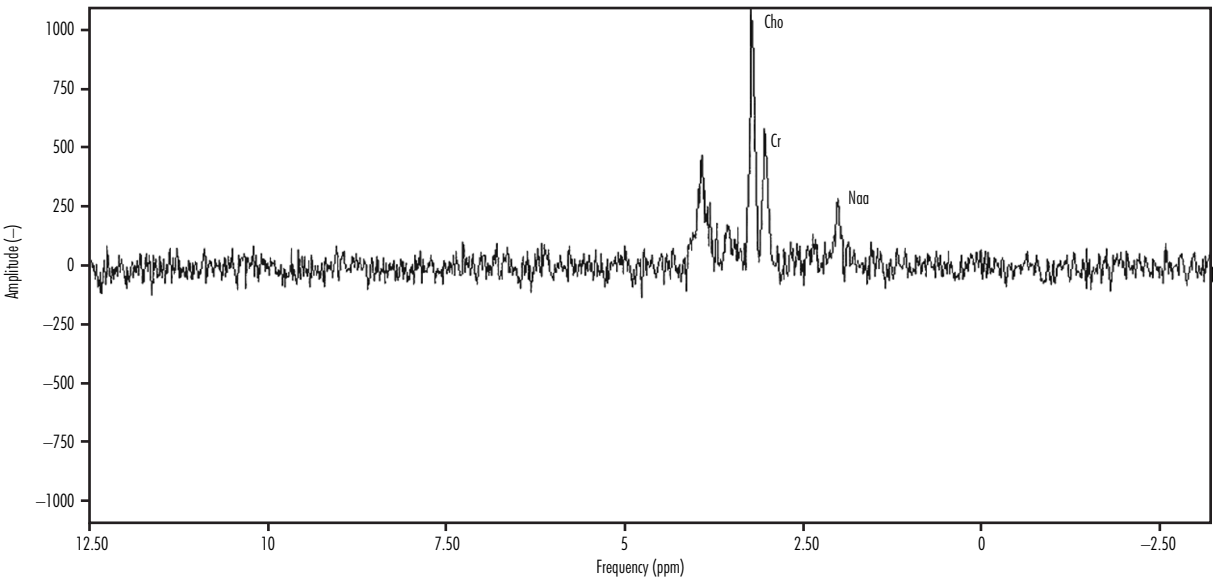

Fig. 6. Long-TE spectrum of fibrillary astrocytoma

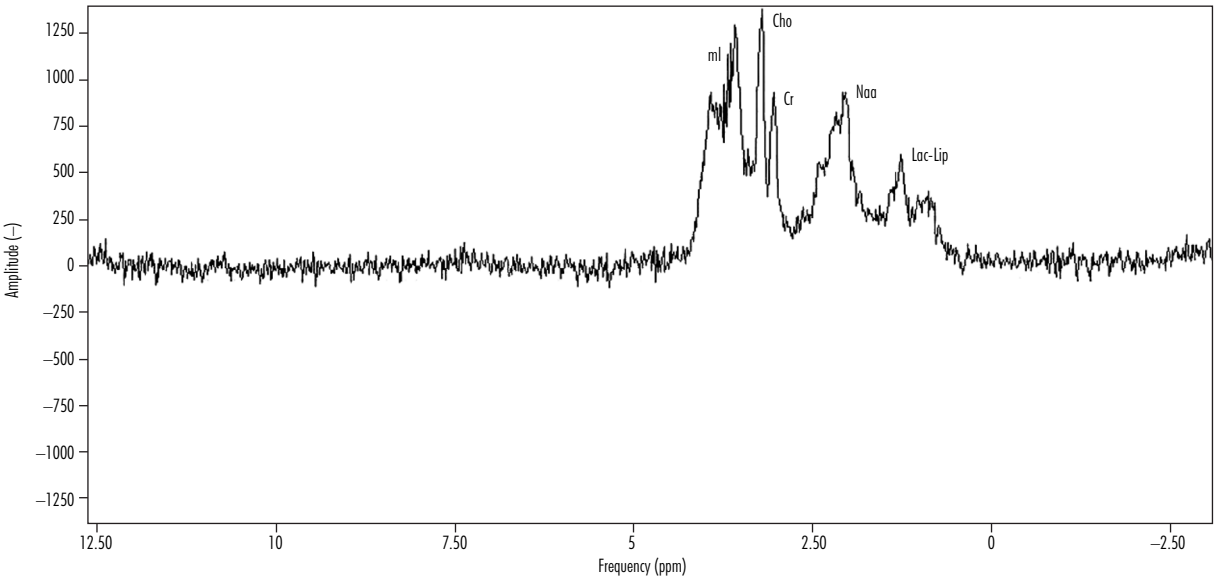

Fig. 7. Short-TE spectrum of fibrillary astrocytoma

[44-46]. However, there are papers claiming that this ratio might be equal or even lesser in WHO grade IV than in astrocytomas grade II-III [36,39,45].

The decrease of Cho/Cr ratio in glioblastoma is explained by the presence of necrosis in these tumours [36]. Our study did not show a statistically significant difference in Cho/Cr ratio between different gliomas.

There were suggestions that the Naa/Cr ratio de - creases with increased malignancy of glioma [44,46,47]. Other authors, however, do not confirm this correlation [35,39,41,42,44,45]. Also in our study, we found no significant difference in Naa/Cr ratio in different types of gliomas. The absence of the expected decrease of Naa/Cr may result from superimposing the lipid signal on the Naa band during the short TE sequence [48].

Despite certain discrepancies, it was suggested that MRS can distinguish astrocytomas of grade II from grade III and IV with the accuracy exceeding 95% [49]. The most difficult task remains to show an MRS difference between anaplastic astrocytomas and glioblas-

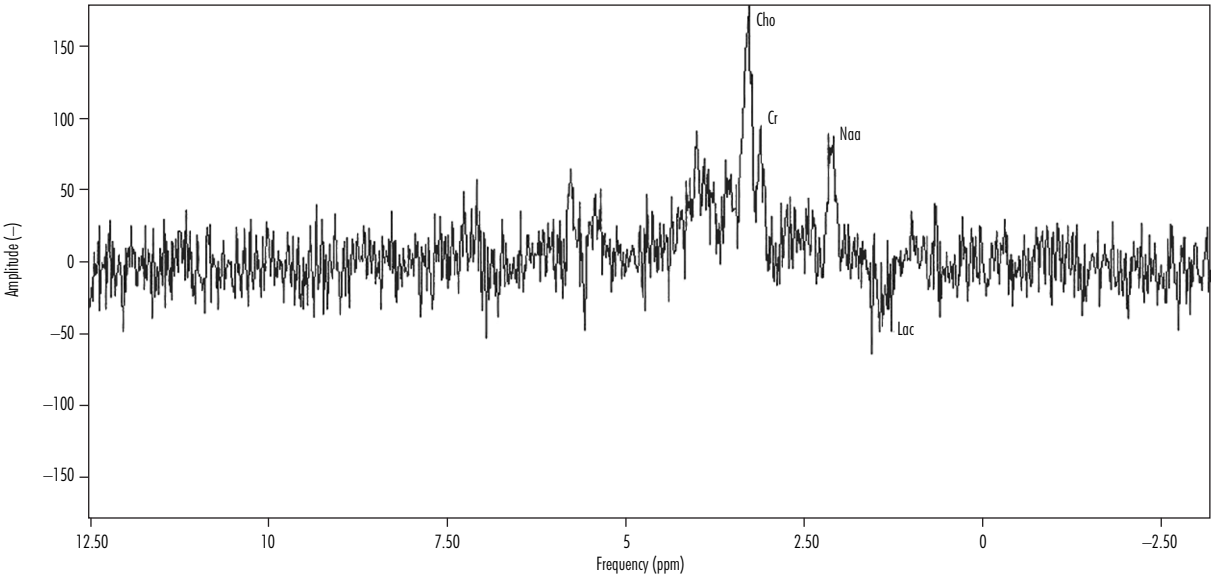

Fig. 8. Long-TE spectrum of pilocytic astrocytoma

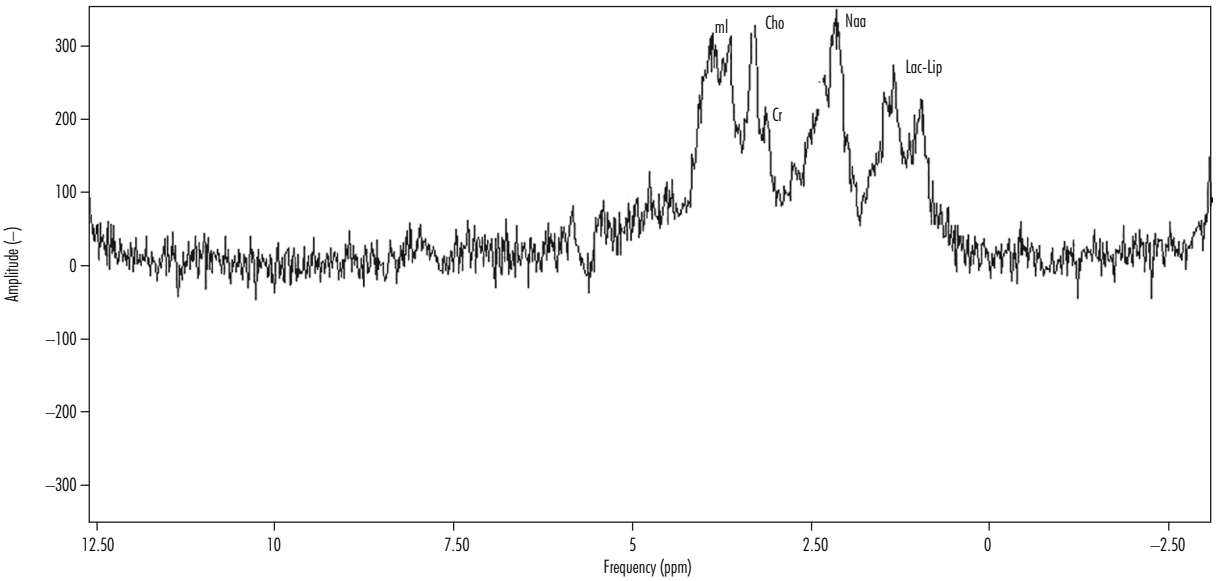

Fig. 9. Short-TE spectrum of pilocytic astrocytoma

toma. This is chiefly due to difficulties in placing the voxel in the representative part of the neoplasm [49].

Another potentially useful metabolite is mI. A study on *in vitro* MRS showed that mI concentration was the only factor discriminating astrocytomas of different grades [50]. It was suggested that mI/Cr level is the highest in WHO grade II astrocytomas [51], whereas the level observed in anaplastic astrocytoma was lower than in normal brain but still greater than the one found in glioblastoma. This was explained by the fact that in malignant gliomas mI is used for synthesis of phosphatidylinositol, whose derivative products (diacylglycerol) are indirect activators of metalloproteinases responsible for spreading of the neoplasm in the brain [52-54]. To date, the increase of mI in grade II astrocytomas has not been explained.

However, also opposite suggestions can be encountered in the literature [55]. One *in vitro* study showed that glioblastomas have a greater mI/Cr ratio than grade II and III astrocytomas [56]. In our study the mI/Cr ratio was greater in grade III than in grade II astrocy-

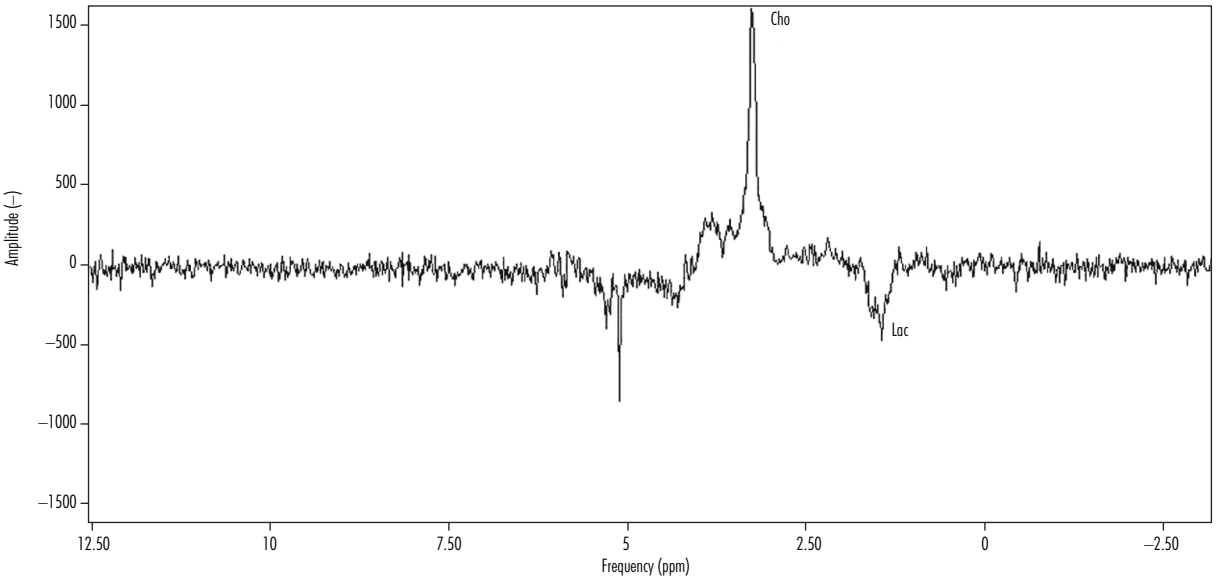

Fig. 10. Long-TE spectrum of oligodendroglioma

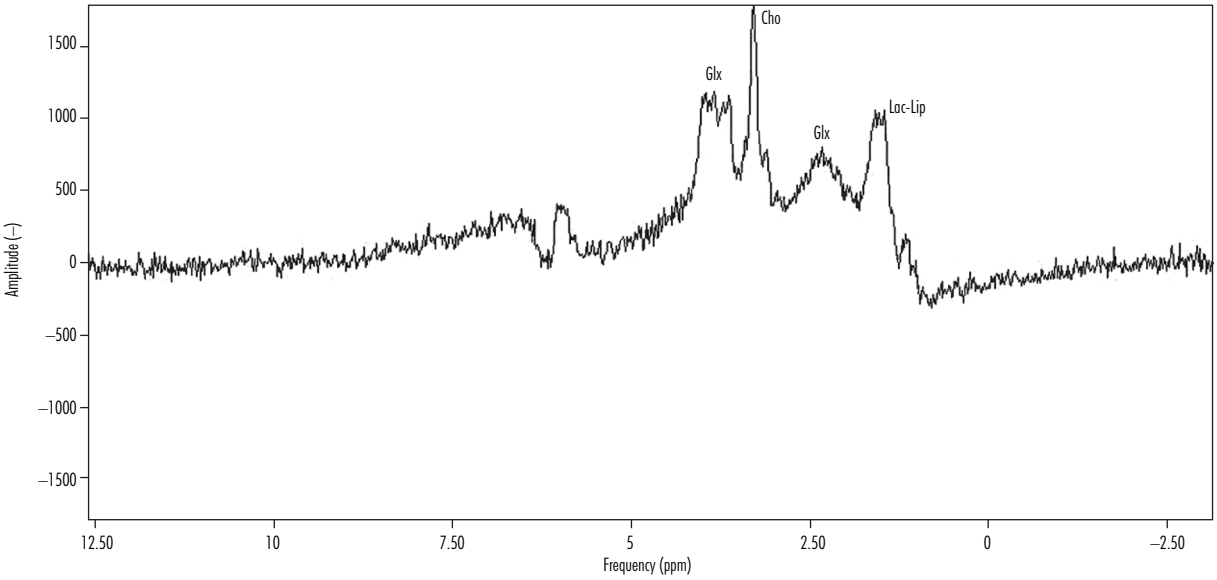

Fig. 11. Short-TE spectrum of oligodendroglioma

tomas and there was no significant difference in mI/Cr between glioblastomas and other types of gliomas. Moreover, all astrocytomas had a significantly greater mI/Cr value than the normal brain. Apparently, our results did not support the data given in the discussed paper. This might be due to the fact that our study involved only patients with newly diagnosed tumours whilst the subjects in the discussed article had been operated on and given radio- and/or chemotherapy 1 to 3 years before the MRS was done.

According to some studies, glycine may be found in the spectra of some WHO grade II gliomas [57]. If present, this metabolite may play some role in the glioma grading. In our study no single glycine peak was identified in any of the glioma spectra.

There are no reports in the literature concerning MRS discrimination of grade I and II astrocytomas. Usually, these neoplasms are placed together in a single group to be compared with other tumours. There is a single paper which gives a brief account of a spectrum of pilocytic

Table 3. Metabolite ratios (mean value and standard deviation) in different gliomas and in the normal brain. Differences between each tumour and the control were recognised as statistically significant if $p < 0.05$

	Cho/Cr		Naa/Cr		Cho/Naa		mI/Cr
	PRESS	STEAM	PRESS	STEAM	PRESS	STEAM	STEAM
Control	1.12 ± 0.25	1.06 ± 0.34	2.1 ± 0.3	1.86 ± 0.54	0.52 ± 0.1	0.59 ± 0.12	0.46 ± 0.07
Glioblastoma	5.65 ± 2.38 $p = 0.0009$	1.91 ± 0.72 $p = 0.0299$	1.65 ± 0.67 $p = 0.0059$	–	4.02 ± 2.09 $p = 0.0006$	–	1.12 ± 0.33 $p = 0.0068$
Fibrillary astrocytoma	4.53 ± 2.71 $p = 0.0106$	1.67 ± 0.65	1 ± 0.65 $p = 0.0059$	–	3.32 ± 1.22 $p = 0.0059$	–	0.92 ± 0.13 $p = 0.0008$
Anaplastic astrocytoma	5.49 ± 3.16 $p = 0.0105$	2.69 ± 1.11 $p = 0.0128$	1.11 ± 0.2 $p = 0.0196$	–	4.1 ± 2.53 $p = 0.0201$	–	2.98 ± 1.83 $p = 0.0068$
Pilocytic astrocytoma	5.41 ± 0.81	4.04	2.58 ± 0.39	–	2.14 ± 0.64	–	3.19
Oligodendroglioma	2.94	4.1 ± 0.47	0.99	–	2.98	–	–
Anaplastic oligodendroglioma	4.96	–	–	–	–	–	–

Cho – choline; Cr – creatinine; mI – myo-inositol; Naa – N-acetyl aspartate; PRESS – Point Resolved Spectroscopy; STEAM – Stimulated Echo Acquisition Mode

astrocytoma located in the cerebral hemisphere [49]. The MRS spectrum obtained during the long TE sequence contained a high Cho signal, somewhat decreased bands of Cr and Naa, with a small negative doublet Lac. The specific feature of this tumour as compared to higher grade gliomas was a relatively high level of both Cr and Naa. In our patients MRS spectra of the pilocytic astrocytomas showed a high Cho signal with low Cr and Naa in the presence of negative Lac and high Lip. The short TE sequence revealed in both our cases a spectrum with a high Cho signal, lower Cr, Naa and mI with a high Lac-Lip doublet. The increase of Cho calculated on the basis of the Cho/Cr ratio was 5.47 ± 0.81; thus it was comparable with glioblastomas and anaplastic astrocytomas but apparently higher than in fibrillary astrocytomas. The high Cho/Cr probably results from a decrease of Cr concentration. This in turn could be explained by concealing a part of the Cr peak in the descending portion of the Cho signal curve or placing the voxel partly over a cystic part of the tumour. The value Naa/Cr = 2.58 ± 0.39 was greater than in other astrocytomas. The increased contents of Naa in comparison to grade II-IV gliomas was confirmed by the Cho/Naa ratio of 2.14 ± 0.64, lower than in other tumours. According to the previously discussed papers, the higher Naa content is characteristic for low grade gliomas. The high Lac-Lip signals are probably caused by the presence of these metabolites in the cystic part of the tumour.

The MRS spectrum of oligodendrogliomas is very similar to that found in astrocytomas: high Cho signal, decreased Naa and Cr with present Lac-Lip and mI. A specific feature is the presence of a glutamate/glutamine peak (Glx). This was confirmed in the literature as the only factor discriminating oligodendrogliomas from astrocytomas [58]. Similar findings were confirmed *in vitro* [59]. In our 6 patients with oligodendrogliomas, a clear Glx signal was identified in all 3 WHO grade II tumours and in 1 out of 3 WHO grade III neoplasms. In contrast, this band was not present in 9 cases with astrocytomas of grade II and III. This is consistent with the literature. The study quoted above [58] showed that

Table 4. Other metabolites found in the examined gliomas

	Glx	Ala	Lac-Lip	
			PRESS	STEAM
Glioblastoma	–	–	15++ 2+	26++ 1+
Fibrillary astrocytoma	–	–	2+ 4–	4+ 2–
Anaplastic astrocytoma	–	–	4+	1++ 3+
Pilocytic astrocytoma	–	–	2+	2++
Oligodendroglioma	3+	–	1+ 2–	1+ 2–
Anaplastic oligodendroglioma	1+	–	1++ 1+ 1–	3++

Ala – alanine; Glx – glutamate; Lac – lactates; Lip – lipids; PRESS – Point Resolved Spectroscopy; STEAM – Stimulated Echo Acquisition Mode absent (–), present (+), high (+++), the numbers indicate how many patients had the respective result

the high Lac-Lip signal is characteristic for anaplastic oligodendroglioma. This was confirmed in our material as the high Lac-Lip peak appeared in all 3 patients with this tumour. In oligodendroglioma (WHO grade II), there was only one instance of a low (+) Lac-Lip signal. Hence, the assessment of concentration of those metabolites may be of assistance in distinguishing oligodendrogliomas from anaplastic oligodendrogliomas.

Conclusions

At present, it seems that *in vivo* MR spectroscopy cannot be used as a reliable method for glioma grading. Nevertheless, it is useful in discrimination between WHO grade I and WHO grade II astrocytomas as well as oligodendrogliomas and other gliomas.

Disclosure

Authors report no conflict of interest.

References

1. Alger J.R., Cloughesy T.F. Structural and functional imaging of cerebral neoplasia. In: Mazziotta J.C., Toga A.W., Frackowiak R.S. [eds.]. Brain-mapping the disorders. *Academic Press*, San Diego 2000, pp. 387-416.
2. Bruhn H., Frahm J., Gyngell M., et al. Noninvasive differentiation of tumors with use of localized H1 MR spectroscopy in vivo: initial experience in patients with cerebral tumors. *Radiology* 1989; 172: 541-548.
3. Fortuniak J., Jaskólski D., Stefańczyk L., et al. Magnetic resonance imaging of rare intracranial neoplasms – role of the in vivo 1 H spectroscopy in the radiological differential diagnostics. *Cent Eur Neurosurg* 2010; 71: 181-188.
4. Horsztyński D., Wawer I., Grieb P. A study of lyophylized human brain tumor tissue using MAS 1H-MRS spectroscopy. *Neurol Neurochir Pol* 2003; 37: 123-132.
5. Kubas B., Tarasów E., Dzienis W., et al. Magnetic resonance proton spectroscopy in neurooncology – preliminary report. *Neurol Neurochir Pol* 2001; 35 (Suppl 5): 90-100.
6. Negendak W., Sauter R., Brown T., et al. Proton magnetic re - sonance spectroscopy in patients with glial tumors. A multicenter study. *J Neurosurg* 1996; 84: 449-458.
7. De Edelenyi F.S., Rubin C., Estève F., et al. A new approach for analyzing proton magnetic resonance spectroscopic images of brain tumors: nosologic images. *Nat Med* 2000; 6: 1287-1289.
8. Bendini M., Marton E., Feletti A., et al. Primary and metastatic intraaxial brain tumors: prospective comparison of multivoxel 2D chemical-shift imaging (CSI) proton MR spectroscopy, perfusion MRI, and histopathological findings in a group of 159 patients. *Acta Neurochir* (*Wien*) 2011; 153: 403-412.
9. Fountas K.N., Kapsalaki E.Z., Vogel R.L., et al. Noninvasive histologic grading of solid astrocytomas using proton magnetic resonance spectroscopy. *Stereotact Funct Neurosurg* 2004; 82: 90-97.
10. Herminghaus S., Pilatus U., Möller-Hartmann W., et al. Increased choline levels coincide with enhanced proliferative activity of human neuroepithelial brain tumors. *NMR Biomed* 2002; 15: 385-392.
11. Meyerand M.E., Pipas J.M., Mamourian A., et al. Classification of biopsy-confirmed brain tumors using single-voxel MR spectroscopy. A*JNR Am J Neuroradiol* 1999; 20: 117-123.
12. Chawla S., Zhang Y., Wang S., et al. Proton magnetic resonance spectroscopy in differentiating glioblastomas from primary cerebral lymphomas and brain metastases. *J Comput Assist Tomogr* 2010; 34: 836-841.
13. Harting I., Hartmann M., Jost G., et al. Differentiating primary central nervous system lymphoma from glioma in humans using localised proton magnetic resonance spectroscopy. *Neurosci Lett* 2003; 342: 163-166.
14. Ishimaru H., Morikawa M., Iwanaga S., et al. Differentiation between high-grade glioma and metastatic brain tumor using single-voxel proton MR spectroscopy. *Eur Radiol* 2001; 11: 1784-1791.
15. Chang K.H., Song I.C., Kim S.H., et al. In vivo single-voxel proton MR spectroscopy in intracranial cystic masses. *AJNR Am J Neuroradiol* 1998; 19: 401-405.
16. Poptani H., Gupta R.K., Roy R., et al. Characterization of intracranial mass lesions with in vivo proton MR spectroscopy. *AJNR Am J Neuroradiol* 1995; 16: 1593-1603.
17. Möller-Hartmann W., Herminghaus S., Krings T., et al. Clinical application of proton magnetic resonance spectroscopy in the diagnosis of intracranial mass lesions. *Neuroradiology* 2002; 44: 371-381.
18. Tarnawski R., Sokol M., Pieniazek P., et al. 1H-MRS in vivo predicts the early treatment outcome of postoperative radiotherapy for malignant gliomas. *Int J Radiat Oncol Biol Phys* 2002; 52: 1271-1276.
19. Czernicki T., Szeszkowski W., Marchel A., et al. Spectral changes in postoperative MRS in high-grade gliomas and their effect on patient prognosis. *Folia Neuropathol* 2009; 47: 43-49.
20. Celda B., Monleón D., Martinez-Bisbal M.C., et al. MRS as endogenous molecular imaging for brain and prostate tumours: FP6 project "eTUMOUR". *Adv Exp Med Biol* 2006; 587: 285-302.
21. Vanhamme L., van den Boogaart A., Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129: 35-43.
22. Fan G., Sun B., Wu Z., et al. In vivo single-voxel proton MR spectroscopy in the differentiation of high-grade gliomas and solitary metastases. *Clin Radiol* 2004; 59: 77-85.
23. Gajewicz W., Papierz W., Szymczak W., et al. The use of proton MRS in the differential diagnosis of brain tumors and tumor-like processes. *Med Sci Monit* 2003; 9: 97-105.
24. Kleinhues P., Cavenee W.K. Pathology and genetics of tumors of the nervous system. *IARC Press*, Lyon 2000.
25. Florian C.L., Preece N.E., Bhakoo K.K., et al. Characteristic metabolic profiles revealed by 1H NMR spectroscopy for three

types of human brain and nervous system tumours. *NMR Biomed* 1995; 8: 253-264.
26. Kinoshita Y., Yokota A. Absolute concentrations of metabolites in human brain tumors using in vitro proton magnetic resonance spectroscopy. *NMR Biomed* 1997; 10: 2-12.
27. Nadler J.V., Cooper J.R. N-acetyl-L-aspartic acid content of human neural tumors and bovine peripheral nervous tissues. *J Neurochem* 1972; 19: 313-319.
28. Wilken B., Dechent P., Herms J., et al. Quantitative proton magnetic resonance spectroscopy of focal brain lesions. *Pediatr Neurol* 2000; 23: 22-31.
29. Lowry O.H., Berger S.J., Chi M.M., et al. Diversity of metabolic patterns in human brain tumors. High energy phosphate compounds and basic composition. *J Neurochem* 1977; 29: 959-977.
30. Kondziolka D., Lunsford L.D., Martinez A.J. Unreliability of contemporary neurodiagnostic imaging in evaluating suspected adult supratentorial (low grade) astrocytoma. *J Neurosurg* 1993; 79: 533-536.
31. Podo F. Tumor phospholipid metabolism. *NMR Biomed* 1999; 12: 413-439.
32. Ruiz-Cabello J., Cohen J.S. Phospholipid metabolites as indicators of cancer cell function. *NMR Biomed* 1992; 5: 226-233.
33. Gotsis E.D., Fountas K., Kapsalaki E., et al. In vivo proton MR spectroscopy: the diagnostic possibilities of lipid resonances in brain tumors. *Anticancer Res* 1996; 16: 1565-1567.
34. Herholz K., Heindel W., Luyten P.R., et al. In vivo imaging of glucose consumption and lactate concentration in human gliomas. *Ann Neurol* 1992; 31: 319-327.
35. Arnold D.L., Shoubridge E.A., Villemure J.G., et al. Proton and phosphorus magnetic resonance spectroscopy of human astrocytomas in vivo. Preliminary observations on tumor grading. *NMR Biomed* 1990; 3: 184-189.
36. Fulham M.J., Bizzi A., Dietz M.J., et al. Mapping of brain tumor metabolites with proton MR spectroscopic imaging: clinical relevance. *Radiology* 1992; 185: 675-686.
37. Heesters M.A., Kamman R.L., Mooyaart E.L., et al. Localized proton spectroscopy of inoperable brain gliomas. Response to radiation therapy. *J Neurooncol* 1993; 17: 27-35.
38. Henriksen O., Wieslander S., Gjerris F., et al. In vivo 1H-spectroscopy of human intracranial tumors at 1,5 Tesla. Preliminary experience at a clinical installation. *Acta Radiol* 1991; 32: 95-99.
39. Kugel H., Heindel W., Ernestus R., et al. Human brain tumors: spectral patterns detected with localized H-1 MR spectroscopy. *Radiology* 1992; 183: 701-709.
40. Liu Z.L., Zhou Q., Zeng Q.S., et al. Noninvasive evaluation of cerebral glioma grade by using diffusion-weighted imaging-guided single-voxel proton magnetic resonance spectroscopy. *J Int Med Res* 2012; 40: 76-84.
41. Ott D., Hennig J., Ernst T. Human brain tumours: assessment with in vivo proton MR spectroscopy. *Radiology* 1993; 186: 745-752.
42. Segebarth C.M., Baleriaux D.F., Luyten P.R., et al. Detection of metabolic heterogenity of human intracranial tumors in vivo by 1H NMR spectroscopic imaging. *Magn Reson Med* 1990; 13: 62-76.
43. Sutton L.N., Wang Z., Gusnard D., et al. Proton magnetic resonance spectroscopy of pediatric brain tumors. *Neurosurgery* 1992; 31: 195-202.
44. Usenius J.P., Kauppinen R.A., Vainio P.A., et al. Quantitative metabolite patterns of human brain tumors: detection by 1H NMR spectroscopy in vivo and in vitro. *J Comput Assist Tomogr* 1994; 18: 705-713.
45. Demaerel P., Johannik K., van Hecke P., et al. Localized H-NMR spectroscopy in fifty cases of newly diagnosed intracranial tumors. *J Comput Assist Tomogr* 1991; 15: 67-76.
46. Gill S.S., Thomas D.G., van Bruggen N., et al. Proton spectroscopy of intracranial tumours: in vivo and in vitro studies. *J Comput Assist Tomogr* 1990; 14: 497-504.
47. Peeling J., Sutherland G. 1H NMR spectroscopy studies of extracts of human cerebral neoplasms. *Magn Reson Med* 1992; 24: 123-136.
48. Hagberg G., Burlina A.P., Mader I., et al. In vivo proton MR spectroscopy of human gliomas: definition of metabolic coordinates for multi dimensional classification. *Magn Reson Med* 1995; 34: 242-252.
49. Herminghaus S., Dierks T., Pilatus U., et al. Determination of histopathological tumor grade in neuroepithelial brain tumors by using spectral pattern analysis of in vivo spectroscopic data. *J Neurosurg* 2003; 98: 74-81.
50. Maxwell R.J., Martinez-Pérez I., Cerdàn S., et al. Pattern recognition analysis of H1 NMR spectra from perchloric acid extracts of human brain tumor biopsies. *Magn Reson Med* 1998; 39: 869-877.
51. Castillo M., Smith K., Kwock L. Correlation of myo-inositol levels and grading of cerebral astrocytomas. *AJNR Am J Neuroradiol* 2000; 21: 1645-1649.
52. Franke T.F., Kaplan D.R., Cantley L.C., et al. Direct regulation of the AKT protooncogene product by phosphatidylinositol 3,4+bisphosphate. *Science* 1997; 275: 665-668.
53. Toker A., Cantley J.C. Signalling through the lipid products of phosphoinisitide-3-OH kinase. *Nature* 1997; 387: 673-676.
54. Uhm J.H., Doley N.P., Villemure J.G., et al. Glioma invasion in vitro: regulation by matrix metalloprotease-2 and protein kinase C. *Clin Exp Metastasis* 1996; 14: 421-433.
55. Candiota A.P., Majós C., Julià-Sapé M., et al. Non-invasive grading of astrocytic tumours from the relative contents of myo-inositol and glycine measured by in vivo MRS. *JBR-BTR* 2011; 94: 319-329.
56. Cheng L.L., Chang I.W., Louis D.N., et al. Correlation of high resolution magic angle spinning proton magnetic resonance spectroscopy with histopathology of intact human brain tumor specimens. *J Cancer Res* 1998; 58: 1825-1832.
57. Bobek-Billewicz B., Hebda A., Stasik-Pres G., et al. Measurement of glycine in a brain and brain tumors by means of 1H MRS. *Folia Neuropathol* 2010; 48: 190-199.
58. Rijpkema M., Schuuring J., van der Meulen Y., et al. Characterization of oligodendrogliomas using short echo time 1H MR spectroscopic imaging. *NMR Biomed* 2003; 16: 12-18.
59. Tugnoli V., Tosi M.R., Barbarella G., et al. Magnetic resonance spectroscopy study of low grade extra and intracerebral human neoplasms. *Oncol Rep* 1998; 5: 1199-1203.