Medicina (Kaunas) 2012;48(10):497–506

Magnetic Resonance Study on Fractional Anisotropy and Neuronal Metabolite Ratios in Peritumoral Area of Cerebral Gliomas

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Key Words: *cerebral glioma; tumor border; fractional anisotropy; magnetic resonance spectroscopy.*

Summary. *Background and Objective. Cerebral gliomas have a tendency to infiltrate the surrounding brain tissue for several centimeters from the core of tumor. The usefulness of structural magnetic resonance (MR) sequences is limited because of their insensitivity for the detection of tumor cells outside the visible tumor border. The aim of this study was to investigate the validity and the repeatability of 2 functional MR methods: fractional anisotropy (FA) and spectroscopy in the assessment of the peritumoral area of cerebral gliomas.*

Material and Methods. Forty-five patients with histologically verified brain gliomas underwent diffusion tensor imaging (DTI) and MR spectroscopy (MRS). Metabolic ratios were calculated from choline (Cho), creatine (Cr), N-acetylaspartate (NAA), lactate/lipids (LL), myo-inositol (MI) spectroscopic values obtained within the tumor center, perifocal edema, and distant and contralateral normal-appearing white matter. DTI maps of FA were calculated at the same locations.

Results. A significant gradual increase of FA and a decrease of LL/Cr ratios from the tumor center to the normal-appearing white matter were observed. The Cho/Cr ratio was significantly lower in the distant normal-appearing white matter than in the perifocal edema and the tumor center. The NAA/Cr ratio was significantly reduced in the tumor center, perifocal edema, and distant normal-appearing white matter compared with the contralateral hemisphere. MRS and DTI measurements of glioma and peritumoral area had a high degree of repeatability.

Conclusions. Our study shows that MRS and DTI measurements are reproducible. The combined use of Cho/Cr, LL/Cr, and FA measurements is a promising MR technique that provides valuable additional information about the location of glioma potential border.

Introduction

Glioma is the most common malignant primary tumor that arises in the brain (1). Despite the development of advanced surgical techniques, new methods of focused radiotherapy, and novel chemotherapy schemes, the majority of glial brain tumors recur (2) due to the invasive growth pattern (3). Malignant infiltrative brain gliomas have a tendency to infiltrate the surrounding white matter for several centimeters from the core of tumor (4). The recognition of gliomas border invasion is a relevant clinical problem (5). The use of conventional structural magnetic resonance (MR) sequences is limited because of their insensitivity for the detection of tumor cells outside the visible tumor border (4, 6).

MR spectroscopy (MRS) is a noninvasive imaging of metabolic changes within the brain (7, 8). *It* can provide quantitative information about the main metabolites, which are involved in different processes in the nervous system (9). N-acetylaspartate (NAA) is as an axonal marker (6). The concentration of NAA decreases in many diseases of white matter, including leukodystrophies, multiple sclerosis, and malignant tumors (10). Choline (Cho) is a marker of cell membrane (6). It increases in brain tumors and inflammation (10). Creatine (Cr) is a marker of energy metabolism (6, 10). Cr is considered a stable metabolite. It is used as a reference for relative metabolite quantification (11). Lactate is a product of anaerobic glycolysis. It increases in brain ischemia, hypoxia, seizures, metabolic disorders and acute inflammation, and necrotic and cystic tumors (10). Lipids are the products of brain destruction. An elevation in lipid levels is observed in infarction, acute multiple sclerosis, or necrosis (12). Myo-inositol (MI) locates in brain glial cells and acts as an osmolyte; the concentration of MI is altered in different disorders of the brain including Alzheimer's disease and tumors (13). The usefulness of various metabolite concentrations or ratios for predicting the grade (14), extent (6),

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and prognosis (8) of glial brain tumors has been reported.

In parallel, the diffusion tensor imaging (DTI) sequence was developed and has an application in the evaluation of white matter pathways by measuring the degree and directionality of water diffusion in the tissue. Fractional anisotropy (FA) is a commonly used DTI measure for the assessment of white matter organization. It is higher in densely packed, parallel white matter fibers and alternates in various diseases of the brain, which impair white matter integrity (15). Combination of MRS and DTI is expected to further improve an assessment of glial tumor infiltration area (16).

Although various studies indicate that DTI and MRS quantitative measurements can provide additional information in the diagnostics and monitoring of glial tumors, it is necessary to determine the repeatability of the tests so that they could be more widely used in clinical practice. However, the reproducibility of DTI- and MRS-derived indices has been not systematically analyzed in the literature yet. Even less repeatability research has been conducted from patients with glial brain tumor. A thorough literature search revealed to us that a few studies established data on the repeatability of the measurements of FA and metabolite ratios. One of these studies tested only the repeatability of the individual metabolites (not metabolite ratios) solely in the central part of the tumor (17). Some authors have studied the individual metabolite measurement repeatability of the brain in healthy subjects (15, 18). However, these studies have not calculated the repeatability index. One of the studies analyzed the repeatability coefficient of FA measurements in patients with glioblastoma (19). However, in this study, measurements were made in the central area of the tumor, but the peritumoral areas and the contralateral white matter were not analyzed (19). To the best of our knowledge, no studies on the repeatability index test in MRS and DTI quantitative measurements of the peritumoral area of cerebral gliomas and the contralateral white matter have been conducted. This is the first study testing for repeatability index in both the FA and the metabolite ratio measurements in several areas of the brain in glial tumor patients included the tumor center, the perifocal edema, the distant and the contralateral normal-appearing white matter.

We hypothesized that the combination of DTI and MRS could add a relevant diagnostic value for the recognition of invasive tumor cells in the peritumoral area of gliomas and a more precise evaluation of the real tumor spread zone, and far exceeds the diagnostic value of structural MR imaging alone.

The objective of this study was a quantitative study of the values of FA and metabolite ratios in the peritumoral zone of brain gliomas, including the repeatability test of MRS- and DTI-derived measures.

Material and Methods

This cross-sectional trial was reviewed and approved by the Ethics Committee of Riga Stradins University and the local institutional review board of the Riga East University Hospital.

Patient Selection. Forty-five patients (24 women and 21 men aged between 20 and 73 years, with an average age of 44 years) with a morphologically confirmed brain glioma underwent MR examination during the period between August 2009 and July 2011. All patients presented with a residual/recurrent glioma after surgical resection and with or without following radiation and chemotherapy. Tumors were classified by using the World Health Organization (WHO) classification. The study included 24 glioblastomas, 9 anaplastic astrocytomas, 6 anaplastic oligoastrocytomas, 2 astrocytomas, 1 oligoastrocytoma, 1 anaplastic oligodendroglioma, 1 oligodendroglioma, and 1 anaplastic pleomorphic xanthoastrocytoma. The patients without a morphologically confirmed brain glioma were excluded from this trial.

MR Protocol. The patients were examined using a 1.5-Tesla MR system (GE Signa EXCITE MR) equipped with an 8-channel head coil. The MR protocol included the standard structural sequences T2-weighted, FLAIR, diffusion-weighted, and T1-weighted imaging before and after the intravenous administration of gadolinium-based contrast medium; DTI (TENSOR 25 directions 1000b) and MRS (8 ch) PROBE-2DSI PRESS 144TE. The imaging parameters for DTI were as follows: scan timing: number of shots, 1; echo time (TE), minimum 97.6; repetition time (TR), 8500 ms; acquisition timing: freq, 128; phase, 128; number of excitations (NEX), 1.00; phase field of view (FOV), 0.80; freq DIR, R/L; shim, auto; scanning range: field of view (FOV), 26.0; slice thickness, 5.0; spacing, 0.0; and number of slices, 27. The time required to acquire the DTI set was 3 minutes and 50 seconds. MRS was performed with 2D multivoxel chemical shift imaging using the following parameters: echo time, 144 ms; repetition time, 1000 ms; NEX, 1; and acquisition time, 4 minutes 20 seconds. The volume of interest of MRS was placed on axial T2-weighted or FLAIR images including as much as possible of the lesion, the distant and the contralateral normal-appearing brain parenchyma, avoiding subcutaneous fat and sinuses according to the previous recommended guidelines (20) as shown in Fig. 1B.

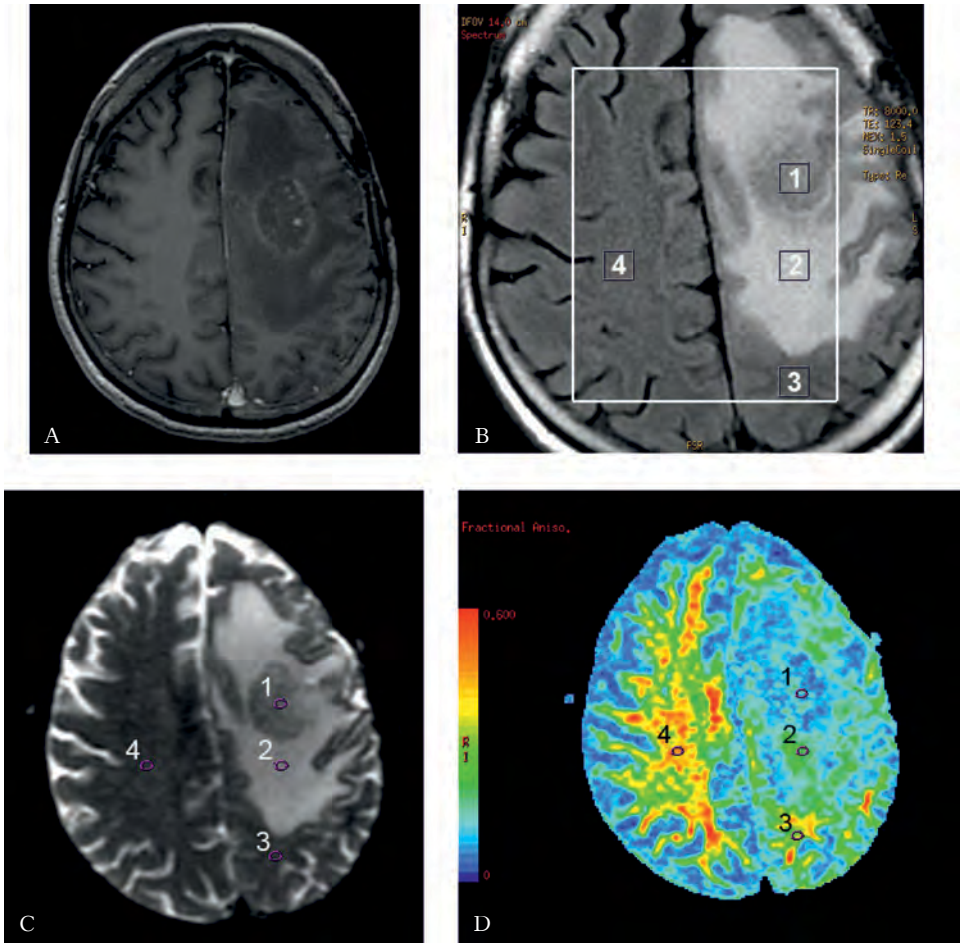

Fig. 1. MR images of a 48-year-old male with a recurrent anaplastic oligodendroglioma in the left frontal lobe

A, axial T1 image after contrast administration demonstrates a well-defined solid tumor portion with inhomogeneous contrast enhancement and perifocal edema; B, the location of the MRS volume of interest in the brain is outlined by a white square. ROIs were placed in the tumor center [1], perifocal edema [2], distant normal-appearing white matter [3], and contralateral normal-appearing white matter [4] for the calculation of the metabolite ratios; C, identical 4 ROIs placements are shown on the diffusion tensor map for the determination of the FA values; and D, the color-coded FA map on which the regions of high anisotropy are shown in red and the regions of low anisotropy are shown in blue.

MRS was acquired before the administration of the contrast agent.

Image Analysis. MR data processing was performed on a MR Gels workstation (General Electric) by using Functool, a program for the visualization and processing of DTI and MRS data.

To assess FA and metabolite changes in the different brain zones, 4 regions of interest (ROIs) were defined: the central part of the tumor, zone of perifocal edema, distant normal-appearing white matter, and contralateral normal-appearing white matter as illustrated in Fig. 1B–D. This selection was based on the previous recommendations (4, 21, 22). The region of tumor center was determined by using the structural MR scans – a region containing a well-defined solid portion, with or without contrast enhancement, and pathological signal intensity on T2-weighted and FLAIR images. The perifocal edema was delineated as a region with higher signal intensity on T2-weighted and FLAIR images, containing no enhancement (Fig. 1A). The region of distant normal-appearing white matter was selected in the distance of 5–10 mm from the visualized zone of perifocal edema. The region of contralateral normal-appearing white matter was selected in the symmetrical white matter zone of the opposite cerebral hemisphere. Both the distant and the contralateral normal-appearing white matter contained normal signal intensity on all structural MR sequences.

DTI measure (FA) was calculated from diffusion tensor maps in each ROIs of similar size (30 pixels). Identical 4 mean metabolites ratios (NAA/Cr, Cho/Cr, MI/Cr, and LL/Cr) were estimated using MRS maps.

To perform the repeatability analysis of DTI and MRS quantitative measurements, 20 patients with

glial tumors were selected. The metabolite ratios and FA measurements were repeatedly measured using the identical methodology – ROIs were placed on the central part of the tumor, zone of perifocal edema, the distant and the contralateral normal-appearing white matter. Repeated measurements were done at approximately 6-month intervals. Overall, 398 repeated measurements were made. In order to create objective data, measurements were made blind regarding to previously chosen ROI localizations in the image slice and the voxel.

Statistical Analysis. Statistical analysis was performed by using the Excel 2003 (Microsoft) and the Statistical Package for the Social Sciences (SPSS, version 20). Descriptive statistics were used for calculating the mean and standard deviations of the samples and the measures. The Kolmogorov-Smirnov test was used to determine if the data were normally distributed. If the data were normally distributed ($P>0.05$), the paired sample *t* test was used to compare the values of means for 2 related samples. If the variables in paired samples did not meet the normal distribution ($P<0.05$), the nonparametric related samples Wilcoxon signed rank test was used to compare the pairs.

The repeatability of measurements was tested with a single factor analysis of variance. The measure of repeatability (r) ranged from 0 to 1. The following terminology was used to describe various degrees of repeatability as recommended Martin and Bateson (1986): slight repeatability ($r<0.2$), low repeatability ($r=0.2–0.4$), moderate repeatability ($r=0.4–0.7$), high repeatability ($r=0.7–0.9$), and very high repeatability ($r>0.9$). This repeatability index was used only in cases when the data were statistically significant (23).

A *P* value of less than 0.05 was considered to indicate a significant difference.

Results

The data of DTI measurements (FA) and MRS parameters (NAA/Cr, Cho/Cr, MI/Cr, and LL/Cr ratios) are displayed in Table 1.

A gradual increase in the FA values and NAA/Cr ratios and a gradual decrease in the Cho/Cr and LL/Cr ratios in the direction from the tumor center to the peritumoral zone were observed (Fig. 2).

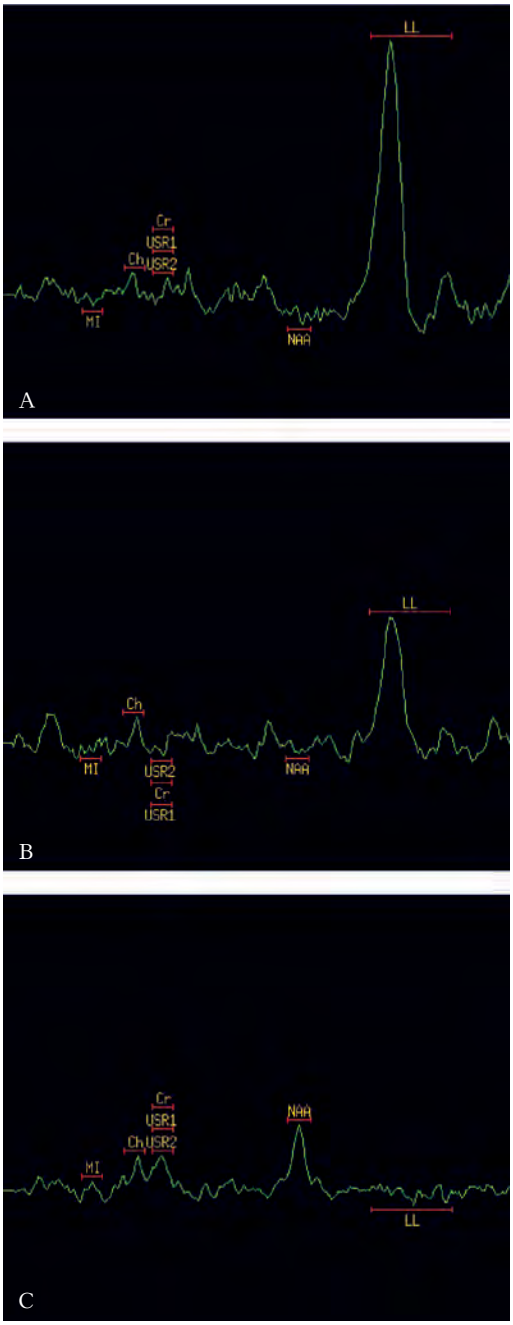

Fig. 2. MRS images of the same case as in Fig. 1

A, typical MR spectrum of the tumor center from the ROIs [1] with an elevated LL peak and a reduced NAA peak; B, MR spectrum in the perifocal edema displays a reduced NAA peak and a gradually reduced LL peak in comparison with the tumor center; and C, MR spectrum in the contralateral normal-appearing white matter shows a high NAA peak. The LL peak is not visible.

Table 1. Fractional Anisotropy and Metabolite Ratios of 4 Selected Brain Regions Among Patients With Brain Glioma

Region of Interest	FA	Cho/Cr	NAA/Cr	MI/Cr	LL/Cr
Tumor center	0.14 (0.05)	2.39 (1.67)	1.48 (1.25)	0.93 (0.64)	4.32 (2.52)
Perifocal edema	0.17 (0.04)	1.77 (1.55)	1.59 (1.01)	0.94 (0.96)	3.48 (2.43)
Distant normal-appearing white matter	0.36 (0.11)	1.11 (0.62)	1.71 (1.04)	0.57 (0.46)	1.94 (1.14)
Contralateral normal-appearing white matter	0.47 (0.08)	1.05 (0.45)	2.31 (1.27)	0.69 (1.10)	1.22 (0.39)

Values are mean (standard deviation).
Cho, choline; Cr, creatine; NAA, N-acetylaspartate; LL, lactate/lipids; MI, myo-inositol.

There were the significant differences ($P<0.001$) in the mean FA values comparing all regions studied, i.e., the tumor center, perifocal edema, distant normal-appearing white matter, and contralateral normal-appearing white matter. Similarly, the significant differences in the mean LL/Cr ratio in all investigated regions were observed; the levels of significance between the tumor center and distant normal-appearing white matter; between the tumor center and contralateral normal-appearing white matter; between the perifocal edema and contralateral normal-appearing white matter; between the perifocal edema and distant normal-appearing white matter, as well as between distant and contralateral normal-appearing white matter were $P<0.001$. The level of significance between the tumor center and the perifocal edema was $P=0.04$.

Analyzing the Cho/Cr ratio, the significant differences between the tumor center and the perifocal edema ($P=0.029$), the tumor center and both the distant and the contralateral normal-appearing white matter ($P<0.001$), the perifocal edema and the distant normal-appearing white matter ($P=0.013$), and the perifocal edema and the contralateral normal-appearing white matter ($P=0.011$) were found. The difference in the mean Cho/Cr ratio between the distant and the contralateral normal-appearing white matter ($P=0.973$) was not significant.

The significant differences between the tumor center and the contralateral normal-appearing white matter ($P=0.002$), the perifocal edema and the contralateral normal-appearing white matter ($P=0.002$), and the distant and the contralateral normal-appearing white matter ($P=0.003$) were recorded. The difference in the mean NAA/Cr ratio between the tumor center and the perifocal edema, the tumor center and the distant normal-appearing white matter, the perifocal edema and the distant normal-appearing white matter was not significant (P values from 0.074 to 0.717).

The significant differences in the MI/Cr ratio were found only between the tumor center and the distant normal-appearing white matter ($P=0.001$) and the tumor center and the contralateral normal-appearing white matter ($P=0.019$). The differences in the mean MI/Cr ratio were not significant between the other investigated regions (P values from 0.077 to 0.843).

Analyzing the repeated measurements in 20 patients, the statistically significant differences were not observed in the tumor center between Cho/Cr measurements ($P=0.180$), NAA/Cr measurements ($P=0.081$), and MI/Cr measurements ($P=0.789$); in the perifocal edema, between Cho/Cr measurements ($P=0.273$), MI/Cr measurements ($P=0.285$), LL/Cr measurements ($P=0.347$); in the distant normal-appearing white matter, between NAA/Cr measurements ($P=0.500$), MI/Cr measurements ($P=0.686$), LL/Cr measurements ($P=0.528$); in the contralateral normal-appearing white matter, between Cho/Cr measurements ($P=0.577$), LL/Cr measurements ($P=0.093$).

In 6 cases, the repeated measurements were statistically significantly different, so the repeatability index r was measured. Table 2 shows the summary of repeatability index analysis for the repeated measurements of MRS- and DTI-derived measures.

Discussion

Understanding the invasion of brain glioma in the normal-appearing white matter still represents a challenge in neurooncological research. The failure of surgical resection in malignant gliomas is thought to be presumably caused by residual tumor cells located peripheral to the surgically removed part of the tumor, and also highly cellular tumor core (1, 24). We reviewed our MR imaging data on patients with histologically confirmed brain glioma. We combined FA and MRS measurements in order to determine whether they provide additional statistically feasible information on the possible gliomas cell infiltration in the peritumoral areas, which appear normal in the structural MR images. We found that in patients with glial brain tumors, the mean FA values increased significantly in the direction from the peritumoral zones toward the tumor

Table 2. Results of Repeatability in MRS- and DTI-Derived Measures on 20 Patients With Glial Brain Tumors Using Single Factor Analysis of Variance

Measurement	LL/Cr Tumor	NAA/Cr Edema	Cho/Cr Distant	NAA/Cr Contralateral	MI/Cr Contralateral	FA Contralateral
Mean square between groups	4.836	0.448	0.193	1.468	0.211	0.019
Mean square within groups	0.851	0.077	0.031	0.365	0.037	0.007
F ratio*	5.679	5.839	6.253	4.023	5.727	2.779
Critical value of F	2.137	2.137	2.137	2.137	2.182	2.137
P	<0.001	<0.001	<0.001	0.002	<0.001	0.01
Index of repeatability (r)	0.701	0.707	0.724	0.602	0.702	0.462
Repeatability	High	High	High	Moderate	High	Moderate

*F was calculated as the ratio of mean square between groups divided by mean square within groups.
Cho, choline; Cr, creatine; NAA, N-acetylaspartate; LL, lactate/lipids; MI, myo-inositol.

center. Theoretically, this finding was expected because FA alterations are associated with the integrity of neurons (6), which decreases closer to the tumor center. Previous studies have shown that the perifocal edema zone of glial brain tumors contains not only pure water, but also invasive tumor cells (4), microglial cells, and reactively altered astrocytes (5).

The nonsignificant differences in the mean NAA/Cr ratio between the tumor center and the perifocal edema, the tumor center and distant normal-appearing white matter, and the perifocal edema and the distant normal-appearing white matter indicate a particularly preserved presence of neurons in tumor and peritumoral zones. That suggests the neuroscientific opinion that infiltrated brain tumor zones are still functioning brain tissues. Although Cr is considered a stable metabolite, some authors have reported that its concentration may vary – Cr level is reduced in tumors, particularly high-grade gliomas (10). Our findings are contrary to those reported by Goebell et al. in their study (6). They found a significantly continuous reduction in the NAA/Cr values from the adjacent white matter to the tumor center. It could be explained by a difference between the present study and that one. Goebell et al. included only WHO grade II and grade III gliomas. The majority of our studied patients had WHO grade IV glioblastomas, which can lead to the decreased Cr concentration. Our study shows that the NAA/Cr ratio in the peritumoral regions provides a low diagnostic significance, because these measurements in the tumor center, perifocal edema, and distant normal-appearing white matter are not different. The NAA/Cr ratio can be used to assess the changes in the distant and contralateral normal-appearing white matter, which appears to be identical in the structural MR images. In the distant normal-appearing white matter around the perifocal edema, the values of NAA/Cr ratio were lower that may indicate the replacement of normal neurons by tumor cells.

Analyzing the mean LL/Cr ratios, a significant gradual decrease in this ratio from the central to the peripheral area was found. This indicates the greater necrotic changes closer to the tumor center. As shown in the previous studies (8, 14), the levels of lipids detected on MRS correlated well with the histological degree of necrosis. Our first finding suggests that the LL/Cr ratio may be even sensitive in the early detection of peritumoral infiltration with a few malignant cells. However, it must be taken in into account that we investigated patients previously treated with radiotherapy and chemotherapy, so the changes in the LL/Cr ratio could also be explained by posttreatment effects. Some authors have reported an increased lipid peak after radiotherapy (14). We detected a statistically significant difference in the LL/Cr ratio between the distant and the contralateral normal-appearing white matter. Our findings are consistent with the previous observations that no abnormal lipid peak is detected in the healthy brain (8). Our results on a gradual reduction in the LL/Cr ratio in the direction from the tumor center to the periphery match with the previous published morphological observations that the majority of gliomas recur within 2.5cm of the resection margin (3), where histologically some invasive cells are identified (6).

We suggest that LL/Cr ratios and FA values may have the superior implications in the detecting of glial tumors extent. In our study, the significant differences in the mean LL/Cr ratio and the mean FA value between tumor and peritumoral regions (perifocal edema and distant normal-appearing white matter) were found.

Similarly, our study showed a gradual decrease in the Cho/Cr ratio in the direction from the tumor center toward the perifocal edema and the distant normal-appearing white matter zone. Whereas there is an increased Cho level in areas with an elevated cell membrane turnover, such as in growing tumors (8), this could indicate a gradual decrease in the concentration of tumor cells in these zones. We suggest that the Cho/Cr ratio is a less sensitive and specific indicator of the distal zone of glial tumor where tumor cells are presented in low concentrations as there was no statistically significant difference in the Cho/Cr ratio between the distant and the contralateral normal-appearing white matter. We suggest that the Cho/Cr ratio could provide additional information about the zone of perifocal edema where the concentration of tumor cells is higher.

In our study, the MI/Cr ratio showed the most inferior diagnostic performance for the evaluation of peritumoral area. Similarly Chernov et al. also did not find a statistically significant difference in the MI/Cr ratio between tumor and perilesional brain tissue (25). Some studies suggested that MI levels might have a role in the grading of cerebral gliomas. Castillo et al. found an elevated level of MI in low grade astrocytomas (26). We found a significant difference in the MI/Cr ratio only between the tumor center and the normal-appearing white matter. The present study indicates that the MI/Cr ratio of the peritumoral area will not improve the accuracy of diagnosis.

Wright et al. developed a novel color mapping technique for the visualization of tumor infiltration zone based on DTI and MRS measurements. The MRS and DTI measurements were investigated in 3 brain areas to determine tumor infiltration. They divided the normal brain zone, but did not specify whether it is localized around the perifocal edema

or in the contralateral cerebral hemisphere (1). We separately investigated distal and contralateral normal signal intensity areas and found differences in the LL/Cr and NAA/Cr ratios and FA measurements. It is consistent with the previous statements that the brain areas of normal signal intensity may have a different degree of tumor cell infiltration, with a gradual decrease in the direction from the tumor core toward the contralateral normal brain. These results indicate that functional MR gives additional information not only on the tumor core and the perifocal edema, but also on functional changes in the brain areas of normal signal intensity. DTI and MRS add a relevant diagnostic value that far exceeds the information of structural MR imaging alone.

Many researchers have studied the role of MRS and DTI quantitative measurements in the diagnosis and monitoring of glial tumors, but only few authors have tested whether these measurements are repeatable. To our knowledge, this is the first study that evaluated the repeatability index of MRS and DTI measures in the peritumoral areas of glial brain tumors. We tested the repeatability of metabolite ratios and FA values in 20 different measurements groups (Cho/Cr, MI/Cr, LL/Cr, NAA/Cr, and FA in 4 different areas). Our study demonstrated the high levels of repeatability in 14 MRS and DTI measures. In 6 cases, the repeated measurements were statistically significantly different, so the repeatability index r was calculated. In 4 cases (LL/Cr ratio in the tumor center, NAA/Cr ratio in the perifocal edema, Cho/Cr ratio in the distant normal-appearing white matter, and MI/Cr ratio in the contralateral normal-appearing white matter), high reproducibility was obtained. Only NAA/Cr and FA measurements in the contralateral area of normal signal intensity showed a moderate repeatability. This could be due to the subjective selection of ROIs, as well as the fact that these measurements depend on various neural densities in the different zones. ROI tracking choices in the contralateral normal-appearing white matter are subjective. Although it is determined according to the area of the opposite hemisphere lesion, there might be variations between adjacent voxels. Both NAA and FA indicate the presence of neurons; however, our data suggest that FA repeatability is lower than that of NAA/Cr ratio. These differences could be explained by differences in MRS and DTI test methodology. MRS is scanned in the one selected image plane, in which the following measurements are also carried out. DTI examination includes all areas of the brain, resulting in approximately 27 axial slices. When the measurements of FA were repeated, the ROIs were selected blindly to the previous localizations of ROIs; therefore, the deviation in ROI selection could have been also between adjacent planes, not only between adjacent voxels, as it is in the MRS measurements. In addition, the course of white matter fibers may vary between adjacent voxels – in the zones where fibers run parallel or neurons have a more dense arrangement, the values of FA and NAA will be higher. Therefore, the differences between adjacent voxels could also be explained by anatomical features, not only by the disease progression or treatment effects. The visual overview of neuron integrity can be obtained by using the color-coded DTI FA maps. The red color visualizes the areas with the largest anisotropy, where most of the fibers run to the same direction, but the blue color shows the areas with the lowest anisotropy. However, in these maps, it is difficult to distinguish the tumor area with an extremely reduced number of neurons from the postoperative cavity – these areas in FA color maps may look identical to the isotropic diffusion (blue). Therefore, ROIs for the FA measurement are easier to draw on the primary gray-scale DTI images, in which the visible tumor mass can be clearly distinguished from the perifocal edema as well as the easily visualized postoperative cavity edge. To improve the measurement accuracy of FA, the ROIs could be draw on the primary DTI images, but at the same time taking into account the color-coded FA map, where defined ROIs appear automatically. Also, NAA/Cr measurements in contralateral cerebral showed a moderate repeatability. Perhaps it could also occur due to different measurement site selection between adjacent voxels. This discrepancy could be reduced if ROIs are chosen in accordance with the DTI color-coded FA maps, which show the areas with different neuronal density. MRS measurements are made either on the axial T2 or FLAIR images of the brain, where white matter produces a homogeneous signal regardless of neuron density. These conventional MR images do not provide information about the amount of neurons in the different areas of white matter. Some authors reported a very high repeatability of FA measurements in the central part of the tumor (r=0.947) (19). In our study, FA from the tumor center did not show a statistically significant difference, indicating the high level of repeatability. However, that study used a slightly different methodology, since the repeated measurements were performed approximately 48 hours apart (19). In our study, the interval was much longer, i.e., about 6 months, which may affect the repeated measurements in terms of when the measurements were not read out in identical measurement areas.

Our study has some limitations. First, we admit that radiation therapy and chemotherapy applied to the patients may have an influence on the FA val-

ues and metabolite ratios that were measured in the tumor and peritumoral areas (6, 27). Recent publications have shown that radiation therapy can alter Cho, Cr, NAA, and LL (28) levels and FA values (29). We assume that further studies involving a patients' cohort with a primary diagnosed glioma before the treatment might expand our results. The second limitation is the lack of separate histological evaluation of the perifocal edema in all cases and in the distant normal-appearing white matter. A previously reported postmortem neuropathological study reported that invasive tumor cells were detected several centimeters beyond the malignant glioma bed and consisted of small anaplastic cells and small fibrillated cells (30).

Considering the additional sequences in the standard examinations, we analyzed a number of advantages and drawbacks of the techniques used (DTI or MRS alone versus DTI plus MRS):

- Additional MR sequences markedly increase the examination expenses. When functional MR series are included in the standard MR protocol, the cost increases by about 44.65%. These expenses do not change regardless of 1 or 2 additional functional MR sequences are used.
- Additional MR sequences prolong examination time. The duration of conventional MR scan in patients with glial brain tumor is approximately 12 minutes 47 seconds. With both DTI and MRS, the examination duration increases by 7 minutes 7 seconds. Accordingly, if we use only one of these sequences, examination extends by 3 minutes 50 seconds (DTI) or 4 minutes 20 seconds (MRS case). In general, patients tolerated the examination well; we did not interrupt examination before the end of the scan.
- DTI or MRS sequences require no additional contrast injection or other invasive manipulation, these examinations are painless. Main unpleasant factors that affect patient comfort are the same as in conventional MR examination sequences – a person must lie motionless in an enclosed tube; MR scanners are usually very noisy.
- MRS and DTI image postprocessing is time-consuming. Using one or more additional sequences the image reconstruction and interpretation time is extending before a radiologist gives the answer. The total scan time and image postprocessing including DTI and MRS analysis take about 1 hour. In comparison, conventional MR scanning and image analysis requires about 30 minutes.
- The combination of MRS and DTI sequences gives more valuable information than only one of the abovementioned MR methods. *DTI* provides a quantitative parameter, *FA,* which implies the integrity of white matter fibers, while MRS provides information about brain metabolism and metabolic changes in pathological processes. The combination of both methods increases the accuracy of measurements – DTI color-coded FA images can be used for ROI selection of both FA and MRS measurements. These FA maps visualize the areas of white matter with different fiber connectivity. Measurements of FA and NAA/Cr may vary within the areas of different neuronal density. Therefore, to get the correct results, comparative measurements must be done in normal white matter areas with similar FA values.

The measurement results must be accurate, repeatable, so they can be used for follow-up in determining the distribution of glial tumors and evaluating the results of treatment and disease progression. We consider 6 conditions relevant when performing MRS and DTI measurements from the different zones of pathological and normal brain tissues in clinical practice. First, select the appropriate slice for MRS examination; it must include a tumor, a perifocal edema area, as well as the distal and the contralateral normal white matter. Second, the MRS plane must be consistent with the DTI selected image slice for FA measurements. Third, choose the same plane in repeated MR scans for follow-up dynamics after the treatment. Fourth, the selected ROIs for both MRS and DTI measurements must be localized in the identical areas. Fifth, one must be very accurate when conducting measurements in the normal contralateral cerebral hemisphere – it is recommended to use DTI color-coded FA maps for ROI selection, which visualize the areas of white matter with different fiber connectivity. Comparative measurements must be done in the areas of normal white matter with similar FA values. Sixth, the repeated measurements should be performed by one experienced neuroradiologist to avoid errors due to the subjective interpretation.

Due to the limitations of DTI and MRS techniques (expensive, time-consuming), it is necessary to clarify the indications for this additional functional imaging. We believe that the main indications are patients with newly diagnosed glial tumors before the planned surgical treatment, as well as previously treated patients with new structural changes around the surgical cavity on conventional MR imaging. In cases when the data of conventional MR examination during follow-up are unchanged compared with the previous data, and the clinical condition is stable, additional functional MR examinations may be omitted. When additional functional sequences are used, we recommend combining both DTI and MRS examinations.

Our data confirmed that an elevation in the Cho/Cr and LL/Cr ratios with the reduction of FA is a reliable indicator of the infiltration of brain glioma cells outside the visible tumor border. These

pathophysiological changes in the peritumoral area demonstrated by MRS and DTI can assist in choosing a biopsy target for brain gliomas, planning the surgical removal or fields and limited target zones of radiation therapy. Our findings have important implications for the use of DTI and MRS sequences in clinical practice. Our study shows that the MRS and DTI measurements are reproducible. The MRS and DTI measurements of cerebral gliomas and peritumoral area have a high degree of repeatability. In order to assess the changes in white matter after received therapy and to compare with the previous MR examinations, it is necessary to follow the accurate methodology. A greater accuracy of measurement is required for comparison with the contralateral normal-appearing white matter.

Conclusions

Our study shows that the MRS and DTI measurements are reproducible. Estimated DTI and MRS data in the peritumoral zone of gliomas detected abnormalities despite the normal-appearing white matter on structural MR images. We conclude that the combined use of Cho/Cr and LL/Cr ratios and FA measurements is a promising MR technique that provides valuable additional information on the extent of real tumor spread along the white matter tracts and location of its potential border.

Acknowledgments

We are deeply grateful to Dr. Daina Apskalne for the carefully conducted morphological analyses and oral consultations. We thank Oskars Rasnacs and Irena Rogovska for excellent technical assistance. This study was supported by the European Social Funds in Latvia (grant No. 2009/0147/1DP/1.1.2.1.2/09/IPIA/ VIAA/009).

Statement of Conflicts of Interest

The authors state no conflict of interest.

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Received 20 January 2012, accepted 30 October 2012