

Early proton magnetic resonance spectroscopy in normal-appearing brain correlates with outcome in patients following traumatic brain injury

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Summary

The long-term clinical outcome following traumatic brain injury (TBI) can be difficult to predict. Proton magnetic resonance spectroscopy (MRS) has previously been used to demonstrate abnormalities in regions of white matter that appear normal on conventional imaging in patients following TBI. We report MRI and MRS studies of 26 patients performed at an early time point following injury (mean 12 days, $n = 21$) and at a later time point (mean 6.2 months, $n = 15$). The proton MRS was acquired from the posterior part of a normal-appearing frontal lobe containing predominantly white matter using stimulated echo acquisition mode to localize, with a relaxation time of 3000 ms and echo time of 30 ms. At both the early and late time points the *N*-acetylaspartate/creatine ratio (NAA/Cr) was significantly reduced ($P = 0.03$, $P = 0.005$, respectively), the choline/creatine ratio (Cho/Cr)

significantly increased ($P = 0.001$, $P = 0.004$, respectively) and the myo-inositol/creatine ratio (Ins/Cr) significantly increased ($P = 0.03$, $P = 0.03$, respectively) compared with controls. There was a small, but significant, further reduction ($P = 0.02$) in the NAA/Cr between the two studies in the 10 patients for whom data was available, at both time points. The NAA/Cr acquired at the early time point significantly correlated with the clinical outcome of the patients, assessed using either the Glasgow outcome scale ($P = 0.005$, $n = 17$) or the disability rating scale ($P < 0.001$, $n = 17$). We conclude that there is a sustained alteration in NAA and Cho. These findings provide possible evidence for cellular injury (NAA loss reflecting neuroaxonal cell damage and raised Cho and Ins reflecting glial proliferation) not visible by conventional imaging techniques. This may be relevant to understanding the extent of disability following TBI.

Keywords: traumatic brain injury; proton magnetic resonance spectroscopy; outcome; *N*-acetylaspartate; choline

Abbreviations: Cho = choline; Cr = creatine; DAI = diffuse axonal injury; DRS = Disability Rating Scale; GCS = Glasgow Coma Scale; GOS = Glasgow Outcome Scale; Ins = myo-inositol; MRS = magnetic resonance spectroscopy; NAA = *N*-acetylaspartate; NAWM = normal-appearing white matter; STEAM = stimulated echo acquisition mode; TBI = traumatic brain injury; TE = echo time; TR = repetition time

Introduction

Diffuse axonal injury (DAI) is recognized to occur in a significant number of patients following mild, moderate and severe traumatic brain injury (TBI) (Mittl *et al.*, 1994). The areas of the brain most affected by DAI are the lobar white matter, corpus callosum and dorsolateral upper brainstem (Gennarelli *et al.*, 1982; Adams *et al.*, 1989). Most DAI tends to be microscopic, producing subtle changes which can be difficult to visualize on conventional imaging (CT or T_1/T_2 -weighted MRI). Consequently, patients may have apparently normal imaging yet remain impaired as a result of a TBI.

This is one possible explanation for the weak correlation between early MRI and late clinical outcome in patients following TBI (Wilson *et al.*, 1988).

Proton magnetic resonance spectroscopy (MRS) enables a non-invasive assessment of intracellular compounds in the brain. This technique may detect abnormalities in areas of the brain that appear normal on conventional imaging. An experimental model of TBI has been developed that selectively damages axons in the white matter (Cecil *et al.*, 1998b; Smith *et al.*, 1998). Proton MRS, in this model of

TBI, detected a reduction of *N*-acetylaspartate (NAA), an amino acid considered to be a marker of neuronal integrity, in regions of white matter that appeared normal on conventional imaging. The reduction in NAA may be an indication that cellular injury had occurred in the white matter tracts. This is in keeping with the subsequent histopathological examination which found diffuse multifocal pathology (Cecil *et al.*, 1998b; Smith *et al.*, 1998).

Proton MRS in patients following TBI has shown reduced NAA in regions of the corpus callosum and occipitoparietal white matter (Cecil *et al.*, 1998a; Friedman *et al.*, 1998, 1999; Ross *et al.*, 1998) as well as the occipitoparietal grey matter (Ross *et al.*, 1998; Friedman *et al.*, 1999). In these studies the patients were generally examined in the chronic stages following TBI (3 weeks to several months). Whilst correlations have been found between the reduction in the grey matter NAA and eventual clinical outcome, no correlation was found between the reduced white matter NAA and outcome (Ross *et al.*, 1998; Friedman *et al.*, 1999). In addition, an increase in the choline (Cho) containing compounds, which are involved in membrane metabolism, has only been found in the grey matter in patients following TBI (Friedman *et al.*, 1998).

We have reported previously on an early reduction of NAA and an early increase in Cho, in normal-appearing frontal white matter, in patients following TBI (Garnett *et al.*, 2000). Both the reduced NAA and the increased Cho correlated with the severity of injury. The aim of the current study was to determine if there was a correlation between this early reduction in NAA and increase in Cho with the neurological outcome of the patients. In addition we sought to determine whether there was any further alteration in the levels of these metabolites over time, which might be indicative of ongoing cellular injury.

Methods

Patient population

Twenty-six TBI patients (mean age 38 years, range 18–66 years) were included in the current study. An early study was performed as soon as the patients were clinically stable (mean 12 days, range 3–35 days) and the late study was performed at 6.2 months (range 2.9–10.6 months) following TBI. The outcome of the patients was assessed at the delayed study using both the Glasgow Outcome Scale (GOS) (Jennett and Bond, 1975; Wilson *et al.*, 1998) and the Disability Rating Scale (DRS) (Rappaport *et al.*, 1982). The GOS attributes a patient with a score in the range 1–5, whereby a patient who dies scores 1, a patient who remains with a severe disability scores 3 and a patient who makes a good recovery scores 5. The DRS attributes a patient with a score in the range 0–30 and then categorizes the patients into 10 levels of disability. A patient who dies is given a category of 9, a severely disabled patient a category of 5 and a patient with no disability a category of 0. Twenty control subjects,

with a mean age of 37 years (range 25–56 years), were studied for comparison, with 11 of 20 studied on two occasions (mean interval between studies 12.5 months, range 5.0–19.1 months).

Ethical approval was obtained for the study from the Central Oxford Ethical Committee, with written consent obtained from the patients or next of kin.

MRI/MRS examinations

Imaging and spectroscopy was performed using a 2 tesla superconducting magnet (Oxford Magnet Technology, Eynsham, UK) interfaced to a Bruker AVANCE spectrometer (Bruker Medical GmbH, Ettlingen, Germany). Conventional imaging, using a purpose built quadrature head coil, consisted of an initial sagittal midline scout image followed by axial T_1 -weighted [gradient echo sequence: echo time (TE), 13 ms; repetition time (TR), 500 ms] and axial T_2 -weighted (fast spin echo sequence: TE, 82 ms; TR, 3000 ms) acquisitions, registered to the transcallosal line. Eight axial images were acquired with a slice thickness of 5 mm and a slice separation of 7.5 mm. Blood–brain barrier integrity was confirmed, at the first study, with the acquisition of a further T_1 -weighted sequence following the injection of gadolinium–DTPA.

Proton spectra were acquired from the posterior aspect of one of the frontal lobes, containing predominantly white matter tracts, but also including some deep grey matter (basal ganglia). The voxel was carefully positioned to avoid any areas of T_1 or T_2 abnormality. Localization of the signal was performed using the stimulated echo acquisition mode (STEAM) sequence (Frahm *et al.*, 1989a) with a short TE and long TR (TE, 30 ms; TR, 3000 ms; mixing time, 47 ms). The initial patients studied had proton MRS to define a $3 \times 2 \times 9$ cm region, which was then subdivided into $3 \times 2 \times 1$ cm voxels by incorporating 16 phase-encoded gradient steps (total number of averages 256) (Brown *et al.*, 1982). The total examination time proved to be at the limit of patient acceptance (spectroscopy acquisition 15 min), so later investigations employed a single-voxel acquisition (voxel size, $3 \times 2 \times 3$ cm; number of acquisitions, 128; acquisition time, 7.5 min). Both methods used the same basic acquisition sequence and timing and hence metabolite peak ratios were unaffected. This was confirmed in five control subjects with <4% difference between the two methods. For the delayed studies the voxel was centred on the same slice as the initial study and with careful repositioning of the voxel the same anatomical tissue was studied. Water suppression was achieved using a chemical shift selective sequence (Haase *et al.*, 1985). Pulse rate and oxygen saturation were monitored throughout the study.

Data analysis

The proton spectra were analysed off-line using the 1D Win-NMR software package (Bruker-Franzen Analytik GmbH, Bremen, Germany). Data were zero filled, Lorentz–Gaussian

Table 1 Demographic data and imaging results in 26 patients following TBI

Case no.	Age (years)/sex	Study 1 (days)	Study 2 (days)	GCS score	GOS score	DRS score	Cause of TBI	Global MRI findings
1	26/M	5	NA	15	5	1	MVA	Unremarkable
2	66/M	14	3.9	15	5	1	Bl inj	Chronic SDH
3	22/M	3	NA	15	5	1	Fall	Unremarkable
4	25/M	18	3.4	15	5	1	Fall	Chronic SDH
5	66/M	5	NA	14	5	1	Fall	Unremarkable
6	39/M	3	NA	14	NA	NA	Fall	Contusion
7	57/M	3	NA	14	5	1	Bl inj	Haemorrhagic contusion
8	27/M	11	5.1	14	5	1	BA	Haemorrhagic contusion
9	49/M	10	NA	14	NA	NA	Fall	Haemorrhagic contusion
10	50/M	35	NA	14	NA	NA	Fall	Haemorrhagic contusion
11	19/F	18	8.1	11	4	1	MVA	Unremarkable
12	34/M	11	5.0	11	4	1	Bl inj	Haemorrhagic contusion
13	59/F	10	10.6	11	4	1	MVA	Unremarkable
14	63/M	4	8.4	9	4	4	Fall	Contusion/previous SDH
15	18/M	12	9.8	9	4	2	Fall	Unremarkable
16	23/M	13	NA	9	5	1	MVA	Unremarkable
17	26/M	4	NA	9	NA	NA	Fall	Contusion
18	28/M	NA	2.9	8	4	3	Fall	Contusion
19	33/M	15	NA	7	4	2	Fall	DAI
20	36/M	NA	6.7	7	5	1	MVA	Haemorrhagic contusion
21	41/M	35	4.4	6	3	5	Fall	Acute SDH
22	31/M	19	NA	6	3	6	MVA	Haemorrhagic contusion/DAI
23	30/M	NA	7.7	5	4	3	MVA	Acute SDH
24	58/F	NA	9.4	5	3	5	MVA	Acute SDH
25	30/F	NA	3.7	3	5	1	Fall	Acute EDH
26	19/M	13	4.9	4	3	5	MVA	Haemorrhagic contusion

NA = not available; MVA = motor vehicle accident; BA = bicycle accident; Bl inj = blunt injury; EDH = extradural haematoma; SDH = subdural haematoma.

converted and Fourier transformed, then phase and base line (automated polynomial) corrected. The spectral peaks from NAA, Cho, creatine (Cr) and myo-inositol (Ins), were fitted to Gaussian line shapes and integrated. Results are expressed in the following metabolite ratios: NAA/Cr, Cho/Cr, NAA/Cho and Ins/Cr. The Mann–Whitney *U* test was used to compare these ratios between the patients and controls, the Wilcoxon Signed Ranks test for paired patient and control data and the Spearman Rank test was used to correlate the outcome with the metabolite ratios.

Results

Proton MRS data were available from 21 of 26 patients at the early study and from 15 of 26 patients at the late study. The early study had to be abandoned in five patients because of restlessness. In the late study four patients were lost to follow-up, two patients were too restless to be studied and five patients were not willing to be rescanned. Outcome data was available on 22 patients, allowing for the four patients who were lost to follow-up. The demographic data for the 26 patients including age, sex, delay from TBI (initial and delayed study), Glasgow Coma Scale (GCS) score following resuscitation (Teasdale and Jennett, 1974), GOS score, DRS score, cause of TBI and global MRI findings are shown in Table 1. The causes of the TBI fell into three major categories:

14 of 26 patients fell (e.g. off a horse, ladder or bicycle), nine of 26 patients were involved in a motor vehicle accident and three of 26 patients were assaulted, sustaining a blunt injury. Reviewing the conventional MRI scans, 12 of 26 patients had evidence of a contusion, three of 26 had an acute subdural haematoma, two of 26 had a chronic subdural haematoma, two of 26 had evidence of diffuse axonal injury (DAI), one of 26 had an acute extradural haematoma and seven of 26 patients had unremarkable conventional MRI. Only one patient had had a previous significant TBI.

An example of a data set from a control subject, together with the early and late data from a patient, is shown in Fig. 1. The patient is a 19-year-old female who was involved in a motor vehicle accident with a GCS score of 11 following resuscitation. She was studied for 18 days following injury and then again at 8.1 months. At both times the conventional MRIs were unremarkable. The spectra obtained at the early study, from a region containing predominantly white matter that appeared normal on conventional imaging (voxel depicted on the image), show a reduced NAA/Cr, increased Cho/Cr and increased Ins/Cr in the patient compared with the control. At the late study the spectra show a further modest reduction in the NAA/Cr compared with the initial study. No lactate was observed at either study.

The metabolite ratios (NAA/Cr, Cho/Cr, NAA/Cho and Ins/Cr) for the controls and the 21 patients at the early study

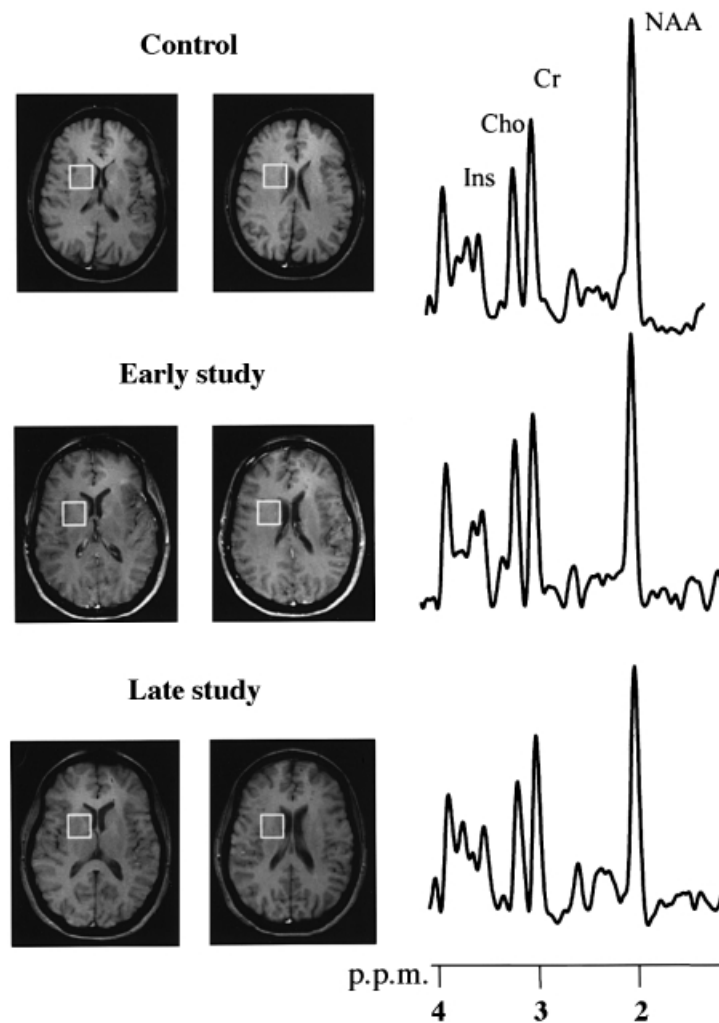


Fig. 1 T₁-weighted images and proton spectra (voxel as shown, STEAM, TR 3000 ms, TE 30 ms, number of acquisitions 128) from a control subject and a patient following TBI. At the patient's early study the image shows no abnormality, the NAA/Cr is reduced and the Cho/Cr increased compared with the control. At the patient's late study the image remains unremarkable and there was a further decrease in the NAA/Cr compared with the early study.

Table 2 Mean (SD) values for NAA/Cr, Cho/Cr, NAA/Cho and Ins/Cr for the controls and the patients at the early and late study

	NAA/Cr	Cho/Cr	NAA/Cho	Ins/Cr
Controls (<i>n</i> = 20)	1.44 (0.12)	0.66 (0.08)	2.20 (0.25)	0.57 (0.08)
Patients (<i>n</i> = 21), early	1.31 (0.21)*	0.84 (0.20)**	1.66 (0.44)**	0.63 (0.09)*
Patients (<i>n</i> = 15), late	1.24 (0.25)**	0.83 (0.23)**	1.60 (0.48)**	0.63 (0.07)*

P* < 0.05; *P* < 0.01.

and 15 patients at the late study are shown in Table 2. At the early study, mean 12 days from TBI, the NAA/Cr was significantly reduced in the patients and the Cho/Cr was significantly increased compared with the controls. As a consequence of this likely decrease in the NAA and increase in the Cho levels in the patients, the NAA/Cho was significantly reduced in the patients compared with the controls. The Ins/

Cr was also significantly increased in the patients compared with the controls. At the late study, mean 6.2 months following TBI, the NAA/Cr was still significantly reduced, the Cho/Cr still significantly increased, with the resultant NAA/Cho still significantly reduced and the Ins/Cr still significantly increased in the patients compared with the controls.

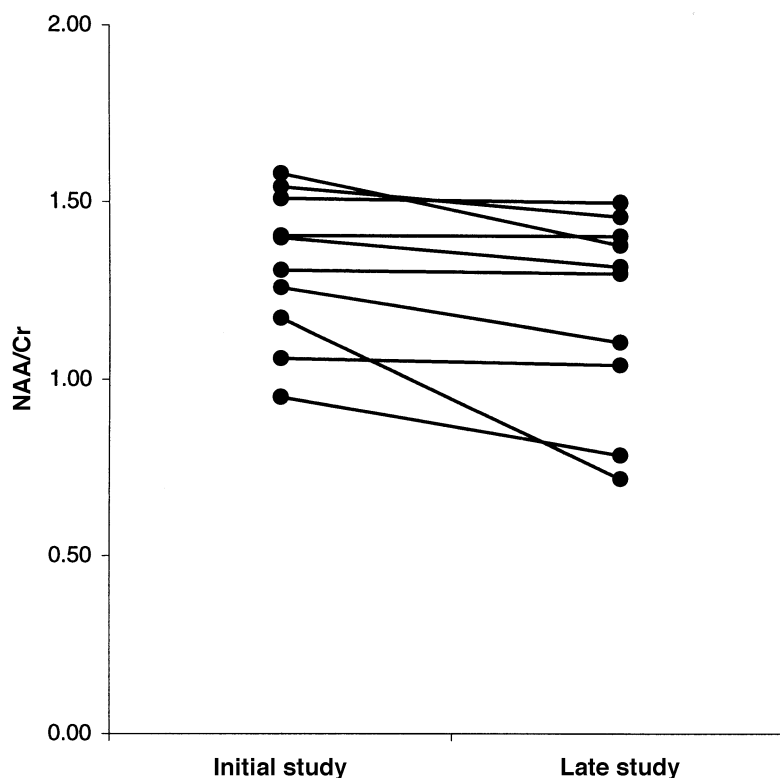


Fig. 2 Data from the 10 patients in whom proton MRS data were available at both the early and late studies. There was a significant decrease in the NAA/Cr between the two studies ($P = 0.02$).

Proton MRS data were available, from both studies, in 10 patients. These 10 patients consisted of three mildly injured patients, five moderately injured patients and two severely injured patients according to their GCS following resuscitation. In this subgroup of 10 patients (Fig. 2) there was a small, but significant ($P = 0.02$) reduction in the NAA/Cr between the initial study at 15 days and the delayed study at 6.3 months. The Cho/Cr, NAA/Cho and Ins/Cr levels remained unchanged between the two studies ($P = 0.80$, $P = 0.84$, $P = 0.29$, respectively). For comparison, 11 of the controls were studied on a second occasion with a mean delay from the first study of 12.5 months. There were no significant differences between the initial study and the delayed study in the controls for NAA/Cr ($P = 0.89$), Cho/Cr ($P = 0.76$), NAA/Cho ($P = 0.93$) and Ins/Cr ($P = 0.41$).

Proton MRS data were available at the early study together with an assessment of clinical outcome, on average 6.7 months from TBI, in 17 patients. In this subgroup of patients, the early NAA/Cr, assessed with a mean delay from TBI of 12 days, significantly correlated with their GOS score ($P = 0.005$, $r_s = 0.65$) and their DRS score ($P < 0.001$, $r_s = 0.76$) as shown in Figs 3 and 4, respectively. Furthermore, there was a significant correlation between the early NAA/Cho and the clinical outcome of the patients using either the GOS ($P = 0.01$, $r_s = 0.58$) or the DRS ($P = 0.005$, $r_s = 0.65$). The early Cho/Cr, despite being abnormal, did not correlate with the GOS score ($P = 0.45$, $r_s = 0.20$) or the DRS score ($P = 0.17$, $r_s = 0.35$). In addition, the

increased Ins/Cr did not correlate with the GOS score ($P = 0.07$, $r_s = 0.51$) or the DRS score ($P = 0.08$, $r_s = 0.51$).

No lactate was visible, above the baseline noise, in any of the spectra at either time point.

Discussion

The principle findings of this study are that when patients were reassessed, on average at 6.2 months following TBI, regions of normal-appearing white matter (NAWM) still had a significant reduction in the NAA/Cr, a significant increase in the Cho/Cr and a significant increase in the Ins/Cr. In addition, between the two studies there was a further significant reduction in the NAA/Cr. The early reduction in NAA/Cr and NAA/Cho, but not the increase in Cho/Cr, significantly correlated with the clinical outcome of the patients assessed using either the GOS or the DRS.

A reduction in NAA has been described previously in experimental models of TBI (Rubin *et al.*, 1997; Smith *et al.*, 1998). Similarly, in patients in the acute stage (Condon *et al.*, 1998), subacute stage (Ross *et al.*, 1998; Garnett *et al.*, 2000) and in the chronic stage (Cecil *et al.*, 1998a; Friedman *et al.*, 1998, 1999) following TBI, a reduction of NAA has been described. The reduction in NAA (reduced NAA/Cr) observed in the current study is therefore in keeping with previous work. In visibly contused brain the reduction of NAA (Condon *et al.*, 1998) is likely to be caused by the primary impact, whereas the reduction of NAA in regions of NAWM

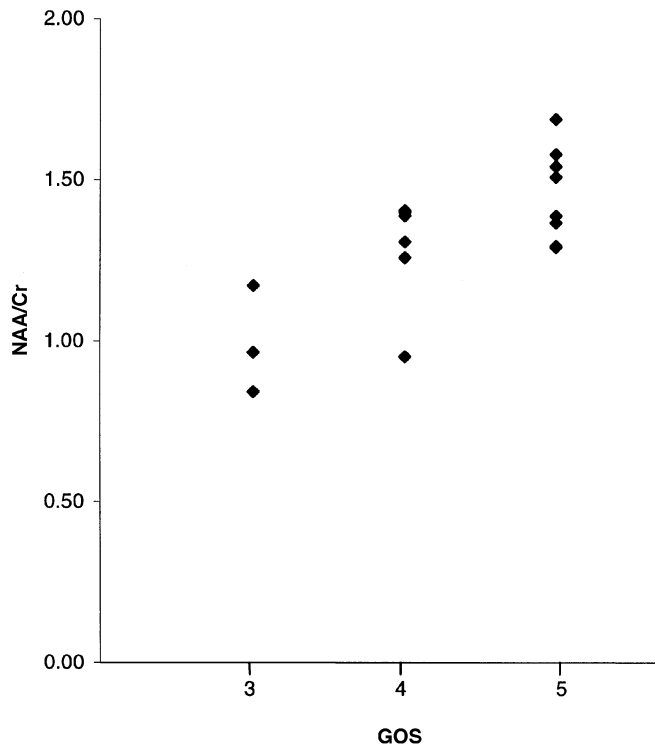


Fig. 3 Data from the 17 patients in whom both proton MRS data were available at the early study and outcome data were available at 6 months. There was a significant correlation between the early NAA/Cr and the GOS score ($P = 0.005$, $r_s = 0.65$). On the GOS, a patient who was severely disabled would have a score of 3 and a patient who had made a good recovery would score 5.

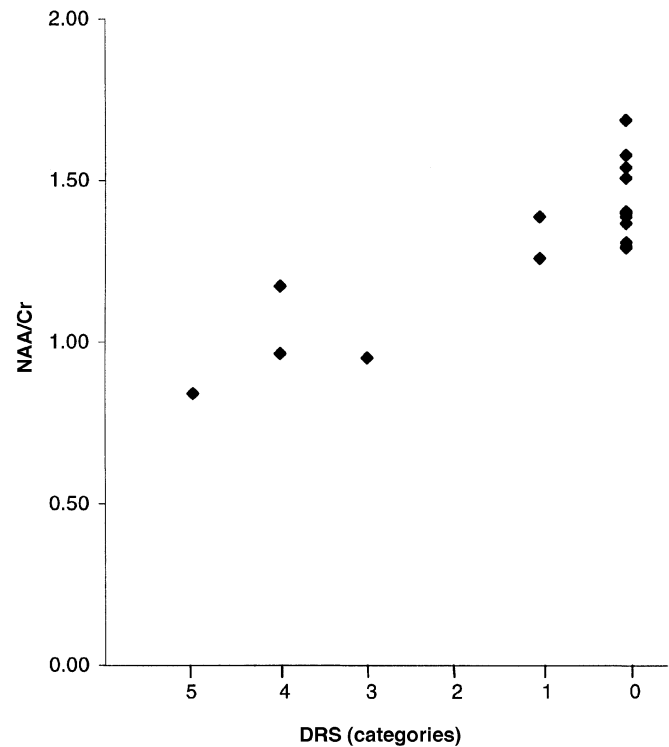


Fig. 4 Data from the 17 patients in whom both proton MRS data were available at the early study and outcome data were available at 6 months. There was a significant correlation between the early NAA/Cr and the DRS score ($P < 0.001$, $r_s = 0.76$). On the DRS, a patient who was severely disabled would score 5 and a patient with no disability would score 0.

may reflect DAI and/or Wallerian degeneration. DAI occurs in patients following mild, moderate and severe TBI (Adams *et al.*, 1989; Mittl *et al.*, 1994). The areas of the brain most affected by DAI are the lobar white matter, corpus callosum and dorsolateral upper brainstem (Gennarelli *et al.*, 1982; Adams *et al.*, 1989). Most DAI tends to be microscopic, thus producing subtle changes which are distinguishable at post-mortem, but are difficult to visualize on conventional imaging. Alternatively, Wallerian degeneration, the anterograde loss of axons that connect to regions of focal damage, has been reported to cause loss of NAA in regions of NAWM in patients with stroke and multiple sclerosis (De Stefano *et al.*, 1998; Pendlebury *et al.*, 1999; Lee *et al.*, 2000). Cortical contusions or secondary ischaemia, both of which are known to occur in patients following TBI, could be the source of the focal injury leading to Wallerian degeneration and hence a reduction in NAA.

The current study found a reduction in NAA at the late time point, mean 6.2 months following TBI. Previous reports have found a reduction in NAA levels in the chronic stage following TBI in the corpus callosum (Cecil *et al.*, 1998a) and parieto-occipital white matter (Friedman *et al.*, 1998, 1999; Ross *et al.*, 1998). However, whilst a reduction of NAA has been observed at 6 weeks following trauma in NAWM (Friedman *et al.*, 1999), when the patients were reviewed at 6 months the white matter NAA was not different

from control values (Friedman *et al.*, 1999), implying a possible recovery of NAA levels. Whilst a recovery of NAA towards normal levels has been observed in conditions such as multiple sclerosis and mitochondrial encephalopathy with lactic acidosis and stroke like episodes (De Stefano *et al.*, 1995), no recovery of NAA was found in the current study. At 6.2 months following TBI, the current study found a significantly reduced NAA in the NAWM. In addition, a small, but significant, reduction in NAA had occurred between the early and late study in a subgroup of patients who had proton MRS available at both time points. Ongoing Wallerian degeneration, and hence axonal loss, could account for this delayed reduction in NAA observed in these patients following TBI. This further small reduction in NAA, normally considered to be a neuronal marker, between the early and late study is in contrast to the subjective improvement in clinical status of this subgroup of patients. A possible explanation is that this further reduction in NAA, after 15 days, reflects a loss of dysfunctional rather than normal functioning neurones.

The current study found an increase in the Cho levels (increase in Cho/Cr) in NAWM in patients following TBI. An increase in Cho has not been documented in experimental models when studied in the first few minutes (Rubin *et al.*, 1997; Cecil *et al.*, 1998b; Smith *et al.*, 1998) or up to 7 days (Cecil *et al.*, 1998b; Smith *et al.*, 1998) following TBI. In

TBI patients an increase in the Cho levels has been reported in NAWM in the early stages following injury (Garnett *et al.*, 2000) and in regions of normal-appearing occipital grey matter in the chronic stages (Friedman *et al.*, 1998, 1999) following TBI. The Cho peak consists of several compounds, principally free Cho, phosphocholine and glycerophosphocholine (Miller *et al.*, 1996). These compounds are involved in membrane metabolism. An increase in the Cho levels could be in keeping with an alteration in membrane metabolism. An alteration in membrane metabolism could be secondary to membrane degradation or membrane synthesis following traumatic cellular disruption (Vance, 1991). There was, however, no evidence for abnormalities (e.g. oedema or parenchymal enhancement) in the regions studied by conventional imaging, which would be suggestive of membrane disruption. The observed increase in Cho could also be associated with an increase in membrane synthesis, similar to that found in tumours (Bruhn *et al.*, 1989; Segebarth *et al.*, 1990; McBride *et al.*, 1995). Alternatively, an alteration in cell population could also account for the increased level of Cho at both the subacute and chronic study. Astrocytic and microglial proliferation occurs in regions of DAI after a few days (Pearl, 1998) and this together with a reduction in neurones would cause a relative increase in the glial population in these areas. Studies on patients with tumours of glial cells (e.g. astrocytomas) have found an increase in the Cho levels (Bruhn *et al.*, 1989; Segebarth *et al.*, 1990; McBride *et al.*, 1995). The observed increase in the Cho levels in patients following TBI could therefore be in keeping with a relative increase in the glial cell population.

The elevated Ins levels could also be in keeping with a relative increase in the number of glial cells in patients following TBI. Ins is present in glial cell cultures but not neuronal cultures (Brand *et al.*, 1993). Clinical studies of patients with dementia have found a glial hypertrophy or gliosis on pathological examination (Brun, 1987, 1993) together with an increase of the Ins levels using proton MRS (Miller *et al.*, 1993; Moats *et al.*, 1994; Shonk *et al.*, 1995; Ernst *et al.*, 1997). Ins is believed to be an organic osmolyte (Lien *et al.*, 1990); the elevation in Ins in these patients may reflect glial hypertrophy with the resultant increase in osmolytes to maintain cellular volume. The increase in both the Ins and the Cho peaks would therefore be in keeping with a relative increase in glial cells in patients following TBI.

The current study found a significant correlation between the reduction in the NAA levels at 12 days following TBI and the clinical outcome at 6.7 months. No correlation was found, however, between the elevated Cho and the clinical outcome. A correlation between proton MRS and clinical outcome in patients following TBI has been reported previously (Ross *et al.*, 1998; Friedman *et al.*, 1999). In these studies the correlation was between a reduction of NAA in occipital grey matter and the clinical outcome of the patients. The current study assessed areas of NAWM in the frontal lobes of patients following TBI. Following TBI patients suffer from a variety of neuropsychological

abnormalities, particularly frontal lobe dysfunction (King *et al.*, 1997; Deb *et al.*, 1998; van der Naalt *et al.*, 1999a, b). In keeping with this, we have found proton MRS evidence of cellular injury in the NAWM of the frontal lobes, measured in the subacute stage following TBI. These proton MRS changes correlated with the clinical outcome at 6.7 months.

The results reported in this study were expressed as ratios and interpreted assuming Cr was present at a constant level. Cr is present in slightly higher concentrations in grey matter than white matter (Pouwels and Frahm, 1998) and is relatively refractive to change. An alteration in Cr levels could not account for the decrease in the NAA/Cr ratio together with the increase in the Cho/Cr ratio. The apparent differences in the ratios of the patient and controls could possibly be explained by a change in the relaxation properties of the metabolites following TBI in the patients. Assuming a T_1 value in normal cerebral white matter of 1500 ms (Frahm *et al.*, 1989b), it would not be possible to explain the observed increase in Cho by alteration of T_1 or T_2 values. However, it would require greater than a 50% increase in T_1 or decrease in T_2 to explain the reduction in NAA observed in this study. Such changes are unlikely in regions of NAWM on conventional MRI. Methods have been described that allow the absolute quantification of the metabolite levels, thus avoiding having to report findings of proton spectroscopy as ratios (Hennig *et al.*, 1992; Ernst *et al.*, 1993; Kreis *et al.*, 1993). This in theory may be desirable; however, we have presented a simple and fast method of both data acquisition and analysis that would be readily accessible to an MRI unit wishing to do clinical proton MRS.

The volume studied for the proton MRS was positioned in patients and controls in the posterior aspect of one of the frontal lobes. The frontal lobes tend to be particularly affected during a diffuse brain injury (Gentry *et al.*, 1988; Ryan *et al.*, 1994; Pearl, 1998); thus, this area was chosen to enable a comparison between injury and global clinical outcome. The voxel was localized to the posterior aspect of a frontal lobe, to avoid susceptibility effects from the frontal sinus, and contained predominantly white matter tracts. Invariably, as in the figures shown, there is some basal ganglia and thalamus included in the voxel. However, DAI, the likely mechanism for the observed changes in the proton MRS in the patients, is known to occur in basal ganglia and thalamus, together with lobar white matter, corpus callosum, brainstem and cerebellum (Hesslink *et al.*, 1988; Gentry, 1994; Gentleman *et al.*, 1995; Parizel *et al.*, 1998).

The current study involved a total of 26 patients; however, at the time of both the early and late study it was not possible to obtain proton MRS data on the whole cohort. At the time of the early study this was usually due to restlessness in the patients either causing significant movement artefact or even leading to abortion of the study. The patients who tended to be the most restless were those who were still suffering from post-traumatic amnesia. At the late study, some patients, particularly the more severely injured patients, were again too restless, thus not allowing for data collection, whilst

others were prepared to have a clinical assessment, but not a repeat MRI or proton MRS examination. Consequently, the data presented involved subgroup analysis.

In conclusion, areas of frontal white matter, which appear normal on conventional MRI, show significant proton MRS abnormalities. These abnormalities are present at both the subacute and chronic stages following TBI, with a significant correlation to the clinical outcome. These results provide evidence of diffuse damage contributing to both the pathology and outcome following TBI.

Acknowledgements

We wish to thank the Trauma Service, Oxford Radcliffe Hospitals for their co-operation with this study, and Dr W. G. Waddington and Mrs M. R. Cooper for providing excellent technical support. This work was funded by the Medical Research Council.

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Received March 27, 2000. Revised May 26, 2000.

Accepted June 26, 2000