# Neonatal Hypoxic-Ischaemic Encephalopathy: Motor Impairment beyond Cerebral Palsy

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Declarations of interest: none

## Abstract

Background: Research investigating neuromotor function in the absence of cerebral palsy (CP) for children who had neonatal HIE is limited.

Aims: To investigate school-age neurological and neuromotor function, and correlations with attention, neonatal Magnetic Resonance Imaging (MRI), and neuromotor assessments at toddler age.

Methods: Twenty-seven children with neonatal HIE without CP who underwent hypothermia treatment and a comparison group of 20 children were assessed at age 5-7 years for Minor Neurological Dysfunction (MND; simplified Touwen), motor skills (Movement Assessment Battery for Children-2; MABC-2), parental concern over motor function (MABC Checklist), general cognition (Wechsler Preschool and Primary Scale of Intelligence-IV, WPPSI), and attention (DuPaul ADHD Rating Scale). Neurological examination and motor development, using Bayley-3 scales, at age 24-months was extracted from the clinical database. Clinical neonatal MRI was assessed for hypoxic-ischaemic injury.

Results: In the HIE group, MND was more prevalent (p=0.026) and M-ABC performance (total score p=0.006; balance subtest p=0.008) was worse; parents were more concerned about children's motor function (p=0.011). HIE group inattention scores were higher (p=0.032), which correlated with lower MABC-2 scores (rs=-0.590, p=0.004). Neurological examination at 24-months correlated with MND (rs=0.437, p=0.033); Bayley-3 motor scores did not correlate with M-ABC-2 scores (rs=368, p=0.133). Neonatal MRI findings were not associated with school-age MND (rs=0.140, p=0.523) or MABC-2 (rs=0.300, p=0.165).

Conclusions: Children with neonatal HIE, without CP, treated with hypothermia may be more likely to develop MND and motor difficulties than typically developing peers. Inattention may contribute to motor performance. In the absence of CP, neonatal MRI and toddler age assessment of motor development have limited predictive value for school-age outcome.



Key words: Hypoxic-ischaemic encephalopathy; Minor Neurological Dysfunction; neuromotor function; attention; magnetic resonance imaging

# 1. Background

Neonatal hypoxic-ischemic encephalopathy (HIE) is a condition which occurs in approximately 1.5 per 1000 live births in high income countries and more frequently in middle- and low-income countries [1,2]. Clinicians had little to offer until therapeutic hypothermia (TH) emerged. Several large randomised controlled trials have shown that TH reduces both mortality and severe neurodisability (Cerebral Palsy, CP), and these effects appear to continue to school age[3]. Commonly, children are discharged from neurodevelopmental follow up at 2 years of age if there is no global developmental or severe neuromotor impairment[4].
However, there is evidence that there is still increased risk for minor neurological, cognitive, or behavioural dysfunctions long term[5]. These typically go unnoticed in early life and manifest at late pre-school or school age. Although they may be subtle, they are likely to impact real life functionality. Overall, long term outcome information about children with neonatal HIE who underwent TH and survived without developing CP is still relatively sparse. Most data come from the period prior to TH, and it is currently not clear whether findings from these studies hold for children treated with TH. In the TH era, a recent study by Jary et al, in school aged children showed that 38% of children who survived HIE following TH without CP had a Movement Assessment Battery for Children-2 (MABC-2) score of ≤15$^{th}$ centile[6]. We have previously shown for a large clinical cohort who underwent TH, that at age 2 years, 12% of children without CP had minor neurological signs (gross or fine motor coordination difficulties, muscle tone imbalance) and this was associated with poorer motor, cognitive, and behavioral function compared to those with normal neurological examination[7].

Minor neurological dysfunction (MND), typically diagnosed at school age, is a description of the neurological profile of a child without CP or major impairments, which describes difficulties with posture, muscle tone regulation, balance, fine manipulative ability, mildly abnormal reflexes, movements, coordination, or cranial nerve function[8]. It can be classified as simple MND or complex MND, depending on the number of dysfunctional domains. Simple MND has limited clinical significance and may represent a normal suboptimal variant of neuromotor development. Complex MND is considered a form of brain dysfunction associated with neuroanatomical deficits and functional impairments such as learning disorders and behavioural problems[9]. Applying the concept of MND to children with neonatal

HIE who have not developed CP, appears an attractive approach to stratify children's risk and target interventions.

Neonatal HIE is associated with brain injury to basal ganglia, thalamus, cortex, watershed zones, hippocampus, or brainstem, and the pattern of injury is dependent on the severity and duration of ischemia and hypoxia, and may correlate with specific neurological impairments[10]. Neonatal MRI is often used for prognostication of neurodevelopmental outcome following HIE, but the value in the context of an absence of CP is currently not well established[11], and findings are inconsistent. For example, in a study by Barnett et al, in children with neonatal HIE, not treated with TH, MND and lower motor scores (assessed with Touwen examination and MABC) were both associated with basal ganglia lesions (focal lesions affecting posterior lentiform nuclei and ventrolateral nuclei of the thalamus, without involvement of the posterior limb of the internal capsule [PLIC]) or focal signal abnormalities in white matter[12]. In contrast, in a study by Perez et al[13], also in children who were not treated with TH, neonatal MRI pattern did not correlate with motor performance on the Zurich Neuromotor Assessment in children without major disability.

In this study, we want to explore neurological examination and motor function, and possible correlations with general cognitive abilities and attention abilities, at school age in children with neonatal HIE who were treated with TH and survived without developing CP. We also aimed to assess whether neurological examination and motor assessments at toddler age correlated with school age outcomes. Finally, we aimed to explore possible correlations of neonatal clinical MRI and school age neurological examination and motor outcomes.

## 2. Materials and Methods

Ethics approval for the study was given by the NHS Health Research Authority National Research Ethics Service Ethics Committee North West – Lancaster REC reference 15/NW/0292. Written informed consent was obtained from the parents, and assent from the children.

## 2.1 PARTICIPANTS

Participants were recruited from a clinical cohort of 95 children who had been admitted for consideration of TH for neonatal HIE at University Hospital Southampton, Southampton UK, a tertiary centre, between 01/08/2009 and 31/05/2013. The criteria for TH were gestational age ≥36 weeks and at least one of the following: Apgar score of 5 or less 10 minutes after birth; continued need for resuscitation 10 minutes after birth; or acidosis (defined as pH <7 or base deficit >15 mmol/L, or both, in umbilical cord blood or any blood sample within 1 hour of birth), signs of moderate to severe encephalopathy (classified using the modified Sarnat and Sarnat staging[14]). After exclusion of infants for whom, after admission, it was decided TH was not indicated, those who had a syndromic or chromosomal disorder, or encephalopathy as a consequence other than perinatal asphyxia, a sample of 70 newborns remained. Fifteen infants died in the neonatal period, leaving a sample of 55 infants who survived. No information after hospital discharge was available for 12 infants, leaving 43 children. Children who had a diagnosis of CP (n=7), were not eligible for this study, 43 children who were eligible to take part. Five had moved out of the region, and of those who could be contacted, 31 families agreed to participate. Three children were excluded after recruitment since it emerged that they had a neurodevelopmental disorder not caused by perinatal asphyxia; one child was excluded since they had an incomplete assessment. The final sample was 27 children.

Supplementary Table 1 compares the subsample that formed the sample of this study and the whole cohort born and treated between 01/08/2009 and 31/05/2013. There were no significant differences in demographic or neonatal characteristics, i.e. this subsample can be regarded as representative of the whole cohort who underwent TH for neonatal HIE in that time period.

For the school age assessments, a comparison group group was recruited using a friends and family approach and from local schools. The recruitment strategy was to achieve a comparison group of typically developing children of comparable age, sex, and post code area. Children who were on the Special Educational Needs Register were excluded.

## 2.2 PROCEDURES AND ASSESSMENTS

Children were invited for a follow-up visit at school age (5-7 years). The assessments included a neurological examination and assessment of motor skills, general cognitive abilities, and attention, as well as parents' views on motor/physical functioning in daily life (Figure 1). For the children with HIE, information on routine clinical neurological and developmental assessments at age two years was retrieved from the clinical database, and clinical neonatal MRI was assessed for signs of hypoxic-ischaemic brain injury. The assessors were not blinded to whether or not the child had HIE.

### 2.2.1 Minor Neurological Dysfunction

A simplified version of the Touwen examination was used for the assessment of MND[15]. This included the evaluation of four domains: reflexes, nerve function of the face and eyes, posture and muscle tone, coordination and balance. The findings were classified on the basis of the number of dysfunctional domains, as normal neurological function, simple MND (one or two domains abnormal), or complex MND (more than two domains abnormal).

### 2.2.2 Motor function

The Movement Assessment Battery for Children, Second Edition (MABC-2) was used to assess motor function[16]. MABC-2 includes three scales: manual dexterity, aiming and catching, and balance. For each of the three scales a standard score is calculated, and a total score is calculated by adding the scores from each subscale. For each scale and the total test score, centiles can be derived, and scores between the 5th-15th centile are indicative of borderline motor problems, scores below the 5th centile are indicative of definite motor problems.

### 2.2.3 Parents' view of children's everyday physical functioning

The MABC Checklist and the Measurement Model and Pediatric Quality of Life Inventory (PedsQL) – Parent Proxy Report Edition were used to infer the real-life impact of motor dysfunction according to parents' perception[16,17]. For this study, the Physical Functioning scale which comprises 8 items, was used. These items include questions on difficulties with walking, running, and activity levels. Scores are transformed on a scale from 0 to 100 and a higher score indicates a better health related quality of life.

### 2.2.4 General cognitive abilities

General cognitive abilities were assessed with the Wechsler Preschool and Primary Scale of Intelligence 4th Edition (WPPSI-IV)[18]. The mean index and IQ scores are 100, with a standard deviation of 15. The Visual Spatial Index (VSI), Verbal Comprehension Index (VCI), and Full-Scale IQ (FSIQ) score were used in this study.

### 2.2.5 Attention

Children's attention was assessed with the DuPaul ADHD Rating Scale Home, i.e. parents, and School version, i.e. teachers. The DuPaul ADHD Rating Scale is an 18-question self-report assessment with 9 items that ask questions about behaviour related to inattention[19]. As recommended by the manual, we used the raw scores for statistical analysis to increase uniformity and precision.

### 2.2.6 Neurodevelopmental assessment at age 24 months

Children with neonatal HIE underwent routine clinical neurological and developmental assessments at age two years (mean 27.3, SD 3.4 months). Information on neurological examination and assessment with the Bayley Scales of Infant and Toddler Development-3 (Bayley-3) were retrieved from the clinical database. Children had been assessed by a Paediatric Neurologist or Neonatologist with experience in neurological and developmental assessments, together with a Physiotherapist. Examiners were not blinded to neonatal course. Neurological examination included assessment of cranial nerve function, movements, posture, reflexes, and muscle tone. Neurological status was categorised as normal (completely normal neurological status), minor neurological signs (gross or fine motor coordination difficulties, muscle tone imbalance, without definite signs of cerebral palsy [CP]), or abnormal (signs of CP present as defined by the Surveillance of Cerebral Palsy in Europe Working Group, SCPE, 2000)[20]. The Bayley-3 is a standardised assessment which consists of a series of developmental play tasks[21]. Composite scores are derived for cognitive, language, and motor development, and scaled to a metric, with a mean of 100, standard deviation of 15, and range of 40 to 160. For this study we only used the motor scales.

### 2.2.7 Neonatal MRI

Information from clinical routine MRI studies was used, which was available for 23 out of the 27 children with HIE, performed at a median of 7 days (min 4 day, max 15 days) after birth. Infants were scanned on a 1.5 T scanner (Siemens Symphony, Siemens AG, Erlangen, Germany); T1-weighted, T2-weighted, and diffusion-weighted MRI images were used. MRI signs of neonatal hypoxic-ischaemic brain injury include signal abnormalities and/or diffusion abnormalities in typically affected brain structures (such as basal ganglia, thalami, perirolandic cortices and watershed areas) were assessed using a scoring system based on Barkovich, 1998[22]. In addition, myelination of the posterior limb of the internal capsules (PLIC) was noted. Furthermore, if the thalami, caudate nuclei, putamina, globus pallidi, PLICs, and hippocampi showed signal abnormalities (including diffusion abnormalities) on any of the MRI sequences, this was noted separately (binary score: affected vs. not affected). This is an in house developed scoring system and it is an attempt to detect more subtle grey matters injuries and/or in locations not specifically specified in the Barkovich scoring system (in which only thalamus and lentiform nucleus are specified as individual structures).

## 2.3 STATISTICAL ANALYSIS

Data were assessed for normality by the Kolmogorov–Smirnov test. The Student's t-test and Mann–Whitney U test were used, as appropriate, to identify differences between the groups in continuous data. The Chi-square test or Fisher's exact test were used, as appropriate, to identify differences between the groups and investigate correlations between neurological classification groups and outcomes for categorical data. Spearman's correlation tests were used for correlations between neurological classification groups and continuous and discrete variable outcomes. The statistical significance level was set at a two-sided p value of less than 0.05. Uncorrected p values were used for this exploratory study, accepting the increased risk of type I errors in exchange for a higher sensitivity in detecting potential group differences and correlations.

# 3. Results

## 3.1 Demographics

Twenty-seven children with HIE without cerebral palsy (CP) were included in the study, along with 20 typically developing children ("comparison group"). The clinical characteristics for both groups are shown in Table 1. Mean age at assessment was significantly different ($p<0.001$) between the groups, but no significant differences in distribution of sex between groups were seen ($p=0.080$). Hearing and visual impairment were more common in children with HIE; this did not affect children's ability to participate in the tests.

## 3.2 MND prevalence and neuromotor function

Of the 27 children with HIE, 22.2% of children presented with MND (4 with simple MND, 2 with complex MND). All 20 children in the comparison group had normal neurological examination.

Children with HIE scored significantly lower on total MABC-2 scores than controls (effect size [r]=-0.398, p=0.006) and significantly lower scores were observed for the manual dexterity (r=-0.428, p=0.003) and balance (r=-0.449, p=0.002), but not for the aiming and catching (r=-0.087, p=0.552) subtests (Table 2); this is shown in Figure 2. Children with HIE had a higher prevalence of clinically significant motor impairment: 6/27 (22%) children with HIE scored below the 15th centile in the total MABC-2 total score, compared with 1/20 (5%) in the control group.

## 3.3 General cognitive abilities

No significant group differences were found in FSIQ scores (r=-0.181, p=0.215), VSI (r=-0.223, p=0.172), or the VCI (r=-0.129, p=0.376) scores of the WPPSI-IV (Table 2).

## 3.4 Parent and teacher rating for attention and motor function

Children with HIE scored significantly higher on the DuPaul ADHD Rating Scale (teacher version) Inattention subscale (r=-0.353, p=0.032) than children in the comparison group, suggesting greater attentional impairment (Table 2). No such differences were found on the parent version of the ADHD Rating Scale. The parents of children with HIE were significantly

more worried about their children's motor function than parents of children in the comparison group, as measured by the MABC Checklist (r=-0.399, p=0.011). However, no difference was found in PedsQL Physical Functioning scores between the groups (r=0.040, p=0.267).

### 3.5 Correlations between MND, neuromotor function, and cognitive function

In the HIE group, there was a weak negative correlation between neurological status (MND) and total MABC-2 scores, suggesting that MND was associated with poorer motor functioning (rs=-0.364, p=0.062). This was, however, limited to the balance subtest (rs=-0.396, p=0.041); no correlation was found with the other subtests. There was also a weak-moderate negative correlation between MND and general cognitive abilities as measured by FSIQ, (rs=-0.482, p=0.011) as well as a weak-moderate positive correlation between MABC-2 total and FSIQ scores (rs=0.459, p=0.016), indicating a correlation between cognitive and motor performance. This was dominated by manual dexterity (rs=0.446, p=0.020); aiming and catching (rs=0.212, p=0.277) and balance subtests (rs=0.290, p=0.142) were not associated with FSIQ. VSI scores did not correlate with overall MABC-2 scores (rs=0.310, p=0.196), nor with any of the subtests, suggesting that motor functioning in the HIE group was not significantly affected by their visual spatial processing skills. A weak correlation between lower VSI scores and MND was found, but this was not significant (rs=-0.415, p=0.077). VCI scores correlated significantly with MABC-2 total scores (rs=0.576, p=0.002) and there was a strong, significant correlation between VCI and teacher-rated Inattention scores (rs=-0.748, p<0.001) in the HIE group.

No correlations between neuromotor function, general cognitive abilities, or attention were seen in the comparison group.

### 3.6 Attention and neuromotor function

Teacher-rated ADHD Rating Scale Inattention scores showed a moderate negative correlation with total MABC-2 score (rs=-0.590, p=0.004), as well as the balance subtest of the MABC-2 (rs=-0.567, p=0.006); this is shown in Figure 3. Furthermore, teacher ADHD Rating Scale Inattention scores were strongly correlated with FSIQ (rs=-0.712, p=0.0002). Parent-rated

Inattention scores also correlated with higher reported parental concerns about motor function, as measured by the MABC-2 Checklist, but this was not statistically significant (rs=0.432, p=0.057). No correlations were found between parent-rated Inattention scores and performance on the M-ABC.

### 3.7 Correlations between 24-month assessments and school age outcomes

Minor neurological signs at 24-months were weakly-moderately correlated with the presence of MND at school age (rs=0.437, p=0.033).

No correlation was found between Bayley-3 motor composite scores and MND or total scores on the MABC-2. However, lower scores in the gross motor and composite motor subtests of the Bayley-3 Scales correlated moderately-strongly with lower scores on the aiming and catching subtest of the MABC-2 (rs=0.695, p=0.001; rs=0.663, p=0.003, respectively). Lower scores in the gross motor and fine motor sections of the Bayley-3 correlated with increased parental concern over children's motor performance at school age, as measured by the MABC-2 Checklist ($p<0.05$).

### 3.8 Neonatal MRI and school age neurological exam and motor outcomes

When applying the scoring system based on Barkovich et al.[22], neither Basal Ganglia score, nor Watershed scores, nor the combined Basal Ganglia-Watershed score was associated with any school-age outcomes. In 10 children, the Barkovich Basal Ganglia scored was 0, and, correspondingly, none of the grey matter structures nor the PLIC showed any signal abnormalities as assessed by the binary score. In the remaining 13 children, all had signal abnormalities in the thalami, 12 in the putamina, 10 in the globus pallidi or PLIC, 7 in the hippocampi, and 3 in the caudate nuclei. The grey matter structures followed the expected pattern of additional injury-involvement from thalami to the lentifrom nuclei, to the hippocampi [22] as well as caudate nuclei. However, only the 3 children with additional signal abnormalities in the caudate nuclei had significantly lower FSIQ scores (r=-0.464, p=0.016) and VSI scores (r=-0.559, p=0.017). Statistically non-significant correlations were seen in total MABC-2 scores (r=-0.387, p=0.066), balance subtest scores (r=-0.401, p=0.052) and VCI scores (r=-0.393, p=0.054), with children with additional caudate abnormalities consistently

scoring lower on all of these measures. In all children, the PLICs were normally myelinated and signal abnormalities in the PLIC, assessed by the binary score, were not associated with school age outcomes.

## 4. Discussion

In this small sample, MND, neuromotor difficulties, in particular manual dexterity and balance skills, and difficulties with inattention in the school setting, were more frequent in children with HIE than in the comparison group. MND was associated with poorer motor skills, and the prevalence of clinically significant motor impairment was higher in the HIE group, which was consistent with parental concerns about motor skills in daily life. Interestingly, although general cognitive abilities, measured by IQ scores, were similar in the two groups, in the HIE group, FSIQ scores were associated with MND and both, FSIQ and VCI scores with motor performance on the M-ABC, in particular in manual dexterity tasks. Of note, motor performance was not affected by visual spatial processing skills, as measured by VSI scores.

Prevalence of motor impairment at school age in the absence of CP in infants with HIE who underwent TH varies in the literature. In the TH arm of the NICHD hypothermia trial, Shankaran et al reported that 5% of children who had TH and had not developed CP had gross and fine motor difficulties[23]. Jary et al., reported that 38% of their cohort of 29 children cooled for moderate/severe neonatal encephalopathy without CP had MABC-2 scores ≤15th centile[6]. In our sample 22% of cooled children without CP scored below the 15$^{th}$ centile on the M-ABC. Studies such as ours and that of Jary et al. can find variable rates of motor impairment, most likely due to the small sample sizes and the potential variation in the severity of neonatal encephalopathy.

Therapeutic hypothermia trials report variable IQ scores above or below the population mean at 6–7 years in cooled children with and without CP[24]. Lee-Kelland reported that school-age children cooled for HIE even in the absence of CP have significantly lower cognitive scores (IQ 14 points lower than their peers)[24]. In our sample, on a group level, children with HIE scored 5 IQ points lower on average than those in the comparison group, and lower FSIQ correlated with poorer neuromotor function, in particular with manual

dexterity. Similarly, Jary et al found significant correlations between MABC-2 manual dexterity scores and WISC-IV perceptual reasoning and working memory scores[6]. Their explanation for the difficulties of children with HIE in timed tests was related to impaired visuo-spatial and perceptual processing of instructions. In contrast, we did not find a relation between motor function and visual-spatial integration scores.

Motor and cognitive impairment in the HIE group was associated with attention problems in the school setting; this correlation was not present in the comparison group. Moreover, the correlation between higher inattention scores and lower verbal comprehension scores could indicate that the correlations between poorer verbal comprehension and neuromotor deficits is compounded by attention problems. This is made even more likely by the fact that no between-group differences were found in VCI scores. Pre-clinical studies have demonstrated the emergence of attentional deficits after neonatal hypoxic and/or ischaemic events in animals. Tissue atrophy and dopaminergic disturbance in the pre-frontal cortex seems to be the reason for this correlation[25]. The pre-frontal cortex is responsible for the ability to plan and execute correct motor performance, an ability essential to the accomplishment of MABC tasks. In our sample, children with HIE with higher scores of inattention were more likely to have poorer motor performance, but also lower IQ scores, two functions in which prefrontal cortex is involved. In this way, our results are consistent with the preclinical data.

Parents of children with MND perceived their children to have motor problems in daily life. In addition, teachers' ratings on the ADHD scale suggest that children with HIE may display inattention in a school setting; inattention was associated with general cognitive and motor abilities, as well as with MND. This indicates that children with MND may be particularly affected at school. Interestingly, parents of children with HIE did not rate their children higher on the inattention scale than parents of children in the comparison group, suggesting that some of the difficulties reported may only be observed in specific daily activities settings. Detecting MND in children with a history of neonatal HIE but without CP may be a way to identify children with a high risk of future learning difficulties.

Minor neurological signs at 24 months were associated with MND at school age. However, gross motor developmental assessment at age 24 months was poorly predictive of motor skills at school age. The lack of a significant correlation between Bayley-3 performance and MND or performance on the MABC-2 have already been reported. Previous reports by Burakevych [26] and Jary [6] confirmed the poor predictive ability of the Bayley-3 for later motor skills. These findings reinforce the importance of serial assessment in children treated with cooling beyond the two years to at least school age, even in those with scores in the typical range at age two years. Additionally, the importance of identifying early minor neurological signs that may be predictive of minor neurological dysfunction in later life is highlighted by our findings.

Neonatal brain MRI provides prognostic information on death or severe disability beyond early infancy in HIE and therapeutic hypothermia does not appear to change its prognostic value[11]. A recent qualitative MRI injury scoring system weighted deep nuclear grey matter injury (thalamus and striatum) as significant predictor of neurodevelopmental outcome (defined as cerebral palsy, development delay and death) at 18-24 months in infants with neonatal HIE[27]. In our study, we found that children who had signal abnormalities within the caudate nuclei, in addition to signal abnormalities in the other deep grey matter structures, had significantly lower FSIQ and visual-spatial processing scores. This, however, must be interpreted with caution, as the number of children with caudate abnormalities was very small (n=3). Poorer cognitive abilities in these children are probably the result of a more extensive brain injury rather than the isolated effects of abnormalities in the caudate nuclei. We used a well established scoring system for MRI in the context of neonatal HIE [22], which considers basal ganglia, thalami, and cortex. However, it does not allow to explicitly focus on the caudate nuclei or the hippocampus, these structures are presumed to be included when assigning the scores that reflect more extensive injury to basal ganglia, watershed areas, or the cortex beyond the peri-rolandic region respectively. This may indicate a limitation of this commonly used scoring system when examining associations between brain injury and motor outcomes beyond cerebral palsy. However, injury to the caudate nucleus is of interest in the investigation of correlations between neonatal HIE and neurodevelopmental outcomes. The caudate nucleus has been demonstrated to be involved in cognition, visuo-spatial processing and motor function[28,29]. A recent study concluded that volume reductions in the caudate

nuclei in children with HIE is associated with impaired motor coordination[30]. However, in that study, the sample was composed of different etiologies of neonatal encephalopathy, MRI was not done according to a standard protocol in the neonatal period, and children were assessed at later age. Future studies should aim to examine in more detail neural substrates for the neuromotor deficits observed in the neonatal HIE population, for example, explore anatomical and functional brain network integrity of the motor and cognitive systems.

Limitations of our work includes sample size and that we examined a clinical convenience sample. It is important to remember that our study was an exploratory one; a more definitive study in the future should ensure that multiple regression analysis is possible, which will require a larger sample. We chose to report uncorrected p values, despite having multiple comparisons and correlations. As an exploratory study, our aim was to detect potential differences between children born with HIE and their typically developing counterparts, as well as correlations between different test measurements. For this, type II errors must be minimised – something that would not be possible if Bonferroni correction were applied. When we repeated our analyses with Bonferroni correction applied, children born with HIE still scored significantly lower on the manual dexterity (p=0.03) and balance subtests of the MABC-2 (p=0.02). Greater levels of inattention in children born with HIE were still significantly correlated with lower FSIQ (p=0.008) and VCI scores (p=0.009) after correction for multiple analyses. A significant positive correlation between Bayley-3 gross motor scores at 24 months and performance on the aiming and catching subtest of the MABC-2 was also detected (p=0.04). Thus, some of our results still remain statistically significant after the application of Bonferroni correction; however, as the purpose of this study requires the prioritisation of minimising type II errors over limiting type I errors, we focused on reporting uncorrected results. We recommend for these to be confirmed in larger scale studies where Bonferroni correction can be applied more readily.

This study also highlights the limitations of visual assessment of standard anatomical MRI sequences used in clinical routine as prognostic tools following HIE in the absence of CP. This study was performed at 1.5T MRI which is in line with concurrent studies at that time. Imaging at higher field strengths, i.e. at 3T in the first week, which is now common, might increase sensitivity to detect hypoxic-ischaemic lesions.

## 5. Conclusions

In the absence of CP, about 1/5 of children with HIE treated with TH had significant motor impairment at school age. This was associated with the presence of MND and parent reports indicate that this has implications for daily functioning. Motor function impairment was associated with general cognitive difficulties and attention problems at school. It is important to keep in mind that early neurodevelopmental assessments may be poor predictors of later motor function. Injury to the caudate nuclei may play a role in neuromotor and general cognitive deficits following neonatal HIE. Our findings emphasize the importance of long-term follow-up, which includes standardized assessment of neurology and motor function.

## 6. Acknowledgements

We thank all the children and parents/caregivers who participated in the study. We also thank Dr M. Schreglmann for contributions to study set-up and data collection.

Funding: This work was supported by the Sir Halley Stewart Trust, UK. The Sir Halley Stewart Trust had no role in the study design, the collection, analysis and interpretation of data, the writing of the report, or the decision to submit the article for publication

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# Tables

Supplementary Table 1 - Comparison of the subsample that formed the sample of this study and the whole cohort born and treated between 01/08/2009 and 31/05/2013 (see supplementary document / online only)

Table 1 – Clinical characteristics of children with a history of neonatal HIE and control children

| | HIE group, n=27 | Control group, n=20 |
|---|---|---|
| Sex, n male/female (%) | 12/15 (44.44%) | 4/16 (20.00%) |
| Age at assessment – mean (SD) | 5 years 0 months (5.2 months) | 5 years 7 months (5.8 months) |
| Gestational age<br>- mean (SD) -weeks<br>- min-max | <br>39.91 (1.43)<br>37.00-41.86 | <br>40.24 (1.12)<br>38.00-42.00 |
| Birth weight<br>- mean (SD) -g<br>- min-max. | <br>3432 (664)<br>2200-4980 | <br>3528 (595)<br>2400-4590 |
| Visual function - no. (%)<br>-Normal:<br>-Corrected with glasses:<br>-Not fully corrected with glasses: | <br>25 (92.60%)<br>1 (3.70%)<br>1 (3.70%) | <br>13 (86.67%)<br>2 (13.33%)<br>0 |
| Hearing – no. (%)<br>-Normal:<br>-Corrected with hearing aids:<br>-Not fully corrected with hearing aids: | <br>24 (87.11%)<br>1 (3.70%)<br>2 (7.40%) | <br>14 (100%)<br>0<br>0 |
| Head circumference<br>- mean (SD) – cm<br>- min-max | <br>49.73 (3.15)<br>37.00-53.00 | <br>51.29 (2.81)<br>46.00-58.00 |

Missing data: 2 HIE children missing head circumference. Control group information was missing for: visual function for 5 children and hearing function for 6 children; 6 missing head circumference; 9 exact gestational age (but all were born full term), and 8 exact birth weight (but all were born with appropriate for gestational age birth weight).

Table 2 – Neuromotor, cognitive, and attention measures in the HIE and comparison groups

| | HIE group, n=27 | Control group, n=20 | p-values* | Effect size (r) |
|---|---|---|---|---|
| Motor function (MABC-2 total score) | 8.96 (3.13) | 11.45 (2.69) | 0.006 | 0.398 |
| Manual dexterity standard score (MABC-2 subtest) | 8.44 (3.43) | 11.25 (2.79) | 0.003 | 0.428 |
| Aiming and catching standard score (MABC-2 subtest) | 9.07 (3.64) | 9.70 (3.37) | 0.552 | 0.087 |
| Balance standard score (MABC-2 subtest) | 9.85 (2.51) | 12.55 (2.61) | 0.002 | 0.449 |
| Parental concern over motor function (MABC-2 Checklist; total score) | 13.71 (13.80) | 3.88 (2.58) | 0.012 | 0.399 |
| Full scale IQ (FSIQ score) | 94.07 (12.52) | 98.65 (11.59) | 0.215 | 0.181 |
| Visuo-spatial processing (VSI score) | 89.89 (17.49) | 97.11 (15.45) | 0.169 | 0.223 |
| Inattention (ADHD Rating Scale Teacher-Rated Inattention Score) | 6.73 (7.11) | 3.05 (4.90) | 0.022 | 0.353 |
| Inattention (ADHD Rating Scale Parent-Rated Inattention Score) | 5.18 (4.99) | 3.27 (2.68) | 0.473 | 0.118 |
| Physical functioning (PedsQL Physical Health Score) | 83.62 (21.00) | 92.29 (7.55) | 0.253 | 0.181 |
| 2-year motor function (BSID-3 composite score) | 108.78 (16.41) | - | | |
| 2-year fine motor function (BSID fine motor scaled score) | 11.11 (3.05) | - | | |
| 2-year gross motor function (BSID gross motor scaled score) | 11.78 (3.57) | - | | |

For each measure, mean scores and standard deviation are reported

*p-values referring to Mann-Whitney U test results

Figure 1 - Timeline of assessments in the HIE and comparison groups

Touwen examination (Touwen) was used for the assessment of Minor Neurologic Dysfunction (MND). Movement Assessment Battery for Children, Second Edition (MABC-2) was used to assess motor function. MABC Checklist (MABC Checklist) and the Measurement Model and Pediatric Quality of Life Inventory (PedsQL) – Parent Proxy Report Edition were applied to infer the real-life impact of motor dysfunction. Wechsler Preschool and Primary Scale of Intelligence 4thEdition (WPPSI-IV) was used to assess general cognitive abilities and children's attention was evaluated with the DuPaul ADHD Rating Scale Home and School version (ADHD). In HIE group, at 2-year-old, routine neurological examination (Neuro Exam) and assessment with the Bayley Scales of Infant and Toddler Development-3 (Bayley-3) were performed.

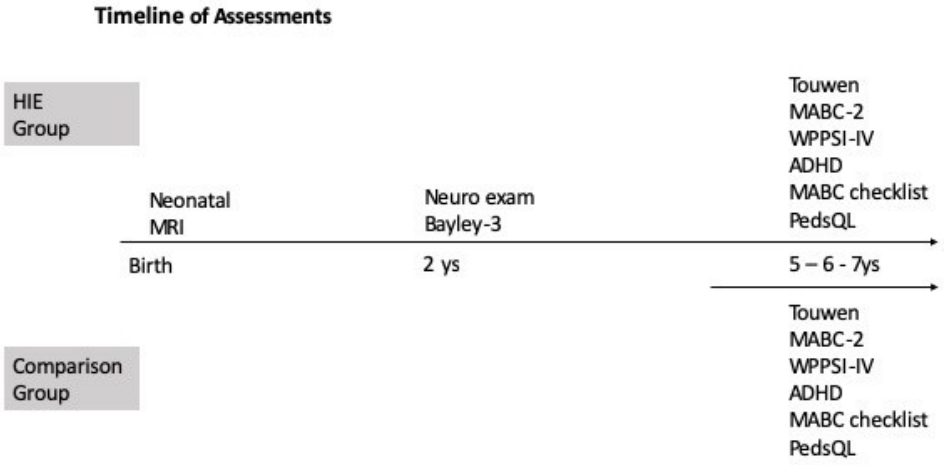
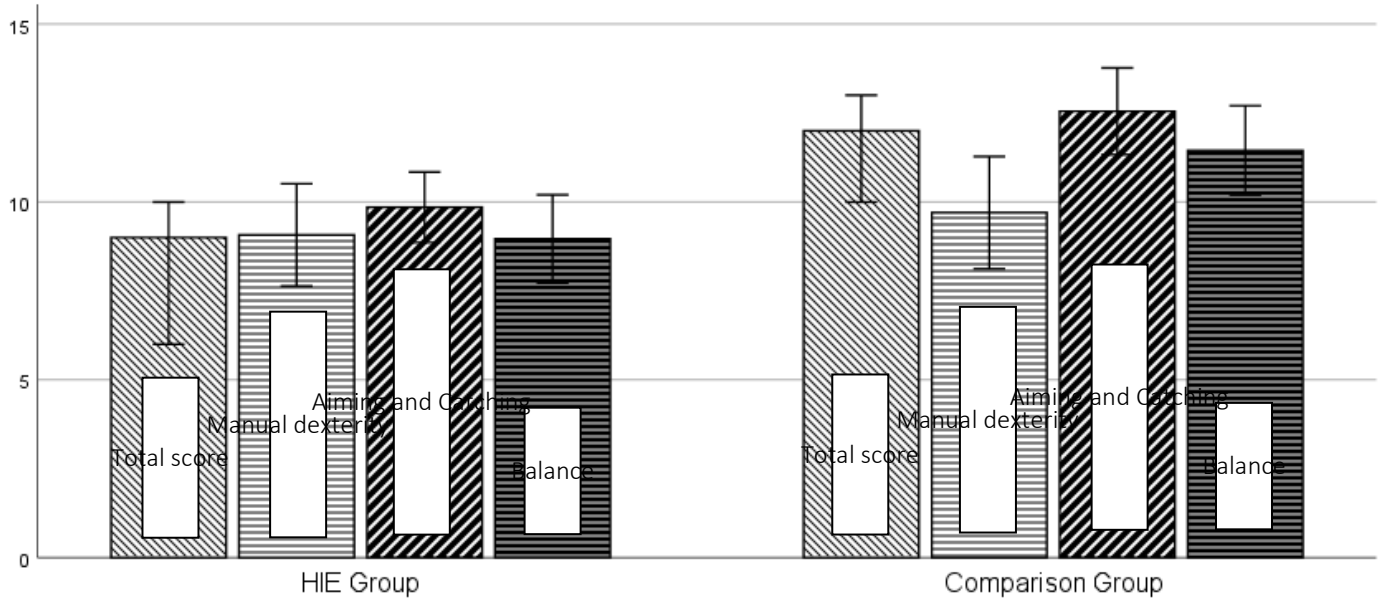


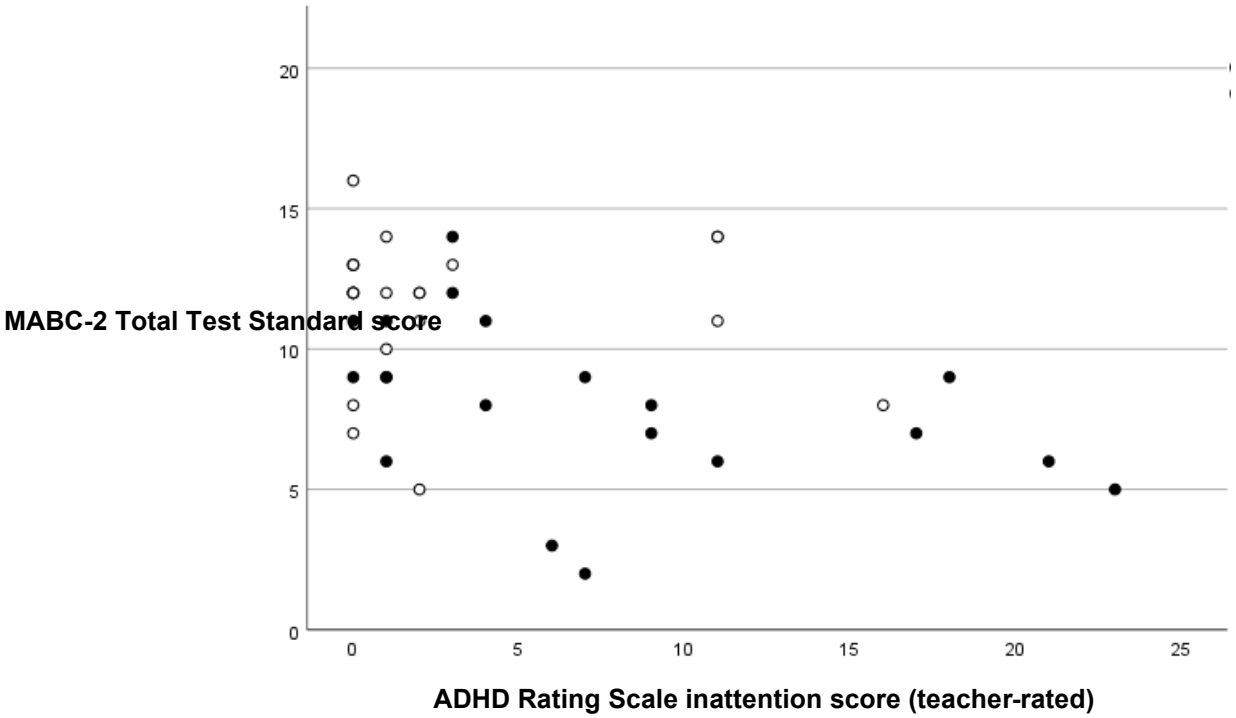
**Figure 2 –** Median values for MABC-2 total score and each MABC-2 subtest for children with HIE and children in the comparison group




Error bars represent 95% confidence intervals

The bars for each group represent, from right to left: 1 - MABC-2 Total Test Standard score; 2 – MABC-2 Manual Dexterity Subtest Standard score; 3 - MABC-2 Aiming and Catching Subtest Standard score; 4 - MABC-2 Balance Subtest Standard score

Figure 3 – Correlation between MABC-2 total score and teacher rated Inattention scores for HIE and comparison groups




Black dots represent children born with HIE

White dots represent children from the comparison group