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The prognostic value of proton magnetic resonance spectroscopy in term newborns treated with therapeutic hypothermia following asphyxia

Paul E. Sijens a,⁎, Katharina Wischniowsky b, Hendrik J. ter Horst b

ABSTRACT

Objective: The purpose of this study was to correlate brain metabolism assessed shortly after therapeutic hypothermia by 1H magnetic resonance spectroscopy (MRS), with neurodevelopmental outcome.

Methods: At the age of 6.0 ± 1.8 days, brain metabolites of 35 term asphyxiated newborns, treated with therapeutic hypothermia, were quantified by multivoxel proton MRS of a volume cranial to the corpus callosum, containing both gray and white matter. At the age of 30 months the Bayley Scale of Infant Development-III was performed.

Results: Infants that died had lower gray matter NAA levels than infants that survived (P = 0.005). In surviving infants (28 of 35) there was a trend of negative correlation between gray matter choline levels and gross motor skills (r = −0.40), and creatine positively with gross motor skills (r = 0.45). In the white matter, choline correlated negatively with fine motor skills (r = −0.45), and creatine positively with gross motor skills (r = 0.58, P = 0.02). There was no relationship between lactate levels and outcome.

Conclusion: MRS of asphyxiated neonates treated by therapeutic hypothermia can serve as predictor of outcome. Unlike previously reported associations in untreated asphyxiates, lactate levels had no relationship with outcome.

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1. Introduction

Birth asphyxia is defined as “a situation of damaging acidemia, hypoxia, and metabolic acidosis” around the time of birth [1]. In term neonates, asphyxia is the most common cause of death [2]. In those who survive, asphyxia may have adverse effects on the cardiovascular, pulmonary, renal, adrenal, gastrointestinal, metabolic, and hematological system of the newborn, but perhaps most importantly, it affects the central nervous system [3]. Possible neurological consequences of birth asphyxia include cerebral edema, and hypoxic-ischemic encephalopathy which may lead to various degrees of disability [3].

If severe enough, the hypoxic state in asphyxia can cause additional ischemia, and as a consequence reduced cerebral blood flow (CBF) [3]. First hypoxia is prevalent and forces the cells into a state of anaerobic energy production, leading to a reduced production of ATP and increased lactate production [4]. Tissue reperfusion after the hypoxic-ischemic insult may further complicate the cell damage: inflammation, increased production of reactive oxygen species (ROS), cell necrosis and vascular endothelial edema further compromise blood flow to the tissues [3–5]. This process of late reperfusion damage is called “secondary energy failure” [5] and contributes to the cell damage which occurs in Hypoxic Ischemic Encephalopathy (HIE). Besides the cerebral cortex, the basal ganglia are the most vulnerable structures to be damaged in term neonates with HIE [3]. To minimize the adverse effects of asphyxia and the resulting HIE in term and late preterm newborns, therapeutic hypothermia has proven to be effective: if started within 6 h postpartum, it reduces mortality without increasing major disability in survivors [6]. The mode of action of therapeutic cooling is based on minimizing the extent of the secondary energy failure [7].

As shown in previous studies, metabolite ratios as lactate/Cr, lactate/NAA, and NAA/Cr are correlated with outcome after neonatal encephalopathy [9,10]. MRS is a non-invasive MRI application measuring the concentrations of brain metabolites such as N-acetyl aspartate (NAA), lactate, choline (Cho), and creatine (Cr).

As shown in previous studies, metabolite ratios as lactate/Cr, lactate/ NAA, and NAA/Cr are correlated with outcome after neonatal encephalopathy [10–15] According to a recent meta-analysis of ten MRS studies, the lactate/NAA ratio has the highest diagnostic accuracy of predicting...
outcome of neonatal encephalopathy [16]. Overall there was a tendency of higher values of lactate and lowered values of NAA to occur in infants with an adverse outcome, whereas Cho and Cr still lead to contradicting results.

Before the introduction of therapeutic hypothermia, it was thus shown that MRS can help to predict the outcome of children with HIE [9–16].

Studies from one group have indicated that an increased ratio of Lactate/NAA relates to adverse outcome in asphyxics patients treated by therapeutic hyperthermia [14,17]. The present study is the first to investigate whether there is a relationship between the relative levels of specific brain metabolites (including but not limited to lactate and NAA) measured by MRS after therapeutic hypothermia and neurodevelopmental outcome at toddler age.

2. Materials and methods

2.1. Study subjects

This retrospective cohort study was conducted with institutional review board approval at the neonatal intensive care unit of University Medical Center Groningen, The Netherlands. UMCG has introduced therapeutic hypothermia for HIE in May 2009, and all related data have routinely been collected and stored in the hospital information system. We included clinical data of the time period in which MRS was part of the routine evaluation after therapeutic hypothermia: from May 2009 until January 2012. The inclusion and exclusion criteria of this study were the same as those for initiation of therapeutic hypothermia at UMCG. Indications and contraindications, the procedure of therapeutic hypothermia, and post-treatment evaluation are defined in a cooling protocol. Included were term infants ≥ 36 weeks of gestation having perinatal asphyxia. The diagnosis of perinatal asphyxia was based on at least one of the following criteria: Apgar score after 5 min of ≤ 5, resuscitation/ventilation within first 10 min after birth, pH below 7, a base deficit over 16 mmol/l, or lactate over 10.0 mmol/l. Besides perinatal asphyxia, further requirements were the initiation of cooling within 6 h, and the presence of clinical signs of HIE. Clinical HIE was defined as a Thompson score [18] of above seven, obtained at one to 3 h postpartum. Contraindications were gestational age below 36 weeks, a weight below 1800 g, and the presence of congenital abnormalities which carry an unfavorable outcome. The dataset consisted of 43 patients with HIE who received therapeutic hypothermia. Two patients died, and did not receive a MRS examination and could therefore not be included. One patient was excluded from MRS examination because of severe motion artifacts on MRI. The final dataset therefore consisted of 35 patients. All MRS data sets were of good quality and therefore all included in subsequent evaluations.

2.2. Therapeutic hypothermia

Whole body cooling was initiated within 6 h after birth. Rectal temperature was continuously monitored and maintained at 33.5 °C for 72 h. 72 h after start of the intervention, the temperature was elevated by 0.2 °C per half hour until the target temperature of 36.8 °C was achieved. After rewarming an MRS was done together with an MRI.

2.3. MRS examination

Multivoxel proton (1H) magnetic resonance spectroscopy was performed together with MRI as part of the cooling protocol at 3–13 days postpartum. A standard 8 channel transmit/receive head coil provided with the MRI system, a single 1.5 T Magnetom Sonata (Siemens AG, Erlangen, Germany) used for all measurements, served for MRI and MRS. During the time span covered by the study there were no significant soft and hard-ware changes to the system and a monthly quality insurance program assured consistent scanner performance and MRS measurement. 2D-chemical shift imaging (CSI) point resolved spectroscopy measurements were performed with a repetition time (TR) of 1500 ms and an TE of 135 ms resulting in T1- and T2-weighting of spectra, and the lactate peaks 180° out of phase relative to the other peaks facilitation quantification [19,20]. A transverse T2-weighted fast spin-echo series was used as guidance for examining an approximately 5 × 5 × 2 cm³ volume of interest cranial to the corpus callosum containing both white and gray matter and little cerebrospinal fluid, in a 16 × 16 phase encoded field of view of 16 × 16 cm² resulting in MRS voxels of 1 × 1 × 2 cm³ (7 minute acquisition time) (Fig. 1). Since 2003, the standard brain MRI measurement protocol for infants following perinatal asphyxia has included MRS measurement according to the above specifications [21,22]. Automated adjustments including localized multiple angle projection shimming, realized water peak line widths of <8 Hz in the volume of interest and chemical shift selective excitation (CHESS) and spoiling of the resultant water signal yielded water suppression by a factor exceeding 10,000. Standardized postprocessing consisted of water reference processing, Hanning filtering (center 0 ms, width 512 ms), zero filling from 512 data points to 1024 data points, Fourier transformation, frequency shift correction, sixth order polynomial baseline correction, phase correction, and frequency domain curve fitting [19]. The curve fitting was set to fit peaks to Gaussian line shapes, including the chemical shift ranges of 3.1–3.3 ppm for Cho, 2.9–3.1 for Cr, 1.9–2.1 for NAA, and 1.2–1.4 for lactate. On the clinical MRI scanner all of these data processing steps were fully automated, allowing for operator-independent quantification. The CSI voxels on the edge of the volume of interest (subject to signal drop-off) were deducted from the total data matrix. The inner 16 voxels were analyzed and separated into the two central columns mainly containing gray matter (GM) (8 voxels; i.e. a central block of 2 × 4 voxels in the examples of Fig. 1c) and the remainder of 8 voxels filled with white matter (WM) (2 periphery columns of 1 × 4 voxels, Fig. 1b), as described elsewhere [23]. The quantifications, also those of the small Lactate peak in the summarized blocks of 8 spectra, were reproducible and, therefore, no manual adjustments were performed. To limit patient examination times, absolute quantification requiring additional CSI measurements without water suppression was not performed. To facilitate tissue signal comparison, we expressed the GM and WM metabolites in percent (%) of summed peak areas requiring additional CSI measurements with percent (%) of summed peak areas of Cho, Cr, and NAA in the 16 analyzed voxels, a method used before [22–24]. Specifically, the sum of these metabolites was set at 1600% for the 16 voxels collectively, after which any metabolite signal per voxel was calculated as (voxel metabolite area) / (total VOI Cho + Cr + NAA area) × 1600%. Within this patient group the summed metabolite peak areas per 2 cm³ MRS voxel, affected by multiple factors such as transmitter and receiver adjustments, shimming, volume of interest size, age, brain size and pathology, met a coefficient of variance of 15%.

2.4. Longterm follow-up

Follow-up was part of the standard post-cooling care, and was carried out between 12 and 30 months of age. Based on neurological exam e classified cerebral palsy (CP) based on the Gross Motor Function Classification System (GMFCS) [25], using scores of one and two for mild CP, and scores of three or more for severe CP. At 30 months of age the Bayley Scale of Infant Development 3rd Edition (BSID-III) was used to assess cognitive and motor development. Outcome was divided into three groups: 1) normal development, 2) mild disability, and 3) severe disability or death (Table1). Normal Development was defined as a BSID-III score ≥ 85 and the absence of CP; mild disability was defined as a CP with a GMFCS 1 or 2 and/or a BSID-III between 70 and 85, and severe disability as a BSID-III <70 and/or a CP with a GMFCS > 3 [26].
2.5. Statistical analysis

We used SPSS version 22.0 software for Windows (IBM Corp., Armonk, NY) for all analyses. First we used the Shapiro-Wilk test to determine whether the metabolites show a normal distribution within each group. To compare means between all groups we used an independent one-way ANOVA in case of normal distribution, and we used Kruskal-Wallis in case non-normal distribution. Group-to-group comparison in data without normal distribution was performed through the Mann-Whitney U test, and for data with normal distribution with an independent t-test. Pearson correlations were calculated for normally distributed data and Spearman correlations for non-normal data between scores of the BSID-III and the metabolites measured by MRS. Bonferroni adjustments were applied in the calculation of the significance of the correlations between multiple MRS parameters (n = 8) and BSID scores. The significance for all tests was defined as \( P < 0.05 \).

3. Results

3.1. MR spectroscopy characteristics

Patient characteristics of the 35 infants are shown in Table 1. The MRS-examination was done at day 6 on average (range: 3–13). Out of 35 patients, 7 patients died for neurologic reason, 3 developed a mild disability and 25 developed normally (Table 2). None of the children developed moderate to severe disability.

Table 2 summarizes the relative peak areas measured by the MRS of both white matter and gray matter metabolites in percent per group. Only gray matter NAA was significantly different between outcome groups, with lower NAA values in the infants that died \( (P = 0.005) \). Specific group-to-group comparison only showed a significant difference of gray matter NAA between the groups of normal development and deceased \( (P = 0.005) \) MRS results for two patients with a normal neurologic outcome and one dying soon afterwards are shown in Fig. 1A and B, respectively. The main difference between the patients’ spectra is the intensity of the NAA peak (observed at 2.0 ppm).

![Fig. 1. A: MRS spectral map (a) and the summed white matter and gray matter spectra (b, c) of a five day old asphyxia patient treated by therapeutic hypothermia with a normal neurologic outcome; B: MRS spectral map (a) and the summed white matter and gray matter spectra (b, c) of a eight day old asphyxia patient treated by therapeutic hypothermia who died three days later.](image-url)
Table 2
Average relative peak areas of metabolites (in %) in both white and gray matter.

<table>
<thead>
<tr>
<th></th>
<th>Normal development (n = 25)</th>
<th>Mild disability (n = 3)</th>
<th>Deceased (n = 7)</th>
<th>P-value of difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho</td>
<td>48 ± 4</td>
<td>49 ± 1</td>
<td>50 ± 6</td>
<td>0.43</td>
</tr>
<tr>
<td>Cr</td>
<td>26 ± 3</td>
<td>24 ± 5</td>
<td>27 ± 4</td>
<td>0.55</td>
</tr>
<tr>
<td>NAA</td>
<td>30 ± 4</td>
<td>28 ± 3</td>
<td>26 ± 2</td>
<td>0.15</td>
</tr>
<tr>
<td>Lact</td>
<td>6 ± 2</td>
<td>6 ± 3</td>
<td>7 ± 3</td>
<td>0.62</td>
</tr>
<tr>
<td>GM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cho</td>
<td>44 ± 5</td>
<td>46 ± 5</td>
<td>46 ± 3</td>
<td>0.50</td>
</tr>
<tr>
<td>Cr</td>
<td>25 ± 3</td>
<td>25 ± 1</td>
<td>26 ± 4</td>
<td>0.93</td>
</tr>
<tr>
<td>NAA</td>
<td>26 ± 3</td>
<td>25 ± 3</td>
<td>22 ± 4</td>
<td>0.017</td>
</tr>
<tr>
<td>Lact</td>
<td>7 ± 2</td>
<td>7 ± 3</td>
<td>6 ± 3</td>
<td>0.54</td>
</tr>
</tbody>
</table>

GM, gray matter; WM, white matter.

3.2. MRS brain-metabolites and the BSID-III scores

In survivors we found moderate correlations between MRS measured metabolites with the BSID-III scores in both gray and white matter (Table 3). In gray matter there was a trend of correlation between Cho and gross motor scores \( (r = -0.45; \text{see Fig. 2}) \), higher values of Cho were associated with lower gross scores. NAA levels correlated positively with cognitive scores \( (r = 0.37) \) although not significantly. In white matter choline showed a trend of correlation with fine motor performance \( (r = -0.40) \), indicating that children with higher values of Cho measured by MRS tended to have lower motor scores at toddler age. White matter Cr correlated positively with gross motor scores \( (r = 0.58, P = 0.02; \text{see Fig. 2}) \).

4. Discussion

Magnetic resonance spectroscopy detectable metabolites include lactate, a marker of anaerobic metabolism; NAA, a marker of neuronal maturation/integrity; Cho, a marker of membrane turnover; and Cr, a measure of energy metabolism. This retrospective cohort study shows that gray matter NAA was the only metabolite significantly differing between the groups with normal outcome and infants that died. In surviving infants we found several trends of relationships between brain metabolites measured by MRS and neurodevelopmental outcome based on BSID-III. Higher white matter Cr is indicative of a better gross motor score \( (P = 0.02) \). In contrast, higher white and gray matter Cho tended to correlated negatively with fine motor outcome or gross motor outcome, respectively, indicating worse outcome. Higher NAA values appear to predict a better cognitive outcome, although this also did not reach significance.

The findings regarding NAA are consistent with those in former research: higher values correlate with better outcome \([9–16]\). NAA is seen as a marker of neuronal health \([27]\), which is in accordance with our findings. The results for lactate contrast with previous studies where in children who did not receive therapeutic hypothermia, the lactate level measured by MRS was indicative of poor clinical outcome \([9–13,16]\). However, our results are in perfect agreement with those few studies associating an increased ratio of lactate/NAA measured after therapeutic hypothermia with therapeutic outcome \([14,17]\). The new aspect here is that our data show that the predictive metabolite is NAA rather than lactate, in other words, NAA decreases rather than lactate increases are predictive of therapeutic outcome after hypothermia.

Previous MRS studies mainly focused on the predictive value of metabolite ratios not allowing for discrimination between lactate increases and NAA decreases. The finding of, according to meta-analysis, increased lactate/NAA being the most powerful indicator of adverse outcome in untreated patients \([16]\) would fit the interpretation that also in the untreated cases of neonatal encephalopathy, NAA might be the more reliable predictor of outcome. However, discrepancy with regard to our own previous finding in untreated neonatal encephalopathy \([9]\), implying lactate as a stronger indicator of prognosis than NAA, may be explained by how the intervention of therapeutic hypothermia works. One of the postulated mechanisms is that therapeutic hypothermia reduces the cerebral metabolic rate by slowing down glycolysis, thereby reducing ATP-usage while maintaining high ATP production \([28,29]\). This would facilitate a more efficient cellular function with less lactate production, explaining our finding of lower lactate values in the brains of the neonates treated with hypothermia with no differences between the outcome groups. Conform the theory of reduced metabolism in therapeutic hypothermia \([19]\), lactate would thus be, unlike in untreated newborns, a less reliable metabolite for predicting outcome after treatment with hypothermia.

Results for Cr and Cho did not correlate with clinical outcome, which was not surprising in view of the contradicting results in the literature \([16]\). However, when looking at the different domains of the BSID-III we found that NAA correlated positively with cognitive outcome, Cho negatively with fine motor skills, and Cr positively with gross motor skills. These findings are consistent with the negative correlations of brain tissue myoinositol/Cr and Cho/Cr ratios with fine motor skills and of the Cho/NAA ratio with gross motor skills observed by Johnson et al. \([30]\) in neonates exposed to chorioamnionitis.

Several limitations apply to our study. First of all, we did not have a control group to compare the results with healthy children. Since MRS-examinations are not routinely done in healthy neonates we did not have such data for comparison. Unfortunately our previous study of asphyxic children \([9]\), could neither serve as control group due to different inclusion criteria. Secondly, the age range at which MRS was performed \((\text{mean age } 6.0 \pm 1.8 \text{ days, range } 3–13, \text{though narrow, poses an additional limitation, because children examined on day three for example might have been in a different phase of brain damage and recovery than children examined on day } 13).\) Thirdly, the location of the MRS volume above the ventricle rather than in the basal ganglia region preferred by several authors, may have affected our outcomes. Fourthly, results are presented in relative metabolite concentrations with the advantage that metabolites are evaluated separately and the limitation that changes in total metabolite level are not accounted for. Finally, Bonferroni adjustments were applied in our correlation analyses of BSID scores with eight MRS parameters, reducing the chances of incorrectly declared differences \((\text{type 1 error})\) at the cost of incorrectly missed true effects \((\text{type 2 error})\). To our knowledge, truly quantitative MRS studies of young infants have not been published to date and are close to impossible because variations in metabolite relaxation rates with aging or after treatment cannot be excluded.

5. Conclusion

This study indicates that MRS might be a good predictor of outcome in children who have been treated with therapeutic hypothermia for
HIE, NAA being higher in infants who survive. In surviving infants several metabolites tend to be associated with outcome; grey matter NAA with cognitive outcome, white matter Cr significantly with gross motor outcome and white and grey matter Cho weakly with fine and gross motor outcome. Lactate values were not associated with outcome, indicating that therapeutic hypothermia works through reducing the metabolic rate.

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**Disclosure statement**

The authors have no competing interests to disclose.

**References**


