Predicting survival of children with CNS tumors using proton magnetic resonance spectroscopic imaging biomarkers

KAREN J. MARCUS[1], LOUKAS G. ASTRAKAS[2], DAVID ZURAKOWSKI[3], MARIA K. ZARIFI[4], DIONYSSIOS MINTZOPOULOS[2], TINA YOUNG POUSSAINT[5], DOUGLAS C. ANTHONY[4], UMBERTO DE GIROLAMI[5], PETER McL. BLACK[6], NANCY J. TARBELL[7] and A. ARIA TZIKA[2]

[1]Department of Radiation Oncology, Children's Hospital Boston, [2]NMR Surgical Laboratory, Department of Surgery, Massachusetts General Hospital, [3]Department of Orthopaedic Surgery, Children's Hospital Boston, [4]Department of Radiation Oncology, Massachusetts General Hospital, [5]Department of Pathology, Children's Hospital Boston, [6]Department of Neurosurgery, Children's Hospital Boston, Harvard Medical School, Boston, MA; [7]Department of Pathology and Anatomical Sciences Missouri-Columbia, MO, USA

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Abstract. Using brain proton magnetic resonance spectroscopic imaging (MRSI) in children with central nervous system (CNS) tumors, we tested the hypothesis that combining information from biologically important metabolites, at diagnosis and prior to treatment, would improve prediction of survival. We evaluated brain proton MRSI exams in 76 children (median age at diagnosis: 74 months) with brain tumors. Important biomarkers, choline-containing compounds (Cho), N-acetylaspartate (NAA), total creatine (tCr), lipids and/or lactate (L), were measured at the 'highest Cho region' and normalized to the tCr of surrounding healthy tissue. Neuropathological grading was performed using World Health Organization (WHO) criteria. Fifty-eight of 76 (76%) patients were alive at the end of the study period. The mean survival time for all subjects was 52 months. Univariate analysis demonstrated that Cho, L, Cho/NAA and tumor grade differed significantly between survivors and non-survivors ($P \leq 0.05$). Multiple logistic regression and stepwise multivariate Cox regression indicated that Cho + 0.1L was the only independent predictor of survival (likelihood ratio test = 10.27, $P<0.001$; Cox regression, $P=0.004$). The combined index Cho + 0.1L was more accurate and more specific predictor than Cho or Cho/NAA. Accuracy and specificity for Cho + 0.1L were 80% and 86%, respectively. We conclude that brain proton MRSI biomarkers predict survival of children with CNS tumors better than does standard histopathology. More accurate prediction using this non-invasive technique represents an important advance and may suggest more appropriate therapy, especially when diagnostic biopsy is not feasible.

Correspondence to: Dr A. Aria Tzika, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, 51 Blossom Street, Room 261, Boston, MA 02114, USA
E-mail: atzika@partners.org

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Introduction

The most common solid malignancies in children, and the highest cause of cancer-related death in this age group, are malignancies of the central nervous system (CNS) (1). Since the 1970s, the incidence of brain tumors in children has increased to >20% of cancers in children under 15 years of age (2). CNS tumors in children differ in histology and outcome from those occurring in adults, with children surviving longer than adults (3). Despite advances in neuroimaging, surgical techniques, radiotherapy, and the availability of newer chemotherapeutic agents used with molecular targeted therapy, the increase in the 5-year survival rate of children with CNS tumors is reportedly 35% (1). We contend that a correct prognosis may lead to more appropriate therapy and improve survival rate in this population. In addition, with no consistent approach to postoperative evaluation and follow-up of these children (4), due to inherent difficulties in performing diagnostic and serial biopsies, there is a clear need for biologically relevant, non-invasive markers if tumors are to be effectively diagnosed and treated.

In addition to histopathology, clinical factors currently used to predict outcome and assist in treatment decisions include the extent of tumor resection, presence of disseminated disease, and patient age (5). Even combined with histopathology, these clinical variables do not accurately predict outcomes. Molecular biomarkers identified through gene expression have shown independent prognostic significance (6). Overexpression of p53, for example, was significantly associated with an adverse outcome in patients with malignant gliomas in the Children's Cancer Group study, and this association was independent of the histologic features, the age of the child, or the location of the tumor (7). Elevated

ras p21 expression was found to be a common characteristic in pediatric brain tumors (8). In a study of 55 children with medulloblastoma, multivariable analysis of DNA microarray gene-expression profiles predicted outcomes independent of the presence of disseminated disease at diagnosis, histologic subtype, or clinical findings (9). Also, expression microarray data suggest that molecular profiles of biomarkers classify malignant gliomas and predict survival better than does standard histopathology (10). Identification of gene-expression profiles, however, requires biopsy, impossible in certain cases, due to tumor location. In such cases, a non-invasive *in vivo* adjunct to MRI - proton magnetic resonance spectroscopic imaging (MRSI) - is the only resource.

Modern diagnostic imaging techniques accurately detect CNS tumors but with limited specificity (11). Multivoxel proton MRSI allows data to be collected simultaneously from within a lesion as well as in adjacent regions, and promises to provide specific, accurate and sensitive biomarkers capable of signaling potential outcomes among pediatric cancer patients (12). Proton MRSI of brain tumors has been used to predict histology, monitor tumor response to treatment, and differentiate tumor from radiation necrosis (12-23). The *in vivo* application of two-dimensional techniques, which have been primarily used *in vitro*, seems promising (24,25). Used on brain tumors, proton MRSI has shown a reduction or absence of N-acetylasparatate (NAA) and total creatine (tCr) and an increase in choline-containing compounds (Cho), lipids and/or lactate (L). There are few reports on the role of MRSI-derived biomarkers as predictors of survival in patients with brain tumors. We believe ours to be the first study designed to test the hypothesis that combining information from biologically important metabolites obtained by MRSI of brain protons in CNS tumors in children will increase our ability to predict survival.

Patients and methods

Patients. Seventy-six (76) children with newly or previously diagnosed brain tumors, 37 boys and 39 girls, were examined with proton MRSI on a 1.5-T MR system. Clinical data were obtained from the Children's Hospital (Boston, MA) tumor registry, and hospital charts, and included sex and patient age at diagnosis. Median age at the time of diagnosis was 74 months (6-188 months). Tumors were classified by the neuropathologists at Children's Hospital, using the current WHO histological brain tumor classification. Each case was also reviewed by two neuropathologists (DCA, UDG). The histopathological diagnosis for the 76 children was as follows: WHO I (3 craniopharyngioma; 3 dysembryoplastic neuroepithelial tumors; 3 ganglioglioma; 9 pilocytic astrocytoma; and 6 optic gliomas); WHO II (6 astrocytomas, 9 brainstem gliomas, 1 ependymoma, 1 mixed glioma, 3 oligodendroglioma, and 3 thalamic astrocytomas); WHO III (3 anaplastic astrocytomas and 3 anaplastic ependymomas) and WHO IV (7 pontine gliomas, 1 choroid plexus carcinoma, 2 glioblastoma multiforme, 2 medulloblastomas, 1 pineoblastoma, 1 poorly differentiated neuroectodermal tumor, 2 atypical teratoid rhabdoid tumors and 1 supratentorial primitive neuroectodermal tumors. Children with disseminated disease at presentation were excluded. Patients received standard treatment based on the tumor histopathology. Survival outcome was determined for each patient. Fifty-eight of 76 (76%) patients were alive at the end of the study period and were therefore included in the survival analysis as censored data. All patients were studied under a protocol approved by the Committee on Clinical Investigations, Children's Hospital, Boston, MA.

Proton MRSI. Proton MRSI was performed using multivoxel chemical shift imaging with point-resolved spectroscopy (PRESS) and volume preselection (26). Shimming and water suppression were adjusted after selecting a 50-100 cc volume. Water suppression was performed using CHESS and volume selection, with 1100 Hz bandwidth RF pulses for the 180-degree pulse and 2000 Hz for the 90-degree pulse. Typically, with phase-encoding gradients in two directions, the following acquisition parameters were used: TR=1s, TE= 65 msec, 16x16 phase-encoding matrix, 160 mm FOV, slice thickness of 10 mm, 1250 Hz spectral width, 2 averages and 512 points. Data sets of 1-1.2 cc nominal resolution were obtained. Because we were primarily interested in detecting lipids, we used a 65 ms TE to reduce contributions from lactate and to increase lipid sensitivity. To separate lactate from lipids, two-dimensional techniques have shown promise in the assessment of brain tumors (24). The prominent peaks of biological importance were found to be NAA at 2.0 ppm, Cho at 2.2 ppm, tCr at 3.0 ppm, and L at 1.3 ppm. Data processing was performed on a Sun workstation (Sun Microsystems, Mountain View, CA) using General Electric spectroscopy analysis software (SAGE) and in-house software developed using IDL 5.3 (Research Systems, Boulder, CO). The data sets were apodized with a 1.0 Hz Lorentzian filter, Fourier-transformed in the time domain and the two spatial domains and phased using SAGE, first automatically and then manually, as necessary. A baseline estimator was then applied to subtract the broad components of the baseline prior to peak area calculations. Finally, the areas of selected metabolite peaks were estimated using the PIQABLE algorithm (27) developed in IDL. Metabolite images were generated and stored as TIFF files on a Sun SPARC workstation and transferred to a Macintosh workstation where image editing software, including NIH Image and Adobe PhotoShop, was used to overlay the metabolite images onto the corresponding anatomical images. Composite metabolite images (i.e., Cho + L images) were created by adding the Cho and L values for every voxel and then assembling the result in a 0-255 red-scale voxel image. The Cho + L voxel image was extrapolated to the MRI image scale (256x256) and superimposed on it.

Biostatistical analysis. Survivors (n=58) and non-survivors (n=18) were compared using univariate analysis with respect to median levels of Cho and L by the Mann-Whitney U test. Tumors were classified as low-grade (WHO grade I or II) or high grade (WHO grade III or IV). Association between tumor grade and survival was determined by Pearson χ^2. Diagnostic characteristics of sensitivity, specificity and accuracy were calculated for each metabolite, using best cut-off values, and biomarker combination, using standard formulas. Briefly, sensitivity refers to the frequency of a positive test result in patients who died, specificity is the frequency of a negative test result in those who survived, and accuracy represents the percentage of all correct classifications (28). The area under the ROC curve was calculated as a

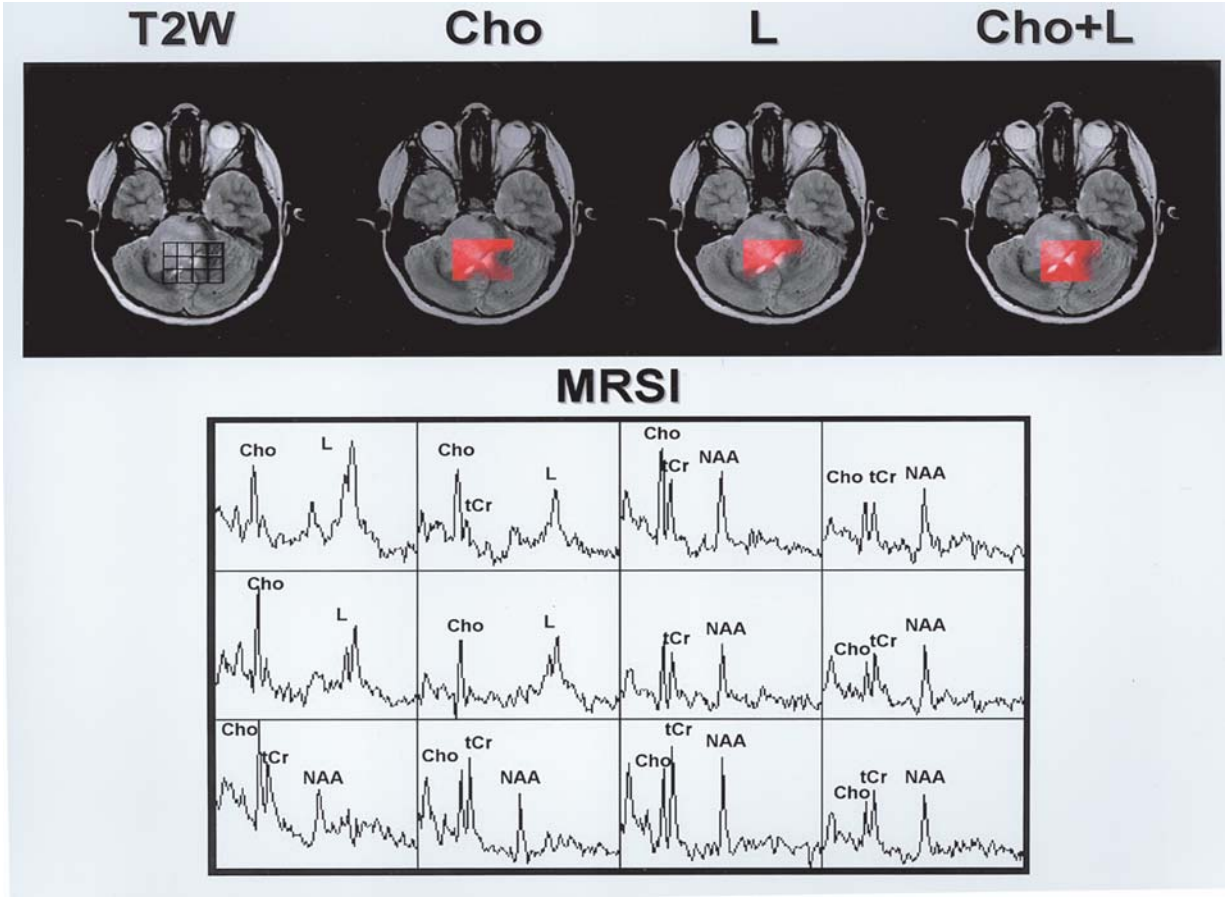

Figure 1. T2-weighted (T2W) image and proton MRSI of a pontine PNET at diagnosis. MR spectra (from within grid areas) indicate three prominent, well-resolved peaks of biological importance, NAA (n-acetylaspartate), tCr (total creatine pool), and choline-containing compounds (Cho). The overlaid Cho, lipids and/or lactate (L), and Cho + L metabolite images show metabolite distributions characteristic of a high-grade tumor.

measure of diagnostic performance for Cho and L (29). We also applied the distribution-free method of Pepe and Thomas (30) to find the linear combination of Cho and lipids, Cho + 0.1L, that maximizes the area under the receiver operating characteristic (ROC) curve to achieve optimal diagnostic accuracy. The ROC curve plots sensitivity (y-axis) versus 1-specificity (x-axis) or false positive rate (FPR) with the points on the curve generated using the cut-off values of the predictors (31). Area under the ROC curve (AUC) is the most widely used index for diagnostic accuracy. The AUC was estimated nonparametrically and used as a measure of test accuracy (32). AUCs were compared using the Z-test (33).

Multiple stepwise logistic regression (backward selection) was applied to determine whether metabolites (i.e., Cho, L, Cho/NAA, Cho + 0.1L) and WHO grade were independent predictors of survival outcome (dead vs. alive). The logistic regression equation includes coefficients, standard errors, adjusted odds ratios, 95% confidence intervals (CI), and the likelihood ratio chi-square test for parameters in the final model obtained by maximum likelihood estimation (34). The probability of a high-grade tumor was estimated for a range of predictor combinations. Patients were categorized on the basis of their Cho (cut-off point ≥1.5), L (cut-off point ≥0.6), Cho/NAA (cut-off point ≥2.0), Cho + 0.1L (cut-off point ≥1.8) and tumor grade values and Kaplan-Meier survival analysis was performed in each category (35). The log-rank test was used to assess differences between survival curves. Multivariate Cox proportional-hazards regression analysis (backward selection) was performed to determine time-related risk factors as independent predictors of survival (36). Statistical analysis was performed using the SPSS software package (version 14.0, SPSS Inc., Chicago, IL). Two-tailed values of $P \leq 0.05$ were considered statistically significant.

Results

To demonstrate the quality of proton MRSI, Fig. 1 shows data for a 9-year-old girl with a primitive neuroectodermal tumor (PNET). The grid overlay on the T2-weighted image indicates the multiple locations for the simultaneous proton MRSI spectral acquisitions. The image labeled 'Cho' depicts the distribution of high Cho, possibly resulting from altered phospholipid metabolism. The image labeled 'L' shows the distribution of primarily mobile lipids (probable contribution from lactate) thought to be a result of cell apoptosis and death in the tumor area. The combined 'Cho + L' image depicts altered phospholipid metabolism and cellular death occurring concomitantly and characteristic of a malignant high-grade tumor.

Univariate analysis was performed to identify whether median levels of metabolites (Cho, L, Cho/NAA), Cho + 0.1L or tumor grade (low versus high) differed between survivors and non-survivors (Table I). Each of these variables was found

Table I. Univariate analysis of variables associated with survival.

Variable	Survivors (n=58) Median (IQR)	Non-survivors (n=18) Median (IQR)	P-value
Cho	1.15 (0.85-1.60)	1.70 (1.20-2.58)	<0.01[a]
L	0 (0-1.23)	1.75 (0.22-3.07)	0.01[a]
Cho/NAA	1.76 (1.17-3.11)	2.82 (1.82-4.22)	0.04[a]
Cho + 0.1L	1.29 (0.92-1.69)	2.03 (1.39-2.90)	<0.01[a]
WHO grade			0.05[a]
Low (I or II)	43 (74%)	9 (50%)	
High (III or IV)	15 (26%)	9 (50%)	

Data for choline (Cho), lipids/lactate (L) and Cho/n-acetylasparate (Cho/NAA) metabolites are medians with the interquartile range (IQR) shown in parentheses with P-values determined by the Mann-Whitney U test. Distribution of WHO grade was compared between survivors and non-survivors by χ^2 analysis. [a]Statistically significant.

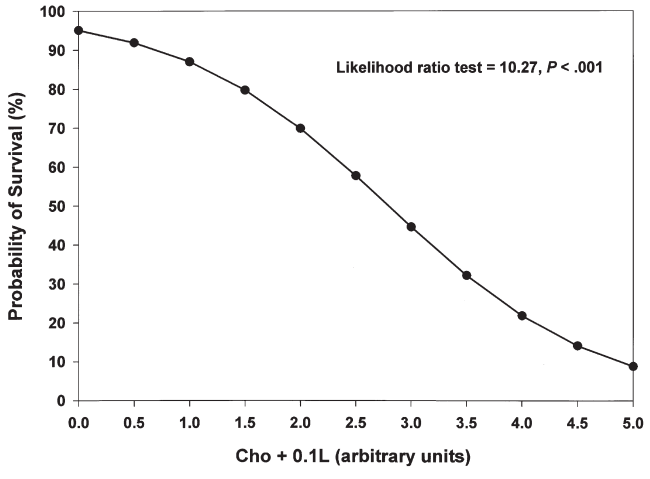

Figure 2. Probability of survival based on Cho + 0.1L value. Logistic regression indicated that among MRSI-derived variables and tumor grade, Cho + 0.1L level was the only independent predictor of survival. Theoretical curve illustrates the probability of survival with increasing Cho + 0.1L.

Table II. Kaplan-Meier analysis for choline, lipids, Cho/NAA, and WHO grade based on chosen cut-off values.

Category	Mean survival time (SE) (months)	Total cases (no. of surviving)	P-value (log-rank test)
Cho <1.5	57.4 (3.0)	47 (41)	0.003[a]
Cho ≥1.5	41.3 (5.2)	29 (17)	
L <0.6	54.8 (3.6)	42 (35)	0.15
L ≥0.6	46.7 (4.4)	34 (23)	
Cho/NAA <2.0	59.0 (2.7)	43 (38)	0.002[a]
Cho/NAA ≥2.0	40.9 (5.1)	33 (20)	
Cho + 0.1L <1.8	57.6 (2.7)	47 (41)	<0.0001[a]
Cho + 0.1L ≥1.8	32.4 (6.2)	29 (17)	
Low WHO grade (I or II)	54.4 (3.3)	52 (43)	0.12
High WHO grade (III or IV)	46.4 (5.4)	24 (15)	

SE, standard error. [a]Statistically significant.

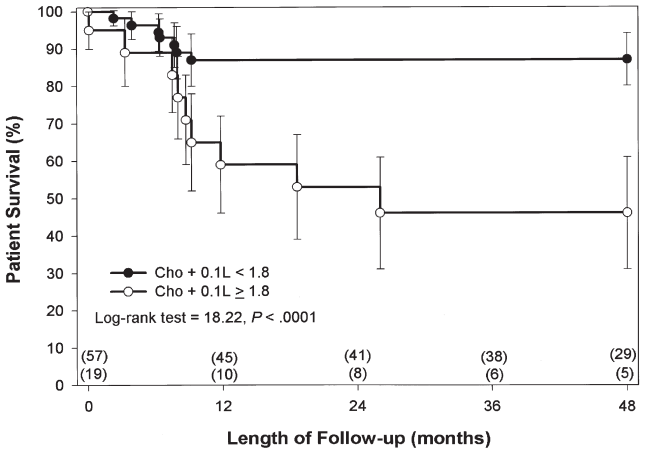

Figure 3. Kaplan-Meier survival curves according to choline (Cho) + 0.1L. Survival was significantly higher in patients with Cho + 0.1L <1.8. Numbers in parentheses represent patients who were in the follow-up with Cho + 0.1L <1.8 (top row) and patients who had not died, although continued to be at risk with Cho + 0.1L ≥1.8 (lower row).

to be associated with outcome, particularly Cho (P<0.01), L (P<0.01) and Cho + 0.1L (P<0.01) in which lower median levels were associated with patient survival.

ROC analysis indicated good discrimination between survivors and non-survivors for Cho (AUC = 0.725, 95% confidence interval = 0.601-0.859) and L (AUC = 0.687, 95% confidence interval = 0.544-0.830). The combined index Cho + 0.1L discriminated survivors from non-survivors better than Cho or L based on area under the ROC curve (AUC = 0.734, 95% confidence interval = 0.592-0.875. Multiple, stepwise, logistic regression analysis indicated that among Cho, L, Cho/NAA, Cho + 0.1L, and tumor grade, only Cho + 0.1L was independently predictive of survival (likelihood ratio test = 10.27, P<0.001). The variables Cho (P=0.42), L (P=0.44), tumor grade (P=0.80) and Cho/NAA (P=0.44) provided no additional information (beyond that of Cho + 0.1L) in terms of differentiating survival versus non-survival. The predicted probability of survival based on Cho + 0.1L level, determined by logistic regression analysis, is shown in Fig. 2. The relationship indicates that a patient with a Cho + 0.1L value of 1.0 having a survival probability of 87%, whereas a patient with a value of 3.0 has a probability of only 22%.

Table II lists the mean survival time for the different Cho, L, Cho/NAA, Cho + 0.1L and WHO grade categories (chosen cut-off levels). The mean survival time for all patients was 52 months (95% CI, 45-57 months). Kaplan-Meier analysis revealed highly significant differences in survival times based

Table III. Diagnostic characteristics of biomarkers with cut-off values chosen to provide maximum accuracy in differentiating patient survival in children with CNS tumors.

Biomarker	Cut-off values	Sensitivity	Specificity	Accuracy
Cho	≥1.5	67 (12/18)	71 (41/58)	70 (53/76)
Cho/NAA	≥2.0	72 (13/18)	66 (38/58)	67 (51/76)
Cho + 0.1L	≥1.8	61 (11/18)	86 (50/58)	80 (61/76)

on Cho + 0.1L ≥1.8, where patients over the cut-off value had earlier mortality and more events (log-rank test = 18.22, P<0.0001, Fig. 3). Similarly, Cox regression analysis confirmed that among all other MRSI-derived variables and tumor grade), only Cho + 0.1L independently predicted survival (likelihood ratio test = 10.27, P<0.001; Cox regression, P=0.004). The hazard ratio for Cho + 0.1L ≥1.8 was 3.9 (95% CI, 1.5-10.5) indicated that the monthly odds of dying are nearly 4 times higher in patients with levels of Cho + 0.1L ≥1.8 compared to those with lower Cho + 0.1L ≥1.8 levels; this finding is independent of patients' tumor grade, L levels, Cho and Cho/NAA. As shown in Fig. 3, more deaths occurred among those with higher Cho + 0.1L ≥1.8 levels. Kaplan-Meier survival at 12 months was 86% for patients with Cho + 0.1L ≤1.8 compared to 69% for those with Cho + 0.1L ≥1.8.

Table III shows the diagnostic ability of each biomarker to predict survival. By choosing cut-off points corresponding to their maximum accuracy, we found that the combined marker Cho + 0.1L, was more accurate (80%) than either Cho/NAA or Cho alone, more specific (86%) than Cho/NAA or Cho alone and equally sensitive as Cho (61%) but less sensitive than Cho/NAA (72%).

Discussion

In the present study, Cho, Cho/NAA and L levels by *in vivo* brain proton MRSI as well as tumor grade by standard histopathology were found to be biomarkers of survival. Based on Cox regression analysis, however, Cho + 0.1L was the only predictor of survival outcome. These results suggest that proton MRSI-derived biomarkers predict survival of children with CNS tumors better than does standard histopathology, with Cho + 0.1L the optimum predictor, a finding that is in agreement with a recent study of malignant gliomas comparing gene-expression of biomarkers with standard histopathology (10). The authors suggested that this might mean that pathologic diagnosis using the WHO classification system remains subjective, a factor which may also have affected our study (10,37). Nevertheless, we do not infer that proton brain MRSI or molecular analysis should replace standard neuropathology.

The present study is an extension of previous reports demonstrating that *in vivo* brain proton MRSI provides biochemical information on tumors that is in agreement with *ex vivo* information obtained from biopsy or resection (38). Our study is also in agreement with a recent report on the ability of proton MRSI-derived Cho to differentiate between non-neoplastic lesions and brain tumors in children, a distinction of importance especially in inoperable cases (21). Fountas *et al* evaluated concentrations of Cho, NAA, tCr, myo-inositol, L, and ratios of these metabolites and compared them blindly to histopathology results following biopsy, and demonstrated that the ratio of Cho to tCr was a statistically significant biomarker in differentiating the grade of astrocytomas (39). In agreement with the report by Astrakas *et al*, (19) who found that the linear combination of Cho and L, Cho + 0.49L was more predictive of tumor grade than Cho alone, we have found that Cho + 0.1L was more predictive of survival than Cho alone.

Indeed, the Cho peak detected by *in vivo* MRSI appears to be the most specific and, as suggested in this study, the most sensitive biomarker in tumors or tissues that are oncogenically transformed and have high proliferative potential. Alternatively, in the absence of compensating apoptotic mechanisms or limitations of vascular supply, Cho may be elevated because: i) the volume of interest is highly cellular (40-42), ii) it includes cells with high PCho, perhaps due to increased proliferative potential (43-48), or iii) it includes oncogenically transformed cells (49-51). The MRS-visible lipids, also important biomarkers, not only correlate with necrosis or apoptosis (42), but also with the proportion of cells in the late S- or G2 phase of the cell cycle (52). This may explain why the combination of Cho and L peaks could be a stronger diagnostic biomarker than either peak alone.

The results of the present study also confirm that MRSI provides important prognostic information in both children and adults (18,23,53). In fact, the Cho/NAA ratio predicts progression in pediatric brain tumors (12,54) and, reportedly, predicted survival in children with recurrent brain tumors (54). As shown in the Kaplan-Meier analysis (Table III), Cho/NAA ≥2.0 predicted poor survival outcome in our study as well.

As noted earlier, there are few reports on biomarkers of survival in children with brain tumors (23). Reports on adult patients with brain tumors suggest that proton MRSI markers predict survival (53,55) and a single study in children with recurrent primary brain tumors suggests that Cho/NAA should be evaluated as a prognostic indicator in newly diagnosed childhood brain tumors (54). Indeed, our study is consistent with these reports, and also reveals novel insights into MRSI-detectible biomarkers as predictors of survival in children with brain tumors. We believe this is the first report to show that non-invasive proton MRSI measurement of biomarkers, in particular Cho combined with L, enhances our ability to predict which children will survive their brain tumors.

Finally, we suggest that brain proton MRSI be used not as a proxy to histopathology but as a non-invasive adjunct to MRI that may gain the role of the simplest, readily available, non-invasive method to make the distinction between favorable and unfavorable outcomes for children with brain tumors under the current standard of care, especially when biopsy or major resection is not possible. Because there is current limitation with standard histopathology, we suggest that proton MRSI-detectible biomarkers, along with other promising molecular profiles of biomarkers be used in addition to standard neuropathology as combined predictors of survival.

To this end, we believe that the future use of combined data, perhaps aided by artificial neural networks or other robust computer-based algorithms that can learn to recognize complex relationships of data will prove useful in subclassifying tumors and will predict survival better (56). Ultimately, we hope that this combined approach will be an important adjunct to evaluate novel therapeutic regimens.

Acknowledgments

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