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Magnetic resonance spectroscopy in the assessment of pilocytic astrocytomas

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ABSTRACT

Background: Pilocytic astrocytomas (PA) are common childhood brain tumours whose management and prognosis vary widely depending on location. ^{1}H magnetic resonance spectroscopy (MRS) measures biochemistry *in vivo* and shows promise for characterising brain tumours and aiding management.

Methods: Single voxel MRS (1.5 Tesla, TE 30 ms, TR 1500 ms) was performed on 27 children with PAs. Cases were designated 'progressors' if tumour progression led to their clinical management plan being altered.

Results: Prior to treatment, supratentorial tumours had significantly higher myo-inositol ($p < 0.01$, *t*-test) and glutamate plus glutamine ($p = 0.02$, *t*-test) than cerebellar tumours. Optic pathway or thalamic tumours that progressed had a significantly ($p = 0.04$, *t*-test) lower myo-inositol at initial MRS than those with stable disease. Myo-inositol levels decreased significantly in progressors between the initial and subsequent MRS ($p = 0.03$, paired *t*-test). Changes in myo-inositol occurred before clinical and radiological progression.

Conclusions: MRS identifies differences with anatomical location in PAs and yields potential non-invasive biomarkers of prognosis.

1. Background

Brain tumours are the most common solid tumours of childhood and are the cause of significant morbidity and mortality.[1–3] Astrocytomas account for 40–55% of tumours and the majority of these are pilocytic astrocytomas (PAs).[4] PAs occur in various locations of the brain and spine but are particularly common in the cerebellum and the optic pathway.[1] The tumours are classified as WHO Grade I and have a low mitotic rate.[1,5,6] More recently a biologically more aggressive variant, pilomyxoid astrocytoma, has been recognised and is WHO grade II. Despite their slow growth, these tumours can

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present a significant management challenge particularly when located in areas not readily amenable to surgery. Furthermore, some tumours invade adjacent tissues and occasionally tumours present with multifocal or metastatic disease, a behaviour which implies that they are not always as benign as their WHO grading suggests.

The 5-year survival rate for patients with cerebellar PAs is as high as 95% if a total resection is achieved and 85% if not.[4,7] Generally a total excision is achievable in lesions occurring in the cerebellum, and hence these tumours are usually cured by surgery alone. PAs that arise in the supratentorial region are commonly sited in the optic pathways, hypothalamus or thalamus and are usually not completely resected. These tumours have a more diverse clinical course, are more likely to progress after surgery and are commonly treated with adjuvant chemotherapy or radiotherapy.[8] It is uncertain whether the difference in prognosis of cerebellar and supratentorial tumours is due to surgical limitations or whether the tumours are inherently biologically different.[8] For supratentorial tumours, complete response to adjuvant treatment is rare, and many patients have a remitting and relapsing course over a period of many years, undergoing several episodes of adjuvant treatment.[8] The decision as to whether there is disease progression requiring further treatment, presently relies on the synthesis of clinical findings, for example, changes in visual function, and conventional MR imaging. Clinical findings are often unreliable in young children particularly when testing requires a high degree of compliance and concentration as in the assessment of visual fields or acuity testing. Furthermore, the aim is to detect changes in tumour behaviour that might threaten vision ideally before such damage occurs. New, non-invasive imaging methods for assessing these patients would be particularly beneficial.

One method of promise in the assessment of brain tumours is ^{1}H magnetic resonance spectroscopy (MRS). This technique may be combined with conventional MR imaging to measure the concentration of a range of metabolites in a pre-selected volume of tissue. A number of studies have shown that MRS metabolite profiles are useful in assessing brain tumours[9–19] providing information on diagnosis[9,10,12,14–17] grading.[11,20,21] and prognostic indicators.[13,16,21,22] MRS has also been used for treatment monitoring,[16,21] and certain changes in metabolite profiles have been linked to apoptosis.[10,21] Changes in metabolism are likely to occur early in response to treatment, and MRS may give an earlier indication of tumour status than size or subtle change in gadolinium uptake measured on conventional MR imaging.[15–18] Given the current difficulties in assessing PAs, non-invasive methods are needed which give reliable repeated measurements throughout the often long course of this condition. Therefore, MRS has the potential to make a particular impact clinically in the management of these tumours.

Previous MRS studies of childhood brain tumours have shown that PAs tend to have low levels of creatine and high levels of *N*-acetyl aspartate when compared with other childhood brain tumours.[14,15,23] Furthermore, these features can be used to distinguish cerebellar PAs from other cerebellar tumours.[14,13] No difference has previously been reported between PAs in the cerebellum and supratentorial tumours. However, improved technology is allowing the quantitation of increasing numbers of potentially important metabolites including myo-inositol, glutamate and glutamine. In one study of MRS, myo-inositol and glutamate plus glutamine were noted to show promise in distinguishing between various childhood brain tumours, and myo-inositol was proposed as a potentially useful marker of treatment response.[12]

This study investigates short echo time MRS for the characterisation of PAs in children. The variation in MRS with location is investigated, and for supratentorial tumours, pretreatment MRS prognostic markers are identified and the changes of metabolite concentrations with treatment developed.

2. Patients and methods

MRS was performed at the time of routine clinical magnetic resonance (MR) imaging on a 1.5 Tesla scanner (Siemens' Symphony Magnetom or GE Signa Excite) as part of a prospective study aimed at determining the ability of MRS to characterise childhood brain tumours and provide biomarkers of prognosis. The initial MRS in each patient was performed prior to any adjuvant chemotherapy or radiotherapy. Where patients underwent surgery as part of their routine clinical management, a histopathological diagnosis was obtained. Where surgery was not performed, the diagnosis was made on clinical and conventional MR imaging characteristics. All diagnoses were agreed as a consensus by the clinical multidisciplinary team. Ethical approval was given for the study and written parental consent obtained.

MRS was acquired using PRESS (echo time 30 ms, relaxation time 1500 ms). A cubic voxel with 1.5 cm or 2.0 cm side length was positioned in the tumour to contain as little cyst as possible. For a 1.5 cm cubic voxel, 256 repetitions were used, and for a 2.0 cm cubic voxel, 128 repetitions were used. Where time permitted, a second acquisition was performed with an echo time of 135 ms. In all cases, a water MRS was acquired for eddy currents correction and as a concentration reference.

Spectra were processed and analysed using LCModel™ (Version 6.1-4).[24] This analysis tool produces a processed spectrum and fits to individual metabolites, macromolecules and lipids. A variable baseline takes account of very broad signals. The resulting spectra with the baseline subtracted were used to calculate mean spectra. Individual metabolite levels were generated automatically by LCModel™ avoiding observer bias in the measurements.

Three quality control measures were selected.

(1) The voxel had to be sited completely within the tumour (Fig. 1).
(2) The signal-to-noise ratio (SNR) determined by LCModel™ had to be greater than 4 to allow accurate quantitation of the major metabolites.
(3) Peak widths, measured by the full-width-half-maximum (FWHM) of LCModel™, had to be less than 0.150 ppm to allow choline (3.2 ppm) and creatine (3.0 ppm) peaks to be separately quantitated.

Spectra were checked visually for artifacts by an expert spectroscopist.

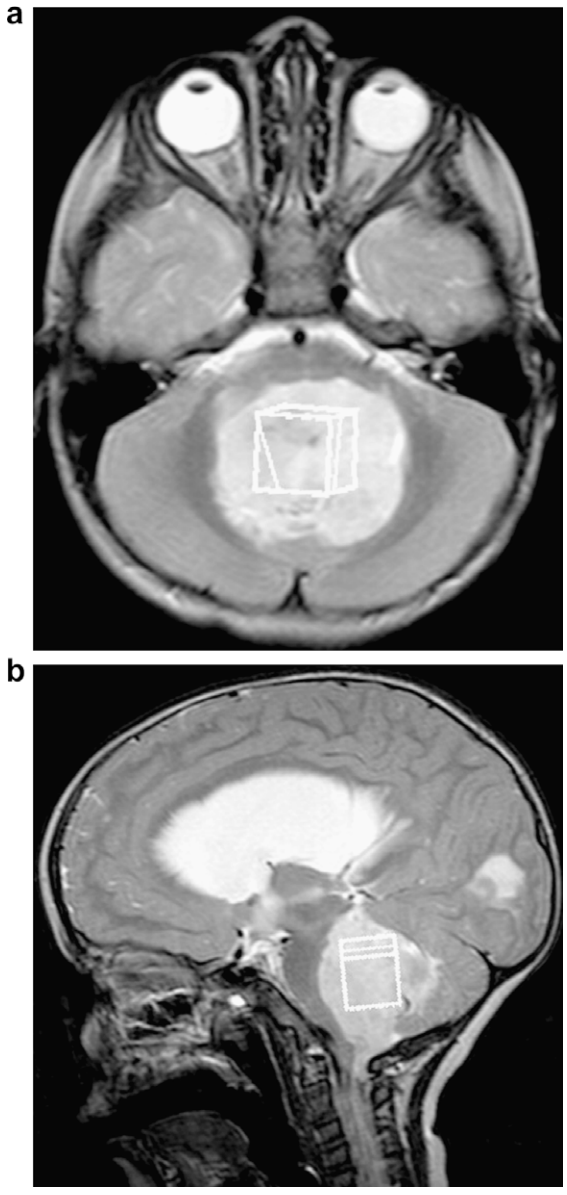

Fig. 1 – MR Images (a) axial (b) sagittal showing the MRS voxel placement in a cerebellar tumour.

Where MR spectral features overlap extensively for two metabolites, the values were combined. This was applied to the following combinations: glutamate and glutamine (Glx), N-acetyl aspartate and N-acetyl-aspartyl-glutamate (tNAA) and phosphocholine and glycerophosphocholine (tCho). LCModel™ calculates Cramer-Rao lower bounds as a measure of accuracy for metabolite levels.[24] The metabolites with consistently high (>50%) Cramer-Rao lower bounds were too poorly determined to provide useful data and removed from further analysis. Mean metabolite concentrations and two-tailed *t*-tests of significance were preformed between the group of cerebellar and supratentorial tumours.

The variability of the pre-treatment MRS metabolite profiles across the cases was determined using a principal component analysis (PCA) performed on the selected metabolite concentrations, normalised to vector length. PCA is an unsupervised method with the metabolite profile from each case represented by a single point on a graph. The closer the points are on the graph, the more similar are the metabolite profiles.

The patients with supratentorial lesions were grouped into 'progressors' and 'stable disease'. Progressors were defined as those in whom a clinical decision, based on a number of clinical factors and appearances on conventional MRI, had been made to give further treatment due to disease progression. Those with stable disease were taken to be all other patients provided they had been followed up for more than 6 months at the end of the study. For these two groups, mean metabolite concentrations were calculated and 2 tailed *t*-tests performed on pre-treatment MRS to determine factors associated with progression. In addition, for patients with follow-up MRS studies on-treatment or during observation, pre-treatment MRS was compared with the next MRS study using paired 't'-tests to determine metabolite changes providing an early marker of future disease progression.

2.1. *Patients*

MRI and MRS were performed between 1st September 2003 and 1st April 2007 on 27 children with PAs prior to any adjuvant treatment. Twelve patients had cerebellar and 15 patients had supratentorial tumours. Six cases were excluded on quality control grounds: two tumours were too small to encompass a 1.5 cm sided voxel, three had poor SNR and one had an inadequate FWHM. All subsequent analysis refers to the reduced cohort of nine cerebellar and 12 supratentorial cases. Of the supratentorial tumours, nine involved the optic pathways including hypothalamus, one was in a cerebral hemisphere and two were thalamic. All tumours had histopathological diagnoses established except for four optic pathway gliomas which were not biopsied, two of which were in children with Neurofibromatosis Type 1 (NF1). Three of the supratentorial cases had NF1. The mean age of the children with cerebellar tumours was 7.9 years and with supratentorial tumours it was 4.9 years. Control MR spectra were recorded from brain distant to the tumour in children with brain tumours who had received no treatment other than surgery.

None of the patients with cerebellar tumour had a post operative residual lesion amenable to MRS. In the group of patients with supratentorial tumours, seven of the eight patients who underwent surgery had significant residual disease. The patient with a cerebral hemisphere tumour had a complete resection. Of the 11 patients with significant residual disease or having no surgery, seven proceeded to treatment with chemotherapy and four were initially observed with no adjuvant treatment. No patients had radiotherapy. Five of the patients had progressive disease before the end of the study follow-up period (1/9/07) leaving six patients classified as having stable disease. Two of the patients did not have a follow-up MRS within the study period leaving four progressors and five with stable disease in the study of treatment monitoring. A graphical summary of patient numbers available for the various aspects of the study is given in Fig. 2.

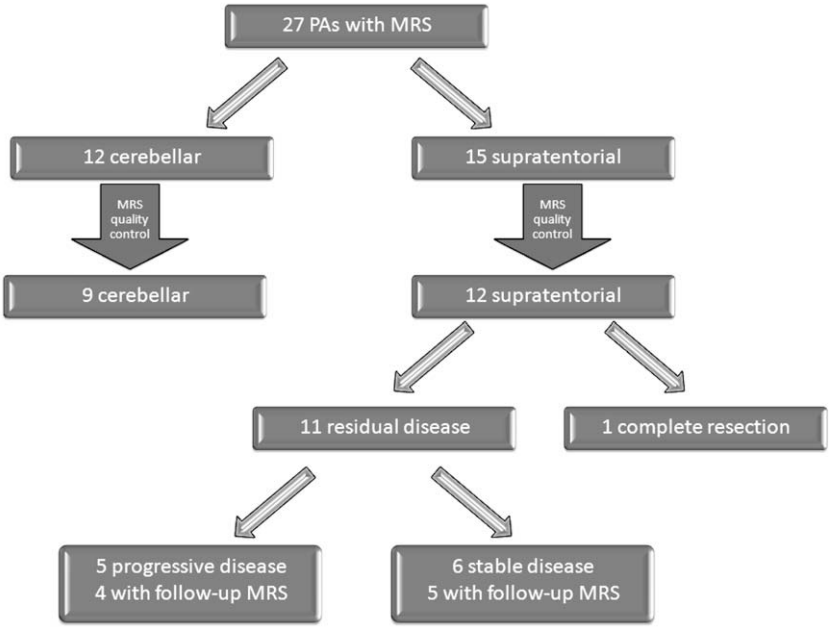

Fig. 2 – Diagram showing patients available in the various sections of the study.

3. Results

3.1. *Differences in MRS metabolite profiles between cerebellar and supratentorial tumours*

Mean MRS for cerebellar and supratentorial tumours is given in Fig. 3. The supratentorial cases have a more prominent peak at 3.65 ppm due to myo-inositol and glycine. In three cases, where MRS was performed at two echo times, there was no significant peak at 3.65 ppm in the long echo time spectra, implying that there is only a small component from glycine in these tumours. The broad component between 2.0 ppm and 2.5 ppm is more pronounced in the supratentorial tumours. This feature has components from glutamate, glutamine, lipids and macromolecules. LCModel analysis was used to quantify the spectral components (Table 1). The supratentorial tumours had significantly greater (two-tailed *t*-test) levels of mIns ($p = 0.002$) and Glx ($p = 0.01$). No other metabolites were significantly different between the two groups although the cerebellar tumours tended to have lower creatine ($p = 0.05$). No significant differences (two-tailed *t*-test) were found in the metabolite profiles of supratentorial tumours between patients with (3 cases) and without NF1 (9 cases). A principal component analysis was performed to assess the variability of the metabolite profiles (Fig. 4) and shows that cerebellar and supratentorial tumours tend to form separate groups with only one outlier.

Brain tissue mIns and Glx were measured in different locations of the brain in controls using 11 MRS taken from a variety of regions both infratentorially (7 cases) and supratentorially (4 cases). Glx was significantly higher in the supratentorial regions ($p = 0.02$), but there was no significant difference in mIns levels ($p = 0.60$).

3.2. *Pre-treatment MRS as a prognostic indicator in supratentorial tumours*

In the cohort with supratentorial tumours, five progressed (mean time to progression 11 months) and six had stable disease throughout the study period (mean follow-up time 30 months). The patients who progressed had significantly (two-tailed *t*-test) lower values for mIns ($p = 0.04$) on their pre-treatment MRS (see Table 2). A cut-off of value of 4.5 mmol/kg maximised sensitivity plus specificity of the test. Patients who progressed also tended to have lower Glx ($p = 0.08$) and tNAA ($p = 0.07$). From the scatter plot of pre-treatment Glx versus mIns (Fig. 5), it is shown that supratentorial tumours cluster into two groups, those with low values of Glx and mIns who tend to progress and those with higher values of these metabolites who tend to have stable disease. One patient with stable disease had low mIns and Glx. This patient had an optic chiasm tumour treated with surgery followed by chemotherapy (vincristine and carboplatin) for 18 months and was 3 months off treatment at the end of the study period. One patient with a completely resected temporo-parietal tumour had been followed up for less than 6 months at the end of the study.

3.3. *Supratentorial tumours: treatment monitoring and early on-treatment predictors of progression*

Nine of the 11 patients with supratentorial tumours followed up for at least 6 months had follow-up scans with MRS of acceptable quality. Four of these patients progressed and five had stable disease. Paired *t*-tests were performed on the MRS metabolite values between the pre-treatment and first on-treatment MRS for each patient to assess any significant

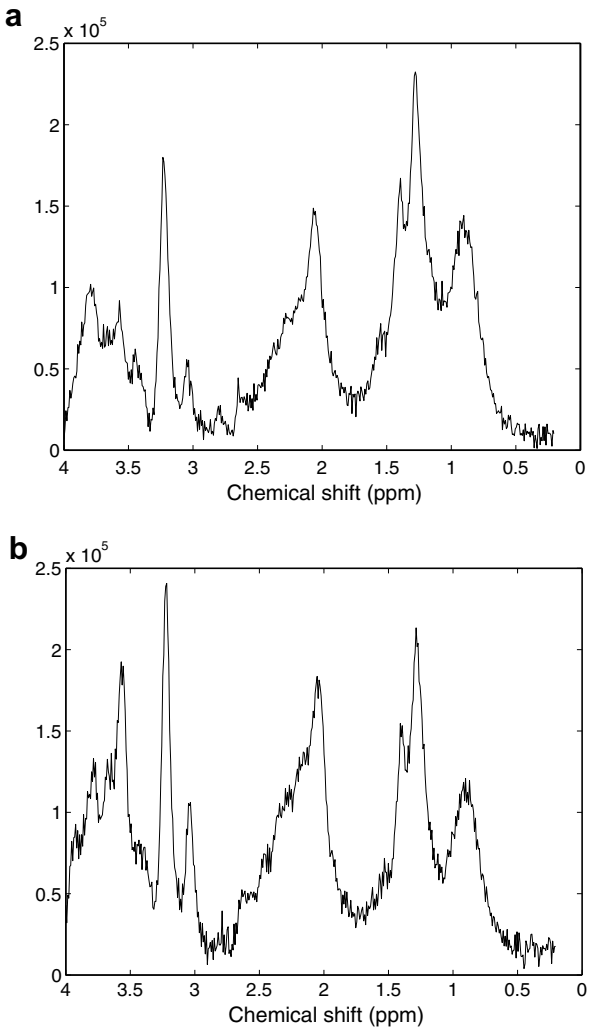

Fig. 3 – Mean MR Spectra for PAs in different locations (a) cerebellar cases (b) supratentorial cases. The main features are lipids (broad peaks at 0.9 ppm and 1.3 ppm), lactate (two narrow peaks around 1.3 and 1.45 ppm), N-acetyl aspartate (narrow peak at 2.0 ppm), glutamate and glutamine (broad feature between 2.0 and 2.5 ppm), creatine (narrow peak at 3.0 ppm), total choline (narrow peak at 3.2 ppm), myo-inositol (narrow peak at 3.65 ppm).

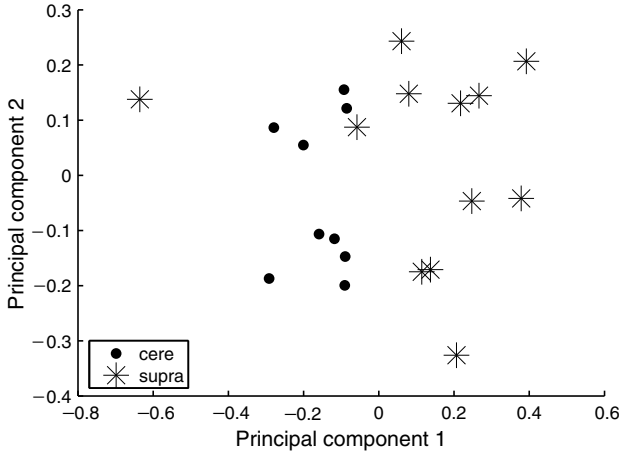

Fig. 4 – Principal component scores plot, cerebellar cases (cere) shown as solid circles and supratentorial (supra) cases as stars.

changes between the two MRS. No significant changes were found in the metabolite profiles between the two MRS in the patients with stable disease. However, in patients who later progressed, there was a significant decrease in mIns, (1.2 mmol/kg, $p = 0.03$). The median time between the initial and subsequent MRS was 2.1 months in the progressors group, with a median time to progression of 12.2 months.

4. Discussion

This study has revealed differences in MR spectra between PAs arising in supratentorial and cerebellar regions and shown that low levels of mIns are significantly associated with adverse clinical behaviour in supratentorial tumours. Two metabolites mIns and Glx are significantly higher in the supratentorial compared to cerebellar tumours, consistent with a differential 'metabolic fingerprint' dependent on tumour location. In controls, no significant difference was found in mIns levels in non-involved brain between these two regions, and a previous study actually found mIns to be higher in normal cerebellum than other brain locations.[25] Higher mIns levels in the supratentorial tumours are therefore likely to arise from tumour-derived tissue. In controls, Glx levels were higher in supratentorial non-involved brain than the cerebellum, but since the tumours studied by MRS

Table 1 – Average metabolite values (mmol/kg) and significance tests (two-tailed t-test) for supratentorial and cerebellar PAs

Metabolite	P-value	Cerebellar PAs		Supratentorial PAs	
		Average	Standard error	Average	Standard error
Ala	0.76	0.948	0.05	0.883	0.04
Cre	0.06	0.498	0.02	1.534	0.14
m-Ins	0.00	1.258	0.08	3.72	0.18
Lac	0.72	2.131	0.06	1.953	0.13
Gua	0.56	1.482	0.06	1.331	0.05
tCho	0.28	0.986	0.03	1.203	0.05
tNAA	0.15	0.816	0.05	1.401	0.1
Glx	0.02	3.571	0.09	5.916	0.23

Table 2 – Average metabolite values (mmol/kg) and significance tests (two-tailed t-test) for supratentorial tumours which later progressed and those which had stable disease

Metabolite	P-value	Stable PAs		Progressors PAs	
		Average	Standard error	Average	Standard error
Ala	0.23	1.002	0.10	0.584	0.09
Cr	0.16	2.326	0.30	0.857	0.27
Ins	0.04	5.089	0.35	2.530	0.25
Lac	0.76	1.798	0.23	2.163	0.44
tCho	0.24	1.463	0.08	0.991	0.14
tNAA	0.07	2.091	0.24	0.742	0.10
Glx	0.08	7.461	0.46	4.600	0.42
(Lip + MM)0.9	0.27	5.295	0.32	7.375	0.67
(Lip + MM)1.4	0.18	8.533	0.76	17.313	2.36

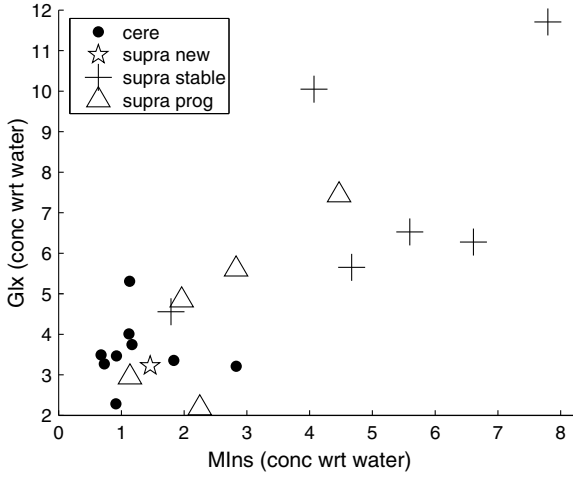

Fig. 5 – Scatter plot of metabolite concentrations (mmol/kg) determined by MRS prior to treatment, glutamate + glutamine (glx) against myo-inositol (mIns). Cases are denoted by cerebellar tumours – solid circles, supratentorial tumours with stable disease – crosses, supratentorial tumours with progressive disease – triangles. The star denotes a supratentorial tumour with short follow-up.

were not diffusely infiltrative, it is unlikely that the differences in Glx levels arise from normal tissue entrapped within the tumour. A principal component analysis of MRS metabolite profiles showed that the cerebellar and supratentorial tumours form two distinct groups with only one outlier who subsequently developed progressive disease. This finding implies that supratentorial tumours are biologically distinct from their cerebellar counterparts. Clear parallels exist here with another glial series tumour, ependymoma, which show key differences in chromosomal abnormalities and gene expression patterns depending on the location.[26] Qualitative review of conventional MR images revealed no difference in major imaging characteristics between the tumours in the different locations including their cystic nature.

All but one of the supratentorial tumours were located in deep structures and as expected these patients presented significant management problems. The use of complex treatment strategies involving various combinations of surgery, and chemotherapy, and active observation was undertaken in this group of patients emphasising the importance of developing non-invasive prognostic markers to aid clinical decision making. In turn, information from MR spectra could be used in the context of multi-disciplinary thinking regarding the optimal management of these tumours. In this study, significantly lower levels of mIns and a tendency to lower Glx and tNAA were found in the group of tumours which progressed. The higher values of mIns in those with stable disease are in keeping with studies on adults with astrocytomas, where higher mIns is seen in the lower grade, better prognosis tumours.[11,20] High levels of NAA have been associated with a good prognosis in a number of studies of brain tumours.[18] The cerebellar tumours had mIns, Glx and NAA values more akin to the supratentorial 'progressors' rather than those with stable disease. Most of the cerebellar cases underwent complete surgical resection leaving minimal residual disease post surgery. None of these tumours recurred, consistent with reports on the outcomes of cerebellar PAs from other studies. This finding implies that the extent of surgical resection is a key determinant of prognosis. Levels of mIns were also found to decrease significantly between the first and subsequent MRS in the group of supratentorial tumours that progressed. Since the median time between the two scans was only 2 months compared with a time to progression of 12 months, these patients could potentially have had earlier intervention for their disease progression.

In this study, the definition of progressive disease was taken as the decision to alter management by the multi-disciplinary team based on the synthesis of several layers of evidence such as clinical findings, visual function and scan appearances, and an understanding of the natural history of these tumours before concluding there had been disease progression. The size of tumour on the MRI scan was an important but not exclusive factor in this decision.

Previous studies of MRS in brain tumours have identified tCho as a useful biomarker of tumour aggressiveness across a range of tumour types,[13] but this was not confirmed in this study for PAs. A potential explanation may be found in the relative proportions of the choline containing metabolites which give rise to the tCho value. Studies in vivo[27,28] and in tissue,[28] have indicated that glycerophosphocholine is the dominant choline containing metabolite in PAs [28] whereas

phosphocholine has been demonstrated to be the main biomarker of tumour aggressiveness.[29] Lipid levels have also been demonstrated to be makers of tumour malignancy,[13] being a measure of necrosis and apoptosis. However, PAs have lipid levels comparable to those found in higher grade tumours.[28] The origin of MRS detectable lipid in PAs requires further investigation.

Whilst mIns has previously been identified as an important metabolite in the characterisation of brain tumours in both children[12,15] and adults,[20] there has been relatively little work to explain this finding. In the study of Castillo et al.,[20] variations in mIns levels were explained in terms of the protein kinase C pathway. Myo-inositol contributes to the formation of a phosphorylated form of phosphatidylinositol which is broken down to form diacylglycerol and inositol 1,4,5-triphospate. The resulting diacylglycerol activates protein kinase C and a cascade of proteolytic enzymes including matrix metalloproteases.[20,30] Matrix metalloprotease-2 has been shown to mediate local aggression in cerebral gliomas and treatment of a glioma, cell line with a metalloprotease inhibitor resulted in a 90% reduction in invasion.[30] Further work is warranted to establish whether these pathways are upregulated in PAs with low levels of mIns and explore their potential as targets for novel agents.

A previous study has shown differences in genetic profiles of PAs between various locations in the brain.[31] Tissue markers of prognosis have also been investigated, and a retrospective study found the MIB-1 index to be a predictor of initial tumour progression in PAs.[32] It will be important to link MRS findings to studies of the tumour biology in order to gain a better insight into MRS findings.

Whilst MRS is a powerful non-invasive method for the characterisation of brain tumours, currently there are important limitations on the size of tumour amenable to the technique. In this study, we excluded tumours which could not encompass a 1.5 cm sided cube. Only two tumours did not meet this criterion at diagnosis, but three other highly cystic tumours had only a small solid component and did not meet the MRS quality control criterion for signal-to-noise ratio since the signal comes almost entirely from the solid component. All of these tumours had a favourable outcome. With longer scan times or improved technology such as higher magnetic field strength, smaller tumours could be studied. However, this will remain an important limitation of MRS for the foreseeable future particularly in the study of more aggressive tumours for which minimal residual disease is of clinical importance.

It is envisaged that after prospective validation in a larger cohort, the findings of this study could be used in conjunction with clinical and other imaging findings to aid clinical decision making in children with supratentorial PAs. A high mIns level at diagnosis would support a clinical decision not to embark on-treatment and this would be further supported by high mIns levels maintained on follow-up. Conversely, a low mIns level at diagnosis could become an indication for treatment or, in combination with other adverse features, lead to intensification of treatment. A falling mIns at follow-up would be one marker of treatment failure. MRS now needs to be tested within a prospective clinical trial where it can be assessed in a robust manner.

5. Conclusions

Key differences have been identified in the MRS metabolite profiles between cerebellar and supratentorial PAs with cerebellar tumours having significantly lower mIns and Glx. Low values of mIns on the initial MRS and decreasing mIns on the subsequent MRS were found to be associated with later disease progression in supratentorial tumours. MRS is a potentially useful additional investigation in assessment of PAs, and gives further insight into the diversity of these important childhood tumours. The findings of this preliminary study should be validated by a prospective multi-centre study with a larger cohort of patients treated according to a specific protocol.

Conflict of interest statement

None declared.

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