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Original Article

Predicting tumor recurrence of astrocytoma by Ki-67 and proton magnetic resonance spectra

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Abstract: *Background:* Astrocytoma is the most common primary cerebral tumor and prediction of its prognosis is crucial. In the study, we aimed to investigate the correlation between the proton magnetic resonance spectroscopy (MRS) spectra of brain astrocytoma and Ki-67 expression and their clinical value in predicting tumor recurrence in astrocytoma after surgery. *Methods:* 73 patients with astrocytoma underwent surgery were retrospectively included from January 2012 to January 2015. Ki-67 expression was examined by immunohistochemical staining. The ratios of tumor parenchyma choline (Cho)/N-acetylaspartate (NAA), Cho/creatine (Cr), NAA/Cr, and occurrence rates of lactate (Lac) and lipid (Lip) signals were obtained from MRS and compared with Ki-67 expression. The prognostic value of the metabolite ratios and Ki-67 for astrocytoma was analyzed. *Results:* Ki-67 expression of astrocytoma was increasing significantly with the ratios of Cho/Cr (r=0.305, P=0.009) and Cho/NAA (r=0.468, P=0.000) but decreasing with NAA/Cr (r=-0.480, P=0.000). Correlation analyses suggested that Ki-67 associated with grade and recurrence, Cho/Cr associated with age and recurrence, Cho/NAA associated with grade and recurrence, NAA/Cr associated with grade, Lac associated with age and recurrence, and Lip associated with grade and recurrence. Multivariate analyses revealed that astrocytoma grade and metabolite ratios Cho/Cr and Lac were independent prognostic factors for recurrence time (Grade, HR=0.337, 95% CI=0.135-0.846; Cho/Cr, HR=0.348, 95% CI=0.145-0.839; Lac, HR=0.457, 95% CI=0.228-0.916). *Conclusions:* MRS data of astrocytoma were associated with Ki-67 expression and can be used to predict tumor recurrence after tumor surgery, thereby helping clinicians make reasonable treatment decisions for astrocytoma patients.

Keywords: 1H-MRS, astrocytoma, Ki-67, recurrence

Introduction

Astrocytoma is the most common primary cerebral tumor. It accounts for 40%-43% of intracranial tumors with an annual incidence rate of approximately 5 per 100,000 and is prone to relapse [1, 2]. Although the treatment of astrocytoma has developed from a surgery-only approach to current treatment strategies that combine surgery and chemoradiation therapy, the prognosis has not significantly improved. In some cases, astrocytoma relapse may occur several months after therapy and results in a poor prognosis [3, 4]. The prognosis of astrocytoma is related to the proliferation and invasiveness of the tumor cells. Evaluating the proliferation and invasiveness of tumor cells prior to surgery can help clinicians predict the likelihood of post-operative recurrence, thereby aiding in the design of more rational treatment solutions that can improve the patient's prognosis. Ki-67 is the most proliferative nuclear antigen and its expression is therefore used as an indicator of proliferation.

Proton magnetic resonance spectroscopy (1H-MR spectroscopy, 1H-MRS; hereinafter, referred to as MRS) is a powerful noninvasive tool to measure a variety of metabolic properties in brain tissue *in vivo* [5], allowing the biological evaluation of astrocytoma at a molecular level. The markers such as choline-containing compounds (Cho), N-acetyl aspartate (NAA), creatine (Cr), lactate (Lac), and lipid (Lip) can be detected. Cho and NAA can be used to distinguish regions of tumor from normal brain tis-

Table 1. Basel characteristics of the included patients with astrocytoma

Clinical features	N
Age (year)	
<60	60
≥60	13
Gender	
Male	35
Female	38
Grade	
1	1
2	24
3	28
4	20
Tumor sites	
1	46
2	18
≥3	9
Therapy	
Surgery alone	15
Surgery + Chemotherapy and/or Radiotherapy	58
Recurrence	
Yes	42
No	31

sue. Cr is involved in ATP metabolism and a marker of energy transfer and storage. Previous work has shown that the parameters obtained from MRS may provide useful information for diagnosis, surgery guiding, prognosis prediction in patients with glioma [6-9]. However, studies of the prognostic value of MRS data in prediction of astrocytoma recurrence are relative less.

Here, we included 73 patients with astrocytoma after surgery alone or plus adjuvant therapy and determined the prognostic value of the metabolic ratios such as Cho/Cr, Cho/NAA, and NAA/Cr obtained from MRS data in tumor recurrence survival. In addition, correlations of the metabolic ratios with proliferation index, Ki-67 expression, were also investigated.

Materials and methods

Subjects

The retrospective study population consisted of 73 patients with astrocytoma who were included from January 2012 to January 2015 at the Second Affiliated Hospital of Zhejiang University. All patients were pathologically confirmed by the stereotactic biopsy. Tumor grade of astrocytoma were classified into grade I-IV according to the 2007 World Health Organization (WHO) Classification of Tumors of the Central Nervous System Standard [10]. The inclusion criteria were as follows: (1) the patient was diagnosed with astrocytoma for the first time; (2) no radiotherapy or chemotherapy was performed prior to MRS; (3) there was no history of brain trauma or surgery; (4) there were no other types of brain tumor, brain metastasis, or brain diseases; (5) routine pre-operative magnetic resonance imaging (MRI) scanning, enhanced MRI scanning, and MRS examination were performed; (6) the patients underwent complete resection or sub-total resection. The study was approved by the Committee on Human Research of the Second Affiliated Hospital of Zhejiang University School of Medicine. Informed consent was obtained from all subjects.

MR examination

MRI data were acquired by the GE 750 3.0T superconducting MR system (General Electric, Fairfield, United States). The MRI was performed by acquiring T1WI, T2WI, and FLAIR sequences. An enhanced MRI scan was performed to acquire axial, sagittal, and coronal T1WI sequences. Gadolinium pentetic acid meglumine (Gd-DTPA) was used as an enhanced contrast agent, with an injection dosage of 0.15 mmoL/kg. MRS scan: Using a standard 8-channel head coil as the transmitting and receiving coil, the two-dimensional acquisition was conducted using point-resolved spectroscopy (PRESS) with the PROBE-P sequence. Each patient underwent single-voxel MRS twice for data collection. Both lactate (Lac) and lipids (Lip) resonate at 1.3 ppm, however, when using a long echo time (TE), such as 135 or 144 ms, Lac appears as an inverted double peak and can be distinguished from the Lip peak. The multi-voxel MRS was performed following single-voxel MRS. For the multi-voxel acquisition, the following parameters were applied: TR/TE =1500 ms/105 ms, field of view (FOV) =240 mm, matrix size =18×18, thickness voxel =10 mm, times of excitation =8, number of scans =128, and duration of each scan =260 s. Pure axial routine T2 images were acquired for positioning.

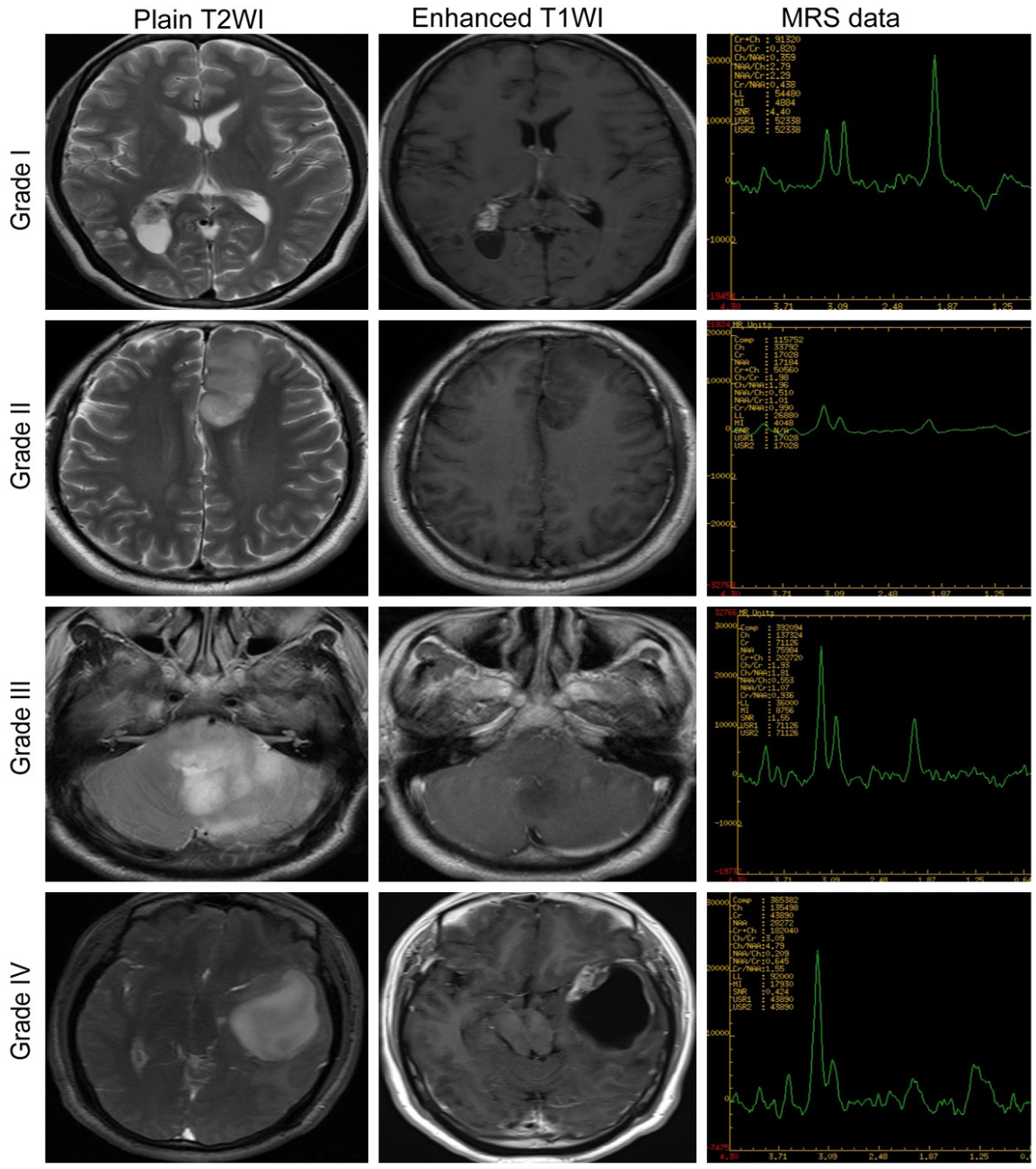

Figure 1. MRI and MRS results in astrocytoma with different tumor grades. Four patients with Grade I-IV tumor were selected as examples. Their MRI and MRS results were presented.

Image processing

To avoid interference from the skull, the fat, and the gas chamber, a region of interest (ROI) was selected that included a maximum portion of the lesion, necrosis, and edema around the lesion and corresponding contralateral regions. Voxel shimming and water suppression were performed by automatic scanning processes. After the spectrum scan was completed, raw data was transferred to the workstation and the GE aw4.6 Functool commercial software package (supplied with the device) was used for data processing. The resulting spectrum was acquired after baseline correction and frequency flips. Semi-quantification of metabolite concentrations was achieved by measuring the area under the peak of each metabolite, and

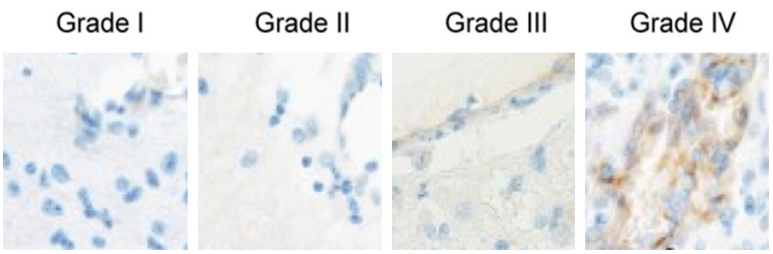

Figure 2. Ki-67 expression in astrocytoma with different tumor grades. Four patients with Grade I-IV tumor were selected as examples. Their ki-67 expressions in tumor tissues were presented (400×).

the creatine (Cr) level was used as an internal control for relative quantitative analysis. The peak values of N-acetylaspartate (NAA), choline (Cho), Cr, Lac, Lip, and myoinositol (mI) and ratios of Cho/Cr, Cho/NAA, NAA/Cr in each ROI were recorded, analyzed, and compared. All MR images were evaluated by two independent neuroradiologists to make a factual comparison among the advanced MR imaging and minimizethe confoundingeffects [11].

Histological examination

After paraffin embedding, the tumor specimens were sliced and subjected to standard H&E staining. The histological structures and cell morphology of tumor tissues were observed using a light microscope. To quantify Ki-67 expression, five fields of view were randomly selected per specimen and Ki-67 staining of 500 tumor cells per field was examined at high magnification (×400). The percentage of positively stained cells in each field of view was calculated and the mean value was defined as the percentage of positive tumor cells.

Follow-up

The follow-up of the patients were performed every three months. The longest follow-up period was three years. The tumor recurrence was evaluated by the Response Assessment in Neuro-Oncology (RANO) standards [12]. The recurrence time was defined as the interval between the first craniotomy for tumor resection and discovery of recurrent lesion during the regular radiographic check.

Statistical analysis

SPSS 18.0 statistical software package was used for all statistical analyzes. Correlations of Ki-67 expression and the metabolite ratios of Cho/Cr, Cho/NAA, and NAA/Cr ratios were evaluated by Spearman analysis. Ki-67 expression and the metabolite ratios were divided into Low groupand high group by the cut-off value obtained by the X-title software according to recurrence time. The associations of clinical features of the patients such as age, gender, tumor grade, tumor sites with Ki-67 expression and the metabolite ratios were analyzed by Fisher's test. Kaplan-Meier analyses and univariate and multivariate analyses were performed to investigate the prognostic value of Ki-67 and the metabolite ratios in prediction of astrocytoma recurrence. Receiver operating characteristics curves (ROC) were also performed to compare the predictive valued of Ki-67 and the metabolite ratios in distinguishing astrocytoma recurrence. A two-tailed value of $P<0.05$ was considered significant.

Results

Patients

73 patients with astrocytoma were retrospectively included (**Table 1**). 35 were male and 38 were female. The average age of the patients was 44.2 (13-77) years. One, 24, 28, and 20 cases were Grade 1, 2, 3, and 4, respectively, according to the 2007 World Health Organization (WHO) Classification of Tumors of the Central Nervous System Standard [9, 10]. Of the 73 patients, 15 cases received operation alone and 58 patients underwent surgery and chemotherapy and/or radiotherapy. After follow up of two to three years, recurrence was found in 42 of 73 patients. MRI and MRS results of all the patients were collected (**Figure 1**).

Correlations of Ki-67 and the MRS results in patients with astrocytoma

The patients were scanned by MRS and the ratios of Cho/Cr, Cho/NAA, NAA/Cr, and the occurrence rates of Lac and Lip were obtained. The Ki-67 expression in the specimens after operation or biopsy was examined by immunohistochemical staining (**Figure 2**). The correlations of Ki-67 expression and metabolite ratios were evaluated by Spearman analysis and the results suggested that Ki-67 was increasing

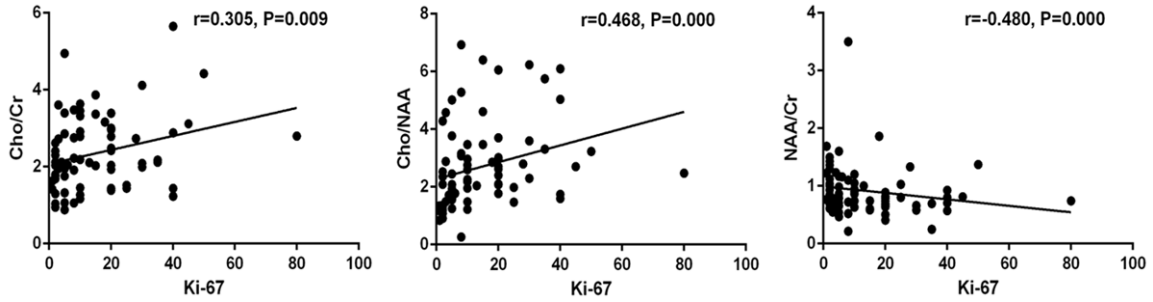

Figure 3. Correlations of Ki-67 expression with Cho/Cr, Cho/NAA, and NAA/Cr in patients with astrocytoma. Ki-67 levels were plot at X-axis and corresponding ratios of Cho/Cr, Cho/NAA, and NAA/Cr, and occurrence rates of Lac and Lip were plot at Y-axis. Correlations were evaluated by Spearman analysis.

significantly with the ratios of Cho/Cr (r=0.305, P=0.009) and Cho/NAA (r=0.468, P=0.000) but decreasing with NAA/Cr (r=-0.480, P= 0.000) (**Figure 3**).

Correlations of clinical features with Ki-67 expression and MRS data

The Ki-67 expression and MRS data of the patients with astrocytoma were divided into "Low" and "High" by X-title according to recurrence in the follow up. And then their associations with clinical features such as age, gender, grade, tumor sites, and recurrence were analyzed. The results suggested that Ki-67 associated with grade and recurrence, Cho/Cr associated with age and recurrence, Cho/NAA associated with grade and recurrence, NAA/Cr associated with grade, Lac associated with age and recurrence, and Lip associated with grade and recurrence (**Table 2**).

Prognostic value of Ki-67 expression and metabolite ratios in predicting recurrence of patient with astrocytoma

To evaluate the prognostic value of the clinical parameters (grade, gender, age, tumor site number, and therapy strategy), Ki-67 expression, and MRS results in predicting recurrence of patients with astrocytoma, Kaplan-Meier analysis using the log-rank test was firstly performed and the analyses indicated that high grade, high age, more tumor sites, high Ki-67 expression, high Cho/NAA, and high Lip were associated with high recurrence while high NAA/Cr was favorable for recurrence (**Figure 4**). Furthermore, univariate and multivariate Cox regression analyses were also conducted. The clinical parameters grade and metabolite ratios Cho/Cr and Lac were identified as independent prognostic factors for recurrence time (Grade, HR=0.337, 95% CI=0.135-0.846; Cho/Cr, HR= 0.348, 95% CI=0.145-0.839; Lac, HR=0.457, 95% CI=0.228-0.916; **Table 3**). In addition, receiver operating characteristic (ROC) analysis revealed that the area under curve (AUC) of Ki-67, Cho/Cr, Cho/NAA, and NAA/Cr in distinguishing astrocytoma recurrence or not was 0.633, 0.614, 0.654, and 0.662, respectively (**Figure 5** and **Table 4**).

Discussion

In the present study, we retrospectively included 73 patients with pathologically confirmed astrocytoma and investigated the correlations of MRS data and Ki-67 and their prognostic value for recurrence free survival in astrocytoma.

Ki-67 antigen is a non-histone protein present in the nuclei of proliferating cells, and its expression level varies at different phases of the cell cycle. It is a representative indicator of cell proliferation and is an excellent biomarker applied in many neurosurgery centers to predict aggressiveness of gliomas and patients' outcomes [13]. It has been shown that Ki-67 is correlated with astrocytomas grade, poor survival [14, 15]. And Varughese et al have found Ki-67/MiB-1 proliferative index was associated poor survival in astrocytomas in a Norway cohort [16]. However, the study number and sample size in the studies are small. In the present study, Ki-67 was found to be associated with the grade and recurrence of astrocytoma. Furthermore, we observed the positive correlation of Ki-67 with the metabolic ratios obtained from MRS results including Cho/Cr,

Table 2. Correlations of clinical features with Ki-67 expression and MRS data in patients with astrocytoma

Parameters	Ki-67			Cho/Cr			Cho/NAA			NAA/Cho			NAA/Cr			Lac			Lip		
	Low	High	P	Low	High	P	Low	High	P	Low	High	P	Low	High	P	Low	High	P	Low	High	P
Age (year)																					
<60	36	24	0.761	49	11	0.003	46	14	0.166	14	46	0.166	19	41	1.000	36	24	0.029	49	11	0.705
≥60	7	6		5	8		7	6		6	7		4	9		3	10		10	3	
Gender																					
Male	21	14	1.000	28	7	0.296	25	10	1.000	10	25	1.000	13	22	0.450	22	13	0.160	28	7	1.000
Female	22	16		26	12		28	10		10	28		10	28		17	21		31	7	
Grade																					
1	1	0	0.000	1	0		1	0	0.000	0	1		0	1	0.049	1	0	0.374	1	0	0.050
2	24	0		21	3		22	2		2	22		3	21		15	9		21	3	
3	16	12		18	10		21	7		7	21		10	18		15	13		25	3	
4	2	18		4	16		9	11		11	9		10	10		8	12		12	8	
Tumor sites																					
1	30	16	0.195	33	13	0.851	35	11	0.684	11	35	0.684	11	35	0.190	25	21	0.943	36	10	0.173
2	10	8		14	4		12	6		6	12		8	10		9	9		17	1	
≥3	3	6		7	2		6	3		3	6		4	5		5	4		6	3	
Recurrence																					
Yes	34	8	0.000	38	4	0.000	39	3	0.000	14	28	0.288	15	27	0.449	32	10	0.000	39	3	0.005
No	9	22		16	15		14	17		6	25		8	23		7	24		20	11	

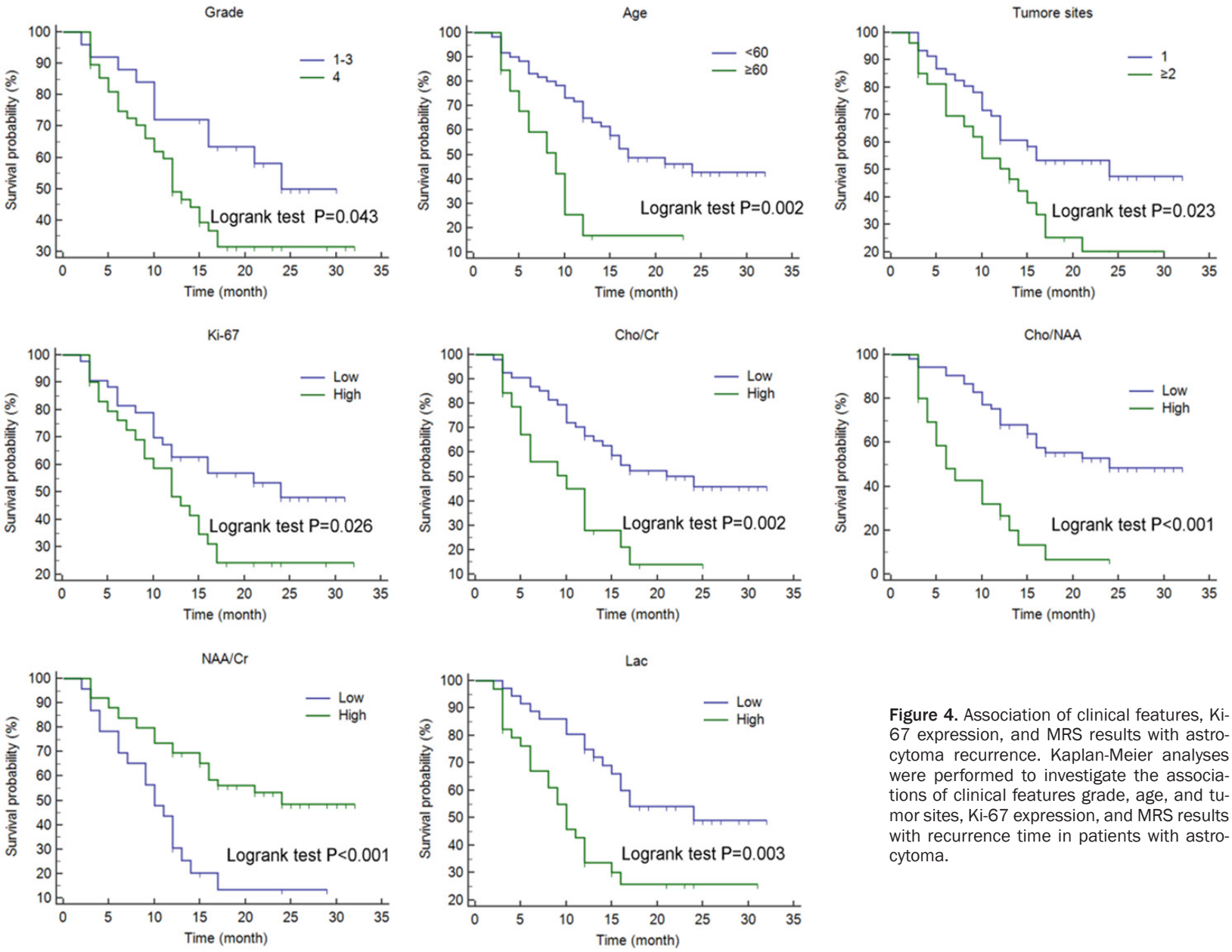

Figure 4. Association of clinical features, Ki-67 expression, and MRS results with astrocytoma recurrence. Kaplan-Meier analyses were performed to investigate the associations of clinical features grade, age, and tumor sites, Ki-67 expression, and MRS results with recurrence time in patients with astrocytoma.

Table 3. Univariate and multivariate Cox regression analyses of Clinical parameters and MRS results in prognosis of recurrence in patients with astrocytoma

Parameters	Univariate analysis			Multivariate analysis		
	P	HR	95% CI	P	HR	95% CI
Grade (1-3/4)	0.001	0.353	0.188-0.661	0.020	0.337	0.135-0.846
Gender (Male/Female)	0.607	1.173	0.638-2.156			
Age (<60/≥60)	0.004	0.335	0.161-0.701	0.133	0.502	0.204-1.235
Therapy	0.879	1.03	0.705-1.503			
Tumor sites (1/≥2)	0.029	0.509	0.277-0.934	0.156	0.603	0.300-1.214
Ki-67 (Low/High)	0.032	0.515	0.280-0.946	0.534	1.318	0.551-3.151
Cho/Cr (Low/High	0.003	0.382	0.201-0.725	0.019	0.348	0.145-0.839
Cho/NAA (Low/High)	0.000	0.252	0.133-0.478	0.402	0.699	0.303-1.613
NAA/Cr (Low/High)	0.001	0.347	0.186-0.648	0.145	1.792	0.818-3.926
Lac (Low/High)	0.007	0.422	0.227-0.786	0.027	0.457	0.228-0.916
Lip (Low/High)	0.120	1.743	0.866-3.508			

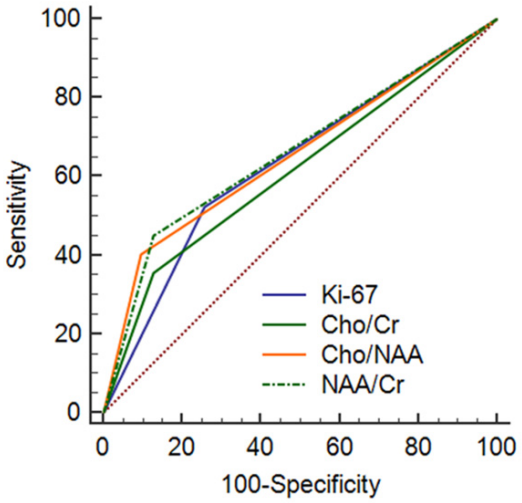

Figure 5. Accuracy of Ki-67, Cho/Cr, Cho/NAA, and NAA/Cr in predicting astrocytoma recurrence. Receiver operating characteristic (ROC) analyses of Ki-67 expression and the ratios of Cho/Cr, Cho/NAA, and NAA/Cr according to astrocytoma recurrence were performed.

Table 4. Area under the curves (AUC) of Ki-67, Cho/Cr, Cho/NAA, and NAA/Cr in distinguishing astrocytoma recurrence or not

Variable	AUC	SE	95% CI
Ki-67	0.633	0.066	0.512-0.743
Cho/Cr	0.614	0.066	0.493-0.726
Cho/NAA	0.654	0.064	0.534-0.762
NAA/Cr	0.662	0.064	0.541-0.768

Note: AUC, area under the curve; SE, standard error.

Cho/NAA, and Lip levels, consistent with the previous reports [17]. The results suggested that MRS data can reflect the expression of Ki-67 and indicate tumor proliferation and degree of malignancy prior to surgery.

MRS data correspond to the histological features of glioma cells and can be used to determine tumor differentiation and grading, as well as in follow-up and radiotherapy planning [9, 18-20]. MRS is also a useful method for identifying early changes in the metabolism of glioma cells and the extent of glioma infiltration [21-23]. Biomarkers such as Cho, Cr, NAA, Lac, and Lip and ratios of Cho/Cr, Cho/NAA, and NAA/Cr can be obtained from MRS data. Heo et al have identified that Cho/Cr and Cho/NAA ratios are high in gemistocytic astrocytomas compared with non-gemistrocytic tumors and associated with progression in astrocytoma [24]. In the current study, elevated Cho, reduced NAA, increased Cho/Cr and Cho/NAA ratios, decreased NAA/Cho and NAA/Cr ratios, and visible Lac and Lip peaks were observed in astrocytoma. These results are consistent with the previous literature [25, 26], which suggest that, with increasing tumor malignancy, cell division and proliferation are accelerated, accompanied by increased destruction of nerve cells and more aggressive tumor cell invasion to surrounding tissues. Further, we identified that Cho/Cr, Cho/NAA, NAA/Cr and Lac were significantly associated with tumor recurrence in astrocytoma through Kaplan-Meier analysis and univariate analysis and low metabolite ratios of Cho/Cr and Lac were favorable for recurrence free survival (Cho/Cr, HR=0.348, 95% CI=0.145-0.839; Lac, HR=0.457, 95% CI=0.228-0.916; **Table 3**). On the other hand,

receiver operating characteristic (ROC) analysis also revealed that the ratios of Cho/Cr, Cho/NAA, and NAA/Cr could distinguish astrocytoma recurrence or not with high accuracy.

This study has some shortcomings. Firstly, patient number was relative small. Secondly, this was a retrospective study and the follow-up duration was short. Thirdly, some other clinical parameters that might affect glioma prognosis such as tumor area, presence of epilepsy, and Kamofsky performance score were not included in the present study. The more prospective studies with large scale should be performed at multiple research centers should be performed to investigate the predictive value of MRS data in astrocytoma recurrence.

In summary, MRS data of astrocytoma is associated with Ki-67 expression, can be used to evaluate astrocytoma cell proliferation, and predict astrocytoma recurrence after surgery. The application of MRS in astrocytoma can provide new evidence for clinical diagnosis and prognosis.

Acknowledgements

Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

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