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CLINICAL INVESTIGATION **Central Nervous System**

^{1}H-MRS *IN VIVO* PREDICTS THE EARLY TREATMENT OUTCOME OF POSTOPERATIVE RADIOTHERAPY FOR MALIGNANT GLIOMAS

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Purpose: To analyze prospectively the prognostic significance of ^{1}H magnetic resonance spectroscopy (MRS) *in vivo* recorded from the tumor bed of patients after surgery for malignant glioma.
Methods and Materials: Fifty-one patients aged 20–68 years were examined using a MRI/MRS system (Elscint 2T Prestige). Of the 51 patients, 33 had Grade 3 gliomas and 18 had glioblastomas. MRI-localized ^{1}HMR spectra were acquired using a single-voxel, double-spin-echo sequence. Relative intensities of the signals (choline, creatine [Cr] *N*-acetyl aspartate [NAA], myo-inositol, lactate, and lipids) were obtained by numeric integration of fitted signals. Two voxels were examined, one located at the tumor bed and the second distant to the tumor bed. All patients were irradiated to 60 Gy using three-dimensional conformal noncoplanar techniques to 60 Gy.
Results: MRS *in vivo* in patients after brain tumor surgery revealed a statistically significant decrease in the NAA/Cr ratio and increases in the choline/creatine (Cr), choline/NAA, and myo-inosytol/Cr ratios. The intensive signals of lactate and lipids appeared in spectrum. Survival correlated strongly with tumor grade and patient age but the strongest prognostic factor was the lactate/NAA ratio. For lactate/NAA values >2.0 (intensive lactate signal) the 1-year survival rate was 20%, and for lactate/NAA values <2.0, the 1-year survival rate was 85%.
Conclusion: A new diagnostic tool demonstrated ability to distinguish between patients with a favorable prognosis and those who will die within 1 year. **© 2002 Elsevier Science Inc.**

NMR spectroscopy, Brain tumors, Prognostic factors, Hypoxia.

INTRODUCTION

Primary brain tumors are a very diverse group of diseases. Despite many clinical trials conducted during the past decades, no improvement has occurred in the treatment outcome (1–3). Both clinical and basic research have shown that the main reason for the poor prognosis is the biologic heterogeneity of brain tumors. This heterogeneity, together with radioresistance and questionable surgical macroscopic or microscopic margins, is the reason most anaplastic tumors are incurable with conventional multidisciplinary treatment. An improvement in patient survival might be achieved by the identification of molecular or metabolic prognostic factors and treatment individualization. Because brain tumors are inaccessible for clinical examination, imaging of the central nervous system with MRI is irreplaceable in the diagnostic workup. Postoperative disruption of the blood–brain barrier limits the value of both CT and MRI in the early differentiation of neoplastic tissue remnants and postoperative changes (4).

Nuclear magnetic resonance (NMR) spectroscopy (MRS) *in vivo* allows for insight in the tissue metabolic profiles by analysis of the NMR spectrum. The pattern of metabolic changes may potentially be helpful in basic and applied research.

The aim of this work was to evaluate MRS findings *in vivo* as predictive or prognostic factors for postoperative radiotherapy (RT) for malignant gliomas. The studies of the prognostic importance of MRS are sparse and fragmentary. Most research has been applied to spectroscopy of brain tumors before surgery. In the case of multidisciplinary treatment, an analysis of the tumor bed might be more informative, because the macroscopic or microscopic remnants of tumor might be present and detected. Localized MRS enables studies of the tumor bed regions that are most suspected of active malignancy.

METHODS AND MATERIALS

The study group consisted of 51 patients (35 men and 16 women, age range 20–68 years, mean 47) treated with

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Table 1. Averaged relative concentration of metabolites for tumor bed and controls

	NAA/Cr	Cho/Cr	mI/Cr	Cho/NAA	Lac/NAA	Lip/NAA
Tumor bed	0.92 ± 0.29*	1.64 ± 0.64*	0.92 ± 0.40†	1.75 ± 0.89*	4.6 ± 5.1*	3.33 ± 3.79*
Control (C1)	1.35 ± 0.21*	0.99 ± 0.17‡	0.75 ± 0.19‡	0.77 ± 0.15†	0.44 ± 0.88	0.73 ± 0.85
Control (C2)	1.56 ± 0.18	0.95 ± 0.18	0.74 ± 0.15	0.66 ± 0.17	0	0

Abbreviations: NAA = *N*-acetyl aspartate; Cr = creatine; Cho = choline; mI = myo-inositol; Lac = lactate; Lip = lipid; (C1) = control group, spectroscopic measurement from the uninvolved opposite brain lobe; C2 = control group of 30 healthy volunteers.

* Highly significant.

† Significant.

‡ Not significant.

postoperative RT at the Center of Oncology Maria Sklodowska-Curie Memorial Institute Branch in Gliwice. All patients had good neurologic status and qualified for radical postoperative RT. Twenty-nine patients underwent total resection and 22 partial resection. Of the 51 tumors, 33 were anaplastic glial tumors and 18 were glioblastomas. Patients were treated with conformal three-dimensional techniques using 6–20 MV photons. The dose per fraction was 2 Gy, and the total dose was 60 Gy for all patients. Patients did not receive adjuvant chemotherapy. All consecutive patients with high-grade gliomas and good performance status who had been referred by neurosurgeons for RT were included in this study. MRI and MRS of the tumor bed were performed before RT. The first control group (C1) consisted of spectroscopic measurements from the uninvolved opposite brain lobe and the second control group (C2) consisted of 30 healthy volunteers (5). The interval between surgery and MRI was 23–63 days (mean 41). Patients were followed using MRI scans 1, 4, and 9 months after treatment. After 9 months of follow-up, MRI was scheduled every 6 months.

The spectra were registered from a region of altered MR signal on pilot imaging sequences, avoiding fluid spaces. MRI-localized ^{1}HMR spectra (using Elscint 2T Prestige operating at the field strength of 2T) were acquired using a single-voxel, double-spin-echo PRESS sequence with the parameters: TR = 1500 ms, TE = 35 ms, 200 acquisitions, for a voxel volume of $1.5 \times 1.5 \times 1.5$ cm^3. The spectra recording was preceded by the global and local shimming procedure. For each patient, the spectra were obtained from at least two regions of interest. The first was located at the tumor site and was placed at the operation field edge or at the region of altered MRI appearance suggestive of tumor remnants. The other control voxel was placed at the opposite uninvolved lobe. The spectra were postprocessed with the automatic fitting in the frequency domain. The relative intensities of the signals caused by *N*-acetyl aspartate (NAA), choline-based compounds (Cho), myo-inositol (mI), lactate (Lac), and lipids (Lip) were obtained by numeric integration of the fitted signals.

Statistical analysis

The statistical difference between the means was calculated using the Mann–Whitney *U* test. The statistical significance between survival curves was calculated using the modified Peto test. Patient survival was scored as the time after the end of RT. Cutoff levels for the metabolite ratios used in the survival analysis were calculated using the maximum-likelihood estimation and logistic model. Cox regression analysis was used for multivariate survival analysis.

RESULTS

All spectrum parameters registered from the tumor bed were statistically significantly different from those obtained from the control uninvolved lobe (control group C1) and from the spectrum of healthy volunteers (control group C2) (Table 1). The NAA/Cr ratio was lower compared with the control values, and the Cho/Cr, Cho/NAA, and mI/Cr ratios were higher. In contrast to the spectra of healthy brain tissue (control group C2), the spectra recorded from the tumor bed reveal strong signals of Lac and Lip, reflected in the high values of the Lac/NAA and Lip/NAA ratios. Interestingly, the NAA/Cr ratio from the uninvolved lobe (control group C1) was also significantly lower than the normal value.

MRI was scored as positive if the contrast enhancement had been registered in the tumor bed (MRI+). For the entire partial resection group, contrast enhancement was observed, but in the total resection group, only 9 patients had MRI scans without contrast enhancement in the tumor bed (MRI−). It should be noted, however, that differentiation between postoperative enhancement and tumor is frequently difficult and may have led to an overestimation of the MRI+ group.

The correlation between the MRS parameters from the tumor bed with tumor grade and contrast enhancement on MRI is shown in Table 2. Lac/NAA and Lip/NAA ratios were significantly increased for Grade 4 tumors and for the patients from the MRI+ group, but the values were widely scattered. No correlation was found between the MRS parameters and the time from surgery.

Patient survival correlated with tumor grade (Fig. 1) and patient age (Fig. 2). However, the extent of resection did not correlate with patient survival (Fig. 3), although it did correlate with the MRI results (Fig. 4).

Table 2. Correlation between MRS parameters from tumor bed with tumor grade and contrast enhancement in MRI

Tumor bed	NAA/Cr	Cho/Cr	mI/Cr	Cho/NAA	Lac/NAA	Lip/NAA
Anaplastic astrocytomas	0.94 ± 0.24	1.61 ± 0.72	1.66 ± 0.83	0.95 ± 0.54	3.22 ± 3.93	2.72 ± 3.37
glioblastoma	0.89 ± 0.36*	1.71 ± 0.45*	1.94 ± 0.98*	0.87 ± 0.31*	7.13 ± 6.07†	4.46 ± 4.33‡
MRI−	0.92 ± 0.31	1.53 ± 0.51	1.53 ± 0.40	0.79 ± 0.34	1.65 ± 1.49	1.63 ± 1.03
MRI+	0.93 ± 0.29*	1.67 ± 0.67*	1.81 ± 0.96*	0.95 ± 0.50*	5.24 ± 5.38‡	3.70 ± 4.07‡

Abbreviations as in Table 1.
* Not significant.
† Highly significant.
‡ Significant.

The cutoff levels used for survival analysis were calculated using logistic regression analysis. It was found that a Lac/NAA ratio of 2 and a Lip/NAA ratio of 2 were the cutoff levels with the best predictive value. Patient survival correlated significantly with the Lac/NAA (Fig. 5) and Lip/NAA (Fig. 6) ratios. To exclude the confounding influence of tumor grade, survival was calculated for Grade 3 tumors and the Lac/NAA ratio (Fig. 7).

The forward stepwise multivariate Cox regression analysis involving all the parameters of the MRS spectrum and the known prognostic factors such as age, grade, and extent of surgery, resulted in finding the Lac/NAA ratio and patient age as the two most important predictors of treatment outcome (Table 3). The other parameters were excluded by stepwise procedure.

DISCUSSION

Patients with malignant gliomas have an extremely bad prognosis. The diffusely infiltrative tumor growth and the site of primary tumor close to important centers of the central nervous system generally preclude radical excision (1). Patient survival depends on both the aggressiveness of the disease and the ability of postoperative treatment to sterilize the tumor. We chose to study the prognostic importance of MRS after surgical tumor excision. Although postoperative changes may potentially change the results of MRS, we did not observe any correlation between the time after surgery and the results of MRS. In this case, MRS may reflect the metabolism of tumor remnants. After a partial resection of tumor, MRS reflects the metabolism of the tumor, and after total resection, it reflects the metabolism of the surgical margins.

MRS *in vivo* for patients after surgery because of brain tumor revealed statistically significant decreases in the NAA/Cr ratio and increases in the Cho/Cr, Cho/NAA, and mI/Cr ratios. The intensive signals of Lac and Lip appeared in the spectrum.

The Lac/NAA ratio was found to be the strongest independent prognostic and predictive factor for patient survival. For values of Lac/NAA >2.0 (intensive Lac signal), the 1-year survival rate was 30%, and none of those patients were alive 2 years after treatment. For the values of Lac/NAA <2.0, the 2-year survival rate was close to 80%. Although the Lac/NAA ratio correlated strongly with the

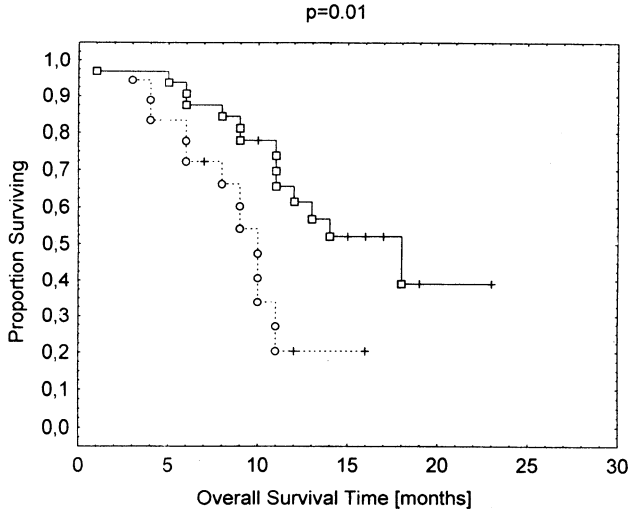

Fig. 1. Overall survival correlated significantly with tumor grade Solid line, Grade 3; dotted line, glioblastoma.

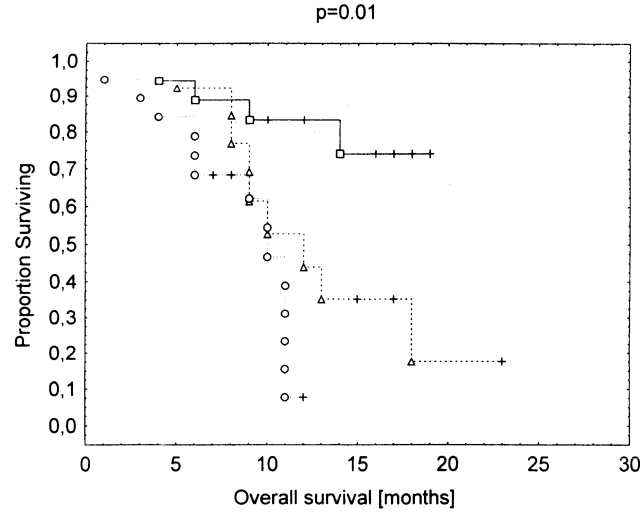

Fig. 2. Overall survival correlated with patient age. Solid line, age <40 years; dashed line, age ≥40 and <55 years; and dotted/no line, age ≤55 years.

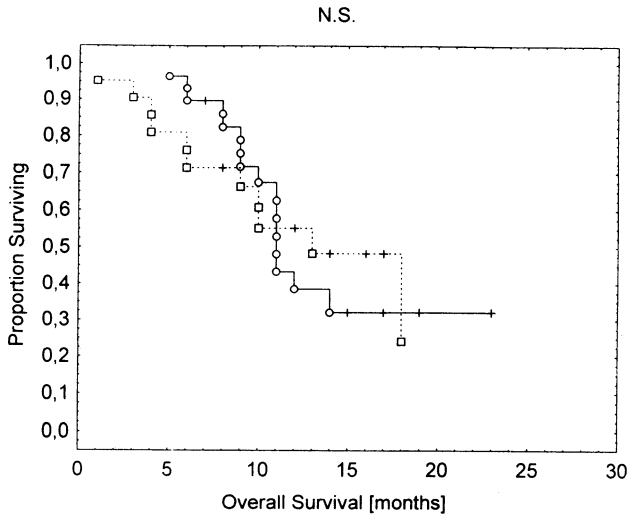

Fig. 3. Extent of operation (total [solid line] vs. partial [dotted line] resection) did not correlate with patient survival.

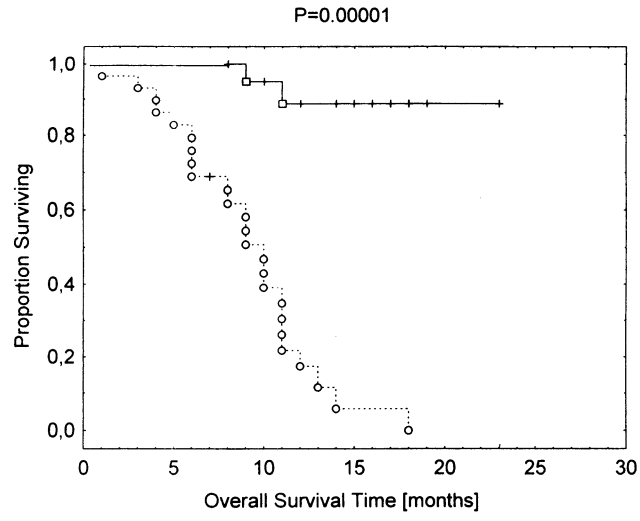

Fig. 5. Survival curves for the patients with different intensities of Lac signal. A Lac/NAA cutoff level of 2 was calculated using logistic regression analysis. Patient survival correlated highly with Lac signal intensity. Solid line, Lac/NAA ≤2; dotted line, Lac/NAA >2.

histologic findings and MRI, its prognostic importance was high in the subgroup of patients with Grade 3 tumors and the group of patients with contrast enhancement on MRI. The forward stepwise multivariate analysis found the Lac/NAA ratio and patient age to be the most important predictors of treatment outcome.

Although the latest MRI techniques are able to provide an excellent depiction of abnormalities in soft tissue morphology, they are frequently unable to distinguish treatment-induced changes from recurrent or residual tumor. For an assessment of the response to therapy, it is therefore important to use functional imaging modalities such as positron emission tomography and MRS (4).

Large randomized trials have established the clinical prognostic factors influencing the survival of patients with malignant gliomas (6–8). The most important factors are age, neurologic performance status, extent of resection, and the presence of enhancement on CT or MRI. Unfortunately, these factors are not sufficient for individualization of treatment. More importantly, functional imaging such as localized MRS may be helpful in delineation of a "boost" region.

Because postoperative MRS studies of the brain are sparse (9, 10), we can only discuss our findings with regard to the preoperative parameters.

^{1}H-MRS of the brain consists of several signals that may be resolved and quantified. The NAA signal derives from the COOCH3 group of *N*-acetyl aspartate, the amino acid

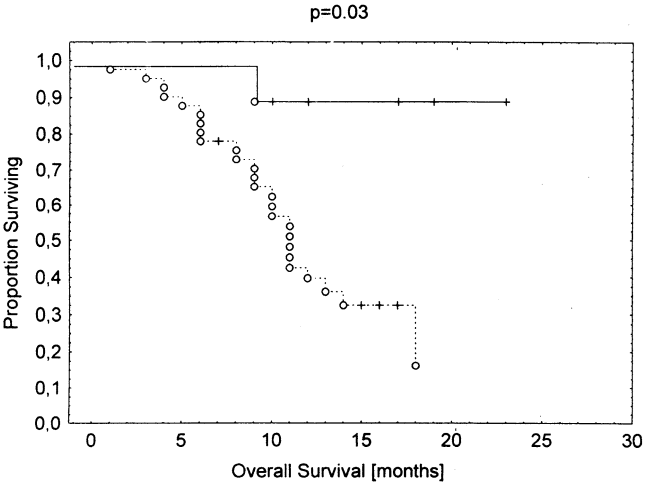

Fig. 4. Patients with contrast enhancement (dotted line) on MRI had significantly shorter survival times compared with those without contrast enhancement (solid line).

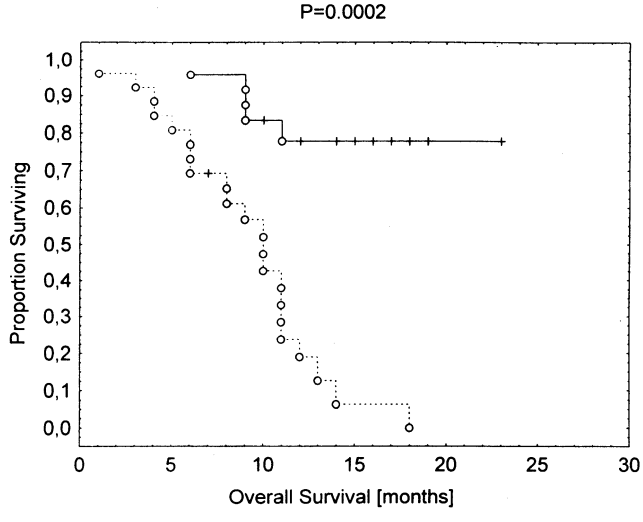

Fig. 6. Survival curves for the patients with different intensities of Lip signal. A Lip/NAA cutoff level of 2 was calculated using logistic regression analysis. Patient survival correlated highly with Lip signal intensity. Solid line, Lip/NAA ≤2; dotted line, Lip/NAA >2.

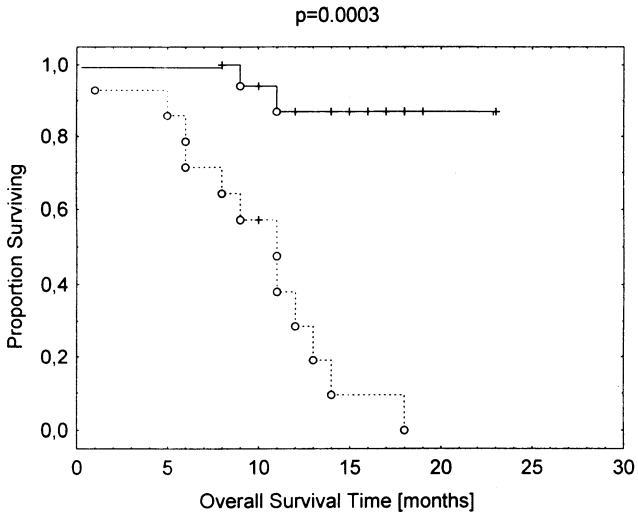

Fig. 7. Survival curves for patients with different intensities of Lac signal in the group with anaplastic astrocytomas (excluding glioblastomas). Patient survival correlated highly with Lac signal intensity. Solid line, Lac/NAA ≤2; dotted line, Lac/NAA >2.

that is present primarily in neurons. A decrease in the NAA level is usually interpreted as due to a reduction in the number of neurons (11). Novel studies have revealed, however, that a decreased intensity of the COOCH3 group signal may reflect lower functional activity of neurons in response to stress or injury (12). On the other hand, the elevation of the Cho/Cr, Cho/NAA, and mI/Cr ratios is explained by the proliferation of glial cells (13). Both Cho and mI are thought to be products of myelin degradation (14). Lac and Lip are not present in normal spectrum; however, the presence of both metabolites in pathologic tissue has been frequently highlighted by many authors (15–19). The Lac signal rises whenever the Lac-producing anaerobic glycolytic pathway exceeds the capacity of the Lac-catabolizing respiratory pathways or when the cellular capacity for exporting Lac to the bloodstream is impaired. Another reason for Lac presence is an impairment of mitochondrial function due to hypoxia. Lac is also thought to be found frequently in malignant gliomas. Similarly, mobile Lip are more likely to be present in higher grade tumors than in lower grade ones (15).

Table 3. Results of forward stepwise multivariate Cox regression analysis

Parameter	Estimate	Standard error	*p*	RR
Lac/NAA ratio	2.61	0.76	0.0001	14
Age	0.52	0.29	0.05	1.7

Abbreviations: Lac = lactate; NAA = *N*-acetyl aspartate; RR = relative risk.

Strongest prognostic factors were Lac/NAA ratio and patient age; the other parameters were excluded by the stepwise procedure.

Lip signals are not seen in normal spectrum because of severe broadening resulting from restricted mobility of Lip molecules involved in cell membrane formation. When cell membranes become disrupted—for any reason—mobile lipids signals may be resolved in spectrum. Recently, it was confirmed that the Lip signals not only increase progressively with tumor grade (16, 17) but that there is also a correlation between the fraction of microscopic necrosis and their integral intensity, as observed *ex vivo* using [1]H-MRS (18). Similarly, it has been shown that Lac signals are present in high-grade astrocytomas and absent in most low-grade gliomas (19). The close correlation of the Lac and Lip concentrations suggests that the mechanisms of their increase are similar.

In summary, we have found an unexpectedly high correlation between patient survival and Lac signal intensity. It is possible that postoperative MRS reflects the metabolism of the postoperative site. Factors such as hypoxia, blood–brain barrier disruption, edema, demyelinization, and inflammatory responses may change brain metabolic function, resulting in changes in the MRS signals (5). The poor outcome of patients with strong Lac signals may be explained by the decreased radiosensitivity in the presence of hypoxia.

Clearly, additional review with a large population will improve the multivariate analysis, important in not only dissecting out the influence of histologic grade and age, but also in the degree of residual tumor seen on MRI.

REFERENCES

1. Sheline GE. Radiotherapy for high grade gliomas. *Int J Radiat Oncol Biol Phys* 1990;18:793–803.
2. Fallai C, Olmi P. Hyperfractionated and accelerated radiation therapy in central nervous system (malignant gliomas, pediatric tumors, and brain metastases). *Radiother Oncol* 1997;43: 235–246.
3. Werner-Wasik M, Scott CB, Nelson DF, *et al.* Final report of phase I/II trial of hyperfractionated and accelerated hyperfractionated radiation therapy with carmustine for adult with supratentorial malignant gliomas. *Cancer* 1995;77:1535–1543.
4. Nelson SJ. Imaging of brain tumors after therapy. *Neuroimag Clin North Am* 1999;9:801–819.
5. Walecki J, Sok&slash;ó M, Pieniążek P, *et al.* Role of short TE 1H-MR spectroscopy in monitoring of post-operation irradiated patients. *Eur J Radiol* 1999;30:154–161.
6. Davis FG, McCarty MJ, Freels S, *et al.* The conditional probability of survival of patients with primary malignant brain tumors. *Cancer* 1999;85:485–491.
7. Nelson DF, Nelson JS, Davis DR, *et al.* Survival and prognosis of patients with atypical or anaplastic features. *J Neurooncol* 1985;3:99–103.
8. Scott CB, Scarantino C, Urtasun R, *et al.* Validation and predictive power of Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis classes for malignant glioma patients: A report using RTOG 90-06. *Int J Radiat Oncol Biol Phys* 1998;40:51–55.

9. Girard N, Wang ZJ, Erbetta A, *et al.* Prognostic value of proton MR spectroscopy of cerebral hemisphere tumors in children. *Neuroradiology* 1998;40:121–125.
10. Lazareff JA, Gupta RK, Alger J. Variation of post-treatment H-MRSI choline intensity in pediatric gliomas. *J Neurooncol* 1999;41:291–298.
11. Bates TE, Stangward M, Keelan J, *et al.* Inhibition of *N*-acetylaspartate production: Implications for ^{1}H MRS studies in vivo. *Neuro Rep* 1996;7:1937–1400.
12. Estève F, Rubin C, Grand S, *et al.* Transient metabolic changes observed with proton MR spectroscopy in normal human brain after radiation therapy. *Int J Radiat Oncol Biol Phys* 1998;40:279–286.
13. Barbarella G, Ricci R, Pirini G, *et al.* In vivo single voxel 1H MRS of glial brain tumors: Correlation with tissue histology and in vitro MRS. *Int J Oncol* 1998;12:461–468.
14. Ross BD. Biochemical considerations in ^{1}H spectroscopy: Glutamate and glutamine, myo-inositol and related metabolites. *NMR Biomed* 1991;4:59–63.
15. Tien RD, Lai PH, Smith JS, *et al.* Single-voxel proton brain spectroscopy exam (PROBE/SV) in patients with primary brain tumors. *Am J Radiol* 1996;167:201–208.
16. Negendank W, Sauter R. Intratumoral lipids in 1H MRS in vivo in brain tumors: Experience of the Siemens cooperative clinical trial. *Anticancer Res* 1996;16:1533–1538.
17. Negendank WG, Sauter R, Brown TR, *et al.* Proton magnetic resonance spectroscopy in patients with glial tumors: A multicenter study. *J Neurosurg* 1996;84:449–458.
18. Kuesel AC, Briere KM, Halliday WC, *et al.* Mobile lipid accumulation in necrotic tissue of high grade astrocytomas. *Anticancer Res* 1996;16:1485–1489.
19. Heesters MA, Kamman RL, Mooyaart EL, *et al.* Localized proton spectroscopy of inoperable brain gliomas: Response to radiation therapy. *J Neurooncol* 1993;17:27–35.