MRI and spectroscopy in (near) term neonates with perinatal asphyxia and therapeutic hypothermia

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ABSTRACT

Background Previous studies have demonstrated the association of abnormalities on diffusion-weighted MRI (DW-MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) in infants with perinatal asphyxia. The use of therapeutic hypothermia might change this association.
Aim To study the association between DW-MRI and ^{1}H-MRS and outcome after perinatal asphyxia and therapeutic hypothermia in infants with a gestational age of ≥36 weeks.
Patients and methods Infants with perinatal asphyxia and therapeutic hypothermia (n=88) were included when an MR examination was performed within 7 days after birth. Apparent diffusion coefficient (ADC) values of the basal ganglia and thalamus were calculated, as were lactate/N-acetylaspartate (LAC/NAA) and N-acetylaspartate/choline (NAA/Cho) ratios. Death or an abnormal neurodevelopment at ≥24 months was considered an adverse outcome. Receiver operating characteristic analysis was performed to determine cut-off levels.
Results Of the 88 infants, 22 died and 7 had an adverse neurodevelopmental outcome. In infants with an adverse outcome, ADC values of the basal ganglia and thalamus were significantly lower, and Lac/NAA ratios were significantly higher than in infants with a normal outcome. Areas under the curve of ADC of the basal ganglia, thalami and Lac/NAA ratio were 0.89, 0.88 and 0.87, respectively. NAA/Cho ratios were in this cohort not associated with outcome.
Conclusions During and after therapeutic hypothermia, low ADC values and high Lac/NAA ratios of the basal ganglia and thalamus are associated with an adverse outcome in infants with perinatal asphyxia.

What is already known on this topic?

► A decreased value of the apparent diffusion coefficient of water in the basal ganglia and thalamus is associated with an adverse outcome in infants with perinatal asphyxia and normothermia.
► Elevated lactate levels in the basal ganglia and thalamus are associated with an adverse outcome in infants with perinatal asphyxia and normothermia.

What this study adds?

► Low apparent diffusion coefficient values and high lactate/N-acetylaspartate (LAC/NAA) ratios in the basal ganglia and thalamus in infants with perinatal asphyxia are associated with an adverse outcome, also during and after therapeutic hypothermia.
► Results of DW-MRI (apparent diffusion coefficient values) and proton MRS (Lac/NAA ratios) can be used as biomarkers of outcome in infants with perinatal asphyxia and therapeutic hypothermia; cut-off levels of these biomarkers differed slightly from those in normothermic infants.
► Apparent diffusion coefficient values were dependent on the MRI sequences.
► Findings of proton MRS in 1.5 T and 3.0 T magnetic fields were comparable.

INTRODUCTION

Perinatal asphyxia followed by hypoxic-ischaemic encephalopathy remains a major cause of mortality and morbidity.[1][2] In the Western world, severe perinatal asphyxia affects 1–2 per 1000 newborns.[3–5] Prediction of outcome is important for parents and caregivers to optimise treatment for the infants beyond the neonatal period. MRI has been used extensively for prediction of outcome in these infants.[6–8] Perinatal asphyxia can lead to cerebral cellular oedema resulting in diffusion restriction, which can be quantified using the apparent diffusion coefficient (ADC) in that region.[9–12] ADC values can be measured by means of diffusion-weighted MRI (DW-MRI). In addition, metabolic changes may occur in affected brain regions, which can be detected using proton magnetic resonance spectroscopy (^{1}H-MRS).[10][13][14]

In a previous study of infants with perinatal asphyxia admitted before the era of hypothermia, we have described the predictive value of both ADC values and ^{1}H-MRS of the basal ganglia and thalamus (BGT).[15] In addition, the association between ADC values of the corpus callosum and outcome was slightly modified by therapeutic hypothermia.[16]

Since 2008, neonates with perinatal asphyxia are treated with therapeutic hypothermia. It is known that therapeutic hypothermia can change the predictive value of biomarkers, like amplitude-integrated EEG (aEEG) or standard EEG.[17] The predictive value of ADC values and ^{1}H-MRS of the BGT in infants with therapeutic hypothermia after perinatal asphyxia has been tested in a limited number of patients only.[15][18] No

definite conclusions could be drawn about differences with nor-mothermic infants.

Therefore, the aim of this study was to investigate whether hypothermia influences the predictive value of ADC values and ^{1}H-MRS obtained in the first week of life in infants with peri-natal asphyxia for death or an adverse neurodevelopmental outcome.

Subjects and methods

Between January 2008 and December 2013, a cohort of 134 neonates with perinatal asphyxia were treated with moderate hypothermia. Inclusion criteria for therapeutic hypothermia were described previously.[3] In all infants, encephalopathy grading according to Sarnat, as well as Thompson scores, was assigned.[19 20] Of the 134 infants, 46 were excluded for various reasons, including gestational age <36 weeks, MRI examination later than 7 days after birth, and congenital malformations, chromosomal abnormalities or syndromes, including inborn errors of metabolism. The remaining 88 (66%) were subjects of the present study.

This retrospective study was approved by the local ethics committee, and the requirement to obtain informed consent for this study with anonymous data analysis was waived.

MRI

MRI was performed on a 1.5 (n=38) or 3.0 T (n=50) MR system (Philips Medical Systems, Best, The Netherlands). The scanning protocol included T1-weighted (3.0 T) or Inversion Recovery (1.5 T), and T2-weighted MRI. In addition, an echoplanar imaging technique was used for diffusion-weighted imaging, 4-mm thick sections, 0 mm section gap and b-values of 0 and 1000 s/mm^2 (1.5 T) or 800 s/mm^2 (3.0 T), as described previously.[16] When patient stability and time permitted, diffusion tensor imaging (DTI) was performed. The DTI protocol (3.0 T) consisted of a single-shot spin-echo echoplanar imaging sequence (echoplanar imaging factor 55, TR/TE 5685/70 ms, field of view 180×146 mm^2, acquisition matrix 128×102 mm, reconstruction matrix 128×128 mm, 50 slices with 2 mm thickness without gap). Images were acquired in the axial plane with diffusion gradi-ents applied in 32 non-collinear directions with a b-value of 800 s/mm^2 and one non-diffusion-weighted image.[21] MRI abnormalities were analysed using an adaptation of the Barkovich score as described previously, ranging from 0 (normal) to 11 (maximal injury).[22]

ADC measurement

ADCs were measured on the ADC map of the DW-MRI scan, and if present, on the ADC map of the DTI scan (see online supplementary figure S1A). Four regions of interest (ROIs) were placed in the left and right basal ganglia and in the left and right medial thalamus because these structures are most severely affected after acute hypoxic-ischaemic events.[1 7 11 23] ROIs were placed by the investigator LCS and reviewed by TA. ROIs were placed in the ADC map on the slice 8 mm (ie, two slices) above the peduncle. ROIs were oval, with a pixel area of around 35 mm^2 in the basal ganglia and 45 mm^2 in the thal-amus. Contact with brain sections other than the BGT, like cere-bral spinal fluid and cerebral white matter, was carefully avoided. All measurements were performed on a Philips MRI operator console. These measurements were performed without prior knowledge of outcome.

^{1}H-MRS

^{1}H-MRS examinations at the level of the left BGT were available in 80 patients. A PRESS sequence was used to select a single volume of interest (mean volume 4 cm^3, range 1.5–9.0 cm^3) located in the left basal ganglia, as reported previously[15] (see online supplementary figure S1B). Contact with cerebral spinal fluid or white matter was carefully avoided. Parameters were TR of 2000 ms, TE 288 ms and 64 measurements were averaged. NAA, Cho and Cr peaks were identified at 2.02, 3.02 and 3.24 ppm, respectively. When present, lactate was identified as a doublet at 1.33 ppm. Automated curve fitting was performed on an MRI operator console using Philips Medical Care proprietary software. If necessary, manual phase adjustment was applied. Ratios of peak areas of N-acetylaspartate/choline (NAA/Cho) and lactate/N-acetylaspartate (LAC/NAA) were calculated.

Clinical data and outcome

Neurodevelopmental outcome was assessed at regular intervals after birth, as described previously.[15] At the age of 24 months, the Bayley Scales of Infant Development-III was used for assess-ment (mean 100, SD ±15). A score of <85 was considered abnormal. In addition, cerebral palsy, severe visual problems or hearing loss were considered a moderate-to-severe disability.

In this study, infants with an adverse outcome (death or dis-ability) were compared with the infants who survived with a good outcome. Clinical findings are presented in table 1.

Statistical analysis

Statistical analysis was performed using SPSS V.21 (SPSS-IBM, Chicago, Illinois, USA). Infants with a good and an adverse outcome were compared using Student's t-tests, Mann-Whitney U test and χ^2 test, where appropriate. ROC curves were gener-ated to create cut-off values for ADC values in the thalamus and basal ganglia, and Lac/NAA ratios in the BGT as described previ-ously.[15] Intraobserver and interobserver variability were deter-mined in 10 subjects.

RESULTS

Of the 88 infants, 22 died and 7 had an adverse neurodevelop-mental outcome (dyskinetic cerebral palsy n=1, cognitive impairment n=5, epilepsy n=1). As expected, patients with an adverse outcome had a more severe degree of encephalopathy, underwent the MR examination earlier after birth and had a

Table 1 Clinical data of the infants

|	Normal outcome N=59	Adverse outcome N=29	p Value
Gender (M/F)	30/29	18/11	0.37
PMA at birth (weeks, mean±SD)	39.9±1.5	40.2±1.7	0.38
Birth weight (g, mean±SD)	3456±601	3605±543	0.26
First pH	6.98±0.20	6.94±0.19	0.39
Apgar 5 min (median, IQR)	4 (3)	3 (3)	0.214
Postnatal age at scan (days, mean±SD)	5.1±0.8	3.6±1.2	<0.001
PMA at scan (weeks, mean±SD)	40.6±1.6	40.7±1.7	0.78
Grade HIE			
1	10	0	<0.001
2	49	13	
3	0	16	
MRI score (median, IQR)	1 (2)	7 (5)	<0.001
Bayley Scales of Infant Development-III cognitive composite score (mean±SD)	110±13	87±10	<0.001
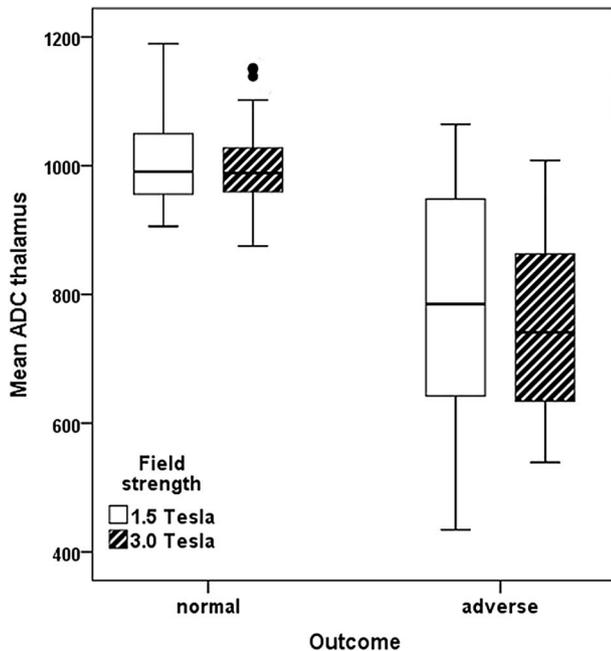
Figure 1 Apparent diffusion coefficient (ADC) values ($\times 10^{-6}$ mm^2/s) of the thalamus versus outcome and field strength (1.5 and 3.0 T). Differences between infants with a good or adverse outcome are significant, but no significant effect of the field strength is seen.
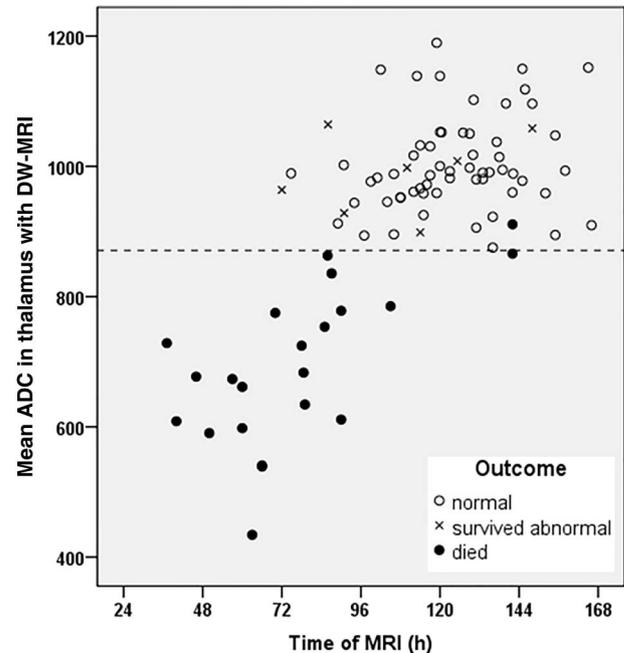

Figure 2 Apparent diffusion coefficient (ADC) values ($\times 10^{-6}$ mm^2/s) of the thalamus versus time after birth in infants with a good outcome (open circles) or adverse outcome (survived: crosses; died: closed circles). The horizontal dotted line indicates the cut-off value of 871×10^{-6} mm^2/s. DWI, diffusion-weighted MRI.

Table 2 Comparison of findings in normothermia obtained from Alderliesten et al[15] and hypothermia cases from the present study

Parameter	Area under the curve	Cut-off	Sensitivity (%)	Specificity (%)
Apparent diffusion coefficient ($\times 10^{-6}$ mm^2/s)				
Normothermia				
Basal ganglia	0.88	1031	78	95
Medial thalamus	0.72	919	48	98
Hypothermia				
Basal ganglia	0.89	974	76	98
Medial thalamus	0.86	871	72	100
Lactate/N-acetylaspartate				
Normothermia	0.81	0.08	96	61
Hypothermia	0.89	0.40	69	94

higher MR abnormality score (table 1). Ten infants of this study were treated with hypothermia but were shown to have grade 1 encephalopathy. Their treatment was based on a high Thompson score or a suppressed aEEG on admission.

ADC in BGT
In the BGT, left and right ADC values were averaged. Intraobserver and interobserver variation in measuring ADC values were respectively 1.2% and 1.5%.

In the basal ganglia as well as the thalamus, infants with an adverse outcome had generally lower ADC values in comparison with infants who survived with a good outcome. Data for the thalamus are presented in figure 1. Mean values for the basal ganglia were $1097 \pm 66 \times 10^{-6}$ mm^2/s for infants with a normal outcome and $870 \pm 169 \times 10^{-6}$ mm^2/s for those with an adverse outcome (p<0.001).

ROC analysis demonstrated cut-off values of 973×10^{-6} mm^2/s for the basal ganglia and 871×10^{-6} mm^2/s for the thalamus (table 2).

In total, 3 of the 22 infants who died had ADC values in the normal range in basal ganglia or thalamus: in one infant, mainly cortical and subcortical abnormalities were seen, with a borderline decrease of ADC in the thalamus (863×10^{-6} mm^2/s); in the second infant, a very low ADC value was measured in the thalamus (598×10^{-6} mm^2/s), but not in the basal ganglia; and in the third infant, low values were measured in the basal ganglia (748×10^{-6} mm^2/s), but not in the thalami (910×10^{-6} mm^2/s). Low ADC values in the BGT were present in the other 19 infants. In addition, five survivors with ADC values in the normal range had an adverse outcome: cognitive problems (n=4) and epilepsy (n=1).

In the group of surviving infants, no effect of time after birth on ADC values could be demonstrated. In the 29 infants with an adverse outcome, however, ADC values tended to increase between day 2 and day 7 after birth (figure 2).

ADC using DW-MRI and DTI
In 59 infants, ADC values were also obtained using DTI. ADC values from DW-MRI were in the same range as those obtained using DTI (figure 3A, B). Linear regression indicated small but consistent differences between both methods.

^{1}H-MRS
^{1}H-MRS of the left BGT with an echo time of 288 ms was performed in 80 infants. Lac/NAA levels were strongly associated with outcome (figure 4). ROC analysis with an area under the curve (AUC) of 0.89 indicated that a Lac/NAA ratio of >0.40 was associated with an adverse outcome. Also, 8 of the 20 infants with an adverse outcome and ^{1}H-MRS data had a Lac/NAA ratio below the threshold levels. In four infants, lactate
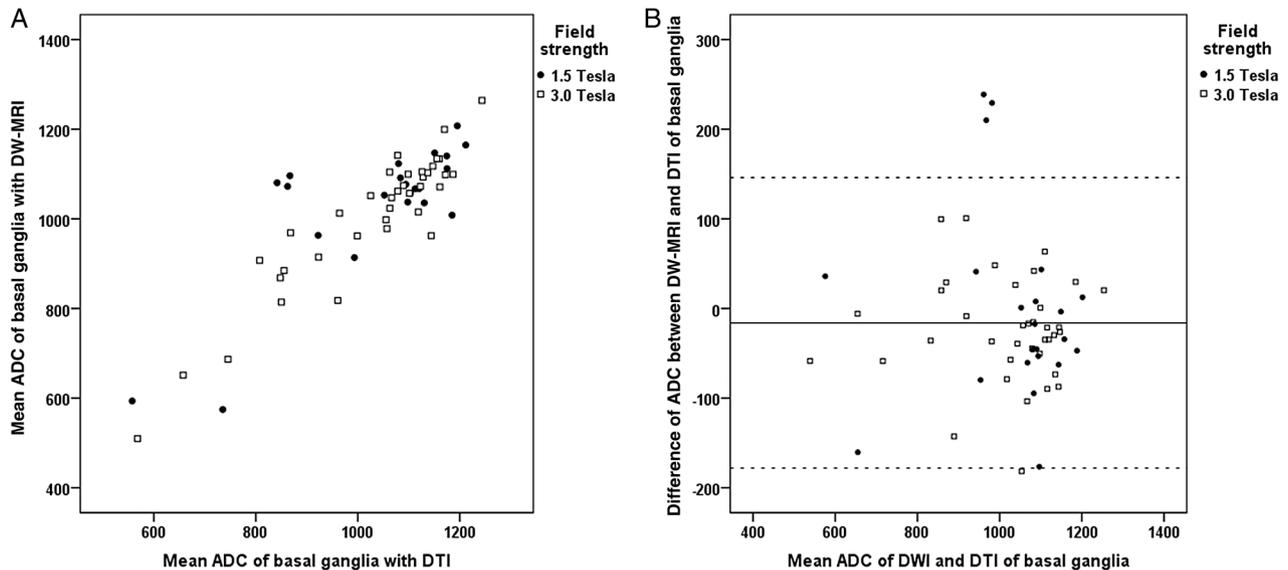
Figure 3 (A) Apparent diffusion coefficient (ADC) values of the basal ganglia measured using diffusion-weighted MRI (DWI) (3 directions) or diffusion tensor imaging (DTI) (32 directions) at two different field strengths (1.5 T, closed circles, and 3.0 T, open squares). (B) Bland-Altman plot of the ADC values of the basal ganglia obtained using DWI and DTI at two different field strengths (1.5 T, closed circles, and 3.0 T, open squares). The dotted lines indicate the upper and lower margins of the 95% CI.
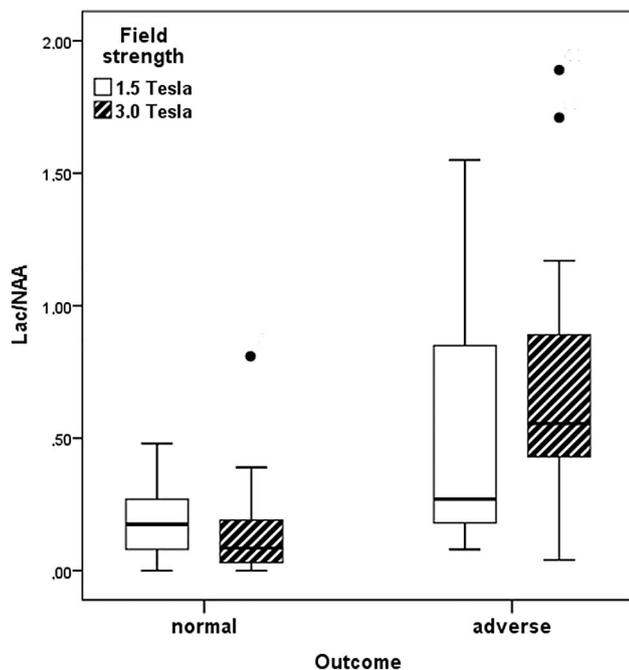

Figure 4 Lac/NAA ratios obtained using proton magnetic resonance spectroscopy of the left-sided basal ganglia and thalamus at two different field strengths (1.5 and 3.0 T). Differences in Lac/NAA ratios between infants with a normal or adverse outcome were significant (p=0.002), and differences in Lac/NAA ratios between field strengths were not significant (p=0.40).

could be demonstrated, but the quality of the spectra was moderate to poor. In two infants, cognition was abnormal and one of them had hearing loss, but MRI and ^{1}H-MRS did not show abnormalities, in one infant MRI showed cortical abnormalities and in one infant mainly basal ganglia abnormalities were seen, but no abnormalities in the thalami. NAA/Cho levels were not associated with outcome in the present study. Although the

difference in Lac/NAA ratios between infants with a good or adverse outcome appeared larger at 3.0 T than at 1.5 T, numbers of patients were too small for further analysis. Reduced ADC values corresponded very well to elevated lactate/NAA levels (see online supplementary figure S2).

DISCUSSION

In this study, we compared the association between ADC values and ^{1}H-MRS in BGT in the first week of life and outcome in infants with perinatal asphyxia treated with hypothermia. A low ADC value of the basal ganglia or thalami, or high Lac/NAA ratios of the same area, was associated with an adverse outcome. Although very low ADC values have been used in the decision to redirect care,[24] ADC values were never the only consideration leading us to redirect care since in all infants an abnormal neurological examination, abnormal aEEG findings (burst suppression, continuous low voltage or flat trace for >48 hours) and cranial ultrasound scans (severe echogenicity of BGT) were always involved in the decision-making process.

The association between previously described low ADC values in basal ganglia and thalamus in infants with perinatal asphyxia and an adverse outcome is confirmed in the present study.[9 10 15 25] Some studies showed no association between ADC values and outcome.[10 26] In one study, the ADC values are very different from those we and others have published.[10]

It should be noted that the cut-off ADC values in the present study were approximately 5% lower than those reported in our previous study, while the AUC of the ROC curve was in the same range (basal ganglia 0.89, medial thalamus 0.86).[15] This is comparable to recently published findings of ADC values in the corpus callosum, where therapeutic hypothermia resulted in lower cut-off ADC values for an adverse outcome.[16]

Bednarek et al[18] have demonstrated that the pseudo-normalisation of ADC is much slower following therapeutic hypothermia compared with normothermia and may take up to 10 days. Because of this relatively slow pseudo-normalisation, and the fact that all our patients were examined within 7 days after birth, we did not take the time of MRI into account to

determine the clinically relevant cut-off level. In fact, multivariable logistic regression models showed that time after birth was not significantly associated with outcome in the present data set with limited variation of time after asphyxia at examination.

In addition, magnetic field strength did not affect ADC or ^{1}H-MRS cut-off levels in the present data set. Nevertheless, ADC values and ^{1}H-MRS ratios may depend on field strength and properties of the coils used, so we suggest that locally obtained normal values should be used for clinical decision-making.

ADC values were obtained using both DW-MRI with diffusion in 3 directions and DTI using diffusion in 32 directions. Although there is an excellent correlation between the two examinations, absolute values are slightly different and cannot be used interchangeably.

Three infants who died and were scanned at 60, 86 and 142 hours after birth had normal ADC values in either the basal ganglia or thalamus. This might be the result of a changing pattern over time of abnormalities following perinatal asphyxia.[11]

It was of interest that all five infants with an adverse outcome and an ADC value above the cut-off value had cognitive problems or epilepsy, rather than motor sequelae. They were scanned between 72 and 148 hours after birth, and none had abnormalities in the basal ganglia or thalamus on T1, T2 or DW-MRI. Two had watershed lesions, one showed a small subdural haemorrhage and two had no abnormalities.

A recent study suggested that lactate levels were slightly lower in the basal ganglia, but not in the thalamus during hypothermia.[27] ^{1}H-MRS has retained the predictive value after perinatal asphyxia and therapeutic hypothermia, although cut-off values were higher than in our previous study. In particular, elevated Lac/NAA ratios are associated with an adverse outcome, which is similar to observations in normothermic infants following perinatal asphyxia.[11 13–15 28–31] In the present study, NAA/Cho ratios of the BGT were not predictive. We cannot exclude that hypothermia delayed the loss of NAA, a neuronal marker, and that infants have been scanned too early after birth to demonstrate a decrease in NAA.

We have demonstrated that DW-MRI and ^{1}H-MRS have predictive properties after hypothermia even prior to rewarming. This is important since other biomarkers, such as early clinical examination or aEEG, showed a delay in predictive power after hypothermia.[17 32] Standard T1-weighted (or IR) and T2-weighted MRI have previously been demonstrated to be useful to detect brain abnormalities after perinatal asphyxia and hypothermia, but >50% of the infants were examined beyond the first week of life.[6] MRI beyond the first week after birth may be late when redirection of care is considered.[24]

For clinical purposes, both DW-MRI and MRS can be recommended. Whereas DW-MRI provides spatial information about ischaemic brain areas and enables discrimination between watershed and BGT lesions, MRS of the BGT has excellent predictive properties, which are less time dependent.[33] The present study has some limitations.

First, infants were examined at a magnetic field of either 1.5 or 3.0 T. Although small differences in ADC values between these magnetic field strengths cannot be excluded, the distribution of ADC values in infants with a normal outcome is remarkably similar, and multivariable analysis including strength of the MR system did not demonstrate an effect of the magnetic field. This is supported by our previous study of ADC values in the corpus callosum, where ADC values were similar at 1.5 and 3.0 T.[16]

Second, some patients who subsequently died were scanned within 72 hours after birth during therapeutic hypothermia.

Kozak *et al*[34] described a 2% decrease of ADC values per °C, resulting in an estimated 7% decrease at 33.5°C. As a consequence, ADC values close to the cut-off levels need to be interpreted with caution when patients are examined during the first few days after perinatal asphyxia. However, others have demonstrated the usefulness of early MRI.[35] When the ADC and ^{1}H-MRS are in the normal range, and clinical signs and aEEG suggest the presence of brain injury, repeating the MRI after rewarming should be considered.

Third, all neonates scanned within 72 hours after birth had an adverse outcome, thereby limiting the predictive value of ADC in this time window. The measured ADC values were very low in our infants with an adverse outcome. In infants with a normal outcome, ADC values are much higher and do not show a trend in the first week as demonstrated by Bednarek *et al*.[18] Therefore, we do not consider this a clinically relevant limitation. In addition, normal ADC in the rewarming period had an excellent negative predictive value.

Finally, in infants with the most severe perinatal asphyxia and meconium aspiration syndrome, no MRI could be made because their condition did not allow us to do so. It is expected that the ADC values in BGT in the infants who died have been very low.

CONCLUSIONS
Low ADC values and high Lac/NAA ratios in the BGT obtained during the first week of life in infants with perinatal asphyxia with therapeutic hypothermia are associated with an adverse outcome. For ADC measurements, both DW-MRI and DTI can be used, although absolute values are slightly different.

REFERENCES
1 Cowan F, Rutherford M, Groenendaal F, *et al*. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 2003;361:736–42.
2 Jacobs SE, Berg M, Hunt R, *et al*. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2013;(1):CD003311.
3 Groenendaal F, Casaer A, Dijkman KP, *et al*. Introduction of hypothermia for neonates with perinatal asphyxia in the Netherlands and Flanders. *Neonatology* 2013;104:15–21.
4 Azzopardi D, Strohm B, Linsell L, *et al*. Implementation and conduct of therapeutic hypothermia for perinatal asphyxial encephalopathy in the UK--analysis of national data. *PLoS ONE* 2012;7:e38504.
5 Chevallier M, Ego A, Cans C, *et al*. Adherence to hypothermia guidelines: a French multicenter study of fulltern neonates. *PLoS ONE* 2013;8:e83742.
6 Rutherford M, Ramenghi LA, Edwards AD, *et al*. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. *Lancet Neurol* 2010;9:39–45.
7 de Vries LS, Groenendaal F. Patterns of neonatal hypoxic-ischaemic brain injury. *Neuroradiology* 2010;52:555–66.
8 Miller SP, Ramaswamy V, Michelson D, *et al*. Patterns of brain injury in term neonatal encephalopathy. *J Pediatr* 2005;146:453–60.
9 Rutherford M, Counsell S, Allsop J, *et al*. Diffusion-weighted magnetic resonance imaging in term perinatal brain injury: a comparison with site of lesion and time from birth. *Pediatrics* 2004;114:1004–14.

10 Zarifi MK, Astrakas LG, Poussaint TY, *et al*. Prediction of adverse outcome with cerebral lactate level and apparent diffusion coefficient in infants with perinatal asphyxia. *Radiology* 2002;225:859–70.

11 Barkovich AJ, Miller SP, Bartha A, *et al*. MR imaging, MR spectroscopy, and diffusion tensor imaging of sequential studies in neonates with encephalopathy. *AJNR Am J Neuroradiol* 2006;27:533–47.

12 Neil JJ, Shiran SI, McKinstry RC, *et al*. Normal brain in human newborns: apparent diffusion coefficient and diffusion anisotropy measured by using diffusion tensor MR imaging. *Radiology* 1998;209:57–66.

13 Groenendaal F, Veenhoven RH, van der Grond J, *et al*. Cerebral lactate and N-acetyl-aspartate/choline ratios in asphyxiated full-term neonates demonstrated in vivo using proton magnetic resonance spectroscopy. *Pediatr Res* 1994;35:148–51.

14 Thayyil S, Chandrasekaran M, Taylor A, *et al*. Cerebral magnetic resonance biomarkers in neonatal encephalopathy: a meta-analysis. *Pediatrics* 2010;125:e382–e95.

15 Alderliesten T, de Vries LS, Benders MJ, *et al*. MR imaging and outcome of term neonates with perinatal asphyxia: value of diffusion-weighted MR imaging and (1)H MR spectroscopy. *Radiology* 2011;261:235–42.

16 Alderliesten T, de Vries LS, Khalil Y, *et al*. Therapeutic hypothermia modifies perinatal asphyxia-induced changes of the corpus callosum and outcome in neonates. *PLoS ONE* 2015;10:e0123230.

17 Thoresen M, Hellstrom-Westas L, Liu X, *et al*. Effect of Hypothermia on Amplitude-Integrated Electroencephalogram in Infants With Asphyxia. *Pediatrics* 2010;126:e131–9.

18 Bednarek N, Mathur A, Inder T, *et al*. Impact of therapeutic hypothermia on MRI diffusion changes in neonatal encephalopathy. *Neurology* 2012;78:1420–7.

19 Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress; a clinical and electroencephalographic study. *Arch Neurol* 1976;33:696–705.

20 Thompson CM, Puterman AS, Linley LL, *et al*. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 1997;86:757–61.

21 Kersbergen KJ, de Vries LS, Groenendaal F, *et al*. Corticospinal tract injury precedes thalamic volume reduction in preterm infants with cystic periventricular leukomalacia. *J Pediatr* 2015;167:260–8.

22 Van Rooij LG, Toet MC, van Huffelen AC, *et al*. Effect of treatment of subclinical neonatal seizures detected with aEEG: randomized, controlled trial. *Pediatrics* 2010;125:e358–66.

23 Rutherford MA, Pennock JM, Murdoch Eaton DM, *et al*. Athetoid cerebral palsy with cysts in the putamen after hypoxic- ischaemic encephalopathy. *Arch Dis Child* 1992;67:846–50.

24 Bonifacio SL, deVries LS, Groenendaal F. Impact of hypothermia on predictors of poor outcome: How do we decide to redirect care? *Semin Fetal Neonatal Med* 2015;20:122–7.

25 Vermeulen RJ, Fetter WP, Hendrikx L, *et al*. Diffusion-weighted MRI in severe neonatal hypoxic ischaemia: the white cerebrum. *Neuropediatrics* 2003;34:72–6.

26 Liauw L, van Wezel-Meijler G, Veen S, *et al*. Do apparent diffusion coefficient measurements predict outcome in children with neonatal hypoxic-ischemic encephalopathy? *AJNR Am J Neuroradiol* 2009;30:264–70.

27 Wisnowski JL, Wu TW, Reitman AJ, *et al*. The effects of therapeutic hypothermia on cerebral metabolism in neonates with hypoxic-ischemic encephalopathy: an in vivo 1H-MR spectroscopy study. *J Cereb Blood Flow Metab* 2015;36:1075–86.

28 Amess PN, Penrice J, Wylezinska M, *et al*. Early brain proton magnetic resonance spectroscopy and neonatal neurology related to neurodevelopmental outcome at 1 year in term infants after presumed hypoxic-ischaemic brain injury. *Dev Med Child Neurol* 1999;41:436–45.

29 Pu Y, Li QF, Zeng CM, *et al*. Increased detectibility of alpha brain glutamate/glutamine in neonatal hypoxic-ischemic encephalopathy. *AJNR Am J Neuroradiol* 2000;21:203–12.

30 Hanrahan JD, Cox IJ, Edwards AD, *et al*. Persistent increases in cerebral lactate concentration after birth asphyxia. *Pediatr Res* 1998;44:304–11.

31 Penrice J, Cady EB, Lorek A, *et al*. Proton magnetic resonance spectroscopy of the brain in normal preterm and term infants, and early changes after perinatal hypoxia-ischemia. *Pediatr Res* 1996;40:6–14.

32 Gunn AJ, Wyatt JS, Whitelaw A, *et al*. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. *J Pediatr* 2008;152:55–8.

33 Robertson NJ, Cowan FM, Cox IJ, *et al*. Brain alkaline intracellular pH after neonatal encephalopathy. *Ann Neurol* 2002;52:732–42.

34 Kozak LR, Bango M, Szabo M, *et al*. Using diffusion MRI for measuring the temperature of cerebrospinal fluid within the lateral ventricles. *Acta Paediatr* 2010;99:237–43.

35 Boudes E, Tan X, Saint-Martin C, *et al*. MRI obtained during versus after hypothermia in asphyxiated newborns. *Arch Dis Child Fetal Neonatal Ed* 2015;100:F238–42.