Magnetic resonance spectroscopic detection of lactate is predictive of a poor prognosis in patients with diffuse intrinsic pontine glioma

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Diffuse brainstem glioma has a poor prognosis, and there are few long-term survivors. We looked for clinical, conventional magnetic resonance (MR), and MR spectroscopic (MRS) findings predictive of the prognosis of patients with brainstem glioma. Our institutional review board approved this retrospective study of 23 patients with diffuse intrinsic pontine or diffuse medullary brainstem glioma treated during the period 2000–2009. To evaluate prognostic values, we performed a Kaplan-Meier survival analysis (log-rank test) that incorporated the patients’ age and sex, symptom duration, the presence or absence of cranial nerve palsy, long tract sign, ataxia, and cysts, the chemotherapeutic regimen, Gd enhancement, longitudinal and cerebellar extension, basilar artery encasement, and MRS parameters. Of the 23 diffuse brainstem gliomas, 19 were located at the pons (ratio of male to female patients, 1.1:1). The mean age of the 23 patients was 15.9 years (range, 4–50 years); 16 were aged <20 years. The duration of overall survival was 19.7 months; in patients with diffuse intrinsic pontine glioma, it was 16.6 months, and in patients aged <20 years, it was 11.8 months. Clinical and conventional MR findings at presentation were not predictive of the prognosis in children with diffuse intrinsic pontine glioma. In addition, a patient age <20 years and the detection of lactate by MRS were poor prognostic factors. The MRS detection of lactate is a prognostic factor in patients with diffuse intrinsic pontine glioma. Additional studies of larger patient populations using other imaging modalities are needed.

Keywords: brainstem glioma, diffuse intrinsic pontine glioma, lactate, MR spectroscopy.
because these metabolites are absent in healthy brain tissue.\textsuperscript{13--15} Therefore, in addition to other 1HMRS parameters, in this study, we closely observed the levels of lactate and lipids. In our retrospective radiological review, we examined whether MRI characteristics were predictive of survival for patients with diffuse brainstem gliomas, including diffuse pontine gliomas, who were treated at our institution. Our results showed that the lactate expression seen in pretreatment 1HMRS is an important prognostic factor in these patients.

Materials and Methods

Patients

This retrospective study was approved by our institutional review board; written patient consent was waived. To protect patient privacy we removed all identifiers from our records at the time of completion of our analyses. This study included 23 patients with pontine or medullary brainstem glioma treated at Hiroshima University Hospital during the period 2000–2009. None of the patients had exophytic-type brain stem gliomas; all pontine gliomas were of the diffuse intrinsic type (ie, diffuse intrinsic pontine glioma).

Of the 23 patients, 12 were male and 11 were female; 19 patients (10 of whom were males and 9 of whom were female) had diffuse intrinsic pontine glioma. The mean age of the 23 patients was 15.9 years (range, 4–50 years); 16 patients were aged <20 years. The mean age of the 19 patients with diffuse intrinsic pontine glioma was 13.5 years (range, 4–36 years). All but 2 patients with medullary glioma had received conventional irradiation therapy (dose, ≤54 G); these 2 patients were treated with chemotherapy alone. Patients with diffuse intrinsic pontine glioma underwent radiotherapy plus the concomitant administration of nimustine (70 mg/m\textsuperscript{2} on days 1 and 29) and vincristine (1.4 mg/m\textsuperscript{2} on days 0 and 1), as well as adjuvant therapy every 8 weeks with nimustine or temozolomide (TMZ) plus radiochemotherapy (the Stupp regimen).\textsuperscript{16} At the time of recurrence, platinum-based chemotherapy, oral etoposide, or supportive care was delivered. At the end-of-life stage, none of the patients underwent endotracheal intubation or tracheotomy.

MRI and single-voxel 1HMRS scans were obtained for all 23 patients immediately before irradiation or surgical biopsy. No patients had a history of previous surgery, chemotherapy, or radiotherapy. Their response rate after radiotherapy was determined based on the criteria of MacDonald et al.\textsuperscript{17} Information on OS and progression-free survival (PFS) rates was obtained from medical records. In compliance with our treatment protocol, the patients were seen on an outpatient basis at least once per month; MR studies were performed every other month. Whole-spine MR data were examined in patients manifesting intracranial dissemination or symptoms suspect of spinal dissemination.

Recurrence was defined on the basis of the criteria of MacDonald et al.\textsuperscript{17}

\textbf{MRI and MRS}

1HMRS was performed with the point-resolved spectroscopic method (Probe-P; repetition time, 2,000 ms (3.0 T); 1,500 ms (1.5 T); TE = 144 ms; data points, 2,048; signals acquired, 64 (3.0 T), 128 (1.5 T); double-spin-echo sequence) on a 1.5 T, versions 5.7 and 12 (Signa Horizon; GE Medical Systems), or a 3.0 T superconducting system, versions 12 and 15 (Signa Excite HD 3.0 T); a circularly polarized head coil was used. Additional spectra with a short TE (30 ms) were also acquired. To guide single-voxel spectroscopic examinations, we used T2-weighted, fluid attenuated inversion recovery (FLAIR), and enhanced T1-weighted images. Under 3D control, the rectangular 1HMRS voxel was placed on the solid tumor area; efforts were made to avoid contamination by surrounding normal-appearing tissue, the skull base, and fourth ventricle. The voxel size was between 15 × 15 × 15 mm (volume, 3.4 cm\textsuperscript{3}) and 20 × 20 × 20 mm (volume, 8 cm\textsuperscript{3}); the estimated disease fraction in the VOI exceeded 70%–100% in all patients. Spatial suppression pulses were applied to the outside of the voxel to reduce spectral contamination; global and localized shimming on the water proton and optimization of water suppression were performed, resulting in water peak-line widths of 2–4 Hz. All MR images covering the VOI selected for MRS were retrospectively assessed by 2 investigators. All spectra were reviewed for quality, and spectra of insufficient quality were excluded from final analyses. Spectra of poor quality were identified by an increased line width of the water resonance (a measure of the field homogeneity in the VOI) of ≥3 standard deviations greater than the mean line-width of all tumor spectra. The line-width of the water signal intensity (SI) was automatically measured and reported by the processing software. The relative SIs of choline (Cho), creatine (Cr), N-acetyl aspartate (NAA), and myo-inositol (ml) were obtained by numeric integration of fitted signals. The expression of apparent citrate was determined by visual inspection. Because the signals of lactate and lipids partially overlap in 1HMRS, their expression was determined by previously reported methods.\textsuperscript{14} In brief, 1HMRS was performed with a TE of 144 ms followed by a TE of 30 ms and/or a TE of 288 ms, if necessary. Long echo-times would suppress the lipid component and therefore allow greater sensitivity for lactate; because the relaxation time of lipids is very short, short echo-time (TE) spectroscopy is suitable for the assessment of lipids. At TE 144 ms, the resonance of lactate exhibits a 180° phase leading to a negative in-phase doublet, whereas TE 30 ms and TE 288 ms give rise to a positive in-phase doublet.

In the clinical setting, if the lactate signal is present and the lipids signal is absent in the VOI of the tumor, a negative doublet peak would appear at 1.3 ppm at TE 44 ms, and a positive doublet peak would appear
at 1.3 ppm at TE 30 ms on 1HMRS. If both lactate and lipids signals are present, there would be a negative peak in the left component of the lactate double peak, whereas the right component of the double peak would be included in the positive lipid peak. This results in a decrease in the right component of the lactate-negative peak at TE 144 ms. The right component of the lactate doublet peak disappears, and the left component of the lactate doublet peak decreases as a result of the greater increase in the positive lipids peak; consequently, the pattern of a reversible negative/positive peak is observed at TE 144 ms. If a large lipids signal is obtained in the VOI, the lactate signal would be hidden in a positive lipids peak at 0.9 ppm and 1.4 ppm; on the left shoulder of the 1.4 ppm lipids peak there would appear a notch at 1.3 ppm; this is indicative of the presence of lactate. If there is no lactate but a lipids signal in the VOI, there will be a positive peak at 0.9 ppm and 1.4 ppm at TE 30 ms; no notch would be observed on the left shoulder of the 1.4-ppm peak.

Statistical Analysis

The duration of survival for patients with brainstem gliomas was measured from the time of diagnosis to the time of death or last follow-up. To evaluate prognostic values, we performed Kaplan-Meier survival analysis (log-rank test) that incorporated the patients’ age and sex, symptom duration, the Karnofsky performance score (KPS) at diagnosis, the presence or absence of cranial nerve palsy, long tract sign, ataxia, the chemotherapeutic regimen, gadolinium (Gd) enhancement, cysts/necrosis, longitudinal and cerebellar extension, basilar artery encasement, and MRS parameters that included the ratio of NAA-to-Cr, Cho-to-Cr, Cho-to-NAA, and mI-to-Cr, and the lactate, lipid, and citrate levels. We also analyzed the relationship between the prognosis and the response to chemo-radiotherapy based on the criteria of MacDonald et al.17 Statistical analyses were performed with StatView computer software, version 5.0 (SAS Institute).

Results

Tumor Location and Prognosis

Of the 23 tumors, 19 were diffuse intrinsic pontine gliomas; the other 4 were located at the medulla (Table 1). At the most recent follow-up, 7 patients were alive, 4 of whom had diffuse intrinsic pontine glioma. OS was 19.7 months (range, 6.5–89.5 months; median, 26.1 months); the OS for patients with diffuse intrinsic pontine glioma patients was 16.6 months (range, 6.5–83.8 months; median, 26.1 months) (Fig. 1); for patients aged <20 years, it was 11.8 months. The tumor location at the pons was a poor prognostic factor.

In subsequent studies, to analyze the prognosis of patients with brainstem glioma more accurately, we excluded the 4 patients with diffuse medullary glioma. We focused on diffuse intrinsic pontine gliomas (n = 19) and reviewed clinical findings and prognostic factors. Representative cases are shown in Figs. 2–5.

Recurrence of Diffuse Intrinsic Pontine Glioma

Of the 19 diffuse intrinsic pontine gliomas, 71% recurred in the radiotherapy fields; 29% developed neuraxis metastasis a median of 7.9 months after the diagnosis of diffuse intrinsic pontine glioma (range, 4.2–36.1 months). Median survival after recurrence was 2.9 months.

Hydrocephalus of Diffuse Intrinsic Pontine Glioma

In the course of the disease, 52.6% of patients developed hydrocephalus, and they tended to have a good prognosis; 30% underwent ventriculo-peritoneal (V-P) shunting, while the others developed hydrocephalus at the terminal stage of the disease and did not undergo V-P shunting. The mean interval between diagnosis and the development of hydrocephalus was 7.9 months.

Clinical Characteristics and Prognosis of Diffuse Intrinsic Pontine Glioma

The relationship between the clinical characteristics and the prognosis of diffuse intrinsic pontine glioma is summarized in Table 2. A patient age of <20 years was a statistically poor prognostic factor and correlated with OS (P < .05) (Fig. 6A). Ataxia and a KPS <70 were also associated with shorter PFS but not with shorter OS. Sex, symptom duration, and the existence of cranial nerve palsy and long tract sign were not associated with OS.

Tumor Response, Chemotherapy and Prognosis of Diffuse Intrinsic Pontine Glioma

Nimustine-based therapy or TMZ treatment based on the Stupp regimen was administered to all patients with diffuse intrinsic pontine glioma. After conventional radiotherapy with concurrent chemotherapy, 9 patients manifested a partial response, and in 5 each, the disease was stable or progressive based on MacDonald criteria.17 Patients with progressive disease had a significantly poor prognosis (P < .05, by log-rank test), and patients with a partial response tended to manifest a good prognosis.

At the time of recurrence, we administered TMZ treatment, platinum-based chemotherapy, or daily oral etoposide treatment. Some tumors responded to chemotherapy; however, we found no statistically significant difference between patients who had and those who had not received any kind of chemotherapy.
Table 1. Summary of brainstem glioma patients

| Patient | Age, years | Sex | Tumor location | Recurrence Status | PFS, months | OS, months | KPS | Gd Necrosis | Lactate | Lipids | NAA/\text{Cr} | Cho/\text{Cr} | Cho/NAA | NAA/\text{Cr} | Cho/\text{Cr} | ml/\text{Cr} | Cho/NAA |
|---------|------------|-----|----------------|-------------------|-------------|------------|-----|-------------|---------|-------|------------|------------|---------|------------|------------|------------|---------|--------|
| 1       | 24         | M   | Pons           | Dead              | 36.10       | 59.93      | 70  | +           | +       | +     | 1.250      | 2.500      | 4.200   | NE         | NE         | NE        | NE       |
| 2       | 23         | M   | Pons           | Alive             | 70.83       | 83.77      | 70  | +           | +       | -     | 2.000      | 2.200      | 1.222   | 2.167      | 1.611      | 1.773     |
| 3       | 36         | M   | Pons           | Dead              | 20.10       | 33.17      | 70  |             | -       | -     | 0.500      | 1.500      | 3.000   | 0.857      | 1.321      | 1.179     |
| 4       | 6          | M   | Pons           | Dead              | 9.40        | 11.83      | 60  | +           | +       | +     | 0.385      | 2.810      | 7.400   | 0.650      | 2.160      | 1.650     |
| 5       | 9          | M   | Pons           | Alive             | 63.10       | 63.10      | 90  |             | -       | -     | 0.870      | 1.660      | 1.944   | NE         | NE         | NE        |
| 6       | 8          | F   | Pons           | Dead              | 4.27        | 10.63      | 70  | +           | +       | +     | 0.780      | 1.680      | 2.083   | 0.990      | 1.390      | 1.120     |
| 7       | 17         | M   | Pons           | Dead              | 10.93       | 16.60      | 80  |             | -       | -     | 1.250      | 1.750      | 1.400   | 1.290      | 1.900      | 0.900     |
| 8       | 6          | F   | Pons           | Dead              | 5.63        | 9.83       | 60  | +           | +       | +     | 1.080      | 5.390      | 4.923   | NE         | NE         | NE        |
| 9       | 6          | M   | Pons           | Dead              | 6.33        | 8.53       | 80  | +           | +       | +     | 0.780      | 1.390      | 1.769   | 0.950      | 1.170      | 1.640     |
| 10      | 6          | M   | Pons           | Dead              | 10.77       | 12.73      | 100 |             | +       | -     | 1.121      | 2.061      | 1.838   | 0.970      | 1.395      | 0.864     |
| 11      | 17         | F   | Pons           | Dead              | 13.30       | 19.67      | 70  | +           | +       | -     | 0.800      | 2.440      | 3.120   | 1.120      | 1.990      | 1.120     |
| 12      | 5          | F   | Pons           | Dead              | 32.53       | 40.10      | 60  | +           | +       | +     | NE         | NE         | NE     | 1.404      | 1.818      | 1.000     |
| 13      | 5          | F   | Pons           | Dead              | 4.93        | 6.50       | 30  | +           | +       | +     | 1.360      | 1.400      | 1.029   | 1.413      | 1.255      | 0.761     |
| 14      | 32         | F   | Pons           | Alive             | 31.30       | 50.87      | 80  | +           | +       | -     | 2.182      | 1.682      | 0.771   | 1.814      | 1.239      | 0.837     |
| 15      | 36         | M   | Pons           | Dead              | 17.43       | 25.17      | 60  | +           | +       | -     | 1.121      | 2.061      | 1.838   | 0.970      | 1.395      | 0.864     |
| 16      | 6          | F   | Pons           | Dead              | 7.70        | 10.90      | 80  | +           | +       | +     | 0.769      | 2.769      | 3.600   | 0.875      | 1.591      | 1.125     |
| 17      | 4          | F   | Pons           | Dead              | 4.17        | 6.50       | 40  | +           | +       | -     | 1.125      | 2.000      | 1.778   | 0.808      | 2.125      | 0.923     |
| 18      | 6          | F   | Pons           | Dead              | 5.43        | 14.37      | 60  |             | +       | -     | 0.821      | 1.607      | 1.957   | 0.857      | 1.385      | 1.349     |
| 19      | 4          | M   | Pons           | Alive             | 11.57       | 11.57      | 100 |             | +       | -     | 1.600      | 1.600      | 1.000   | 1.190      | 1.238      | 1.143     |
| 20      | 50         | M   | Medulla        | Dead              | 4.47        | 11.27      | 100 | +           | +       | +     | NE         | NE         | NE     | 0.900      | 1.750      | 0.930     |
| 21      | 49         | F   | Medulla        | Alive             | 84.27       | 89.47      | 80  |             | -       | -     | 1.600      | 2.000      | 1.250   | 1.246      | 1.230      | 0.852     |
| 22      | 8          | F   | Medulla        | Alive             | 23.57       | 23.57      | 40  | +           | -       | +     | 1.190      | 1.476      | 1.240   | 1.261      | 1.043      | 0.500     |
| 23      | 3          | M   | Medulla        | Alive             | 38.17       | 38.17      | 40  |             | -       | -     | 0.143      | 2.714      | 19.000  | 0.057      | 1.043      | 1.171     |

Cho indicates choline; Cr indicates creatine; KPS indicates Karnofsky performance status; Gd indicates gadolinium; ml indicates myo-inositol; NAA indicates N-acetyl aspartate; NE indicates not examined; OS indicates overall survival; PFS indicates progression free survival; TE indicates echo time.
Conventional MR Findings and Prognosis of Diffuse Intrinsic Pontine Glioma

The relationship between the clinical characteristics and the prognosis of diffuse intrinsic pontine glioma is shown in Table 3. Gd enhancement at presentation was not predictive of the prognosis (Fig. 6B), nor were other conventional MR findings.

MRS Parameters and Prognosis of Diffuse Intrinsic Pontine Glioma

Among the MRS parameters, lactate expression was statistically correlated with shorter OS ($P < .01$) (Fig. 6C), as was a high Cho-to-NAA ratio at TE 30 ms ($P < .05$). No other parameters exhibited an association with OS (Table 4). In 26% of the patients, we detected an apparent citrate peak, and these patients tended to have a good prognosis.

Discussion

We found that the MRS detection of lactate is a poor prognostic factor in patients with diffuse intrinsic pontine glioma. To our knowledge, ours is the first documentation of the power of 1HMRS for predicting the prognosis of patients with this tumor. Fischbein et al.\textsuperscript{18} reported that the differentiation between diffuse and focal medullary tumors may be difficult. Therefore, in our detailed study of the prognostic value of 1HMRS, we excluded diffuse medullary gliomas.

Some clinical characteristics have been reported to be associated with the prognosis of diffuse brainstem gliomas. For example, younger age may predict a better outcome,\textsuperscript{19,20} and hydrocephalus at presentation may be related to shorter PFS;\textsuperscript{6} a lower KPS was reported to be a poor prognostic factor.\textsuperscript{21,22} It has been suggested that symptom duration correlated with improved survival; however, the tumors in those studies were low grade, and symptom duration may not be a true prognostic factor in patients with diffuse brainstem glioma.\textsuperscript{3,6,21,23–26}

Most recurrent tumors were found in the radiotherapy field,\textsuperscript{27} and neuraxis metastasis was observed in the course of disease in 5%–30% of patients with...
Fig. 3. Magnetic resonance (MR) findings for a 6-year-old girl with an enhancing diffuse infiltrative pontine glioma (patient 8). Note the presence of a lactate peak and a large lipid peak. (A) Axial T2-weighted image. (B) Axial T1-weighted image after gadolinium infusion. (C) Sagittal T1-weighted image after gadolinium infusion. (D) 1H MRS at 144 ms echo time. (E) 1H MRS at 30 ms echo time. With TE 30 ms, lactate signals are almost hidden by lipid signals and only a small notch (arrowhead) is observed.

Fig. 4. Magnetic resonance (MR) findings for an 8-year-old girl with an enhancing diffuse infiltrative pontine glioma (patient 6). Note the presence of a lactate peak and a small lipid peak. (A) Axial T1-weighted image. (B) Axial T2-weighted image. (C) Axial T1-weighted image after gadolinium infusion. (D) 1H MRS at 144 ms echo time. (E) 1H MRS at 30 ms echo time. With a TE of 30 ms, lipids and lactate peaks overlap, resulting in a drop (arrowhead).
Fig. 5. Magnetic resonance (MR) findings for a 6-year-old boy with a non-enhancing diffuse infiltrative pontine glioma (patient 10). Note the presence of a lactate peak and a large lipid peak. (A) Axial FLAIR image. (B) Axial T1-weighted image after gadolinium infusion. (C) Sagittal T1-weighted image after gadolinium infusion. (D) 1HMRS at 144 ms echo time. (E) 1HMRS at 30 ms echo time.

Table 2. Summary of clinical findings and prognosis of diffuse intrinsic pontine glioma

| Characteristic              | Rate, % | Progression-free survival | Overall survival |
|----------------------------|---------|---------------------------|-----------------|
|                            |         | Median duration, months   | P               | Median duration, months | P |
| Age, years                 |         |                           |                 |
| 0–20                       | 73.7    | 7.7                       | .0405           | 11.8                     | .0280 |
| ≥20                        | 26.3    | 36.1                      |                 | 59.9                     | .0280 |
| Sex                        |         |                           |                 |
| Male                       | 52.6    | 17.4                      | .0589           | 25.2                     |
| Female                     | 47.4    | 5.6                       |                 | 10.9                     |
| KPS                        |         |                           |                 |
| 70–100                     | 63.2    | 13.3                      | .0405           | 19.7                     |
| 10–60                      | 36.8    | 5.6                       |                 | 11.8                     |
| Symptom duration           |         |                           |                 |
| ≤1 month                   | 52.6    | 9.4                       | .0405           | 11.8                     |
| >1 month                   | 47.4    | 17.4                      | .9576           | 25.2                     |
| Cranial nerve palsy        |         |                           |                 |
| Absent                     | 21.1    | 9.4                       | .9422           | 12.7                     |
| Present                    | 78.9    | 13.3                      |                 | 19.7                     |
| Long tract sign            |         |                           |                 |
| Absent                     | 26.3    | 20.1                      | .514            | 33.2                     |
| Present                    | 73.7    | 9.4                       |                 | 14.4                     |
| Ataxia                     |         |                           |                 |
| Absent                     | 36.8    | 36.1                      | .0264           | 59.9                     |
| Present                    | 63.2    | 7.7                       |                 | 12.7                     |

KPS indicates Karnofsky Performance Status
brainstem gliomas. Of our patients, 29% manifested neuraxis metastasis at a median of 8 months after diagnosis, possibly reflecting our close MRI surveillance or a changing pattern of recurrence. In earlier studies, the incidence of neuraxis metastases may have been underestimated because autopsy findings were not available for most patients with brainstem glioma. The management of leptomeningeal dissemination at tumor recurrence is of increasing concern.

MRI studies involving patients with diffuse brainstem glioma demonstrated disease progression and therapeutic effects; responders to completed concurrent radiotherapy had better outcomes than did patients with a lower response. Our observation that patients with progressive disease after the completion of concurrent radiotherapy had a poor outcome is consistent with previous reports. In our study, pretreatment conventional MRI studies failed to predict the prognosis of patients with diffuse pontine glioma, as they did in patients with brainstem gliomas.

1H MRS is a noninvasive technique for the spatial characterization of biochemical markers in tissues. Metabolites that can be identified by standard brain proton MRS include NAA, Cho, Cr, and mL NAA, located primarily in neurons, peaks at 2.0 ppm. It is a marker for neuronal density and viability and is decreased in disease processes involving neuronal dysfunction or death or the replacement of neurons by other cells. Cho peaks at 3.2 ppm with contributions from glycerophosphocholine, phosphocholine, and phosphatidylcholine. Because Cho reflects the metabolism of cellular membrane turnover, increased Cho levels are seen in processes with an elevated cell-

Table 3. Summary of conventional magnetic resonance findings and prognosis of diffuse intrinsic pontine glioma

| Characteristic                  | Rate, % | Progression-free survival | Overall survival |
|--------------------------------|---------|---------------------------|------------------|
|                                |         | Median months |  P   | Median months |  P   |
| Cyst                           |         |               |     |               |     |
| Absent                         | 57.9    | 17.4          | .1676 | 25.2          | .1274 |
| Present                        | 42.1    | 6.3           | .127 | 10.6          | .1254 |
| Gd                             |         |               |     |               |     |
| Absent                         | 36.8    | 20.1          | .127 | 16.6          | .1254 |
| Present                        | 63.2    | 7.7           | .127 | 10.9          | .1254 |
| Cerebellar extension           |         |               |     |               |     |
| Absent                         | 57.9    | 17.4          | .127 | 25.2          | .1254 |
| Present                        | 42.1    | 7.7           | .7929| 10.9          | .7354 |
| Medulla/midbrain extension     |         |               |     |               |     |
| Absent                         | 63.2    | 10.8          | .6525| 14.4          | .7522 |
| Present                        | 36.8    | 13.3          | .6525| 19.7          | .7522 |
| BA encasement                  |         |               |     |               |     |
| Absent                         | 15.8    | 20.1          | .1803| 33.2          | .1995 |
| Present                        | 84.2    | 9.4           | .1803| 12.7          | .1995 |

BA indicates basilar artery; Gd indicates gadolinium enhancement.
membrane turnover—for example, in proliferating tumors and in the developing brain. Cr peaks at 3.03 ppm; the peak is composed of resonances from Cr with contributions from creatine phosphate. It serves as a marker for energy-dependent systems in brain cells. In the normal brain, no abnormal lactate peak (1.32 ppm) is observed. Lactate, which is produced under conditions of anaerobic glycolysis, resulting from a mismatch between glycolysis and the oxygen supply, indicates hypoxic conditions and hypermetabolic glucose consumption. In the healthy brain, no abnormal lipid peak is observed physiologically. The amount of lipids detected by spectroscopy correlates well with the histologic degree of micro- and macro-necrosis.

The accurate measurement of lactate and lipids is difficult because their peaks frequently overlap. When lipid and lactate signals overlap, peaks with many different shapes are formed because lactate signals reverse and point in the negative direction within the TE of 135–144 ms. Although it may be possible to optimize lactate and eliminate lipid signals by using a long TE of 270–288 ms, long TEs have several drawbacks. For example, the signal magnitude of all peaks is decreased because of T2 relaxation during the long echo interval and by signals from rapidly relaxing compounds. Moreover, it is sometimes difficult to eliminate lipid signals completely in clinical settings. Others reported that, by varying the TE, lactate and lipids can be qualitatively differentiated in the brain and that their expression correlates with the World Health Organization grade for the gliomas.

1HMRS was useful for differentiating between tumors and nonneoplastic lesions, including brainstem lesions, and between tumor recurrence and treatment-related changes. It has been used to determine the malignancy grade of brain tumors, to guide biopsy, to monitor the treatment response, and for radiosurgical planning. It has been reported that the higher the Cho-to-NAA ratio, the higher the grade of brainstem tumors; also, in high-grade gliomas, the level of Cho and lactate/lipids was increased. MRS has been used to observe the response in patients with diffuse pontine gliomas; increases in Cho and the Cho-to-NAA ratio or in the Cho-to-Cr ratio were predictive of subsequent disease progression.

According to Li et al., who studied MRS parameters for their prognostic value in patients with malignant glioma, higher Cho-to-Cr and Cho-to-NAA ratios, higher lactate and/or lipid levels, and a lower Cr-to-NAA ratio were predictive of a poor outcome. Warren et al. suggested that the Cho-to-NAA ratio predicted the outcome in children with recurrent primary brain tumors, and Marcus et al. used the combined Cho and lactate value as a specific predictor of survival. According to Tarnawski et al., the ratio of lactate-to-NAA on pretreatment MRI studies was the strongest prognostic factor in patients with malignant glioma scheduled for radiotherapy. Our study showed that lactate expression on 1HMRS studies was correlated with a poor prognosis in patients with diffuse brainstem glioma and that lactate tended to be observed in high-grade gliomas. The expression of lactate may be indicative of hypoxia, a factor in the poor response of these tumors to radiotherapy. According to Berger, there was no correlation between the prognosis of brainstem gliomas and their histologic malignancy grade. Prospective studies that include histologic information are needed to resolve this issue.

Seymour et al. reported that in their 1HMRS study, citrate concentrations were highest in patients with diffuse intrinsic brainstem gliomas, did not differ in patients with anaplastic and low-grade astrocytomas, were low in those with pilocytic astrocytomas, and were higher in patients with ependymomas than in those with anaplastic ependymomas. They speculated that the observed decrease in the citrate level after radiotherapy was attributable to malignant transformation.

### Table 4. Summary of proton magnetic resonance spectroscopy findings and prognosis of diffuse intrinsic pontine glioma

| Characteristic | Rate, % | Progression-free survival | Overall survival |
|---------------|--------|---------------------------|----------------|
|               |        | Median months | P     | Median months | P     |
| Cho/Cr (TE 30) |       |               |       |               |       |
| ≤2            | 75.0   | 10.9           | 16.6  |               |       |
| >2            | 25.0   | 5.6            | .9343 | 9.8           | .7569 |
| NAA/Cr (TE 30) |       |               |       |               |       |
| ≤1            | 50.0   | 7.7            | 11.8  |               |       |
| >1            | 50.0   | 13.3           | .0854 | 19.7          | .086  |
| mL/Cr (TE 30) |       |               |       |               |       |
| ≤1            | 31.3   | 10.9           | 16.6  |               |       |
| >1            | 68.8   | 10.8           | .805  | 14.4          | .9644 |
| Cho/NAA (TE 30) |     |               |       |               |       |
| ≤2            | 81.3   | 13.3           | 19.7  |               |       |
| >2            | 18.8   | 7.7            | .1163 | 10.9          | .0272 |
| Cho/Cr (TE 144) |     |               |       |               |       |
| ≤2            | 64.7   | 10.9           | 16.6  |               |       |
| >2            | 35.3   | 9.4            | .4587 | 11.8          | .3817 |
| NAA/Cr (TE 144) |     |               |       |               |       |
| ≤1            | 52.9   | 10.8           | 14.4  |               |       |
| >1            | 47.1   | 10.9           | .9346 | 16.6          | .756  |
| Cho/NAA (TE 144) |     |               |       |               |       |
| ≤2            | 52.9   | 10.9           | 16.6  |               |       |
| >2            | 47.1   | 9.4            | .8283 | 11.8          | .8101 |
| Lactate       |       |               |       |               |       |
| Absent        | 42.1   | 20.1           | 33.2  |               |       |
| Present       | 57.9   | 6.3            | .0061*| 10.9          | .0065*|
| Lipids        |       |               |       |               |       |
| Absent        | 63.2   | 13.3           | 19.7  |               |       |
| Present       | 36.8   | 6.3            | .174  | 10.6          | .124  |
| Apparent citrate |   |               |       |               |       |
| Absent        | 73.7   | 10.8           | 12.7  |               |       |
| Present       | 26.3   | 32.5           | .2013 | 40.1          | .131  |

Cho indicates choline; Cr indicates creatine; mL indicates myo-inositol; NAA indicates N-acetyl aspartate; TE indicates echo time.
As an alternative, they proposed that the long-term administration of steroids, radiation therapy, and chemotherapy may affect a change in the citrate level. Citrate was apparent in 26% of the patients in our study with diffuse intrinsic pontine glioma; however, its detection was not strongly associated with a good prognosis.

Our study has some limitations. First, because of their location, we were unable to examine an area contralateral to the site of the brainstem gliomas. Second, MRS studies cannot replace histopathological findings in these tumors. Brainstem gliomas including diffuse intrinsic pontine gliomas exhibit a heterogenic pathology that may affect treatment results. None of our patients had exophytic-type brainstem gliomas, such as pilocytic astrocytomas, that may manifest the presence of lactate.14,57 Earlier reports revealed no relationship between lactate expression and a poor prognosis in patients with brainstem glioma, although those findings may have been affected by the inclusion of patients with pilocytic astrocytoma in the study population. Third, in a considerable number of TMZ-treated patients, a diagnosis of pseudoprogression may be returned. Because this would affect PFS data, in the latest response criteria for glioma, T2/FLAIR images are taken into account. However, newer imaging techniques that provide functional information, such as PET, MRS, and perfusion- and diffusion-weighted imaging, may be more reliable in the assessment of tumor activity.

Fourth, the intensity of end-of-life care has an effect on OS data. Fifth, our data are qualitative, and our study population was small. We are planning to perform larger prospective studies that include quantitative data obtained with Spectroscopy Analysis by General Electric (SAGE) or LC models to test the results obtained in our current retrospective investigation.

In conclusion, the MRS detection of lactate is a poor prognostic factor in patients with diffuse intrinsic pontine glioma. To address issues not resolved in this investigation, we are planning to perform additional studies of larger study populations using other imaging modalities.

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