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Reliability of early MRI and necessity of repeating MRI in non-cooled and cooled infants with neonatal encephalopathy

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Abbreviations:
ADC : Apparent diffusion coefficient
DWI : Diffusion Weighted Imaging
FOV : Field of View
MRI : Magnetic Resonance Imaging
NE : Neonatal Encephalopathy
T1 : T1 weighted imaging
T2 : T2 weighted imaging
TE : Time of Echo
TR : Time of Relaxation

ABSTRACT

In cooled newborns with encephalopathy, although late MRI scan (10-14 days of age) is reliable in predicting long-term outcome, it is unknown whether early scan (3-6 days of life) is. We compared the predominant pattern and extent of lesion between early and late MRI in 89 term neonates with NE. 43 neonates (48%) were cooled. The predominant pattern of lesions and the extent of lesion in the watershed region agreed near perfectly in non-cooled (kappa=0.94; k=0.88) and cooled (k=0.89; k=0.87) infants respectively. There was perfect agreement in the extent of lesion in the basal nuclei in non-cooled infants (k=0.83) and excellent agreement in cooled infants (k=0.67). Changes in extent of lesions on late MRI occurred in 19/89 infants, with higher risk in infants with hypoglycemia and moderate-severe lesions in basal nuclei. In most term neonates with NE, early MRI (relative to late scan) robustly predicts the predominant pattern and extent of injury.

KEY WORDS: newborn, neonatal encephalopathy, magnetic resonance imaging, brain injury, hypothermia, asphyxia.

INTRODUCTION

In term infants with neonatal encephalopathy (NE) of suspected hypoxic-ischemic origin, therapeutic hypothermia (33.5 ± 0.2ºC) for 72 hours commenced within 6 hours of life decreases death and moderate to severe disability at 18-22 months,[1,2] and improves neurologic outcome in survivors.[3] The benefits of treatment persist at 6-7years of age[4,5] and it has now become standard of care.[6]

The predominant pattern of lesions in MRI brain, including basal ganglia, watershed, total, and focal and multifocal (stroke and white matter injury)[7] is strongly predictive of later neurodevelopmental outcome.[8] The extent of lesion in the basal nuclei (basal ganglia and thalamus) is associated with poorer long-term motor outcome,[9] whereas the extent of lesion in the watershed region is associated with lower verbal IQ.[10] In non-cooled infants with NE, the pattern of MRI brain lesions remains consistent between early scan (day of life 3-6; determined by changes in diffusion weighted imaging or apparent diffusion coefficient maps) and late scan (day of life 10-14; determined by changes in corresponding regions on T1 and T2 sequences).[7,11]

Studies from cooled infants have demonstrated that late MRI brain (day of life 6-15) is predictive of neurodevelopmental outcome at 18- 24 months.[12-14] It is important to be able to prognosticate soon after the completion of therapeutic hypothermia, as this is the time frame when critical decisions are made regarding the direction of clinical care. Although MRI brain undertaken during cooling before 3 days of age was predictive of later brain injuries in a small cohort of infants,[15] it remains unknown whether the changes oin brain MRI performed after rewarming (day of life 3-6) are consistent with later MRI. Moreover, it is not known which group

of cooled infants should routinely have a repeat MRI following an early (day 3-6) scan.

In a retrospective cohort of infants with NE, we aimed to determine the agreement of the predominant pattern of brain lesions and the extent of basal nuclei and watershed lesions between the early and late MRI in non-cooled and cooled infants, and to identify the risk factors associated with disagreement in the late MRI compared with early MRI.

METHODS

Human subjects

The Children's and Women's Health Centre of British Columbia/University of British Columbia Clinical Research Ethics Board approved this study.

Inclusion criteria

Term newborns (gestational age >36 weeks) admitted to the level III Neonatal Intensive Care Unit at British Columbia Children's and Women's Hospital between June 2004 and December 2012 were included in this study if they had clinically recognizable encephalopathy and at least one of the indicators of peripartum hypoxia-ischemia including: (1) intrapartum fetal distress; (2) requirement for resuscitation for > 10min of age; (3) Apgar score ≤ 5 at 10 min; or (4) metabolic acidosis (umbilical artery pH<7.1 or base deficit >10).

From 2008, therapeutic hypothermia was standard of care in our NICU. Term infants with moderate or severe NE[1] underwent therapeutic hypothermia (whole body cooling for 3 days) within 8 hours of life if they had one or more acute perinatal events (e.g. placenta abruptio), indicators of peripartum hypoxia-ischemia, and clinical or amplitude integrated electroencephalogram evidence of encephalopathy. No other neuroprotective therapies were used in this cohort. Infants with metabolic or genetic syndromes or structural brain malformations were excluded.

MR imaging

MRI was performed on day of life 3-6 (early scan) and on day of life 10-14 (late scan) under anesthetic sedation in a specialized neonatal head coil (Sree Medical, Cleveland, Ohio, USA) as part of protocolized clinical care. Late scan was undertaken in infants who continued to have abnormal neurological examination beyond the first week of life ~~that was not explained by the findings on the early scan~~.

All MRIs were acquired on a Siemens (New Jersey, USA) 1.5 Tesla Avanto using VB 13 software and included the following sequences: 3D volumetric T_1-weighted images (TR, 36; TE, 9.2; FOV, 200mm; slice thickness, 1mm; no gap), axial fast spin echo T_2-weighted images (TR, 4610; TE, 107; FOV, 160mm; slice thickness, 4mm; gap, 0.2mm), and DWI b=600 and 700s/mm^2 with ADC maps (TR, 3300; TE, 82; FOV 210mm; average 4; slice thickness 4mm; gap, 0.5mm). An experienced pediatric neuroradiologist, blinded to the clinical information, scored the patterns and extent of lesions, as previously published (intra-observer reliability: >0.9).[7,8,18]

Using the Barkovich system, the extent of lesions in the basal nuclei (basal ganglia and thalamus) was scored 0 to 4 denoting the spread of abnormal signal from normal (0), to thalamus (1), to lentiform nucleus (2), to peri-rolandic cortex (3), to more extensive involvement (4). The extent of lesions in the watershed region was scored as: normal (0); focal infarction (1); abnormal signal in anterior or posterior watershed cortex (2); extending to white matter (3); involving both anterior and posterior watershed zones (4), and more extensive involvement (5).[7,18] The four predominant patterns of lesions were normal, watershed (scores of extent of lesion in the watershed region higher than the basal nuclei), basal ganglia (scores of extent of lesion in the basal nuclei equal or higher than watershed region), total (maximal injury in the basal nuclei and watershed region), and multifocal (focal and multifocal white matter lesions and stroke).[7,8] The extent of basal nuclei and watershed lesions were classified to normal to mild (score ≤1) and moderate to severe (score 2 to 4). The predominant patterns of lesions on the early MRI were identified primarily from the DWI and ADC maps followed by T1 and T2 weighted images. The predominant patterns of lesions on late MRI were scored primarily from the T1 and T2 weighted images, as the diffusion changes undergo pseudo-normalization by then. ADC values

<800mm^2/sec were defined as restricted diffusion. The imaging features of neonatal hypoglycemia were considered to be abnormal signals in the posterior white matter, optic radiations and pulvinar.[16] Infants with reduced or worsened extent of lesions on late scan compared with early scan were defined as infants with change in lesions on late scan.

Clinical data

Antenatal and perinatal factors including gestation, birth weight, gender, head circumference, Apgar score, cord pH, encephalopathy score,[19] electrical and clinical seizures, and clinical hypoglycemia (<46mg/dL) were collected by retrospective chart review.

Statistics

We used student's t-test (normally distributed data) or Mann-Whitney U test (data with skewed distribution) to compare continuous variables and Fisher's exact test or chi-squared test for categorical variables. We assessed the agreement on the pattern of lesions and the extent of lesions in the basal nuclei and watershed region between the early and late MRIs in the non-cooled and cooled infants with the Kappa (κ) coefficient. Using established cut-offs, we described the strength of agreement as poor (0.00-0.40), good (0.41-0.60), excellent (0.61-0.80) and perfect (0.81-1.00).[20] The clinical factors were compared between the infants with and with out change in lesions on late scan. All statistical analyses were performed using Stata 9.0 (Statacorp, College Station, Texas) and SPSS statistics v19.0 (North Harbour Portsmouth, Hampshire, England). P values less than 0.05 were considered significant.

RESULTS

Eighty-nine infants with NE comprised the cohort with 46 non-cooled infants and 43 cooled infants. There was no significant difference in the gestational age, head circumference, gender distribution, cord pH, encephalopathy score on day 1 and neonatal seizures between the cooled and non-cooled infants. Non-cooled infants (29%) and cooled infants (69%) underwent late MRI. The non-cooled infants compared with cooled infants were significantly lighter (mean (SD), 3.25Kg (0.58) versus 3.52Kg (0.47); P=0.016) and had higher Apgar score at 10 min of age (Median (IQR), 7 (4,8) versus 5 (3,6); P=0.003) (Table 1). Nearly 30% of MRIs were undertaken under sedation and the drugs used for sedation included morphine or fentanyl bolus, propofol, ketamine and midazolam depending on the preference of physician. Infants who received bolus morphine or fentanyl were already receiving the respective infusion prior to MRI. Infants who are well sedated either due to the infusion of analgesics, sedatives or recent anticonvulsant use in the NICU did not receive additional sedation during MRI.

Pattern of lesions

There was perfect agreement of pattern of lesions between the early and late MRI in the non-cooled (κ=0.94) and cooled (κ=0.89) infants. Among the non-cooled infants, one infant with normal early MRI developed multifocal lesions on late MRI and another infant with total pattern of lesion on early MRI downgraded to watershed pattern of lesion on late MRI (Table 2). Among the 23 cooled infants with normal early MRI, one infant developed basal nuclei pattern of lesion and another infant developed multifocal pattern of lesion on late MRI. One cooled infant with total pattern of lesion on early MRI downgraded to basal nuclei pattern of lesion on late MRI (Table 2).

Basal nuclei extent of lesions

There was perfect agreement of basal nuclei extent of lesions between the early and late MRI in the non-cooled infants (κ=0.83) and excellent agreement in the cooled infants (κ=0.67).

Non-cooled infants

The extent of lesions reduced in 5/46 non-cooled infants on late MRI. Two non-cooled infants with abnormal signal in thalamus on early scan (score 1) normalized on late scan (score 0). Of 3 non-cooled infants who had abnormal signal in basal nuclei with extensive cortical involvement on early scan (score 4) and then had reduced extent on late MRI, one infant normalized (score 0), another infant had abnormal signal in thalamus (score 1) and the third infant had abnormal signal in thalamus and lentiform nucleus (score 2) (Table 3).

Cooled infants

The extent of lesions reduced in 3/43 and worsened in 4/43 cooled infants on late MRI. One infant with abnormal signal on thalamus on early scan (score 1) normalized on late scan (score 0). Out of two cooled infants who had abnormal signal in basal nuclei and extensive cortical involvement on early scan (score 4), one infant normalized (score 0) and another infant had abnormal signal in thalamus and lentiform nucleus (score 2) on late scan. One cooled infant with normal early scan (score 0) worsened to having abnormal signal in thalamus and lentiform nucleus on late scan (score 2); three cooled infants with abnormal signal in thalamus and lentiform nucleus on early scan (score 2) worsened to having abnormal signal in basal nuclei and extensive cortical involvement, particularly the peri-rolandic cortex, on late scan (score 4) (Figure 1) (Table 3).

Watershed extent of lesions (supplement table)

There was perfect agreement of the extent of lesions in the watershed region between the early and late scan in the non-cooled (κ=0.88) and cooled (κ=0.87) infants.

Non-cooled infants

The extent of lesions worsened in 4/46 non-cooled infants on late scan. Two infants with normal early scan (score 0) worsened on late scan; one infant developed abnormal signal in anterior or posterior watershed cortex (score 2) and another infant developed abnormal signal in both anterior and posterior watershed zones (score 4). Two infants with focal infarction on early scan (1) developed abnormal signal in the watershed zone extending to white matter on late scan (score 3).

Cooled infants

The extent of lesions reduced in 2/43 and worsened in 1/43 cooled infants on late scans. One cooled infant with abnormal signal in the anterior and posterior watershed zones on early scan (score 4) evolved to having normal late scan (score 0) and another infant with extensive cortical involvement on early scan (score 5) reduced to having abnormal lesions in the white matter on late scan (score 3). One infant with normal early scan (score 0) developed abnormal signal in white matter on late scan (score 3).

Clinical factors associated with change in lesion on late scan (Table 4)

There were no differences in the demographics, ~~occurrence of seizures,~~ cooling, severity of encephalopathy, occurrence of sentinel events, resuscitation, occurrence of seizures, distribution of neonatal EEG abnormalities and anticonvulsant therapy and age of scan between infants with and without changes of lesions (new or disappearance) on late MRI. Infants who had change in lesions compared with infants who did not have change in lesions on late MRI had significantly higher incidence of

clinical hypoglycemia within 72 hours of age (52.6% versus 17.9%; relative risk 3.2, 95% CI 1.51, 6.91), MRI features of hypoglycemia on early MRI (47.3% versus 17.1%; relative risk 2.9, 95% CI 1.37, 6.21). Note that most infants with change in lesion extent from early to late scans had moderate-severe extent of lesions in the basal nuclei on early MRI (52.6% versus 28.5%; relative risk 2.2, 95% CI 1.0, 4.80), with some infants worsening (21%) and others improving (31.6%) (Table 2). Of the infants with changes in the extent of lesions on late MRI and delayed neurodevelopment diagnosed at a median (IQR) age of 19.0 months (10.8, 37.8), early MRI basal ganglia extent score was associated with outcome in 3/9 while late MRI was associated with outcome in 2/9 infants. As for the watershed extent score, early MRI was associated with outcome in 2/9 while late MRI was associated with outcome in 1/9 infant.

DISCUSSION

In newborn infants with neonatal encephalopathy, the predominant pattern of lesions and the extent of lesions in the watershed region on early MRI performed between day 3-6 of life were reliably consistent with late scans performed between day 10-14 of life irrespective of therapeutic hypothermia. The extent of lesions in the basal nuclei was less reliable in a minority of infants on the early scan. The infants who had clinical hypoglycemia within 72 hours of age or MRI features of hypoglycemia or moderate to severe extent of lesions in the basal nuclei on the early scan will benefit from a repeat late MRI to confirm the full extent of lesions.

Early MRI in a small cohort of cooled NE infants was reported to predict later brain injuries.[21]However, determining brain lesions within day of life 3 (cooling period) on DWI can be challenging, as brain water ADC values vary with brain temperature.[22] As the MRI in our cooled babies was performed after re-warming, the effect of low brain temperature during cooling influencing the ADC values was eliminated, perhaps explaining the lack of influence on pattern classification. The observation that the predominant pattern of brain injury is not altered by therapeutic hypothermia has useful clinical implications given its predictive value for long-term neurodevelopmental outcome.[8,13,23] Pattern of brain lesions on MRI performed after day of life 6 in cooled infants was predictive of long-term outcome.[12,13,24] As our study shows that the pattern of lesions did not change on the late scan (days of life 10-14) compared to the early scan (days of life 3-6) in the majority of cooled infants, MRI can be undertaken in cooled infants early after rewarming to predict long-term neurodevelopmental outcome.

The extent of watershed lesion is associated with language related disabilities.[10] Few infants (3/7), who demonstrated changes in the extent of lesion on late MRI,

developed watershed white matter lesions on late MRI. Early MRI reliably predicted the extent of lesion in the watershed region compared with late scan.

A minority of cooled infants developed perirolandic lesions on late scan, a worsening of the basal nuclei pattern from that observed on the early scan. This progression of cortical lesions is likely to be genuine as the first MRI was undertaken between day of life 3-6, which is well within the time window to develop and maintain diffusion changes. Clinically, identification of the peri-rolandic cortex involvement is relevant because of the presence of the motor cortex. The extent of lesions in the basal nuclei, in addition to being associated with long-term motor outcome at 24 months,[9] is also predictive of severity of communication impairment.[25]

Early scans are important for prognostication as critical decisions may need to be taken within the first few days of life. However, it is also crucial to identify the infants who may require a repeat scan due to potential suboptimal accuracy of the early scan. Our data indicates that hypoglycemia, both diagnosed clinically and on MRI, may alter the reliability of early scans. Clinical hypoglycemia is common in infants with neonatal encephalopathy of presumed hypoxic-ischemic origin and is associated with adverse neurodevelopmental outcome.[26] MRI features of hypoglycemia including abnormal signal in the posterior white matter, pulvinar and anterior medial thalamic nuclei can be superimposed on the hypoxic-ischemic pattern of lesions.[16] The infants with clinical hypoglycemia within the first 3 days of life and the infants with MRI features of hypoglycemia are at increased risk of both worsening and improving in the extent of brain lesions on late MRI. In 33% of infants with MR features of hypoglycemia identified as more extensive cortical involvement in the early ADC scans, the lesions did not translate into T1 or T2 signal abnormalities on the late scan. It is not known if these early changes in ADC scan will lead to long

term neurodevelopmental consequences. Relying on just the early scan in this group will lead to overestimation of the extent of lesion. These findings stress the importance of close monitoring of blood glucose in newborns suspected for hypoxic-ischemic brain injury. The cut off for blood sugar level used to define hypoglycemia did not differentiate between symptomatic and asymptomatic infants. It is unknown if hypoglycemia has a direct effect on changes in the extent of lesions on late MRI or merely reflects the metabolic difference in sick infants. However the lack of difference in the severity of asphyxia and encephalopathy score between the infants with and without changes on the extent of lesions on late MRI suggests a likely direct effect of hypoglycemia on the brain.

Additionally, our data confirmed that in infants with moderate to severe extent of lesions in the basal nuclei on the early scan, there is some risk that the injury could alter at the time of the late scan.

The strengths of our study include the standardized acquisition parameters for the MRI involving infants from a single center. The serial MRI scans enabled us to identify the intra-individual progression of lesions and characterize the groups of infants who will require repeat scan after early MRI. A limitation of our study is that the late MRI was only undertaken in non-cooled neonates with NE if they had persistent abnormal neurology ~~signs~~ on clinical examination beyond the first week of life. Given this we may have selected non-cooled infants with a more severe extent of lesions in whom to compare early and late MRI findings. Importantly however, only few non-cooled infants had mild encephalopathy as indicated by the higher Apgar scores. All cooled neonates with NE had early and late scans. With the comparable findings across these groups, we are confident that we captured the full extent of NE.

CONCLUSION

In infants with neonatal encephalopathy, the predominant pattern of lesions on MRI performed between days of life 3-6 can be reliably interpreted irrespective of cooling. The extent of lesions in the watershed region is also stable from scans on day 3-6 of life to scans on day 10-14 of life. In infants with clinical hypoglycemia, MRI features of hypoglycemia, or moderate to severe extent of basal nuclei lesions on the early MRI, repeat MRI between 10-14 days of life is required to confidently know the full extent of lesions,

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AUTHOR CONTRIBUTIONS
Elavazhagan Chakkarapani: carried out acquisition, analysis, and interpretation of data, performed statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted.

Kenneth J Poskitt: conceptualized and designed the study, carried out acquisition of neuroimaging data, critically reviewed and revised the manuscript for important intellectual content, and approved the final manuscript as submitted.

Steven P. Miller: conceptualized and designed the study, contributed to determining outcome variables and obtaining funding, carried out acquisition, analysis, including statistical analysis, and interpretation of data, participated to drafting of the initial manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Jill Zwicker: contributed to determining outcome variables, critically reviewed and revised the manuscript for important intellectual content, and approved the final manuscript as submitted.

Helen Qi Xu: carried out acquisition of data, reviewed and approved the final manuscript as submitted.

Darren S.T. Wong: carried out acquisition of data, reviewed and approved the final manuscript as submitted.

Elke H. Roland: contributed to determining outcome variables, critically reviewed and revised the manuscript for important intellectual content, and approved the final manuscript as submitted.

Alan Hill: contributed to determining outcome variables, critically reviewed and revised the manuscript for important intellectual content, and approved the final manuscript as submitted.

Vann Chau: provided supervision throughout the study, conceptualized and designed the study, carried out acquisition, analysis, including statistical analysis, and interpretation of data, participated to drafting the initial manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted.

DECLARATION OF CONFLICTING INTERESTS
The authors have no conflict of interests to declare.

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ETHICAL APPROVAL

The Children's and Women's Health Centre of British Columbia/University of British Columbia Clinical Research Ethics Board approved this study.

REFERENCES

1. Shankaran, S., *et al.* Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* **353**, 1574-1584 (2005).
2. Gluckman, P.D., *et al.* Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* **365**, 663-670 (2005).
3. Azzopardi, D.V., *et al.* Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* **361**, 1349-1358 (2009).
4. Shankaran, S., *et al.* Childhood outcomes after hypothermia for neonatal encephalopathy. *N Engl J Med* **366**, 2085-2092 (2012).
5. Azzopardi, D., *et al.* Effects of hypothermia for perinatal asphyxia on childhood outcomes. *N Engl J Med* **371**, 140-149 (2014).
6. Edwards, A.D., *et al.* Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* **340**, c363 (2010).
7. Chau, V., *et al.* Comparison of computer tomography and magnetic resonance imaging scans on the third day of life in term newborns with neonatal encephalopathy. *Pediatrics* **123**, 319-326 (2009).
8. Miller, S.P., *et al.* Patterns of brain injury in term neonatal encephalopathy. *J Pediatr* **146**, 453-460 (2005).
9. Ferrari, F., *et al.* General movements in full-term infants with perinatal asphyxia are related to Basal Ganglia and thalamic lesions. *J Pediatr* **158**, 904-911 (2011).

10. Steinman, K.J., *et al.* Neonatal watershed brain injury on magnetic resonance imaging correlates with verbal IQ at 4 years. *Pediatrics* **123**, 1025-1030 (2009).

11. Welch, K.M., *et al.* A model to predict the histopathology of human stroke using diffusion and T2-weighted magnetic resonance imaging. *Stroke* **26**, 1983-1989 (1995).

12. Cheong, J.L., *et al.* Prognostic utility of magnetic resonance imaging in neonatal hypoxic-ischemic encephalopathy: substudy of a randomized trial. *Arch Pediatr Adolesc Med* **166**, 634-640 (2012).

13. Rutherford, M., *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* **9**, 39-45 (2010).

14. Shankaran, S., *et al.* Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* **97**, F398-404 (2012).

15. Wintermark, P., *et al.* Early versus late MRI in asphyxiated newborns treated with hypothermia. *Arch Dis Child Fetal Neonatal Ed* **96**, F36-44 (2010).

16. Wong, D.S., *et al.* Brain Injury Patterns in Hypoglycemia in Neonatal Encephalopathy. *AJNR Am J Neuroradiol* **34**, 1456-1461 (2013).

17. Gano, D., *et al.* Evolution of pattern of injury and quantitative MRI on days 1 and 3 in term newborns with hypoxic-ischemic encephalopathy. *Pediatr Res* (2013).

18. Barkovich, A.J., *et al.* Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR Am J Neuroradiol* **19**, 143-149 (1998).

19. Miller, S.P., *et al.* Clinical signs predict 30-month neurodevelopmental outcome after neonatal encephalopathy. *Am J Obstet Gynecol* **190**, 93-99 (2004).

20. Landis, J.R. & Koch, G.G. The measurement of observer agreement for categorical data. *Biometrics* **33**, 159-174 (1977).

21. Wintermark, P., *et al.* Early versus late MRI in asphyxiated newborns treated with hypothermia. *Arch Dis Child Fetal Neonatal Ed* **96**, F36-44 (2011).

22. Zhang, Y., *et al.* On the accuracy of noninvasive thermometry using molecular diffusion magnetic resonance imaging. *Int J Hyperthermia* **8**, 263-274 (1992).

23. Cheong, J.L., *et al.* Prognostic utility of magnetic resonance imaging in neonatal hypoxic-ischemic encephalopathy: substudy of a randomized trial. *Arch Pediatr Adolesc Med* **In press**(2012).

24. Shankaran, S., *et al.* Brain injury following trial of hypothermia for neonatal hypoxic-ischaemic encephalopathy. *Archives of disease in childhood* **97**, F398-404 (2012).

25. Martinez-Biarge, M., *et al.* Feeding and communication impairments in infants with central grey matter lesions following perinatal hypoxic-ischaemic injury. *Eur J Paediatr Neurol* **16**, 688-696 (2012).

26. Nadeem, M., Murray, D.M., Boylan, G.B., Dempsey, E.M. & Ryan, C.A. Early blood glucose profile and neurodevelopmental outcome at two years in neonatal hypoxic-ischaemic encephalopathy. *BMC Pediatr* **11**, 10.

27. Tam, E.W., *et al.* Occipital lobe injury and cortical visual outcomes after neonatal hypoglycemia. *Pediatrics* **122**, 507-512 (2008).

Figure 1 Example of new brain abnormalities on the late MRI in a cooled infant compared with a non-cooled infant.

Images A, B, C, D are from a non-cooled infant and images E, F, G, H are from a cooled infant.

The top row (A, C, E, G) represents the first (ADC) and the bottom row (B, D, F, H) represents the second (T1) MRI. In non-cooled infant, restricted diffusion is initially seen in the ventrolateral thalamic nucleus, lentiform nucleus, optic radiation, hippocampus (A) and peri-rolandic cortex (C) on the early MRI. Subsequently, T1 shortening in the corresponding regions can be seen in the late MRI (B, D). In the cooled infant, there is restricted diffusion in the ventrolateral thalamic nuclei, lentiform nucleus and optic radiation (E), with corresponding T1 shortening on the second MRI (F). There is T1 shortening on the late MRI in hippocampus (F, white arrowhead) and peri-rolandic cortex (H, white arrow head) with no corresponding restricted diffusion in the ADC map of early MRI (E and G) (ADC value right, left in the peri-rolandic cortex: 950,1003).

Table 1: Baseline demographics between non-cooled and cooled infants.

Demographic features	**Non-cooled infants** **N = 46**	**Cooled infants** **N = 43**
Gestation weeks mean (SD)	39.5 (1.51)	39.5 (1.49)
Male, n (%)	19 (41.3%)	18 (41.8%)
Birth weight, Kg mean (SD)	3.25 (0.58)*	3.52 (0.47)*
Head circumference, cm mean (SD)	34.4 (1.81)	34.6 (1.17)
10min Apgar score, median (IQR)	7 (4, 8)*	5 (3,6)*
Cord pH, mean (SD)	7.03 (0.13)	7.03 (0.17)
Encephalopathy score on day 1, median (IQR)	6 (5, 7)	7 (5,7)
Neonatal seizures, n (%)	6 (13%)	12 (27.9%)

Table 2: Predominant pattern of lesions: comparison between early and late MRI in non-cooled and cooled infants

Early scan pattern of lesion	**Late scan pattern of lesion**					**Total**
Non-cooled infants	Normal	Watershed	Basal nuclei	Total	Multifocal	
Normal	7	0	0	0	1	8
Watershed	0	10	0	0	0	10
Basal nuclei	0	0	11	0	0	11
Total	0	1	0	2	0	3
Multifocal	0	0	0	0	14	14
Total	7	11	11	2	15	46
Cooled infants	Normal	Watershed	Basal nuclei	Total	Multifocal	
Normal	23	0	1	0	1	25
Watershed	0	4	0	0	0	4
Basal nuclei	0	0	9	0	0	9
Total	0	0	1	1	0	2
Multifocal	0	0	0	0	3	3
Total	23	4	11	1	4	43

Table 3: Extent of lesions in the basal nuclei: comparison of early and late MRI in non-cooled and cooled infants.

Early scan basal nuclei extent of lesion	**Late scan basal nuclei extent of lesion**					**Total**
Non-cooled infants	0	1	2	3	4	
0	23	0	0	0	0	23
1	2	3	0	0	0	5
2	0	0	6	0	0	6
3	0	0	0	1	0	1
4	1	1	1	0	8	11
Total	26	4	7	1	8	46
Cooled infants	0	1	2	3	4	
0	28	0	1	0	0	29
1	1	1	0	0	0	2
2	0	0	2	0	3	5
3	0	0	0	2	0	2
4	1	0	1	0	3	5
Total	30	1	4	2	6	43

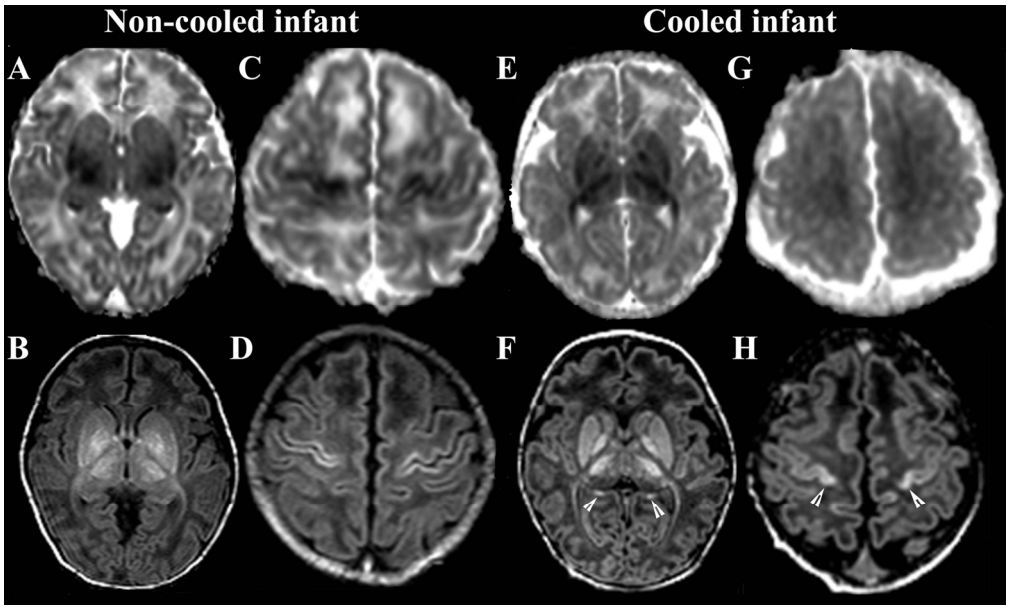
Table 4: Comparison of clinical and MRI factors between infants with and without change in lesions on late MRI. MRI features of hypoglycemia and moderate to severe extent of lesions in the basal nuclei and watershed region were identified on early MRI.

Variable	**No change in lesion on late MRI N=70**	**Change in lesion on late MRI N=19**
Gestational age weeks, Mean (SD)	39.5 (1.41)	39.4 (1.79)
Birth weight Kg, Mean (SD)	3.38 (0.54)	3.39 (0.59)
Male N (%)	44 (62.8%)	8/19 (42.1%)
Head circumference cm, Mean (SD)	34.4 (1.57)	34.8 (1.29)
Apgar 10 min, Median (IQR)	6 (4,7)	5 (2,8)
Cord pH, Mean (SD)	7.03 (0.16)	7.03 (0.15)
Encephalopathy score day1, Median (IQR)	7 (5,7)	7 (6,7)
Encephalopathy score day3, Median (IQR)	5 (4,6)	5 (4,6)
Cooling N (%)	33/70 (47.1%)	10/19 (52.6%)
Seizures N (%)	54/70 (77.1%)	17/19 (89.4%)
Clinical hypoglycemia <72hrs of age N (%)	12/67 (17.9%)*	10/19 (52.6%)*

MR features of hypoglycemia N (%)	12/70 (17.1%)*	9/19 (47.3%)*
Moderate-severe Baal nuclei lesion N (%)	20/70 (28.5%)*	10/19 (52.6%)*
Moderate-severe Watershed lesion N (%)	28/70 (40%)	9/10 (47%)
Age of early MRI hours, Mean (SD)	84.0 (16.3)	90.9 (28.0)
Age of late MRI hours, Mean (SD)	264.3 (42.1)	264.3 (41.3)

Supplementary table. Extent of lesion in the watershed region: Comparison of early and late MRI in non-cooled and cooled infants.

Early scan watershed extent of lesion	**Late scan watershed extent of lesion**						**Total**
Non-cooled infants	0	1	2	3	4	5	
0	13	0	1	0	1	0	15
1	0	3	0	2	0	0	5
2	0	0	2	0	0	0	2
3	0	0	0	12	0	0	12
4	0	0	0	0	1	0	1
5	0	0	0	0	0	11	11
Total	13	3	3	14	2	11	46
Cooled infants	0	1	2	3	4	5	
0	28	0	0	1	0	0	29
1	0	3	0	0	0	0	3
2	0	0	2	0	0	0	2
3	0	0	0	3	0	0	3
4	1	0	0	0	2	0	3
5	0	0	0	1	0	2	3



Example of new brain abnormalities on the late MRI in a cooled infant compared with a non-cooled infant.

Images A, B, C, D are from a non-cooled infant and images E, F, G, H are from a cooled infant. The top row (A, C, E, G) represents the first (ADC) and the bottom row (B, D, F, H) represents the second (T1) MRI. In non-cooled infant, restricted diffusion is initially seen in the ventrolateral thalamic nucleus, lentiform nucleus, optic radiation, hippocampus (A) and peri-rolandic cortex (C) on the early MRI. Subsequently, T1 shortening in the corresponding regions can be seen in the late MRI (B, D). In the cooled infant, there is restricted diffusion in the ventrolateral thalamic nuclei, lentiform nucleus and optic radiation (E), with corresponding T1 shortening on the second MRI (F). There is T1 shortening on the late MRI in hippocampus (F, white arrowhead) and peri-rolandic cortex (H, white arrow head) with no corresponding restricted diffusion in the ADC map of early MRI (E and G) (ADC value right, left in the peri-rolandic cortex: 950,1003).
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