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Ability of Early Neurological Assessment and Continuous EEG to Predict Long Term Neurodevelopmental Outcome at 5 Years in Infants Following Hypoxic-Ischaemic Encephalopathy

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Signed: _____ Date: _____

Dedication

This thesis is dedicated to the memory of my dear father, Dr. Joseph Rex O'Connor, who instilled in me a belief that anything is possible with loving support, determination and hard work.

It is also dedicated to the memory of my wonderful esteemed former colleague and great friend, Ann Moloney, former Principal Psychologist, Enable Ireland and Lecturer, Dept. of Applied Psychology, UCC. She lived and breathed psychology.

I know that both of you would appreciate the 'diagnostic' element of the thesis.

Dad, because as a pathologist you always wondered how best to diagnose and Ann, because you always wondered how a person could best adapt to a diagnosis.

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Glossary of Terms

ACOG	American College of Obstetrics and Gynecology
ADD	Attention deficit disorder
ADHD	Attention deficit hyperactivity disorder
aEEG	Amplitude integrated EEG
ARICD	Association for Research in Child Development
ASD	Autism spectrum disorder
ATNAT	Amiel-Tison Neurological Assessment at Term
ATP	Adenosine triphosphate
AUROC	Area under the receiver-operated characteristic curve
BD	Base deficit
BCVA	Best corrected visual acuity
BGT	Basal ganglia thalamic
BS	Burst suppression
CBCL	Child Behavior checklist
CMS	Children's memory scale
CNS	Central nervous system
CP	Cerebral palsy
CS	Caesarean section
CSF	Cerebro-spinal fluid
D	Diopter
DCD	Developmental co-ordination disorder
DQ	Developmental quotient
DSM	Diagnostic and Statistical Manual of mental disorders
EEG	Electroencephalogram
EF	Executive function
FSIQ	Full scale intelligence quotient

GDQ	Griffiths global developmental quotient
GLC	General language composite
GMDS-R	Griffiths Mental Development Scales - Revised
GMFCS	Gross motor function classification system
HI	Hypoxic-ischaemic
H/I	Hypoxia-ischaemia
HIE	Hypoxic ischaemic encephalopathy
IBI	Interburst interval
ICF	International Classification of Diseases
ID	Intellectual disability
IQ	Intelligence quotient
IQR	Inter-quartile range
K	Radius of corneal curvature
Lang D/O:	Language disorder
Md	Median
MD EI	Multi-disciplinary early intervention
MRI	Magnetic resonance imaging
NE	Neonatal encephalopathy
NEPSY-II	Neuropsychological Assessment Tool – Second Edition
NICHD NRN	National Institute of Child Health and Human Development – Neonatal Research Network
NICU	Neonatal intensive care unit
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NP	Neuropsychological
NPV	Negative predictive value
OFC	Occipito-frontal circumference
PA	Perinatal asphyxia

PIQ	Performance intelligence quotient
PLIC	Posterior limb of the interior capsule
PPV	Positive predictive value
PSQ	Processing speed quotient
RCT	Randomized controlled trial
SE	Spherical equivalent
SER	Spherical equivalent refraction
SES	Socioeconomic status
SGA	Small for gestational age
SLI	Specific language impairment
SS	Standard Score
SWC	Sleep-wake cycle
TH	Therapeutic hypothermia
VIQ	Verbal intelligence quotient
WHO	World Health Organisation
WMI	White matter Injury
WPPSI-III^{UK}	Wechsler Preschool and Primary Scale of Intelligence – 3rd UK edition
WS	Watershed brain region

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Abstract

Introduction

Hypoxic-ischaemic encephalopathy (HIE) is experienced by 1-6 neonates per thousand in the developed world. Untreated, it results in death in 15-20% of cases, and significant disability in a further 25%. These rates have been reduced since the introduction of therapeutic hypothermia (TH) treatment over the past decade. HIE symptoms evolve during the first days of life and close monitoring is critical for treatment decisions and for the prediction of long-term outcome in the infant. Neurological assessment, neuroimaging and continuous electroencephalography (EEG) are used to track symptom evolution. Severity of encephalopathy grade is usually assigned using the Sarnat and Sarnat system.

Protocols to measure childhood outcomes following neonatal HIE must consider when best and how best to assess neurodevelopment. By school-age, neuropsychological and educational difficulties emerge in a significant minority of children post-HIE with no motor disability. Research studies have typically reported outcome by HIE grade. Severe HIE results in significant mortality and morbidity, with variable outcome for moderate HIE suggestive of a heterogenous diagnostic category. Little is known about outcomes following mild HIE, but the consensus until recently has been of a benign condition. Thus, only infants with moderate to severe neonatal HIE were included in the TH RCTs that took place during the early 2000s.

To progress knowledge of the neonatal and long-term course, all grades of HIE in children require repeated monitoring into school age to capture the non-linear pattern of development. TH treatment decisions are made by 6 hours of age in the infant, requiring a continued focus on how HIE manifests in the first hours and days of life, and the value of tools such as EEG for prognostication.

The aims of this research study were to assess the neurodevelopmental outcome, at age five years, of a prospective cohort of infants following all grades of neonatal HIE. It further sought to:- (i) Determine the predictive value of neonatal neurological assessments and outcome at 24 months; (ii) Investigate stability of development at 6, 12 and 24 months of age and 5-year outcome; (iii) Compare the age 5 outcomes of the HIE group with a comparison group across Sarnat and EEG grades and analyse their predictive value; (iv) Examine neuropsychological

profiles of children with and without intact outcome; (v) Record comprehensive vision outcomes.

Methods

Participants were recruited at birth from two prospective cohorts and followed up at age five years. The HIE cohort (n=65) was recruited in the pre-TH era. All newborns with HIE and born between May 2003 and December 2005 from the three Cork regional maternity centres were invited. The comparison cohort of healthy newborns (n=80) were recruited between October 2005 and August 2007, for a neonatal EEG sleep study. The HIE cohort underwent neurological assessment using the Amiel-Tison Neurological Assessment at Term (ATNAT) on days 1, 2 and 3 of life, and a NICU discharge exam. Sarnat grade was assigned at 24 hours, and continuous video-EEG monitoring commenced before the sixth hour of life and continued for up to 72 hours. EEG recordings were also available for the comparison cohort. EEGs were assigned background pattern severity scores at 6, 12, and 24 hours of age, and a seizure burden score. The HIE cohort were assessed at 6, 12 and 24 months of age using the Griffiths Mental Development Scales (0-2) – revised (GMDS-R).

In the current study, at age five years, the parents/carers of children from the HIE cohort and from the comparison cohort who met the target age criteria (n=42 of the 80) were invited to participate for follow-up. Intellectual abilities were assessed using the Wechsler Preschool and Primary Scale of Intelligence 3rd UK version (WPPSI-III^{UK}). Neuropsychological processes were assessed using the NEPSY-II, and a verbal working memory task from the Children's Memory Scale (CMS). Neurological examination assessed tone, balance and reflexes. Detailed demographic, and clinical information was obtained via parent/carer interview. HIE cohort children also attended ophthalmic and orthoptic examinations. An 'overall outcome' category (normal/abnormal) was assigned to each child. Abnormal outcome was defined as death, cerebral palsy (CP), IQ >1SD below the norm, sensory impairment, clinical diagnosis of a neurodevelopmental disorder (NDD) and/or in receipt of multidisciplinary early intervention. Correlations and predictive values between the ATNAT scores and 24 months outcome were calculated. Stability of GMDS-R quotients at 6, 12 and 24 months was examined, as was their associations with five-year outcome. Prediction of neonatal Sarnat, and EEG grades for 5-year IQ and overall outcome were calculated using AUROCs. The neuropsychological profiles of children from the HIE cohort with an otherwise intact

outcome at 5 years was compared with the comparison cohort, and differences by Sarnat and EEG grade was examined. Detailed vision data was compared between children with intact and non-intact outcome.

Results

At five years, outcome was available for 81.5% (53/65) of children from the total HIE cohort and 71.4% (30/42) of children from the comparison cohort who met the age criteria. In the HIE cohort, 47.2% (25/53) had an abnormal outcome: 6 had died, 8 had CP, 1 had hearing impairment, 4 had IQs > 1 SD below the mean, 4 had NDD, and 2 were attending early intervention. This contrasted with 3.3% (1/30) of the comparison cohort. Processing speed and verbal short-term memory was significantly below the norm for the HIE cohort.

ATNAT scores in the first three days of life were associated with 24 month outcome, with best predictions for day 3 ATNAT (AUROC(95% CI)=0.75(0.58-0.93), *p*=.012) and discharge examination (AUROC(95% CI)=0.79(0.64-0.96), *p*=.005). ATNAT correlated with age 5 Full-Scale IQ (FSIQ) score (r=-0.47, p<.01) as did 9 of the test items which assessed neonatal passive muscle tone, neurosensation and palmar grasp.

GMDS-R developmental quotients at 6, 12 and 24 months of age correlated with 5-year FSIQ, Verbal and Performance IQ, with correlations improving over time (best correlation: GMDS-R and FSIQ (ρ=0.64, *p*<.001)). Within-child GMDS-R scores were inconsistent across 24 months. All children with normal GMDS-R at 24 months (n=30) had a normal 5-year FSIQ. All children with abnormal GMDS-R at 6 months (n=6) had an overall abnormal 5-year outcome.

Rates of abnormal outcome differed by Sarnat HIE grade, with 27% for mild, 47% for moderate and 83% for severe. No differences between the IQ scores of those following mild or moderate Sarnat HIE were found, and both grades, though normal, were significantly below comparison group IQs. Intact survival at 5 years varied across neonatal EEG grade at 6 hours of life: 75% for EEG mild grade 1, 46% for moderate grade 2, 43% for major Grade 3 and 0% for inactive grade 4. An EEG grade of >=2 at 24 hours of age had superior positive predictive value (74%; AUROC(95%CI)=0.70(0.55-0.85) for abnormal outcome at age five than EEG of >=2 at 6 hours (68%; AUROC(95%CI)=0.71(0.56-0.87).

NEPSY-II scores revealed that children post-HIE had finger dexterity, design fluency and memory for names skills in the borderline range. Of 25 tasks, 52% were significantly below the comparison group, with a higher burden of low-scoring tests per child. Furthermore, 28% of tasks remained lower for children post-HIE with otherwise intact outcome, principally in language, sensorimotor and specific memory areas. Finger dexterity remained in the borderline range. No differences in the scores were found between children following mild and moderate Sarnat groups, or EEG grades 1 and 2.

Ocular biometry at age 5 was not affected by the presence of HIE. Incidence of strabismus however was higher than expected and occurred despite normal motor outcomes.

Conclusion

This thesis has confirmed the importance of detailed follow-up at age 5, not only to identify children with abnormal outcomes across ALL grades of HIE, but also, to document patterns of learning which may suggest differential impacts of HIE. In this study, children post-HIE presented with lower dexterity skills, verbal short-term memory, and graphomotor processing speed, and a higher burden of low scores on neuropsychological tasks.

The importance of detailed serial neurological and continuous EEG recording across the neonatal period has been demonstrated to track the evolution of HIE symptomatology. Scores either remained constant or improved in the first three days of life. Early neonatal scores are now crucial for treatment decisions, whilst later neonatal scores have higher predictive value for outcome in early childhood. This was shown for day 3 ATNAT, and 24-hour age background EEG scores. Repeated development assessments in the first two years were inconsistent but are warranted to track the non-linear nature of developmental progression. Normal development at 24 months was the most reassuring for a normal IQ at five years but not necessarily for an overall intact outcome.

Finally, newborns with mild HIE have lower rates of intact survival at five years than the comparison group and have cognitive and neuropsychological outcomes similar to children with moderate HIE. This research adds to the emerging evidence that mild HIE is not a benign diagnosis for the neonate, and supports the evidence for a rationale to examine the potential of TH treatment for these infants.

CHAPTER 1

1 Introduction

1.1 Encephalopathy in the Newborn – Important Definitions

Neonatal encephalopathy (NE) is a heterogenous condition which is clinically defined and describes the evolution of symptoms of disturbed neurology in the new-born period. It is tracked clinically and electrographically in the first hours and days of life. Neonatal hypoxic-ischaemic encephalopathy (HIE) is a subset of NE thought to demonstrate that a significant period of oxygen deprivation (hypoxia) and/or blood flow (ischaemia) to the brain – termed perinatal or birth asphyxia (PA) - has occurred either before or during labour and birth of the infant and is severe enough to cause altered physiological and neurological signs in the infant.

1.1.1 Neonatal Encephalopathy

NE symptoms include “difficulty with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness and … seizures" (1). The largest studies of NE to date have included a Western Australia population cohort recruited between 1993-95, and the Vermont Oxford network register from 2006 onwards. These and others used a broad definition of moderate to severe grade NE, which didn’t assume an asphyxial cause. Results showed, that in addition to hypoxic-ischaemic episodes such as an acute sentinel event (8-25%) and other markers such as bradycardia (35%), NE symptoms were also found to be preceded by maternal fever and inflammation (16-27%) (2, 3), small-for-gestational-age (SGA) (16-17%) (3, 4), and non CNS birth defects (4-11%) (3). All of these signs can lead to long term adverse outcome. The authors cautioned that HI aetiology may be under-represented, because more specific measures - such as cord blood to measure acidosis and base deficit - were unavailable. Notwithstanding this, the cause of NE in many cases remains unclear, and hence the importance of clearly defining NE Vs. HIE and the avoidance of using the terms interchangeably.

This leads to much debate about the validity of the terms used to describe PA, HIE and NE, and their commensurate aetiologies (5-9). Some argue for NE as the preferred term as it is not yet possible to isolate out all causes of NE although those of HI origin are the best understood (9), whilst others argue for the importance of using the term HIE, albeit only when appropriate assessment tools evidenced to detect hypoxia-ischaemia are used (5). What is not in debate is the fact that a proportion of children, whether they experience NE or it’s subset HIE, are at risk of developing cerebral palsy (CP) and other neurodevelopmental

disorders. For example, in a recent outcome study using a population cohort of children with CP following moderate to severe NE, it was found that 41% met the criteria for HIE. Those who did, had greater evidence of deep grey matter injury on MRI with higher rates of severe quadriplegic CP, levels of physical disability and non-verbal communication, than those who developed CP without birth asphyxia (10).

There are many routes to the development of CP and non-CP neurodevelopmental outcomes. It is important to acknowledge that NE, HIE and PA in isolation or combination with one another, may lead to a normal outcome, or CP or other adverse neurodevelopmental outcomes (see Figure 1.1) (1, 7).

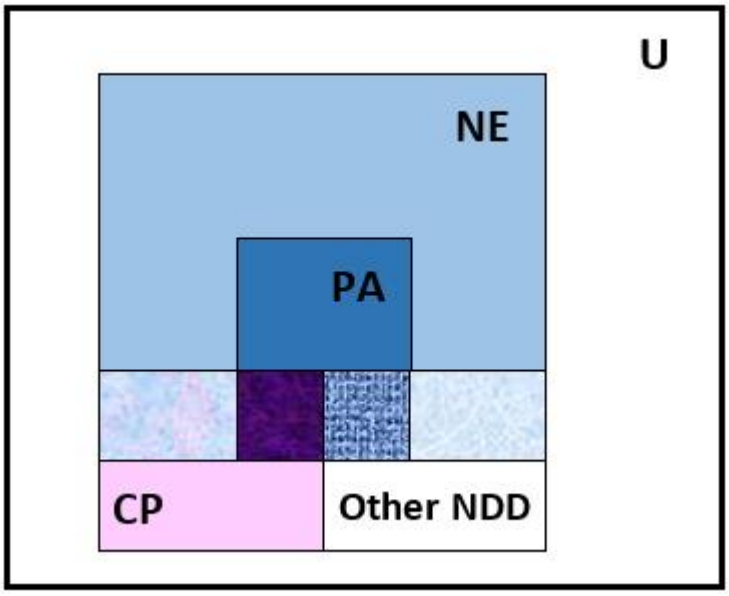

Figure 1.1 Venn diagram outlining the associations between neonatal encephalopathy (NE), perinatal asphyxia (PA), cerebral palsy (CP), and other non-CP adverse neurodevelopmental sequelae (Other NDD), and normal outcome (solid blue shapes). Reprinted from Seminars in *Paediatric* Neurology, 11(1), M.I. Shevell, The "Bermuda Triangle" of neonatal neurology: Cerebral palsy, neonatal encephalopathy, and intrapartum asphyxia, pp 24-30, Copyright (2004), with permission from Elsevier.

The focus in this thesis is limited to encephalopathy that has arisen from a presumed HI insult or episode in the perinatal period. Our studied cohort was recruited prospectively and had EEG and clinical signs of an evolving encephalopathy in keeping with HIE. Other causes, such

as sepsis or inborn errors of metabolism were excluded. The remainder of this introduction will thus focus on HIE studies.

1.1.2 Hypoxic-Ischaemic Encephalopathy

HIE affects between 1 to 6 infants per thousand born full-term (11, 12), and is caused by a presumed acute hypoxic-ischaemic event. According to the 2013 Global Burden of Diseases report, 'neonatal encephalopathy (due to birth asphyxia/trauma)' (i.e. HIE) remains as one of the top ten causes of death in infants under 1 month of age globally and is one of the top ten contributors to years of life lost in this age group (13). When left untreated, between 15-20% of affected infants die in the neonatal period, and a further 25% will have permanent neurological disabilities such as cerebral palsy (CP) or intellectual disability (ID). The reported outcomes for children following neonatal HIE varies widely, primarily due to the severity of encephalopathy but also due to research design variables. Severity is most usually graded using the clinical Sarnat Score (see Section 1.4.1.2) (14). However, the incidence of HIE varies depending on the criteria used to define it (15).

Clinical agreement about the specific criteria for the measurement of HIE has changed over the past decade and has been latterly influenced by the diagnostic criteria used for the therapeutic hypothermia (TH) treatment randomised controlled trials (RCTs) (16-18). It is also suggested that previous consensus criteria may have been restrictive, and also confounded by the timing of the injury, and whether or not late preterm (born at 35-36 weeks) babies were included in cohorts, where hypotonia due to immaturity could be misconstrued as a clinical sign of HIE (8). Some of the most common markers include abnormal foetal heart rate, poor umbilical cord gases, and low Apgar scores (19).

The most recent consensus statements on the measurement of HIE published jointly by the American Pediatric Association and the American College of Obstetrics and Gynaecology (ACOG) (20) are outlined in Table 1.1.

Table 1.1 ACOG consensus statement of the measurement of HIE

Neonatal Signs of an Acute Event	Signs which increase the likelihood that a peripartum HI/H event has occurred that leads to NE	Signs which decrease the likelihood that a peripartum HI/H event has occurred that leads to NE
Low Apgar Score at 5 and 10 minutes	Apgar score <5 at 5mins and 10mins Increase relative risk of cerebral palsy	Apgar >=7 at 5 minutes
Foetal umbilical artery acidaemia	Artery pH < 7.0, or base deficit >= 12 mmol/L, or both increase likelihood of hypoxic episode but does not define timing of onset	Arterial cord gas pH > 7.2 – hypoxia unlikely
Evidence of acute brain injury on brain MRI or MRS	Best define nature and extent of cerebral injury in NE	Cranial US and CT lack sensitivity
	Patterns of abnormality observed >=35 weeks gestation, and have prognostic value for outcome	No injury observed by neuroradiologist after 24 hours of life. However, full injury may not be evident until after first week
	MRI at 24-96 hours of life more sensitive to timing of injury, whilst at 10 days (7-21days) better describes extent of injury	Measurement of precise timing remains challenging
Multisystem organ failure (includes renal, hepatic, haematologic, cardiac, metabolic, gastrointestinal injury/abnormality	Presence increase risk of HIE	Doesn't always correlate with severity of injury seen on neuroimaging.

Information in this table adapted from ACOG 2014. HI = hypoxic-ischaemic; H = hypoxic; mmol/L = millimoles per litre

1.1.3 Perinatal Asphyxia

The term perinatal asphyxia (intrapartum/late antepartum asphyxia) describes an interruption to the normal gas exchange resulting in (i) hypoxia – low oxygen levels, hypercapnia (high CO_2 levels) and causes metabolic acidosis – measured in blood by the base deficit, and (ii) ischaemia – inadequate blood supply to meet the system's needs. Ischaemia initially affects the nonessential organs, but if it persists the brain, heart and adrenal glands

become affected (8). If acute, it affects the brain very quickly, in particular the basal ganglia and thalamus.

Despite a large body of research dedicated to the topic, there is still no gold standard for the diagnosis of PA in the newborn, although a plethora of neonatal signs have been implicated. The difficulty remains that single clinical signs, for example the Apgar score, used in isolation do not exclusively mark for PA (7).

Many children who experience PA in the neonatal period have a normal outcome, but if PA leads to encephalopathic signs in the first days of life, then the long-term prognosis becomes poorer. This is also true for accuracy of prediction of outcome (21-23).

1.2 Mechanism of Hypoxic Ischaemic Injury

1.2.1 Pathogenesis of HIE

Due to detailed studies in the domains of animal research, and neuroimaging, the pathophysiology of brain injury in the neonate with HIE is becoming better understood. It is generally conceptualised as injury that evolves over days and weeks (24). However, much remains to be learned from this complex process. Pioneering studies based on Vanucci's animal-model of brain damage observed following induced unilateral H/I injury in rat pups (25) described the following: (i) ipsilateral damage was found in the cortical, subcortical and periventricular regions, (ii) the damage was not observed in hypoxia alone (iii) reperfusion injury is different in the adult and immature brain (26). An understanding of how, where and when the damage occurs is critical to the ongoing development of novel treatments.

Since the 1980s, neonatal brain injury has been conceptualised in two phases. The first phase is the injury caused by the primary hypoxic-ischaemic insult and is seen in the initial hours. This is followed by a latent phase of apparent recovery and homeostasis is obtained. This however is quickly followed in severe H/I by a secondary stage, which happens 6-12 hours after birth, and is the sequential cascade of biochemical processes in the nerve cells triggered by the initial injury, which leads to eventual, mainly programmed cell death. This is a multi-faceted complex process and is summarised below. TH is administered before 6 hours of age to suppress the secondary stage cascade of injury.

Glucose metabolism is altered by H/I and works differently in the immature brain, not least because of higher metabolic load demanded by the growing brain at this time (especially the subcortical white matter), and the ability of the immature brain to recruit energy from ketones in addition to glucose (27). Low energy supply in cells leads to a build-up of calcium and to de-polarisation of the neurones and glia causing seepage of excitatory amino acids into the extracellular space. Excitotoxicity results from high levels of glutamate, due to reuptake failure and the overactivation of N-methyl-D-aspartate (NMDA) receptors which increases the calcium levels between cells and lowers the blood pH (lactic acidosis). This sets in motion the cellular processes leading to cell death (26). NMDA plays an important role in brain development, facilitating the increase and migration of nerve cell precursors and later synaptic development and plasticity. Because of this, there is a higher density of glutamate receptors on NMDA in the neonate, thus compounding vulnerability to damage from the excitotoxicity process described above (27). In human neonates, higher glutamate levels can be measured via proton magneto-spectroscopy and is observed in the CSF (cerebrospinal fluid) of babies with severe HIE. In addition, excitotoxicity of the neurons can lead to seizures as measured by the EEG (electroencephalogram).

A separate source of excitotoxicity is also gaining attention, with evidence that de-polarisation of neurons also leads to the release of ATP (adenosine triphosphate) into the synaptic cleft, causing increased adenosine receptor activation on the synapse. Evidence is accruing that this may be implicated in inhibited axonal growth and white matter formation (27).

The pathway to cell death arising from excitotoxicity from overactive NMDA receptors results from the activation of neuronal nitric oxide synthase (nNOS) which produces nitric oxide. This leads to mitochondrial malfunction, oxidative damage and cell death. Calcium also builds up in the mitochondria of the cell (28). The immature brain is particularly vulnerable to this dysfunction due to its sensitivity to oxidative stress (26). Oxidative stress can be defined as increased free radical production arising from oxidative metabolism due to pathology. This is seen in H/I injury due to the primary ischaemia which reduces the delivery of oxygen and other nutrients transported in the blood, and the secondary damage caused by reperfusion of blood to hypoxic cells thus inducing oxidative stress.

The cellular response to H/I, reperfusion, infection and inflammation, is guided by cytokines which play a systemic and local role in this response.

Two processes of cell death – necrosis and apoptosis, are seen in neonatal H/I injury. Necrotic death is often seen after severe injury and occurs as a result of a weakening of the cell membranes, leaking of cytoplasm outside the cell, and a secondary inflammatory response. There is also emerging evidence that some necrosis can also become programmed in some situations termed necroptosis (29). Apoptosis is a regulated process of programmed cell death via nuclear shrinkage. Cell remnants then break off as apoptic bodies and are absorbed by adjacent cells (26). Apoptosis in the neonatal brain behaves differently following H/I injury compared with the mature brain, and appears to be the principal mode of cell death in neonates (27).

A recent review suggests that pathogenesis of perinatal brain injury should include ongoing processes termed tertiary forms defined as '*those that worsen outcome, predispose a patient to further injury, or prevent repair or regeneration after an initial insult to the brain. (p.557*)' (30). Thus far, these processes have been suggested in the main from animal studies, but knowledge is accruing from specialised neuroimaging techniques. The processes suggested includes long-term microglial activation, (although not directly studied in relation to HIE), altered inflammatory processes, and epigenetic changes which are suggested to lead to gliosis, delayed maturation of oligodendrocyte progenerator cells (OPC), altered proliferation and synaptogenesis and epigenetic dysfunction. If true, this could help explain the long-term memory and learning difficulties experienced by children due to myelination, white matter and connectivity issues, even following mild HI insults (30).

As knowledge and techniques become more sophisticated in the quest for a model to explain perinatal insults, multifactorial aggregate ('multiple hit') models are attracting interest such as the 'sensitisation' model which purports that susceptibility may have its origins in the antenatal phase where inflammation, maternal stress, hypoxia, malnutrition and genetic factors may sensitise the foetus to experiencing a hypoxic-ischaemic insult, with postnatal factors (pain, drug administration, inflammation, genetic factors) intensifying the injury impact (31). Gender is also thought to play a role with evidence from a small pool of studies suggesting male susceptibility to worse injury and prognosis (32).

1.2.2 Pattern and Timing of Brain Injury in HIE

The pathophysiology described in the previous section affects brain areas in different ways reflecting underlying vulnerabilities, gestational age and type of insult. The brain regions

most vulnerable, are those with the highest metabolic load. MRI studies have contributed greatly to an understanding of the topology of same. This knowledge has accumulated since the 1980s, when early MR studies identified two principal patterns of neonatal brain injury – (i) 'BGT': deep grey matter nuclei (i.e. thalami, basal ganglia and peri-rolandic cortex) and (ii) 'WS': white matter and cortex damage in the intravascular boundary zones – the regions between the territories served by the major cerebral arteries (i.e. 'watershed' region) (33). When infant MRIs are repeated in childhood, injury patterns tend to be consistent (34). Over time, scoring systems which correlated with early childhood outcome were devised, which met best accuracy if administered after the first week of life. Many use the Barkovich scoring system or an adapted version (see Table 1.2). Other brain areas implicated include the posterior limb of the internal capsule (PLIC) (35), hippocampus and brain stem (in severe cases) often associated with type (i) pattern, and the cerebellum. BGT are generally associated with acute, severe, sentinel events such as placental abruption. The WS injury pattern is associated with a more chronic partial asphyxia, with uni- or bi-hemispheric involvement, attributed to hypotension, hypoglycaemia, and infection (19). However, further studies cast doubt on this clear dichotomy, with later MRIs showing that most post-HIE injuries with WS injury also have evidence of BGT injury. Also, BGT injury may also display abnormal signal intensity in the central sulcus, interhemispheric fissure and insula cortex regions. Those with cortical and white matter injury (WMI) alone are often caused by perinatal stroke with no HIE signs (36).

Recent evidence from term-births, suggests another pattern of injury, (previously more often attributed to prematurity) that demonstrates periventricular WMI associated with milder encephalopathic signs. A recent study of 49 infants with HIE revealed that 11 (23%) presented with WMI on MRI (37).

Theories on the precise timing of damage acquisition in the encephalopathic neonate have vacillated and have medicolegal implications. A large cohort study analysed MRI findings for two groups of infants, the first who met the criteria for neonatal encephalopathy following presumed perinatal insult and the second group who presented with seizures in the first three postnatal days. Results found 80% and 69% of MR evidence of perinatal cause of injury in the respective groups, with little evidence of antenatal causation (38). Comparison of results such as these across studies is confounded by the configuration of neonatal signs used for inclusion criteria.

In term infants, patterns of MR injury have proved a useful tool to compare brain injuries between infants cooled and not cooled in TH trials. In addition the administration of TH does not appear to affect accuracy of predictive value of MR for later outcome (39). In summary, MRI is an excellent tool to determine the pattern of injury and for prediction of outcome if the MRI is acquired after the first week of age.

Table 1.2 The Barkovich MRI Scoring System (Adapted from Barkovich et al., 1998) (33).

Score	Finding
Basal Ganglia	
0 =	Normal or isolated focal cortical infarct
1 =	Abnormal signal in thalamus
2 =	Abnormal signal in thalamus and lentiform nucleus
3 =	Abnormal signal in thalamus, and lentiform nucleus, and perirolandic cortex
4 =	More extensive involvement
Watershed	
0 =	Normal
1 =	Single focal infarction
2 =	Abnormal signal in anterior or posterior watershed white matter
3 =	Abnormal signal in anterior or posterior watershed cortex and white matter
4 =	Abnormal signal in both anterior or posterior watershed zones
5 =	More extensive cortical involvement
Basal ganglia/ watershed	
0 =	Normal
1 =	Abnormal signal in basal ganglia or thalamus
2 =	Abnormal signal in cortex
3 =	Abnormal signal in cortex and basal nuclei (basal ganglia or thalami)
4 =	Abnormal signal in entire cortex and basal nuclei
Summation	
Arithmetic sum of BG and W	
Enhancement	
0 =	No enhancement
1 =	Enhancement in white matter only
2 =	Enhancement in deep grey matter nuclei
3 =	Enhancement in cerebral cortex
4 =	Enhancement in cortex and deep grey matter or white matter

Adapted from: Barkovich AJ, Hajnal BL, Vigneron D, Sola A, Partridge JC, Allen, F, Ferriero DM. Prediction of neuromotor outcome in perinatal asphyxia: Evaluation of MR scoring system. AJNR Am J Neuroradiol; 1998; 19: 143-9.

1.3 Treatment for HIE

The only recommended treatment for neonatal HIE is therapeutic hypothermia (TH). This developed from the discovery that the two-stage model of pathogenesis in HIE (see section 1.2.1) provides a therapeutic window of opportunity in the first hours of life, before the secondary wave of energy failure brain damage occurs. First described in animal research, a

number of small pilot studies led to large RCTs in the 2000s which confirmed the efficacy and safety of this treatment in human babies. The NICHD NRN (National Institute of Child Health and Human Development Neonatal Research Network) (40), Toby (17), CoolCap (41), 'China' study (42), neo.nEuro.network (43) and ICE (44) RCTs have all published broadly similar findings, which are outlined in detail in Section 1.5.2. Despite significantly lower morbidity and mortality rates, the proportion of children who die and are long term disabled is still considerable and new treatments are being pursued (45).

Little is known about whether TH in the first days of life has an impact on the long term subtle neuropsychological outcomes for children. Those RCTs which did include NP measures were not designed to have power to show significant effects for these secondary measures. At this point, the American Academy of Pediatrics recommends cooling therapy solely for moderate and severe encephalopathy grades (46).

There are a number of RCTs currently underway to increase the armament of treatments for neonatal HIE. These include Xenon administration in conjunction with TH, melatonin, allopurinol, erythropoietin, and free radicals. Stem cell research also holds promise for future therapies (30, 47).

1.4 Tracking the Course of Neonatal HIE

Clinical assessment at birth of the compromised infant will typically include the Apgar Score (48). Developed and standardised by Dr. Virginia Apgar in the 1950s, it includes five elements: (i) colour, (ii) heart rate, (iii) reflexes, (iv) muscle tone, (5) respiration. Each is allocated a score of 0, 1, or 2. Thus it '*quantifies clinical signs of neonatal depression such as cyanosis or pallor, bradycardia, depressed reflex response to stimulation, hypotonia, and apnea or gasping respirations' (pg.e52)* (49). It is recorded at 1, and 5 minutes and thereafter every five minutes if the score < 7. This valuable score however was not designed to predict HIE, albeit a lower score at 5 and 10 minutes confers a greater relative risk of the incidence of CP in population studies (49, 50). Notwithstanding, many infants with low Apgar scores will have a normal neurological outcome, for example 20.8% of infants with a 10-minute Apgar score of 0 in the NICHD TH trial had intact outcome (51). If a HI insult is suspected, additional assessment is required, and because of the variable timing of HI insults, most of which are intrauterine, it is not possible to directly measure hypoxia-ischaemia in terms of oxygenation and blood flow. Instead, markers of these events are taken such as the pH of

arterial cord blood (52), and serum lactate level which marks the cell change from aerobic to anaerobic metabolism. Two measures may be used, lactate level and time to lactate normalisation (53).

There is a tremendous interest in the identification of useful new biomarkers for HIE, and a host of lab-based biomarkers hold promise for the future. These include investigating umbilical cord blood, urine and CSF for metabolomics etc. (54). However, the focus of this PhD thesis is not to investigate the value of single markers of outcome, and a discussion of these biomarkers is beyond the scope of this review.

HIE is an evolving condition, which requires accurate and frequent measurement in the first week of life. The next section will principally provide a review of some of the most commonly used assessment measures to track HIE over time. These include neurological assessment, HIE grading systems - in particular Sarnat grade - and electroencephalography (EEG).

1.4.1 Neurological Assessment

1.4.1.1 Summary of Neurological Assessment

The fundamental aim of the neurological assessment of the full-term newborn is to identify the CNS maturity or neurobehavioural state of the baby. Classical measures focus on muscle tone and eliciting primitive reflexes, but many also include other additional behavioural signs. Some of the most common include those devised by Dubowitz, Brazelton and Amiel-Tison (55-57). Infant behaviours are included in some systems, such as orienting to environmental stimuli such as sound and sights, and behavioural states (alertness) (57). The value of neonatal neurological assessments for the prediction of outcome has been investigated, and assessment at discharge has been reported to predict outcome (58). It has been suggested that tracking early neurological signs may have better prognostication value than other biomarkers such as Apgar and blood gas pH (59).

Claudine Amiel-Tison and her colleagues developed a neurological assessment which has evolved from the initial studies of her predecessors Andre-Thomas and Saint-Anne Dargassies, and by further work by Sarnat, who reviewed anatomic and physiologic correlates of early neurological development (60). She suggested that the assessment of cortical control in the infant is a useful add-on (61), and that the assessment should focus on

the usual locus of damage – the cerebral hemispheres and the integrity and maturation of the upper control system - rather than reflexes elicited by the brainstem.

Cortical control can be clinically elicited through passive and active tone in the axis, alertness tested by visual fixation and pursuit, and cranial signs. Brainstem functions are elicited by primary reflexes and passive tone in limb flexor muscles. Neural maturation can be observed through the gradual uptake of control by the upper system in the first two years of life (62). Amiel-Tison purports that the stage of maturation of the two motor control systems (subcorticospinal and corticospinal) can be clinically evaluated in the term infant (63). Serial assessments have been recommended for two reasons: (i) to reduce the confounder of transient clinical signs caused by medical treatments, and metabolic processes (64); (ii) to discriminate between a 'static' neurological profile more usually seen in infants with prenatal damage, and an 'evolving' profile more commonly seen in HIE and 'normal' babies (65). Evidence suggestive of an old prenatal insult includes a high-arched palate, 'cortical' thumb, and cranial ridges on every suture (64).

The Amiel-Tison Neurologic Assessment at Term (ATNAT) (60) has been developed to provide a framework for observing the above. There are 35 items clustered into 10 groups. It has been shown to predict cerebral palsy following birth asphyxia (61, 62), to correlate with outcome at 12-15 months (62, 65) and is easy to administer, with no expensive equipment needed. Mild and severe abnormal scores are good predictors of normal and later disability respectively, and the moderate abnormal score less so. There can be a tendency for false positives, however, in one study, negative predictive value of a normal examination in the first two days was 100% for one year outcome (62).

Neonatal examination used in combination with a modified Sarnat score at 6 hours of age with amplitude integrated EEG (aEEG) has been found to enhance the ability of the aEEG to predict neurological outcome at one year (66). Amess et al. also found that neonatal neurological assessment was a useful aide to magnetic resonance spectroscopy, with late examinations (> 7 days) having superior predictive sensitivity of 12-month outcome than early examinations (< 48 hours) (62).

A system such as the ATNAT holds the advantage that it can also track the infant into the first and second year of life using the same system. This allows the assessment of later-elicited responses (e.g. postural reactions) which may possess superior predictive qualities (67).

Further analysis of the individual items of the ATNAT may reveal markers for later outcome, because it includes behavioural and communication items, which share construct validity with later developmental assessments (68). The 'Amiel-Tison triad' of signs seen in the first 18 months, for example, may suggest damage due to a focal lesion. The signs are uni- or bilateral phasic stretch reflex, imbalance of passive axial tone, and cranial signs on the squamous ridge of the skull (69).

A main advantage of the use of neonatal neurological assessment is that it can be used in low-resource environments, that have no access to more technically-sophisticated modes of assessment.

1.4.1.2 Sarnat and other Grading Systems

In 1976, Sarnat and Sarnat published their seminal paper '*Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study*' (14). Four decades later, it's staging system for NE is the most comprehensively studied neurological status grading system in neonatal HIE. Its predictive value has also been well scrutinised. The system aims to stage the evolving encephalopathy using three grades, - mild, moderate and severe. This grade may change or remain constant across the first days of life. It comprises clinical and electroencephalographic elements, and the components are presented in Table 1.3. A considerable drawback of the Sarnat grading outcome studies is the neonatal age at which the infant is graded using Sarnat – typically 24 hours. However, Sarnat (or other reliable grading tools) are required by six hours of age if they are to be used for decisions around suitability for TH (70). In addition, advances in the NICU include ventilation, sedative and anticonvulsive drugs which can interfere with accurate Sarnat scoring (19). The way in which the individual neurologic signs are assessed continue to frustrate standardised inclusion of children into these categories (71, 72).

Table 1.3 Summary of Sarnat stages of encephalopathy (Sarnat & Sarnat, 1976)

	Stage 1	Stage 2	Stage 3
Level of consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Neuromuscular control			
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decreased or absent
Segmental myoclonus	Present	Present	Absent
Complex reflexes			
Suck	Weak	Weak or absent	Absent
Moro	Strong; low threshold	Weak; incomplete; high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or absent
Tonic neck	Slight	Strong	Absent
Autonomic function	Generalized sympathetic	Generalized parasympathetic	Both systems decreased
Pupils	Mydriasis	Miosis	Variable; often unequal; poor light reflex
Heart rate	Tachycardia	Bradycardia	Variable
Bronchial and salivarysecretions	Spars	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased; diarrhea	Variable
Seizures	None	Common; focal or multifocal	Uncommon (excluding decerebration)
Electroencephalogram findings	Normal (awake)	Early low-voltage continuous delta and theta. Later, periodic pattern (awake). Seizures: focal 1- to 1.5-Hz spike- and -wave	Early: periodic pattern with isopotential phases. Later: totally isopotential
Duration	Less than 24 h	2-14 d	Hours to weeks

From Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress, a clinical and electroencephalographic study. Arch Neurol. 33:696, 1976; with permission).

Until recently it was generally accepted that infants with a Sarnat grade I (mild) score at birth would have a good outcome, without long term neurological deficit. Those with Sarnat grade III (severe) HIE progress to severe neurological deficit and/or death, and that the prognosis for those with a Sarnat grade II (moderate) score was less predictable, with between 20-40% progressing to neurological disability. It remains difficult to predict which infants in this group might experience negative outcomes, and what forms of difficulty might arise (71, 73), however the solution may lie in the differential power of the individual neurological signs within this category to predict outcome, the most obvious being the presence of seizures (71).

Long-term follow up studies, such as those devised by Robertson and Finer (74), and Marlow et al. (75), which also included comparison/control groups utilised more detailed neurological, cognitive, educational attainments and behavioural assessments to observe outcome. These studies allowed for more specific indicators of neuropsychological sequelae within each grade of encephalopathy severity. One pertinent finding was that those within the moderate group (both with *and* without motor impairment) had lower cognitive and educational attainments functioning when compared with the mild group or the control group. Research is thus required to further analyse predictors for cognitive difficulties in the absence of motor impairment, to improve prognostic data (76).

There had been a consensus in the literature that Sarnat mild grade encephalopathy is a benign condition, hence it was not included in initial TH RCT trials, and are an understudied group compared to the moderate and severe grades. In Robertson and Finer's cohort some differences were found between the mild encephalopathy group and the control group. However, this is a dated cohort. More recently, in a retrospective study, children following mild HIE had greater than expected rates of specific memory and learning difficulties (77, 78), and white matter changes on MRI have also been reported (36, 79, 80). Robertson and Perlman (2006) (8) recommend follow up of children with mild HIE who experienced hypotonia at any stage (especially low shoulder girdle hypotonia), because they noted that studies in the 1990s that reported adverse outcome for those following mild encephalopathy had transient hypotonia and/or suppressed reflexes (71). Overall outcome findings will be reviewed in further detail in Section 1.5 – childhood outcomes.

Sarnat and Sarnat's grading system has remained the most commonly reported in research studies. However, no matter what grade is chosen, definitional difficulties exist (72). An alternative system, the Thompson grade has also gained considerable interest. It has the advantage of a simple scoring system (0-3, where 0 is normal) comprising nine items to be assessed: tone, level of consciousness, fits, posture, moro, grasp, suck, respiration, and fontanelle (81). It has been shown to have high sensitivity and specificity for death and severe outcome (such as the development of severe epilepsy) in the short-term in both uncooled and cooled infants (82).

To conclude, current views of observing dysfunction focus on a continuum or spectrum model of disability from normal to severe. It could be argued that encephalopathy is no different, and that there is insufficient evidence-based rationale to discriminate individual grades such as 'mild', 'moderate', and 'severe' (75, 83, 84).

1.4.2 Electroencephalography

The EEG, developed by Hans Berger in 1929, is a recording of summated post-synaptic electrical activity in the cerebral cortex close to the skull surface of the scalp (85). This is achieved with the application of electrodes to the scalp in specified patterns (i.e. the montage). For infants, typically 9 are positioned using the 10-20 pattern (see Figure 2.1 in the methodology chapter). In order to standardise practice, the American Clinical Neurophysiology Society has recommended adherence to this 10-20 pattern (86). For

accuracy, it is important that electrodes have good contact and symmetry, and in a new-born sick baby, this can take up to an hour to achieve (87).

Continuous multichannel EEG recordings are the gold standard (86) as they provide comprehensive spatial information about newborn brain function and provide information about synchronous/asynchronous patterns between the cerebral hemispheres. Amplitude integrated EEG (aEEG) has become more popular because of its ease of use but it does have limitations. Until recently it was argued that aEEG pattern recognition was easier to use and interpret than continuous EEG, however studies have shown poor inter-rater reliability (88). aEEG originally relied on cerebral functional monitoring from 1 channel, developed for use with adults, although used in a number of the TH trials (87). Information about spatial patterns, synchrony/asynchrony and lower amplitude seizures can be lost. Two channel recordings (either C3 and C4 but more often P3 and P4) allows the detection of interhemispheric asymmetries not detected with one channel (86). aEEG is now used concurrently with access to at least two channels of continuous EEG trace, especially for surveying background activity. For seizure detection, continuous multi-channel EEG remains the best choice where available (86).

Factors that affect the accuracy of the EEG reading include artefact from the electrical noise of the NICU, other physiological processes in the infant including movement, ocular, cardiac and breathing, and administration of drugs to sick babies including phenobarbitone, diazepam, pethidine, morphine, midazolam and lignocaine (87, 89).

There is a burgeoning interest in the potential of machine learning algorithms to detect EEG patterns. Quantitative analysis using mathematical models can, for example, compute the area of burst suppression (90), grade abnormality in EEG (91), and provide a detection system for the evolution of EEG which discriminates mild and moderate instances (92). These programmes continue to be developed to improve prognostic utility. Some studies are limited by the number of samples, and the length of EEG epochs under scrutiny (93).

In newborns, EEG is assessed in qualitative and quantitative ways. Qualitative is generally used by clinicians, using visual pattern recognition. Quantitative is being developed but not yet in clinical practice (94). Typically, the background activity is characterised by the neurophysiologist, sleep and wake states are identified, and seizures if present are identified and described in terms of amplitude, frequency, location and morphology (87). The

background features of the EEG have received most attention. They are the baseline actions of the brain at rest, when awake and during sleep. The principal features analysed are continuity, amplitude, frequency, synchrony and symmetry, maturational characteristics, state differences and reactivity (89). The pattern of the sleep-wake cycle (SWC) is important. There are three phases in a cycle (quiet sleep, active sleep and wakefulness). A study of the SWCs of 91 healthy term infants suggest that an EEG recording of 2.5 hours is required to capture the entire SWC duration in neonates (95), with active sleep contributing to, on average, 52.1%,of active and 38.6% quiet sleep per SWC.

A normal neonatal EEG has a unique pattern, different to all other stages of life. It will have a background frequency of 0.5-12Hz, and amplitude range of 50-100μV, a continuously synchronous and symmetrical discharge pattern across the hemispheres, show a definitive SWC in the immediate postnatal period and react to tactile and auditory stimuli. The maturational features that can be identified at term are anterior slow dysrhythmia (first appears from 35 weeks) and frontal sharp transients (89).

1.4.2.1 EEG and HIE

After hypoxia-ischaemia, early presence of a normal background EEG recording usually predicts positive outcome, whilst severely abnormal readings are associated with major adverse neurological sequelae. The predictive value of a moderately abnormal EEG is affected by time of recording, and recovery on serial recordings (96). Holmes and Lombroso (1993) in a review of studies observed that the background EEG patterns were more important in prognosis than EEG discharges (97). One study cited that 85% of children with neonatal seizures combined with normal background had normal outcome.

In a more recent systematic review of the ability of background features of EEG and aEEG to predict outcome in infants following HIE, it was found that low voltage, burst suppression and flat trace predict adverse outcome with high sensitivity (0.78-0.92) and specificity (0.82-0.99) (98). However, heterogeneity of background definitions confound the findings highlighting the need for a universal definition, such as that proposed by the American Clinical Neurophysiology Society (99). In addition, the background grades were dichotomised for statistical purposes into ‘normal’ (normal/minor/mildly abnormal) and ‘abnormal’ (moderate/severe/death). Thus, the analysis precluded investigation at the finer grade level.

EEG continues to offer the best option for temporal tracking of HIE evolution and recovery in neonates, with the potential to enable a better understanding of when to treat. Although few in number, studies demonstrate that repeated recordings at regular intervals enhance prognostication (96, 97, 100-102), and are recommended at least every 24 hours (99). In a detailed review (up to 2011), Walsh et al. (103) found that only six studies commenced continuous EEG at or before 24 hours (14, 101, 104-107) of age. In two of the studies, an exact commencement time was not stated, but EEG was initiated, 'as soon as possible', or shortly after birth (14, 106). A comparison of the outcomes was confounded by the different EEG montages and grading systems employed with the infants. Grade descriptions differed, and the number of grades per system ranged from two 'normal/abnormal' to six. Outcome for these children was measured at between 12-30 months. This review also highlighted that time of recording had a significant impact on prediction accuracy. In the cohort of infants used in this thesis, this point of timing was clearly demonstrated when EEG epochs taken at 6, 12, 24 and 48 hours were compared with 24 month outcome (105). A normal EEG achieved by 6 hours of age always predicted a normal developmental score at 2 years.

In addition to the importance of the background pattern, the EEG is also essential for seizure detection. This has been recognised for over sixty years. Neonatal seizures are often subclinical, with no outward behavioural signs (108). EEG allows earlier and more accurate diagnosis of same. Other clinical markers of HIE such as acidosis and Apgar scores are ineffective predictors of seizure compared to EEG (108).

The association between EEG and MRI for outcome has been investigated, and findings suggest a high correlation between normal/abnormal findings on EEG at day 3 and MRI in the first month with later outcome (109). Weeke et al., were the first to investigate these associations in infants that had undergone TH (110). In this study, serial EEGs at 24, 36 and 48 hours, and MRI scans at the end of the first week were compared and prognostic values with two-year outcome were presented. Results showed no associations with EEG at 24 hours, but strong correlations between EEG at 36 and 48 hours, and MRI findings with 2-year outcome. Background activity was a stronger predictor than seizure burden on EEG. However, methodological limitations include the use of a small sample and incomplete 24-month standardised development scores (using the Bayley III scales) for a number of the children measured at follow-up. This study also highlighted that, similar to aEEG, continuous EEG may have lower predictive values in cooled compared to non-cooled infants (111-113).

Two explanations for this observation have been noted in the literature: (i) the delayed recovery of burst suppression in TH on aEEG (113, 114) and (ii) that seizure burden may be reduced by TH (115).

In summary, EEG is particularly useful for monitoring brain function in neonates with HIE because (i) it is very sensitive to physiological changes associated with alterations in oxygenation and blood pressure (87), (ii) it confirms the presence of subclinical seizures activity (108), and (iii) it demonstrates excellent prognostic properties for later outcome. EEG can be used sooner than other neuroimaging techniques and, critically, in the first six hours after birth.

1.4.3 Conclusion:

This section sought to provide a summary review of some of the most commonly used assessment methods for tracking HIE progress in the neonatal period. In addition to tracking progress, the second major use of neonatal markers is in the prognostication of outcome for the infant. The next section will provide a review of childhood outcomes following HIE and their association with neonatal assessment measures.

1.5 Childhood Outcomes following HIE

There has been a substantial body of research undertaken in comparative, experimental and clinical areas to investigate the long-term effects of HIE. In general, studies have progressed from an initial emphasis on the outcomes of perinatal asphyxia (PA) focusing on mortality and severe CP, to now include a broader assessment of the extent of outcome following HIE. Short-term outcomes primarily at 12, 18 and 24 months have been extensively reported, and the focus has now extended to observational studies into childhood and beyond. Most is known about the prevalence of survivors of HIE who develop motor (namely cerebral palsy), intellectual and sensory difficulties. Less is known, but interest has increased in subtle sequelae including social-emotional and neuropsychological outcomes. This represents a departure from the now outdated 2003 statement from the American College of Obstetrics and Gynecology (ACOG) (116) that adverse cognitive patterns did not occur in the absence of a child developing CP following a perinatal asphyxial event (76).

Dilenge et al. undertook a comprehensive review of longitudinal studies following PA in the pre-TH treatment era (117). Having reviewed the prognostic indicators of PA they concluded that children in studies which employed a diagnosis of HIE had higher rates of moderate and severe outcome than those studies that limited themselves to using one or more markers of PA. For example, one study reported lower than expected rates of poor outcome but only a single marker (acidosis) was used (117, 118).

Moster et al. (21), in a Norwegian cohort looked retrospectively at low 5 min Apgar scores in combination with 3 signs of neonatal encephalopathy. Those children with PA and NE signs who were nonimpaired had significantly higher minor signs than those with low Apgar alone or the reference group. Feeding difficulties were found to have the best predictive value of the three neonatal signs measured. However, the NE was not graded and could not in all cases be presumed to have been caused by a HI episode. Additionally, standardised measures were not used for outcome assessment.

Thus, individual markers of perinatal asphyxia such as pH or base deficit (BD) alone remain of limited value due to the nonspecific nature about what they can predict (76).

1.5.1 HIE Outcome Studies in the Pre-Hypothermia Epoch

The principal cohorts of children who were followed into school age in the pre therapeutic hypothermia era include the influential work of Robertson and Finer of children born in Canada in the 1970s and 1980s (58, 71, 74, 106, 119, 120), the Hammersmith cohort (121, 122) the Utrecht/Hammersmith cohort born in the 1990s (38, 77, 78, 83, 123, 124), and a number of smaller significant cohorts. Well-designed retrospective studies have also reported outcomes following neonatal encephalopathy (75, 125). These studies were among the first to focus on outcomes other than cerebral palsy and intellectual disability, and of particular interest, reported adverse minor outcomes in the absence of motor (and sometimes cognitive) deficit (23, 74-76, 121, 126).

Overall rates of mortality and morbidity following HIE vary across studies. For those that followed all grades of encephalopathy, mortality ranged from 11-16% (12, 58, 121), and in one study, as high as 28% (77). By grade, rates were 0% mild, 4-5% moderate, and 75-100% severe (58, 77, 127). Rates of CP ranged from 10-40% (58, 122) (35-50% in moderate grade (12, 128), and rates of intellectual disability (variable diagnostic criteria employed) were 25-

58% (58, 122), (30-50% in moderate grade) (117). Sensory (hearing and vision) assessments revealed abnormal vision outcomes as 5.5% (58) (vision <20/60) or legal blindness (vision < 20/200) in best eye following correction; Hearing 6.5% (58). Epilepsy rates are 10% (8, 58). Overall morbidity rates in those that survived were 11-19% (12, 58, 129) (mild 0%, moderate 21%, severe 100%) (58). Even before the onset of TH as standard of treatment for HIE, there is evidence across studies over the past 40 years suggesting minor improvements in these mortality and morbidity rates (129), presumably arising from improvements in antenatal and perinatal medical care where available. At first glance, it appears that children following mild HIE survive unscathed. This is true for mortality and for major morbidity such as motor or intellectual disability. However, as will be shown in this section, when detailed studies of learning processes and educational attainment are reviewed it is seen that mild HIE and contemporaneous control groups do not always obtain similar results.

With regards to the measurement of IQ, many post-HIE children will obtain IQs within the 'average' range. Those studies that used comparison groups, typically reported mean IQs in the comparison group approaching the high average range (71, 74, 75, 77, 119, 124), rather than the test norm mean of 100. This denotes the importance of using an ecologically valid comparison group especially with respect to the study of outcomes of mild HIE, to evaluate any group score discrepancies. For example, in the Utrecht cohort, significantly lower estimated IQ scores (derived from four subtests) were found for those with mild and moderate NE (without CP) than for the comparison group, with no significant difference between NE grades (77, 124). In relation to verbal IQ, one study described an association between the severity of brain injury to the watershed area in the neonate and lower verbal IQ at age four years (130).

A pattern of learning domains vulnerable to HI damage has emerged in studies reporting long-term cognitive and neuropsychological outcome. Tables 1.4 and 1.5 summarise the design and outcomes from research in the pre-TH era that followed children longitudinally and reported outcome in relation to HIE grade after the age of three years. These findings are grouped by overall IQ, verbal/language, auditory, non-verbal/performance, visual motor, executive functioning, memory areas, and the impact on rate of educational progress. Significant differences between mild, moderate and comparison children (where referred to in studies) are highlighted in Table 1.5.

Auditory and language skills acquisition has been identified as one area vulnerable to HIE injury. This includes significantly lower scores in children following moderate HIE (with and without motor impairment) compared to peer comparisons in the areas of verbal concepts (119), receptive language (119, 131), auditory attention/discrimination, sound blending (131) and for the language domain of the NEPSY (neuropsychology test) (75). Impaired verbal learning in 9-10 year-old children following mild and moderate grade NE has also been reported, with no discernible differences between the grades found (77).

Visual-motor and visual perceptual skills, many of which depend on underlying fine motor skills are a second area vulnerable to HIE damage, and with a higher proportion of mild as well as moderate HIE scoring lower than their peers. Lower skills in the moderate group included visual motor skills and perceptual speed (58, 74, 119, 131), which is more pronounced in younger than older children, sensorimotor neuropsychological skills (75), and delayed fine motor skills (58). Skills below peers for mild and moderate grade include performance IQ (measured on the Wechsler but NOT seen using the British Ability Scales), visual attention (131), general motor performance, and manual dexterity (123).

Memory has been the most comprehensively studied domain due to (i) early identified trends of difficulty in studied children and (ii) the known association between memory loss and hippocampal damage. Retrospectively studied isolated case studies of children and adolescents with specific memory difficulties in the area of episodic (but not semantic) memory shared a neonatal experience of asphyxia (125, 132, 133). Other specific areas of memory where children post-NE have displayed lower scores than peers across studies are verbal working memory, sentence repetition, narrative memory (remembering story content), and a broader range of memory difficulties in moderate NE with no CP including verbal and visual long term episodic memory and verbal working memory (23, 75, 124, 131). Of note, the memory difficulties were not wholly explained by differences in IQ between the groups (124).

Interestingly, other areas of neuropsychological function have not, to date, been consistently associated with HIE damage and include areas of cognitive control and inhibition with mixed results (23, 75). However, as Maneru points out, these executive functions rely on the prefrontal cortex, which had not reached maturity when some children were assessed, and the children may therefore have been too young to be reliably assessed for these functions (23).

Table 1.4 Summary of longitudinal studies from the pre-therapeutic hypothermia era, with neonatal encephalopathy grades that measured long-term (over 36 months) cognitive, neuropsychological and academic outcome following neonatal HIE.

Study	Age at Outcome	Design	Inclusion Criteria	Encephalopathy Grade Definitions
1(i): Robertson & Finer (1985) (58)	3.5yrs Born 1974-79	Prospective. N=167/226. Mild=66, mod=94; severe=7	Abnormal neurological exam AND one of - foetal distress (abn HR); Apgar<5 at 1 or 5 mins; birth resuscitation (bag and mask or intubation with ventilation)	Mild (hyperalert/ hyperexcitable); Moderate (hypotonia and suppressed primitive reflexes); Severe (stuporous, flaccid and absent primitive reflexes). The most severe grade in the 1st week was assigned (seizures in hyperalert infants without suppressed reflexes assigned mild grade) Clinical staging similar to Sarnat grading.
1(ii): Robertson & Finer (1988) (119)	5.5yrs Born 1974-79	Prospective. n=149/167 seen at 3yrs N=127/149 (nondisabled) Controls=167 Mild=56, mod=71 Controls: neonatal recruits = 71 age 5 recruits = 188	As above. At 5.5yrs only non-disabled children were examined.	As above
1(iii): Robertson et al. (1989) (74)	8yrs Born 1974-79	Prospective N=145/226. Mild=56; mod=84 (non-dis=66, imp=18); severe=5 Control=155	As above. All study children examined.	As above
1(iv) Robertson & Grace (1992) (131)	8yrs Born 1974-79 & 1982-86.	Prospective Mild=56, Mod non-dis=66 Control=61.	As above Non-disabled subgroup examined.	As above
2. Marlow et al., (2005) (75)	7yrs Born: 1992-94.	Retrospective N=68/130; 50/68 non-disabled	>=35 weeks gestation with neonatal seizures OR 'encephalopathy'. Outcomes focussed on those with no CP.	Moderate: Seizures alone or any 2 (lasting >= 24 hours) of: abn. consciousness, difficulty maintaining respiration (central origin), abn. tone and reflexes.

Study	Age at Outcome	Design	Inclusion Criteria	Encephalopathy Grade Definitions
		Mod (no CP)=32; Severe=18 Matched Control=49.		Severe: At least one of: ventilation for >24hrs, 2 or more anti-convulsant treatments, comatose or stuporous Grade criteria based on Badawi et al. criteria for NE (134), not HIE.
3(i) Van Kooij et al. 2010 (77, 123)	9-10yrs Born 1993-97	Prospective 80/118 survivors Controls=52	37-42 weeks gestation AND 3 of: signs of -foetal distress; Apgar <7 at 5mins; arterial umbilical pH<7.10; delay in onset of spontaneous respiration; multiorgan failure.	Mild (NE1): Symptoms for <24 hrs. Hyperalertness, uninhibited Moro and stretch reflexes, sympathetic effects and normal EEG. Moderate (NE2): Obtundation, hypotonia, strong distal flexion, multifocal seizures. Main distinction between NE1 and NE2 was presence of neonatal (clinical/subclinical on aEEG) seizures. Highest Sarnat score achieved in 1st week
3(ii) Van Handel et al. 2012) (124)	9-10yrs Born 1993-97	Prospective 81/170 survivors Controls=53	As above.	As above.
4. Maneru et al. (2001) (23)	15yrs Born 1978-86	Retrospective 28/401. Mild=8; Mod=20 all nondisabled. Matched controls=28	At least 2 of:- umbilical pH artery <=7.15; Apgar <=6 at 5mins; meconium stained; presence of intrapartum bradycardia; respiratory distress in 1st 24 hrs of life; O_2 supply after delivery; neurological anomalies in 1st 48 hours (abnormal tone, neurological depression, primitive reflex anomalies, seizures, etc.) AND history of 'good outcome' (no CP, ID or neurosensory deficit)	Mild and moderate PA based on Sarnat grade

Abn=abnormal; HR=heart rate; mod=moderate grade; hrs=hours; mins=minutes; imp=impaired; non-dis=non-disabled; CP=cerebral palsy; ID=intellectual disability.

Table 1.5 Summary of outcomes from long term studies (identified in Table 1.4) that include control, mild and moderate grade HIE/NE groups for intellectual ability, verbal/language, nonverbal, memory, neuropsychological and educational areas.

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
	INTELLECTUAL ABILITIES				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(i)	Global IQ	Stanford Binet L-M (Terman Merrill, 1973)	Composite	100(16)		102[D]		92[B]	
1(ii)*	Global IQ	Stanford Binet L-M (Terman Merrill, 1973)	Composite	100(16)	108[C]	106[C]	99[A,B]		
1(iii)	Full Scale IQ	WISC-Revised (1974)	Composite	100(15)	112[C,D,E]	106[C,D,E]	102[A,B]	95[A,B]	68[A,B]
1(iv)	Full Scale IQ	WISC-Revised (1974)	Composite	100(15)	115[B,C]	106[A]	102[A]		
2	General Cognitive Ability	British Ability Scales (BAS II) 1994	Composite	100(15)	114		112		
3(i)	IQ estimation	WISC-III NL (2005) short form 4 subtests	Composite	100(15)	109[B,C,E]	99[A,E]	92[A,E]		70[A,B,C]
	VERBAL IQ and SUBTESTS				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(iv)	Verbal IQ	WISC-R (1974)	Composite	100(15)	111[C]	105	100[A]		
2	Verbal Domain Score	BAS II (1994)	Composite	100(15)	116		114		
1(iv)	Information (general knowledge)	WISC-R subtest – Information	Scaled	10(3)	11	10	10		
1(iv)	Similarities (verbal concepts)	WISC-R subtest – Similarities	Scaled	10(3)	13[C]	12[C]	10[A,B]		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
1(iv)	Arithmetic (working memory)	WISC-R subtest – Arithmetic	Scaled	10(3)	11	11	10		
1(iv)	Vocabulary (verbal concepts)	WISC-R subtest – Vocabulary	Scaled	10(3)	12[C]	12[C]	10[A,B]		
1(iv)	Comprehension	WISC-R subtest – Comprehension	Scaled	10(3)	12	11	10		
	LANGUAGE – receptive, expressive, auditory				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(i)	Receptive vocabulary	Peabody Picture Vocab Test (PPVT) 1965, mean IQ	Standard	100(15)		104[D]		93[B]	
1(ii)	Receptive Vocabulary	PPVT, (1965), mean IQ	Standard	100(15)	107[C]	104[C]	97[A,B]		
1(ii)	Receptive Language	PPVT and Zimmerman Verbal abilities subtest and/or Carrow Test of auditory comprehension					[A,B]		
1(iii)	Receptive Vocabulary	PPVT (Dunn et al., 1981)	Standard	100(15)	100[D,E]	99[D,E]	95	87[A,B]	62[A,B]
1(iv)	Receptive vocabulary	PPVT (1981)	Standard	100(15)	102	99	95		
1(i)	Expressive language	Mean Length of morpheme Utterance (MLU)(1973)	SD away from predicted CA score	0		1[D]		0[B]	
1(ii)	Expressive Language	Zimmerman, MLU, Brown's Post-stage V.							
1(iv)	Producing sentences	CELF (1969)	Raw		38[C]	35	30[A]		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
1(ii)	Auditory Attention for related symbols	Detroit Tests of Learning Aptitude, rev, (Baker, 1967)	Standard	100(15)	97[C]	94[C]	90[A,B]		
1(iv)	Auditory attention for related symbols (sentences)	Detroit auditory attention for related symbols (sentences)			88[C]	77	72[A]		
1(iv)	Auditory attention for unrelated symbols	Detroit auditory attention for unrelated symbols			86[C]	79	71[A]		
1(ii)	Auditory discrimination of sounds in words	Auditory Discrimination Test, (Wepman 1973)	SD away from predicted CA norms	0	+.73[C]	+.23[C]	-.23[A,B]		
1(iv)	Auditory discrimination	Wepman Auditory Discrimination	Raw	out of 40	28[C]	28[C]	26[A,B]		
1(ii)	Sound blending	Gates McGinnitie Reading Test	STen	5 (1.96)	5.9[C]	5.1[C]	4.8[A,B]		
1(iv)	Sound blending	Illinois Psycholinguistics sound blending	Scaled	36	45[C]	43	41[A]		
1(iv)	Phonetics	Roswell-Chall Phonetics (1962)	Raw		82[C]	80[C]	67[A,B]		
1(iv)	Auditory closure	Illinois Psycholinguistics auditory closure			34[C]	33	31[A]		
1(iv)	Free associations	Detroit (1967) Free associations			124[C]	114	104[A]		
1(iv)	Verbal absurdities (comprehension)	Detroit Verbal absurdities (comprehension)			135[C]	128	116[A]		
2	Language	NEPSY (1997) Domain	Composite	100(15)	120[C]		109[A]		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
	NON-VERBAL/VISUAL IQ				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. To**
1(ii)	Mental maturity and detail recognition	Goodenough-Harris Drawing Test, (Harris, 1963)	Standard	100(15)	96 [C]	91 [C]	86 [A,B]		
1(iv)	Performance IQ	WISC-R (1974)	Composite	100(15)	114 [B,C]	106 [A]	103 [A]		
1(iv)	Picture Completion	WISC-R subtest	Scaled	10(3)	13 [C]	11	10 [A]		
1(iv)	Picture Arrangement	WISC-R subtest	Scaled	10(3)	13 [B,C]	11 [A]	11 [A]		
1(iv)	Block Design	WISC-R subtest	Scaled	10(3)	13 [B,C]	10 [A]	10 [A]		
1(iv)	Object Assembly	WISC-R subtest	Scaled	10(3)	12	11	11		
1(iv)	Coding	WISC-R subtest	Scaled	10(3)	11	10	10		
2	Non-verbal (visual) composite	BAS II (1997)	Composite	100(15)	110		109		
2	Non-verbal reasoning	BAS II	Standard	100(15)	110		110		
2	Spatial reasoning	BAS II	Standard	100(15)	109		106		
	VISUAL-MOTOR SKILLS				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. To**
1(i)	Visual Motor/Perceptual motor skills	Visual Motor Integration Test (VMI) 1967	Months below CA	0		-1 [D]		-5 [B]	

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
1(ii)	Visual motor/perceptual motor	Test of VMI (Beery, 1967)	Scaled	10(3)	9.1 [C]	9.4 [C]	7.7 [A,B]		
1(iii)	Perceptual-Motor	Test of VMI (Beery, 1982)	Scaled	10(3)	9.3 [D]	9.0 [D]	8.7	7.4 [A,B]	3.9
1(iv)	Visual perceptual/visual motor	Dev. Test of VMI (Beery) (1982)	Scaled	10(3)	10	9	9		
1(iv)	Visual motor	Bender VM Gestalt (1946)	Standard	100	99	97	92		
1(iv)	Visual field independence/ dependence	Embedded Figures (Benton, 1963)			23	21	19		
1(iv)	Perceptual Speed (visual matching & spatial relations)	Woodcock-Johnson Perceptual Speed (1977)	Raw	Out of	482 [C]	476	472 [A]		
1(iv)	Visual attention – letters	Detroit Tests visual attention (1967)			114 [B,C]	104 [A]	98 [A]		
1(iv)	Visual attention - objects	Detroit Tests visual attention (1967)			122 [B,C]	100 [A]	98 [A]		
4	Perceptual motor speed	Digit symbol (WAIS/WISC-R)	Scaled	10(3)	13.3 [C]	12.0	11.2 [A]		
2	Visuospatial reasoning	NEPSY (1997) Domain	Standard	100(15)	118		113		
	FINE and GROSS MOTOR				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
4	Motor performance	Purdue Pegboard Test			36.3 [C]	32.6	31.9 [A]		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
4	Motor performance	Hand sequencing (Luria)			45.9	41.0	39.9		
3(i)	Motor performance (Total impairment score)	Movement ABC (Dutch instructions, 1998) (Sum of 3 subtests below)	Sum of raw scores	0-15 (0=highest score)	8.0 [B,C]	11 [A]	11.5 [A]		
3(i)	Motor performance (manual dexterity)	Movement ABC (Dutch instructions, 1998)	Raw	0-5 (0=highest score)	4.0 [B,C]	5.0 [A]	4.5 [A]		
3(i)	Motor performance (ball skills)	Movement ABC (Dutch instructions, 1998)	Raw	0-5 (0=highest score)	1.0 [C]	1.0	2.5 [A]		
3(i)	Motor performance (static and dynamic balance)	Movement ABC (Dutch instructions, 1998)	Raw	0-5 (0-highest score)	2.25 [C]	2.5	4.3 [A]		
3(i)	Motor score	Proportion of abnormal/borderline/normal (excluded children with CP)	Proportion abn/borderline/ normal	n/a	7/10/33 [B,C]	9/9/13 [A]	12/9/11 [A]		
2	Sensorimotor	NEPSY (1997) Domain	Composite	100(15)	111 [C]		104 [A]		
1(i)	Fine motor		% with >6mth delay	n/a		2 [D]		15 [B]	
1(i)	Gross motor		% with >6mth delay	n/a		5 [D]		18 [B]	
	SHORT TERM & WORKING MEMORY				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
1(iv)	Auditory Digit Span subtest	(WISC-R)(1974)	Scaled	10(3)	11 [C]	10	9 [A]		
1(iv)	Visual-auditory short-term memory	Visual Aural Digit Span Test (STM((1977)			100 [C]	96	93 [A]		
3(ii)	Visuospatial ST memory	Spatial Memory (Kaufman-ABC, 1983)			16.11	15.90	14.95	n/a	12.68
4	Verbal short-term memory	WISC-R/WAIS Digit span forwards			6.4 [C]	5.8	5.5 [A]		
3(ii)	Verbal short-term memory	Digit Span forwards (WISC-III NL, 2005)			7.43	7.39	6.96	n/a	6.24
2	Sentence repetition	NEPSY (1997) Mem & Learn subtest	Scaled	10(3)	11.1 [C]		9.0 [A]		
4	Verbal working memory	WISC-R/WAIS Digit Span Backwards			5.1	4.6	4.9		
3(ii)	Verbal working memory	Digit Span Backwards (WISC-III NL, 2005)			4.80	4.88	4.82	n/a	3.67
3(ii)	Verbal working memory	Memory Search Using Letters (Amsterdam NP tasks, ANT, 2005): Load	Raw (Reaction time in seconds)		1322 [C]	1336 [C]	1600 [A,B]	n/a	1759
3(ii)	Verbal working memory	Memory Search Using Letters (Amsterdam NP tasks, ANT, 2005): Distraction	Raw (Reaction time in seconds)		1644 [C]	1658	1912 [A]	n/a	2331
3(ii)	Verbal working memory	Memory Search Using Letters (ANT, 2005): Load	Raw(No. of errors)		5.65	6.52	7.97	n/a	11.64

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
	MEMORY and LEARNING VERBAL MEMORY				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
2	Memory and Learning	NEPSY (1997) Domain	Composite	100(15)	107		105		
4	Auditory verbal learning	Rey's Auditory Verbal Learning Test (RAVLT) Immediate word list recall	Sum of words		55.9 C	52.1	52.0 A		
4	Delayed word list recall	RAVLT (delayed recall)			12.9 C	12.0	11.5 A		
3(ii)	Verbal LT episodic memory	Learning capacity (15 Words Test, 1986)			11.7 C	10.7 C	9.3 A,B,E		7.1 C
3(ii)	Verbal LT episodic memory	Recall (15 words Test, 1986)			9.7 C	9.3 C	6.2 A,B		4.9
3(ii)	Verbal LT episodic memory	Learning words (15 Words Test (1986)			B,C	A	A		
3(ii)	Verbal LT episodic memory	Recognition (15 Words Test (1986)			29.2 C	29.0 C	28.2 A,B		27.3
3(ii)	Verbal associative memory learning – learning capacity	Learning Names (RAKIT, 1993)			9.3 C	9.1 C	6.5 A,B		5.5
2	Memory for Names	NEPSY (1997) Memory & Learning subtest	Scaled	10(3)	10.1		10.0		
2	Narrative memory	NEPSY (1997) Memory & Learning subtest	Scaled	10(3)	12.4 C		10.7 A		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
	VISUAL MEMORY				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
4	Visual memory	WMS-R: Visual reproduction test – immediate			38.2	37.1	37.3		
4	Visual memory	WMS-R: Visual reproduction test - delayed			36.9 [C]	37.3 [C]	32.4 [A,B]		
3(ii)	Visuospatial LT episodic memory	Rey Visual Design Learning Test (RVDLT, 1991) – Learning capacity			9.5 [C]	9.1 [C]	6.5 [A,B]		4.7
3(ii)	Visuospatial LT episodic memory	RVDLT (1991) – Recall			9.5 [C]	9.3 [C]	5.8 [A,B]		5.1
3(ii)	Visuospatial LT episodic memory	RVDLT (1991) – Recognition			28.3 [C]	28.2 [C]	25.8 [A,B]		24.7
3(ii)	Visuospatial LT episodic memory	Rey Complex Figure Test (RCFT)(1995) – Copy			26.7 [C]	25.0 [C]	20.8 [A,B]		17.8
3(ii)	Visuospatial LT episodic memory	RCFT (1995) – Recall			15.2 [C]	13.6 [C]	8.3 [A,B]		6.8
2	Memory For Faces	NEPSY (1997) Mem & Learn subtest	Scaled	10(3)	9.95		10.6		
2	Orientation	NEPSY (1997) Mem & Learn subtest	Scaled	10(3)	11.8		11.5		
2	Everyday memory	Everyday Memory Impairment Score. (Sunderland and Baddeley (1983))			17.8		19.0		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
	EXECUTIVE FUNCTIONING				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(iv)	Freedom from distractibility	WISC-R (1974)	Composite	100(15)	106 [C]	100	96 [A]		
4	Word generation (by letter)	Controlled Oral Word Association Test (COWAT)			42.7	34.3	34.4		
4	Executive functions	Wisconsin Card Sorting Test			5.7	5.3	5.2		
4	Inhibition	Stroop Test Word			101.8	98.1	92.0		
4	Inhibition	Stroop Test Colour			69.4	68.4	62.4		
4	Inhibition	Stroop Test Colour-Word			44.4 [C]	40.8	35.1 [A]		
4	Attentional control	Continuous Performance Test adapted (identical pairs)	Median Rxn. Time (Secs)		554	654	589		
4	Attentional control	Continuous Performance Test	No. of errors		2.3	3.7	3.9		
2	Attention and Executive Function	NEPSY (1997) Domain	Standard	100(15)	115		109		
	EDUCATIONAL ATTAINMENTS	**Psychometric Test (Year Published)**	**Score Metric**	**Test Norms**	**Control Group**	**Mild HIE**	**Moderate HIE: No Disability**	**Moderate HIE: All**	**Moderate HIE: With Disability**
	READING SKILLS				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(ii)	Reading	Metropolitan Reading Test – Composite score	Stanine	5(1.96)	4.3	4.4	3.8		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
1(ii)	Language reading skills	Metropolitan Reading Test (auditory memory, rhyming, school language and listening, quantitative language)	Stanine	5(1.96)	4.0	4.1	3.7		
1(ii)	Visual reading skills	Metropolitan Reading test (letter recognition, visual matching)	Stanine	5(1.96)	4.4	4.9	4.2		
1(iii)	Word reading and reading passages	McCracken Standard Reading Inventory (1966)	% ≥ 1 grade below		15 [C]	13 [C]	35 [A,B]	n/a	78
2	Reading	UK education curriculum level	≈% failed to reach UK peer level		16 [C]		39 [A]		
2	Speaking and listening	UK education curriculum level	≈% failed to reach UK peer level		16		35		
	SPELLING SKILLS				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(iii)	Spelling	Edmonton Spelling Abilities Test (1968)	% ≥ 1 grade below		8 [C]	2 [C]	18 [A,B]	n/a	72
2	Spelling	UK education curriculum level	≈% failed to reach UK peer level		29 [C]		50 [A]		
2	Writing	UK education curriculum level	≈% failed to reach UK peer level		20		44		

Study no. (Table 1.4)	Domain/Skill	Psychometric Test (Year Published)	Score Metric	Test Norms	Control Group (A)	Mild HIE (B)	Moderate HIE: No Disability (C)	Moderate HIE: All (D)	Moderate HIE: With Disability (E)
	MATHEMATICS SKILLS				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(iii)	Arithmetic	Keymath Diagnostic Arithmetic Test (Connolly et al. 1982)	% ≥ 1 grade below		12 [C]	16	20 [A]	n/a	83
2	Shape, size and measurement	UK education curriculum level	≈% failed to reach UK peer level		15		25		
2	Numbers and algebra	UK education curriculum level	≈% failed to reach UK peer level		18		27		
2	Using and applying	UK education curriculum level	≈% failed to reach UK peer level		22		30		
	EDUCATIONAL PROGRESS				**A Signif. to**	**B Signif. to**	**C Signif. to**	**D Signif. to**	**E Signif. to**
1(iii)	Educational attainments (reading, spelling, mathematics	McCracken Reading, Edmonton spelling, Keymath	Delay in at least one of reading, spelling, maths (%)		19 [D]	21 [D]	n/a	36 [A,B]	n/a
1(iii)	Curriculum/School	Educational support - special class, resource help	%		20	11	n/a	38	n/a
3(ii)	Curriculum/School	Requires special education	%		0	9.3	21.6		54.5
1(iii)	Curriculum/School	Repeated a school grade	%		5	12	n/a	21	n/a

NOTE: It was unclear from some research articles whether or not they investigated significant differences between mild and moderate HIE grade, or whether there were simply no significant differences to report for the study. Severe HIE groups and some impaired moderate HIE groups were not analysed in some of the studies due to obvious lower scores and low 'n'. *Study 1(ii) did not report significance values for any mild NE vs comparison group scores.
The between-group significance from research studies are presented using the letters A to E to indicate which columns differ. For example, the 'control group' column has been assigned the letter 'A', and labelled 'A sig. to' ('A' significantly different to..). Letters in superscript denote the column(s) to which the current column differs significantly. For example, 15^{C} denotes that '15' (the control group score) is significantly different to column 'C' (Moderate HIE group with no disability score). A Signif. to= Column A is significantly different to..; B Signif. to = Column B is significantly different to..; C Signif. to = Column C is significantly different to..; D Signif. to = Column D is significantly different to..; E Signif. to =Column E is significantly different to..; 'A'=Control Group column; 'B'=Mild HIE column, 'C'=Moderate HIE: No Disability column, 'D'=Moderate HIE: All, 'E'=Moderate HIE: With Disability. Mod=moderate grade; 'Composite', 'scaled', 'standard' relate to age-corrected standard scores; No.=Number; IQ=intelligence quotient; WISC=Wechsler Intelligence Scale for Children; BAS=British Ability Scales; R, rev=Revised; PPVT=Peabody Picture Vocabulary Test; MLU=mean length of utterance; SD=standard deviation; CA=chronological age; CELF=Comprehensive Evaluation of Language Function; Discrim=discrimination; STen=standard ten score; NEPSY=Neuro-Psychological Test Battery for Children; VMI=Visual Motor Integration; Dev.=Developmental; excl.=excluding; CP=cerebral palsy; STM=short term memory; WAIS=Wechsler Adult Intelligence Scale; NL=Netherlands; ANT=Amsterdam Neuropsychology Test; Mem=memory; Rxn=reaction; RAVLT=Rey's Auditory Verbal Learning Test; LT=Long term; WMS-R=Wechsler Memory Scale - Revised; RVDLT=Rey Visual Design Learning Test; RCFT=Rey Complex Figures Test; COWAT=Controlled Oral Word Association Test; UK=United Kingdom.

Many would argue that the real-life implications for the altered learning styles of children following neonatal HIE are to be found in the analysis of their educational and school performance. To this end, a pattern of delayed school readiness, increased educational resource support and lower attainments in reading, spelling and mathematics are found when compared with peers at age 7 (75), or are at least one grade below peers by age 8 (74). A more recent study reported the following rates for children in receipt of special education for mild (9%), moderate (22%) and severe (55%) NE, compared with the comparison group (0%). See Table 1.5 for more detail. These educational delays are considerable, although this pattern is based on too few studies.

The behavioural and emotional wellbeing of children following HIE has not received sufficient attention amongst researchers to date. Studies that have reported these outcomes have suggested that, when asked, parents and teachers reported higher rates of overall significant behavioural difficulties in the moderate (parents: 16%; teachers: 14%) and severe HIE (parents: 50%; teachers: 40%) groups compared with the comparison groups (parent: 9%; teacher: 11%). This reached significance for the severe group for parental reported hyperactivity and teacher reported hyperactivity and social/emotional areas (75). Attention difficulties were also rated higher in a Dutch cohort, although not high enough to warrant clinical diagnosis. Elevated (though not diagnostically significant) scores were also seen in social, anxiety/depression - and for mild HIE - thought problems (78). A Swedish study reported behavioural patterns in an adolescent cohort (aged 15-19 years) following moderate neonatal HIE, and found significantly higher scores on inattention and a screening tool for Asperger's syndrome compared with peers (128).

Some outcome studies controlled well for demographic variables, for example the Utrecht cohort controlled for maternal education (124), and the Robertson & Finer studies analysed confounding effects using regression analyses. Factors which contributed to the variance of scores included gender (74), family socio-economic status (SES) (74), maternal education (74), and also clinical confounding effects including low-birthweight for gestational age (119), abnormal discharge exam, and neonatal convulsions (58, 74, 119). Design issues that frustrated clear comparisons between studies were the different inclusion criteria, the retrospective assignment of encephalopathy grade, the exclusion of mild encephalopathy in some studies, and the varied reporting of moderate encephalopathy. Some studies provided

global outcome studies for moderate grade whilst others subdivided the moderate group into normal motor and/or IQ and abnormal IQ and/or motor outcome.

Small and uneven numbers of groups reduce the validity of using inferential statistics. For example, in one study of only 8 children in the mild group, there were no statistically significant differences reported between the neuropsychological scores of mild HIE and a comparison group despite a consistent pattern of lower scores in the mild group (23). Some retrospective studies who sought recruitment in middle childhood have had lower uptake rates of children enrolled. For example, in one group of 7-year-olds, 58% of the potential study group were included (75).

1.5.2 Childhood outcomes in the hypothermia trials

The cohort data published from the major randomised controlled trials (RCTs) of the treatment effects of therapeutic hypothermia (Head cooling - 'China' study (42), CoolCap- (head cooling in conjunction with milder body cooling) (18, 41); Total body cooling - NICHD NRN (16, 135-138), Toby (17, 41, 139), NEO.nEURO (43); ICE (out-born whole body cooling) (44)) allow not only the observations of treatment but also more up to date mortality and morbidity rates from their 'normothermic' control groups. RCTs recruited infants presenting with moderate or severe HIE within 6 hours of birth (with some analysis of mild HIE in the China and ICE studies), and randomly assigned them to TH for 72 hours or normothermic treatment conditions. Early childhood outcomes including comparison between normothermic and cooled babies are summarised in Table 1.6. Mortality rates in the cooled infants ranged from 20-38% compared with 27-57% in the control groups. Overall, when the primary outcome of combined morbidity and mortality were studied, reduced rates were reported for cooled infants, significantly so in the majority of cases (see Table 1.6). Unfortunately, TH does not appear to be as an effective treatment for the most severely affected infants. Meta-analytic review of these studies report significant reductions in the mortality rates for moderate and severe HIE, however only those with moderate HIE also displayed significant reductions in morbidity (45).

Table 1.6 Mortality and morbidity rates at 18-24 months from the principal therapeutic hypothermia RCTs.

Study	Description of RCT	Outcome measurement	Cooled	Normo-thermic	*p*-value (sig level)
CoolCap (41)	1999-2002: >=36 weeks, clinical and aEEG dx, Sarnat grade II & III; Cooled at 6hrs for 72hrs. Cooled n=108; Control n=110	Primary outcome: Death or severe disability at 18mths	48%	66%	0.02 (sig<.01)
NICHD (40)	2000-2003: >=36 weeks, clinical signs to dx, mod/severe HIE; Cooled at 6hrs for 72 hrs. Cooled n=102; Control n=106	Primary outcome: Death or mod-severe disability at 18-22mths	44%	62%	0.01 (sig<.05)
Toby (17)	2002-2006>=36 weeks, clinical and aEEG signs for dx, cooled n=163, controls n=162	Primary outcome: Death or severe neurodisability at 18mths	45%	53%	0.17
NEO.n* (43)	2001-2006, >=36 weeks, clinical and aEEG/ EEG for dx, TH n=53, controls n=58. Whole body TH and morphine	Primary outcome: Death or severe disability at 18-21mths	51%	83%	0.001
China (42)	2003-2005: >=37 weeks, clinical dx, Sarnat grades, head cooling. TH n=100, Controls n=94	Primary outcome: Death or severe disability at 18mths	31%	49%	0.01 (sig<.05)
ICE* (44)	2001-2007 >=35 weeks, clinical signs for dx, mod/severe HIE using Sarnat. Out-born infants treated with temporary ice pack in transit. TH n=107, Controls n=101	Primary outcome: Death or major sensorineural disability at 24mths	51%	66%	0.03 (sig<.05)
Study	**Domain assessed**	**n/a**	**Cooled**	**Normo-thermic**	**p-value**
CoolCap (41)	Death		33%	38%	0.48
NICHD (40)	Death		24%	37%	0.08
Toby (17)	Death		26%	27%	0.78
NEO.n* (43)	Death		38%	57%	0.09
China (42)	Death		20%	29%	0.16
ICE* (44)	Death		25%	39%	0.04

Study	Domain assessed	Outcome measurement tool	Cooled	Normo-thermic	p-value
CoolCap	Motor	Severe Neuromotor (GMF 3-5)	19%	31%	0.12
NICHD	Motor	Disabling CP	19%	30%	0.20
Toby	Motor	Severe Neuromotor (GMF 3-5)	20%	31%	0.06
NEO.n	Motor	Disabling (GMF 3-5)	13%	48%	0.007
China	Motor	Cerebral palsy	N=10	N=19	n/a
ICE	Motor	Mod-severe CP	20%	22%	0.8
CoolCap	Development global	Bayley overall <70	30%	39%	0.27
NEO.n	Development global	Griffiths <2SDs	21%	57%	0.009
China	Development global	Gesell <70	5%	21%	0.01
NICHD	Development mental	Bayley II <70 (mental)	25%	39%	0.18
Toby	Development mental	Bayley II <70 (mental)	24%	35%	0.09
ICE	Development mental	Bayley (II/III) <70 mental	23%	28%	0.55
NICHD	Development motor	Bayley II <70 (psychomotor)	27%	35%	0.39
Toby	Development motor	Bayley II <70 (psychomotor)	24%	34%	0.09
ICE	Development mental	Bayley (II/III) <70 mental	n=26	N=28	0.81
CoolCap	Vision	Cortical blindness	10%	17%	0.22
NICHD	Vision	Blindness	7%	14%	0.20
Toby	Vision	No useful vision	7%	11%	0.30
NEO.n	Vision	Bilat. cortical visual deficit	3%	5%	n/a
ICE	Vision	Legal blindness	1	0	0.99
NICHD	Hearing	Severe hearing impairment	4%	6%	0.47
Toby	Hearing	Hearing loss not corrected by aids	4%	6%	0.31
NEO.n	Hearing	Severe hearing loss	0%	12%	n/a
ICE	Hearing	Deafness req. amplification	2.5	3.4	0.75

[42]

*NEO.nEURO and ICE studies were abandoned early due to introduction of TH treatment for neonatal HIE during the course of this RCT. These studies had higher proportions of severe HIE due to out-born births, and treatment with opioids. Dx=diagnosis. Column header (sig level) refers to the significance level (shown in brackets) set for the RCT where reported.

School age outcomes from the TH trials were recorded in three studies – the NICHD study (16, 135), the Toby Trial (139) and the CoolCap trial (18). The NICHD and Toby trials used the Wechsler scales to assess intellectual abilities and different versions of the NEPSY to assess neuropsychological functioning. The results are presented in Table 1.7. The 'CoolCap' trial reported 7-8 year outcome in the context of the predictive value of a standardised developmental score at 18 months of age (using the Bayley scale) and WeeFIM parent ratings of adaptive behaviour at 7-8 years, with a high correlation being reported for many of the adaptive behaviour domains measured at school age (18).

Table 1.7 School age cognitive and neuropsychological outcome reported in the NICHD and Toby therapeutic hypothermia RCTs.

TH RCT	Test used	Domain measured	Score Metric	Hypo-thermia	Normo-thermic Controls	P value
NICHD	WPPSI III/WISC IV	Full Scale IQ	Mean	81.9	75.3	0.22
Toby	WPPSI III/WISC IV	Full Scale IQ	Mean	103.6	98.5	0.07
NICHD	WPPSI III/WISC IV	Full Scale IQ	Percentage of scores < 70	27%	33%	0.51
Toby	WPPSI III/WISC IV	Full Scale IQ	Percentage of 'normal' scores	77%	63%	0.05
NICHD	WPPSI III/WISC-IV	Verbal IQ	Mean	85.9	86.4	NS
Toby	WPPSI III/WISC-IV	Verbal IQ	Mean	105.2	101.1	0.16
NICHD	WPPSI III/WISC-IV	Performance IQ	Mean	91.3	90.5	NS
Toby	WPPSI III/WISC-IV	Performance IQ	Mean	101.1	96.7	0.12
NICHD	WPPSI III/WISC-IV	Processing Speed Q	Mean	93.2	92.4	NS
Toby	WPPSI III/WISC-IV	Processing Speed Q	Mean	98.7	95.3	0.22
NICHD	NEPSY	Attention /Executive Function	Mean	94.5	92.6	NS
Toby	NEPSY-II	Attention /Executive Function	Mean: 10(3)	9.6	8.6	0.03
NICHD	NEPSY	Visuospatial	Mean	98.5	96.5	NS
Toby	NEPSY-II	Visuospatial	Mean: 10(3)	10.4	9.6	0.17
Toby	NEPSY-II	Memory & Learning	Mean: 10(3)	10.0	9.4	0.18
Toby	NEPSY-II	Sensorimotor	Mean: 10(3)	8.1	7.1	0.06
Toby	Working memory Test Battery for Children	Digit recall (auditory short-term memory)	Mean	104.1	106.1	0.54

TH RCT	Test used	Domain measured	Score Metric	Hypo-thermia	Normo-thermic Controls	P value
Toby	Block recall	Visual working memory	Mean	97.0	94.7	0.49
Toby	Digit span backwards	Verbal working memory	Mean	96.1	95.3	0.81
Toby	Manual ability classification system	Manual ability	Percentage of 'normal' scores	77%	61%	0.04

*Standard scores have a mean(SD) of 100(15) unless otherwise stated, NEPSY subtest scores of 10(3). TH RCT = Therapeutic hypothermia randomised controlled trial; NS= not statistically significant.

1.5.3 Conclusion

This section provides a review of the major studies describing long term outcome in children following HIE, categorised by encephalopathy grade. In sum, van Handel et al.'s recommendations remain relevant a decade after they were written (83) – that is, that a better grading system is needed which is sensitive enough to capture the gradual dose response effects of neonatal HIE, and further studies are required to determine whether HIE injury has a global dampening effect across the intellectual spectrum as measured by lower IQs and/or a distinct configuration of areas of intact and impacted thinking skills. Moreover, to associate these with severity and pattern of neural injury. Post the introduction of TH treatment we can add the questions of how and to what extent TH will alter these patterns.

Investigation of the ways in which an initial hypoxic/ischaemic episode leads to brain injury which may have long term manifestations for the child, requires an acknowledgement of the complex nature of brain growth and development in childhood. The next section seeks to present features of brain development considered relevant to this area of inquiry.

1.6 (Brain) Development in Childhood

The study of the brain in childhood, especially from a psychological perspective has been heavily influenced by broader developmental theories of growth in childhood. The quality and relevance of longitudinal research involving children depends on the scientific value placed on the outcome measures that are chosen to represent stability and change over time. These outcome measures, in various guises, attempt to measure a subset of external behaviours that represent the internal thought and memory processes of the brain of the

individual. These measures have been influenced by the prevailing psychological paradigms and theories of their time.

The enormous influence of 'nature' and 'nurture' theories have been central to the field. Sameroff, in a 'rough history' of the nature-nurture debate has summarised how this pendulum has swung over the past 130 years: 1880s-1940s – nature – research on inherited differences and instincts; 1920s-1950s - nurture – behavioural learning theories (behavioural reinforcement) and psychoanalytic theories; 1960-1970s – nature – ethology (species differences), behavioural genetics and cognitive psychology; 1980s-1990s – nurture – studies on poverty and social ecology; 2000-2010s – nature – molecular biology and neuroscience (140). The current state of play is that most researchers realise the integrated and bi-directional influences between nature and nurture.

1.6.1 Traditional Stage Theories of Development

A number of stage models have been developed to describe the increasingly complex cognitive behaviours of humans across the lifespan. The most influential cognitive stage model of child development is that proposed by Jean Claude Piaget. Piaget's central contention (based on child observation) was that if the child is provided with a stimulating environment in which to explore, they will construct their own knowledge of the world, constrained only by the maturational stage of their brain at any time. This promoted the idea of the child as an active agent in their own learning rather than as a passive recipient of 'taught' knowledge. Table 1.8 summarises the cognitive stages of the model. Children achieve learning through mental representations of the world, called schema. When presented with a novel experience in the environment, the child either assimilates the experience into an existing schema, or makes accommodations to their schema to account for the new information (141).

Table 1.8 Piaget's stages of cognitive development (adapted from Santrock, 1998) (142).

Stage	Age Range	Description
Sensorimotor	Birth to 2 years	The infant progresses from reflexive, instinctual actions at birth to the beginning of symbolic thought. The infant constructs an understanding of the world by coordinating sensory experiences with physical actions

Stage	Age Range	Description
Preoperational	2 to 7 years	The child begins to represent the world with words and images; these words and images reflect increased symbolic thinking and to beyond the connection of sensory information and physical action.
Concrete Operational	7 to 11 years	The child can now reason logically about concrete events and classify objects into different sets.
Formal Operational	11 to 15 years	The adolescent reasons in more abstract and logical ways. Thought is more idealistic.

Adapted from: Santrock, JW. Child Development. 1998. Columbus, Ohio: McGraw Hill Publishers.

The three main criticisms of his approach are, firstly, that the role of the adult or others in nurturing development was not fully considered, that Piaget relied on outward signs of behaviour as evidence for task mastery at any stage, and that there was insufficient evidence on the qualitative differences between each discrete stage of the theory to merit a stage model. Newer, more sophisticated research paradigms such as the measurement of eye-tracking devices to study infant habituation to novel stimuli, suggest that Piaget underestimated the cognitive potential of infants aged 0-2 whose intent could not yet be fully demonstrated through intentional movement or speech (143). Additionally, neuroimaging studies can now show the intention of infants in the first year of life by observing evoked potentials of the brain areas responsive to environmental stimuli.

1.6.2 Systems Theories of Development

Systems theory has widened the lens through which child development is studied. Urie Bronfenbrenner's influential social ecological systems theory (1977) (144) has been at the forefront in the social sciences, and has helped to articulate an argument away from a medical towards a social model of disability in the context of child development.

This leads to the conceptualisation of the brain, whether intact or damaged, being nested within a baby, within a family, within a neighbourhood, within a society and the interactions between all elements of this system represents the totality of the child's experience. It is argued that complex models such as these are required to account for the large number of confounding variance factors in predictive longitudinal studies (140).

In regard to HIE, as outlined earlier in Section 1.5, a handful of longitudinal studies have analysed the role played by demographic variables for child IQ, with gender, particularly maternal education level, and family income contributing to variance (74, 124). In the NICHD NRN and Toby TH trials, the interaction effects of maternal education for child IQ were investigated with mixed results. Whilst in the Toby trial no interaction effect was found for maternal high school completion on the primary outcome of survival with intact IQ (139), in the NICHD trial, a prediction model using linear regression reported that children whose mothers had completed high school had adjusted IQs that were, on average, 7.5 points higher ($p = .02$), than for those children whose mothers hadn't completed high school (135). These studies broadly follow what is already known about the influence of social and educational disadvantage on IQ scores.

There is a dearth in HIE outcome studies in relation to the contribution that other family factors related to emotional connectedness, such as the availability to the infant of quality early warm reciprocal attachment relationships, the role of maternal mental health and parental coping styles will have on the wellbeing and subsequent outcomes for the child. However, there is a clear rationale for considering these issues given the knowledge base of how these issues can affect brain architecture, and lead to poorer social and cognitive outcomes for the child (145).

In attempting to devise a unified theory of development, Sameroff has overlaid Bronfenbrenner's social ecological model with a deeper within-child paradigm whereby the (nested) child's self, comprises of psychological and biological processes that interact with each other. The specific biological and psychological elements are outlined in the grey and black spheres in Figure 1.2. This process model is developmental, in that the elements of the model undergo quantitative and qualitative shift and growth from birth throughout childhood into adulthood.

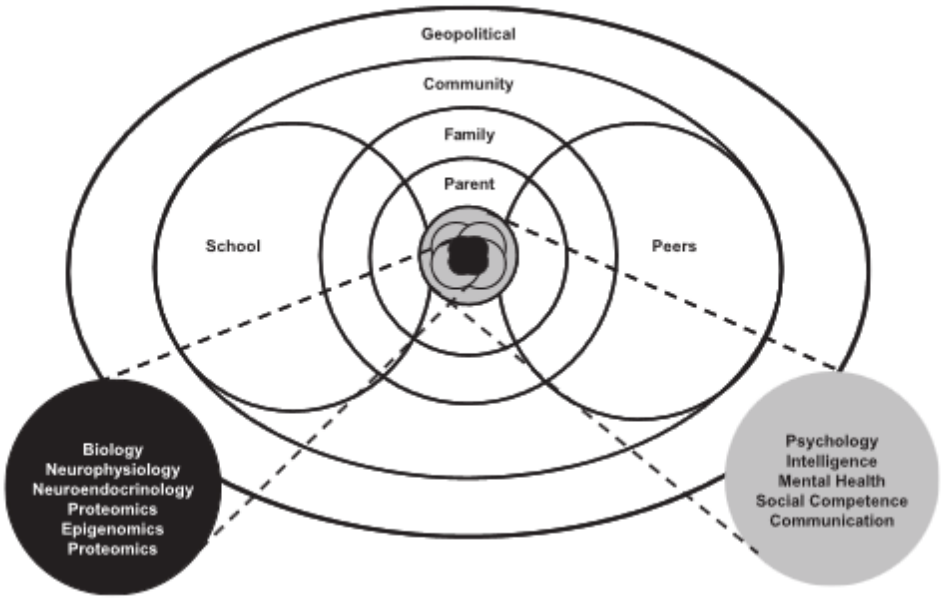

Figure 1.2 Sameroff's Biopsychosocial Ecological model. Reprinted from Child Development, 81(1), A Sameroff, A Unified Theory of Development: A Dialectic Integration of Nature and Nurture, pp 6-22, Copyright (2010), with permission from Wiley Publishers 2010, © the Author(s). Journal Compilation_2010, Society for Research in Child Development, Inc. All rights reserved.0009-3920/2010/8101-0002.

In the words of Sameroff:

> "Over time the body changes, the brain changes, the mind changes, and the environment changes along courses that may be somewhat independent of each other and somewhat a consequence of experience with each other." (2010, p. 20) (140).

1.6.3 Neonatal Brain Injury and Future Development

Structural definitions of injury (i.e. site of damage) are not sufficient to explain learning outcomes. The consequent experience of HIE also plays a role, and there are individual differences (146) to injury response.

In order to understand the processes by which early damage affects later outcome there are a number of issues to be considered: (i) the skill being assessed at one time point represents a location on the assumed trajectory path for that skill (e.g. language - see Figure 1.3); (ii) this trajectory is influenced by the maturational status of the CNS (see Figure 1.4); (iii) early damage alters the course of these trajectories. Therefore, the call for repeated measures assessment in longitudinal designs is crucial. In the case of children with HIE, these

considerations have taken on a new light with emerging theories of HIE damage now including a tertiary phase of injury extending weeks if not years into life with richer evidence of, for example, delayed myelination and poorer white matter connectivity (24, 30). If true, these considerations about developmental trajectories become more important to the field.

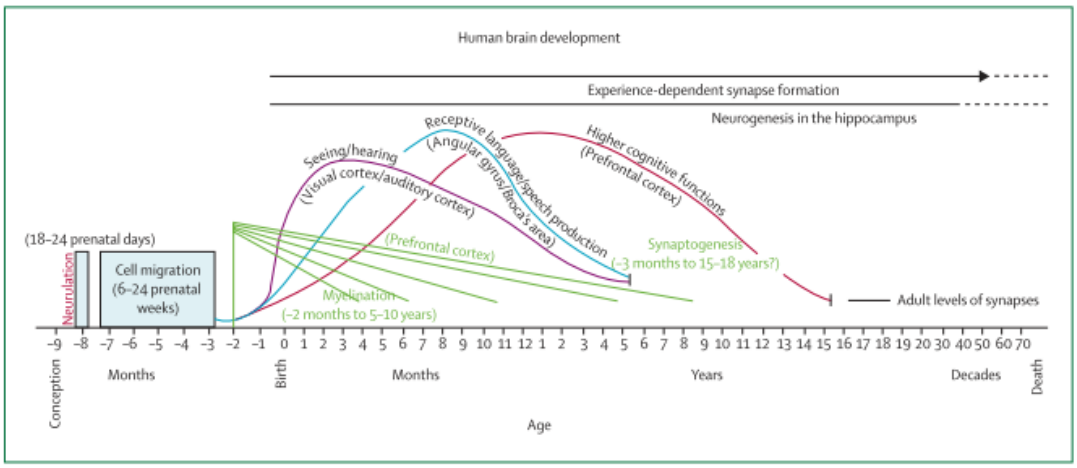

Figure 1.3 Human brain development. Reprinted from The Lancet, 369(9555), S Grantham-McGregor, Y.B. Cheung, S. Cueto, P Glewwe, L Richter, B Strupp et al., Developmental potential in the first 5 years for children in developing countries. pp 60-70, Copyright (2007), with permission from Elsevier Publishers. Adapted from original graph by Thompson and Nelson 2001, p.8. (147). APA granted permission for reuse.

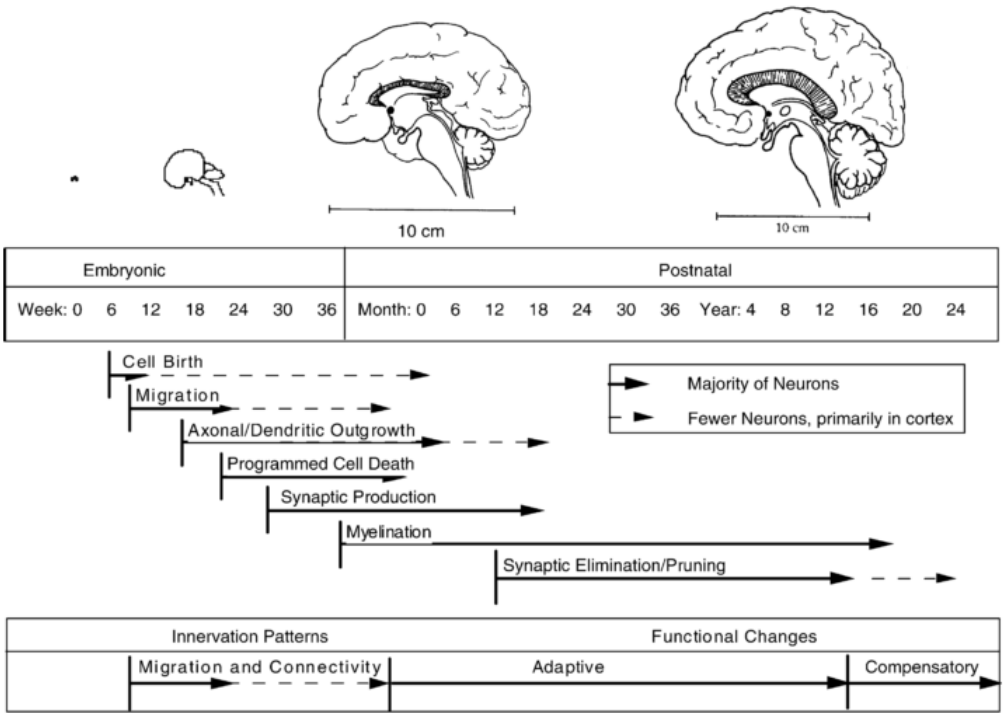

Figure 1.4 "The stages of brain development (top) and different windows of vulnerability (bottom). Developmental processes occur in phases, setting the stage for potential periods of vulnerability. Insults early in life (bottom) will be assimilated into innervation patterns, whereas a later pre-pubertal insult will cause functional changes that are more adaptive." Reprinted from Neuroscience & Biobehavioral Reviews, 27(1-2), S. L. Andersen, Trajectories of brain development: point of vulnerability or window of opportunity?, pp 3-18, Copyright (2003), with permission from Elsevier Publishers *(148).*

1.6.3.1 Early Plasticity or Early Vulnerability?

The role that neurodevelopmental processes play in the eventual consequences for a child who has experienced brain injury is unquestionable (149). There are conflicting theories in the field of developmental cognitive neuroscience in relation to whether brain injury, inflicted early in life is 'preferable' in developmental terms when compared to injury acquired later in childhood.

This role was initially investigated in the early studies of Kennard in the 1930s, and Hebb in the 1940s with conflicting results. Kennard compared the effects of acquired unilateral injury to the motor cortex of monkeys in infancy to that acquired in adulthood. These studies demonstrated better recovery for early onset lesions, and Kennard was one of the first to posit that this may be due to functional abilities arising from cortical reorganisation, perhaps recruitment of intact brain tissue in the contralateral hemisphere ('Kennard's principle')

(149-151). This suggests that injury sustained earlier in life has a better outcome than injury acquired later, due to brain plasticity. Studies of children following left-sided perinatal stroke show considerable development in language skills that would not be observed for adult-onset stroke. However, whilst many language skills do develop, they are not equivalent to those of their peers in many subtle ways, and they reflect a pattern of delay and catch up throughout childhood. Furthermore, language skills may demonstrate more potential for plasticity than, for example, spatial cognition (152, 153). For activities that require recruitment of different functional brain regions, for example in the case of emotion labelling, less functional recovery from focal lesions is observed (152). Of late, the suggestions of an interactional effect of plasticity has emerged which stresses how the injury 'becomes a factor in the developmental trajectory of the individual child' p.20 (153).

In contrast to the plasticity thesis, the work of Hebb suggests, based on studies of children with frontal damage, that early injury may be deleterious because damage is occurring at a time when the brain is in the initial stages of organisation (151). This alternative early vulnerability model has re-emerged and recently gained support, as seen in the work of Vicki Anderson and colleagues (154). In a well-designed study that sought to compare the developmental impact of focal brain injuries at different times in childhood, it was found that injuries acquired at six different time points – early prenatal (1st-2nd trimester), perinatal (3rd trimester to month 1 of life), infancy (2 months to 2 years), preschool (3 to 6 years), middle childhood (7 to 9 years), and later childhood (10 years and over), could be collapsed into those that sustained injury before and after three years of age. For cognitive skills measured in adolescence, age at insult was the strongest predictor of outcome and an injury acquired before 3 years of age was associated with more severe and diffuse learning difficulties. However, the findings are confounded by other differences between the groups other than age, including the cause of injury, which tended to be developmental in those under three years, and traumatic after 3 years with higher burden of seizures in the younger group (155, 156). These results are interpreted in the context of the early vulnerability model suggesting that early injury disrupts neural pathways which have not met a level of maturity to avail of sparing of what has already been learned. This damage may also impact the future pattern and quality of neural circuitry.

In the context of HIE, a recent 'tertiary injury' model has proposed that inflammatory responses lead to long term epigenetic and other difficulties that alters the brains ability to

learn and remember, because it interferes with the brain's neurogenerative capacities (30, 31). This supports the early vulnerability model described above.

Although much remains unknown about the specific damage processes of HIE, three results are of relevance to the current study. Firstly, diffuse early injury to an immature brain is not good, especially in the perinatal period during cell migration, proliferation, axonal/dendritic growth and myelination. Secondly, similar outcome behaviours may mask recruitment of different brain locations and networks to achieve the behaviour, and, tracking this over a longer period of time can reveal compromised functioning in higher order tasks. Thirdly, the environment and the child's experiences modulate plasticity of the damaged brain. Thus research models need to track functional reorganisation at different developmental stages of childhood (157, 158).

1.6.3.2 Protracted Development

Recent advances in the discipline of developmental neuroscience have revealed patterns of protracted development in some areas. Previous estimates of the age at which different brain regions reach full maturity have now been extended. In the case of prefrontal cortex for example, full neural maturity is not completed until well into adulthood. This has implications for the requirement to continue assessing different domains of learning until maturity is reached. Animal studies have shown that if a skill is assessed too soon in development, the results merely reflect immaturity of the skill in both the brain-injured and the comparison group. Once the brain area under study has matured, the comparison group will display expected skill competence and this can be used as a benchmark for the brain-injured group (76). This implies that follow-up of children up to 24 months as described in most studies, precludes valid assessment of future emerging cognitive and neuropsychological processes especially those dependent on prefrontal cortex maturity such as executive functions like planning, attention and inhibition.

The time course for synaptic proliferation and later pruning differs per brain region, and, consequently for the behavioural outcome being measured. For example, synaptic density in the visual cortex reaches adult levels by preschool age. In the prefrontal cortex this is not complete until adulthood. Similarly, myelination is greatest in the first two years, but continues in the white matter until adolescence (159). Longitudinal studies of children following a perinatal injury revealed that the children's IQs were significantly lower after the

age of seven years than at an earlier time-point. Importantly this finding did not reflect loss of knowledge but rather a slower rate of development than in the normative population (152).

The next section will review pertinent longitudinal study design issues in light of the aforementioned discussion of explanatory models of brain damage in a developmental context.

1.7 Outcome Studies – Design Issues

1.7.1 Neurodevelopmental Outcome Research: Theoretical and Design Considerations

Neurodevelopmental outcome research is inherently complex because the constructs being measured are evolving over time. Schmidt and Teti (2006) contend that longitudinal research identifies age-related developmental change and employs the following variables: age, cohort and time. These are often given different emphasis especially as different disciplines have different theoretical preferences (e.g. psychologists might focus on 'age'). It is argued that these phenomena cannot be isolated to constitute true independent variables, and require multi-disciplinary expertise and complex models for investigation (160).

The non-linearity of human development calls for multiple repeated measures over time to capture differential effects of growth and the effects on changing developmental trajectories and to tap differential developmental outcomes. This is especially true to capture brain areas with more protracted maturity as outlined in the previous section.

Prospective studies can lead to an understanding of the mechanisms by which a brain that is damaged **before** a cognitive system has been realised (e.g. language), can adapt by generating alternative neural organisations for that behavioural function (152), and whether (or not) this leads to a functional compromise in another cognitive system. These can also track the optimum times for plasticity factors and the brain's ability to accommodate for the loss by changing the underlying sites for which the purpose was developed. For example, children with left hemispheric lesions develop language but at a cost to the normal development of the spatial areas of the right hemisphere.

Annaz et al. (161), describe the importance of tracing developmental trajectories using a 'neuroconstructivist' approach, because of the possible 'cascading' effects of a small impairment on future mental attainments. They argue that a phenotype measured at one time point is limited in the information it can provide about predictive validity because what is ideally required is to observe (i) how the phenotype originated at the beginning of a developmental trajectory as well as (ii) knowing where it will lead in the future trajectory. This is especially true of an atypically developing brain, where a small insult could alter the usual interconnectivity of early cortical development later permeating all parts of the cortex.

Neurodevelopmental outcome studies benefit from being theory and method driven. Friedman et al. caution against the temptation to be driven by the availability of outcome assessment tools (162). Choosing ecologically valid, psychometrically sound, standardized tools enhance comparisons across disciplines and between research protocols. The need for uniformity in test methods across studies is particularly important, because the use of outcome measures can be a major source of bias in research trials (163). The challenge lies in finding measures that allow for meaningful description of continuity and change (164), that are reliable and valid (see Figure 1.5)

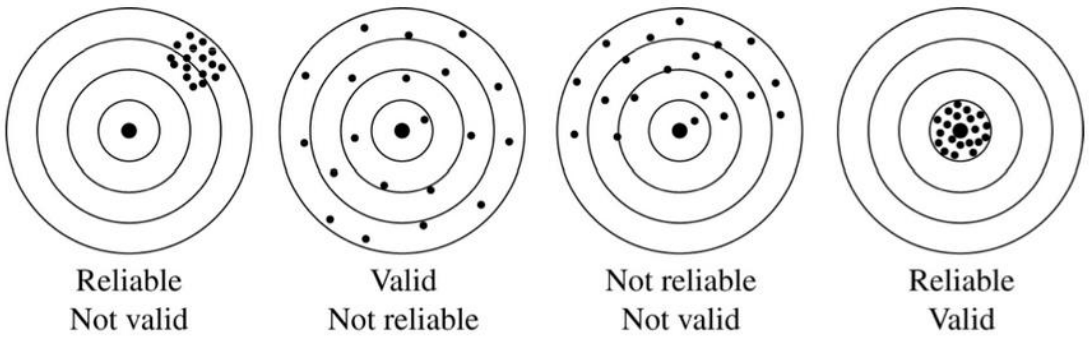

Figure 1.5 Pictorial representation of reliability and validity measures, where the bulls eye represents the concept being assessed. (original source unknown). Available at © Nevit Dilmen [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0) or GFDL (http://www.gnu.org/copyleft/fdl.html)], via Wikimedia Commons

In conclusion, well designed, long-term developmental outcome studies in conjunction with electrophysiological and scanning techniques may enable us to better link neurocognitive outcomes to lesion areas, or timing of injury if demonstrable on scanning. Interpreting "neuroimaging of the developing brain involves a theoretical attitude of mind, a truly

developmental framework, and a focus on progressive change." (Karmiloff-Smith, 2010, p.937) (165).

In order to improve our understanding of the utility and validity of the assessment of neurodevelopmental outcome, it has become clear that a more unified model to help link the progression of knowledge and skills acquisition through childhood needs to exist. From neurological assessment in the neonatal period through developmental assessment in early childhood to assessment of cognitive and neuropsychological processes in school age children, prediction will only improve if the theoretical links are strengthened through the inclusion of behavioural criteria that can be measured over time (166). This begins with primitive reflexes and ends with higher order thinking (167).

These types of assessment will now be reviewed in more detail.

1.7.2 Developmental Assessment

Some of the earliest well defined longitudinal studies of infants resulted in the publication of childhood development scales. These include Arnold Gesell's (1880-1960) work on motor development at Yale and Bayley's work on intellectual, social and motor development at Berkeley in the late 1940s. These were, in the main, descriptive in nature (168). Gesell was in conflict with the main influence of behaviourist theories of the time, and he believed that child development was a maturational process that unfolded in a predetermined sequence. In addition, he was one of the first to recognise that different domains of child development are interdependent (168). Scales developed from these works including the Gesell and especially the Bayley scales are in widespread use to this day. The majority of the early childhood developmental tests assess the following domain areas – gross motor, fine motor/eye-hand coordination, language, cognitive and personal/social/adaptive functioning.

Dr. Ruth Griffiths was a pioneering psychologist and medic who systematically observed the natural development of hundreds of babies, infants and young children. This work resulted in the publication of the Griffiths Mental Development Scales. In her 1954 publication, 'The Ability of Babies' she outlined a sophisticated 'avenues of learning' model (see Figure 1.6) which acknowledged the intrinsic and environmental factors which influence infant developmental milestones acquisition (169).

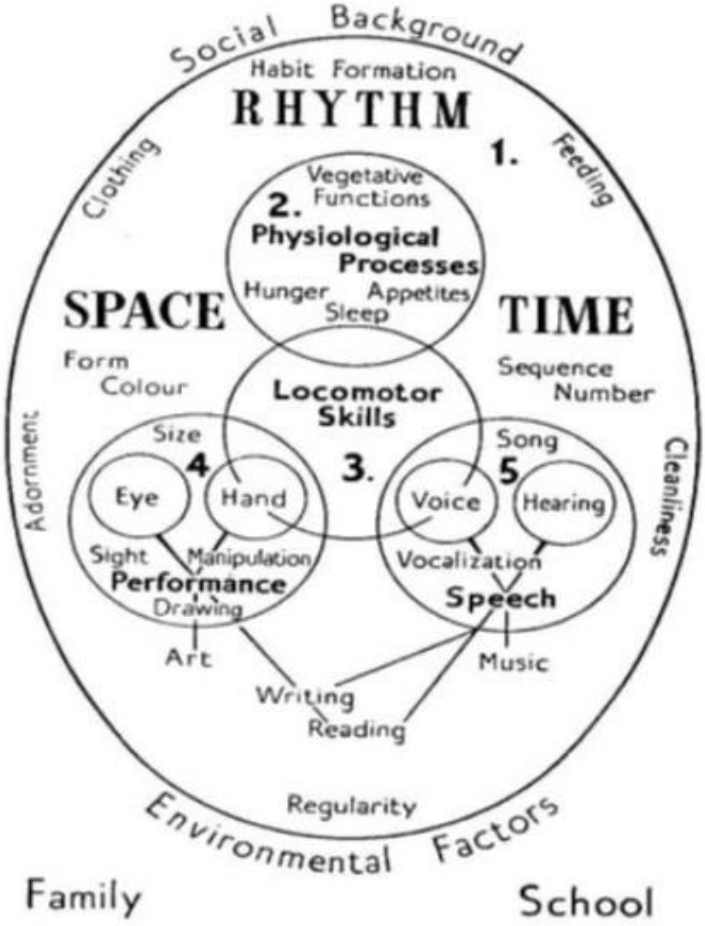

Figure 1.6 Ruth Griffiths' 'Avenues of Learning' Model. Reprinted from R. Griffiths. The Abilities of Babies. Copyright (1954). Permission granted from ARICD (Association for Research in Child Development). © ARICD.

The literature demonstrates that in the general population the ability of early developmental assessment to predict school age cognitive outcome is relatively poor. However, in the clinical population, the predictive value improves significantly (170).

Early developmental assessment may not be sensitive enough to capture the later-emerging deficits that, for example in our case, mild grade HIE might be associated with.

It has been suggested that for developmental tests, assessments at 6 months capture genetic data but as a child grows into the second year the impact of the quality of the environment plays a larger role as the child must use their potential skills in real settings (171). Test-retest reliability values are much better in the second year than the first year, showing the individual rates of development in normally developing infants (172).

Some criticisms of developmental assessments are that they have high false positive rates in early childhood, that some difficulties persist whilst others are transient, and that there is high variability and discontinuity in early development (57).

1.7.3 Assessment of Intellectual Ability

Intelligence is a social construct, that since the turn of the 20th century has been bound up in the notion of how it is assessed. The original rationale for assessing intelligence emerged from the focus on methods of quantification and diagnostics in medicine, education and psychology at the turn of the 20th century and for the measurement of the suitability of children for education.

In its current form, 'intelligence' - as assessed by the most commonly used IQ tests - is based on an underlying general mental ability potential, called 'g', which is a well-supported construct based on Spearman's factor analysis. IQ tests measure 'fluid' (our inherent potential) intelligence and 'crystallised' intelligence (acquired knowledge). The notion that the totality of human intellect can be found in the results on an intelligence test is a contentious one. The principal criticisms relate to this being too reductionist, and the fact that significant cultural bias may exist in this type of assessment.

IQ tests reflect a more traditional model of intelligence which favours analytical and quantitative skills, which are generally highly valued by many societies. This contrasts with more recent models of intelligence proposed in the main by Robert Sternberg and Howard Gardner, which use a broader model including creativity and other domains (173, 174).

The content of intelligence tests differs but typically include verbal tasks such as word vocabulary, comprehension, general verbal knowledge, visual tasks such as block design, matrices; processing speed tasks and working memory tasks. The quality and accuracy of intelligence tests depend upon their underlying psychometric properties and the extent to

which the standardisation sample represents the actual population. The most commonly used tests for children are the Stanford-Binet, Wechsler, British Ability Scales and Kaufman tests. Over the past twenty years, revised versions of IQ tests have included indexes and subtests that have been more theory-driven. Most notably, they have been influenced by the Cattell-Horn-Carroll (CHC) theory of multiple cognitive abilities, which, through detailed factor analytic studies, have developed a set of broad and narrow abilities. Broad abilities include fluid intelligence, crystallised intelligence, visual processing, short-term memory, long-term storage and retrieval, auditory processing, processing speed, and quantitative knowledge. Researchers have suggested that the CHC model of abilities provides a common language or taxonomy that can be used across different IQ tests to describe the underlying constructs measured and as an important tool for interpretation of test scores (175).

Results on IQ tests are associated with genetic, environmental and epigenetic factors. One study suggests that a child whose parents are educated, and who is nurtured both physically and intellectually is 24 times more likely to have a higher IQ than a child with 4 or more environmental risk factors such as low parental education, parental mental health difficulties, command-led discipline, and/or stressful life events (Sameroff, 1989) (176).

1.7.4 Neuropsychological Processes

The functional correlates of neurological changes are not simple and need to be contextualized in terms of the impact of neurological damage on active developmental trajectories. This forms the basis for the need for neuropsychological assessment which is unique in its ability to track functional domains, which can allow for the observation of the impact of a lesion on complex cognitive processes such as the ability to remember something or selectively attend to something.

In Alexander Luria's influential text, 'the Working Brain', he described the importance of neural networks between brain regions, and how the brain processes information. "*In early childhood a lesion of a brain area responsible for relatively elementary mental activities inevitably provokes a secondary 'system' effect manifested in underdevelopment of higher superposed structures*" (Luria, 1973a, p.75, cited in Glozman (2013) pg11 (177)). His writings have influenced the development of a variety of neuropsychological assessment instruments, because he emphasised the importance of qualitative clinical observations, and believed much could be gleaned about a person's learning, through the observation of the

error patterns made on cognitive tasks, thus revealing difficulties with underlying processes (178).

Neuropsychological follow-up can also contribute to the measurement of 'high prevalence/low severity dysfunction' (p.234) termed by Aylward to describe the myriad of attention and executive behaviours that are sensitive to neonatal brain damage. It allows the measurement of mental activities at the process level. These mental activities have systemic effects across different domains of learning (177), and are less influenced by environmental factors as they more directly observe underlying physiological functions (179). Neuropsychological tasks have the advantage of measuring skills at a more basic level than IQ subtests (180), however, subtest content will require different sensory, motor and cognitive demands (the aforementioned '*elementary mental activities*') which recruit different brain regions and influence a child's performance. It is therefore critical to consider the contribution of these underlying basic element skills to the child's score (181). Indeed, these elements will have cross-domain influence. For example, if processing speed is the skill of interest, scores are dependent on the task demands, which may include memory, scanning, auditory, verbal, or visual-motor demands.

One test developed specifically developed for use with children is the NEPSY tool. It is based on a process-based Lurian model (178), within a developmental theoretical context, and not as a downward extension of an adult scale (182, 183). It measures components of attention and executive functioning, language, memory and learning, sensorimotor, visuospatial processing and social perception. The subtests contributing to each domain contain both basic and complex components. Some elements of these components will affect performance on tasks across other domain areas. The intra-domain subtest reliability correlations are also low as different tasks within the same domain areas recruit different brain regions, for example memory for faces compared with memory for names.

Patterns of deficit are age-dependent. The age at which neuropsychological outcomes are measured reflect the task performance at a certain time-point for a dynamically maturing brain, capturing measurements of brain processes at varying stages of maturation. Different brain regions are recruited at different ages for the same cognitive task, as learning becomes more automated with maturity (184). Low scores may reflect transient delay which catches up with time (71, 185), or more pervasive difficulties. Likewise, a 'normal' score may reflect a robust normal score, or a score measuring a developmentally immature skill which is not

yet sensitive to later emerging difficulties when the load on this skill increases (24, 149, 186). This example demonstrates the need for interpretation in a developmental context and the importance of repeated assessments across childhood.

1.7.5 Conclusion

This section has provided an overview of important design issues to consider for longitudinal research that aims to capture neurodevelopmental skills in a developmental context. This acknowledges the challenges faced in determining patterns of learning in a dynamically growing brain. This subsection was followed by a description of standardised developmental assessments, IQ assessments and neuropsychological tests, and their potential for the measurement of neurodevelopmental outcome.

1.8 The Current Project – Rationale and Aims

1.8.1 Rationale

The long-term outcome for children who have experienced neonatal HIE has attracted considerable research interest because of the devastating neurodevelopmental consequences experienced by a considerable proportion of these children. The era of therapeutic hypothermia hopefully marks the beginning of a new wave of potential treatments for neonatal HIE. In the meantime, ongoing research that can contribute to an enhanced understanding of the neurodevelopmental vulnerabilities in the long term can support infants and their families/carers with informed knowledge of the condition, make recommendations for the type, frequency and age at which ongoing assessment and monitoring should be offered during early and middle childhood, and may enhance understanding of the neurocognitive effects of the neural damage caused by HIE.

Much is known about the mortality and severe morbidity caused by HIE, but less is known about the long term subtle neurodevelopmental adverse sequelae in this population. This casts a light on the importance of using sensitive psychometrically valid cognitive tools that can identify these difficulties over developmental time. In addition, the time-sensitive nature of therapeutic intervention increases the importance of assessing the evolving nature of HIE in the neonatal period, especially when treatment decisions such as the administration of TH are only currently offered to those infants who meet a moderate or severe encephalopathy criteria within six hours of birth. The ability of early assessment tools such as continuous EEG

and Sarnat grading systems have taken on a new importance, and their predictive ability for later outcome becomes more valuable. This is especially true for moderate grade encephalopathy which appears heterogenous in nature, and emerging isolated long-term studies which have found neuropsychological difficulties in children diagnosed with mild HIE at birth. Because infants with mild HIE were not recruited to the recent TH RCT cohorts, their long-term outcomes have not been as well documented. Thus, the importance of the continued surveillance of **all** children with HIE is warranted.

1.8.2 Aims

The aim of this PhD thesis is to assess the long-term neurodevelopmental outcome in a cohort of term infants who have experienced neonatal HIE in an Irish-born population. These infants, who were born shortly before the widespread introduction of TH treatment, have previously received (i) detailed neurological assessments shortly after birth; (ii) early continuous EEG monitoring for up to 72 hours, commenced within 6 hours of birth; (iii) serial detailed neurological and developmental assessments up to the age of 2 years. The specific objective is to examine the association of the neurological, cognitive and neuropsychological outcome data with the early Sarnat grading and the continuous early EEG background grading. In doing so it is hoped to identify the utility of these grades in the prediction of outcome at five years. A community comparison sample of children with normal neonatal neurological and EEG findings will be assessed at five years with an identical battery of the neurodevelopmental assessments.

The specific aims of the thesis are:

1) To investigate the predictive value of serial neurological assessments in the first neonatal days with developmental outcome at 6, 12 and 24 months. It is hypothesised that later neurological assessment will better stage neonatal HIE and therefore provide more accurate prediction of outcome. Closer inspection of the ATNAT items will be undertaken in relation to their associations with five-year outcome.

2) Track the neurodevelopmental course of these children across early childhood, using detailed assessments at 6, 12, 24 and 60 months. Furthermore, to compare the predictive values of standardised developmental assessment at 6, 12 and 24 months with cognitive and overall outcome at 5 years. It is hypothesised that assessment at

24 months will provide the best prediction due to the higher order cognitive processes that are available to measure at this age.

3) To compare the five-year outcomes between the HIE and comparison cohorts of children, and to investigate the role of mild, moderate, and severe HIE assignation, graded with both early EEG and clinical assessment. Furthermore, to compare the predictive value of an EEG background feature grade assessed at 6 hours and 24 hours of age with five-year outcome.

4) (a) To describe the neuropsychological abilities of children at five years following neonatal HIE, and to investigate whether the scores of those with normal motor and IQ outcome will differ from the comparison group. It is hypothesized that neuropsychological difficulties will be present in children following HIE with otherwise normal motor and intellectual outcome;

(b) To compare the neuropsychological findings at five years of all children with all Sarnat and EEG grades with the comparison group. It is hypothesised that each Sarnat and EEG grade of the HIE group will have poorer outcome than the comparison group in a 'dose-response' fashion.

5) To improve understanding of visual outcome following HIE, by describing comprehensive vision outcome in five-year-old children to include ophthalmic and orthoptic measures, and to compare the findings with developmental and cognitive assessment.

CHAPTER 2

2 Methodology

2.1 Participants

2.1.1 HIE Sample

2.1.1.1 Neonatal Measures

The HIE sample was comprised of a prospective cohort of 65 newborn term infants (gestational age (GA) ≥ 37 weeks) who experienced neonatal HIE. They were recruited to a previous study based in three Cork maternity hospitals between May 2003 and December 2005. The inclusion criteria at birth for HIE was 2 or more of the following:

- Initial pH < 7.1;
- Apgar score < 5 at 5 minutes;
- Lactate > 7mmol/L;
- Abnormal neurology or seizure.

Infants were excluded if they presented with co-existing congenital anomalies, or significant co-morbidity.

The previous researcher (Dr. Deirdre Murray) (187) recorded comprehensive clinical data in the neonatal period. Abnormal neurology was assessed using the Amiel-Tison Neurological Assessment at Term (ATNAT). This was evaluated daily for the first three days (see Appendix J). A separate neurological examination by NICU staff at discharge was completed. This exam was graded as follows: '0' for normal, '1' for moderate with any suspect findings such as head lag, and '2' for severe signifying significant neurological abnormalities (increased peripheral tone, poor suck, marked head lag). Time to commencement of oral feeding was also recorded.

A modified Sarnat encephalopathy grade was assigned at 24 hours. The modified version was used to exclude EEG criteria as this was separately evaluated. These grades are summarised in Table 2.1 (14, 188).

Table 2.1 Modified Sarnat Score based on Levene et al. criteria (188)

Grade I (mild)	**Grade II (moderate)**	**Grade III (severe)**
Irritability	Lethargic	Comatose
'Hyperalert'	Seizures	Prolonged seizures

Grade I (mild)	Grade II (moderate)	Grade III (severe)
Mild hypotonia	Marked abnormalities of tone	Severe hypotonia
Poor sucking	Requires tube feeding	Failure to maintain spontaneous respiration

Early continuous video-EEG (v-EEG), was recorded from within the first 6 hours of life and continued until 24-72 hours. A bedside 16 channel Viasys NicOne Video-EEG system was used to record multi-channel EEG using the 10-20 system of silver-chloride electrodes placement modified for neonates (189) (See Figure 2.1). The electrodes were placed at F3, F4, C3, C4, T3, T4, O1, O2 and CZ.

The EEGs were graded according to background activity, as follows: Grade 0 (normal) - continuous background pattern with normal physiologic features; Grade 1 (normal/mild abnormalities) - continuous background pattern with slightly abnormal activity (mild asymmetry, mild voltage depression, or poorly defined sleep wake cycle (SWC); Grade 2 (moderate abnormalities) – discontinuous activity with inter-burst interval (IBI) of <10s, no clear SWC, or clear asymmetry or asynchrony; Grade 3 (major abnormalities) – discontinuous activity with IBI of 10-60s, severe attenuation of background patterns or no SWC; Grade 4 (inactive EEG findings) – background activity of < 10μV or severe discontinuity with IBI of >60s (105).

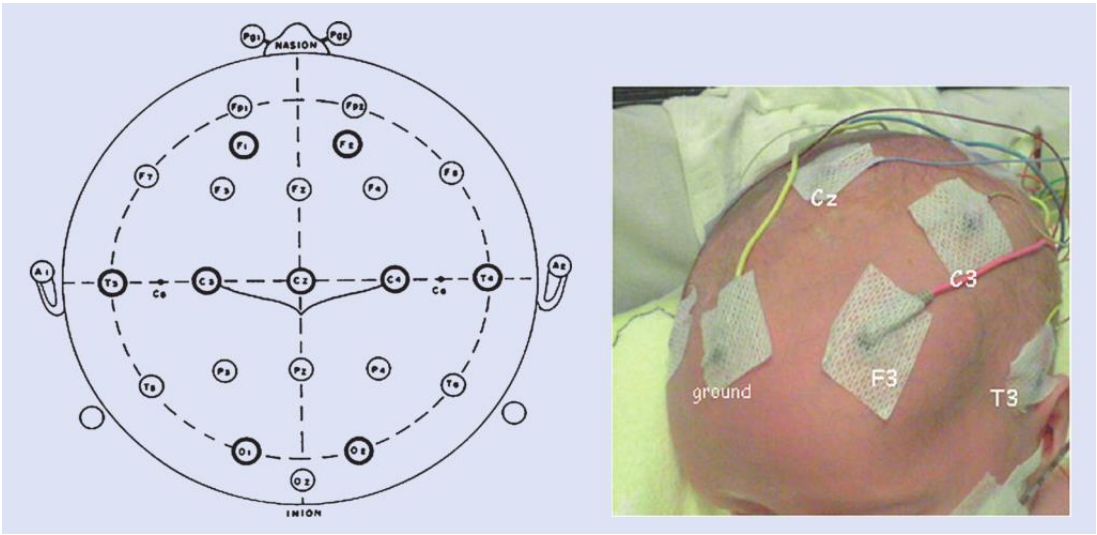

Figure 2.1 Recommended modification of 10-20 montage for neonatal EEG (189). The electrodes in bold mark the placements for the neonate. Figure from Boylan, GB. Principles of EEG. In JM Rennie, NJ Robertson, CF Hagmann (eds) Neonatal Cerebral Investigation.2008. p.3-21. Cambridge: Cambridge University Press (87). With permission.

Clinical markers for HIE were recorded. These included initial pH, base deficit and lactate levels (mmol/L) within 30 minutes of birth. Time to normal lactate, Apgar scores, and nucleated red blood cell counts were taken. Clinical data pertaining to the time of injury comprised cardiotocograph (CTG) (foetal heart trace readings) readings from the maternal file notes, and maternal antenatal data was reviewed retrospectively by Obstetricians.

2.1.1.2 Follow up at 6, 12, and 24 months

Long term neurodevelopmental outcome was measured at 6, 12, and 24 months using the Griffiths Mental Developmental Scales (0 - 2) – Revised version (GMDS-R) (172). It was used to obtain standardised developmental assessments. Children were seen by Dr. Deirdre Murray, a Griffiths' certified trained paediatrician either at home or at the outpatients' clinic. A neurological examination assessing peripheral tone, axial tone and deep tendon reflexes was completed on each occasion (see Fig 2.4 for flow-chart of HIE cohort).

The Griffiths is a standardised assessment of infant and child development and was chosen for its clinical utility, psychometric properties and ecological validity. The scales were scientifically constructed based on item analysis and developmental theory. Items are categorised into five subscales, 'Locomotor', 'Hearing and Language', 'Personal-Social', 'Eye-Hand Co-ordination' and 'Performance', with standardised subscale means ranging 100.2–101.1 (15.9–16.3) for the standardisation sample (172). The overall Griffiths' developmental quotient (GDQ) mean is 100.5 (11.8). An 'abnormal' score in this study was considered a GDQ ≤88.

2.1.2 Comparison Sample

The comparison sample was originally recruited to a birth cohort study (n=80) of healthy neonates born in the same Cork maternity hospitals as the HIE cohort between October 2005 and August 2007. These infants had been recruited in the postnatal wards at birth (by Dr. Irina Korotchikova) and the study aimed to examine normal neonatal brain functioning as measured on continuous video EEG (94). The inclusion and exclusion criteria are outlined in Table 2.2. For outcome, all children from the cohort who met the age criteria at the time of testing were contacted for follow-up (n=42). A flow chart outlining the cohort flow from birth to five can be found in Figure 2.4.

Table 2.2 Inclusion and exclusion criteria for recruitment of the comparison sample at birth.

Inclusion Criteria (comparison group)	Exclusion Criteria (comparison group)
Gestation ≥ 37 weeks	Maternal epilepsy or diabetes
No requirement for resuscitation	Birth weight < 2.5 kg
Apgar scores > 8 at 5 minutes	Congenital anomalies
Normal cord pH (>7.1)	Admission to the neonatal unit for special or intensive care

2.1.3 Sample Characteristics

The hospitals where both cohorts were born were the sole maternity service-providers to the Cork City and County areas in Southern Ireland, and served public and private patients. This was beneficial because all children resided in the hospitals' catchment area representing mixed urban/rural and SES areas, and it also controlled for potential differences in obstetric and neonatal care services. Furthermore, the comparison cohort had EEG confirmed normal neonatal neurology from which to compare our HIE group.

To examine the representativeness of the study samples, Table 2.3 outlines key neonatal and demographic variables for the HIE and Comparison groups at 5 years. Relevant information from the total HIE cohort was also available. Additionally, data from Ireland's largest national birth cohort study, the 'Growing Up in Ireland: National Longitudinal Study of Children' (190) was included, which recruited 11,100 families to their infant cohort in 2008. This provided a national reference for the comparison cohort. Of note, our Comparison group is generally consistent with the national cohort, however boys are underrepresented, and more of the births followed an unassisted delivery. The proportion of mothers who had completed secondary level education was very consistent across the groups. A detailed comparison between the HIE and Comparison group children at age five-years on clinical and demographic factors can be found in Table 5.2 in Chapter 5.

Table 2.3 Birth and demographic variables for the HIE cohort, Comparison cohort, and Growing Up in Ireland National Infant Cohort

Variable	**HIE Cohort at 5 years (n=47)**	**HIE Birth Cohort: Total (n=65)**	**Comparison Birth Cohort at 5 years (n=30)**	**Growing Up in Ireland National Infant Cohort (n=11,100)**
Gender: male n (%)	28 (59.6%)	37 (56.9%)	13 (43.3%)	(51.3%)
Birth Weight: mean (SD)	3431.0 (618.3)	3411.2 (615.8)	3567.7	3470.0
First Born n(%)	36 (78%)		11 (37%)	(41%)
Mode of delivery (NVD)	8 (17%)	14 (22%)	22 (73%)	(58%)
Age assessed at 5-year outcome: mean (SD)	64.1 (5.7)	n/a	67.6 (6.8)	n/a
Has siblings at 5 years n (%)	32 (73%)	n/a	29 (97%)	n/a
Maternal Education (completed secondary school) n (%)	35 (79%)	n/a	25 (84%)	(82%)
Parent Occupation (highest): n (%) - Professional/Manager: - Semi-professional/Technical/Clerical: - Skilled manual: - Semi-/Unskilled:	 15 (34%) 8 (18%) 8 (18%) 13 (30%)	n/a	 12 (40%) 8 (27%) 5 (17%) 5 (17%)	 (53%) (11%)

The last column contains information from the largest Irish national cohort – the Growing Up in Ireland Study. Findings were presented as percentages in the research report thus 'n' values not included in the cells of this column. Parent occupation categories were not directly relatable to the 'social class' categories in the national cohort so only equivalent category information is included. SD = standard deviation, NVD = normal vaginal delivery.

As with all research including a control or comparison group, the individual characteristics of parents/carers who are more likely to consent to participate in research cannot be controlled for but needs to be acknowledged. Additionally, a confounding variable is the willingness and/or motivational factors of parents of sick infants versus healthy newborn babies in the differential impact of decision making around informed consent (191, 192).

2.2 Materials

This section outlines the materials used for the five-year follow-up study which forms the basis for this PhD thesis. The term '*current researcher*' or '*I*' is used to clarify which aspects of the work that I (i.e. Catherine O'Connor) independently undertook.

The materials were researched and chosen by the current researcher for use in this thesis. The psychometric tools chosen for the five-year outcome are described in detail below in order to acknowledge the importance of reliability and validity issues when considering their use and interpretation in the research setting.

2.2.1 Consent Forms

The parent/carer consent forms consisted of a description of the purpose of the consent form, the nature and duration of the research procedures, potential risks and benefits and possible alternatives. Two versions of the consent form were created, one for the parents of the HIE cohort (Appendix D), and a second version for the parents of the comparison cohort (Appendix H).

2.2.2 Parent Questionnaire

The Parent Questionnaire (see Appendix I) was devised by the current researcher to obtain information in relation to each of the participant's (i) relevant medical information, (ii) need for early intervention services such as occupational therapy, physiotherapy, speech and language therapy, and/or psychology and (iii) demographic data. The medical section sought to gather information about adverse medical experiences in the intervening period since the child was last seen by the research team at 24 months of age. Questions about hospital admissions, experience of head injury, seizures/epilepsy, current medications and health conditions such as asthma and allergies were included. The demographic questions related to number of siblings and birth order, nationality/ethnicity, first language, marital status at

birth and currently, maternal education level, occupation and household income. The demographic questions were adapted from questions used by the Irish Government's Central Statistics Office national census 2006 questionnaires (193).

2.2.3 The Wechsler Preschool and Primary Scale of Intelligence – 3rd Edition (UK version) (WPPSI-III^{UK})

2.2.3.1 Introduction to the WPPSI-III^{UK}

The WPPSI-III (Figure 2.2) is an individually administered clinical instrument for assessing the intelligence of children aged 2 years 6 months to 7 years 3 months, and is used worldwide (194). The third version is a major revision of the test, providing updated standardised norms and adhering more closely to a hierarchical model of intelligence based on the Cattell-Horn-Carroll (CHC) model (182). There are two age-range bands (2-6 to 3-11, and 4-0 to 7-3), which use different subtests to obtain composite quotients. The administration of the core subtests yields a Verbal IQ (VIQ), Performance IQ (PIQ) and Full-Scale IQ (FSIQ). An additional Processing Speed Quotient (PSQ) is calculated for the older age band. A General Language Composite (GLC) is calculated using supplemental/optional subtests.

The complete test for the 4-0 to 7-3 age band comprises core, supplemental and optional subtests which are summarised in Table 2.4. The core subtests are used to calculate the VIQ, PIQ, and FSIQ. The FSIQ = VIQ subtests (information, vocabulary, word reasoning) + PIQ subtests (block design, matrix reasoning, picture concepts) + one PSQ subtest (coding). There are two verbal supplemental subtests (comprehension, similarities), two performance supplemental subtests (picture completion, object assembly) and one processing speed subtest (symbol search). The optional subtests - receptive vocabulary and picture naming – are used to calculate the GLC.

The WPPSI-III^{UK} was chosen for a number of reasons in addition to its strong psychometric properties. Firstly, for its clinical utility as it is the most commonly used IQ test for young children in Irish educational and clinical contexts. Secondly, it includes a domain for processing speed which an important skill shown to be vulnerable in children with neurological conditions. Thirdly, for comparison purposes with other research studies that use this tool including the Toby TH trial. The continued use of the Griffiths scales at age five was decided against because although the extended revised version (2006) can be used with

children to 8 years, it essentially comprised two tests where the 2-8 year section was restandardised at a different time and with a different sample to the 0-2 year version. In addition, an IQ rather than a DQ (developmental quotient) was deemed more useful for this age cohort.

Figure 2.2 WPPSI-III^{UK} - Wechsler Preschool and Primary Scale of Intelligence - 3rd UK Edition. Image from Pearson Education Ltd. Available at: https://www.pearsonclinical.com/psychology/products/100000422/wechsler-preschool-and-primary-scale-of-intelligence--third-edition-wppsi-iii.html

2.2.3.2 Administration and Scoring of WPPSI-III^{UK}

The WPPSI-III^{UK} is individually administered test of IQ, and a qualified psychologist is required for its interpretation. The complete battery with supplemental tests takes approximately 90 minutes to complete and includes a mixture of verbally presented and visually presented materials. Answers are given orally, through pointing, or the fine motor manipulation of blocks and pencil and paper tasks. The standard subtest administration order was used. It was scored by transferring raw scores to the summary score sheet (see Appendix K) and hand scored to calculate scaled and standardised scores from the reference group tables provided in the manual.

Table 2.4 Domains and subtests of the WPPSI-III^{UK}

Domain (bold) and Subtest	Type	Description and how assessed
Verbal (VIQ)		
Information*	Core	Learn and remember general factual knowledge. Child answers questions that tap wide general knowledge
Vocabulary*	Core	Word knowledge and verbal concept formation. Child provides definitions for words read aloud
Word Reasoning*	Core	Verbal reasoning. The child asked to identify the common concept being described in a set of clues.
Comprehension	Supl.	Verbal reasoning and ability to use past information and demonstrate practical behaviour; verbal comprehension and expression. The child answers questions based on knowledge of general knowledge and social principles.
Similarities	Supl.	Verbal reasoning and concept formation. Child is asked how 2 concepts are similar or share a common characteristic. Ability to categorise.
Performance (Visual/non-verbal) (PIQ)		
Block Design*	Core	Analyse and synthesise abstract visual stimuli. The child uses blocks to recreate designs presented first in 3D and then 2D.
Matrix Reasoning*	Core	A matrix analogy test measuring fluid intelligence. The child is asked to complete various pattern matrixes.
Picture Concepts*	Core	Abstracts, categorical reasoning ability. The child chooses a picture from each row of pictures which share a common characteristic.
Picture Completion	Supl.	Visual perception and organisation; attention. The child views a picture and then points to or names the missing part.
Object Assembly	Supl.	Visual-perceptual organisation. The child is presented with puzzle pieces, and is timed to assemble the whole.
Processing Speed (PSQ)		
Coding*	Core	Processing speed, short-term memory, visual perception, visual-motor coordination. The child, in a timed task, copies symbols that are paired with geometric shapes.
Symbol Search	Core for PSQ	Processing speed, short-term visual memory, visual-motor coordination, cognitive flexibility, visual discrimination. The child scans a search group and marks whether a target symbol is represented in the search group.
General Language Composite (GLC) – optional		
Receptive Vocabulary	Opt.	Comprehend verbal directives, auditory discrimination and processing. The child looks at group of four pictures and points to the one presented aurally.
Picture Naming	Opt.	Expressive language ability, word retrieval from memory and visual association. The child is asked to name a sequence of pictures.

*subtests used to calculate the full-scale IQ (FSIQ) score. Supl = supplemental; Opt = optional. Table adapted from WPPSI-III^{UK} Administration Manual Table 1.2 p. 3 (194) and WPPSI-III^{UK} Technical Manual pp. 25-30 (195).

2.2.3.3 Psychometric Properties of WPPSI-III^{UK}

The WPPSI-III was normed on a United States sample of 1,700 children, the 'UK' version normed on a representative sample of 805 children, which closely approximated the UK 2001 Census data on similar demographic variables. Reliability and validity information is outlined extensively in the WPPSI-III Technical and Interpretative Manual (195). The average internal consistency values for individual coefficients are .95 for VIQ, .93 for PIQ, .89 for PSQ, and .96 for FSIQ (196). Test-retest reliability is fairly stable (VIQ 0.91; PIQ 0.86; FSIQ 0.92; PSQ 0.86; GLC 0.91). Construct validity is supported by factor-analytic studies and with comparisons with other assessments of cognitive functioning. One criticism of the test is that it doesn't include a robust measure of working memory for young children (197).

2.2.4 The NEPSY II

Figure 2.3 The NEPSY-II neuropsychological assessment tool. Image from Pearson Education Ltd. Available from: https://www.pearsonclinical.co.uk/Psychology/ChildCognitionNeuropsychologyand Language/ChildGeneralAbilities/NEPSY-SecondEdition(NEPSY-II)/NEPSY-SecondEdition(NEPSY-II).aspx

2.2.4.1 Introduction to the NEPSY-II

The NEPSY-II assesses neuropsychological development in children aged 3 to 16 years (198). It originated in the 1980s in Finland and evolved throughout the 1980s and 1990s leading to the publication of the NEPS in Finland and the NEPSY in America in 1998. This assessed children aged 3-12 years on a broad range of tasks assessing five neuropsychological

domains, namely, attention and executive functioning, language, memory and learning, sensorimotor and visuospatial processing (182). What distinguishes it from other children's neuropsychological assessment batteries is that it was designed exclusively for children, rather than depending on downward extensions of adult-based tasks (182). Based on Lurian theory, it uses a process-oriented approach to explain cognitive development (178). Cognitive areas are viewed as complex capacities mediated by interacting functional systems. When one subcomponent is impaired then complex functions can be impacted upon and sometimes performance can be affected across functional domains.

It was designed to detect subtle difficulties both across and within subtest domains; to track effects of congenital and acquired brain damage, and the lower age limit allows the study of development in preschool aged children (199).

The NEPSY-II was published in 2007 and constitutes a major revision of the NEPSY. It covers a broader age range of 3-16 years. It includes a new domain – social perception - in addition to the original theoretically derived five domains outlined above. There are 32 subtests in total. Subtests with lower psychometric properties have been replaced by subtests with greater clinical sensitivity and construct validity. Primary, combined, process and contrast scores can be calculated, depending on the selection of subtests chosen for administration.

The rationale for including the NEPSY-II, was that in addition to its sound psychometric properties it was created within a child developmental context. It allows for cross comparison with other longitudinal studies that have used NEPSY and NEPSY-II including the NICHD and Toby TH trials (16, 139). A large set of subtests are included in this study (see Section 2.2.4.2 below) representative of the Attention & Executive Functioning, Language, Memory & Learning, Sensorimotor and Visuospatial Processing domains to provide a detailed age-specific NP profile of children at age five years. It was important to include not only neuropsychological processes previously shown to be associated with HIE, such as memory (see Chapter 1 for review), but equally to include less well understood areas for this age group to provide a cross-domain profile. These detailed profiles are necessary at different ages to improve our understanding of the emerging nature of these skills across childhood, and to build knowledge of potential phenotypes following HIE. Currently, the knowledge base is reliant on a mere handful of well-designed studies in this area.

2.2.4.2 Administration and Scoring of NEPSY-II

The NEPSY-II is individually administered test, and a qualified psychologist is required for its interpretation. There is a core battery of subtests, and also specific batteries recommended for a variety of referral queries, for example suspected dyslexia or attention difficulties. The subtests administered in this study are described in Table 2.5, which include all subtests of the core battery and additional subtests with the highest clinical sensitivity. Due to a lack of published data on the social perception scale it's subtests were excluded.

It was scored by transferring the raw scores from the record form (see Appendix L) to the NEPSY-II scoring assistant software (see Appendix M).

Table 2.5 NEPSY-II domains and subtests

Domain (bold) and Subtest	**Abbr.**	**Description and how assessed**
Attention and Executive Functioning		
Auditory Attention	AA	Selective auditory attention and the ability to sustain it (vigilance). The child listens to a series of words and touches the appropriate circle when he/she hears the target word.
Design Fluency	DF	Behavioural productivity in the child's ability to generate unique designs by connecting up to five dots, presented in 2 arrays: structured and random. The child draws as many designs as he/she can on each array within a specified time limit.
Inhibition	IN	inhibit automatic responses in favour of novel responses and the ability to switch between response types. The child looks at a series of shapes or arrows and names either the shape or direction or an alternate response, depending on the colour of the shape or arrow.
Statue	ST	Motor persistence and inhibition. The child is asked to maintain a body position with eyes closed during a 75-second period and to inhibit the impulse to respond to sound distractors.
Language		
Comprehension of Instructions	CI	Receive, process, and execute oral instructions of increasing syntactic complexity. The child points to appropriate stimuli in response to oral instructions.
Phonological Processing	PH	Phonemic awareness such as identifying words from word segments; Phonological Segmentation - phonological processing at the level of word segments (syllables) and letter sounds (phonemes). The child is asked to repeat a word and then to create a new word by omitting a syllable or a phoneme, or by substituting one phoneme in a word for another.
Repetition of Nonsense Words	RN	Phonological encoding and decoding. The child repeats nonsense words presented aloud.
Speeded Naming	SN	Rapid semantic access to and production of names of colours, shapes, and sizes. The child is shown an array of colours and shapes; and

		colours, shapes, and sizes. He/she names them in order as quickly as possible.
Word Generation	WG	Verbal productivity - ability to generate words within specific semantic and initial letter categories. The child is given a semantic category and asked to produce as many words as possible in a limited time.
Memory and Learning		
Memory for Designs Memory for Designs Delayed	MD MDD	Spatial memory for novel visual material. The child is shown a grid with four to eight designs on a page, which is then removed from view. The child selects the designs from a set of cards and places the cards on a grid in the same location as previously shown. A delayed task assesses long-term visuospatial memory.
Memory for Faces Memory for Faces Delayed	MF MFD	Encoding of facial features, face discrimination and recognition. The child looks at a series of faces and then is shown three photographs at a time from which he/she selects a face previously seen. A delayed task assesses long term memory for faces.
Memory for Names Memory for Names Delayed	MN MND	Learn the names of children over 3 trials. The child is shown six cards with drawings of children on them while being read the child's name. The cards are shown again, and the child is asked to recall the name of the child on the card. A delayed task assesses long term memory for names.
Narrative Memory	NM	Memory for organised verbal material under free recall, cued recall and recognition conditions. The child listens to a story and is then asked to repeat the story. The child is then asked questions to elicit missing details from his or her recall of the story.
Sentence Repetition	SR	Repeat sentences of increasing complexity and length. The child is read a series of sentences and asked to recall each sentence immediately after it is presented.
Sensorimotor		
Fingertip Tapping	FT	Finger dexterity and motor speed. The second part assesses rapid motor programming. The child copies a series of finger motions demonstrated by the examiner as quickly as possible.
Imitating Hand Positions	IH	Imitate hand/finger positions. The child imitates various hand positions demonstrated by the examiner.
Manual Motor Sequences	MM	Imitate a series of rhythmic movement sequences using one or both hands. The child repeats a series of hand movements demonstrated by the examiner until the required number of movements is completed.
Visuomotor Precision	VP	Graphomotor speed and accuracy. The child uses his/her preferred hand to draw lines inside of tracks as quickly as possible.
Visuospatial Processing		
Design Copying	DC	Motor and visual-perceptual skills associated with the ability to copy two-dimensional geometric figures. The child copies figures displayed in the Response Booklet.
Geometric Puzzles	GP	Mental rotation, visuospatial analysis, and attention to detail. The child is presented with a picture of a large grid containing several shapes. For each item, the child matches 2 shapes outside of the grid to two shapes within the grid.

Amended version of Table 2.1 in NEPSY-II Clinical and Interpretive Manual pp 21-23 (200).

2.2.4.3 Psychometric Properties of NEPSY-II

The NEPSY-II aimed to improve and expand the cognitive domains, enhance the clinical utility, improve the psychometric properties, and simplify its administration (201). It was standardised on a single, well-stratified sample of 1,200 U.S. citizens with exclusion criteria including neurological disorders (200). A Finnish standardisation has also been completed. Most, but not all subtests underwent re-standardisation for the 2nd version. Separate clinical samples were also investigated for the revision. Its principal strength being one of the only paediatric neuropsychological tests with wide domain coverage using subtests that are co-normed and can be cross-compared (201).

The internal reliability overall is very good. Across the age groups they range from adequate to very high (200). In the 5-6 year group, six subtests have very high reliability. One subtest – memory for faces – was low for this age. For children of this age with a clinical diagnosis, reliability coefficients of r= 0.80 and higher are given (201). Test-retest correlations range from adequate to high. Concurrent validity has more evidence with comparisons with intelligence (WISC-IV), and neuropsychological tests (CMS and D-KEFs), and enhanced evidenced for discriminant validity with its analysis of clinical samples (202).

2.2.5 Children's Memory Scale (CMS)

The Children's Memory Scale (203) was published in 1997 and was developed as a downward extension of the adult Wechsler Memory Scale (WMS). It assesses learning and memory in those aged 5 – 16 years. It evaluates the processes of the learning and subsequent retention of information in different modalities. There are nine subtests which assess functioning in three domains: (i) auditory/verbal learning and memory (verbal), (ii) visual/nonverbal learning and memory (visual), and (iii) attention/concentration. It covers short-term memory, working memory and long-term memory which include immediate and delayed recall and recognition tasks. It acknowledges the central importance of attention to this process. The working memory model assumed in this test is that of Baddeley and Hitch (1995) which postulates a short-term memory system which allows for the temporary retention and manipulation of a limited amount of information, in order to complete a cognitive task (204).

Recent research related to children's intellectual functioning has acknowledged the important role 'working memory' in children's development. Despite the many strengths of the choice of WPPSI-III^{UK} as the intellectual abilities measure for this research, some have commented on the removal of subtests from the previous edition that assessed working memory ('arithmetic' and 'sentences'), resulting in no assessment of working memory in the WPPSI-III^{UK} as a weakness of this tool (196). To redress this, the 'numbers' subtest from the attention/concentration domain of the CMS was administered as a replacement. This subtest in addition to the *memory and learning* subtests of the NEPSY-II provide a comprehensive assessment of memory.

The numbers subtest assesses the ability to repeat random digit sequences of graduated length. In the 'Forwards' portion, the child repeats the digits in the same sequences as heard. In the 'Backward' portion, the child repeats the digits in the reverse order of that presented (203) (See Appendix O). The subtest measures verbal short-term and working memory.

The CMS was standardised on a stratified U.S.A. nationwide sample of 1,000 'normally functioning' children using the 1995 US Census information. The CMS has been linked to the Wechsler intelligence scales using a sample of 300 children. This allows for discrepancy analysis between intellectual and memory needs and abilities (195). The reliability coefficient for the Numbers total score is 0.79 (forwards 0.74 and backwards 0.82). Test retest stability coefficients were only provided for indexes. In this case attention/concentration corrected for variability was 0.85. In comparison/discrimination studies with other psychometric tools, the attention/concentration index correlated highly with other measures of complex attention, moderately with measures of general cognitive ability, and a strong relationship to academic achievement more so than the verbal and visual long-term memory tasks. Convergent validity was demonstrated in the studies using other childhood memory tests on equivalent subtests (203).

Special group studies carried out with clinical samples suggest that the CMS is very sensitive to dysfunction caused by brain anomalies. The attention/concentration index unsurprisingly was most affected by epilepsy, ADHD and specific language impairment.

The CMS Numbers raw score is converted to an age-related standard scaled score of 10(3).

2.2.6 Interpretative Features of Psychometric Tests

When using psychometric tools - such as the use of WPPSI-III^{UK}, NEPSY-II and CMS in this study - it is important to remain cognizant of the issue of test purity. Standardised tests measure constructs (such as memory) by developing tasks that typically require input (e.g. the instructions), 'thinking', and output ('the answer') by the examinee. Although tests with high validity seek to measure constructs in a pure sense, all tests are burdened with measurement error and pure assessment of a mental construct in isolation to other constructs is nigh on impossible (e.g. in order to remember something it must first be attended to). A second feature of test content is to include a set of items that spans a full range of age-related performance in order to achieve satisfactory floor and ceiling levels. This often necessitates a set of items that differ in some way in their task demands. For example, the vocabulary subtest of the WPPSI-III^{UK} includes picture items and verbal items. Finally, scaled and standardised scores are comprised of raw scores accrued from different tasks. For example, the CMS Numbers scaled score represents the scores of two tasks, namely, digit forwards (a short-term memory task) and digit backwards (a working memory task). Thus, the Numbers scaled score masks the individual contributions made by the underlying tasks, which may include individual areas of impairment. An awareness of these issues is important for the understanding of the limitations of test interpretation, and an acknowledgement of which underlying skills are influencing subtest or domain level performance.

2.2.7 Neurological Assessment

The neurological assessment was undertaken by one of three consultant paediatricians (Dr. Deirdre Murray, Dr. Evonne Low, Dr. Louise Gibson). They assessed muscle tone, power and deep tendon reflexes. A GMFCS (Gross Motor Function Classification System) score (205), was assigned to children with cerebral palsy.

2.2.8 Vision Assessments

Vision assessments were undertaken by a consultant ophthalmologist (Dr. Mark James) and demographic, clinical, visual, orthoptic, biometric and refractive data was measured. See Information Letter in Appendix P.

Clinical data additional to that already described included: family history of ocular disorders; and anterior segment and fundal findings on slit lamp biomicroscopy. Visual data included: visual acuity from a Sheridan-Gardner or Snellen chart, and logMAR chart if appropriate; and confrontation visual fields assessment using a target gradually moved in from the periphery until detected by the child, while maintaining fixation on a central target. Orthoptic data was assessed by an experienced paediatric orthoptist (Beatrix Haskins), and included: ocular alignment on cover testing; angle of squint as determined by prism cover testing; ocular motility, including convergence; and presence or absence of stereopsis as determined by the Lang II stereo test, scored out of four.

All biometric and refractive measurements were performed by Dr. James. Cycloplegic autorefraction was performed 30 minutes following instillation of a drop of cyclopentolate 1% to each eye, with the use of one of three autorefractors available at different times during the study. These were (i) the NIDEK Auto Refractometer AR-630A (Nidek CO., Japan), (ii) the Topcon RM-A3000 Auto Refractometer (Topcon, Tokyo, Japan), or (iii) the NIDEK Auto Refractometer AR-800 (Nidek CO., Japan). Data reliability was checked by performing cycloplegic streak retinoscopy using a Welch Allyn retinoscope (Welch Allyn Medical Products, Skaneateles Falls, New York). Results recorded in sphero-cylindrical form, including axis, were used to calculate the spherical equivalent (SE) refractive error for each eye. Ocular biometry using the IOLMaster (Carl Zeiss Meditec, Jena, Germany) recorded the average of 10 valid readings of axial length and 15 keratometry readings for each child. Keratometric values were the mean of the steepest and flattest meridian in dioptres (D) in each eye.

Visual impairment was defined as a presenting vision (i.e. unaided or with glasses if worn) of less than 6/12 in the better seeing eye. Ametropia was defined as the presence of one or more of the following in either eye: myopia if SE refraction ≤ -0.5 D; hyperopia if SE refraction ≥ +2.0 D; and astigmatism if cylinder power ≥ 1.5 D. Anisometropia was defined as an interocular difference in SE of ≥ 1.0 D.

2.3 Experimental Design

This quantitative follow-up study employed a prospective cohort observational design. Children were followed longitudinally from birth to 5 years. For temporal reasons, the researcher was aware of the group membership (i.e. HIE vs comparison) of each child, but importantly was blinded to ALL other data collected from previous studies except for the

child's name, parent(s)' name, contact details, and date of birth. The current study assessed neurological status, intellectual ability, neuropsychological functioning, visual function and behavioural and executive functioning carer ratings of the HIE and comparison groups at age five.

This researcher was responsible for all aspects of the five-year assessment protocol, chose the neurodevelopmental assessment tools, designed the parent questionnaires, recruited the children and families at age five and assessed, scored and interpreted all assessments with the exception of the orthoptic/ophthalmic and neurological assessments.

2.4 Proposed Data Analysis

Quantitative descriptive and inferential statistics, and clinical prediction measures will be the principal forms of data analysis employed in this thesis. Statistical analysis was completed using IBM SPSS Statistics V12.0, 20.0, 22.0 for MS Windows (IBM Corporation, NY, USA)s and VassarStats: Website for Computational Statistics (Lowry, R 1998-2018, Vassar College, NY, USA). Some of the ocular data in Chapter 7 was analysed using Stata package V8.0 (StataCorp. 2005; Stata Statistical Software: Release 9.0; College Station, TX, USA).

The relationship between repeated administrations of the ATNAT (ordinal) in the first three days of life, neonatal discharge exam and time to oral feeding with 24 months GMDS-R outcome (continuous) and with overall outcome (ordinal) will be examined using Spearman's rank order correlation.

The relationship between repeated administrations of the GMDS-R at 6, 12 and 24 months (continuous) with 5-year WPPSI-III^{UK} outcome (continuous) and with overall outcome (ordinal) will be examined using Spearman's rank order correlations.

Between-group differences in scores of outcome dependent variables at age five years across categorical independent variables assigned in the neonatal period will be analysed using the non-parametric Kruskal-Wallis H Test. These include:

i. WPPSI-III^{UK} domain scores, NEPSY-II subtest scores and the CMS Numbers subtest scores at five years across Sarnat grades assigned at 24 hours of age;

ii. WPPSI-III^{UK} domain scores, NEPSY-II subtest scores and the CMS Numbers subtest score at five years across EEG grades assigned at 6 and 24 hours of age.

Pair-wise post hoc comparisons of the significant results will be analysed using Mann-Whitney U Tests. All *p* values are 2-tailed with statistical significance level set at $p<.05$ except for the NEPSY-II subtest scores. To reduce the chance of Type 1 error caused by multiple comparisons (potentially 25), a Bonferroni correction for post hoc testing is made for the subset of subtests within each NEPSY-II domain (Attention and Executive Functioning: .05/5=.01; Language: .05/5=.01; Visuospatial Processing (.05/2=.025); Memory and Learning: .05/8=.006); Sensorimotor: (.05/5=.01).

Mann-Whitney U Tests were additionally used for the investigation of differences between: NEPSY-II subtest scores in the HIE and Comparison cohorts; NEPSY-II subtest scores in the HIE intact-at-five-years group and Comparison cohort; Griffiths and WPPSI-III scores in the HIE group with normal and abnormal 5 year ophthalmic outcome.

Clinical utility of prediction will be analysed firstly by creating a set of binary 'normal/abnormal' variables, by collapsing the scores by cut-off range (see Table 2.6). Next, ATNAT classification, and neonatal discharge exam for predicting 2-year outcome, and Sarnat, EEG Grade, and GMDS-R scores for predicting 5-year outcome will be calculated using PPVs (Positive Predictive Values), NPVs (Negative Predictive Values), sensitivity, specificity, and AUROC (area under the receiver operated characteristic curve) with 95% confidence intervals. (IMPORTANT NOTE: In Chapters 3 and 5 these clinical utility measures are used in the conventional fashion, i.e. PPV to analyse proportions of children with an abnormal early score that have an abnormal later score (206). However, in Chapter 4 these measures are inverted, i.e. to determine the value of an early normal score for later normal score. To reduce confusion this will be highlighted again for the reader in Chapter 4).

Table 2.6 Definitions of normal and abnormal cut-off levels for each test used and for the overall outcome variable.

Assessment	'Normal' category	'Abnormal' category
ATNAT	= 0	= 1 or 2
Sarnat	= 0	= 1-3
EEG Grade	= 0-1	= 2-4

Assessment	'Normal' category	'Abnormal' category
Neonatal discharge exam	= 0	= 1-2
GMDS-R	DQ ≥ 88	DQ < 88
WPPSI-III^{UK}	FSIQ ≥ 85	FSIQ <85
Overall 5yr outcome	None of: death, CP, IQ<85, sensory impairment NDD, EI multidisciplinary team	At least one of: death, CP, IQ<85, sensory impairment NDD, EI multidisciplinary team

ATNAT = Amiel-Tison Test of Neurological Assessment at Term, GMDS-R = Griffiths Scale of Mental Development – Revised, WPPSI-III^{UK} = Wechsler Preschool and Primary Scale of Intelligence 3rd UK version, CP = cerebral palsy, FSIQ = Full-scale IQ, DQ = Griffiths developmental quotient, NDD = neurodevelopmental disorder, EI = early intervention.

The birth data (e.g. birthweight, mode of delivery) and demographic data gathered at age five for the HIE and Comparison cohorts will be described using means and standard deviations for continuous variables and frequencies for categorical variables. Differences between these cohorts will be explored using parametric pairwise comparisons using independent t-tests for continuous variables and Chi-square test for independence for categorical variables. One-sample t-tests are used to compare observed WPPSI-III^{UK} scores to the test norms.

Fisher's exact (and Freeman Halton extension) statistics will be used when minimum cell frequency is violated in any of the Chi-square calculations. Effect sizes in this study are calculated as follows: Cohen's d for independent Samples T-Test, Phi coefficient and Cramer's V for Chi-square, $r = z/\sqrt{N}$ where N = no. of cases for Mann-Whitney U tests (207).

2.5 Procedure

2.5.1 Funding and Ethical Approval

The previous researcher (Deirdre Murray) had identified the potential importance of the follow-up of this HIE cohort of children beyond 24 months. It provided a unique opportunity to investigate the long-term emerging subtle neurological sequelae of this group as identified in other long-term follow up studies (71, 75, 117). A submission was made, and funding granted from the Health Research Board of Ireland (HRB), grant number: RP/2008/238. The research protocol, entitled "*Ability of early continuous EEG to predict long-term neurological*

outcome at 4.5 years in infants following neonatal hypoxic-ischaemic encephalopathy" was produced and submitted to the Clinical Research Ethics Committee of the Cork Teaching Hospitals, University College Cork. Ethical approval was granted in January 2008 (reference: ECM4 (hhh) 05/02/08, see Appendix A). When the current researcher was recruited to the study an ethics amendment was submitted in November 2008 (see Appendix B) due to changes I made to the tests to be used in the five-year protocol (namely the addition of WPPSI-III^{UK} and NEPSY-II). In 2010, an ethics amendment submission was made to a separate birth cohort study (protocol title: "*Sleep patterns of full-term babies in the early postnatal period*" - related to EEG, sleep-wake cycles in the 'normal' neonate) to seek permission to recruit these children at age five as a comparison sample (see ethics proposal Appendix E, and approval (reference ECM 3 (bb) 06/12/11), Appendix F).

Confidentiality of the participants was assured by anonymising identifying data through the use of a numeric unique identifier for each participant. Hard copies of record forms were stored in locked filing cabinets within a secure office at UCC accessible only by secure ID cards. The electronic SPSS database file was similarly pseudonymised and stored on an encrypted drive.

2.5.2 Recruitment for 5-year follow-up

In the HIE cohort, there were 65 children recruited to the original study. Of those, 6 died and 2 were excluded, with 6 lost to follow-up by age 24 months (see Figure 2.4 which provides a flowchart of cohorts from birth). The remainder of the cohort of 51 infants with HIE were invited to participate in the current study by letter updating parents/carers about the status of the study and explaining that funding had been received to continue the follow-up. All families had been previously informed at 24 months follow-up that there was a possibility that the study would be continued pending appropriate funding (see letter in Appendix C). It explained that vision assessments would be offered in the forthcoming weeks and that neurodevelopmental and neurological assessments would be offered when the child turned five years. A follow-up telephone call was then made by this researcher to each of the 51 families. In cases where parents reported that they had changed residence, a copy of the information was sent to the new address. In cases where telephone numbers were obsolete, the child's hospital number was used to contact the family's general practitioner for current contact details.

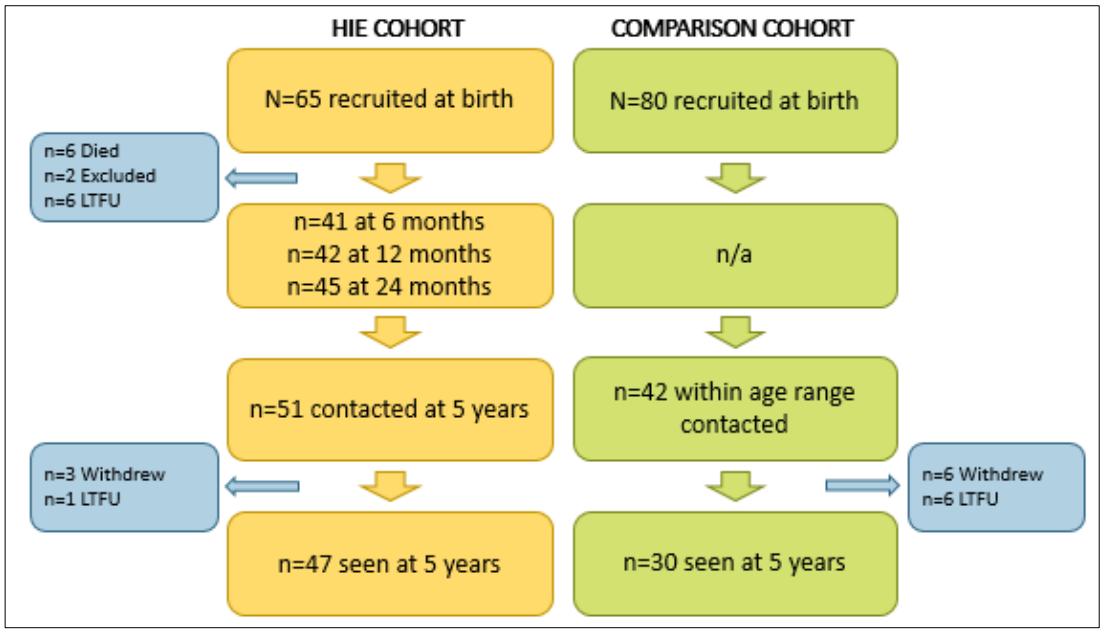

Figure 2.4 Flowchart for the HIE and Comparison cohorts from birth to five-year outcome assessment. LTFU=lost to follow-up.

The comparison birth cohort (see Figure 2.4) comprised 80 infants. A subset received a separate letter (from the original researcher to protect anonymity) describing the purpose of the HIE study and outlining the function and importance of a comparison group for longitudinal research. A summary of the proposed commitment and assessment details were included with an invitation for inclusion of the child (see Appendix G).

For both groups, parents were asked whether the child had been assessed by a psychologist in the preceding year. If this was the case, the researcher sought permission to contact the child's psychologist to ascertain which if any standardised measures had been used with the child. In cases where the WPPSI-III^{UK}/other IQ assessment (n=6) or NEPSY-II (n=0) had been administered the researcher used the results from the previous assessment to avoid the practice effects of re-administration (see Appendix N).

Once verbal consent was received, two initial appointments were made at a suitable time with a third organised if required. Each family was given the choice to have the assessment carried out in a clinical room in the health sciences building at University College Cork or at the child's home. Funding was made available to support transport and parking costs.

2.5.3 Assessment Process

At the initial appointment, the researcher read the written consent form to the parent, and time was given to clarify information and answer questions. Once informed consent was agreed, and researcher signed two copies of the consent form, one for the parent to keep. The parent was then invited to complete the parent questionnaire with the researcher. Parallel to this process, the researcher sought to engage the child in order to build rapport.

The focus then turned to the child and time was spent building rapport and asking social questions including whether the child was in preschool/school. A brief description of the expected activities was described. Although no formal informed assent was collected from the child, the current researcher asked the child if he/she wished to be involved and was assured that they could stop at any time. The current researcher only continued if she felt the child was comfortable based on her many years working as a psychologist assessing young children. The parent(s) were encouraged to leave the room for the process of the assessment. However, as is typical for this young age group, many parents remained in the room for some, or all of the testing. In these cases, it was explained that due to the nature of the way in which standardised tests are structured that no assistance from the parent could be given to the child.

During session one, the complete WPPSI-III^{UK} was administered in strict accordance with the administration guidelines in the WPPSI-III administration manual (194). The subtests were administered in the order in which they appeared in the assessment form, as recommended by the authors. The 'Numbers' subtest from the CMS was then administered. At the end of the session brief verbal feedback was given to the parent to describe a subjective evaluation of the child's test behaviours, and a rough estimate of the child's intellectual performance on the assessment.

During session two, verbal feedback from the first session was given to the parent re the WPPSI-III^{UK} and CMS scores. Selected subtests from the NEPSY-II were then administered to the child. The following order was used: (i) Memory for Names (immediate), (ii) Auditory Attention, (iii) Repetition of Nonsense Words, (iv) Memory for Designs (immediate), (v) Memory for Names (delayed), (vi) Comprehension of Instructions (vii) Design Copying, (viii) Visuomotor Precision, (ix) Memory for Designs (Delayed,) (x) Memory for Faces (immediate), (xi) Fingertip Tapping, (xii) Geometric Puzzles, (xiii) Imitating Hand Positions, (xiv) Memory

for Faces (Delayed), (xv) Inhibition, (xvi) Manual Motor Sequencing, (xvii) Narrative Memory, (xviii) Speeded Naming, (xix) Statue, (xx) Sentence Repetition, (xxi) Phonological Processing, (xxii) Design Fluency, (xxiii) Word Generation.

The Memory & Learning subtests that contained an immediate and a later administered delayed component (typically 15-25 minute interval) were administered early to ensure successful administration of the delayed subtests in cases where the session finished early. In cases where the child began to lose interest or tired of the verbal or longer activities the administration order was altered to maintain rapport. When required, a third session was organised to complete NEPSY-II administration. The NEPSY-II technical manual reports on subtest order studies that demonstrate that the administration order has no significant association with the NEPSY-II results (200).

At the end of session two, the research paediatrician completed a neurological assessment with the child. At the final session, the parent and child were thanked for their time and contribution, and the parent was provided with feedback or informed that a future telephone feedback session would be completed, with a written copy to follow in the post where requested. The parent was encouraged to contact the researcher by telephone at any time in the future if further queries arose. In cases where there were areas of difficulty assessed for the child, a one-to-one feedback appointment was arranged with information about and referral to an appropriate clinical and/or educational follow-up service.

To minimize the effect of fatigue in the children, sessions were broken up (e.g. time for a snack) to suit the children to ensure maximum chance of achieving the best performance of a child without compromising the standardised administration procedures. An important factor in the project was the ample time afforded to the researcher to organise additional appointments and times that suited the families and the option of doing home-based assessments.

CHAPTER 3

3 The Predictive Value of Early Neurological Examination in Neonatal Hypoxic Ischaemic Encephalopathy and Neurodevelopmental Outcome at 24 Months.

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3.1 Abstract

Aim:

The clinical and electrographic signs of hypoxic-ischaemic encephalopathy evolve over the first days of life. We examined the evolution of neurological signs over the first 3 days of life, and determined whether serial administration of the Amiel-Tison neurological assessment at term (ATNAT) would predict neurodevelopmental outcome at 24 months. Associations between Individual ATNAT items on day 3 and five-year IQ outcome were also explored.

Methods:

Term neonates born with suspected HIE between May 2003 and May 2005 in a Cork maternity unit were recruited prospectively. Modified Sarnat grading was assigned. The ATNAT was administered on days 1, 2 and 3 of life and a discharge neurological examination. Time to oral feeding and demographic variables were recorded. Developmental status was assessed using the Griffiths Mental Development Scales-Revised at 6, 12, and 24 months. IQ was assessed at five years using the WPPSI-III^{UK} scales.

Results:

From a birth cohort of sixty five children, n=57 infants who had reached 24 months, were invited for follow-up. Of these, 4 were lost to follow up and 2 excluded. Thus, 51 (31 male and 20 female) were included. Of these 6 had died, resulting in 76% (45/59) assessed that had survived from the birth cohort. Neurological examination evolved and normalised over the first three days of life in many cases. At 24 months, 41% (21/51) children had an adverse outcome, (i.e. developmental delay, CP or death). Examination at all time points correlated significantly with neurological outcome at 24 months. The best correlations were found to be (i) neurological examination at discharge (r=0.65, p<0.001), (ii) Sarnat grading (r=0.64, p<0.001) and (iii) ATNAT on day 3 (r=0.46, p<0.001). The best predictive value was seen with neurological examination at discharge (PPV and NPV of 72% and 86%) for 24-month outcome. Day 3 ATNAT correlated with five-year IQ (r=-0.47, p<0.01) as did 9 of the individual items, the highest being palmar grasp and crying.

Interpretation:

Persistence of abnormal neurological signs correlates significantly with adverse outcome. The later a neonatal neurological examination was performed, the better its predictive ability. Four ATNAT items correlated as well or better than the overall ATNAT score for five-year IQ outcome. This study demonstrates that neurological symptoms quickly evolve in the neonatal period, with the requirement for repeated measurement and a future focus for research to better understand which early neurological test items remain constant and which evolve in the first hours and days of life.

COMMENT: It is important to note that this chapter is partially based on the previous original work of Dr. Deirdre Murray for her PhD thesis. Dr. Murray undertook the analysis at 24 months and these findings were previously included in chapter 7 of her thesis (187). Additionally, Ms. Claire Thompson, Medical Student UCC supported the preparation of the individual item ATNAT scores and the preliminary statistics including the preparation of the scatterplot diagrams. My role in the production of this chapter was as follows: I prepared the manuscript for publication and wrote-up the content of Dr. Murray's chapter for article submission, double-scored all Griffiths results, and expanded some of the statistical interpretation and presentation of the results at 24 months. I recruited and assessed all of the infants at age five years, and summarised and interpreted all of the five-year outcome data outlined later in the chapter.

This is described in further detail in Chapter 8, Section 8.1: Summary and Impact of the Thesis Findings.

3.2 Introduction

HIE affects 3-5 per 1000 live births. In the severe form, it remains one of the leading causes of neonatal death, and approximately 25% will experience severe neurological disability including cerebral palsy, epilepsy, intellectual disability, and sensory loss (208). Outcome can be difficult to predict and is traditionally described using the clinical Sarnat score assigned at 24 hours post-delivery (14). Whilst the Sarnat score is useful at the extremes (mild and severe grades), it is more difficult to predict outcome in those infants with a moderate Sarnat grade of encephalopathy. Although the Sarnat grade provides a single summary score, the neurological signs observed in HIE continue to evolve over the first 72 hours of life.

The Amiel-Tison Neurologic Assessment at Term (ATNAT) (60) has been developed to provide a framework for observing the development of cortical control in the infant at term and has been shown to predict the occurrence of cerebral palsy following birth asphyxia (61, 62). Previous studies have not examined repeated measurements over the first days of life. The central tenet of the Amiel-Tison is that the stage of maturation of the two motor control systems (subcorticospinal and corticospinal) can be clinically evaluated in the term infant (63). Amiel-Tison, author of the ATNAT, recommends serial assessments for two reasons: (i) to reduce the confounder of transient clinical signs caused by medical treatments, and metabolic processes; (64), (ii) to discriminate between a 'static' neurological profile more usually seen in infants with prenatal damage, and an 'evolving' profile more commonly seen in HIE and 'normal' babies (65).

In a NICU setting, the clinician requires quick, reliable and valid tools to assess the sick infant. The ATNAT takes approximately 15 minutes to complete and contains 35 individual items. Interobserver reliability values from two separate studies suggest excellent agreement for 16 items (209, 210), with one study reporting good agreement for the remaining items also (209), whilst the second report the majority of the remaining items with fair to good reliability with lower reliability on two final items (210). Amiel-Tison reported that three items – called the 'triad' may be particularly useful for prediction because they may mark lower cognition in later childhood (69). In a study of babies following preterm birth, those children with abnormalities in the AT triad displayed poorer IQ scores at age five (211).

The aims of this study were (i) to examine the evolution of neurological signs in term infants with HIE over the first three days of life, (ii) to investigate the ability of these repeated serial

assessments to predict neurodevelopmental outcome at 2 years, and (iii) to analyse the relative importance of items of the ATNAT for outcome at five years.

3.3 Method

This prospective study was carried out in a large maternity service with approximately 6000 deliveries per annum as part of a study into early continuous video-EEG monitoring (212). Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Between May 2003 and May 2005, term infants (> 37 weeks gestation) with HIE were recruited if they fulfilled 2 or more of the following criteria: initial capillary or arterial pH < 7.1, Apgar score < 5 at 5 minutes, initial capillary or arterial lactate > 7 mmol/L or abnormal neurology/clinical seizures. Neurology was assessed by dedicated research paediatricians not involved in the clinical care of the infant. The parents of infants fulfilling the criteria were approached and written informed consent was obtained. Infants were typically recruited within 6 hours of birth, with the remainder recruited at the onset of clinical seizures. Participants were excluded if they had co-existing congenital anomaly, or significant co-morbidity.

Initial neurological assessment was administered using the Amiel-Tison Neurologic Assessment at Term (ATNAT) (60). Early neurological status continued to be monitored with repeated administration of the ATNAT daily for three consecutive days. The ATNAT consists of examination of 35 items (see Table 3.1) describing cranial characteristics, alertness, behaviour and spontaneous activity, passive tone in the limbs and axis, active tone, and primary reflexes (see Appendix J for a copy of the ATNAT assessment form). Scores are assigned for each item as follows: 0 = 'within normal range', 1 = 'a moderately abnormal result', 2 = 'a definitely abnormal result', and are assigned each time the ATNAT is administered. The scores are not used in a quantitative way, but rather are designed to develop an opinion of the infant's neurological status based on the most commonly observed scores. The final synthesis is described as normal in the absence of neurological signs, or presence of minor abnormalities (without CNS depression), moderate (with CNS depression) or severe (deep CNS depression and repeated seizures for > 30mins) for the term infant (64).

Table 3.1 Amiel-Tison Neurological Assessment at term (ATNAT): List of items for assessment (64).

Domain	Item for measurement	
Cranial Assessment	Head circumference	
	Anterior fontanelle	
	Squamous sutures	
	Other sutures	
Neurosensory Function and Spontaneous Motor Activity	Fix and track	
	Ocular signs	
	Response to voice	
	Social interaction	
	Crying	
	Excitability	
	Convulsions	
	Spontaneous motor activity	
	Spontaneous thumb abduction	
Passive Muscle Tone	Upper Limbs:	Recoil
		Scarf
	Lower Limbs:	Recoil
		Popliteal angle
	Right-left	Asymmetry
	comparisons:	Ventral incurvation (flexion)
	Body axis:	Dorsal incurvation (extension)
		Comparison of curvatures
Axial Motor Activity (active tone)	Righting reaction (lower limbs and trunk)	
	Raise to sit (neck flexor muscles – head forward)	
	Reverse manoeuvre (neck extensor muscles – head backward)	
Primitive Reflexes	Non-nutritive sucking	
	Palmar grasp	
	Automatic walking	
	Moro reflex (only used when other reflexes asymmetrical/absent)	
	Asymmetric tonic neck reflex (ATNR)	
Palate and Tongue	High arched palate	
	Fasciculations of tongue (peripheral, at rest)	
Adaptedness to Manipulations During Assessment	Stability	
Feeding Autonomy	Term newborn	
Medical Status at the Time of Assessment	Assisted ventilation	
	Anticonvulsant drugs	
	Phototherapy	
	Other	

The majority of the ATNAT assessments were undertaken by the research Paediatrician (Dr. Deirdre Murray), with the remainder completed by a designated experienced neonatal

paediatric registrar (Dr. Pronab Bala). In the event that some of the items could not be administered (e.g. sucking, Moro reflex, axial tone assessment) due to endotracheal intubation, difficulties were recorded, and the assessment attempted again at a later stage.

Additional neurological assessment consisted of a designation of a modified Sarnat score assigned at 24 hours (Table 3.2) (188, 213).

Table 3.2 Modified Sarnat Score.

Grade I **(mild)**	**Grade II** **(moderate)**	**Grade III** **(severe)**
Irritability	Lethargic	Comatose
'hyperalert'	Seizures	Prolonged seizures
Mild hypotonia	Marked abnormalities of tone	Severe hypotonia
Poor sucking	Requires tube feeding	Failure to maintain spontaneous respiration

Source: Levene et al. (1985) (188).

Each infant underwent a final neurological examination by the neonatal staff at discharge from the neonatal intensive care unit (NICU). The examination was graded as '0' for normal if there were no abnormalities seen, '1' for moderate if there were any suspect findings, such as mild head lag, or '2' for severe if there were significant neurological abnormalities (increased peripheral tone, poor suck, marked head lag). The date that oral feeding was fully established was also recorded.

3.3.1 Long-term neurodevelopmental follow-up

Neurodevelopmental follow up was carried out at 6, 12, and 24 months using the Griffiths Mental Development Scales – Revised (GMDS-R) (172) for children aged 0-2 years. The scales were administered by the Griffiths' trained paediatricians (DM, and some by PB), either at the outpatients' clinic, or in the participant's home, in addition to a neurological examination assessing peripheral, axial tone and deep tendon reflexes. Maternal educational level and socio-economic status were recorded. At five years, children were invited back for neurocognitive assessment including IQ testing using the WPPSI-III^{UK}. This tool measures overall IQ (FSIQ), Verbal IQ, Performance IQ, Processing Speed quotient (PSQ) and General

Language Composite (GLC). A more comprehensive description of the five-year outcome assessments is described in Chapters 2 (methodology) and Chapter 5 (main 5-year outcome chapter).

For this study, an abnormal outcome at 24 months was defined as developmental delay (GDQ<=87, (mean score minus 1SD), cerebral palsy or death.

3.3.2 Statistical Analysis

Statistical analysis was completed using IBM SPSS Statistics V12.0 and V20.0 for MS Windows (IBM Corporation, NY, USA) and VassarStats: Website for Computational Statistics (Lowry, R 1998-2009, Vassar College, NY, USA). Descriptive statistics (means and standard deviations, or frequencies) were calculated for birth weight, gestation, and mode of delivery. Evolution of ATNAT scores across the first 3 days was presented in stacked bar charts. Correlations between neonatal signs and 24-month outcome were assessed using non-parametric Spearman's rho rank correlation in preference to Chi-square test for independence, to analyse the ordinal nature of the categories. Their predictive utility were assessed using the area under the receiver operator characteristic curve (AUROC), positive predictive value (PPV), negative predictive value (NPV), specificity and sensitivity of binary values. For associations between ATNAT items and five-year IQ, Pearson's Product Moment correlation was used. Statistical significance was taken as $p < 0.05$.

3.4 Results

From a birth cohort of 65, n=57 infants who had reached 24 months of age at the time of the study, were included. Of these, 4 were lost to follow up, giving a two year follow up rate of 82%. In addition, two infants were excluded due to uncertainty regarding diagnosis of HIE. One had concurrent congenital diaphragmatic hernia repaired and one was investigated for dysmorphic features and hypotonia. Figure 2.4 in Chapter Two provides a flowchart of the cohorts over time.

The remaining 51 infants consisted of 31 males and 20 females, with a mean (SD) birth weight of 3,416 (634) grammes, and a mean (SD) gestation of 40.1 (1.5) weeks. The mode of delivery was normal vaginal delivery in 12 (23.5%), forceps in 5 (9.8%), vacuum in 10 (19.6%), combined vacuum and forceps in 5 (9.8%), and emergency caesarean section in 19 (37%).

Their assigned Sarnat grades were I (mild) in 24 cases, II (moderate) in 18 cases and III (severe) in 9 cases.

3.4.1 Early neurological examination and 24-month outcome.

The results of the early neurological examinations, observed for each participant and his/her diagnostic and developmental outcome at 24 months, are displayed in Table 3.3 (children following an abnormal NICU discharge exam) and Table 3.4 (children following a normal NICU discharge exam).

Table 3.3 Neurological examination and outcome details of participants who died or obtained an abnormal discharge examination (Case 1-22).

Case no	***ATNAT Day 1***	***ATNAT Day 2***	***ATNAT Day 3***	***Oral Feed***	***Discharge exam***	***Sarnat Grade***	***GDQ - 24mth***	***Outcome***
1	2	1	n/a	2	n/a	3	86	LD
2	2	died	died	No	Died	died	died	Died
3	2	died	died	No	Died	died	died	Died
4	2	2	2	No	Died	3	died	Died
5	2	2	2	No	2	3	50	SQ
6	1	0	0	3	2	1	95	MD
7	1	2	2	11	2	3	68	hemi/GDD
8	1	1	1		2	2	68	hemi/GDD
9	2	2	1	7	2	2	102	Hemi
10	2	2	1	6	2	2	52	GDD
11	2	1	1	8	2	2	84	GDD
12	1	1	1	4	2	2	99	Diplegia
13	2	2	2	No	2	3	died	Died
14	2	2	2	No	2	3	died	Died
15	2	2	2	No	2	3	died	Died
16	NMB	NMB	1	5	2	2	113	0
17	2	2	1	7	2	2	116	0
18	2	2	2	6	1	2	103	Hemi
19	1	1	1	5	1	2	50	GDD
20	2	2	1	3	1	2	102	0
21	1	1	1	2	1	1	112	0
22	1	1	1	2	1	1	121	0

ATNAT = Amiel-Tison Neurologic Examination at Term, GDQ = Griffiths Developmental Quotient, NMB = neuromuscular blockade, n/a = not available, oral feed = days from birth to establishment of oral feeding, SQ = spastic quadriplegia, MD = motor delay, GDD = global developmental delay, LD = language delay, hemi = hemiplegia, 0 = normal result/outcome.

Table 3.4 Neurological examination and outcome details of participants who obtained a normal discharge examination (Case 23-51).

Case no	*ATNAT Day 1*	*ATNAT Day 2*	*ATNAT Day 3*	*Oral Feed*	*Discharge exam*	*Sarnat Grade*	*GDQ - 24mth*	*Outcome*
23	2	2	2	3	0	2	71	GDD
24	2	2	1	11	0	2	83	GDD
25	1	0	0	6	0	1	74	GDD
26	1	0	0	2	0	1	87	GDD
27	2	2	1	5	0	2	119	0
28	2	2	0	5	0	2	117	0
29	2	1	0	3	0	2	98	0
30	2	1	0	3	0	1	113	0
31	2	1	0	2	0	2	101	0
32	1	1	1	6	0	2	120	0
33	1	1	1	4	0	2	109	0
34	1	1	1	4	0	1	109	0
35	1	1	1	1	0	1	112	0
36	1	1	0	5	0	1	103	0
37	1	1	0	4	0	1	109	0
38	1	1	0	4	0	1	111	0
39	1	1	0	3	0	1	109	0
40	1	1	0	3	0	1	100	0
41	1	0	0	5	0	1	110	0
42	1	0	0	3	0	1	113	0
43	1	0	0	3	0	1	119	0
44	1	0	0	3	0	1	112	0
45	1	0	0	2	0	1	102	0
46	1	0	0	2	0	1	113	0
47	1	0	0	2	0	1	113	0
48	0	1	0	3	0	1	113	0
49	0	0	0	3	0	1	108	0
50	0	0	0	1	0	1	117	0
51	0	0	0	1	0	1	110	0

ATNAT = Amiel-Tison Neurologic Examination at Term, GDQ = Griffiths Developmental Quotient, oral feed = days from birth to establishment of oral feeding, GDD = global developmental delay, 0 = normal result/outcome.

In the first day of life, 21/51 (41%) had a 'severely abnormal' ATNAT score of 2. Of this group, by day 2 of life, 2/21 had died, and of the surviving infants, 14/19 continued with a score of 2, whilst 5/19 had improved to a score of 1. By day 3, only 7/19 continued with a score of 2, and all of these experienced a poor outcome (4 deaths, 3 with cerebral palsy, and 1 with global developmental delay). These findings are summarised in Figure 3.1. An additional child who had an ATNAT score of 1 on day 1 had deteriorated to a score of 2 for day 2 and 3. This child was later diagnosed with CP and developmental delay.

On day 1, 25/51(49%) had a 'moderately abnormal' ATNAT score of 1. By day 2, of these, 14/25 remained with ATNAT = 1, and by day 3, 9/25 continued to have an ATNAT = 1. These findings are summarised in Figure 3.2. By day 3 for the whole group, 17 infants had ATNAT = 1 (9 from the Day 1 ATNAT=1 group and 7 from the Day 1 ATNAT=2 group and 1 without a day 1 ATNAT score). Of these, 7/17 had abnormal outcomes at 24 months; 4 children with global developmental delay, 1 with hemiplegia and developmental delay, 1 with diplegia and 1 with an isolated hemiplegia.

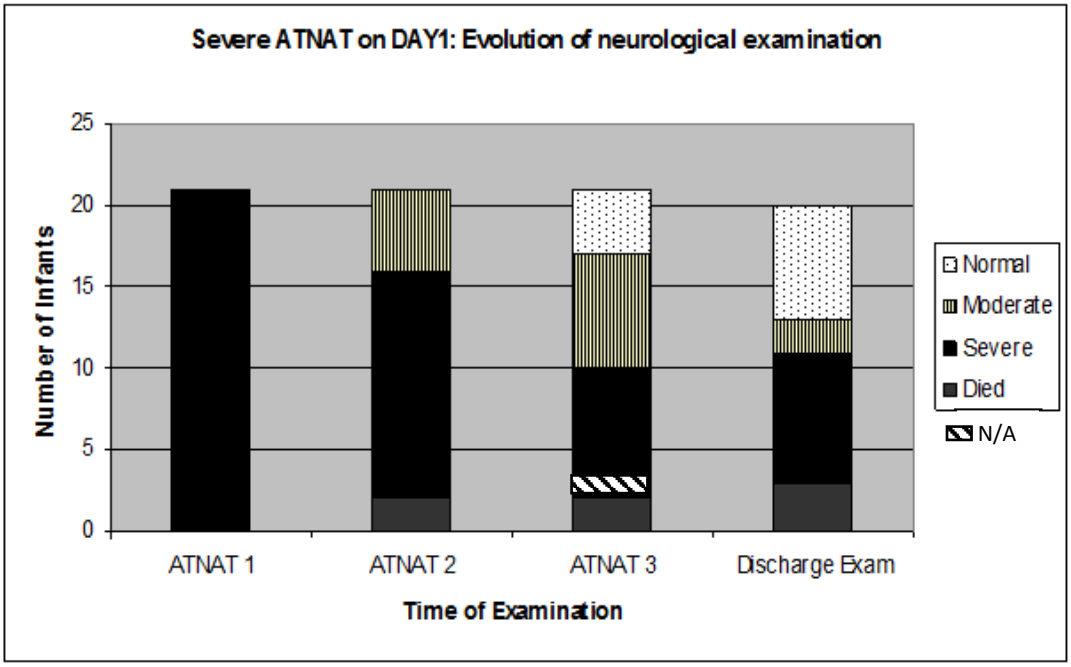

Figure 3.1 The evolution of infants with a severe neurological abnormality on Day 1, assessed using the ATNAT. ATNAT1, ATNAT2, ATNAT3 = Amiel-Tison neurological assessment at Term day 1, 2 and 3 respectively. Normal = ATNAT score = 0, moderate = ATNAT score = 1, Severe = ATNAT score = 2. Discharge exam = neurological examination at discharge from the NICU.

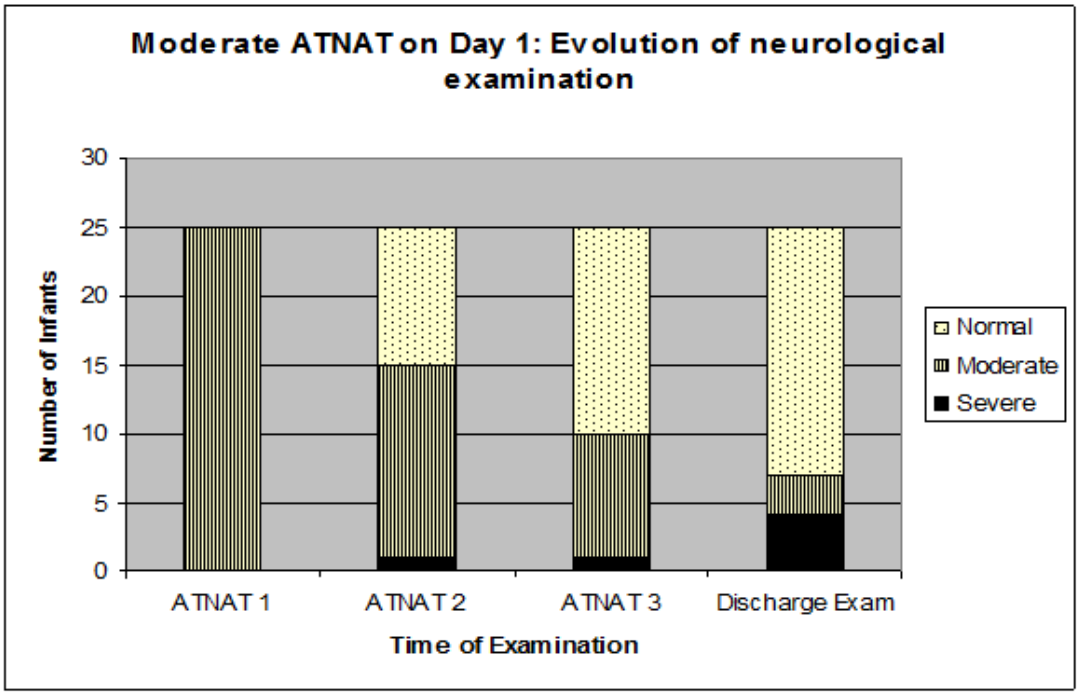

Figure 3.2: The evolution of infants with a moderate grade neurological abnormality on Day 1, assessed by ATNAT. ATNAT1, ATNAT2, ATNAT3 = Amiel-Tison neurological assessment at Term day 1, 2 and 3 respectively. Normal = ATNAT score = 0, moderate = ATNAT score = 1, Severe = ATNAT score of 2. Discharge = neurological examination at discharge from NICU.

A small group of infants, 4/51 (8%) had normal ATNAT assessments on day 1, all with normal 24 month outcomes.

In the whole series, by day 3 of life, 23/49 (47%) of the surviving infants had normal ATNAT neurological assessments. Of this group, 3/23 had mild developmental delay at 24 months.

Examination at discharge was performed on the final day of the infant's stay in NICU by research or medical staff. This assessment did not use the structured ATNAT and each baby was assigned a grade of normal, moderate or severe based on their clinical findings. The age of the infants varied from 2 to 27 days, with the sicker infants remaining hospitalized longer. Data was missing for one infant, and three infants died prior to NICU discharge. Of the remaining 47, discharge neurological examination was entirely normal in 29/47 (62%), in 5/47 (11%) moderate abnormalities were seen. The remaining 13 infants (28%) continued to have severely abnormal examinations on discharge from NICU.

There was a significant correlation ($p < 0.01$) between neurological outcome at two years and ATNAT on days 1, 2 and 3 of life. In addition, outcome correlated with Sarnat score, and examination at discharge (Table 3.5). The best correlation was seen between outcome and examination at discharge, followed by Sarnat score assigned, and finally ATNAT on day 3. GDQ scores at 24 months differed significantly depending on ATNAT score on day 3. The mean (SD) GDQ scores were 106.4 (10.5) in the normal ATNAT day3 group, 98.3 (22.9) in the moderately abnormal ATNAT day3 group, and 61.5 (18.96) in the severely abnormal ATNAT day3 group.

Table 3.5 Correlation between early neurological assessment and 24 month outcome.

Examination	**Correlation with Outcome Status***		**Correlation with GMDSR Dev. Quotient**		**AUROC for Outcome Status**	
	***ρ* value (n=47)**	***p*-value**	***ρ* value (n=44)**	***p*-value**	**(95% CI)**	***p*-value**
ATNAT day 1	0.45	0.001	0.43	0.002	0.652 (0.47-0.84)	0.132
ATNAT day 2	0.42	0.003	0.40	0.004	0.652 (0.45-0.86)	0.132
ATNAT day 3	0.46	0.001	0.59	0.001	0.754 (0.58-0.93)	0.012
Sarnat grade	0.64	0.001	0.61	0.001	0.744 (0.57-0.92)	0.016
Discharge exam	0.65	0.001	0.50	0.001	0.786 (0.64-0.96)	0.005

*Outcome status = Normal or abnormal 24 month outcome, ρ = rho value = correlation coefficient using Spearman's Rank correlation, GDQ = general quotient of GMDS-R at 24 months, ATNAT day 1 = Amiel-Tison Test of Neurological Assessment at Term on day 1 of life, ATNAT day 2 = ATNAT score on day 2 of life, ATNAT day 3 = ATNAT score on day 3 of life. AUROC = area under the receiver operated characteristic curve.

AUROC curves were statistically significant for ATNAT day 3, Sarnat score, and highest for discharge examination. In summary, the later that a neurological assessment was performed, the more reliable was its prediction of outcome.

To determine whether differences existed between the correlation coefficients of the neonatal tests (that had significant AUROC values) and the 24 month GDQ and overall

outcomes, the t-statistic was used as the comparison between dependent coefficients (214). No differences were found in the correlation strengths between the neonatal tests and 24-month GDQ scores, however, Sarnat grade (z=3.52, p<.01) and the discharge exam (z=2.26, p=.012) had stronger correlations than ATNAT day 3 for 24-month outcome. Within each of the individual neonatal tests, only ATNAT day 3 had a stronger correlation for one of the 2 year outcomes, suggesting a better ability to correlation with GDQ than with overall 24-month outcome (z=2.53, p<.01).

Neurological outcomes at 24 months correlated with discharge examination (ρ = 0.65, p < 0.001). Of the 13 severely abnormal participants, 11/13 had abnormal outcomes at follow up. Of the 5 moderately abnormal, 2/5 had abnormal outcomes. Of the 29 infants with normal examinations at discharge, 25/29 (86%) were normal at 24 months. This gave a positive predictive value (PPV) of an abnormal examination at discharge of 72% (95% C.I.: 0.46-0.89), and a negative predictive value (NPV) of 86% (95% C.I.: 0.67-0.95). Sensitivity was calculated at 77%, and specificity at 83%.

Within the subgroup of 18 infants with Sarnat Grade II, or moderate encephalopathy, examination at discharge was still moderate or severely abnormal in 10/18. An abnormal examination at discharge predicted an abnormal outcome with a PPV and NPV of 70% (95% C.I.: 0.35-0.92) (7/10) and 75% (95% C.I.: 0.36-0.92) (6/8) respectively.

3.4.1.1 Oral feeding and 24-month neurological outcome

Time to establishment of oral feeding (days from birth) correlated significantly with neurological outcome (ρ=0.40, p=0.008). In 7/51 (14%) infants oral feeding was never established; 3/7 died in the first week of life, prior to NICU discharge. The 4 surviving infants were discharged with nasogastric feeding tubes. Of these 4 children, 3 have died and the sole survivor has severe cerebral palsy (spastic quadriplegia). Three further children took more than 7 days to establish feeding, and all of these infants had abnormal outcomes; 1 with isolated hemiplegia, and 2 with global developmental delay.

3.4.2 ATNAT items and five-year outcome

We were interested to further analyse the ATNAT day 3 findings, and to examine whether they were related to five-year cognitive outcome in our HIE cohort. The outcome

measurement used was the Full-Scale IQ (FSIQ) from the WPPSI-III^{UK}. From the cohort, at the time of preliminary analysis, 34 were included in this arm of the study.

Each of the individual 35 items of the ATNAT were analysed using Pearson's Product Moment correlation statistics to assess associations between ATNAT items and five-year FSIQ score. Of these, only nine items correlated significantly (p<0.05) with FSIQ. Table 3.6 provides a summary of these findings.

Table 3.6 ATNAT total score and items that correlate significantly with 5-year WPPSI-III^{UK} Full Scale IQ

ATNAT vs. FSIQ	Pearson coefficient (r)	Level of significance (p-value)	Shared variance r^2
ATNAT Total	-0.47	<0.01	0.22
Palmar Grasp	-0.65	<0.01	0.42
Crying	-0.63	<0.01	0.40
Lower Limb Recoil	-0.49	<0.01	0.24
Upper Limb Recoil	-0.47	<0.01	0.22
Upper Limb Scarf	-0.45	<0.01	0.20
Fix and Track	-0.43	<0.05	0.18
Response	-0.40	<0.05	0.16
Social Interaction	-0.40	<0.05	0.16
Lower Limb Angle	-0.35	<0.05	0.12

For the ATNAT total, and the individual items that had a shared variance of at least 20%, the results are displayed graphically in Figure 3.3 and Figure 3.4.

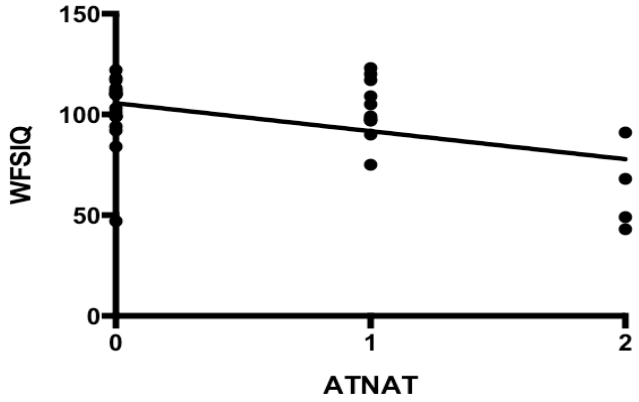

Figure 3.3 Correlation between the global ATNAT score measured on day 3 of life in the HIE cohort and global IQ outcome at age five years.

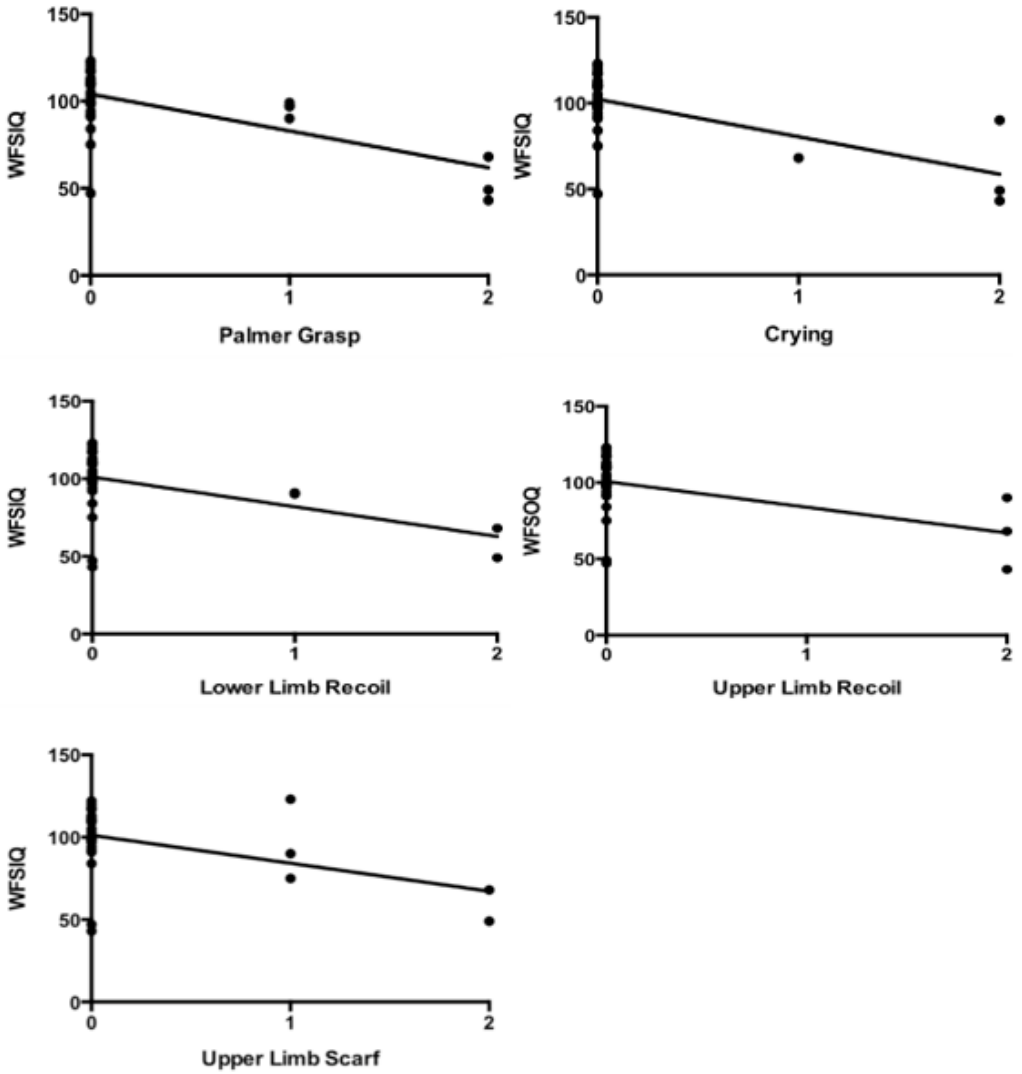

Figure 3.4 Correlation between ATNAT individual items (palmar grasp, crying, lower limb recoil, upper limb recoil, upper limb scarf) measured on day 3 of life in the HIE cohort and global IQ outcome at age five years.

With respect to the ATNAT scores, the 'palmar grasp' item had the highest shared variance for all but one (PIQ) of the WPPSI-III^{UK} domains – (r^2 values were 0.42 for FSIQ, 0.33 for VIQ, 0.40 for PIQ, 0.40 for PSQ, 0.37 for GLC). With respect to the FSIQ and the other IQ domains, Performance IQ had the highest shared variance for all but one (palmar grasp) of the nine significant ATNAT items.

3.5 Discussion

This study demonstrates the pattern of neurological evolution in infants with hypoxic-ischaemic encephalopathy over the first three days of life. Neurological examination normalised in many infants with 47% having a normal examination by day three. A normal early assessment predicted a normal outcome at 24 months. Those infants with persistent neurological abnormality on day three were more likely to progress to neurodevelopmental disability. This risk was 100% in those with severely abnormal ATNAT rating, and 41% in those with a moderately abnormal rating

With respect to five-year outcome, the ATNAT total score and nine of the individual items measured on day 3 of life correlated negatively with IQ at five years.

A number of methods to assess neonatal neurological status have been described (56, 57, 215). The Amiel-Tison method was chosen because it is quickly and easily administered, examines forebrain function and has been previously shown to correlate with outcome at 12-15 months, in particular to outcome following perinatal asphyxia (62, 65). A further strength of the ATNAT, is the inclusion of items that assess early observations of infant behaviour such as sucking. Neonatal examination used in combination with a modified Sarnat score at 6 hours of age with amplitude integrated EEG (aEEG) has been found to enhance aEEG's ability to predict neurological outcome at one year (66). Amess et al. also found that neonatal neurological assessment was a useful adjunct to magnetic resonance spectroscopy, with late examinations (> 7 days) having superior predictive sensitivity of 12-month outcome than early examinations (< 48 hours) (62).

The current study found that the later that a neurological examination was performed, the greater the correlation with neurological status at 24 months. In terms of correlation strength, the ATNAT day 3 was on a par with Sarnat and the discharge exam for mental

development at 24 months. An abnormal examination at the time of discharge placed the infant in a high-risk group, with 72% progressing to developmental delay. In the moderate subgroup, the discharge examination was particularly useful. In infants with moderate Sarnat scores, the overall rate of abnormal outcome was 50%. However, this was 25% in those with a normal discharge examination, and 70% in those with an abnormal discharge examination. Thus, a clinician may find the neurological status at discharge useful in counselling parents and guiding long-term follow-up. Sarnat scoring, or ATNAT assessment at 24 hours combined with a detailed examination at discharge could provide outcome indicators to physicians who do not have access to more sophisticated methods of prognostication. The utility of detailed neonatal neurological assessment in these situations has been previously demonstrated in the developing world (81, 216). Our data confirms the importance of structured neonatal neurological examination and its ability to aid prognostication.

Why does the discharge examination appear to be more reliable in predicting outcome? A number of factors may interfere with accurate neurological assessment in the first few days of life. Sicker infants may be intubated, and/or sedated. Hypotension, shock and multi-organ failure may lead to abnormalities of tone and movement (217). Many of our infants received anticonvulsants and sedatives. One infant who had meconium aspiration syndrome was ventilated and received neuromuscular blockers for the first 2 days of life, making neurological assessment impossible. By discharge most of these confounders will have abated, helping to explain the increased sensitivity (77%) of the abnormal examination at discharge, compared with earlier examinations. Notably, one limitation was that the discharge examination was performed and recorded by different medical staff. These examinations were also performed at different ages. This highlights the importance of a detailed, consistent discharge examination of the neurological status of these high-risk infants.

The individual contribution of each item of the ATNAT for the prediction of outcome deserves further scrutiny. The nine items found to be significant in this study were related to passive muscle tone (upper and lower limb recoil, upper limb scarf and lower limb angle), neurosensory elements (crying, visual fix and track, response and social interaction) and the palmar grasp primitive reflex. The cranial items were not significant, and this is consistent with the finding from others that it is too early to detect cranial dysmorphisms or a head which may not grow as expected postnatally (218). As outlined earlier, NICU care and

sedation can affect the manifestation of reflexes, however palmar grasp was more easily elicited and significantly associated with five-year IQ outcome. The set of items with the greatest correlation were for passive muscle tone and neurosensation (crying, fix and track, response to voice and social interaction). Hypotonia is a central feature of neurological assessment and differences in severity between upper and lower limbs can aide diagnosis and prognostication (127). The infants' alertness indicated by the neurosensory observations also correlated with five year IQ and may reflect intact cortical control (64). In this study upper and lower limb recoil scores had similar if not higher correlation than the ATNAT global score for five-year outcome. Active tone, Gosselin et al. argue is a better marker for higher control (basal ganglia and hemispheres) and descends with time, than for lower motor control of the brain stem and cerebellum. These axial motor activities were not significant with IQ outcome, however axial tone can be affected by transient abnormal posture (64).

A limitation of the administration of the ATNAT exclusively in the neonatal period is that it precludes access to later-elicited responses (e.g. postural reactions) which may possess superior predictive qualities (67). Further analysis of the individual items of the ATNAT may reveal markers for later outcome, because it includes behavioural and communication items, which share construct validity with later developmental assessments (68). The 'Amiel-Tison triad' of signs, administered in the first 18 months, for example, can suggest damage due to a focal lesion. There have been promising observations in relation to premature infants and clusters of ATNAT signs that may predict subtle neurological outcome (219). Detailed analysis of early clinical signs is dependent on what can be elicited in a neurological examination. At birth this is limited by the level of maturation of the baby at that time, and what their nervous system can display. A recent study of low risk infants assessed serially at 3, 6, and 48 hours of age, differentiated items that remained stable over time, and those which marked maturity in the infant (e.g. visual tracking) in this short period (220). Future study will be required to replicate these findings and especially to isolate those items most likely to be transiently affected by the birth process in the first hours of life.

Similar to the argument made for the importance of detailed follow-up in premature babies (210), the clinical criteria for neurodevelopmental follow-up in the HIE population deserves attention. Earlier studies have demonstrated neuropsychological deficits in older children, (75, 121) and our cohort are currently being followed up into the school years. This study has highlighted eight children at age 2 with developmental delay with no motor disability. It is

this group of children, if not appropriately screened, who are at risk of not being identified for early interventions. Fortunately, interesting models of serial developmental screening in young children are being developed, for example, the 'developmental pathways study' (221), which may go some way towards resolving this issue.

In conclusion, we have shown that neurological signs evolve rapidly over the first three days following perinatal asphyxia. Persistence of abnormal neurological signs correlates significantly with adverse outcome. The later a neonatal neurological examination was performed, the better its predictive ability, with examination at discharge having a PPV and NPV of 72% and 86% respectively.

CHAPTER 4

4 The Ability of Early Serial Developmental Assessment to Predict Outcome at 5 Years Following Neonatal Hypoxic-Ischaemic Encephalopathy.

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4.1 Abstract

Background:

Neurodevelopmental difficulties in children following hypoxic-ischaemic encephalopathy (HIE) may not emerge until school age.

Aims:

To evaluate the value and stability of early serial developmental assessments in predicting long-term outcome.

Study Design:

Prospective study of 65 infants with neonatal HIE and early continuous EEG at birth. Participants: Term infants with HIE were recruited at birth. Development was measured at 6, 12 and 24 months using the Revised Griffiths' Scales (GMDS-R). Outcome Measures: Intellectual abilities at age five were measured using the Wechsler Preschool & Primary Scale of Intelligence (WPPSI-III^{UK}) and the 'numbers' subtest from the Children's Memory Scale. Overall five-year outcome was also reported.

Results:

IQ outcome was available in forty-seven surviving children (28 male, 19 female: mean (SD) age 64.0(5.7) months). Mean processing speed (p=0.01) and short-term verbal memory (*p*=0.005) were below the norm. Global development (GDQ) at 6, 12 and 24 months correlated (*p*<0.01) with five-year global, verbal and performance IQ with improved correlation over time. Normal GDQ throughout early childhood predicted normal IQ at 5 years (24-month AUROC value=0.941, *p*=0.001). All children who had an abnormal 6-month or 12-month GDQ had an overall abnormal outcome at age five, as did 80% of abnormal GDQ at 24mths. GDQ predictions were superior to neonatal Sarnat and EEG grading for abnormal outcome at age five.

Conclusions:

Normal early development predicts normal 5-year IQ with prediction increasing over time. Repeated measurement is warranted due to instability of findings across the first two years. Follow-up for children with abnormal early development is warranted given high sensitivity for school-age global abnormal outcome.

4.2 Introduction

Hypoxic-ischaemic encephalopathy (HIE) occurs following approximately 3 per 1000 term births. It remains one of the leading causes of neonatal death, with survivors at risk of significant neurological disability including cerebral palsy, epilepsy, intellectual disability, and sensory loss (127). Longer term outcome studies have reported more specific neurodevelopmental sequelae that may not emerge until after 24 months (71, 74, 75). These include delayed educational attainment and specific deficits in language, sensorimotor and selected memory domains (130, 222). In particular, working memory, long-term episodic and specifically verbal learning and retention are lower in children with moderate and severe HIE (124, 223).

The challenge lies in the early identification of children for whom later-emerging deficits are more likely to occur. Research studies have outlined the importance of follow up of affected children into their school years to capture the full impact of early injury (224).

The ability of early standardised developmental assessment to predict later cognitive outcome is clear at the extremes of developmental progress because a severe insult has a suppressor effect across multiple development domains (225). However, the prediction of more subtle deficits, most amenable to intervention, poses a greater challenge. Prediction studies observing clinical samples of infants tend to report low sensitivity and high specificity for later intellectual difficulties (226-228). Development progresses rapidly in the first years of life and the range of "normal development" is wide. Little information is available regarding the temporal stability of repeated early developmental assessments (229). Stability is likely to depend upon the patterns of development in the underlying domains of motor skills, language and cognition. The optimal time to assess these domains in early childhood remains unclear and the importance of serial assessment is acknowledged (230).

The use of the Bayley Scales (231), predominates in this field, and knowledge of predictive properties of other standardised development assessments such as the Griffiths scales is less well understood, especially for infants following neonatal HIE.

This study sought to record the temporal stability of serial developmental assessment in children following HIE at 6, 12 and 24 months and to determine the role of age at assessment for the prediction of (i) cognitive abilities and (ii) overall outcome at five years.

4.3 Materials and methods

4.3.1 Participants

Infants with hypoxic-ischaemic encephalopathy (HIE) were prospectively recruited between May 2003 and December 2005, from a maternity service with approximately 6,000 deliveries per annum (212). Children were recruited prior to the introduction of therapeutic hypothermia and were therefore not cooled. Ethical approval was granted from the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Cork, Ireland. Inclusion criteria were infants of >=37 weeks gestation who met at least two of the following: (i) initial capillary or arterial pH <7.1, (ii) Apgar score <5 at 5 minutes, (iii) initial arterial or capillary lactate >7 mmol/L, (iv) abnormal neurology or clinical seizures. Infants were excluded if they had co-existing congenital anomalies, or significant co-morbidity. Written informed consent was obtained from the parent(s) within 6 hours of birth, or where relevant, with the onset of clinical seizures. Serial administration of neurological assessments, and continuous EEG monitoring was performed for 24-72 hours duration. The EEGs were graded according to background activity, as follows: Grade 0 (normal) - continuous background pattern with normal physiologic features; Grade 1 (normal/mild abnormalities) - continuous background pattern with slightly abnormal activity (mild asymmetry, mild voltage depression, or poorly defined sleep wake cycle (SWC); Grade 2 (moderate abnormalities) – discontinuous activity with inter-burst interval (IBI) of <10s, no clear SWC, or clear asymmetry or asynchrony; Grade 3 (major abnormalities) – discontinuous activity with IBI of 10-60s, severe attenuation of background patterns or no SWC; Grade 4 (inactive EEG findings) – background activity of < 10µV or severe discontinuity with IBI of >60s. A modified Sarnat encephalopathy grade was assigned at 24 hours (14, 188). These methods have been described previously (232).

4.3.1.1 Outcome data

The Griffiths Mental Development Scales (0–2), 1996 revision (GMDS-R) (172), was used to obtain standardised developmental assessments. Children were seen at 6, 12 and 24 months by a Griffiths' certified trained paediatrician (Dr. Deirdre Murray) either at home or at the outpatients' clinic. A neurological examination assessing peripheral tone, axial tone and deep tendon reflexes was completed.

The Griffiths is a standardised assessment of infant and child development and was chosen for its clinical utility, psychometric properties and ecological validity. The scales were scientifically constructed based on item analysis and developmental theory. Items are

categorised into five subscales, 'Locomotor', 'Hearing and Language', 'Personal-Social', 'Eye-Hand Co-ordination' and 'Performance', with standardised subscale means ranging 100.2–101.1 (15.9–16.3) for the standardisation sample(172). The overall Griffiths' developmental quotient (GDQ) mean is 100.5 (11.8). An 'abnormal' score in this study was considered a GDQ ≤88.

At age five years, participants were contacted, and those that provided written consent for inclusion in the research study, were seen either at home or at a research facility, for intellectual assessment by a clinical psychologist (the current researcher) blinded to the neonatal data and early Griffiths' assessments. Neurological assessment was undertaken by a research paediatrician who assessed muscle tone, power and deep tendon reflexes. A GMFCS (Gross Motor Function Classification System) score (205), was assigned to children with cerebral palsy. Medical and demographic information was included in a parent questionnaire. Intellectual ability was assessed using the Wechsler Preschool and Primary Scales of Intelligence – 3rd UK Edition (WPPSI-III^{UK}) (194). Scaled scores for subtests and Full-Scale IQ (FSIQ), Verbal IQ (VIQ), Performance (non-verbal) IQ (PIQ), Processing Speed Quotient (PSQ), and General Language Composite (GLC) were calculated. All composite scores are standardised with a mean of 100(15). An 'abnormal' IQ was defined in this study as <=84. The 'Numbers' subtest of the Children's Memory Scale (CMS) (203), was also administered to include a measure of verbal working memory, and scaled scores calculated.

An 'abnormal' outcome at age five was defined as death, cerebral palsy (CP), significant hearing/vision loss, FSIQ >= 1SD below the mean (i.e. IQ <=84), a DSM-IV diagnosed neurodevelopmental disorder (autistic spectrum disorder (ASD), developmental co-ordination disorder (DCD), attention deficit hyperactivity disorder (ADHD), Language Disorder (Lang D/O)) and/or requirement for multidisciplinary early intervention (MD EI) team. Referrals to relevant individual health professionals were also noted.

4.3.2 Statistical Analysis

Statistical analysis was completed using IBM SPSS Statistics 20.0 for MS Windows (IBM Corporation, NY, USA) and VassarStats: Website for Computational Statistics (Lowry, R 1998-2012, Vassar College, NY, USA). Mann-Whitney (continuous) and Chi-square (categorical) correlations were used to explore normal/abnormal 5-year outcome data with birth and social data. Fisher's exact (and the Freeman Halton extension) statistics were used when the

minimum expected cell frequency assumption was violated, i.e. cell numbers less than 5. Spearman's rank correlation coefficients were analysed for all IQ quotients. One sample t-tests were used to compare observed scores to the WPPSI-III^{UK} test norms. PPV, NPV, sensitivity, specificity, and AUROC curves with confidence intervals were calculated. It is important to note that in this chapter the use of prediction statistics (PPV, NPV, sensitivity and sensitivity, AUROCS) were not used in the conventional way, but rather to predict normality rather than diagnosis. Consequently, 'PPV' refers to the predictive value of a NORMAL early score for later NORMAL score, and 'NPV' refers to the predictive value of an early abnormal score to predict later abnormal score. All *p* values were two-tailed with statistical significance cut-off set at < 0.05.

4.4 Results

In total, 65 children were recruited to the study. The modified Sarnat grades assigned at 24 hours were: 27 mild, 21 moderate, and 12 severe. Of these, by 24 months, 6 (all severe) had died, 6 (4 mild, 2 moderate) were lost to follow-up and two had been excluded due to confounding medical conditions – one (mild) with a repaired congenital diaphragmatic hernia and the other (moderate) with a suspected genetic syndrome due to dysmorphic features and generalised hypotonia. The remaining 51 children were contacted at age five. Of these, 1 (moderate) was lost to follow up and 3 (2 mild, 1 moderate) withdrew from the study. See Figure 4.1 for flowchart.

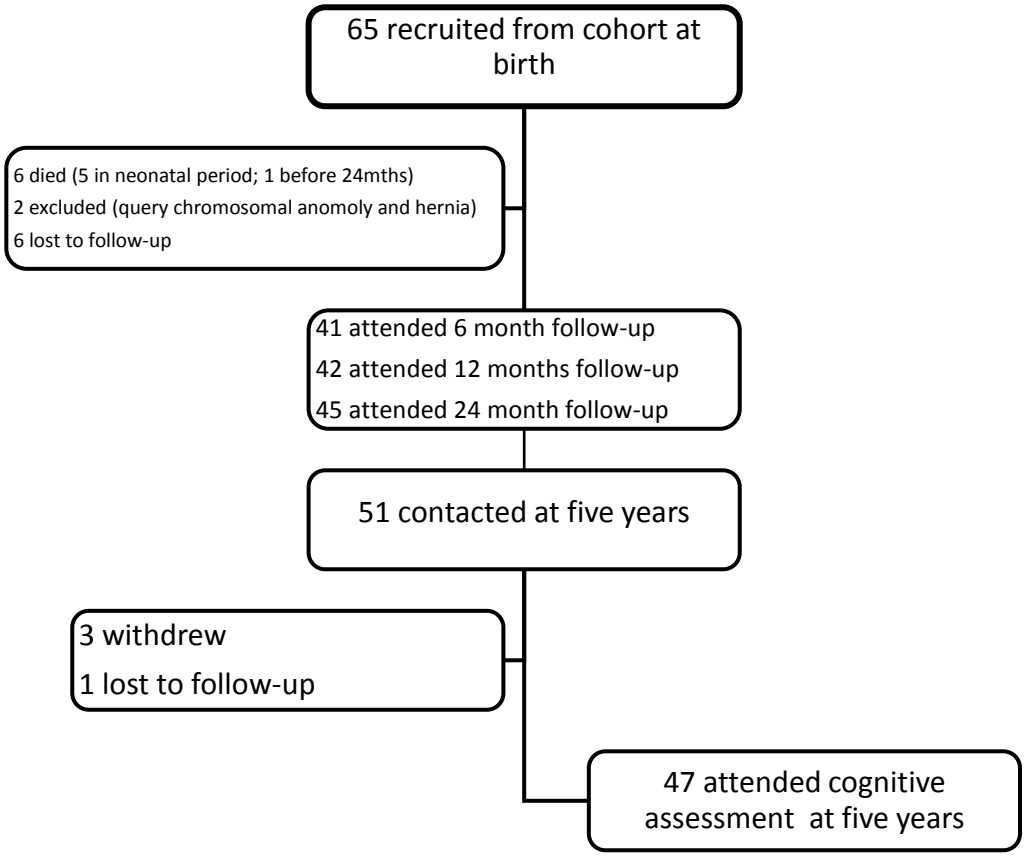

Figure 4.1 Flowchart of HIE Birth Cohort to Five Year Assessment.

At five years 47/59 (79.6%) surviving children (28 male, 19 female) attended for IQ assessment. Mean(SD) age at assessment was 64.0(5.7) months. To avoid practice effect, the IQ data for six of these children already enrolled in local health/disability services was taken from assessments completed within the previous six months by external psychologists. Three children were unable to complete the WPPSI-III^{UK} due to severe physical disability. Of these, two were assigned basal IQ outcomes at age five, and one was inconclusive. Therefore, IQ scores were available for 46 children.

The relevant birth, clinical and demographic data for the children who attended at age five years is presented in Table 4.1.

Table 4.1 Clinical and Demographic Characteristics of the Children who attended for five-year outcome.

PERINATAL DATA	**n=47**
Birth weight (mean(SD)) g	3431.02 (618.26)
Gestational age (mean(SD)) weeks	40.15 (1.48)*
First Born n(%)	36 (78)*
Mode of delivery n(%)	
NVD	8 (17)
Instrumental	22 (47)
Emergency CS	17 (36)
Apgar Score at 1 min	3.70 (2.28)
Apgar Score at 5 mins	6.00 (1.91)
Male Gender n(%)	28 (60)
First documented pH (venous) mean(SD)	6.99 (0.18)
First documented BD (venous) mean(SD)	-13.8 (4.9)
Sarnat Grade n(%)	
Mild	22 (47)
Moderate	19 (40)
Severe	6 (13)
EEG at 24 hours n(%)	**
Grade 0 (normal)	5 (11)
Grade 1 (normal/mild abnormalities)	26 (55)
Grade 2 (moderate abnormalities)	7 (15)
Grade 3 (major abnormalities)	6 (13)
Grade 4 (inactive)	1 (2)
DEMOGRAPHICS	n=47
Age assessed (Mean(SD)) months	64.00 (5.74)
Has sibling(s) n(%)	32 (73) †
Commenced Primary Schooling n(%)	36 (78)*
Maternal Education Level‡ n (%)	†
Primary or Lower Secondary	9 (21)
Secondary School Completed	19 (43)
Third Level Qualification	16 (36)
Household Occupation‡ n(%)	*
Professional/Managerial/Technical	18 (39)
Non-manual	9 (20)
Skilled/Semi-skilled/Unskilled Manual	19 (41)
Race: White n(%)	43 (92)
Region: n(%)	
Urban	15 (32)
Small Town	20 (43)
Rural	12 (26)

SD = standard deviation, NVD= normal vaginal delivery, CS=Caesarean Section, BD= base deficit, n/a = not available. *1 missing value; **2 missing values; †3 missing values; ‡ Category definitions modelled on Irish Census (2006) data.

4.4.1 HIE Birth Cohort - Outcome at Five Years

Of the original cohort of 65 children born with HIE, the outcome of 53 children was known at 5 years, with 47.2% (25/53) having an abnormal outcome. Six died (5 in the neonatal period and 1 before 24 months), eight had cerebral palsy (3 with intellectual disability), one had hearing impairment, four had an IQ>1SD below the mean (3 with concurrent diagnoses: 1 ADHD, 2 Lang D/O) four further diagnosed neurodevelopmental disorders (1 ASD, 1 ADHD, 1 DCD, 1 Lang D/O) and two attending MD EI teams. Categorised by Sarnat grade, the rates of abnormal outcome were: mild - 6/22 (27.3%); moderate - 9/19 (47.4%); severe - 10/12 (83.3%).

Intellectual abilities assessed at five years revealed mean scores in keeping with the mean standardised test norm of 100(15) for Full-Scale (FSIQ) 99.2(18.0), Verbal (VIQ) 100(17.9), Performance (PIQ) 101.3(17.1) and Language (GLC) 97.7(16.3) domains. However, Processing Speed (PSQ) was significantly reduced at 92.5(18.3) (t=-2.69, p=0.010), due to lower than expected scores on the 'Coding' subtest (one of two subtests used to calculate PSQ). The mean scaled score of 8.11(3.41) and median score of '8' were significantly below the expected norm of 10(3), (t=-3.68, p=0.001).

To display further the influence of the underlying individual WPPSI-III^{UK} subtests for each of the quotients, Table 4.2 provides outcome subtest-level scores for the HIE cohort as a whole, and for those with no diagnosis of CP. The patterns of central tendency and dispersion are broadly similar within the sets for the Verbal IQ and General Language Composite. However, within the Performance IQ subtests, the mean Picture Concepts scaled score is higher than the Block Design and Matrix Reasoning subtests.

Table 4.2 Mean WPPSI-III^{UK} and CMS subtest scaled scores for (i) the HIE cohort at age 5 (ii) the HIE cohort without cerebral palsy.

Subtest scaled scores	HIE Cohort: All (n=42)		HIE Cohort: No CP (n=38)	
WPPSI-III^{UK}	**Mean**	**S.D.**	**Mean**	**S.D.**
Verbal IQ				
Information	10.31	2.67	10.32	2.65
Vocabulary	11.20	2.22	11.27	2.16
Word Reasoning	11.00	2.68	11.0	2.62
Performance IQ				
Block Design	10.48	2.97	10.66	2.84
Matrix Reasoning	10.36	2.02	10.37	1.99
Picture Concepts	11.71	2.23	11.73	2.17
Processing Speed Quotient				
Coding	8.11	3.41	8.70	3.04
Symbol Search	9.33	3.46	10.00	2.92
General Language Composite				
Receptive Vocabulary	9.59	2.45	9.54	2.48
Picture Naming	10.49	2.47	10.51	2.50
CHILDREN's MEMORY SCALE				
Verbal Working Memory	8.45	4.02	8.74	3.72
Digit Span Forwards	8.21	3.78	8.53	3.47
Digit Span Backwards	9.97	3.56	10.03	3.50

CP = cerebral palsy, WPPSI-III^{UK} = Wechsler Preschool and Primary Scale of Intelligence 3rd UK version, IQ = intelligence quotient; S.D. = standard deviation.

The impact of Sarnat grade of HIE on IQ scores is presented in Table 4.3. The effect of a cerebral palsy diagnosis is also displayed. As expected, the infants with severe HIE had the worst outcomes, with the mean FSIQ = 73.8(33.3). However, the two children with severe HIE and no cerebral palsy at follow-up had normal IQs, compared to three children with CP (IQs - 68, 43, 43).

Table 4.3 Means and standard deviations of WPPSI-III^{UK} Full-Scale and domain quotients for all children at five years, and subdivided by Sarnat Grade (mild, moderate, severe) and/or diagnosis of cerebral palsy.

WPPSI-III^{UK} scores	***All Children***					***No cerebral palsy***			***Cerebral palsy***		
	N	***Min***	***Max***	***Mean***	***S.D.***	***N***	***mean***	***S.D.***	***N***	***mean***	***S.D.***
FSIQ total	46	43	125	99.2	18.0	39	102.9	12.4	7	78.6	29.3
FSIQ mild	22	84	125	102.8	11.2	22	102.8	11.2	0	NA	NA
FSIQ mod	19	75	123	101.7	14.5	15	102.4	14.5	4	99.0	16.6
FSIQ severe	5	43	118	73.8	33.3	2	107.5	14.8	3	51.3	14.4
VIQ total	46	48	131	100.0	17.9	39	103.0	13.6	7	82.9	28.8
VIQ mild	22	83	131	104.2	11.1	22	104.2	11.1	0	NA	NA
VIQ mod	19	67	127	100.8	16.5	15	100.3	16.8	4	102.8	17.6
VIQ severe	5	48	121	78.0	32.1	2	110.5	14.8	3	56.3	13.6
PIQ total	46	49	131	101.3	17.1	39	105.0	11.2	7	80.6	28.5
PIQ mild	22	81	131	104.1	11.5	22	104.1	11.5	0	NA	NA
PIQ mod	19	79	127	104.7	13.0	15	106.0	11.3	4	100.0	19.6
PIQ severe	5	49	116	75.6	30.2	2	107.0	12.7	3	54.7	9.8
PSQ total	43	49	137	92.5	18.3	37	95.8	15.6	6	71.8	21.2
PSQ mild	21	71	125	95.4	13.3	21	95.4	13.3	0	NA	NA
PSQ mod	17	60	137	94.8	19.0	14	96.1	20.0	3	88.7	14.6
PSQ severe	5	49	104	72.6	25.0	2	99.0	7.1	3	55.0	7.9
GLC total	42	45	128	97.7	16.3	36	100.2	11.9	6	82.5	29.2
GLC mild	21	83	128	102.5	12.6	21	102.5	12.6	0	NA	NA
GLC mod	17	78	113	98.5	10.1	13	98.0	9.8	4	100.3	12.7
GLC severe	4	45	103	68.8	27.2	2	90.5	17.7	2	47.0	2.8

WPPSI-III^{UK}: Wechsler Preschool and Primary Scale of Intelligence – 3rd UK Edition. FSIQ: Full Scale Intelligence Quotient. VIQ: Verbal Intelligence Quotient. PIQ: Performance Intelligence Quotient. PSQ: Processing Speed Quotient. GLC: General Language Composite. Mild: mild Sarnat grade. Mod: moderate Sarnat grade. Severe: severe Sarnat Grade. N: number of children. S.D.: standard deviation

The mean FSIQ outcome scores for mild (102.8(11.2)) and moderate (101.7(14.5)) HIE grade, were similar and did not differ significantly from expected norms. In addition, scores for mild and moderate VIQ (104.2(11.1), 100.8(16.5)), PIQ (104.1(11.5), 104.7(13.0)), PSQ (95.4(13.3), 94.8(19.0)) and GLC (102.5(12.6), (98.5(10.1)) were similar in both groups. The greatest difference was seen in processing speed (PSQ) across all grades.

Verbal short-term memory ('digit-span forwards' task) scores for all Sarnat grades were lower than the normative values. For the sample, the Numbers Forward task was 8.33(3.85); significantly below the expected norm of 10(3) (t=-2.85, *p*=0.007). Furthermore, we were unable to assign verbal working memory scores, as an insufficient number of children met the baseline criteria for the 'Numbers backwards' task on the Children's Memory Scale.

4.4.2 Correlation between early developmental assessment and five-year outcome.

We next examined the strength of association between the Griffiths' developmental assessments undertaken at 6, 12 and 24 months with IQ outcome at five years. GDQ at 6, 12 and 24 months correlated significantly with FSIQ, VIQ and PIQ at five years (Table 4.4), and the strength of correlation increased over time. The highest correlation was found between the composite Griffiths DQ at 24 months and FSIQ at 5 years (ρ=0.64, *p*<0.001), explaining 41% of the shared variance.

Table 4.4 Correlation between developmental quotients at 6, 12 and 24 months and intellectual outcome at five years.

WPPSI-III^{UK} at 5 years	**Griffiths DQ at 6 months**			**Griffiths DQ at 12 months**			**Griffiths DQ at 24 months**		
	ρ-value	***p* value**	**N**	**ρ-value**	***p* value**	**N**	**ρ-value**	***p* value**	**N**
Full Scale IQ	0.45	0.006	36	0.58	<0.001	37	0.64	<0.001	40
Verbal IQ	0.48	0.003	36	0.52	0.001	37	0.58	<0.001	40
Performance IQ	0.42	0.010	36	0.51	0.001	37	0.51	0.001	40
Proc. Speed Q	0.20	0.263	33	0.52	0.002	34	0.53	0.001	37
Gen. Lang. Com	0.57	0.001	33	0.30	0.081	34	0.42	0.011	36

WPPSI-III^{UK} at 5 years	Griffiths DQ at 6 months			Griffiths DQ at 12 months			Griffiths DQ at 24 months		
ST Verbal Mem	0.34	0.05	33	0.16	0.371	34	0.27	0.106	37

WPPSI-III^{UK}: Wechsler Preschool & Primary Scale of Intelligence (3rd Edition); Griffiths DQ: Griffiths Mental Development Scales (0-2)-Revised Developmental Quotient; IQ: intelligence quotient; Proc Speed Q: processing speed quotient; Gen Lang Com: General Language Composite; ST Verbal Mem: short-term verbal memory ρ-value: Spearman's rho correlation coefficient.

Given that processing speed (PSQ) was reduced at 5 years in this group, the Griffiths five domain level subscales at 24 months and association with 5-year PSQ was considered. Although the five domains correlated with overall GDQ, only Locomotor (ρ=0.50, *p*=0.002), Eye-Hand Coordination (ρ=0.54, *p*=0.001), and Performance (ρ=0.60, *p*<0.001) subscales were significant for PSQ. Additionally, all children with abnormal Eye-Hand Coordination (n=4) and Locomotor subscale scores (n=6) at 24 months, had abnormal WPPSI-III^{UK} coding scaled scores at age five years. Locomotor and Eye-hand Coordination subscales did not correlate with the other WPPSI-III^{UK} subquotients.

4.4.3 Temporal stability of early developmental assessments

The stability of Griffiths developmental quotients (GDQ) gathered at 6, 12 and 24 months for each infant were tracked across time. The mean (SD) developmental scores achieved at 6, 12 and 24 months are presented in Table 4.5. There were 27 children who attended on all of the three occasions. The total number of children who attended at each time point are also included.

For the 27 children who attended for assessment on all 3 occasions, there was a dip in the Locomotor subscale at 12 months (mean=82.97, 1SD below test norms), which had recovered to within the normal range by 24 months, albeit with a wider dispersion (SD=27.41) than expected. This trend was mirrored when all of the cohort who attended at each time point were included. A similar dip at 12 months was observed for the Personal-Social domain, however scores remained within the normal range at all time points. Hearing & Language subscale dropped approximately 12 points between 6 and 24 months of age. The Performance subscale was the lowest of the domains tested at 24 months (Table 4.5).

Table 4.5 Mean Griffiths Mental Development Scale (0-2) Revised's Global and Subscale scores achieved at 6, 12 and 24 months of age for (i) the 27 children (with 5-year outcome) who attended on all three occasions and (ii) the total set of children (with 5-year outcome) who attended at each time point.

	Children who attended at all ages (n=27)						**Children who attended at each age**					
Griffiths Mental Development Scales (0-2) Revised.	**6 months**		**12 months**		**24 months**		**6 months (n=37)**		**12 months (n=37)**		**24 months (n=40)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Griffiths DQ	**99.89**	14.13	**94.26**	18.05	**96.59**	19.95	**98.38**	17.45	**94.78**	16.61	**98.05**	19.21
Locomotor	**95.78**	16.80	**82.07**	20.78	**99.59**	27.41	**94.31**	18.07	**83.03**	20.67	**102.15**	24.58
Personal-Social	**101.63**	16.02	**95.22**	20.65	**102.78**	24.51	**101.43**	17.43	**97.51**	20.15	**109.11**	15.70
Hearing & Language	**107.37**	16.46	**98.48**	18.56	**95.52**	20.95	**109.11**	15.70	**100.00**	17.11	**97.23**	20.22
Eye & Hand Coord.	**97.63**	17.15	**99.96**	22.28	**97.56**	18.94	**98.26**	17.93	**100.22**	21.62	**100.95**	17.32
Performance	**95.11**	18.77	**97.93**	18.00	**92.56**	20.67	**94.97**	19.49	**98.05**	18.21	**91.73**	20.88

Overall, twenty-nine children tested 'normal' on the Griffiths - either on all three occasions (n=16) or on all of the occasions that they attended (n=13) in early childhood. This was 100% predictive of a normal cognitive (IQ) outcome. However, despite a normal FSIQ, 3/29 (10.3%) of these had an overall abnormal outcome: one had mild ataxic CP, one had ADHD and one had significant hearing impairment.

Nineteen children in the cohort were assigned an abnormal Griffiths GDQ score on at least one of the early developmental assessments. Of these, 17/19 (89.5%) had an overall abnormal outcome at 5 years, including 6/19 (31.6%) with an abnormal IQ. Of those, 5/19 children achieved an abnormal GDQ on *all* occasions that they attended, and all had an abnormal outcome at 5 years. Notably, all children whose Griffiths score was abnormal at 6 or 12 months, and all children whose Griffiths score was >=2SD below the mean at any time point from 6-24 months had an abnormal five-year outcome (Table 4.6).

Table 4.6 Profile of children assessed at 6, 12, or 24 months and five-year outcome.

Age assessed (n)	Griffiths GDQ	Total No. of children	No. with Normal 5yr O/C	No. with Abnormal 5yr O/C	Abn 5yr O/C with Abnormal IQ
6 months (n=38)	Normal	32	21	11	3
	1SD below	2	0	2	1
	2SD-4SD below	4	0	4*	2
12 months (n=38)	Normal	26	21	5	2
	1 SD below	6	0	6	1
	2SD-4SD below	6	0	6*	3
24 months (n=40)	Normal	30	22	8	0
	1 SD below	4	2	2	0
	2SD-4SD below	6	0	6	6

*1 death before 24mths. GDQ = Griffiths Global Developmental Quotient, No. = number, O/C = outcome, Abn = abnormal.

4.4.4 Predictive Power of normal early childhood assessment at each time point for normal 5-year IQ

We examined the predictive power of a normal GDQ score for a normal five-year IQ at each time point (Table 4.7). As expected, the best prediction was seen at 24 months with an AUROC = 0.941 (p=0.001). The 24-month normal GDQ was 100% predictive of normal 5-year IQ. At all time-points the early GDQ scores displayed moderate to high AUROC accuracy

values (see Figure 4.2), with higher sensitivity and lower false negative rates. A higher proportion of children with a normal five-year IQ, had normal early Griffiths scores, than had mild Sarnat or normal Early EEG at 24 hours. All children with an abnormal IQ at five years had an abnormal 24mth GDQ. When we examined all perinatal and demographic factors listed in Table 4.1, it was found that in addition to early GDQ scores, Sarnat grade was the only characteristic that differed significantly between those children with and without normal five-year IQ (ρ=0.46, *p*=0.02).

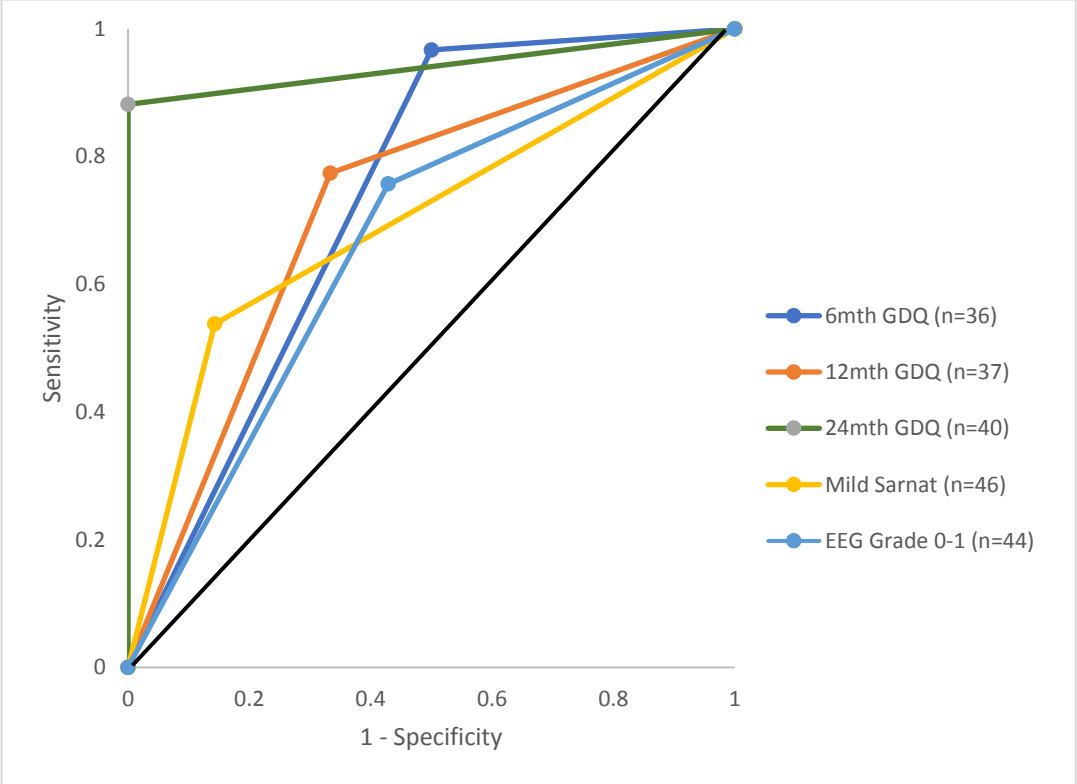

Figure 4.2 AUROC values for the prediction of normal 5 year IQ from normal development scores at 6, 12 & 24 months, and from a mild Sarnat grade, or from an EEG Grade 0-1. AUROC, area under the receiver operated curve; mth, month; GDQ, Griffiths Global Development Quotient.

Table 4.7 The ability of normal early serial assessments to predict a normal WPPSI-III^{UK} FSIQ at age five years.

GDQ	PPV (95% CI)	NPV (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	AUROC (95% CI)	*p* value
6 MTH (n=36)	90.63 (73.83-97.55)	75.00 (21.94–98.68)	96.67 (80.95-99.83)	50.00 (13.95-86.05)	0.733 (0.469–0.998)	0.075
12 MTH (n=37)	92.31 (73.40–98.66)	36.36 (12.37–68.39)	77.42 (58.46–89.72)	66.67 (24.11–94.00)	0.720 (0.482–0.959)	0.091
24 MTH (n=40)	100.00 (85.87–100.00)	60.00 (27.37–86.31)	88.24 (71.61–96.16)	100.00 (51.68–100.00)	0.941 (0.870–1.00)	0.001**
Sarnat grade I (n=46)	95.46 (75.12-99.76)	25.00 (10.60-47.05)	53.85 (37.38-69.57)	85.71 (42.01-99.25)	0.698 (0.505-0.890)	0.099
EEG Grade 0-1 (n=44)	90.32 (73.10–97.48)	30.77 (10.36–61.12)	75.68 (58.45–87.63)	57.14 (20.24–88.19)	0.664 0.431–0.897	0.173

GDQ: Griffiths Developmental Quotient; PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUROC: Area Under the Receiver Operated Curve; MTH: months. Sarnat grade I: Mild Sarnat grade. EEG Grade 0-1: Normal/mildly abnormal EEG grade at 24 hours.

4.4.5 *Predictive Power of early childhood assessment at each time point for overall outcome*

For overall outcome, all children with a normal five-year outcome had normal 6 and 12-month GDQ. Furthermore, an abnormal 6- and 12-month GDQ score was 100% predictive of abnormal outcome. Surprisingly GDQ at 12 months was most closely related to overall outcome suggesting that this was a more global measure of injury (see Figure 4.3 and Table 4.8), whilst the 24-month assessment was more closely linked to 5-year IQ.

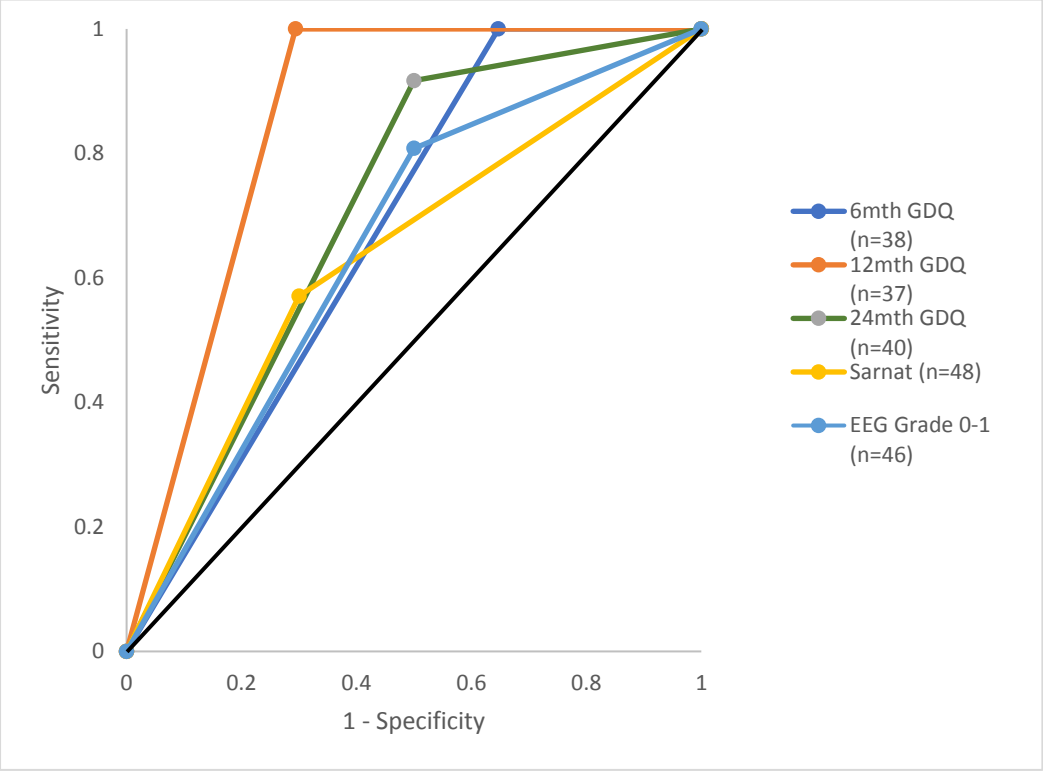

Figure 4.3 AUROC values for the prediction of normal overall 5 year outcome from a normal Griffiths score at 6, 12, or 24 months, from a mild Sarnat Grade, or from an EEG Grade 0-1. (AUROC, area under the receiver operated curve; mth, month; GDQ, Griffiths Global Developmental Quotient.

Table 4.8 The ability of normal early serial assessments to predict a normal overall outcome at age five years.

GDQ	**PPV (95% CI)**	**NPV (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**AUROC (95% CI)**	***p* value**
6 MTH (n=38)	65.63 (46.78–80.83)	100.00 (51.68–100.00)	100.00 (80.76- 100.00)	35.29 (15.2-61.38)	0.676 (0.50-0.86)	0.064
12 MTH (n=37)	80.78 (60.02–92.70)	100.00 (69.88–100.00)	100.00 (80.76–100.00)	70.59 (44.05–88.62)	0.853 (0.72-0.99)	<0.001**
24 MTH (n=40)	73.33 (53.83–87.03)	80.00 (44.22–96.46)	91.67 (71.53–98.54)	50.00 (25.51-74.49)	0.708 (0.53-0.88)	0.027*
Sarnat* grade (n=48)	72.73 49.56-88.39	53.85 (33.75-72.86)	57.14 37.43-74.97	70.00 45.67-87.16	0.636 (0.48-0.80)	0.112
EEG* grade 0-1 (n=46)	67.74 (48.54–82.68)	66.67 (38.69–87.01)	80.77 (60.02–92.69)	50.00 (27.85–72.15)	0.654 (0.490-0.818)	0.076

GDQ: Griffiths Developmental Quotient; PPV: Positive Predictive Value; NPV: Negative Predictive Value; AUROC: Area Under the Receiver Operated Curve; MTH: months. Sarnat grade I=mild Sarnat grade. EEG Grade 0-1: Normal/mildly abnormal EEG grade at 24 hours. *only children who survived the neonatal period were included in the analysis.

4.5 Discussion

We have described the five-year IQ and overall outcome in a cohort of children following neonatal HIE. We confirmed the importance of undertaking repeated developmental assessments in the early years since one-third of children with HIE in our cohort had unstable development scores across early childhood. We have also confirmed the utility of early developmental assessment in the prediction of overall outcome at five years, and the importance of long-term monitoring for those with abnormal early development scores.

At five years, similar to findings for other populations of children with perinatal injury such as stroke (233), the Processing Speed quotient was the lowest score, and was depressed for all grades due to poor performance on a 'coding' pencil task, even in those with no motor impairment. Therefore, this reduction may be due to either poor fine motor coordination or actual poor processing speed. The motor data collected in the early Griffiths assessments and the observation that 100% of children with lower than average locomotor and eye-hand coordination at 24 months had lower coding scores at age five suggests a motor coordination explanation.

Verbal working memory is an important skill to assess at school-going age, and the 'Numbers' subtest from the CMS was included to measure this construct. The CMS verbal working memory subtest comprises two tasks – an aggregate of digit-span forwards and digit-span backwards. However, it transpired that for the HIE cohort, the floor items were set too high on the digit-span backwards task for accurate interpretation. This meant that a child (aged 5) with a raw score of 0 achieved a scaled score of 7 (16^{th} percentile), thus risking the inflation of the verbal memory score. Consequently, only digit forwards was interpreted, which yielded instead a short-term memory score which was significantly below the expected level. This finding raises the psychometric issue of testing children at the extreme ends of the age range for a test, (the CMS is aimed at 5 to 12 years) and also to have a sufficient range of tasks at the lower end to allow for a spread of low scaled score results at this level. Executive functions such as processing speed and working memory are critical for effective thinking and educational attainment. In our cohort, lower than expected scores in these areas were not easily predicted by early assessment and were often seen in children with normal IQ at five years. Similarly, we have shown that infants with mild grade HIE do not survive unscathed, and follow-up at 6 months of age with a structured developmental assessment may be useful in detecting those with potential future difficulties.

Although previous studies have reported the ability of the GMDS-R to predict outcome assessed using the previous WPPSI-R version, this is the first study to examine the prediction of serial GMDS-R in early childhood to predict outcome assessed using the third edition of the WPPSI (122, 234). In this HIE population, early developmental assessment correlated with later IQ outcome. GMDS-R scores at all time-points correlated with Full-Scale, Verbal and Performance IQs, with the relationship strengthening over time. Although our cohort was recruited before the introduction of therapeutic hypothermia, the high sensitivity values of an early normal development score for school-age normal outcome is commensurate with those reported from randomised controlled trials of therapeutic hypothermia (18, 137, 139).

In the current study, the importance of assessing children serially in the first two years was observed, as different children demonstrated abnormal development patterns at different ages. The developmental stage of the child at the time of measurement may affect the ability of an assessment tool to detect delay (171). Milne et al., suggest that changes in score stability over time may represent natural developmental trajectories rather than any inconsistency in the developmental tool itself (170). At twelve months, in our study, children were more likely to obtain an abnormal GMDS-R score, typically due to low attainments on the Locomotor subscale, which often normalised by 24 months. This provides an example of the differential role of specific domain areas, and how the age at assessment plays an important role in their predictive ability (235). Consistent with our findings, Barnett et al., in a previous neonatal encephalopathy cohort, demonstrated higher prediction of a Griffiths score at 12 months for later motor competence than for IQ, with IQ prediction improving by 24 months (122). Similarly, we found the 12-month assessment to be a poor predictor of IQ, although it did correlate well with overall outcome. This may be a reflection of the test battery used at this time point, with the 12-month battery more heavily influenced by the global function (fine motor, gross motor, behavioural function) of the child at that developmental stage. Motor milestones vary considerably at this stage due to the influence of the musculoskeletal system, rather than cerebral function alone (236). Some children, particular those who “bottom shuffle” may not crawl, or walk unaided until up to 18 months despite normal cognitive outcome. By 24 months the range of motor skills seen is less and so motor function is less likely to sway the reliability of the test.

Previous studies reporting the ability of abnormal early development assessments to predict abnormal cognitive outcome consistently show a pattern of low sensitivity and high

specificity. This has led some researchers to question the cost-effectiveness of the continued use of developmental assessment tools by clinicians. However, these studies have been constrained by limiting their focus to outcome based on IQ tests. Our study suggests that early developmental assessments are helpful to predict later IQ but perhaps more interesting is their greater predictive value for *overall outcome.* Seventeen of the nineteen (89.5%) children who experienced at least one abnormal Griffiths score in early childhood had an abnormal overall outcome at five years of age.

In our research, we were interested to determine whether serial early assessment provided added value when compared with other markers for outcome. The only other variable included in our analysis that proved to be significantly different for children who had a normal compared to an abnormal five-year cognitive outcome was Sarnat grade. The predictive properties of neonatal Sarnat and EEG grades were lower than Griffiths with poorer sensitivity, and higher false negative rates. Thus, assignation of a mild vs. a moderate or severe Sarnat or a Grade 0-1 vs. a higher grade EEG was not reassuring for predicting outcome. Recently, Tusor et al. similarly found that the Thompson scoring grade for encephalopathy was a poor predictor (237). Findings from the current study indicate the need for repeated assessment in the early years to increase the sensitivity for both later IQ outcome and overall outcome. Assessment at six months, allows for the early identification of abnormal development, which is likely to remain severe and persistent, whilst assessment at 12 and 24 months, tracks those children who initially do well but show variable results over time. Serial assessment may help to control for the wide normal variability seen in the acquisition of developmental milestones, and to reduce the dependence on sensory-motor tasks as children grow. It allows us to plot trajectories of developmental change over time, and to highlight emerging cognitive deficits.

A significant limitation of this study is the small sample size, however, this cohort remains one of the largest prospectively recruited with clearly defined HIE of all grades; mild, moderate and severe. This cohort was recruited immediately prior to the era of therapeutic hypothermia and so the overall rate of long-term disability will differ from contemporary cohorts. However, the validity of early neurological assessments and the high rate of subtle disability found in the group with mild HIE (not currently cooled) will remain unchanged. In terms of interpretation a further limitation of this study is the unconventional way in which predictive testing was used in this study. PPV was developed to investigate the accuracy of

diagnostic tests, i.e. the extent to which a positive diagnostic test, in fact predicts a diagnosis. NPV predicts the accuracy of a negative test score for absence of disease. In this study, the PPV was used to examine the prediction of normal early developmental tests for normal 5-year outcomes, with NPV examining the prediction of abnormal score for later abnormal outcome. The current researcher had sought to define these clearly, but because the use of PPV and NPV was inverted in this study, the threat of causing confusion to the reader is regrettable. On a separate point, our conclusions are limited by the low numbers of children who had abnormal early and five-year outcomes which reduced the accuracy of our NPV rates, with wide confidence intervals noted.

To conclude, in the first two years of life, early developmental assessment using the GMDS, even at 6 months can accurately identify those children at risk of later difficulties, although they are poor predictors of exact IQ score at 5 years. A 24-month developmental assessment is the best time to predict later intellectual outcome in a population of children with HIE. Measures of early development are inconsistent in the first two years and merit repeated administration.

COMMENT: My contribution to this chapter is described in further detail in Chapter 8 Section 8.1: Summary and Impact of the Thesis Findings.

4.6 Appendix 4.A

Summary of HIE Cohort data including assigned Sarnat and EEG grade at 24 hours of age, global development scores at age 6, 12 and 24months, IQ and overall outcome at five years of age.

Table 4.9 Summary of HIE cohort data including assigned Sarnat and EEG grade at 24 hours of age, global scores at 6, 12 and 24months, IQ and overall outcome at five years.

Case No.	Sarnat Grade	EEG Grade	GDQ– 6mth	GDQ- 12mth	GDQ– 24mth	WPPSI-III^{UK} – FSIQ at Age Five	Outcome at Age Five
01	1	1	Normal	Normal	Normal	Normal*	Normal
02	2	1	Normal*	Normal*	Normal	Normal*	CP Ataxic: GMFCS Level I
03	2	2	Normal	Normal	Normal*	Normal*	Hearing impairment
04	1	2		Normal	Normal*	Normal*	Normal
05	1	1	Normal	Normal	Normal	Normal*	Normal
06	3	1	Normal			Normal*	Normal
07	1	1	Normal	Normal	Normal	Normal*	Normal
08	2	1	Normal*	Normal*	Normal*	Normal*	Normal
09	2	3	Normal*			Normal*	Normal
10	1	1	Normal	Normal	Normal*	Normal	Normal
11	2	1		Normal	Normal*	Normal	Normal
12	2	1	Normal		Normal	Normal	Normal
13	1	0	Normal	1SD Below	Normal	Normal	DCD; (SLT, OT, Psy)
14	1	0	Normal	Normal	Normal*	Normal	Normal
15	1	1	Normal*	Normal	Normal	Normal	Normal
16	2	1	Normal	Normal	Normal	Normal	Normal
17	2	1	Normal*	Normal	Normal*	Normal	Normal
18	2	0	Normal*	Normal	Normal	Normal	Normal
19	1	1	Normal	Normal	Normal	Normal	Normal
20	2	1	Normal	Normal		Normal	Normal
21	1	1	Normal*	Normal	Normal	Normal	Normal
22	1	0		Normal	Normal	Normal	Normal
23	1	1		1SD Below	1SD Below	Normal	MD EI Team (SLT, OT, Psy): Referred to CAMHS, Paed Neuro
24	1	1	Normal	Normal	Normal*	Normal	Normal
25	1	1	Normal	1SD Below	Normal	Normal	ASD
26	1	1		Normal	Normal	Normal	Normal
27	1	0	Normal	Normal*	Normal	Normal	Normal (SLT)
28	2	2			Normal	Normal	Normal (OT)
29	2	3	Normal	Normal	1SD Below	Normal	S/L D/O. MD EI Team: (SLT, OT, Psy)
30	2	3	Normal	2SD Below		Normal	CP Dyskinetic: GMFCS Level 3
31	3	2	Normal	Normal	1SD Below	Normal	Normal

Case No.	Sarnat Grade	EEG Grade	GDQ– 6mth	GDQ- 12mth	GDQ– 24mth	WPPSI-III^{UK} – FSIQ at Age Five	Outcome at Age Five
32	1	1	Normal	3SD Below	Normal	Normal	MD EI Team: (SLT, Psy, PT)
33	1	N/A		Normal	Normal	Normal	Normal
34	1	N/A			Normal	Normal	Normal
35	1	1	Normal*			Normal	Normal (OT)
36	2	2	Normal		1SD Below	Normal	Normal (OT)
37	2	1	1SD Below	1SD Below	Normal	Normal	CP Sp Unilateral (left): GMFCS Level 1
38	1	1			Normal	Normal	ADHD
39	2	2	Normal	1SD Below	Normal	Normal	CP Sp Unilateral (right): GMFCS Level 1
40	1	1	Normal	1SD Below	2SD Below	1SD Below	IQ 1SD Below; MD EI Team (SLT, OT, Psy) LST.
41	2	1	1SD Below	Normal	2SD Below	1SD Below	IQ 1SD Below; Severe SL D/O, SpLC (SLT)
42	2	1	Normal	3SD Below	4SD Below	1SD Below	IQ 1SD Below; SL D/O: (SLT, Psy)
43	2	2		Normal	3SD Below	1SD Below	IQ 1SD Below; ADHD
44	3	3	Normal	2SD Below	2SD Below	2SD Below	CP Sp Unilateral (left): GMFCS Level I; I.D.
45	3	3	4SD Below	4SD Below	4SD Below	Assigned 4SD Below	CP Sp Bilateral (quad): GMFCS Level V; I.D.
46	3	4	4SD Below			Assigned 4SD Below	CP Sp Bilateral (quad): GMFCS Level V. I.D.
47	1	1	Normal*	LTFU	LTFU	LTFU	LTFU
48	1	0		Normal	Normal*	Withdrew	Normal at 24mths
49	1	2	Normal	Normal*		Withdrew	Normal at 12 mths
50	1	2		1SD Below	Normal	LTFU	Normal at 24mths
51	2	1	Normal*	Normal*	Normal*	Withdrew	Normal at 24mths
52	3	4	4SD Below	4SD Below	Died		Died
53	3	3	Died				Died
54	3	3	Died				Died
55	3	N/A	Died				Died
56	3	4	Died				Died
57	3	3	Died				Died
58	3	3	4SD Below			Test inconclusive	CP Dyskinetic. GMFCS Level V
59	2	2					Excluded – dysmorphic features
60	1	N/A					Excluded – congenital diaphragmatic hernia
61	1	2	LTFU	LTFU	LTFU	LTFU	LTFU
62	1	1	LTFU	LTFU	LTFU	LTFU	LTFU
63	2	N/A	LTFU	LTFU	LTFU	LTFU	LTFU
64	2	N/A	LTFU	LTFU	LTFU	LTFU	LTFU
65	1	N/A	LTFU	LTFU	LTFU	LTFU	LTFU

No.=number; GDQ=Griffiths Mental Development Scales–Revised developmental quotient; 6mth= six months of age; 12mth= twelve months of age; 24mth=twenty four months of age; WPPSI-III^{UK}=Wechsler Preschool and Primary Scale of Intelligence 3rd UK version; FSIQ=full scale intelligence quotient; N/A=Not Available; SD=Standard Deviation; Normal*=IQ score 1SD above the mean; CP=cerebral palsy; GMFCS=gross motor functioning classification system; DCD=developmental coordination disorder; SLT=speech & language therapy; OT=occupational therapy; Psy=psychology; MD EI Team=multi-disciplinary early intervention team; CAMHS=child/adolescent mental health service; paed neuro=paediatric neurology; ASD=autistic spectrum disorder; PT=physiotherapy; ADHD=attention deficit hyperactivity disorder; Sp=spastic; LST=learning support teaching; SL D/O=speech/language disorder; SpLC=special language class; I.D.=intellectual disability; LTFU=lost to follow up; mths=months

.

CHAPTER 5

5 Early EEG Grade and Outcome at 5 Years After Mild Neonatal Hypoxic-Ischaemic Encephalopathy

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5.1 Abstract

Objective:

More than half of all infants with neonatal hypoxic ischaemic encephalopathy (HIE) are graded as mild and do not meet current criteria for therapeutic hypothermia (TH). These infants are often not enrolled in follow up and hence our knowledge of their long-term outcome is sparse. We wished to compare 5-year outcomes in a group of infants with mild, moderate, and severe HIE, graded with both early EEG and clinical assessment, none of whom were treated with TH.

Methods:

Term infants with HIE and a healthy comparison group were recruited at birth. Both groups had early continuous EEG recordings. Cognitive and motor outcome was assessed at 5 years.

Results:

Outcome was available in 53/65 infants with HIE and 30/42 infants in the comparison group at 5 years. Infants with mild HIE at birth (n=22) had significantly lower full-scale IQ, verbal IQ, and performance IQ than comparison infants (n=30) at 5 years (p=0.001, 0.001 and 0.004 respectively). No difference in cognitive measures was seen between infants with mild and moderate grades HIE. Intact survival – with no requirement for early intervention at 5 years - varied across EEG grade HIE at 6 hours after birth; 75% in mild, 46% in moderate, 43% in major abnormalities and 0% with inactive EEGs, compared to 97% in the comparison group.

Conclusion:

Survivors of mild HIE, graded clinically or by early EEG have higher rates of non-intact outcome than a non-HIE comparison group with neonatal normal EEG, and they have cognitive outcomes similar to that of children with moderate encephalopathy in an uncooled HIE cohort.

5.2 Introduction:

Neonatal hypoxic ischaemic encephalopathy (HIE) remains a leading cause of newborn death and long-term neurodisability. It causes 23% of global neonatal deaths, totalling 1 million annually. Twenty-five percent of surviving children, are left with significant long-term neurological disability (127), placing HIE in the top ten diseases with the highest lifelong global burden (13). The significance of non-motor disability is increasingly recognized, with reported rates of learning and neuropsychological difficulties, autism, epilepsy, and sensory loss contributing to adverse long-term quality of life. The more subtle disabilities may not be apparent in early childhood where assessment is focused on developmental milestones.

Outcome is generally aligned with clinical grading, with moderate and severe grades having the highest rates of motor deficit and mortality (127). The outcome in mild HIE is generally considered to be normal. Due to a fear of serious adverse events, prior to the establishment of the safety profile of induced hypothermia, infants with mild HIE were not enrolled in trials of therapeutic hypothermia (TH), and therefore do not meet current criteria for therapeutic intervention (139). Few prospective cohorts have included infants with mild encephalopathy, and have focused instead on moderate and severe grades. We wished to determine the cognitive and motor outcome of a prospective cohort of infants across all grades of HIE, with both clinical and early continuous EEG grading.

5.3 Participants and Methods:

5.3.1 Initial recruitment

This prospective study was conducted in a maternity service with 6000 deliveries per year. Ethical approval was obtained from the clinical research ethics committee of the Cork Teaching Hospitals. Between May 2003 and December 2005, term infants (≥37 weeks of gestation) with HIE were recruited if they fulfilled two of the following criteria: initial capillary or arterial pH of <7.1, Apgar score at 5 minutes of <5, initial capillary or arterial lactate level of >7 mmol/L, and abnormal neurologic features/clinical seizures. Broad inclusion criteria were used to ensure recruitment of all grades (mild, moderate and severe) of HIE. Neurologic condition was assessed by a research paediatrician (Dr. Deirdre Murray), not involved in the clinical care of the infants within the first 6 hours and was performed using a standardized method (60). Infants were excluded if alternative diagnoses were suspected; sepsis, intracerebral infarction, congenital abnormalities or metabolic encephalopathy.

The parents of infants fulfilling the criteria were approached and written informed consent was obtained within 6 hours of birth, or with the onset of clinical seizures. After recruitment, silver-chloride EEG electrodes were applied to the scalp at F3, F4, C3, C4, T3, T4, O1, O2, and CZ (according to the international 10–20 system of electrode placement, as modified for neonates). A Viasys NicoletOne EEG monitor (Viasys International, Madison, WI) was used to record continuous video-EEG recording for a 24-72 hours duration. Recordings were commenced as soon as possible after birth, generally within 6 hours. Measurements of heart rate, respiration, and oxygen saturation were also recorded. A modified Sarnat encephalopathy grade was assigned at 24 hours as described by Levene in 1985 (14, 188). All EEGs were assigned a grade based on background activity which has been previously described (see Table 5.1) (105). All seizures in each EEG recording were annotated visually by an experienced neonatal neurophysiologist (Prof. Geraldine Boylan). Electrographic seizure was defined as a sudden repetitive, stereotyped discharge of minimum 10 seconds duration on one or more EEG channels with evolving frequency, amplitude and morphology.

Table 5.1 Classification of EEG background activity (105).

Grade	Findings	Description
0	Normal EEG Findings	Continuous background pattern with normal physiologic features such as anterior slow waves
1	Normal/mild abnormalities	Continuous background pattern with slightly abnormal activity (e.g., mild asymmetry, mild voltage depression, or poorly defined SWC)
2	Moderate abnormalities	Discontinuous activity with IBI of < 10s, no clear SWC, or clear asymmetry or asynchrony
3	Major abnormalities	Discontinuous activity with IBI of 10-60s, severe attenuation of background patterns, or no SWC
4	Inactive EEG findings	Background activity of < 10μV or severe discontinuity with IBI of >60s

EEG = Electroencephalogram; IBI = Inter-burst interval; SWC = Sleep wake cycle

5.3.2 Outcome assessment

Outcome assessment was performed at 6, 12, 24 and 60 months. The 24 month outcome has been previously reported (105). Outcome at 5 years was assessed by a clinical psychologist (Catherine O'Connor) blinded to the neonatal clinical course, EEG findings and early outcome assessments of the HIE group. Attendance at early intervention services (physiotherapy, speech therapy, psychology and/or occupational therapy) were also recorded.

A parental questionnaire was designed to capture relevant medical, clinical and demographic information. Intellectual ability was assessed using the Wechsler Preschool and Primary Scale of Intelligence – 3rd Edition(UK) (WPPSI-III^{UK}) (194). The Full-Scale IQ (FSIQ), Verbal IQ (VIQ), Performance IQ (PIQ), Processing Speed Quotient (PSQ), and General Language Composite (GLC) were calculated. All quotients are standardized with a mean(SD) of 100(15). The children were then administered the 'Numbers' subtest from the Children's Memory Scale (CMS) (203), to include an additional measure of short-term auditory memory, due to known vulnerability of infants with hippocampal injury (223). The 'Numbers' Subtest has a mean(SD) of 10(3). The WPPSI-III^{UK} was not administered to those children whose IQ had been assessed in their clinical setting within the previous six months, and the clinical IQ scores were used instead. Neurological assessment of muscle tone, power and deep tendon reflexes was completed (Dr. Deirdre Murray). A GMFCS (Gross Motor Function Classification System) score was assigned to children with cerebral palsy (205).

An overall abnormal outcome at age five was defined as follows (at least one present): cerebral palsy, Full-Scale IQ >= 1 SD below the mean (IQ < 85) (with intellectual disability (Full-Scale IQ > 2SD below the mean, IQ < 70)), other diagnosed neurodevelopmental disorder (i.e. attention deficit (hyperactivity) disorder (ADD/ADHD), developmental coordination disorder (DCD), autistic spectrum disorder (ASD), specific language disorder (SLI)); hearing or vision loss or death.

5.3.3 Comparison group

Comparison infants were recruited from an age-relevant subset (n=42) from a prospective study examining normal newborn EEG in 80 healthy term infants, born between October 2005 and August 2007, who were recruited in the first postnatal day following delivery, with Apgar scores >8 at 5 minutes and a normal result from standardized neurological exam (60).

A baseline EEG was recorded for a maximum of 2 hours in each infant. Seven scalp electrodes were positioned over the frontal, central, temporal and parietal areas using the 10-20 system of electrode placement, modified for neonates (F4, F3, Cz, T4, T3, P4, P3). Exclusion criteria were: maternal epilepsy or diabetes; birth-weight(BW) <2.5kg; congenital anomalies; admission to neonatal unit for special or intensive care (94). Parents/Carers whose children were currently aged between 60 and 78 months (n = 42), were sent an information letter and a follow-up telephone call to describe the study.

5.3.4 Statistical Analysis

Statistical analysis was completed using IBM SPSS Statistics 22.0 for MS Windows (IBM Corporation, NY, USA) and VassarStats: Website for Computational Statistics (Lowry, R 1998-2012, Vassar College, NY, USA). The between-group differences of clinical and demographic data for the HIE and Comparison groups were explored using Independent Samples T-Test (Cohen's d for effect size) for continuous variables, and Chi-Square Test for Independence (Phi co-ef. and Cramer's V for effect size) for categorical variables. Kruskal-Wallis and post hoc Mann-Whitney U tests, PPVs, NPVs were analyzed to explore differences between neonatal clinical HIE and EEG grades and outcome. Effect sizes for Mann-Whitney U tests were calculated using the formula '$r = z/\sqrt{N}$' where N = no. of cases.

5.4 Results

In total, 107 children were recruited to the study - 65 children with neonatal HIE, and 42 comparison infants. Of the 65 children with HIE recruited at birth, 2 were excluded from outcome analysis due to co-existing diagnoses; 1 (mild) with congenital diaphragmatic hernia, and 1 (moderate) with unexplained dysmorphic features. Of the remaining 63 infants, 7 (5 mild, 2 moderate) were lost to follow up, and 3 (2 mild, 1 moderate) withdrew from the study resulting in outcome data for 53 children at five years of age. Of these, 6 (all severe) children had died either in the neonatal period or early childhood. The remaining 47 children were assessed at five years. In the comparison group, 42 children were contacted and 6 were lost to follow up and 6 withdrew, resulting in n=30 seen at five years. A flowchart depicting cohort numbers from birth to five years can be found in Figure 2.4 in Chapter Two.

Clinical and demographics characteristics of the HIE and comparison cases seen at five years are displayed in Table 5.2. No significant difference in socio-economic status was evident between the groups.

Table 5.2 Clinical and Demographic Characteristics of the Children Included at Five Years.

Perinatal data	**HIE Cohort (n=47)**	**Comparison Cohort (n=30)**	***P* Value**	***R* Value**
Birth weight (mean(SD)) g	3431.02 (618.26)	3567.67 (423.71)	0.29	0.12
Gestational age (mean(SD)) weeks	40.13 (1.48)	40.05 (1.17)	0.82	0.03
First Born n(%)	36 (78)	11 (37)	0.001*	0.42
Mode of delivery n(%)			<0.001*	0.56
NVD	8 (17)	22 (73)		
Instrumental	22 (47)	2 (7)		
Elective CS	0 (0)	2 (7)		
Emergency CS	17 (36)	4 (13)		
Male Gender n(%)	28 (60)	13 (43)	0.25	0.16
First documented pH (venous) mean(SD)	6.99 (0.18)	n/a	n/a	
First documented BD (venous) mean(SD)	-13.8 (4.9)	n/a	n/a	

Demographics	**HIE Cohort (n=47)**	**Comparison Cohort (n=30)**	***P* Value**	***R* Value**
Age assessed (Mean(SD)) months	64.11 (5.73)	67.57 (6.80)	0.03*	0.30
Has sibling(s) n(%)	32 (73)	29 (97)	0.02*	-0.31
Commenced Primary Schooling n(%)	36 (78)	20 (69)†	0.53	0.10
Maternal Education Level (n (%))			0.67	0.10
Primary or Lower Secondary	9 (21)	5 (17)		
Secondary School Completed	19 (43)	11 (37)		
Third Level Qualification	16 (36)	14 (47)		
Hollingshead occupation criteria* n(%)			0.57	0.17
Professional/Managerial (Codes 7-9)	15 (34)	12 (40)		
Semi-Professional/Technician/ Clerical (Codes 5-6)	8 (18)	8 (27)		
Skilled Manual (Code 4)	8 (18)	5 (17)		
Semi-skilled/Unskilled Manual (Codes 1-3)	13 (30)	5 (17)		
Race: White n(%)	43 (92)	30 (100)	0.15	-0.19
Region: n(%)			0.71	0.09
Urban	15 (32)	7 (23)		
Small Town	20 (43)	14 (47)		
Rural	12 (26)	9 (30)		

SD = standard deviation, NVD= normal vaginal delivery, CS=Caesarean Section, BD= base deficit, n/a = not available. *Based on Hollingshead Occupational Scale (238) R value = correlation with Full scale IQ at 5 years.

5.4.1 Cognitive Outcome:

5.4.1.1 Clinical Sarnat Grade

Of the 47 children with HIE who were assessed at 60 months, three children with severe CP were unable to complete the assessment. Based on available psychological, medical and educational placement information, two were assigned the basal IQ score, and the third was inconclusive. Cognitive assessment was therefore available for 46 children with HIE and in all 30 comparison children. The median FSIQ, VIQ PIQ, PSQ and GLC scores for each grade of HIE are displayed in Figure 5.1 and Table 5.3.

There were statistically significant differences in all WPPSI-III^{UK} cognitive domains across Sarnat grades (Comparison, n=30; Mild, n=22; Moderate, n=19; Severe, n=5). For Full Scale IQ, $\chi^2\ (3, n = 76) = 21.93,\ p < 0.001$. Median FSIQ, VIQ, PIQ, PSQ and GLC differed significantly between comparison and mild (p<0.001, <0.001, 0.004, 0.048 and 0.01 respectively) infants. Median FSIQ, VIQ, PIQ, and GLC differed significantly between comparison and moderate (p=0.001, <0.001, 0.02, and 0.001 respectively) infants, with PSQ at (0.06). No significant difference was seen between mild and moderate HIE infants in any of the WPPSI outcome parameters measured. Surviving infants with severe encephalopathy who were able to complete assessments had a variable outcome. The three children with CP had FSIQ indicating intellectual disability (= 68, 43, 43) and those without CP (n=2) had scores in the normal range. Similarly, for the CMS Numbers subtest, children with mild HIE had significantly lower scores compared to comparison children (p=0.004).

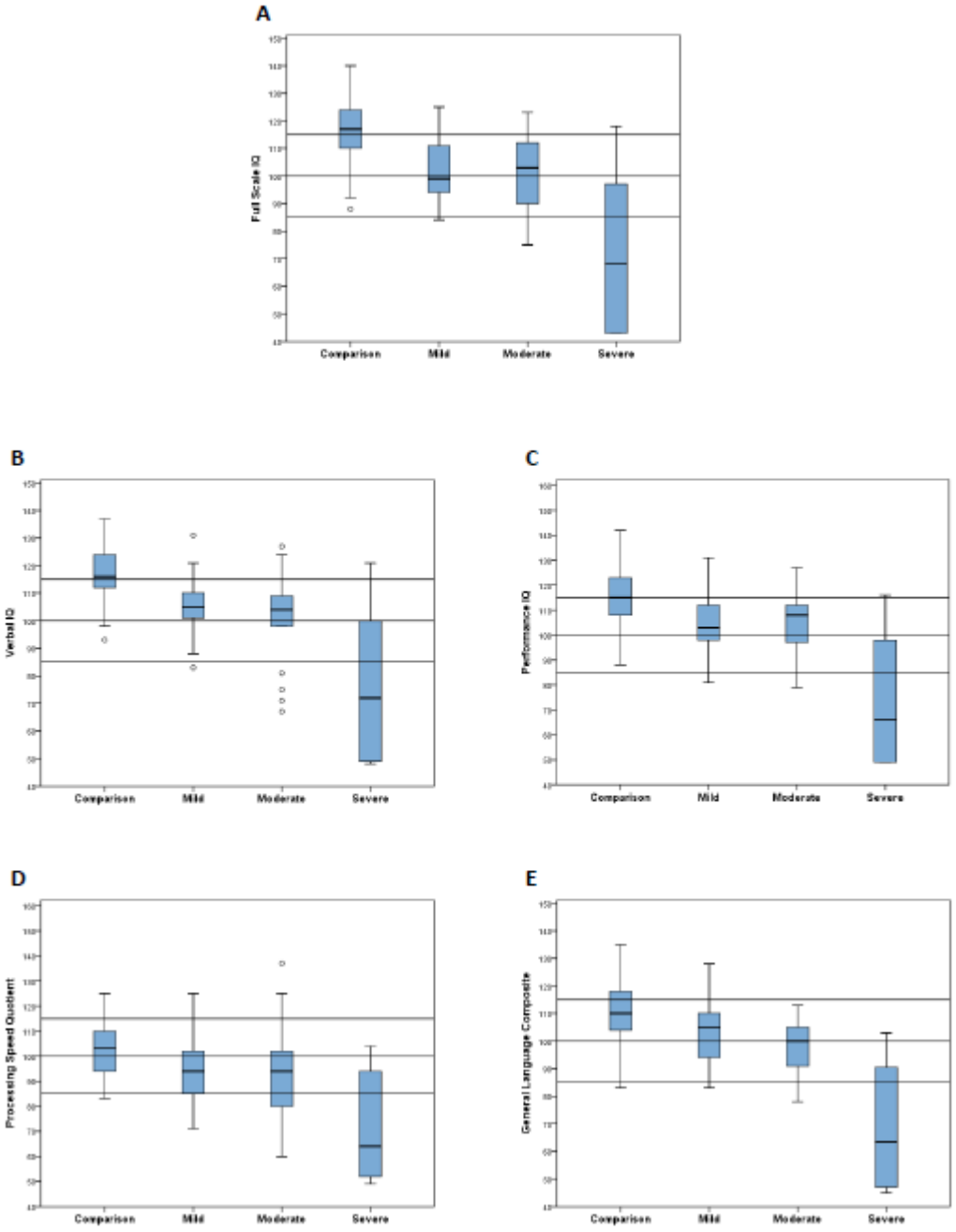

Figure 5.1 Boxplot representations of (A) WPPSI-III^{UK} Full Scale IQ and subquotient scores (B=Verbal IQ; C=Performance IQ; D=Processing Speed IQ; E=General Language Composite) for the HIE Sarnat grade groups and the comparison group. Horizontal lines set at mean(SD) expected = 100(15).

Table 5.3 Cognitive outcome assessment categorized by Sarnat grade at 24 hours.

WPPSI-III^{UK} Score	Comparison N=30	Mild N=22	Moderate N=19	Severe N=5	Comparison vs. Mild		Comparison vs. Moderate	
	Median (Interquartile Range)				*p*-value (effect size (r))			
Full Scale IQ	117 (110-124)	99 (94-112)	103 (90-112)	68 (43-108)	<0.001*	(0.51)	0.001*	(0.49)
Verbal IQ	116 (112-125)	105 (99-111)	104 (98-110)	72 (49-111)	<0.001*	(0.55)	<0.001*	(0.55)
Performance IQ	115 (107-124)	103 (98-112)	108 (96-114)	66 (49-107)	0.004*	(0.40)	0.02*	(0.34)
Processing Speed Quotient	103 (94-110)	94 (85-105)	94 (80-103)	64 (51-99)	0.048*	(0.28)	0.06	(0.27)
General Language Composite	110 (104-118)	105 (93-109)	100 (90-107)	64 (46-97)	0.01*	(0.35)	0.001*	(0.49)
CMS Numbers Subtest	11 (9-12)	7 (6-11)	9.5 (7.8-12.5)	5 (1-10.5)	0.004*	(0.42)	0.28	(0.17)

Q – Quotient, C – Composite, CMS = Children's Memory Scale. $p<0.05$=significant difference. r=0.1 -small effect, r=0.3 -medium effect, r=0.5 -large effect. No significant difference was seen between mild and moderate in outcome parameters measured. WPPSI-III^{UK} Standardized mean (S.D.) = 100(15); Numbers subtest mean(S.D.) = 10(3).

5.4.1.2 EEG grades at 6 and 24 hours

In all cases EEG grades either remained constant or improved between 6 and 24 hours. All comparison infants had grade 0 baseline EEGs recorded, at a median(IQR) age of 9.00(6.75-12.25) hours. EEG grades and Sarnat grading is displayed in Figure 5.2.

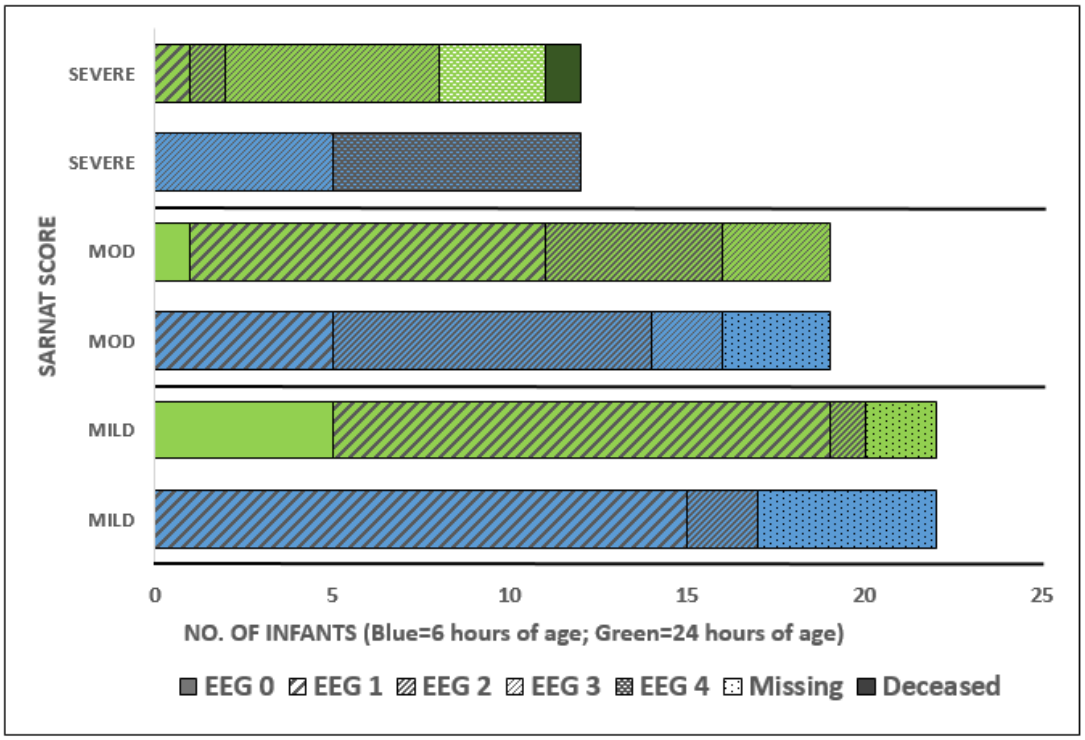

Figure 5.2 Distribution of EEG grades at age 6 and 24 hours for categorised by the infants Sarnat encephalopathy grade. Mod = moderate; Missing = EEG grade not available for infant

The evolution of EEG grades from 6 to 24 hours of age for infants with mild Sarnat grade are more closely seen in Figure 5.3 below.

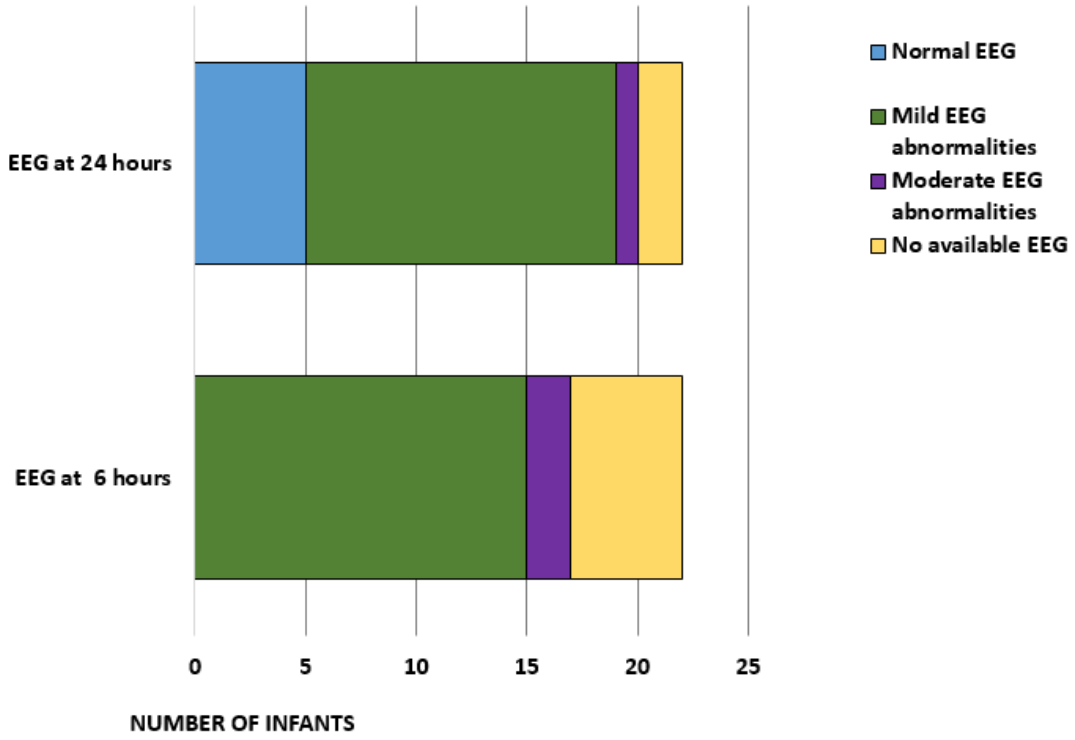

Figure 5.3 EEG gradings at 6 and 24 hours of age for infants with a Sarnat grade of mild HIE.

A Kruskal-Wallis Test revealed statistically significant differences in WPPSI domains across EEG grades 0-4. For the Full-Scale IQ, $\chi^2\ (4, n = 68) = 23.32, p < 0.001$ at 6 hours, and $\chi^2\ (4, n = 74) = 18.76, p = 0.001$ at 24 hours. At both 6 and 24 hours, those infants with Grade 0 EEG readings had the highest median IQ scores, and FSIQ scores decreased as EEG abnormalities worsened, but did not differ significantly between EEG grades 1-3 (see Table 5.4 for median(IQR) IQ scores, and Table 5.5 for IQ differences between grades 1-3). Infants with Grade 4 EEGs at either time point who survived, had severe to profound developmental delay, and due to very low numbers were excluded from further analysis.

Infants with a normal (Grade 0) EEG reading within the first 24 hours had significantly higher Full Scale, Verbal and Performance IQs than infants with abnormal (grades 1 to 3) recordings at either 6 or 24 hours.

Table 5.4 Five-year cognitive outcome for case and comparison infants with EEG Grades assigned at 24 hours of age.

WPPSI-III[UK]	EEG Grade 0 n=35	EEG Grade 1 n=26	EEG Grade 2 n=7	EEG Grade 3 n=9	EEG Grade 4 n=3	EEG Grade 0 vs.1	EEG Grade 0 vs. 2	EEG Grade 0 vs. 3
	Median (Interquartile Range)					*P* value (Effect Size (r))		
Full Scale IQ	117 (109-124)	104 (93-114)	97 (86-120)	97 (56-107)	43 (43-43)	0.002* (0.39)	0.02* (0.36)	0.008* (0.42)
Verbal IQ	114 (108-124)	106 (102-112)	100 (81-106)	98 (60-116)	49 (49-49)	0.001* (0.44)	0.01* (0.40)	0.03* (0.34)
Performance IQ	112 (105-123)	108 (98-113)	98 (90-108)	101 (58-110)	49 (49-49)	0.03* (0.29)	0.03* (0.34)	0.03* (0.35)
Processing Speed Q	102 (94-110)	97 (85-104)	94 (78-113)	73 (58-84)	49 (49-49)	0.08 (0.23)	0.41 (0.13)	0.001* (0.55)
General Language Composite	110 (102-117)	105 (96-110)	93 (78-101)	98 (57-107)	49 (49-49)	0.08 (0.23)	0.006* (0.44)	0.06 (0.31)

WPPSI-III[UK] = Wechsler Preschool and Primary Scale of Intelligence 3rd UK Edition, EEG 0 = normal EEG findings, EEG 1 = normal/mild abnormalities, EEG 2 = moderate abnormalities, EEG 3 = major abnormalities, EEG 4 = inactive EEG (see table 5.1 for detailed EEG grade descriptions), IQ = Intelligence Quotient, Q = Quotient, *p*=0.05 significance level for post hoc Mann-Whitney U Tests, r=effect size where 0.1=small effect; 0.3=medium effect; 0.5=large effect. Interquartile ranges rounded to nearest whole number.

Table 5.5 : Significance levels and effect sizes of median cognitive score differences at age five years between normal EEG Grade 0 and EEG Grades 1, 2 and 3 assigned at age 6 and 24 hours, and between Grades 1 and 2, 1 and 3, and 2 and 3 at age 6 and 24 hours.

WPPSI-III[UK] Quotients at five years	Age at EEG grade reading	Grade 0 Vs Grade 1	Grade 0 Vs Grade 2	Grade 0 Vs Grade 3	Grade 1 Vs Grade 2	Grade 1 Vs Grade 3	Grade 2 Vs Grade 3
		P value (effect size (r))					
Full Scale IQ	6 Hours	0.001**	0.002**	0.01*	0.18	0.11	0.65
		(0.45)	(0.48)	(0.43)	(0.24)	(0.32)	(0.11)
	24 hours	0.002**	0.02*	0.008**	0.36	0.11	0.37
		(0.39)	(0.36)	(0.42)	(0.16)	(0.29)	(0.26)
Verbal IQ	6 Hours	<0.001**	0.001**	0.02*	0.11	0.14	0.69
		(0.56)	(0.53)	(0.41)	(0.29)	(0.29)	(0.10)
	24 hours	0.001**	0.01*	0.03*	0.12	0.23	0.52
		(0.44)	(0.40)	(0.34)	(0.27)	(0.22)	(0.19)
Performance IQ	6 Hours	0.02*	0.01*	0.02*	0.32	0.28	0.61
		(0.34)	(0.39)	(0.39)	(0.18)	(0.22)	(0.13)
	24 hours	0.03*	0.03*	0.03*	0.24	0.21	0.57
		(0.29)	(0.34)	(0.35)	(0.20)	(0.23)	(0.16)
Processing Speed Quotient	6 Hours	0.37	0.40	0.02*	0.92	0.06	0.14
		(0.13)	(0.13)	(0.40)	(0.02)	(0.39)	(0.39)
	24 hours	0.08	0.41	0.001**	0.92	0.005**	0.06
		(0.23)	(0.13)	(0.55)	(0.02)	(0.52)	(0.54)
General Language Composite	6 Hours	0.001**	0.03*	0.006**	0.78	0.06	0.25
		(0.48)	(0.35)	(0.49)	(0.05)	(0.39)	(0.32)
	24 hours	0.08	0.006**	0.06	0.02*	0.23	0.59
		(0.23)	(0.44)	(0.31)	(0.42)	(0.23)	(0.17)

IQ=Intelligence Quotient, EEG 0 = normal EEG findings, EEG 1 = normal/mild abnormalities, EEG 2 = moderate abnormalities, EEG 3 = major abnormalities, EEG 4 = inactive EEG (see Table 5.1 for detailed EEG grade descriptions), *p*=0.05 significance level for post hoc Mann-Whitney U Tests, r=effect size where 0.1=small effect; 0.3=medium effect; 0.5=large effect. *=significance at .05 level; **=significance at .01 level.

5.4.2 Overall Outcome

A detailed picture of overall outcome at five years is displayed in Figure 5.4. When all types of disability and/or clinical requirement for multidisciplinary early intervention team support are examined, infants with a grade 1 EEG at 6 hours had a 75% chance of intact survival. Those with grade 2 EEG abnormalities had a 46% chance of intact survival and those with EEG grade 3 or 4 at 6 hours had only 21% intact survival. Overall at 6 hours a moderate/major/inactive EEG (grade 2 or more) had a PPV of 68% (95%CI; 0.465-0.843) and a NPV of 75% (95%CI; 0.506-0.904), AUROC = 0.712 (95%CI; 0.558-0.867) in predicting an abnormal outcome at 5 years. At 24 hours the ability of an EEG grade of 2 or more to predict abnormal outcome was PPV= 74% (95%CI; 0.486-0.899), NPV 68% (95%CI; 0.485-0.827) and AUROC = 0.696 (95%CI; 0.546-0.845). The improved PPV at 24 hours was due to the natural evolution of HIE, with normalization between 6 and 24 hours. Thus, EEG recordings which remained abnormal at 24 hours held a worse prognosis (Figure 5.4).

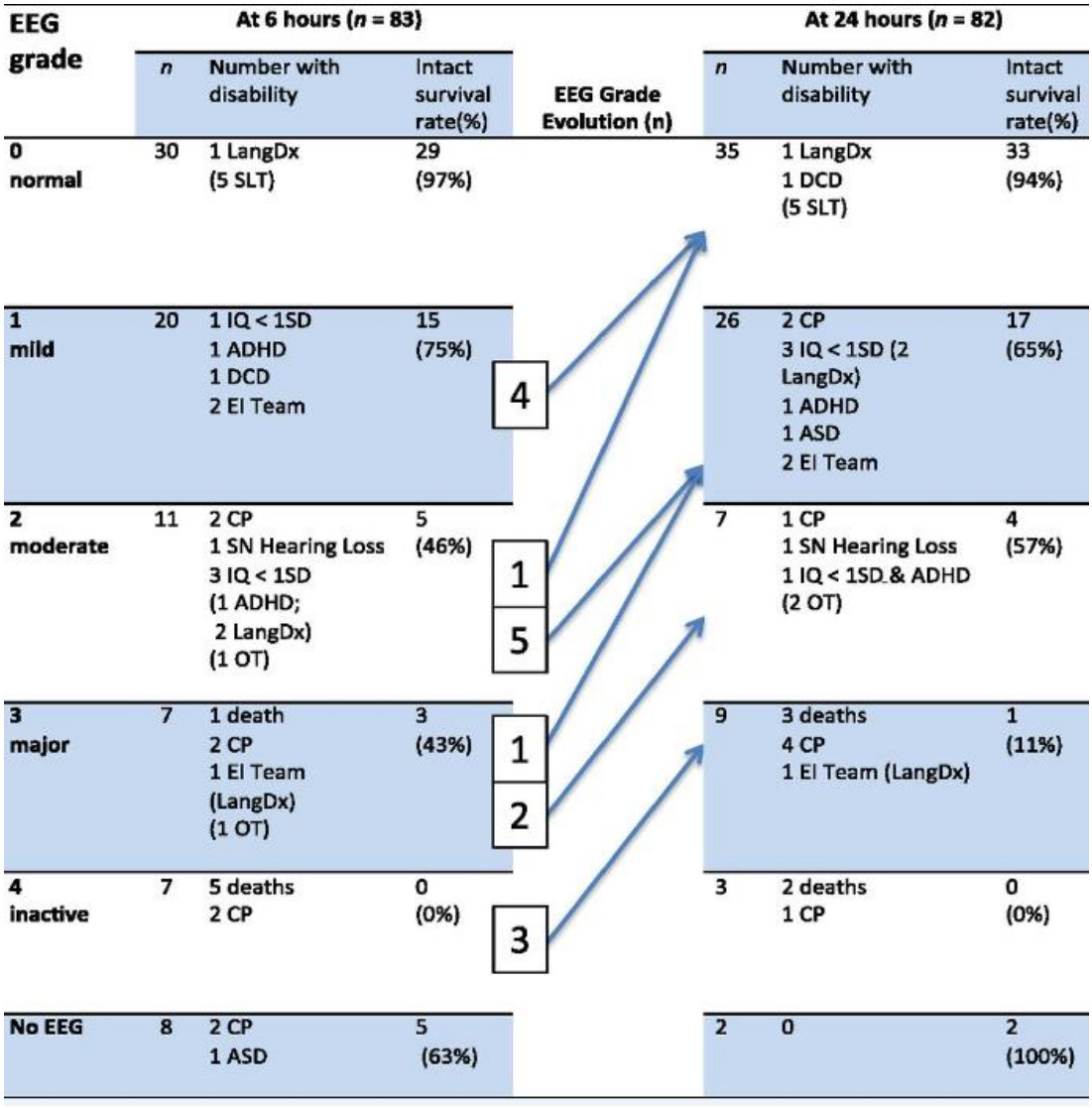

Figure 5.4 Detailed outcome data for children with outcome available at 5 years, categorised by EEG grade at 6 hours, and 24 hours after delivery. CP = cerebral palsy, OT = occupational therapy, SLT = speech & language therapy, EI team = attending multidisciplinary early intervention team, IQ < 1SD (<2SD, <3SD) = FSIQ > 1SD (>2SD, >3SD) below the mean at 5 years. ADHD = attention deficit hyperactivity disorder, ASD = autistic spectrum disorder, DCD = developmental coordination disorder, Language D/O, Lang = Diagnosed language disorder. Single therapy interventions (SLT, OT) are displayed in brackets, but did not define disability if child's outcome was otherwise intact.

5.5 Discussion

We have shown that whilst median IQ scores were within age-expected average levels, there were significant reductions in FSIQ, VIQ, PIQ, PSQ and GLC at five years in children who had mild HIE at birth compared to a comparison group. Children with mild HIE had higher rates of overall intact survival, but on detailed IQ assessments, children with a history of mild HIE

did no better than children with moderate HIE at birth. Greater differences were evident between the mild and moderate grades when a detailed analysis of outcome was examined. Intact survival following mild EEG abnormalities at 24 hours was 65%, and only 57% with moderate HIE. Both groups compared poorly to the comparison groups where 97% had an intact survival.

Current care strategies are based on the premise that outcome in infants with mild encephalopathy is normal, with studies reporting good outcome (74). However, a number of retrospective studies have recently shown rates of memory and learning impairment to be greater than expected, and somewhere closer to that of infants following moderate HIE (77, 124). In the era of hypothermia, studies have shown that infants classified as mild on clinical scoring alone have higher rates of morbidity in the neonatal period (239), but direct comparison to uncooled, moderate grade infants is no longer possible due to universal introduction of hypothermia in high-income countries. Our finding builds on growing evidence that some children with mild HIE do not follow a normal developmental trajectory. Significant, subtle disability can become more frequent with age as children 'grow into' their deficits (77, 124). The FSIQ's of children from Robertson & Finer's Canada cohort with mild-like HIE did not differ from controls at age 5, but did by age 8 (71). Recent retrospective cohorts suggest that up to 70% of adolescents have learning and behavioural difficulties which affect their everyday lives (240, 241). Unfortunately, most long-term follow-up studies either exclude those with mild HIE, or do not have local contemporary control populations.

Management of infants with mild HIE remains supportive. Current protocols for TH include infants with moderate to severe HIE only (139). These protocols are based on clear data from large randomized controlled trials of efficacy in moderate and severe HIE showing improved rates of intact survival (16, 18, 139). Even within these grades benefit is not universal with a number needed to treat of 7-8 infants for one intact survival (45). In infants with mild HIE, the risk:benefit ratio is likely to be even less balanced in favour of aggressive intervention. However, although the economic cost of NICU care and cooling is high, it may not be so when compared to lost academic achievement. Furthermore, preliminary indications from the roll-out of hypothermia in clinical settings report that infants with mild HIE are being treated in many centres (242). Recent studies highlight the difficulty in assigning HIE grade with many infants misclassified based on clinical grading alone (239). We have confirmed the accuracy

of our clinical grading at 24 hours by assessing simultaneous EEG recordings. This has allowed us to examine the ability of both clinical and EEG grade to predict long term outcome.

Our data support the need for future clinical trials of neuroprotective agents to include infants with mild HIE, both to ascertain the effect of cooling and to examine adjunct low cost medical therapies (243, 244). As 50% of infants with HIE have mild grade encephalopathy, the burden of disability with loss of academic potential and decreased employment prospects may be greater on a global scale, with greater numbers, but similar cognitive outcomes to that of moderate HIE (245).

It remains unclear why such an apparently mild injury may lead to adverse outcome. The answer may lie in *in vitro* and animal studies suggesting that an initial insult permanently alters the brain's ability to repair and develop. This "tertiary brain injury" appears to initiate an altered inflammatory or epigenetic response within the brain affecting oligodendrocyte development and synaptogenesis, leaving the brain more susceptible to further injury (30).

Our data shows the heterogenous profile of disability following HIE and the need for multidisciplinary early intervention. Additional multi-disciplinary developmental support was required in 35% of children with mild EEG abnormalities at 24 hours, compared to 3% in the comparison group. Although previous reports have estimated an increased rate of ASD in HIE (241), our rates of ASD and ADHD were not higher than expected (246-248).

Our data confirms the importance of long term follow up to school age, and the use of contemporaneous local controls. At our two year outcome assessment these children were compared to standardized norms and almost all mild infants scored within normal ranges (105). Unfortunately, we did not assess a comparison group at this stage. Longer term follow-up, with the availability of a comparison group has revealed significant differences at school age. Our comparison group performed better than expected on WPPSI-III^{UK} assessment, with median FSIQ, VIQ and PIQ scores one standard deviation above the mean. Scores approaching this level of attainment have been recorded in control groups of children in other studies using the WPPSI-III (249). For HIE studies, mean IQ scores in controls of 109 (Utrecht cohort) and 115 (Canada cohort) in 8-10 year-olds were recorded using the WISC (Wechsler Intelligence Scale for Children) scales (see also Chapter 1, Table 1.4 and Table 1.5) (77, 131). This may be due to local population differences, the socio-economic profile of the group, or the Flynn effect of IQ drift over time, estimated by the test publishers to be a 0.33

score increase per annum (250). This would equate to a 2.3 to 3.6 point increase for our study.

Our comparison group was used because of the availability of neonatal EEG for them and were recruited based on an uneventful perinatal course. No significant differences in socio-economic status (SES) or maternal education existed between our case and comparison groups However, this group may be 'healthier' than children chosen from the normal population. We reviewed a number of our comparison groups' clinical and demographic variables to an Irish national cohort – the 'Growing Up in Ireland' study – which randomly recruited a national infant cohort of 11,100 families in 2008 (see Chapter 2 Table 2.3). Data from this study suggests that in our comparison group, males are underrepresented (43.3% Vs. 51.3%), and that our cohort had more infants born after an unassisted delivery (73% Vs. 58%). Both of these factors may have contributed to inflated scores. However, rates of maternal education were surprisingly consistent (84% Vs. 82%). This points to the critical need for further studies which include designs that carefully case-control for demographic determinants of lower IQ such as male gender, age and educational level, SES and maternal education.

The major limitation of the study is the small numbers available with outcome at 5 years. However, recruitment of infants with HIE is difficult and few cohorts are studied prospectively from birth. Fewer still include children with mild HIE. Our cases have been carefully categorized using both EEG and Sarnat grading, and our comparison group uniquely had early EEG and clinical assessment of their neurological status at birth. Follow up was detailed and blinded to neonatal data in both the cases and the comparison group. Very little prospective data on non-cooled cohorts comparing mild to moderate HIE is available. Although rates of disability in infants with moderate HIE will now be altered due to therapeutic hypothermia, there are no approved and effective therapies for those with mild or severe infants. Our data has shown that significant disability occurs within these grades at 5 years, and supports continued research in these cohorts to develop adjunct therapies suitable and effective in all grades of HIE.

In conclusion, we have shown that cognitive outcome, measured by the WPPSI-III^{UK} is age-expected but significantly reduced following mild, moderate or severe HIE compared to a comparison group, whether graded by EEG or clinical Sarnat grade. Although mild HIE had the highest rates of intact survival; no difference in cognitive ability was evident between

mild and moderate grades of HIE. Our data supports the need for future studies of neuroprotection to include infants with mild HIE.

COMMENT: My contribution to this chapter is described in further detail in Chapter 8 Section 8.1: Summary and Impact of the Thesis Findings.

CHAPTER 7

7 Ophthalmic and Neurodevelopmental Outcomes Following Neonatal Hypoxic-Ischaemic Encephalopathy; Oculomotor, Biometric, and Refractive Data in Early Childhood

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7.1 Abstract

Objectives:

To investigate the functional and structural impact of neonatal hypoxic-ischaemic encephalopathy (HIE) on childhood visual development and neurodevelopmental outcomes.

Methods:

In a prospective study, the neurocognitive outcomes of 42 children with a history of neonatal HIE, graded at birth by clinical Sarnat score and early continuous EEG, were assessed serially up to 24 months with the Griffiths' mental development scale (0-2) revised and at 5 years using the WPPSI-III^{UK}. Visual, refractive, orthoptic and ocular biometry measurements were obtained in 32 children, with axial length measurements estimated using the IOLMaster.

Results:

Of the 32 children who completed the ophthalmic section of the study, the severity of HIE grade was determined to be mild, moderate, or severe in 18 (56.3%), 13 (40.6%), and 1 (3.1%) cases, respectively. One (3.1%) child was classed as visually impaired. Twelve (37.5%) were found to have ametropia. Mean (± SD) axial length was 22.09 (± 0.81) mm, within the normal range for the age of this cohort. Seven of the 42 (16.7%) children who were involved in the larger neurodevelopmental arm of the study had clinical evidence of a squint. There was no correlation between the severity of HIE grade at birth and axial length, occurrence of squint, or motor outcome.

Conclusions:

Ocular biometry measurements were not adversely impacted by HIE, showing no correlation with the severity of HIE grade. Neonatal HIE is associated with a higher incidence of squint compared with the general paediatric population. This occurred despite normal motor outcome and in both mild and moderate to severe HIE grades.

7.2 Introduction

Neonatal hypoxic-ischaemic encephalopathy (HIE) remains one of the commonest causes of acquired neonatal brain injury, affecting approximately 3 per 1000 term births in the developed world with a rate ten times higher in the developing world (127). It causes 23% of all neonatal deaths, and surviving infants remain at risk for significant neurological disability including cerebral palsy (CP), epilepsy, intellectual disability and sensory loss. Recent research has also reported learning and behavioural difficulties often not detected until school age (222).

Visual loss is frequently associated with neurological disability making perinatal HIE, in term and preterm infants, the most common cause of visual disability in developed countries (258). While damage to the primary visual cortex, visual associative cortex, optic radiations, optic nerves or visual attention pathways can occur (258), the mechanism of injury and prognosis for recovery is usually unclear. Cortical occipital lesions do not correlate with the degree of visual loss, and recovery of cortical blindness can occur in the first few years of life (259-261).

Head growth in early life can be related to the severity of the initial hypoxic insult, and result in suboptimal growth and consequent microcephaly (218). This may have ophthalmic implications as generally the growth pattern of the eye mirrors that of the brain (262, 263). Most postnatal growth of the eye occurs within the first 3 years of life, with a rapid phase acceleration evident from birth to 6 months of age (264-268). It is uncertain whether perinatal ischaemic injury has a negative effect on early ocular growth rate similar to the restriction in brain growth. There is a correlation between the impact HIE has on early visual function and subsequent neuromotor development (269), with increased rates of strabismus usually explained in terms of concomitant motor difficulties and/or CP (270).

Few studies have followed the visual outcome of children with neonatal HIE to school age so limited outcome data is available, and, to our knowledge, none have previously reported detailed ocular biometry. In this paper we report on the ophthalmic outcomes, including visual, refractive, orthoptic and ocular biometry measurements of a carefully defined prospective cohort of infants with neonatal HIE and compared this with detailed neurocognitive outcome from infancy through to school age.

7.3 Methods

Term infants with suspected HIE were prospectively recruited between May 2003 and December 2005 from the regional maternity service. Inclusion criteria for the study were term (≥37 weeks) infants who met at least two of the following: (a) initial capillary or arterial pH <7.1, (b) Apgar score <5 at 5 minutes, (c) initial arterial or capillary lactate >7 mmol/L, (d) abnormal neurology or clinical seizures. The exclusion criteria were evidence of co-existing congenital anomaly, or significant co-morbidity. Written informed consent was typically granted within 6 hours of birth, but outside this timeframe if later seizures emerged. Comprehensive neurological assessments and continuous early EEG was recorded. Each infant was assigned a modified Sarnat encephalopathy grade at 24 hours and HIE grade was confirmed on serial EEG recordings (14, 188). Early developmental progress was assessed at 6, 12 and 24 months of age using the Griffiths Mental Development Scales – Revised, (172) and administered by a registered user of the test (Dr. DM Murray). This standardized test provides an overall development quotient (GDQ) and subdomain quotients for locomotor, personal/social, hearing/language, hand/eye co-ordination, and performance. The mean GDQ is 100.5 (11.8). An abnormal score is ≤88. This is an 'uncooled' cohort due to being recruited in the pre-therapeutic hypothermia era (17). The two year and five year neurodevelopmental outcome of this cohort has been previously reported (105, 252).

The current study was part of this longitudinal study which followed the neurodevelopmental progress of these children to five years and was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Research protocols were in keeping with the tenets of the Declaration of Helsinki. Parents or carers of the children in the HIE birth cohort from age three years onwards were contacted by telephone by CM O'Connor who explained the nature of the vision tests and to obtain consent to forward contact details to the ophthalmic team. Written informed consent for the ophthalmic component of the study was obtained from parents/carers by Dr M James at the ophthalmic appointment.

During their ophthalmic assessment, data recorded for each participant was classed as demographic, clinical, visual, orthoptic, biometric, and refractive. Clinical data included: general medical history; family history of ocular disorders; birth weight (kg); occipito-frontal circumference (OFC) (cm); HIE grade; and anterior segment and fundal findings on slit lamp biomicroscopy. Visual data included: Snellen, logMAR, or Sheridan-Gardiner visual acuity as

appropriate; and confrontation visual fields assessment by the use of a target gradually moved in from the periphery until detected. Orthoptic data was assessed by an experienced orthoptist (B Haskins), and included: ocular alignment on cover testing; angle of squint by prism cover testing; ocular motility, including convergence; and stereopsis determination by the Lang II stereo test.

All biometric and refractive measurements were performed by a single ophthalmologist (Dr. M James). Cycloplegic autorefraction was performed 30 minutes following instillation of a drop of cyclopentolate 1% to each eye, with the use of one of three autorefractors which were available at different times through-out the duration of the study: the NIDEK Auto Refractometer AR-630A (Nidek CO., Japan), the Topcon RM-A3000 Auto Refractometer (Topcon, Tokyo, Japan), or the NIDEK Auto Refractometer AR-800 (Nidek CO., Japan). Reliability of data was checked by performing cycloplegic streak retinoscopy in all cases using a Welch Allyn retinoscope (Welch Allyn Medical Products, Skaneateles Falls, New York). Results recorded in sphero-cylindrical form, including axis, were used to calculate the spherical equivalent (SE) refractive error for each eye. Ocular biometry using the IOLMaster (Carl Zeiss Meditec, Jena, Germany) was used to obtain average axial length (mm) and keratometry values (expressed as the mean of the steepest and flattest meridian in dioptres (D)).

Visual impairment was defined as a presenting vision (i.e. unaided or with glasses if worn) of less than 6/12 in the better seeing eye. Anisometropia was defined as an interocular difference in SE of ≥ 1.0 D. As there were no statistically significant differences between refractive and ocular biometry data between right and left eyes, all subsequent analyses used data from a single eye in each case following randomization.

At 5 years of age, the study participants were seen either at home or at the university by a clinical psychologist (C O'Connor). Each child's intellectual abilities were assessed using the Wechsler Preschool and Primary Scale of Intelligence - III (UK version) (WPPSI-III^{UK}), which yields a full-scale IQ score (FSIQ), verbal IQ (VIQ), performance IQ (PIQ), processing speed quotient (PSQ) and general language composite (GLC). The mean score is 100 (15). An abnormal score is <85.

7.3.1 Statistical analysis

Ocular data were analysed using Stata package ver. 8.0 (StataCorp. 2005; Stata Statistical Software: Release 9.0; College Station, TX). The associations between the categorical variables were examined using the Chi-square test. IBM SPSS Statistics (version 22.0.0.1) was used to explore differences between normal/abnormal vision outcome and clinical, developmental and IQ data using Chi-square test for independence for categorical variables and Mann-Whitney U tests for continuous variables as these were not normally distributed. Statistical significance was set at p value < 0.05.

7.4 Results

The study flow and attrition of the cohort is displayed in Figure 7.1. Of the 42 children included in the study group, the parents of 33 children agreed to allow them to participate in detailed ophthalmological examinations, while the remaining nine did not wish to return for assessment, but agreed to a review of their existing ophthalmology notes. Two (22.2%) of these nine children had known ophthalmic issues (both with moderate HIE and previously diagnosed squint), while there were no visual concerns reported for the other seven.

Of the 33 children available for detailed ophthalmological examination, one infant with severe HIE at birth and severe CP was unable to co-operate with biometric measurements; however, no gross squint or significant refractive error was noted. Thus, complete ophthalmological assessments were available in 32 infants. The mean age (± SD) at ophthalmic examination was 61.0 (± 9.2) months, (range: 40 to 76 months). The ratio of boys (n=21) to girls (n=11) was 1.9:1 (see Table 7.1). Ethnicity of children were Caucasian (31; 96.9%), and Asian (1; 3.1%). HIE severity, assigned by Sarnat grade, was mild, moderate, or severe in 18 (56.3%), 13 (40.6%), and one (3.1%) cases, respectively (a detailed break-down of Sarnat grade proportions for each part of the study is displayed in Figure 7.2). Median (IQR) birth OFC by Sarnat grade were as follows: mild = 36.0cm (34.0-37.05), moderate = 36.0cm (34.35-36.3), severe = 36.8cm (n=1), with no significant difference between mild and moderate HIE grade (p=0.53). There were 2/32 (6.3%) children with an OFC below the 3rd centile, one with mild and one with moderate HIE.

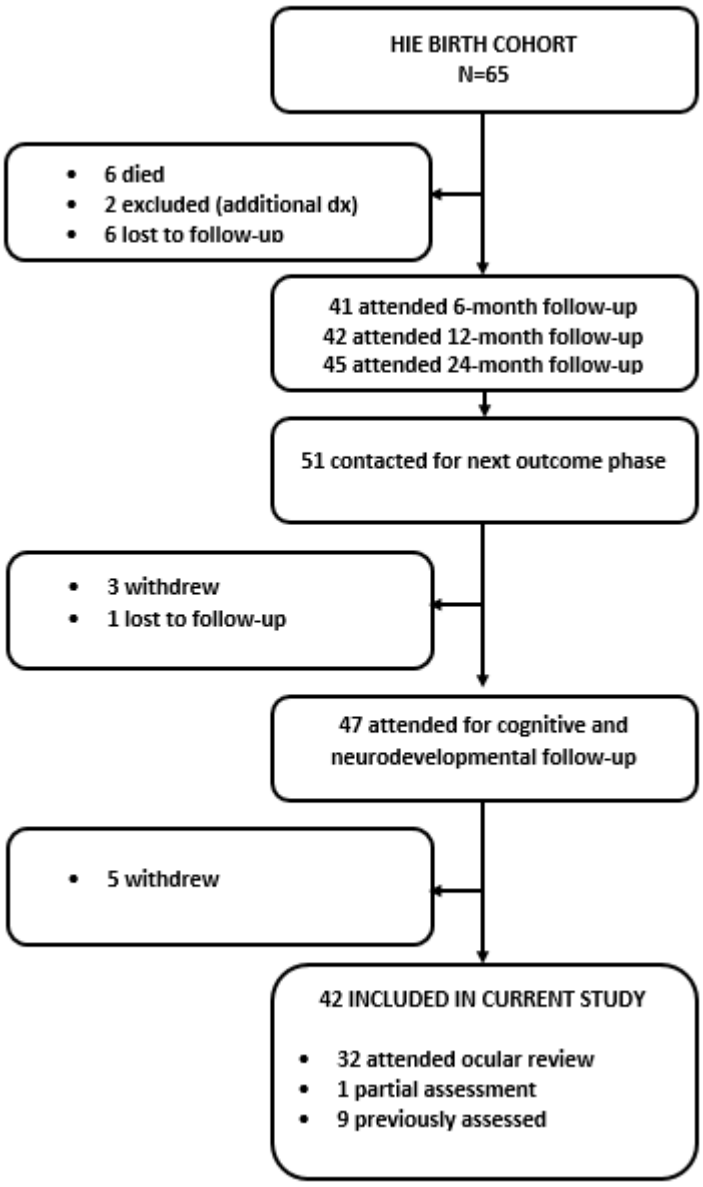

Figure 7.1 Flowchart of HIE birth cohort and attrition over time to 5 years.

Table 7.1 Details of HIE grade and birth parameters by gender for children who attended for ophthalmic examination.

	Boys (n=21)	Girls (n=11)	Total (n=32)
HIE Grade, n (%)			
Mild	13 (61.9)	5 (45.5)	18 (56.3)
Moderate	7 (33.3)	6 (54.5)	13 (40.6)
Severe	1 (4.8)	0 (0.0)	1 (3.1)
Birth weight (kg)			
Mean (± SD)	3.5 (± 0.46)	3.16 (± 0.73)	3.38 (± 0.58)
Median (range)	3.59 (2.44 – 4.20)	3.21 (1.83 – 4.18)	3.45 (1.83 – 4.20)
Low BW* n (%)	1 (4.8)	2 (18.2)	3 (9.4)
Birth OFC (cm)			
Mean (± SD)	35.72 (± 1.85)	35.53 (± 2.31)	35.65 (± 1.99)
Median (range)	36 (31.5 – 40)	35.5 (32 – 40)	36 (31.5 – 40)

*Birth weight less than 2.5kg; OFC=occipito-frontal circumference (head circumference)

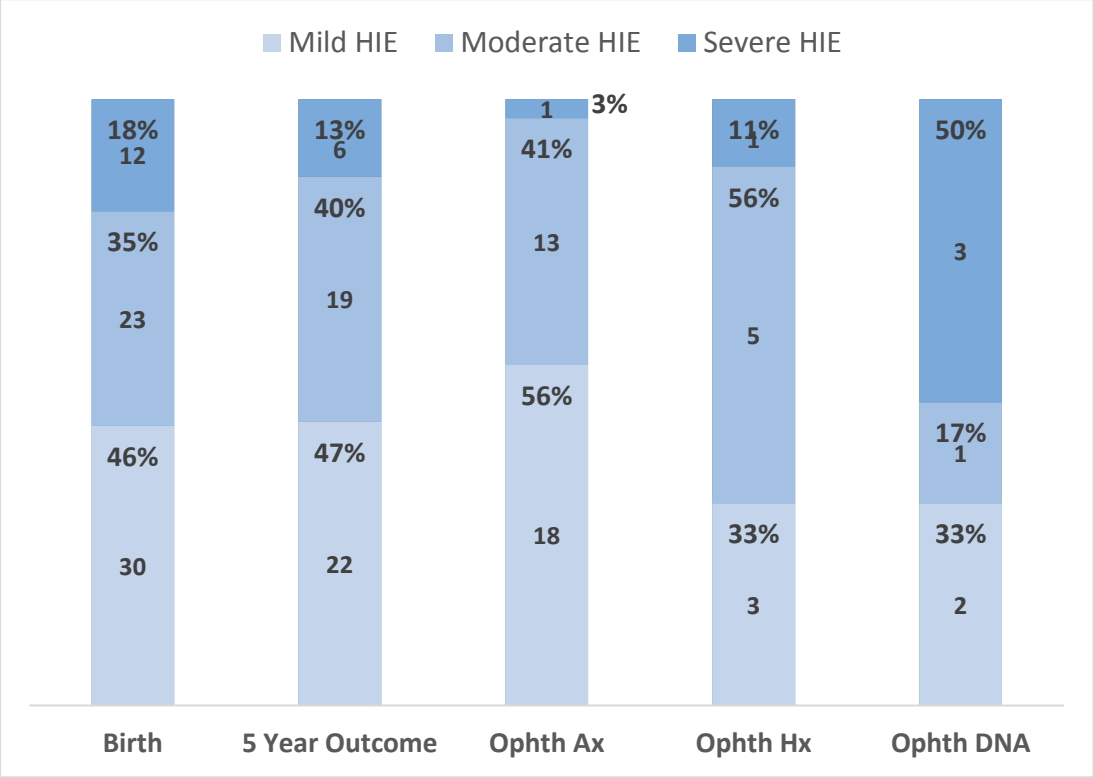

Figure 7.2 Proportion of children in the HIE cohort with mild, moderate and severe Sarnat grade at birth, five year cognitive outcome and for the current study. Ophth Ax – children who attended ophthalmology assessment; Ophth Hx – children whose parents consented to obtaining vision history but did not attend ophthalmology assessment; Ophth DNA – children who withdrew/did not attend ophthalmology assessment.

Normal visual fields were found in 27 (84.4%) children, while the remaining five (15.6%) had no obvious defect, but had insufficient co-operation to allow reliable assessment. A presenting visual acuity in either eye of 6/12 or better was documented in 31 (96.9%) children, and 6/6 or better in 29 (90.6%). One (3.1%) child was classed as visually impaired, with a visual acuity of 6/18 in each eye in the absence of any significant refractive error or ocular pathology. Two (6.3%) children were noted to have an amblyopic eye, with a visual acuity of 6/12 or worse, secondary to anisometropia.

A summary of the abnormal ophthalmic outcomes by HIE grade is displayed in Table 7.2. Five (15.6%) children had evidence of a manifest squint: two had accommodative esotropia with hyperopia; another three had divergent strabismus with no significant refractive error but had a positive family history of squint. There was no statistically significant difference in strabismus rates between the HIE grades (Table 7.2).

Table 7.2 Distribution of visual impairment, refractive error, and squint by severity of HIE grade for children who attended for the full ophthalmic examination section of the study.

HIE Grade†	No. of children	Visual acuity of 6/18 or worse in better eye n (%)	Ametropia in either eye* n (%)	Strabismus** n (%)
Mild	18	1 (5.6)	9 (50)	3 (16.7)
Moderate	13	0 (0)	2 (15.4)	2 (15.4)
Severe	1	0 (0)	1 (100)	0 (0)
Total	32	1 (3.1)	12 (37.5)	5 (15.6)

*: Spherical equivalent refraction of ≥ +2.0 D or ≤ -0.5 D in either eye, astigmatism of ≥ 1.5 D.
**: Strabismus defined as the presence of a manifest deviation on cover testing.
†: There were no statistically significant differences in the ocular parameters by HIE grade.

The refractive error, angle of squint, and other notable associations are detailed in Table 7.3. This table also shows the neurodevelopmental outcomes of two additional children who had a diagnosis of squint documented in their medical/orthoptist notes from the larger cohort of 42, but did not partake in the full ophthalmic assessment arm of the study. Thus, the total number of children with confirmed squint from the whole cohort was seven (16.7%).

Table 7.3 Subgroup characteristics of children who had manifest strabismus.

No. and gender	Age (mths)	HIE grade	Squint type	Angle of squint at 1/3m (prism diopters)	Lang stereo test	SE refractive error (D)*	Other visual findings	Overall outcome
1. Female	74	Mild	Exo	12	4/4	+1.125	Convergence insufficiency	Early Intervention
2. Male	76	Mild	Eso	20	4/4	+1.875		ADHD; VIQ >1SD below mean
3. Male	47	Mild	Exo	45	4/4	+2.375	Saccades initiation failure	ASD; PSQ>1SD below mean
4. Female	61	Mod	Exo	20	4/4	+1.75		Normal
5. Male	60	Mod	Eso	12	4/4	+4.625		ADHD; FSIQ >1SD below mean
6. Female**		Mod	Exo					Normal; PSQ >2SD below mean
7. Female**		Mod	Exo					FSIQ>1SD below mean; language disorder

D = diopters; SE = spherical equivalent; Eso = esotropia; Exo = exotropia; SD = standard deviation; Mod = moderate; ADHD = attention deficit hyperactivity disorder; ASD = Autism spectrum disorder; FSIQ = full scale IQ; VIQ = verbal IQ; PSQ = processing speed quotient: Early Intervention = receiving multidisciplinary clinical intervention. *: SE refractive error of the eye with the greater refractive error. **: Two additional children with confirmed history of squint from orthoptist notes were participants of the main HIE study group, but were not from the 32 who attended for the full ophthalmic assessment part of the study.

No statistically significant difference between refractive data achieved through autorefraction and that from retinoscopy was noted ($p > 0.05$). The mean (± SD) SE refractive error as calculated by cycloplegic autorefraction was +1.82 (± 1.74) D for right and +1.79 (± 1.44) D for left eyes. The overall range of SE refractive error was -0.625 D to +7.25 D. Mean astigmatism was +0.44 (± 0.42) D for right and +0.57 (± 0.69) D for left eyes, with a range in either eye from 0 D to 3.75 D. In total, 12/32 (37.5%) children were found to have ametropia; one child (3.1% of sample) was classed as myopic, nine (28.1%) as hyperopic, three (9.4%) as having astigmatism (of whom one was also hyperopic). Two (6.3%) children were noted to have anisometropia (both were also in the hyperopic group).

Ocular biometry assessment showed the mean (± SD) axial length was 22.04 (± 0.88) mm for right and 22.06 (± 0.81) mm for left eyes (overall range, 19.74 – 24.77 mm). The mean (± SD) keratometric measurement was 43.18 (± 1.42) D for right and 43.18 (± 1.45) D for left eyes (equivalent to a mean corneal radius of curvature of 7.81 mm). All subsequent analyses used data from a single eye in each case following randomization, a summary of which is given in Table 7.4. There was a positive correlation between axial length and age (rho=0.4919, *p*=0.0042), with children over 60 months of age having statistically significant longer median axial length than those under 60 months ($p = 0.039$). Otherwise, there was no statistically significant differences between the ocular biometric parameters (axial length, SER, or K) and sex or HIE grade (mild Vs. moderate) on univariate analysis.

Table 7.4 Mean (± SD) refractive and biometry measurements of the randomized eye by gender, age, race, and HIE grade.

	No. of children	Axial length (mm)	Keratometry (D)	SE refractive error (D)
Gender				
Male	21	22.20 (± 0.92)	43.00 (± 1.47)	+1.74 (± 1.73)
Female	11	21.87 (± 0.47)	43.57 (± 1.39)	+1.60 (± 0.97)
Age (months)				
40-60	15	21.74 (± 0.64)	43.38 (± 1.29)	+2.11 (± 1.94)
61-76	17	22.40 (± 0.82)*	43.04 (± 1.60)	+1.32 (± 0.85)
Race				
Asian	1	22.04	43.25	-0.625
Caucasian	31	22.09 (± 0.82)	43.20 (± 1.47)	+1.77 (± 1.46)

HIE Grade				
Mild	18	22.12 (± 0.99)	43.15 (± 1.66)	+1.99 (± 1.72)
Moderate	13	22.05 (± 0.53)	43.26 (± 1.24)	+1.46 (± 0.97)
Severe	1	22.04	43.25	-0.625
Total	32	22.09 (± 0.81)	43.20 (± 1.45)	+1.69 (± 1.50)

*p = 0.039 with respect to the difference in mean axial length between the age groups. D = diopters; SE = spherical equivalent; SD = Standard deviation.

We next analysed the developmental progress of children post-HIE with a normal (assessed or reported) versus abnormal ophthalmic outcome (i.e. those with visual impairment, strabismus and/or ametropia). We found no significant differences in median Griffiths' development scores obtained at six, 12 or 24 months or five-year WPPSI-III^{UK} IQ scores between those with normal and abnormal ophthalmic outcomes. At five years, Performance IQ, which consists of visually-based tasks, was approaching significance (p=0.06). In addition to IQ, there was no significant increase in the proportion of children with an overall abnormal five-year neurodevelopmental outcome between those with normal and abnormal ophthalmic outcomes.

Whilst no children in the cohort with confirmed strabismus had a diagnosis of CP, there was evidence of delayed early motor development at 12 and 24 months of age. Global development quotients (DQs) were significantly lower at 12 months (p = 0.038) and 24 months (p = 0.025) in children with strabismus. These global differences were influenced at a domain level by lower locomotor scores at both ages (See Table 7.5).

Table 7.5 Comparison of early development scores at 12 and 24 which significantly differed between children with and without strabismus.

Griffiths Score	Strab. Md (IQR)	Strab. 'n'	No strab. Md (IQR)	No strab. 'n'	Z score	*P* value
Global (12m)	84 (68-91)	4	100 (88-109)	31	-2.08	0.038
Global (24m)	87 (52-101)	7	109 (99-113)	31	-2.25	0.025
Locomotor (12m)	63 (51-72)	4	87 (80-100)	31	-2.16	0.031
Locomotor (24m)	88 (49-102)	7	116 (90-126)	31	-2.40	0.016

Personal-Social (24m)	89 (52-106)	7	112 (99-119)	31	-2.07	0.038

Md = median; IQR = inter-quartile range; Global = Griffiths Mental Development Scales global development quotient.

At five years, there were no significant differences between the children with and without strabismus for measured IQ. While the incidence of abnormal overall outcome (defined as at least one of the following: CP, vision/hearing loss, neurodevelopmental disorder, need for early intervention, or IQ < 85) was higher in the strabismus group compared to children without strabismus (five out of seven (71.4%) versus 12 out of the 35 (34.3%), respectively), the result was not statistically different (see Table 7.3).

7.5 Discussion

We have described the detailed ophthalmic outcomes of a prospective cohort of infants following neonatal HIE. The infants were born prior to the introduction of therapeutic hypothermia treatment for those with moderate-severe HIE. In a recent Cochrane review of the effects of cooling (therapeutic hypothermia) infants with neonatal HIE, seven of the clinical trials reported visual outcome rates (45). Fifty-eight of 749 survivors (i.e. 7.74%) were legally blind. Meta-analysis across the trials suggested a non-significant reduction in blindness in infants who were cooled (typical RR 0.62 (95% CI 0.38 to 1.01). Thus, adverse sequelae relating to vision remains a significant outcome for infants in this population. It is important to note that these randomized controlled trials have focused on infants with moderate to severe encephalopathy only. Our data adds significantly to the limited knowledge that is available for outcome following mild HIE.

HIE causes brain injury through a process of inflammation and cell death involving oxidative stress and excitotoxicity, which can result in death or permanent neurologic deficits such as cerebral palsy, epilepsy, and learning disorders (271). The ophthalmic effects are characterized by restriction of the visual fields and may include severe visual loss, with neuroimaging studies suggesting that a greater trend towards visual dysfunction in infancy has been shown when lesions involve the basal ganglia (272). The visual impairment noted in early life interferes with visual exploration at a critical time of neuronal maturation in the

occipital lobes, and has been shown to have a negative impact on visual function at school age (79, 273).

The prevalence rate of visual impairment in Caucasian children of seven years of age in a British population-based study was found to be 0.5% (273). While higher than this, the rate of visual impairment in our study would still be considered low, with no child having clinically observable restriction of their visual fields, and only one (3.1%) child found to have visual impairment where acuity was worse than 6/12 in both eyes. This child had mild HIE, but had no significant refractive or other ocular cause found for reduced vision, raising the possibility of cortical impairment or injuries to the inner retina, where chronic functional and structural changes have been found in experimental models of induced HIE (274, 275). The rate of adverse visual outcomes in our study was lower than those found by Mercuri et al. which involved 39 children affected by HIE of a similar age group, where seven (17.9%) children were classed as having severe visual impairment, and a further six (15.4%) found to have narrow visual fields (79). This may be due to differences between the cohorts with respect to Apgar criteria, classification and severity of HIE grade. Our cohort included those with mild HIE who would be expected to have a better outcome. In addition, at 5 years, very few infants with severe grade HIE were still alive, or able to co-operate for a complete examination due to severe motor or intellectual difficulties.

Our incidence of manifest squint of 16.7% was similar to that reported by Mercuri et al. (79), and exceeds the number that would be expected in a healthy childhood population of between 1.5 and 4.0% (273, 276-279). Consistent with our findings, a retrospective Norwegian population based study of infants with a combination of low Apgar score (0-3) and neonatal signs of encephalopathy found that school-aged children had significantly increased risk of strabismus and requirement for glasses (21). One explanatory hypothesis proposes that adverse intrauterine and perinatal events during a critical period of brain development may disrupt the prenatal programming of coordination and control of eye movements (279). Cerebral visual impairment of hypoxic-ischaemic origin is associated with higher rates of ocular motility disorders, including saccadic initiation failure, absence of pursuit, nystagmus, and variable angle squint (280). The co-occurrence of strabismus and problems with specific areas of early development such as locomotion in premature infants (281), and eye-hand coordination and depth perception has been previously recorded (282). None of the children with strabismus in our study had CP and yet had significantly lower

locomotor skills at 12 and 24 months of age, contributing to significantly lower global development scores at these ages. We did not find significant differences for eye-hand coordination at these early ages or visuomotor processing speed at age five in our cohort, and it remains to be seen whether this was due to the low number of tasks requiring depth perception skills on the measures used. The role strabismus plays in the acquisition of these developmental tasks is an important focus for future study; for example, motor improvements have been observed post-esotropic surgery (283).

Our cohort was predominantly white European, whose mean SE refractive error of +1.69 D is close to that found in both a Spanish and an American study involving healthy Caucasian children of an equivalent age (+1.49 D and +1.53 D, respectively), with ethnicity being an important consideration as there are lower rates of myopia reported in Caucasian children (284, 285). Additionally, rates of myopia and hyperopia in our study were similar to that found in population-based data involving Northern Irish children with a comparable age and race profile (3.1% vs. 2.8%, and 28.1% vs. 26% respectively) (286). Therefore, it appears that mild and moderate HIE does not confer any additional risk with respect to the development of refractive error in early childhood.

Axial length increases with age in the paediatric population (264-266), so it is unsurprising that there was a significant difference in axial length comparing those under and over the age of five years. Axial growth rates in infants have been found to be similar between the genders (268, 287). In keeping with the findings of previous studies (265-267), we found that boys generally had longer axial lengths than girls, but not significantly so. Along with rapid growth in the eye that occurs within the first three years of life, there are compensatory mechanisms such as flattening of the cornea which can offset refractive errors which would otherwise be incurred with increasing axial length (288). Consistent with patterns of axial length in our study, mean keratometric measurements were also similar to what would be expected in this age group; the mean corneal radius of curvature in our study of 7.81 mm is very close to the 7.78 mm found in the study by Ojaimi et al. involving school children in the 6- to 7-year-old age group (289).

Mercuri et al. found that a correlation exists between visual impairment in early life and subsequent abnormal outcomes with respect to neurological and neurodevelopmental evaluation, suggesting that impacts on maturation of visual function may mirror those of the brain in general (269). There are also well-established links between biometric

measurements of the eye and brain, with respect to size and growth rates (262, 263). However, we found no correlation between axial length measurements and severity of HIE grade in our cohort of infants with mostly mild and moderate HIE. Indeed, the mean axial length in our cohort of 22.09 mm is close to the expected normative value from a published algorithm for early eye growth (264), and similar to the 22.22 mm noted in a group of healthy Spanish Caucasian children of an equivalent age (285). One of the key drivers of axial growth within the first year of life is the degree of initial hypermetropia, in support of an active model of emmetropization linked to feedback from a defocused visual input (287). It is possible that if early ocular growth rates were retarded in our cohort, the resulting excess hypermetropia and visual blur may have acted to reverse this trend and stimulate growth. There are other birth parameters such as birth weight, head circumference, and gestational age which have an impact on ocular biometry, but these did not have any significant impact on our results; only full-term babies were included in our cohort, and the numbers with head circumference below the 15th centile (n=3) or having low birth weight (n=3) were small.

To our knowledge, this article represents the first report which has detailed ocular biometry data in early childhood complementing ophthalmic and neurodevelopmental outcomes in a cohort who were previously diagnosed with HIE. One of the strengths of the study was the inclusion of ocular biometry data measured with the IOLMaster, which is reported to be superior to ultrasound, and should be the standard for the precision and repeatability of axial length measurements in children (290). A limitation of our study was the absence of serial axial length measurements from an earlier age in each child. However, cooperation with detailed ocular examinations at a younger age is difficult to achieve and less acceptable to parents (267). Therefore, we cannot rule out a delay in early ocular growth rates before reaching this end point.

Another limitation of our study is the relatively small numbers involved in the cohort. This is due to difficulties in recruiting and retaining a cohort of children with HIE and the high rate of mortality and disability in the severe group. However, this remains, to our knowledge, the largest prospective study of ophthalmic outcomes in a HIE cohort, and the only one with detailed ocular biometric data. In conclusion, we found no increase in refractory error or change in ocular axial length measurements in surviving children with a history of neonatal HIE. We have confirmed a higher rate of squint in this cohort, in both mild and moderate HIE.

Therefore, we suggest that infants with HIE of any grade should undergo an ophthalmic assessment in early childhood as part of their overall neurodevelopmental follow-up.

COMMENT: Dr. James and I worked closely on this piece of work. I recruited the children to the vision arm of the study, Dr. James completed all of the ophthalmic assessments, and I completed the neurodevelopmental assessments. We analysed different aspects of the data. Dr. James focussed on the vision correlates and I focussed on the outcomes of children with strabismus (assessed by Ms. Beatrix Haskins), and investigated whether differences existed between children with normal vs abnormal vision outcome at five years. Further detail can be found in Chapter 8 Section 8.1: Summary and Impact of the Thesis Findings.

CHAPTER 8

8 Summary, Impact and Conclusions

8.1 Summary and Impact of the Thesis Findings

In this thesis, I have presented detailed five-year outcome data of a birth cohort of infants following HIE of all grades. This data included demographic, clinical, intellectual, visual and neuropsychological measures that provided an in-depth analysis of each child's neurodevelopment. I have also compared these outcomes to a unique community comparison group who experienced a normal neonatal period as measured by neurological examination and electrophysiology in the perinatal period. I have calculated the associations and predictive value of neonatal neurological assessment, Sarnat grading and background EEG patterns, along with 6, 12 and 24-month developmental status for five-year IQ and overall outcome. In so doing, this thesis has confirmed the importance of serial tracking of children across early childhood to capture the non-linear acquisition of skills, and to discriminate those skills that are transient or permanent across early childhood. In addition, this thesis has described the pattern of abnormal outcome in a small cohort of infants with mild encephalopathy adding a strong clinical rationale for investigating the potential of TH for mild HIE to protect later outcome.

This PhD focussed on five main objectives, the results of which are summarised below.

Firstly, the capacity of a clinical neurological exam to trace the dynamic nature of neonatal hypoxic ischaemic brain injury in the initial hours and days of life remains critical for treatment onset, and to understand which items, at which time, may hold predictive value. Amiel-Tison recommends that a consensus may not be reached until the end of the first week, but clinicians don't have the luxury of waiting in order to make treatment decisions. Previous research shows that some items such as eye tracking show maturation within hours (220), whilst others take days and all are interrupted by NICU care (60). Our work on the ATNAT and two-year outcome confirms the prognostic value of a persistent abnormal score. I prepared this work for publication and have examined correlations of individual ATNAT items with 5-year IQ outcome, and demonstrated that early visual and behavioural signs of alertness, passive tone, and palmar grasp correlated with five-year IQ. However, cranial signs, active tone and the majority of primitive reflexes measured on day 3 of life were not helpful for prediction. Observations of patterns such as these in different neonatal cohorts may contribute to a better understanding of the neuro-anatomical (127) and neuro-physiological (291) correlates in the neonate that may lead to the ideal constellation of signs

to be assessed in a neonatal neurological battery which could be used in non-specialised NICU settings.

Secondly, longitudinal research designs must consider how best to (i) measure the unfolding of developmental pathways beyond infancy into childhood, and (ii) examine how the young brain dynamically adapts to early brain insult across developmental time. A robust body of knowledge demonstrates that developmental follow up to 24 months will capture principally sensori-motor, linguistic, social-emotional and emerging cognitive skills, but continued assessment into childhood will identify children who 'grow into their deficits', when higher order cognitive skills are required for task mastery. This is the first study that we are aware of that gathered comprehensive data at birth, 6, 12, 24 and 60 months in a HIE cohort, with EEG grading.

Standardised developmental tests were previously serially administered at 6, 12 and 24 months of age, and I analysed how these skills were associated with intellectual and overall outcome at five years. In addition to 5-year full-scale, verbal and performance (non-verbal) IQ domains correlating with development at 6, 12 and 24 months, the data demonstrated that a consistent early normal development score from 6 to 24 months lead to a normal IQ at five years. Of those with at least one abnormal early development score, 89.5% had an abnormal overall outcome at five years. The need for serial assessment is established because scores are inconsistent across early childhood (33% in our case) as the qualitative differences in difficulties in the underlying domains are realised at different ages. In our cohort, delay in the locomotor domain was evident at 12 months but not 6 or 24. I have confirmed the importance of access to early intervention for children following HIE with abnormal 6 or 12 month Griffiths scores, all of whom presented with at least one of cerebral palsy, low IQ, diagnosed neurodevelopmental disability, sensory loss or ongoing need for early intervention by school age.

Thirdly to describe the pattern of five-year outcome across all grades of hypoxic-ischaemic encephalopathy diagnosed in the neonatal period, and examine the predictive value of early Sarnat and EEG grade assignation in the first day of life for outcome at this age. I demonstrated that as a group, these children had significantly lower verbal short-term memory and processing speed skills that require visual motor coordination skills, than the test norms. Unique to this study, I was able to recruit a comparison sample with EEG confirmed normal neurological birth and neonatal period and compare the five-year

outcome results with same. I found no difference between the IQ scores of those children who experienced mild or moderate neonatal HIE, and although within the average ranges, critically these grades of HIE were performing significantly worse than the comparison group. I further showed the evolution of background EEGs which remained constant or improved between 6 and 24 hours of age. I have described the outcomes at these two ages, and investigated the predictive power of an EEG assigned at 24 hours. IQs at five years decreased as EEG grade worsened, and all infants with grade 4 EEG died or had profound neurodevelopmental disabilities. IQ scores did not differ significantly between grades 1-3 but all were significantly lower than those with a normal EEG reading at 24 hours (i.e. all of the comparison group and 5 HIE infants).

In relation to the ability of EEG background pattern to predict outcome at five years there was a clear pattern of increased propensity for abnormal outcomes as EEG grade increased. At six hours of age an infant with a slightly abnormal EEG background activity (mild asymmetry, mild voltage depression, or poorly defined sleep-wake cycle) had a 25% chance of experiencing later abnormal outcome. Similar to Sarnat grade, the trend of increased burden of pathology as measured by EEG confirms the importance of this measurement tool for clinical decision making in the hours immediately after birth.

The fourth aim was to investigate the neuropsychological processes found in these children at 5 years. A consistent finding in the literature is that children following HIE can experience educational difficulties despite otherwise intact survival. In this study I used 25 tasks of the NEPSY-II, and found that although children had scores within the average range (except for fingertip tapping, design fluency and memory for names) the accumulation of low scores across the tasks was significant. Per child, 28% of median scores were below average, compared with 8% in the comparison sample. For children from the HIE cohort with an otherwise intact outcome, scores in over a quarter of the subtests were significantly below the comparison group. Similar to IQ, I found that children who had Sarnat and EEG grade 0 at 24 hours of age had higher scores across all neuropsychological domains, with no differences between mild and moderate grades. This pattern of lower scores across domains despite normal IQ may determine which child may require additional educational support in school, and merits further study.

Finally, I was fortunate to work closely with an ophthalmologist colleague (Dr. Mark James) to describe detailed vision outcome in this HIE cohort. Vision outcomes for children post HIE

have reported visual field difficulties and, we found an increased incidence of strabismus even in those children without cerebral palsy. Furthermore, of the 7 children with strabismus, 5 had abnormal outcomes at age five, and a sixth had a low processing speed index score. In addition, this group of children had significantly lower developmental scores at 12 and 24 months possibly due to delayed locomotor skills. This is a tentative finding due to low numbers. There were no differences found for vision outcome between mild and moderate encephalopathy grades, or between children with normal or abnormal five year outcome.

8.2 Strengths of the Research

A significant strength of this study was the availability of two prospectively recruited cohorts of infants from previous studies born at the same regional maternity services, both of whom had been comprehensively neurologically assessed in the neonatal period. The HIE cohort had well-defined clinical and EEG confirmed signs of mild, moderate and severe hypoxic ischaemic encephalopathy. The community comparison cohort with normal neonatal EEG findings. Demographic analysis demonstrated that the two cohorts had similar distributions of clinical and demographic factors including gender, birthweight, gestation, maternal education, parent occupation, ethnicity, and rural/urban background. Inter-rater variability was minimised by the same clinician recruiting the infants at birth (although different for two cohorts), the same specialist neonatal neurophysiologist who graded the EEGs, and the same Griffiths registered paediatrician who carried out the majority of the GMDS-R assessments at all ages.

At five years, I assessed all children from both cohorts, including intellectual and neuropsychological assessment. Apart from knowing which cohorts the children came from I was blind to the neonatal and early developmental status of the infants. In the HIE cohort, a high retention rate was achieved, and this was enabled by the flexibility I was given to assess the children at times and locations (home or university) that suited families. This minimised the loss of children who lived a significant distance from the assessment centre. I scored all assessments and an assistant psychologist was available for double scoring to ensure minimal errors in the raw data. The use of software for scoring and exporting scores to excel and then to SPSS assured minimisation of error in the standardised data for the

NEPSY-II test which produced 107 output scores per child. As an experienced clinical psychologist with a history of working with children and families in early intervention I was familiar with the importance of building rapport and communicating empathically to reduce stress in the assessment setting. In addition, although I wasn't involved in the project for the early years assessments, I am a registered user of the Griffiths scales and was very familiar with the scoring criteria. I re-scored all Griffiths record forms to ensure cross-study accuracy.

Much is understood about the motor and intellectual effects of neonatal HIE. There has been less focus on other neurodevelopmental disorder categories. In our study, I collated rates of clinically diagnosed disorders including autism, ADHD, DCD and language disorders. Our rates of all of these were higher than for the comparison group recruited at birth. Some studies suggest higher rates of birth asphyxia in population studies of children who develop ADHD than those who don't (292).

Because this research was undertaken in a multidisciplinary research setting, the availability of highly specialised professionals was an important strength, especially in relation to neonatal EEG analysis by Prof. Geraldine Boylan.

The most significant strength of our study was the inclusion of infants with all grades of HIE which meant that I could undertake detailed analysis of children with mild HIE at a time when emerging literature was suggesting that this level of encephalopathy may not always be benign. Our cohort also allowed for the comparison of outcome of mild and uncooled moderate HIE which will no longer be possible due to the introduction of TH for moderate HIE.

8.3 Limitations of the Research

The principal limitations of this study are the small cohort sizes of the HIE and comparison groups. This is a common feature of longitudinal research that recruits individuals with rare conditions. With an annual birth rate of 6,000, it took 30 months to recruit 65 infants at birth. The small numbers limited choices for statistical analysis, especially regression models, and the control of co-variates. Potential confounding factors such as gender, maternal education level, family income, non-native language status and child IQ have shown to hold predictive value in HIE cohorts (58, 124), and were not statistically controlled for in our study. When compared with a national Irish cohort of children (national 'Growing Up in Ireland Study'

(190)), our Comparison group had significantly fewer males and infants following unassisted birth. However, there were no differences between maternal education level between our cohorts, and coincidentally, all children and their parents were fluent English speakers.

A second important limitation of this study relate to certain characteristics of the Comparison group chosen. Although detailed neonatal data was available including, uniquely, EEG recordings, the potential for bias was introduced because (i) caregivers who provide consent to allow their healthy newborns to be monitored with EEG, may not be representative of a community sample, and (ii) comparison with the national 'Growing Up in Ireland Study' (190) suggests that male gender and firstborns were underrepresented, and the mothers were more likely to have had unassisted deliveries. On a positive note, maternal education level and parent occupations appeared equivalent. The inclusion of a matched case control group, which could have matched for important demographic factors such as gender and age would have mitigated their potential influence on childhood outcome. Consequently, it would have enhanced the validity removed the threat of the competing hypothesis that the reason for the differences found between our cohorts was not solely due to lower group scores due to HIE, but also to inflated scores achieved by our Comparison group. However, achievement of mean upper average range IQs in control groups has been previously recorded in other well designed longitudinal outcome studies (Utrecht study: (109); Canada study: (74, 77)).

At a broader level, our cohorts were recruited from one region in Ireland, and to recruit greater numbers, and improve generalisability of the findings requires multi-centre recruitment to encompass a wider population, different hospital structures and approaches to neonatal care.

A significant challenge in longitudinal research arises from how outcomes are measured. New knowledge and the passage of time demand updates to psychometric tools (293). Since the commencement of this thesis, revised editions of two of the assessment tools have been published. ARICD published a major revision of the GMDS-R/GMDS-ER - the Griffiths III - in 2016. The latest revision of the WPPSI tool, the WPPSI-IV was published in 2012. This alters the underlying definition of domain scores, as different constellations of tasks and factors used to calculate them are updated. Some longitudinal studies use a combination of versions of tests across time, but we felt it more important to remain consistent with the older tests to ensure that cross comparison would remain valid.

An important aspect of the study that was insufficiently explored was that of the ecological validity of the outcome assessments. It is argued for example that the functional implications of IQ measurement, bar the extreme scores, may be limited (294). This could be improved by exploring in more detail how the findings relate to adaptive skills in real life. In relation to developmental outcomes in the early years, Castro et al., explored a mapping structure for items from the Griffiths Mental Development Scale with how they relate to everyday life by comparing them to capacity areas of the WHO's ICF (International Classification of Functioning) model of disability (295). This more closely aligns assessment scores to domains of quality of life. Many measures have been developed to evaluate these adaptive skills relevant to young children (296), and there is a paucity of studies using these outcome measures in HIE research. One exception was the CoolCap RCT study, which employed the 'WeeFIM' quality of life measure with parents of children at age 7-8 years. This measures a range of skills within self-care, mobility and cognition. Results found predictive value of 18mth Bayley (BSID-II) scores for outcome on the WeeFIM (18). Measures such as these include important contextual and environmental factors which could have more closely linked our study to a systems based model as was proposed by Sameroff (140). Furthermore, the inclusion of an adaptive behaviour scale such as the Vineland Adaptive Behavior Scales (297) would have allowed me to examine whether the children in the cohort with an IQ < 70 met the ICF and DSM criteria for intellectual disability.

Educational outcomes for this cohort were not included in this study and reduce the findings' practical implications. The outcomes would have greatly benefitted from the inclusion of feedback from early years' and primary school educators. Additionally, further analysis of differences between children who had and had not commenced formal schooling is warranted.

8.4 Focus for Future Research

8.4.1 The Neonate

The techniques available to measure and observe the neurology of the neonate continues to improve. For the neonate at risk of evolving HIE, the aspiration must be to improve the tool(s) that can pinpoint the infants that will benefit from treatments such as TH in the hours after birth to prevent secondary and tertiary injury mechanisms. This is a tall order, and will only be realised with large well-designed multi-centre studies, that can standardise their clinical observation procedures. One focus for research must be on the differential contributions

that items in grading systems make to marking the injury, otherwise their predictive worth will remain diluted within the umbrella of their grade. This is not to suggest a return to the pursuit of the magical 'single marker'. A set of time-specific markers will still be required, but these markers need to be weighted based on the ratio of contribution they make towards a valid diagnosis and/or prognosis. Whether these markers should remain within grading systems, or on a continuum of severity remains to be seen.

In the current TH climate, the underlying cut offs for mild and moderate grade are of specific importance. Amiel-Tison (60) notes that some mild grading systems show no CNS depression whilst other systems allow for some.

To give one example from our cohort, is the item '*tone*'. The original Sarnat and Sarnat (1976) (14) criteria defined tone in mild grade to be '*normal tone*'. In Levene et al's (1985) (188) adaptation of the Sarnat grade used in our study, mild grade allows for '*mild alterations of tone*'. Within the category of tone, Volpe (127) among others has observed the poorer prognosis for infants with 'shoulder girdle low tone' than for low tone in other body regions. Future research could observe the individual contributions of tone, and other items for prognostic value, similar to our preliminary work on the ATNAT items. This requires further scrutiny with larger samples.

> "Researchers agree that to define the limit between optimal central nervous system function and mild degree of central nervous system dysfunction is the only real difficulty. Moreover, in the early stages of extra-uterine adaptation, it is difficult to separate the transient effects of cardiorespiratory and metabolic problems from the specific expression of brain damage." (Amiel-Tison, 2002: p. 198)

A similar argument can be made for future research in regard to EEG grade to elucidate which features of the background EEG hold best predictive value. In our cohort and other post TH cohorts recruited in our research centre, detailed analysis of EEG background features and seizure burden for machine learning algorithms are being developed to enhance the clinical value of EEG to examine the course of neonatal HIE. In relation to this HIE cohort, an EEG grading algorithm for HIE to predict 5-year outcome has been initiated by engineering colleagues.

Measures of neonatal signs have never been more timely now that abnormal MRI findings have been reported in mild HIE (251, 254), and surveys of current TH practice (242, 298) reflect 'therapeutic drift' to include babies with borderline mild HIE for cooling. Furthermore, RCTs currently recruiting for the efficacy of TH for mild grade encephalopathy are underway. A critical goal for future work will be to agree a consensus definition of what is meant by mild HIE as articulated by Prempunpong et al. (254), and its measurement pre 6 hours of life (299).

8.4.2 The Child

Distinct patterns of neurocognitive difficulties are emerging in children post neonatal HIE. Therefore, the identification of early signs in infants for whom developmental trajectories may be impacted is crucial. For example, one neurocognitive difficulty seen in children post-HIE is in the area of memory (in this thesis, verbal short-term memory, and memory for names), and associated damage to the hippocampus has been established. A recent comparative study further suggests a specific HIE vulnerability to the expected development of connective pathways between prefrontal cortex and the hippocampus at birth in neonatal rat models. Brockwood et al. (300) showed that following mild/moderate HIE the neural networking was not as sophisticated in young rats between the prefrontal cortex and hippocampus. In human infants, a well-designed study presented data on poorer attention to faces in the first year of life, as observed by lower preferential looking at faces in 12 month old infants following neonatal HIE (301). These potentially exciting research studies require replication but demonstrate how future studies could align themselves with new evidence from the discipline of developmental cognitive neuroscience. The end goal here would be to track early specific injury with early developmental markers that cascade to later 'domain relevant' (302) difficulties such as the specific memory impairment difficulties seen in later childhood. This is similar to the model proposed by Bornstein et al. in relation to infant behavioural markers for later cognition (143). One potential trajectory that merits future examination is the longitudinal set of observations presented in this thesis that in children post-HIE with no diagnosed motor disability, there were indications of compromised locomotor signs at 12 months, locomotor and eye-hand coordination skills at 24 months which correlated with low visuo-motor processing speed at 5 years, and lower coding and finger dexterity at five years. Neuroscience may provide the answer to the perinatal causes of this potential phenotype.

In terms of optimising the early detection of the developmental impacts of neonatal HIE, the major questions remain – when and how often do we bring children in for assessment during infancy and childhood? Recent trends in developmental research point to the importance of intervening at specific developmental points with probable treatments to target the predicted later pathology (303). Efforts to devise early screening markers for infants with early signs of ASD (304) is one example of a community based initiative with this aim. Although speculative at this stage, it may be that for HIE, early motor delay that doesn't merit diagnosis may be an important marker. This work needs to continue to develop a profile of patterns of difficulty linked to different developmental timepoints to ensure timely early intervention.

Finally, future high-quality prospective studies should include designs that pay greater heed to the examination of systems level factors that influence childhood outcomes post-HIE such as, for example, family coping and parenting style. Consequently, parent interventions and supports could be developed to mitigate the factors other than HIE that may be contributing to adverse childhood outcomes. This has been successfully researched with children following other conditions such as congenital heart disease and prematurity (305).

8.5 Conclusion

We have added to the emerging body of knowledge in relation to infants with mild HIE. We were able to undertake a thorough comparison of the natural history and outcome of untreated children with mild and moderate HIE, which is no longer possible due to the introduction of TH for all children with moderate HIE in high income countries. Our pattern of evolution of HIE signs in the first 24 hours supports the notion of a 'dose response' burden of HIE. A grading system, no matter how sophisticated cannot replace a continuum or spectrum model and there will always be infants who are on the borderline grades. Our data strongly suggests that infants with mild HIE are heterogenous. At 24 hours of age in infants with mild grade encephalopathy, EEG grading demonstrated a group of infants where some EEG traces normalised and other which didn't. Our follow up to 5 years further shows how the EEG grades can discriminate between normal and abnormal outcome at five years.

The intellectual and neuropsychological skills at age five years are similar for children following mild vs. moderate neonatal HIE, and display a significant burden of injury in comparison to their neurologically normal-at-birth peers.

8.6 Academic outputs arising from this PhD thesis

Peer reviewed publications

Murray, DM, Bala, P, O'Connor, CM, Ryan CA, Connolly, S, Boylan, GB *The predictive value of early neurological examination in neonatal HIE and neurodevelopmental outcome at 24 months.* Dev Med Child Neur. 2010;52(2):e55-e59.

Murray, DM, O'Connor, CM, Ryan, CA, Korotchikova, I, Boylan, GB. *Early EEG grade and outcome at 5 years after mild neonatal hypoxic ischemic encephalopathy.* Pediatrics. 2016;138(4):e20160659.

O'Connor, CM, Ryan, CA, Boylan, GB, Murray, DM. *The ability of early serial developmental assessment to predict outcome at 5 years following neonatal hypoxic-ischaemic encephalopathy.* Early Hum Dev. 2017;110:1-8.

James, M, O'Connor, CM, Cullinane, A, Murray, DM, Boylan, GB. *Ophthalmic outcomes following neonatal hypoxic ischaemic encephalopathy; Oculomotor, biometric and refractive data in early childhood.* Eye. 2019 Mar 5; [Epub ahead of print].

Papers in preparation

O'Connor, CM, Boylan, GB, Ryan, CA, Korotchikova, I., Wrigley, C., Murray, DM *No difference between mild and moderate encephalopathy in neuropsychological outcome.*

Conference Platform Presentation

O'Connor, CM, Boylan, GB, Murray, DM. *Correlation between Repeated Early Developmental Assessment and Intellectual Abilities at 5 years Following Neonatal Hypoxic-Ischemic Encephalopathy (HIE).* Presented at the Pediatric Academic Societies Annual Meeting 4th-7th May 2013, Washington Convention Center, Washington DC, USA. Session 4330: Developmental/Behavioral Pediatrics: Outcomes. 7th May 2013.

Figure 8.1 Conference logo for Pediatric Academic Societies Annual Meeting 2013.

Poster:

O'Connor, CM, Boylan, GB, Murray, DM *Can Standardised Developmental Assessment at 6, 12 & 24 months predict five year intellectual outcome following neonatal hypoxic-ischaemic encephalopathy?* Presented at the 3rd UK Paediatric Neuropsychology Symposium 23rd-27th April 2012, UCL, ICH, London (2012) Scientific Posters. Sp1. Dev Med Child Neur. 2012;54:9-36.

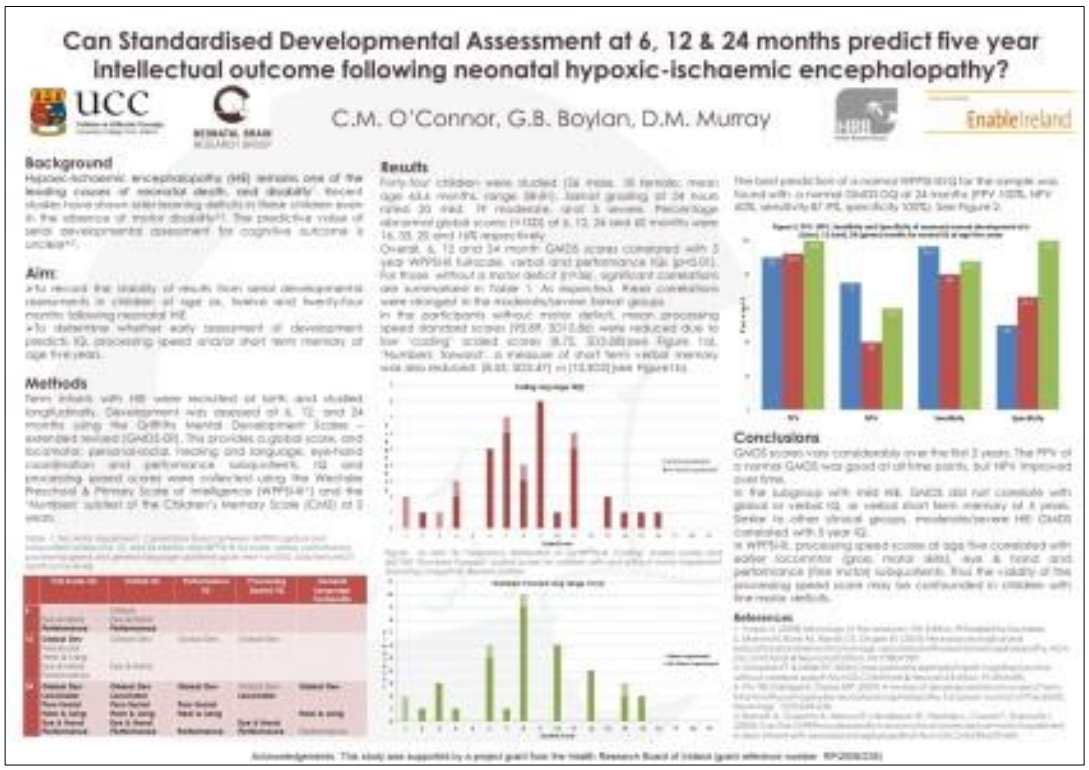

Figure 8.2 Scientific poster presented at the 3rd UK Paediatric Neuropsychology Symposium 23rd-27th April 2012.

CHAPTER 9

9 Bibliography

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Appendix A
Ethical approval for long-term outcome of the HIE cohort

UCC
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COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee
Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our Ref: ECM 4 (hhh) 05/02/08

22nd January 2008

Dr Geraldine Boylan
Senior Lecturer
School of Medicine
Brookfield Health Sciences Complex
University College Cork
College Road
Cork

Re: Ability of early continuous EEG to predict long term neurological outcome at 4.5 years in infants following neonatal hypoxic ischaemic encephalopathy.

Dear Dr Boylan

Expedited Approval is granted to carry out the above study at the following site:

- Cork University Hospital

We note the following co-investigators will be involved:

- Dr Deirdre Murray
- Professor Anthony Ryan

Chairman's approval is granted

- To extend developmental assessment to 4.5 years
- Parental Questionnaire & visual audiiology & visual acuity assessments.

Yours sincerely

Dr Michael Hyland
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

Appendix B
Ethical amendments for HIE long-term outcome study

This submission requested a change of psychometric tests (as recommended by the current researcher) to the protocol and the recruitment of the current researcher.

Dept of Paediatrics and Child Health
University College Cork

Dr Michael Hyland,
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospital

24th November, 2008.

RE:Ability of early continuous EEG to predict long term neurological outcome at 4. 5 years in infants following neonatal hypoxic-ischaemic encephalopathy.

Dear Dr Hyland,

We are writing to request an amendment to the above mentioned study which received expedited ethical approval on 22nd January 2008. Your reference number is ECM 4 (hhh) 05/02/08.

We are proposing to amend the assessment tests used to determine neurodevelopmental outcome at 4.5 years. Further literature reviews and consultation with research colleagues has led us to propose the replacement of the *Griffiths Scale of Mental Development* with the *Wechsler Preschool and Primary Scale of Intelligence 3rd Edition (UK version)* and selected subtests from the *NEPSY-II*, which is a neuropsychological battery for children aged 3 to 16 years. Both of these tools have been extensively psychometrically evaluated and contain test materials specifically designed for use with preschool aged children. It is hypothesised that the use of these tests will provide better predictive value for later cognitive and educational outcome, and more clinically sensitive results. They will assess some of the subtle underlying cognitive processes, including 'executive functioning' which appears particularly sensitive to neurological trauma.

The interpretation of these assessments must be completed by a qualified psychologist and we have recruited Catherine O'Connor as a co-investigator. She is a senior clinical psychologist who has worked in Enable Ireland Cork Services (Lavanagh Centre) for the past 14 years. It is proposed that she will assess, interpret and provide feedback on these assessments to the children and parents/carers of this cohort. In individual cases where the assessments yield below average intellectual performance, she will advise families in

consultation with Dr. Deirdre Murray, Consultant Paediatrician, of the most appropriate referral pathways to clinical/educational services.

It has also been agreed that the *'Strengths and Difficulties' questionnaire* is the most appropriate general parental questionnaire to use to augment the use of the *Behavior Rating Inventory of Executive Functioning – Preschool Version*. It is quick and easy for parents to complete and includes questions about a child's strengths in addition to areas of difficulty. It has been widely used in epidemiological studies.

We would be grateful if you could consider our request for ethical approval for these amendments to this project. Included in the submission is 'Form 2', and amended versions of the original research proposal and letter of informed consent.

Yours sincerely,

Dr Geraldine Boylan

Dr Deirdre Murray

encl.

Introduction:

Neonatal hypoxic-ischaemic encephalopathy remains an important neurological process. It is a major cause of both acute mortality, and long-term severe neuro-disability. HIE affects between 1 and 6 per thousand infants born at full term and is caused by a significant period of oxygen deprivation (hypoxia) and/or blood-flow (ischaemia) to the brain. When blood flow is restored, further damage to brain tissue can occur as toxins from injured cells are circulated. Between 15 and 20% of affected infants die during the newborn period, and a further 25% have permanent neurological deficits. Thirteen percent of survivors have cerebral palsy, and these children usually have more significant disability than children with cerebral palsy due to other causes.

Outcome varies considerably, depending on clinical severity. Clinical signs and symptoms allow staging of encephalopathy at 24 hours of age (Sarnat grade I, II or III), as first described by Sarnat and Sarnat in the 1970's. This clinical method of staging and assigning prognosis remains the most widely used today. Accurate recognition of these clinical stages is therefore vital. Frequently, the disabilities are multiple and can include spastic cerebral palsy, severe or profound intellectual disability, blindness, and/or seizure disorder.

Whist most children who suffered grade I (mild) encephalopathy have a good outcome, the outcome in grade III(severe) encephalopathy is extremely poor, with all survivors progressing to severe neurodisability. The prognosis for those with (grade II) moderate encephalopathy is less certain. Twenty to forty percent of those with Grade II encephalopathy will have an adverse outcome (death or disability). It is difficult, however to accurately predict which of the infants with moderate encephalopathy will fall into this category.

Previous retrospective studies have shown that even in children without demonstrable cerebral palsy, long-term academic performance may be affected, with significantly more of those with moderate encephalopathy having a greater than one year delay in their reading, spelling, and/or arithmetic skills.

A more recent retrospective study by Neil Marlow in Nottingham used multiple cognitive and neuropsychological tests in 65 "neurologically normal" survivors of moderate and severe encephalopathy, using matched controls at 7 years of age. They also evaluated behaviour and memory. Children in the severe encephalopathy group had significantly lower overall scores and significantly underachieved in educational attainment scores. The children with moderate encephalopathy had lower educational attainment scores for spelling and reading and a higher prevalence of behavioural problems particularly hyperactivity. In many centres these children would not have been followed up and would have been discharged as "normal outcomes". These patients were identified retrospectively using the medical chart diagnoses; the response rate was low (56%) as many children were lost to follow-up. Thus the results may not fully reflect the full extent of adverse cognitive outcomes in children with HIE. However, there is a growing body of evidence which suggests that perinatal asphyxia leads to significant cognitive impairment in the absence of cerebral palsy.

Parents, as well as treating clinicians, will want to know the implications of the child's condition as early as possible, and thus an evidence-base for the use of prognostic tools such

as electro-encephalograms (EEG) needs to be provided with appropriate prospective cohort studies.

The prognostic value of the EEG was first described by Monod and colleagues in 1972 and since then it has become an important prognostic tool. A good outcome is generally seen in those babies with normal background EEG activity following a hypoxic event. Severely abnormal background recordings (inactive or isoelectric) are generally associated with major neurological sequelae or death. There is still uncertainty about the predictive value of moderately abnormal EEGs. However the predictive ability differs depending on the time of the recording, with better reliability seen in those studies performed in the first week following birth. Those EEGs which recovered on serial recordings over the first weeks of life were associated with an improved prognosis.

However, although the evolution of the EEG appears to be important, very few studies have looked at EEG changes in the first days following a hypoxic injury. Most of our knowledge base is built on EEG studies performed at 1-3 weeks of age. The evidence suggests that for accurate prognosis the initial EEG must be performed as close as possible to the peak of symptomatic neurological impairment. No previous study has used continuous early EEG data.

The individual features of the EEG which will best correlate with long-term outcome are unknown. Previous features which have been linked with outcome are background amplitude, discontinuity, interburst interval length, disturbance of sleep-wake cycling and the presence of electrographic seizures. The relative importance of these factors is unknown. The ideal time to perform the EEG, and the important of EEG recovery are questions yet to be answered.

Outcome studies have not been performed on this group of infants in Ireland. Since the prevalence and outcome of HIE can vary from country to country, the availability of up-to date, locally relevant outcome figures are important to Irish physicians and can directly influence treatment. Early detection of at risk children is vital so the appropriate referral to early intervention services can be made.

None of the studies already performed have evaluated the early use of continuous EEG monitoring and the evolution of the EEG in predicting prognosis. Finally, the predictive value of EEG has not been examined in long-term follow-up studies. While good outcome data is available for the mild and severe forms of encephalopathy, infants in the moderate grade have an uncertain future, and while many have been deemed “normal” in the past because no serious neurological sequelae occurred, more subtle cognitive, behavioural and motor deficits may be missed.

Methods:

The infants previously recruited for the EEG study will undergo surveillance until the age of 4.5, to assess long-term outcomes of neurological and cognitive function, as well as behaviour and executive function. The assessments will be done at 4.5 years. At this time a consultant paediatrician (Deirdre Murray) will perform a full neurological assessment. A

clinical psychologist (Catherine O'Connor) will perform a neurodevelopmental assessment using the Wechsler Preschool and Primary Scale of Intelligence – 3rd Edition (UK) (WPPSI-III^{UK}) and parts of the NEPSY. Prior to this assessment the parents will be sent a BRIEF-P (Behaviour Rating Inventory of Executive Function – Preschool version) form and the 'Strengths and Difficulties' Questionnaire to complete. Ms. O'Connor will assist the parents in completing the form. In cases where parents do not speak English, or having difficulty with literacy, the form may be completed by the child's teachers, or playschool carer, with the parents consent. Following these assessments, appointments will be arranged for assessment of hearing and visual acuity which will take place on different dates to avoid fatigue in the children. Parents will be asked to provide details of occupation residence and education for the determination of socio-economic grouping.

Subjects and setting:

The parents of babies, who have already been enrolled in our initial EEG study up to the age of two years, will be invited to participate in further follow-up assessments when they have reached the age of 4.5 years. Those who consent to ongoing participation in the follow-up study will be contacted by our project officer and appointments for assessment arranged. These assessments will be carried out adjacent to the paediatric clinic of Dr. Deirdre Murray, Senior Lecturer in Paediatrics and Child Health. An assessment using the WPPSI-III^{UK} and selected subtests from the NEPSY will be administered by Catherine O'Connor, Senior Clinical Psychologist. Following their psychological assessment, each child will have a neurological examination to look for specific motor deficits. The neurological assessment will be carried out by Dr. Murray. Additional appointments for audiology and assessment of visual acuity will be arranged for each child in the Audiology departments of the South Infirmary hospital, and ophthalmology departments of the Cork University Hospital.

Wechsler Primary and Preschool Scale of Intelligence (UK version) WPPSI-III^{UK}

The WPPSI-III^{UK} is an individually administered clinical instrument for assessing the intelligence of children aged 2 years 6 months to 7 years 3 months, and is used worldwide. The third version is a major revision of its predecessors, providing updated standardised norms and adhering more closely to a hierarchical model of intelligence based on the Cattell-Horn-Carroll (CHC) model. There are two age-range bands (2-6 to 3-11, and 4-0 to 7-3), which use different subtests to obtain composite quotients. The administration of the core subtests yields a Verbal IQ, Performance IQ and Full-Scale IQ. An additional Processing Speed Quotient can be calculated for the older age band. A general Language Composite can be calculated using supplemental/optional subtests.

The WPPSI-III was normed on a United States sample of 1,700 children, the 'UK' version was normed on a representative sample of 805 children, which closely approximated the UK 2001 Census data on similar demographic variables. Reliability and validity information is outlined extensively in the WPPSI-III Technical and Interpretative Manual. The average internal consistency values for individual coefficients are .95 for V-IQ, .93 for P-IQ, .89 for PSQ, and .96 for FS-IQ. Test-retest reliability is fairly stable. Construct validity is supported by factor-analytic studies and with comparisons with other assessments of cognitive functioning.

NEPSY-II

The NEPSY-II assesses neuropsychological development in children aged 3 to 16 years. It originated in the 1980s in Finland, and evolved throughout the 1980s and 1990s leading to

the publication of the NEPS in Finland and the NEPSY in America in 1998. This assessed children aged 3-12 years on a broad range of tasks assessing five neuropsychological domains, namely, attention and executive functioning, language, memory and learning, sensorimotor and visuospatial processing. What distinguished it from other children's neuropsychological assessment batteries was that it was designed exclusively for children, rather than depending on downward extensions of adult-based tasks. It is based on Lurian theory which uses a process-oriented approach to explain cognitive development. Cognitive areas are viewed as complex capacities mediated by interacting functional systems. When one subcomponent is impaired then complex functions can be impacted upon and sometimes performance can be affected across functional domains.

It was originally designed to detect subtle deficiencies both across and within subtest domains; to increase understanding of effects of congenital and acquired brain damage to enhance treatment strategies and to provide long-term follow up; to study development in preschool aged children.

The NEPSY-II was published in 2007 and constitutes a major revision of the NEPSY. It covers a broader age range of children aged 3-16 years. It includes a new domain – social perception – in addition to the original five domains. Subtests with lower psychometric properties have been replaced by subtests with greater clinical sensitivity and construct validity. Primary, combined, process and contrast scores can now be calculated, depending on the selection of the 32 subtests chosen for administration. It has been standardised on a single well stratified sample of U.S. citizens and includes clinical groups and a Finnish standardisation sample is currently underway.

The Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a brief screening tool that asks about the behavioural/emotional symptoms and positive attributes for 4-16 year olds. For children, the 25-item questionnaire is completed by a parent or teacher. It has been validated against other widely used behavioural rating scales and has been normed on a UK representative sample numbering 10,438. It yields five scales – emotional symptoms, conduct problems, hyperactivity, peer problems and prosocial. The summation of the first four scales yields a 'total difficulties' score. It is quickly administered and considered more acceptable by informants because of the inclusion of positive attributes. The SDQ has been used extensively for research and epidemiological purposes.

Behaviour Rating Inventory of Executive Function- Preschool Version

The Brief-P is the first standardised measure of executive functioning available for the pre-school child. It was designed to measure the range of behavioural manifestations of executive function in pre-school-aged children. The Brief-P consists of a single Rating form used by parents, teachers and day-care providers to rate the child's executive function within the context of their everyday environments. The rating form consists of 63 items that measure various aspects of executive functioning: inhibit, shift, emotional control, working memory and plan/organise. The clinical scales form three broad indexes (Inhibitory self-control, Flexibility and Emergent metacognition) and one composite score (Global Executive Composite).

This method of assessment has been shown to demonstrate good internal consistency, reliability, and high test-retest reliability. The normative data used is based on child ratings from 460 parents and 302 teachers from across differing socioeconomic groups, and in urban, suburban and rural areas in the United States. Discriminant validity was demonstrated

with other measures of inattention, hyperactivity-impulsivity, depression, anxiety and somatic complaints.

EEG

The EEG of the neonate is best described in terms of the background activity or pattern. This is the ongoing activity of the brain at rest during wakefulness or sleep.

All Neonatal EEG data will be analysed by clinical Scientist, Dr Geraldine Boylan and Consultant Clinical Neurophysiologist, Dr Sean Connolly, both of whom will be blind to clinical status and 2 year follow up information in each infant.

The following sub-tasks will be carried out for each neonatal EEG:

- Characterisation of each multimodal recording in terms of background EEG activity (non seizure EEG)
    - Amplitude
    - Dominant frequency
    - Presence of specific features
    - Continuity
    - Interburst interval duration

- Distinguishing the presence or absence of sleep states
- Identifying and marking of electrographic seizures.
- Characterisation of seizure in terms of amplitude, duration, frequency, location and morphology
- Representative 1 hour segments of each EEG at 6, 12, 24, 48 and 72 hours will exported to an EDF format for quantitative EEG analysis using time and frequency domain analysis measures.
- Our previous research has allowed us to identify the most useful quantitative features for EEG analysis in the neonate. We propose to measure Spectral Edge frequency, Spectral Entropy, Total Power and dominant frequency measures to quantify the neonatal EEG.

In addition full diagnostic audiology assessment will be carried out at 4-5 years of age. This will include:

- Tympanometry
- Pure tone audiometry

In children with learning disability who can not easily comply with these procedures, we will perform free field audiometry or test oto-acoustic emissions and speech discrimination tests.

Visual acuity assessment will be performed by a registered optometrist who has experience in the assessment of children with neuro-disability.

We will examine associations between measures of cognitive, neurological, and behavioural outcome, at 4.5 years, and early EEG findings (visual and quantitative analysis). Socioeconomic status will be determined for each family and correlated with outcome. Results will be compared using a robust non-parametric analysis to allow for correlations between repeated measures.

The Potential benefits of the study:

This is the first study which proposes to prospectively follow the cognitive, neurological and behavioural outcome of the survivors of neonatal encephalopathy to age 4.5 years. The outcomes of these children will be correlated with their early EEG and neurological assessments. Never before has a neonatal cohort of survivors of HIE been followed up prospectively in Ireland, so no Irish outcome data is available to clinicians.

New treatments such as head-cooling are showing promising results in the treatment of HIE. However without accurate prognostic data, it will be difficult to identify those infants who would most benefit from treatment. Accurate follow-up data may help to guide surveillance practices in this high risk group and will ensure that referral to specialised centres for early intervention will occur in the appropriate groups, to maximise the child's long-term functional outcome.

It will provide parents with important information which will allow them to plan for the future for their child and their family as a whole. It may also provide them with evidence to support the withdrawal of treatment in the rare cases where the prognosis is hopeless.

Appendix C
Invitation letter for HIE Cohort at Age 5

Neonatal Research Centre

University College Cork

Dear Parents,

Once again we would like to express our gratitude to you for allowing your child to take part in the Neonatal Encephalopathy Study when they were born, and for attending their follow up assessments. With your help we have been able to gain very important information about what happens to babies soon after birth, and what this might mean for their future development. This information will help doctors caring for babies in the first few days of life, and help their parents by increasing the information that we have to give them at this time. This will not only help children in Ireland, but our findings will be helpful to parents and children in many different countries. It is very important for us to follow all of the children involved, and see how they develop and gain skills over time.

The Health Research Board of Ireland has recognised the importance of this research and has recently agreed to fund the Neonatal Research Centre to study the progress of all the children involved in the study again at 5 years of age. This funding will allow us, with your permission, to carry out detailed assessments of your child's development, hearing and vision. We could not continue this research without your help.

Over the next few days, you will be contacted by one of our research team and asked to take part in our ongoing follow up study. We will arrange the assessments to times which suit you, and you will be reimbursed for any travel expenses. Some of the assessments can be arranged to take place in your home, if this is more convenient for you.

If you have any queries, or need to change the appointment time, please contact Ms Brenda O'Flynn on 021 4901628 between the hours of 9am to 1pm or Senior psychologist, Ms Catherine O'Connor 087 6435049 after hours.

Thank you again for your help.

Dr Deirdre Murray,

Consultant Paediatrician/Senior Lecturer

Department of Paediatrics and Child Health,

University College Cork.

Appendix D
HIE Cohort Informed Consent Form

UNIVERSITY COLLEGE CORK
Clinical Research Ethics Committee Of The Cork Teaching Hospitals

CONSENT BY SUBJECT FOR PARTICIPATION IN RESEARCH PROTOCOL

Section A

Protocol Number:_____Patient Name:_____

_____

Title of Protocol: Long term follow up of children post neonatal encephalopathy.

Research directed by:
Prof G. Boylan; Dr D Murray.

You are being asked to participate in a research study. The doctors at University College Cork study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent. This consent form gives detailed information about the research study and this will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

Section B

I. NATURE AND DURATION OF PROCEDURE(S):

As you know your child was involved in an important research project which looked at brainwaves of babies who required resuscitation at birth. With your consent we were able to closely follow your child's development up to 2 years of age. This study has given us very valuable information about what happens to children such as yours. It has allowed us to discover the types of brainwave changes that can predict whether a baby is likely to have any long term problems. This information is now being published in international medical journals and will help other babies and their families in the future, both in Ireland and worldwide.

To give us extra information we would now like to see you and your child again at the age of 4.5 years. We wish to do a further assessment of your child to see how they are developing and learning. This will involve your child being asked to carry out tasks such as answering questions, and using pictures and blocks. We will ask you to fill in

two questionnaires about how they act when they are at home. We also wish to perform eye tests on them to look at their ability to see.

II. POTENTIAL RISKS AND BENEFITS:
There are no potential risks. The study will take some of you and your child's time. We will pay for any travel or food costs which you will need to attend the follow up sessions in the Cork University Maternity Hospital. The assessments will be reported to you if you wish and will give you a detailed picture of how your child is progressing compared to other children their age.

III. POSSIBLE ALTERNATIVES:
The clinical management of your baby will not be affected if you do not want to participate in this research.

Section C AGREEMENT TO CONSENT

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I understand that the sponsors and investigators have such insurance as is required by law in the event of injury resulting from this research.

I, the undersigned, hereby consent to participate as a subject in the above described project conducted at the Cork Teaching Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Clinical Sciences Building, Cork University Hospital, Wilton, Cork.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Doctor: _____ _____

Signature of Subject, Parent or Guardian
(include a separate line for assent of minor, if applicable)

Witness: _____ Date: _____ Time: _____ AM
(Circle) PM

Appendix E
Ethical amendment application for recruitment of comparison group at age five years.

Dept of Paediatrics and Child Health
University College Cork

Dr Michael Hyland,
Clinical Research Ethics Committee of the Cork Teaching Hospitals
1st Floor,
Lancaster Hall
6 Little Hanover Street,
Cork

25th October, 2011

Dear Dr Hyland,

We are requesting extended ethical approval for further neurodevelopmental follow up of our original cohort of children recruited to the study: ***Sleep patterns of full-term babies in the early postnatal period.***

In October 2005, we commenced a study to describe and quantify normative EEG patterns during sleep in healthy term newborns in the first twelve hours after birth(1). A further aim was to enhance the normative reference data for use in studies of babies with hypoxic-ischaemic encephalopathy (HIE). This study had full ethical approval of the clinical research ethics committee of the Cork teaching hospitals.

Fifty-nine of the babies are now at least five years of age. We now wish to follow-up this unique cohort of healthy babies with documented normal neurological assessments and EEG readings, to school age. We hope to invite this cohort to be included in a longitudinal follow-up study at age 5. Comprehensive assessments of intellectual, neuropsychological, executive functioning and behavioural screening data will be provided by a registered clinical psychologist, and a screening neurological assessment by a neonatologist. Parents/carers will obtain detailed feedback about their child's profile, and where a child is assessed as requiring interventions, relevant feedback and referrals will be made to community services by the psychologist.

These results will provide very valuable culturally relevant normative data of a representative cross-section of five-year old children in the Cork geographical region who experienced a normal neonatal period. This data will provide a vital research tool to control for the extraneous variables of birth experience and demographics when compared with cohorts of results for at risk children, such as the cohort of 66 infants who experienced neonatal HIE who are now being studied to age 5 years in an associated study.

Further investigation of these groups may also elucidate subtle differences between EEG readings of neurologically normal neonates and the children in the HIE cohort

with ‘mild’ encephalopathy, who have been shown in the research to experience normal outcome.

We wish to request the continued ethical approval of the Clinical Ethics Committee of the Cork University Teaching Hospitals. The cohort of children will be turning five years from October 2010 onwards. We will endeavour to reduce the possibility of causing stress to potential families by reviewing the ‘Patient Information Monitoring System’ to remove contact details of any children who may have died in the intervening period, and to obtain updated contact details for each potential participant.

We would be very grateful if you would consider our request for ethical approval for the amendments to this project. Included in the submission are (i) ‘Form 2’; (ii) an amended version of the original research proposal outlining the specific aims and detailed information about the proposed assessment tools; (iii) amended letter of informed consent.

Yours sincerely,

C Anthony Ryan, MD DCH D Obst FRCPI FRCPCH FRCPC FAAP(Ped Crit Care)
Consultant Neonatologist, HSE
Professor in Paediatrics and Child Health, UCC.

encl.

Reference List

(1) Korotchikova I, Connolly S, Ryan CA, Murray DM, Temko A, Greene BR, et al. EEG in the healthy term newborn within 12 hours of birth. Clin Neurophysiol 2009;120(June):1046-53.

Form 2

UNIVERSITY COLLEGE CORK

Clinical Research Ethics Committee Of The Cork Teaching Hospitals

AMENDMENT SUBMISSION FORM

When any revision to an approved research protocol, written consent form and/or advertisement for subject recruitment is desired, an amendment must be filed with the Ethics Committee. The amendment submission form must be completed indicating the changes; revisions may be within the protocol itself, the written consent form or the advertisement. The form should explain what changes have been made and the rationale for the change. Eight copies of the revised pertinent original documents (protocol, consent form, and/or advertisement) should also be submitted with the changes identified using a blue highlighter pen. A cover letter or additional information may also be attached, as necessary.

Amendments to approved protocols may not be initiated until Ethics Committee approval has been obtained, except when necessary to eliminate apparent immediate hazards to the subject. Amendments usually require full Board review at the scheduled monthly meetings; therefore, the submission deadlines must be met. The Ethics Committee reserves the right to determine whether proposed changes are substantive and to request further information or a new protocol submission, as appropriate.

Chief Investigator: Prof C Anthony Ryan, Consultant Neonatologist and Senior Lecturer

Department: Department of Paediatrics and Child Health, College of Medicine and Health

Protocol Title: Sleep patterns of full-term babies in the early postnatal period.

The following changes are proposed for this protocol:

Chief Investigator	☐	Co-investigator(s)	X
Dosage	☐	Treatment Procedures	☐
Drug/Device	☐	Study Population	☐
Number of Subjects	☐	Risks	☐
Advertisement	☐	Editorial Corrections .	☐

Other 5 year neurodevelopmental follow-up

Is a revised protocol necessary as a result of this amendment? If yes, please attach a revised protocol to this amendment.	Yes	X	No	
Is a revised consent form necessary as a result of this amendment? If yes, please attach a revised consent form to this amendment.	Yes	X	No	
Is a revised advertisement necessary as a result of this amendment? If yes, please attach a revised advertisement to this amendment.	Yes		No	X

Please list the specific changes from the previously approved protocol and provide sufficient rationale for each change to allow the committee to make a decision. Use additional pages as necessary.

- We wish to undertake a 5 year neurodevelopmental follow-up of a sample (n=55) of this cohort of neurologically normal neonates previously recruited to this prospective observational study investigating normal EEG sleep patterns in the twelve hours following birth. This will provide a comparison group for the research protocol 'Ability of early continuous EEG to predict long term neurological outcome at 4.5 years in infants following neonatal hypoxic-ischaemic encephalopathy.'

Please see attached cover letter, amended consent form and amended research proposal outlining these alterations in further detail.

Investigator _____ Date _____

(This form must bear the original signature of the chief investigator)

Study Proposal

Sleep patterns of full-term babies in the early postnatal period– five year follow up.

Introduction

EEG is one of the most important investigations in the neurologically compromised newborn baby and is the only investigation currently available for accurate seizure diagnosis. The EEG excels in the early diagnosis and prognosis of neonatal encephalopathy and recent studies suggest that the evolution of changes in the neonatal EEG soon after a hypoxic ischaemic insult can provide information on timing of injury (1). More recently, neonatal EEG data obtained within 6 hours of birth has been used to identify babies with hypoxia-ischaemia that could benefit from neuroprotective therapies, in particular brain cooling (2). There is very little data available on the neonatal EEG in hypoxia-ischaemia early in the newborn period i.e. between 0-12 hours of birth and virtually nothing is known about the EEG of the normal neonate during this time period.

The influence of factors such as labour, delivery method and maternal anaesthesia have been measured in this research. This information is essential to accurately identify the severity of hypoxia ischaemia early in the newborn period, provide accurate prognosis in this period and correctly identify those babies that will benefit most from neuroprotective therapies.

The normal neonatal EEG in the early newborn period needs to be studied in sufficiently large numbers, in order to ensure that EEG information from so very early in life can be used for more accurate diagnosis and prognosis. The current study recruited and has accrued normal EEG and sleep pattern data and recorded the effects of labour, delivery methods and maternal anaesthesia on EEG readings in a cohort of 92 healthy full-term neonates born in the Cork region from October 2005. This large cohort ensures inclusion of normal variation. These patterns provide a valuable template of 'normal' neonatal patterns and can be compared and contrasted with EEG patterns of babies with hypoxic-ischaemic encephalopathy and other anomaly cohorts.

In the normal newborn babies, continuous Video-EEG data was recorded using the Viasys NicOne video-EEG system for two hours. Data was collected as soon as possible, preferably within 6 hours post delivery but any data collected up to 12 hours was valuable. A modified system of electrode placement limited to 8 scalp electrodes was used. ECG leads recorded a single channel heart rate signal. Respiratory movements were measured using a thoracic respiratory band. Simultaneous video was collected for the assessment of sleep patterns and artefact elimination.

This study availed of the specialist expertise of our Neurophysiologist, Prof. Geraldine Boylan in addition to a collaboration with the digital signal processing departments of University College Cork and University College Dublin, who have developed quantitative analysis techniques for the interpretation of neonatal EEG. This has allowed specific analysis of small changes in the EEG that may not be quantifiable visually, and may identify common patterns or trends in the data. Results for 30 babies has recently been published (3).

Work which has led up to this project:

Accurate assessment of the neurological status of the neonate in the first days of life is difficult but extremely important. Foetal distress, hypoxia and acidosis may lead to encephalopathy

and seizures. Hypoxic-ischaemic encephalopathy (HIE) occurs in 3-5 per 1000 live births and may have far-reaching consequences including severe neurological disability or death. Clinical features range from mild irritability and hyper-alertness to severe coma and death. The outcome depends on the severity of the encephalopathy, with mild encephalopathy having an excellent outcome, moderate encephalopathy having a 20-40% risk of neurological disability and severe encephalopathy almost invariably leading to severe neurological impairment or death (4;5).

The accurate grading of encephalopathy is important. This is achieved by clinical scoring, or by using the analysis of electroencephalographic (EEG) patterns (6). Early serial EEG and amplitude integrated EEG are currently the best available tools with which to predict long-term neurological outcome (7;8). Early accurate prediction of outcome is becoming more important with the advent of neuroprotective therapies such as induced hypothermia, glutamate receptor antagonists and calcium channel blockers (9). To be effective, these therapies need to be instituted early, usually in the first 6 hours following delivery before secondary reperfusion injury occurs (10). Due to possible side effects they will need to be targeted at those infants who will benefit most. For these reason the accurate, early diagnosis and grading of hypoxic ischaemic encephalopathy is essential.

Early sleep patterns

It is well known that EEG matures and sleep patterns change rapidly in the first few weeks of life (11). The healthy term neonatal EEG shows alternating patterns of high and low amplitude signals and differing frequency content which are greatly dependent on sleep state. In infants who have suffered stress or hypoxic injury a burst suppression pattern may evolve (12). The degree of burst suppression is used, along with other factors such as overall voltage and the presence or absence of seizures, in the EEG grading system of HIE. It is unclear how much suppression of EEG signals might occur following the stress of an eventful labour compared to a normal labour, or following instrumental or caesarean deliveries. The effects of different methods of maternal analgesia have not been examined. It is known that newborn baby's sleep patterns are different from those of an older infant, with higher percentages of quiet sleep very early on in life (13;14). Increased quiet sleep has also been documented in neonates who have experienced excessive stimulation or pain (15). Power spectral analysis has been performed in small numbers of healthy infants and in those post intra-partum asphyxia (16). However, the control infants (n=9) in this group were studied at 24 hours of age when the effects of birth stress had probably resolved. Kim et al looked at spectral analysis of the EEG in 27 term neonates in the immediate post-natal period following vaginal deliveries (n=10) and caesarean sections (n=17). They found some differences in amplitude patterns between the two groups (17). However, EEG recordings were limited to only 10 minutes in each infant; a timeframe too short to provide meaningful information regarding EEG patterns during differing sleep states.

Strong links with the Engineering Department of our affiliated University is the advantage of this research study. Successful collaboration of two departments on several projects in the past makes it possible to continue the multidisciplinary aspect of this research (8). The methodology used in the analysis of the EEG data has been tested and verified in the prior research collaborations. To date, early continuous EEG within 3-4 hours of birth for up to 72 hours post delivery was collected in 55 infants. Recruited infants also have serial neurological examination, cerebral Doppler flow velocity measurements, MRI scanning and long term neurological follow up to 2 years of age. This work has confirmed the view that normal control data are essential. To this end, a pilot study collecting EEG data from 15 normal term infants has been conducted. The results have impressed the need to extend this study in order to clearly identify what is 'normal' and 'abnormal' in the early neonatal EEG.

Methods

The infants previously recruited for the normal neonatal prospective observational EEG study will be invited to participate in a longitudinal study at age 5 years, to assess neurological and cognitive function, as well as behaviour and executive function outcomes. At this time a neonatologist (Irina Korotchikova) will perform a full neurological assessment. A clinical psychologist (Catherine O'Connor) will perform a neurodevelopmental assessment using the Wechsler Preschool and Primary Scale of Intelligence – 3rd Edition (UK) (WPPSI-III^{UK}) (18), the majority of the NEPSY-II (19), and the 'numbers' subtest from the Children's Memory Scale. Prior to this assessment the parents will be sent a BRIEF-P (Behaviour Rating Inventory of Executive Function – Preschool version) (20) form and the 'Strengths and Difficulties' Questionnaire (21) to complete. Ms. O'Connor will assist the parents in completing the forms where required. Following these assessments, appointments will be arranged for assessment of visual acuity which will take place on different dates to avoid fatigue in the children. Parents will be asked to provide details of occupation, residence and education for the determination of socio-economic grouping and demographics, and a brief medical history will be taken for each child to rule out acquired neurological damage since the normal neonatal period.

Subjects and setting:

The parents of babies, who have already been enrolled in our normal EEG study, will be invited to participate in further follow-up assessments when they have reached the age of 5 years. Those who consent to ongoing participation in the follow-up study will be contacted by our project officer and appointments for assessment arranged. These assessments will be carried out in an appropriate clinical room in UCC's Brookfield Health Sciences Building or in the Cork University Hospital. An assessment using the WPPSI-III^{UK} and selected subtests from the NEPSY-II will be administered by Catherine O'Connor, senior clinical psychologist. Following psychological assessment, each child will have a routine neurological examination to look for tone, power and reflexes. The neurological assessment will be carried out by Dr. Korotchikova. Additional appointments will be arranged for assessment of visual acuity in the ophthalmology departments of the Cork University Hospital.

Wechsler Primary and Preschool Scale of Intelligence (UK version) WPPSI-III^{UK}

The WPPSI-III^{UK} is an individually administered clinical instrument for assessing the intelligence of children aged 2 years 6 months to 7 years 3 months, and is used worldwide (22). The third version is a major revision of its predecessors, providing updated standardised norms and adhering more closely to a hierarchical model of intelligence based on the Cattell-Horn-Carroll (CHC) model (23). There are two age-range bands (2-6 to 3-11, and 4-0 to 7-3), which use different subtests to obtain composite quotients. The administration of the core subtests yields a Verbal IQ, Performance IQ and Full Scale IQ. An additional Processing Speed Quotient can be calculated for the older age band. A General Language Composite can be calculated using supplemental/optional subtests.

The WPPSI-III was normed on a United States sample of 1,700 children, The 'UK' version was normed on a representative sample of 805 children, which closely approximated the UK 2001 Census data on similar demographic variables. Reliability and validity information is outlined extensively in the WPPSI-III Technical and Interpretative Manual (22). The average internal consistency values for individual coefficients are .95 for V-IQ, .93 for P-IQ, .89 for PSQ, and .96 for FS-IQ. Test-retest reliability is fairly stable (24). Construct validity is supported by factor-analytic studies and with comparisons with other assessments of cognitive functioning.

NEPSY-II

The NEPSY-II assesses neuropsychological development in children aged 3 to 16 years (19). It originated in the 1980s in Finland, and evolved throughout the 1980s and 1990s leading to the publication of the NEPS in Finland and the NEPSY in America in 1998. This assessed children aged 3-12 years on a broad range of tasks assessing five neuropsychological

domains, namely, attention and executive functioning, language, memory and learning, sensorimotor and visuospatial processing. What distinguished it from other children's neuropsychological assessment batteries was that it was designed exclusively for children, rather than depending on downward extensions of adult-based tasks (23). It is based on Lurian theory which uses a process-oriented approach to explain cognitive development (25). Cognitive areas are viewed as complex capacities mediated by interacting functional systems. When one subcomponent is impaired then complex functions can be impacted upon and sometimes performance can be affected across functional domains.

It was originally designed to detect subtle deficiencies both across and within subtest domains; to increase understanding of effects of congenital and acquired brain damage to enhance treatment strategies and to provide long-term follow up; to study development in preschool aged children (26).

The NEPSY-II was published in 2007 and constitutes a major revision of the NEPSY. It covers a broader age range of children aged 3-16 years. It includes a new domain – social perception - in addition to the original five domains. Subtests with lower psychometric properties have been replaced by subtests with greater clinical sensitivity and construct validity. Primary, combined, process and contrast scores can now be calculated, depending on the selection of the 32 subtests chosen for administration. It has been standardised on a single, well-stratified sample of U.S. citizens and includes clinical groups and a Finnish standardisation sample is currently underway.

Numbers subtest from the Children's Memory Scale

The Children's Memory Scale was published in 1997 and is a comprehensive standardised assessment designed to evaluate learning and memory functioning in individuals aged 5 through 16 years. It evaluates the learning and subsequent retention of information in different modalities. There are nine subtests, covering tasks related to short-term memory, working memory and long-term memory which include immediate and delayed recall and recognition tasks. The scales aim to reflect current theoretical knowledge of memory and learning, and to remain sensitive to the developmental changes.

The 'numbers' subtest from the attention/concentration domain of the CMS will be administered to obtain a measure of verbal short-term and working memory, an important component of cognitive functioning not included in the WPPSI-III.

The CMS was standardised on a U.S.A. nationwide sample of 1,000 'normally functioning' children and has been linked to the Wechsler intelligence scales using a sample of 300 children. This allows for discrepancy analysis between intellectual and memory needs and abilities. The reliability coefficient for the Numbers total score was .79 (forwards .74 and backwards .82).

The Strengths and Difficulties Questionnaire (SDQ)

The SDQ is a brief screening tool that asks about the behavioural/emotional symptoms and positive attributes for 4-16 year olds. For children, the 25-item questionnaire is completed by a parent or teacher. It has been validated against other widely used behavioural rating scales and has been normed on a UK representative sample numbering 10,438. It yields five scales – emotional symptoms, conduct problems, hyperactivity, peer problems and prosocial. The summation of the first four scales yields a 'total difficulties' score. It is quickly administered

and considered more acceptable by informants because of the inclusion of positive attributes. The SDQ has been used extensively for research and epidemiological purposes (21;27).

Behaviour Rating Inventory of Executive Function- Preschool Version
The BRIEF-P is the first standardised measure of executive functioning available for the pre-school child. It was designed to measure the range of behavioural manifestations of executive function in pre-school-aged children. The Brief-P consists of a single Rating form used by parents, teachers and day-care providers to rate the child's executive function within the context of their everyday environments. The rating form consists of 63 items that measure various aspects of executive functioning: inhibit, shift, emotional control, working memory and plan/organise. The clinical scales form three broad indexes (Inhibitory self-control, Flexibility and Emergent metacognition) and one composite score (Global Executive Composite).

This method of assessment has been shown to demonstrate good internal consistency, reliability, and high test-retest reliability. The normative data used is based on child ratings from 460 parents and 302 teachers from across differing socioeconomic groups, and in urban, suburban and rural areas in the United States. Discriminant validity was demonstrated with other measures of inattention, hyperactivity-impulsivity, depression, anxiety and somatic complaints (20).

Data analysis:
Each EEG recording will be visually assessed by an experienced neonatal electroencephalographer and the following tasks completed.

- EEG will be graded for background and sleep activity.
- Each EEG will also be assessed for the degree of continuity and the presence of focal features. A discontinuity index is displayed on the EEG system.
- Segments of EEG with obvious movement or muscle artefact will be eliminated.
- The EEG will then be converted to a format suitable for digital signal processing. The NicOne EEG system allows us to export all EEG data to ASCII or EDF format which can be processed more easily using digital signal processing techniques.

To characterise the EEG, two major signal processing tasks will then be carried out.

1. The automatic elimination of other artefacts (not visually identifiable) in the data set using Independent Component Analysis.
2. Analysis and classification of EEG data in neurologically normal term infants within 12 hours of birth using time and frequency domain techniques.
3. Power spectral analysis of heart rate variability to determine 'normal' autonomic nervous system functioning within the first 24 hours of birth.

Independent Component Analysis (ICA) [ICASSP] is a technique for separating an observed set of signals into a set of statistically independent sources, or independent components (ICs). A popular analogy is that of following a particular conversation at a noisy party; the human brain is able to follow the voice of the person you are trying to listen to and filter out the other conversations, music and background noise in the room. In the area of EEG analysis the set of signals can consist of multiple EEG channels. It is assumed that the information in the EEG, i.e. artefact EEG and background EEG, arises from a number of independent sources. Therefore, applying an ICA method to the multichannel EEG can separate these various signals into separate ICs. One method particularly suited to the extraction of artefacts is Constrained ICA (CICA). These methods use some a prior knowledge of the artefact in question to find the sources which contain them. The initial task for the signal processing section of this research will be the development of a Constrained Independent Component

Analysis system specific to the analysis of EEG data in neurologically normal term infants in the first 6, 12 and 24 hours of life.

Time-frequency analysis and processing allows analysis and processing of signals with a time-varying frequency content. Such signals are best represented by a time-frequency distribution (TFD). These convey how the energy of a multi-component or mono-component signal is distributed over the two-dimensional time-frequency space. Common uses for TFD's are the analysis of raw signals in the (t,f) domain so as to identify characteristics of the signal e.g. time and frequency variations, the number of components and the influence of harmonics on those components. It is also very useful for tracking of the instantaneous frequency, the instantaneous amplitude and the instantaneous bandwidth. The advantages of the time-frequency domain clearly surpass the advantages of both the time and frequency domains individually as neither the time nor the frequency information need be sacrificed at the cost of the other, as the team found in the analysis of automated seizure detection techniques. In this project the proposal is to use time-frequency analysis to carry out the classification of the EEG signals. Some initial research will be required to identify the "best" TFD method to use in this application.

We will examine associations between measures of cognitive, neurological, and behavioural outcome at 5 years, and early EEG findings (visual and quantitative analysis). Socioeconomic and demographic variables will be correlated with outcome. Results will be compared with outcomes from the HIE cohort and dependant outcome variables will controlled for by the observation of extraneous variables in the 'normal' group.

The Potential Benefits of the Study:
This is the first study which proposes to prospectively follow the cognitive, neurological and behavioural outcome of a cohort of full-term healthy neonates at age 5 years who have documented normal EEG recordings and neurological assessments in the first 12 hours of life in addition to comprehensive labour and birth data. The outcomes for these children will be correlated with their early EEG and neurological assessments. These patterns can be used as a unique comparison group for a prospective cohort of children with documented hypoxic-ischaemic encephalopathy born in Cork who have/are due to receive an identical battery of assessments at age five.

Specifically, this study design will (i) allow the normal neonatal EEG data to control for the possible confounders of labour, delivery type and maternal analgesics on EEG readings; (ii) control for demographic, socioeconomic and cultural variables for five-year outcomes in the Cork geographical area. This should provide a more accurate measure for the predictive validity of neonatal EEG readings in the HIE cohort.

Protective hypothermia treatment has shown promising results in the treatment of HIE, and is now being used at Cork University Maternity Hospital. Improved prognostic and accurate follow-up data will help to identify those neonates most likely to benefit from treatment, based on more meaningful discrimination between normal and abnormal early EEG analysis.

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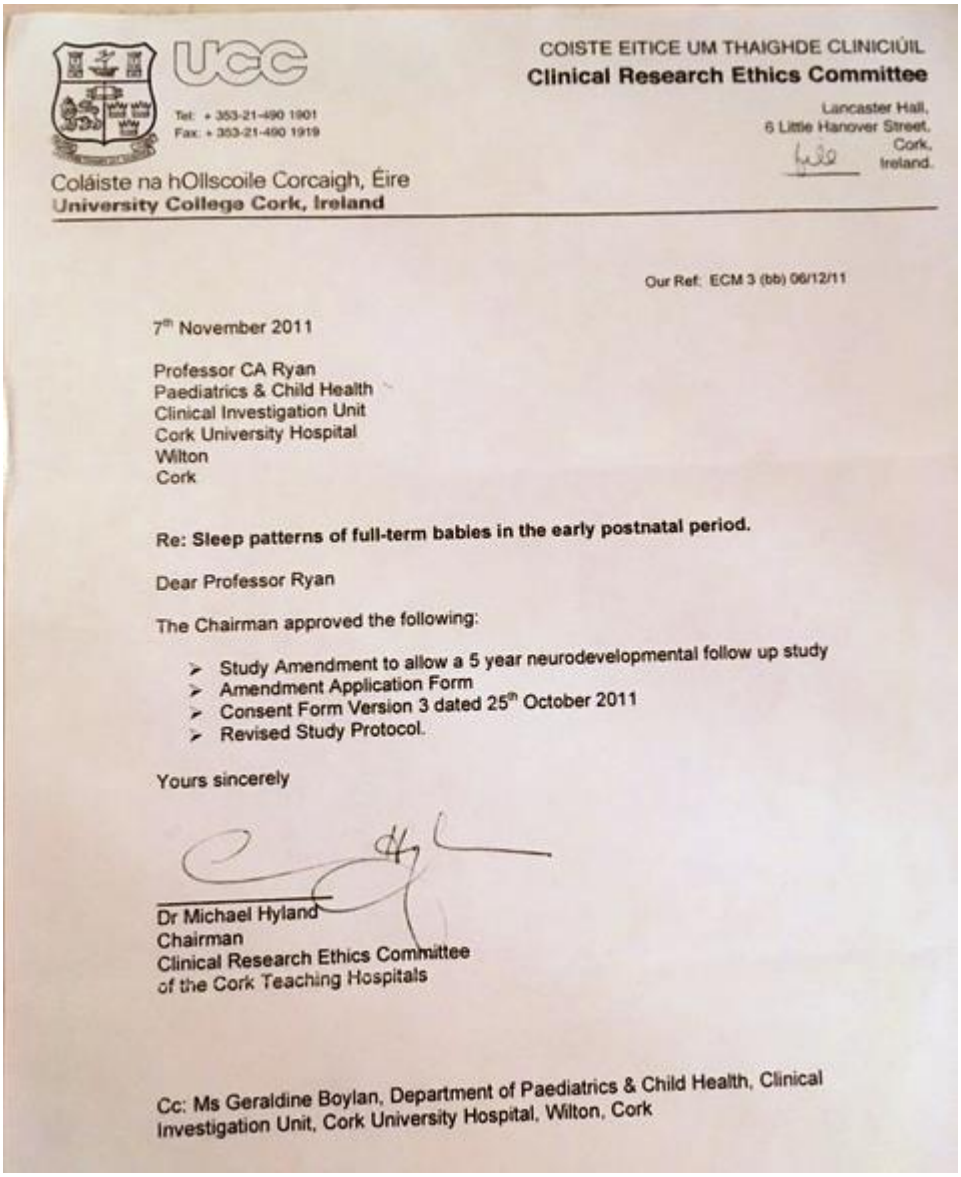
Appendix F

Ethics Approval letter for five-year follow-up of the 'Sleep patterns of full-term babies in the early postnatal period' study



UCC

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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee

Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our Ref: ECM 3 (bb) 06/12/11

7th November 2011

Professor CA Ryan
Paediatrics & Child Health
Clinical Investigation Unit
Cork University Hospital
Wilton
Cork

Re: Sleep patterns of full-term babies in the early postnatal period.

Dear Professor Ryan

The Chairman approved the following:

- Study Amendment to allow a 5 year neurodevelopmental follow up study
- Amendment Application Form
- Consent Form Version 3 dated 25th October 2011
- Revised Study Protocol.

Yours sincerely

Dr Michael Hyland
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

Cc: Ms Geraldine Boylan, Department of Paediatrics & Child Health, Clinical Investigation Unit, Cork University Hospital, Wilton, Cork

Appendix G
Comparison group study information letter

Neonatal Research Centre
Neonatal Unit
Cork University Maternity Hospital
Wilton, Cork, Ireland

Date here.

Dear Parent,

Once again we would like to express our gratitude to you for allowing your child to take part in the EEG (electroencephalogram) sleep study when they were born, and also to those of you who took part in filling out questionnaires last summer. With your help we have been able to gain very important information about the sleep patterns of babies soon after birth, and what this might mean for their future development. This information helps doctors caring for sick babies because they can compare the EEGs with a normal baby's EEGs.

The Health Research Board of Ireland has recognised the importance of this research and we recently completed a study funded by them that allowed us to see the sick babies again at age five years. As part of this research, we wish to include children who were healthy at birth for comparison. The aim of this project will be to compare healthy children at birth, to children who were very sick at birth, at approximately five years of age. This will be achieved by carrying out detailed assessments of your child's development and learning. This will allow us, with your permission to understand a ***typical*** child's development outcome AND to be able to see if a baby's EEG is linked to their development later in life. This information will be used with future research with sick babies.

Over the next few weeks, you will be contacted by Catherine O'Connor, child psychologist to invite you to take part in our ongoing follow up study. We will try to arrange the assessments to times which suit you, and you will be reimbursed for any travel expenses. The assessments can be arranged to take place in your home, if this is more convenient for you. If you have any queries, please contact Ms Catherine O'Connor on 087 ------- at any time, or Ms. Brenda O'Flynn on 021 -------|between the hours of 9am to 1pm.

Thank you again for your help.
Yours sincerely,

_____

Dr. Irina Korotchikova
Neonatal Brain Research Group

Appendix H
Participant information and consent form for the comparison group.

UNIVERSITY COLLEGE CORK
Clinical Research Ethics Committee of the Cork Teaching Hospitals

CONSENT BY SUBJECT FOR PARTICIPATION IN RESEARCH PROTOCOL

Section A

Protocol Number:_____Child's Name:_____

9.1.1.1.1 Title of Protocol: Sleep patterns of full-term babies in the early postnatal period.

Research directed by:
Prof. C.A. Ryan (Tel.021-4920525); Prof. G. Boylan; Dr B. Murphy; Dr D Murray.

You are being asked to participate in a follow up research study. The doctors at University College Cork study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgement. This process is known as informed consent. This consent form gives detailed information about the research study and this will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

Section B

I. NATURE AND DURATION OF PROCEDURE(S):

You may recall that your baby was involved in an important research project which studied sleep patterns of normal healthy babies soon after birth. This study used an EEG which measured your baby's brainwaves using small attachments to the scalp. This allowed us to gather important information about sleep patterns in newborn babies shortly after birth. This helped us to compare the information about normal sleep in babies to the sleep patterns of sick babies in the neonatal intensive care unit. This information has now been published in international medical journals and will help other babies and their families in the future, both in Ireland and worldwide.

We would now like to meet you and your child again at the age of 5 years in order to gather data about children who we know had a full-term healthy birth. This data will allow us to compare healthy children with 5 year old children who spent time in the intensive care unit at birth. We wish to organise 2 or 3 assessment appointments to see how your child is developing and learning. This will involve tasks such as answering questions, remembering things and using pictures and blocks. This will be carried out by an experienced child psychologist. We will ask you to fill in two questionnaires about how they act when they are at home. We will also ask you general information about your child's health, age, family, and your education and occupation. All information will be kept in the strictest confidence.

II. POTENTIAL RISKS AND BENEFITS:
There are no potential risks. The study will take some of you and your child's time. We will pay for any travel, parking and food costs which you incur while attending the follow up sessions in University College Cork. The assessments will be reported to you if you wish and will give you a detailed picture of how your child is developing compared to other children their age.

III. POSSIBLE ALTERNATIVES:
The clinical management of your child will not be affected if you do not want to participate in this research.

Section C **AGREEMENT TO CONSENT**

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I understand that the sponsors and investigators have such insurance as is required by law in the event of injury resulting from this research.

I, the undersigned, hereby consent to participate as a subject in the above described project conducted at the Cork Teaching Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Clinical Sciences Building, Cork University Hospital, Wilton, Cork.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Doctor/Psychologist _____

_____
Signature of Subject, Parent or Guardian

(include a separate line for assent of minor, if applicable)

Witness:_____ Date: _____ Time:_____AM
(Circle) PM

Appendix I
Parent Interview Questionnaire

Parent Interview Questionnaire

We would be very grateful if you could complete the following questionnaire. All of the information will be kept in the strictest of confidence.

Please state your relationship to the child participating in the study (e.g. mother, father, carer): _____ Date questionnaire completed: _____

1. Since last seen by Dr. Deirdre Murray at age 24 months, has your child:

   a) Been admitted to hospital? ☐ YES ☐ NO
   If yes, please describe **each** occasion and include where seen, when, reason, outcome
   _____
   _____
   _____
   _____
   _____

   b) Experienced any head injuries or head trauma? ☐ YES ☐ NO
   If yes, please describe
   _____
   _____
   _____

   c) Had any seizures or been diagnosed with epilepsy? ☐ YES ☐ NO
   If yes, please describe when diagnosed and date of last seizure:
   _____
   _____
   _____

   d) Experienced health conditions that require medical monitoring (such as diabetes, asthma, allergies etc). Please list:
   _____
   _____
   _____

   e) Attended a psychologist/SLT/OT/Physiotherapist/early intervention?
   ☐ YES ☐ NO
   If yes, when was he/she last seen and for what reason? Please include any names of assessment tests used:
   _____

_____
_____

f) Is your child currently taking any medications? ☐ YES ☐ NO
If yes, please list names and dosages per day:
_____
_____
_____

2. If your child has brothers and/or sisters, what gender and age are they? (e.g. Boy - 7 years; Girl - 6 months): _____

_____
_____

_____

3. What is your nationality? _____

What ethnic group best describes your child? (please tick)

White:

☐ Irish

☐ Irish Traveller

☐ Any other white background

Black or Black Irish

☐ African

☐ Any other black background

Asian or Asian Irish

☐ Chinese

☐ Any other Asian background

Other, including mixed background:

_____

Is English your first language? ☐ YES ☐ NO

4. **At the time of your child's birth**, were you:

☐ Single (never married)

☐ Married (first marriage)

☐ Re-married (following widowhood)

☐ Re-married (following divorce/annulment)

☐ Separated (including deserted)

☐ Divorced

☐ Widowed

Has your marital status changed since then? ☐ YES ☐ NO

If yes, please describe current marital status (if you have since married, please also state whether the marriage is to the child's birth father):

_____

5. **At the time of your child's birth**, what was the **highest** level of education (part-time or full-time) completed? Please tick one box only.

☐ No formal education

☐ Primary education

Second Level

☐ Lower secondary education
Junior/Intermediate/Group Certificate, 'O' Levels/GCSEs, NCVA Foundation Certificate, Basic Skills Training Certificate or equivalent.

☐ Upper secondary education
Leaving Certificate (including Applied and Vocational Programmes), 'A' Levels, NCVA Level 1 Certificate or equivalent.

☐ Technical or Vocational Qualification
Completed Apprenticeship, NCVA Level 2/3 Certificate, Teagasc Certificate/Diploma or equivalent.

☐ Both Upper Secondary and Technical or Vocational Qualification

Third Level

☐ Non-degree:
National Certificate, Diploma NCEA/Institute of Technology or equivalent, Nursing Diploma

☐ Primary Degree *(Third Level bachelor degree)*

☐ Professional qualification (of Degree status at least)

☐ Both a Degree and a Professional Qualification

☐ Postgraduate Certificate or Diploma

☐ Postgraduate Degree (Masters)

☐ Doctorate (Ph-D)

6. Have you attended any further education/training since your child's birth?
If yes, please describe briefly:

_____

_____

_____

7. **Before** your child's birth, had you been working outside of the home?

☐ YES ☐ NO

If yes, please define your job title as clearly and in as much detail as possible (e.g. part-time assistant project manager; full-time contract trainee accountant; permanent registered nurse; Sessional Grade III clerical officer, etc.)

_____

_____

8. Are you currently working outside of the home? ☐ YES ☐ NO

Is your **current** job the same as described above? ☐ YES ☐ NO
If no, please describe new job title: _____

_____

9. Are you currently living with your child's birth father? ☐ YES ☐ NO

If NO, does the child's birth father have regular contact? ☐ YES ☐ NO

Please state birth father's occupation as fully as possible: _____

10. **At the time of your child's birth** was your **annual household** income:

☐ < €9,000 ☐ €9,001 – €10,000

☐ €10,001 – €15,000 ☐ €15,001 – €20,000

☐ €20,001 – €25,000 ☐ €25,001 – €30,000

☐ €30,001 – €35,000 ☐ €35,001 – €40,000

☐ €40,001 – €45,000 ☐ €45,001 – €50,000

☐ €50,001 – €60,000 ☐ €60,001 – €70,000

☐ €70,001 – €80,000 ☐ €80,001 – €90,000

☐ €90,001 – €100,000 ☐ >€101,000

11. Is your **current annual household** income:

☐ < €9,000 ☐ €9,001 – €10,000

☐ €10,001 – €15,000 ☐ €15,001 – €20,000

☐ €20,001 – €25,000 ☐ €25,001 – €30,000

☐ €30,001 – €35,000 ☐ €35,001 – €40,000

☐ €40,001 – €45,000 ☐ €45,001 – €50,000

☐ €50,001 – €60,000 ☐ €60,001 – €70,000

☐ €70,001 – €80,000 ☐ €80,001 – €90,000

☐ €90,001 – €100,000 ☐ >€101,000

Many thanks again for the help and time that you have given in completing this questionnaire.

Appendix J
Amiel-Tison Neurological Assessment at Term: Record Form

AMIEL-TISON NEUROLOGICAL ASSESSMENT AT TERM

Claudine Amiel-Tison, Julie Gosselin, Françoise Lebrun and Sheila Gahagan

Name _____ Birth date M D Y

Mother's name _____ Gestational age (wk)

Chart number _____ Sex M ☐ F ☐

Assessments

Number	1	2	3	4
Date of assessment				
Day of life				
Corrected age (wk)				
Weight (g)				
Height (cm)				
Head circumference (cm)				

INSTRUCTIONS

For whom?
Term neonates within the first days of life and preterm neonates closest to the term period (between 37 and 42 weeks corrected).

How to code?
A numerical system is proposed to code the observations . Level of severity in abnormal responses is defined.
0 indicates a typical result, within normal range
1 indicates a moderately abnormal result
2 indicates a definitely abnormal result
X indicates examination results when scoring is considered inappropriate because the normal or abnormal character of the observation cannot be defined with certainty.

This coding system is not quantitative. Thus, any computation of quotient or total score is inappropriate.

Pregnancy and birth

Single ☐

Multiple : twin ☐

higher ☐

Vaginal delivery : cephalic ☐

breech ☐

Cesarian section planned or repeated ☐

emergency cs ☐

Apgar 1' ☐ 5' ☐

Growth parameters at birth

Weight	☐	g	☐	centiles
Height	☐	cm	☐	centiles
Head circumf (HC)	☐	cm	☐	centiles
Mid arm circumf.	☐	cm	☐	centiles

Postural deformities (acquired in utero or postnatally)

Skull ☐

Neck ☐

Body axis ☐

Upper limbs ☐

Lower limbs ☐

Describe:

Mechanical consequences of birth process

Caput succedaneum ☐

Cephalohematoma ☐

Severe cranial molding ☐

Facial ecchymosis ☐

Bruising from forceps (if extensive, asymmetrical or abnormally located) ☐

Facial paralysis ☐

Brachial plexus paralysis ☐

Hematoma of SCM ☐

Fracture of the clavicle ☐

Other ☐

Socioeconomic data

Maternal age ☐

Maternal education ☐

Presence of the father at home yes ☐ no ☐

Parental growth parameters

	Mother	Father
HC	☐	☐
Height	☐	☐

CRANIAL ASSESSMENT

		1	2	3	4
Head circumference	± 2SD	0	0	0	0
	> 2SD	X	X	X	X
	< 2SD	X	X	X	X
Anterior fontanellel	Normal	0	0	0	0
	Tense	X	X	X	X
Squamous sutures	Edge-to-edge	0	0	0	0
	Separated	X	X	X	X
	Overlapping	X	X	X	X
Other sutures	Edge-to-edge	0	0	0	0
	Separated	X	X	X	X
	Overlapping	X	X	X	X

NEUROSENSORY FUNCTION AND SPONTANEOUS MOTOR ACTIVITY DURING THE ASSESSMENT

Fix and track	Easy to obtain 4 times	0	0	0	0
	Difficult to obtain	1	1	1	1
	No response	2	2	2	2
Ocular signs	Absent	0	0	0	0
	Present, describe_____	X	X	X	X
Response to voice	Easy to obtain	0	0	0	0
	Difficult to obtain	1	1	1	1
	No response	2	2	2	2
Social interaction	Easy and spontaneous	0	0	0	0
	Poor and limited	1	1	1	1
	No interaction	2	2	2	2
Crying	Normal pitch, easy to calm	0	0	0	0
	Monotoneous, abnormal pitch	1	1	1	1
	Absent	2	2	2	2
Excitability	Consolable, normal sleep	0	0	0	0
	Excessive crying, insufficient sleep	1	1	1	1
	Tremors and/or clonic movements	1	1	1	1
Convulsions	Absent	0	0	0	0
	Present (1 or 2)	2	2	2	2
	Repeated for more than 30 min.	2	2	2	2
	Describe variety				
Spontaneous motor activity	Varied, harmonious	0	0	0	0
	Insufficient, stereotyped	1	1	1	1
	Absent or barely present	2	2	2	2
	Asymmetrical (pathological side)	R L	R L	R L	R L
Spontaneous thumb abduction	Active thumb	0	0	0	0
	Inactive thumb	2	2	2	2
	Fixed thumb in adduction	2	2	2	2
	Asymmetrical (pathological side)	R L	R L	R L	R L

PASSIVE MUSCLE TONE

			1		2		3		4	
			R	L	R	L	R	L	R	L
UPPER LIMBS	Recoil	Quick, reproducible	0	0	0	0	0	0	0	0
		Slow, not reproducible	1	1	1	1	1	1	1	1
		Absent	2	2	2	2	2	2	2	2
	Scarf	Elbow does not reach midline	0	0	0	0	0	0	0	0
		Elbow slightly passes midline	1	1	1	1	1	1	1	1
		No resistance	2	2	2	2	2	2	2	2
LOWER LIMBS	Recoil *	Quick, reproducible	0	0	0	0	0	0	0	0
		Slow, not reproducible	1	1	1	1	1	1	1	1
		Absent	2	2	2	2	2	2	2	2
		Value of the angle								
	Popliteal angle*	70 - 90°	0	0	0	0	0	0	0	0
		100 - 120°	1	1	1	1	1	1	1	1
		130° or more	2	2	2	2	2	2	2	2
	* No coding in cases of breech delivery									
RIGHT-LEFT COMPARISONS	Asymmetry	Absent or not categorized	0		0		0		0	
		Right side more relaxed	X		X		X		X	
		Left side more relaxed	X		X		X		X	
BODY AXIS	Ventral incurvation (flexion)	Moderate, easy to obtain	0		0		0		0	
		Absent or minimal	1		1		1		1	
		Unlimited	2		2		2		2	
	Dorsal incurvation (extension)	Absent to moderate	0		0		0		0	
		Opisthotonos (excessive)	2		2		2		2	
	Comparison of curvatures	Flexion ≥ extension	0		0		0		0	
		Flexion < extension	1		1		1		1	
		Flexion & extension unlimited	2		2		2		2	

AXIAL MOTOR ACTIVITY (active tone)

	Righting reaction (Lower limbs + trunk)	Present, complete or not	0	0	0	0
		Excessive with arching	1	1	1	1
		Absent	2	2	2	2
	Raise to sit (neck flexor muscles →head forward)	Easy, in the axis	0	0	0	0
		Muscle activity but no passage	1	1	1	1
		No response	2	2	2	2
	Reverse maneuver (neck extensor muscles →head backward)	Easy, in the axis	0	0	0	0
		Brisk, excessive response	1	1	1	1
		No response	2	2	2	2

PRIMITIVE REFLEXES

		1	2	3	4
Non nutritive sucking	Rythmic movements, efficient	0	0	0	0
	Few movements, inefficient	1	1	1	1
	No movements	2	2	2	2
Palmar grasp	Strong finger flexion	0	0	0	0
	Weak, short duration	1	1	1	1
	Absent	2	2	2	2
	Asymmetrical (pathological side)	R L	R L	R L	R L
Automatic walking	A few steps, easy to obtain	0	0	0	0
	Difficult to obtain or absent (no concern if isolated finding)	X	X	X	X
Moro reflex**	Brisk, with opening of the hands	0	0	0	0
	Incomplete	1	1	1	1
	Absent	2	2	2	2
	Asymmetrical (pathological side)	R L	R L	R L	R L
Asymmetric tonic neck reflex (ATNR)	Absent	X	X	X	X
	Present	X	X	X	X

** to assess only when other primitive reflexes are asymmetrical or absent

PALATE AND TONGUE

High arched palate	Absent	0	0	0	0
	Present	2	2	2	2
Fasciculations of tongue (peripheral, at rest)	Absent	0	0	0	0
	Present	2	2	2	2

ADAPTEDNESS TO MANIPULATIONS DURING ASSESSMENT

Stability	Excellent	0	0	0	0
	Transient changes	1	1	1	1
	Severe destabilisation	2	2	2	2

FEEDING AUTONOMY

Term newborn	Immediate, easy	0	0	0	0
	Incomplete	1	1	1	1
	Absent until day 7	2	2	2	2
Preterm infant close to term	Present, easy	0	0	0	0
	Incomplete	1	1	1	1
	Absent	2	2	2	2

MEDICAL STATUS AT THE TIME OF ASSESSMENT

		1	2	3	4
Term neonate (within the first week)	Assisted ventilation	X	X	X	X
	Anticonvulsant drugs	X	X	X	X
	Phototherapy	X	X	X	X
	Other	X	X	X	X
Preterm infant at the time of examination Persisting extraneurological pathology	Cardiac problems	X	X	X	X
	Respiratory problems	X	X	X	X
	Digestive problems	X	X	X	X
	Retinopathy	X	X	X	X
	Other (describe)	X	X	X	X

UNFAVORABLE CIRCUMSTANCES AT THE TIME OF EXAMINATION

Condition(s)	Has just been fed	X	X	X	X
	Too hungry	X	X	X	X
	Noisy environment	X	X	X	X
	Other (describe)	X	X	X	X

COMPLEMENTARY INVESTIGATIONS

	Date	Results
CRANIAL ULTRASOUND		
CT-SCAN OR MRI		
CSF		
Optic fundi		
EEG		
BAER		
Other		

HOW TO ACHIEVE A SYNTHESIS OF THE DATA

For the term newborn infant (page 49):

In the absence of any abnormality at the first assessment (day 1 or 2), synthesis relies on this single assessment.

In the presence of abnormalities at the first assessment, synthesis relies on repeated assessments within the first week of life.

For the preterm infant around 40 weeks corrected (page 50):

Synthesis is based on a single assessment performed as close as possible to 40 weeks.

SYNTHESIS FOR TERM NEWBORN INFANTS

ABSENCE OF ANY NEUROLOGICAL SIGN	☐

PRESENCE OF NEUROLOGICAL SIGNS, VARIABLE DEGREES	☐
Minor degree, without CNS depression	
Hyperexcitability	☐
Various abnormalities of passive tone	☐
Normalized by day 3	**Yes** ☐ **No** ☐
Normalized by day 7	**Yes** ☐ **No** ☐
Moderate degree, with CNS depression	
Lethargy poor fix and track	☐
Hypoactivity	☐
Passive hypotonia in limbs	☐
Poor activity in neck flexors	☐
Primary reflexes poor or absent	☐
Seizures (1 or 2)	☐
Normalizedby day 7	**Yes** ☐ **No** ☐
Severe degree, with deep CNS depression and repeated seizures for more than 30 minutes	
Duration of status epilepticus	_____ hours
Duration of assistive ventilation	_____ days
Duration of absence of feeding autonomy	_____ days

EVOLVING PATTERN BASED ON REPEATED EXAMINATIONS	
Dynamic (tendency to aggravation followed by improvement)	☐
Static (few or no changes)	☐

SIGNS IN FAVOR OF A PRENATAL INSULT (present at birth)	
Cortical thumb	☐
High-arched palate	☐
Overlapping sutures (with or without microcephaly)	☐

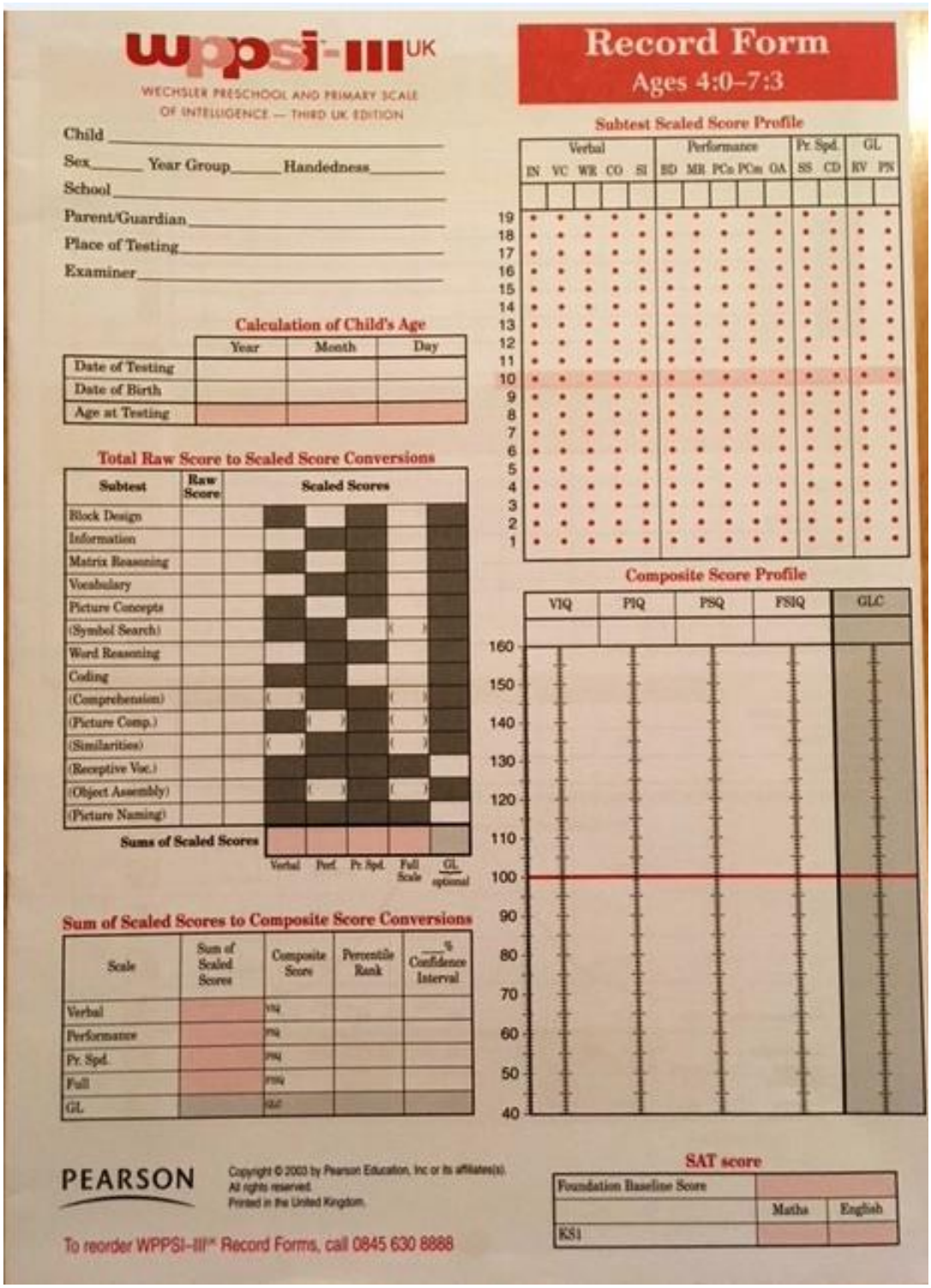
UNCONCLUSIVE RESULTS	
Due to unfavorable circumstances for examination	☐

PROVISIONAL CONCLUSION ON THE PROBABLE CAUSE OF NEUROLOGICAL IMPAIRMENT	
Brain malformation	☐
Genetic syndrome	☐
Hypoxic-ischemic encephalopathy	☐
prenatal	☐
intrapartum	☐
postnatal	☐
White matter damage	☐
Infarction of an arterial territory	☐
Infectious process	☐
Other (describe)	☐

RECOMMENDED FOLLOW-UP

Reprinted from Gosselin, J, Gahagan, S, Amiel-Tison, C. The Amiel-Tison Neurological Assessment at Term: Conceptual and Methodological Continuity in the Course of Follow-Up. *Mental Retardation and Developmental.Disabilities Research Reviews* 11: 35-51 (2005).

Appendix K
WPPSI-III[UK] Record Form Cover Page



wppsi-III UK

WECHSLER PRESCHOOL AND PRIMARY SCALE OF INTELLIGENCE — THIRD UK EDITION

Record Form
Ages 4:0–7:3

Child _____

Sex_____ Year Group_____ Handedness_____

School _____

Parent/Guardian _____

Place of Testing _____

Examiner _____

Calculation of Child's Age

	Year	Month	Day
Date of Testing			
Date of Birth			
Age at Testing			

Total Raw Score to Scaled Score Conversions

Subtest	Raw Score	Scaled Scores
Block Design		
Information		
Matrix Reasoning		
Vocabulary		
Picture Concepts		
(Symbol Search)		
Word Reasoning		
Coding		
(Comprehension)		
(Picture Comp.)		
(Similarities)		
(Receptive Voc.)		
(Object Assembly)		
(Picture Naming)		
Sums of Scaled Scores		Verbal / Perf. / Pr. Spd. / Full Scale / GL optional

Sum of Scaled Scores to Composite Score Conversions

Scale	Sum of Scaled Scores	Composite Score	Percentile Rank	___% Confidence Interval
Verbal		VIQ		
Performance		PIQ		
Pr. Spd.		PSQ		
Full		FSIQ		
GL		GLC		

Subtest Scaled Score Profile

	Verbal					Performance					Pr. Spd.		GL	
	IN	VC	WR	CO	SI	BD	MR	PCn	PCm	OA	SS	CD	KV	PN
19	•	•	•	•	•	•	•	•	•	•	•	•	•	•
18	•	•	•	•	•	•	•	•	•	•	•	•	•	•
17	•	•	•	•	•	•	•	•	•	•	•	•	•	•
16	•	•	•	•	•	•	•	•	•	•	•	•	•	•
15	•	•	•	•	•	•	•	•	•	•	•	•	•	•
14	•	•	•	•	•	•	•	•	•	•	•	•	•	•
13	•	•	•	•	•	•	•	•	•	•	•	•	•	•
12	•	•	•	•	•	•	•	•	•	•	•	•	•	•
11	•	•	•	•	•	•	•	•	•	•	•	•	•	•
10	•	•	•	•	•	•	•	•	•	•	•	•	•	•
9	•	•	•	•	•	•	•	•	•	•	•	•	•	•
8	•	•	•	•	•	•	•	•	•	•	•	•	•	•
7	•	•	•	•	•	•	•	•	•	•	•	•	•	•
6	•	•	•	•	•	•	•	•	•	•	•	•	•	•
5	•	•	•	•	•	•	•	•	•	•	•	•	•	•
4	•	•	•	•	•	•	•	•	•	•	•	•	•	•
3	•	•	•	•	•	•	•	•	•	•	•	•	•	•
2	•	•	•	•	•	•	•	•	•	•	•	•	•	•
1	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Composite Score Profile

	VIQ	PIQ	PSQ	FSIQ	GLC
160					
150					
140					
130					
120					
110					
100					
90					
80					
70					
60					
50					
40					

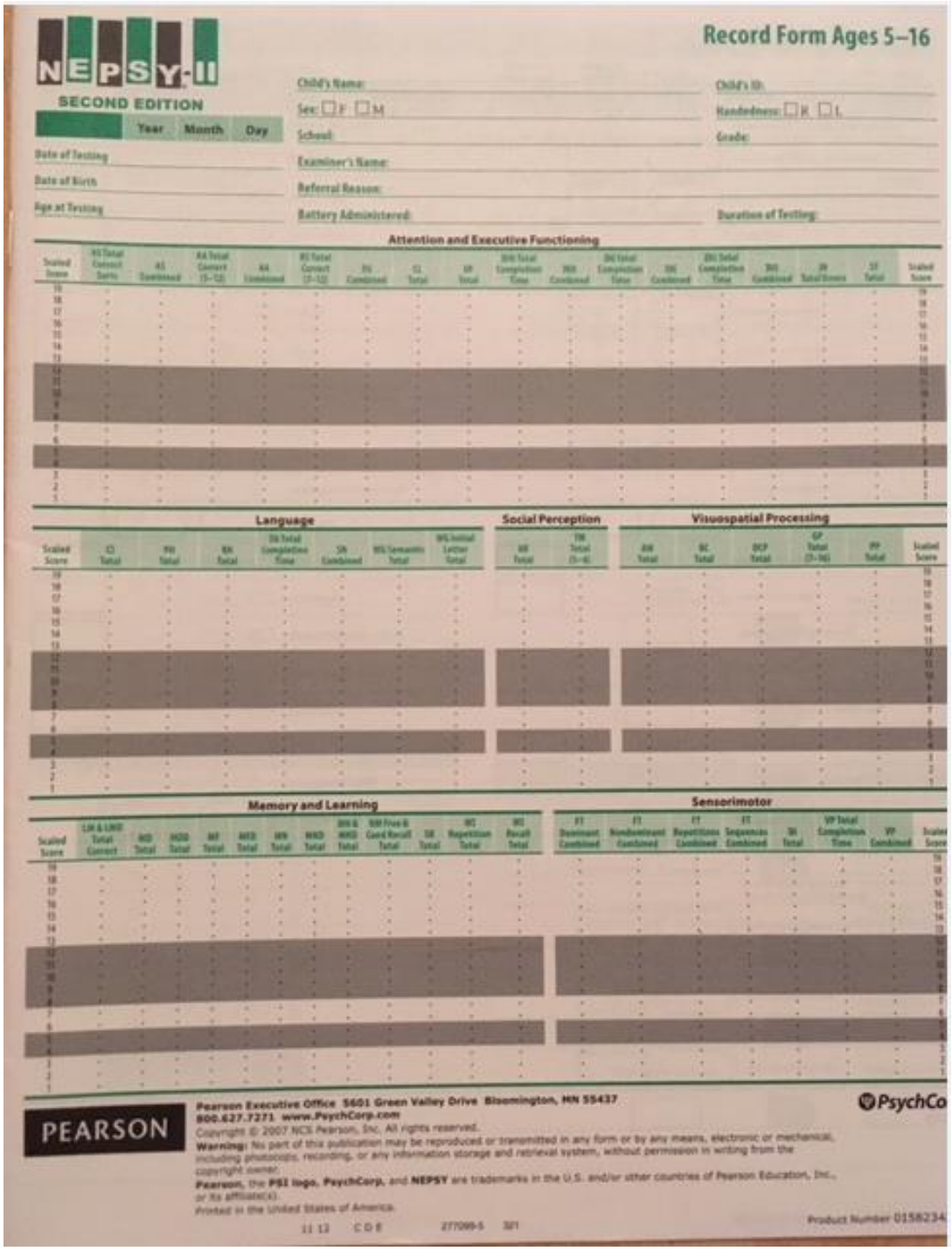
PEARSON

To reorder WPPSI-III™ Record Forms, call 0845 630 8888

SAT score

Foundation Baseline Score		
	Maths	English
KS1		

Appendix L
NEPSY-II Record Form Cover Page



NEPSY-II
SECOND EDITION
Record Form Ages 5–16

Child's Name:
Child's ID:
Sex: ☐ F ☐ M
Handedness: ☐ R ☐ L
School:
Grade:
Year Month Day
Date of Testing
Examiner's Name:
Date of Birth
Referral Reason:
Age at Testing
Battery Administered:
Duration of Testing:

Attention and Executive Functioning

Language

Social Perception

Visuospatial Processing

Memory and Learning

Sensorimotor

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Appendix M
NEPSY-II Scoring Software and Report Generator

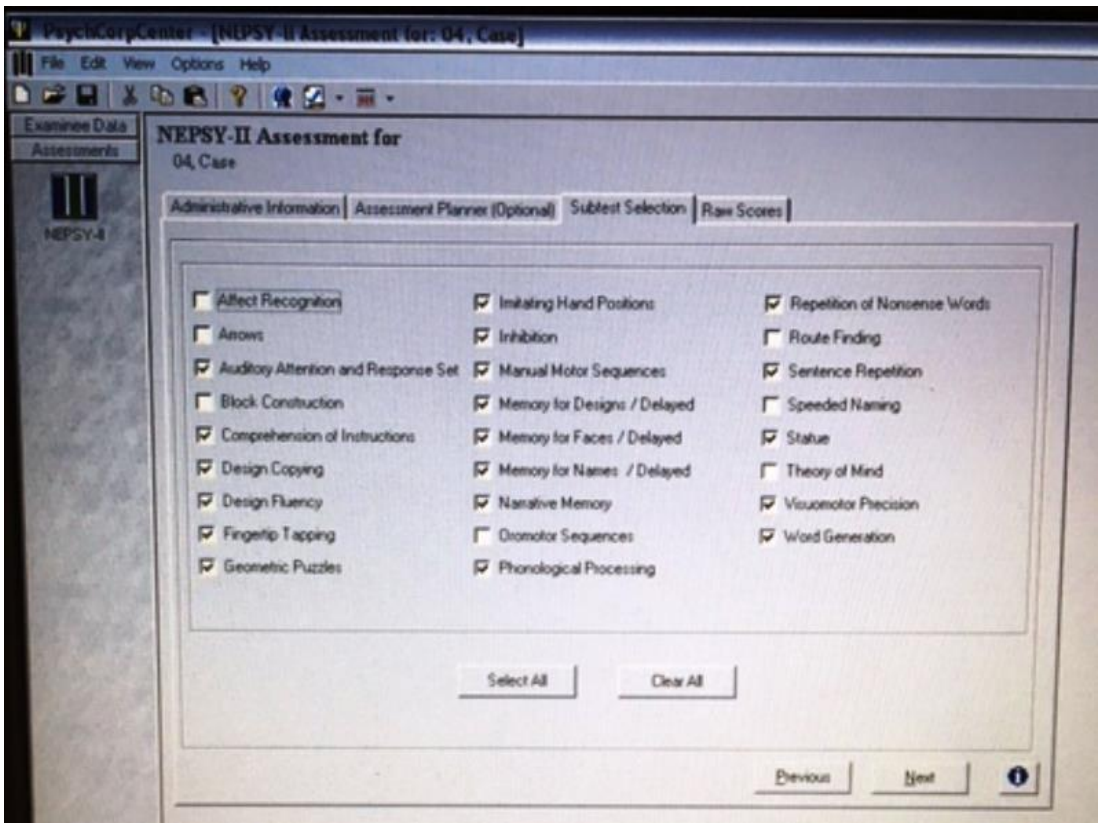

Figure M 1 Screenshot of NEPSY-II scoring software

Appendix N
Consent Form to obtain clinical copies of psychoeducational reports completed within previous 6 months.

Neonatal Brain Research Group
Neonatal Encephalopathy Study – 5 year follow-up.

CONSENT FORM

Name: _____ D.O.B. _____

Address: _____

We/I consent to the psychologist working on the Neonatal Brain Research Group's follow up study to contact and obtain relevant information/most recent psychology report from the psychologist who has been involved in the treatment and/or management of my child.

Signed _____.

Relationship to Child _____.

Research Psychologist: CATHERINE O'CONNOR _____

Date _____.

Appendix O
The Numbers subtest of the Children's Memory Scale

Figure M 2 Logo of the Children's Memory Scale. Ref: Cohen M. Children's Memory Scale. San Antonio, TX: The Psychological Corporation; 1997.

The Numbers subtest of the Children's Memory Scale (Cohen, 1997) was used to assess short-term verbal memory. It consists of a measure of short-term verbal memory using a digit recall task, and a measure of short-term verbal working memory using a backwards digit recall task. The two raw scores are combined to obtain the Numbers Total Raw Score. An age-related scaled score is then calculated.

Appendix P
Vision Appointment information letter

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

CUH
Ospidéal Ollscoil Chorcaí
Cork University Hospital

Tel: 087

OUT PATIENT DEPARTMENT
Appointment Offer

19th August 2009

[child name]
[Address line 1],
[Address line 2],
[Address line 3]

Participant's name:	[Child's name]
Examiner:	Dr Mark James
Speciality:	Ophthalmology
Suite:	Eye Outpatient Department, Cork University Hospital
Appointment:	**Friday 28th August 2009 at 16:45**
Clinic:	**Hypoxic Ischaemic Encephalopathy Study Cohort – Ophthalmic Section**

Location of department:

- On entering the main hospital gates from Wilton Road, patients turn right and enter through the door with the sign for Admissions / Out-Patients / A&E.
- The Wilton Shopping Centre, which includes restaurants, etc. is situated opposite the hospital.

On arrival to department:

- Please check in with the secretary at the front desk, so they can contact me on my mobile to let me know you have arrived. There may be a queue of people checking in for clinic, so please ignore this and head straight for the front desk, as our study is separate.
- The nurse will then check the vision, and put dilating drops into the eyes. These sting the eyes for a few seconds, and when the pupils are large, are likely to blur the vision somewhat until they wear off (their effect usually wears off by the next day).
- If you wish, you may leave the department for about 15 minutes to give the drops time to work. I will then call your name to begin the examination.

What does the examination involve?

- Firstly, we will be doing some basic measurements of the eye, which involves looking at a light in a machine for about 10 minutes. This is pretty straight-forward, but can be a little boring!
- Next, we will use another machine to measure whether the eyes are short-sighted or long-sighted. This involves looking at another target (a balloon) for a few seconds.
- After that, we will examine the front and back of the eye with a moderately bright light.
- Lastly, after the drops have been in for about 40 minutes, we manually determine whether the eyes are short-sighted or long-sighted by placing a few lenses in front of the eyes and shining another light through them.
- We are aiming to be finished within an hour of your arrival, although this may not always be possible if I am called away for an emergency (though this should rarely happen).

Follow-up:

- If any problems are found with the eyes, such as the need for glasses, this will be followed up.
- An orthoptist who is involved in detailed examination of squints and vision in children has also been invited to take part in the study, and is likely to arrange her own appointment with you on a different day. She will be looking at different aspects of the vision.

If you cannot make the appointment, or have any queries, please contact me on the number above.
Many thanks for your participation in this study.

Yours sincerely,

_____
Dr Mark James MB BCH BAO MRCPI MRCOphth