
# Neurodevelopmental Outcome in Perinatal Asphyxia: Prediction and Measurement

Author: Dr Caroline Ahearne MB BCh BAO MRCPCH

Department of Paediatrics and Child Health

University College Cork

Thesis submitted to National University of Ireland, Cork, in candidature for the degree of Doctor of Philosophy

Date of Submission: October 2016

Head of Department: Professor Jonathan Hourihane

Supervisors:

Dr Deirdre Murray

Professor Geraldine Boylan

# Table of Contents

## Declaration

The thesis submitted is the candidate's own work and has not been submitted for another degree, either at University College Cork or elsewhere.

_____ _____

Dr Caroline Ahearne Date

## Acknowledgements

When I came to Cork University Hospital as a paediatric SHO, I had no idea the journey that lay ahead of me. My supervisors, Dr Deirdre Murray and Prof Geraldine Boylan, gave me the chance to join the exciting world of clinical research and have inspired me every step of the way. Their passion for improving healthcare for the most vulnerable babies and their pursuit of excellence in that quest will continue to influence me in my future career. I'd like to sincerely thank them for the knowledge they have shared, the enthusiasm and the guidance that made this thesis possible. I've gained so many new skills, had so many experiences and achieved things I would never have thought possible without them.

I'd also like to thank my colleagues who shared the late nights on call as well as the ups and downs of the research process; Dr Ann Marie Looney, Dr Niamh Denihan, Mr Rhodri Lloyd, Mr Robert Goulding, Dr Liudmila Kharoshankaya and Dr Elena Pavlidis. The collaboration, cooperation and dedication of this unique group have helped keep me going and I am grateful beyond measure to you all.

There are so many people I've met through the various studies I've been involved with that I'd like to thank but who are too numerous to mention here. In particular, to everyone involved in the BiHIVE Study, especially Mrs Jean Conway, the BASELINE Study, the Babyscreen App project, especially Mr Sean Murray and Mr Steven Burgess, who made the app a reality, and all the medical students who brought fresh eyes to our work, thank you for everything. Thank you to everyone in the Irish Centre for Foetal and Neonatal Translational Research for their ongoing hard work that always made me feel I was part of something really special.

My involvement in research has been funded by a grant from the Health Research Board Ireland. I greatly appreciate their support of this work.

I am particularly grateful to the parents and children who so altruistically participated in this research. I so often had to ask people at the most difficult time of their lives to turn their attention to research for the benefit of others. I am in awe of their generosity and bravery. I would also like to extend a special thank you to all the staff in Cork University Maternity Hospital working tirelessly in the delivery suite and neonatal unit to care for all the newborns and who supported this research at every step.

Finally I would like to thank my friends and family, particularly my parents, Patricia and Eamonn, for their never-ending support in every aspect of this process. And to my best friend and partner, Aidan, your patience, support and love has kept me going at every turn and always made me believe I could do this. I wouldn’t be here without you.

# List of Figures

# List of Tables

# List of Abbreviations

AAP = American academy of paediatrics

ACVR2B = activin-A receptor type IIb

ADC = apparent diffusion coefficient

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANOVA = analysis of variance

ASD = autistic spectrum disorder

ASQ = ages and stages questionnaire

ATNAT = Amiel-Tison neurological assessment at term

ATP = adenosine tri-phosphate

BD = base deficit

BGT = basal-ganglia thalami

BSID = Bayley scales of infant and toddler development

$Ca^{2+}$ = calcium

CANTAB = Cambridge neuropsychological testing automated battery

CAT/CLAMS = clinical adaptive test/clinical linguistic and auditory milestone scale

CDI = child development inventory

CDQ = cognitive development questionnaire

CI = confidence interval

CNS = central nervous system

CP = cerebral palsy

CPR = cardiopulmonary resuscitation

CSF = cerebrospinal fluid

CTG = cardiotocograph

DNA = deoxyribonucleic acid

EEG = electroencephalopraph

- aEEG = amplitude integrated EEG
- cEEG = continuous EEG

EFM = electronic Foetal monitoring

ELISA = enzyme-linked immunosorbent assay

GDD = global developmental delay

GFAP = glial fibrillary acid protein

HIE = hypoxic-ischaemic encephalopathy

HRV= heart rate variability

ID = identification

IDI = infant development inventory

IGFR = insulin growth factor receptor

IL = interleukin

IPPV = intermittent positive pressure ventilation

IQ = intelligence quotient

IQR = interquartile range

$K^+$ = potassium

MDI = mental developmentindex

MHC = major histocompatibility complex

MRI = magnetic resonance imaging

fMRI = functional MRI

MRS = magnetic resonance spectroscopy

MS = mass spectrometry

MSEL = Mullen scales of early learning

$Na^+$ = sodium

NICU = neonatal intensive care unit

NE = neonatal encephalopathy

NMDA = N-methyl-D-aspartate

NPV = negative predictive value

OR = odds ratio

PA = perinatal asphyxia

PARCA = parent report of children's abilities

PCR = polymerase chain reaction

qRT-PCR = quantitative reverse transcription PCR

PKU = phenylketonuria

PLIC= posterior limb of internal capsule

PPV = positive predictive value

QTL = quantitative trait loci

RNA = ribonucleic acid

miRNA = micro RNA

nRNA = messenger RNA

ROC = receiver operator characteristic (curve)

AUROC/AUC = area under the ROC curve

RR = risk ratio

SD = standard deviation

SOP = standardised operating procedure

SVD = spontaneous vaginal delivery

TBI = traumatic brain injury

TGF = transforming growth factor

TH = therapeutic hypothermia

TNF = tumour necrosis factor

UCB = umbilical cord blood

UCHL-1 = ubiquitin carboxy-terminal hydrolase L1

ZPD = zone of proximal development

$^{1}$H-NMR = proton nuclear magnetic resonance

# Publications and Presentations

## Publications

- *Cord blood IL-16 is associated with 3-year neurodevelopmental outcomes in perinatal asphyxia and hypoxic-ischaemic encephalopathy.*
  Ahearne C, Chang R, Walsh B, Boylan G, Murray D. Developmental Neuroscience. 2017 (In Press)
- *Early Cord Metabolite Index and Outcome in Perinatal Asphyxia and Hypoxic-Ischaemic Encephalopathy.*
  Ahearne CE, Denihan NM, Walsh BH, Reinke SN, Kenny LC, Boylan GB, Broadhurst DI, Murray DM. Neonatology. 2016 Aug 3; 1110 (4): 296-302.
- *Touch-screen Technology Usage in Toddlers.*
  Ahearne C, Dilworth S, Rollings R, Livingstone V, Murray D. Arch Dis Child 2016; 101:181-183.
- *Short and Long Term Prognosis in perinatal asphyxia: An update.*
  Ahearne C, Boylan G, Murray D. World J Clin Pediatr 2016 February 8; 5 (1): 67-74
- *Downstream mRNA target analysis in neonatal hypoxic-ischaemic encephalopathy identifies novel marker of severe injury: a proof of concept paper.*
  Looney AM, Ahearne C, Hallberg B, Boylan G, Murray D. Molecular Neurobiology. 2016 Dec 12 (Epub ahead of print)
- *Glial Fibrillary Acidic Protein is not an early marker of injury in perinatal asphyxia and hypoxic ischaemic encephalopathy.*
  Looney A-M, Ahearne C, Boylan GB, Murray DM. Frontiers in Neurology. 2015; Dec 21;6:624.
- *Early EEG findings in tuberous sclerosis complex presenting with apneic seizures soon after birth.*

Kharoshankaya L, Murray DM, Bogue C, Ahearne C, Murphy BP, Boylan GB. Clin Neurophysiol. 2016 Aug 4;127(10):3265-3267.

- *Seizure burden and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy.*
  Kharoshankaya L, Stevenson NJ, Livingstone V, Murray DM, Murphy BP, Ahearne CE, Boylan GB. Developmental Medicine and Child Neurology. 2016 Dec;58(12):1242-1248.

## Presentations

- 10th Hershey Conference on Developmental Brain Injury, Equevilly, France, June 8th-11th 2016. *Cord Blood IL-16 predicts neurodevelopmental outcome at 3 years in perinatal asphyxia and HIE.* Ahearne C, Chang R, Walsh B, Boylan G, Murray D (Karger Young Investigators Travel Award Winner)
- 1st Congress of joint European Neonatal Societies (jENS), 56th ESPR/ESN Annual Meeting, 5th International Congress of UENPS. Budapest, September 16th-20th 2015 *Bayley Scales of Infant and Toddler Development (Edition 3) in a low risk healthy population* Ahearne CE, Hannon G, Kiely M, Kenny LK, J O'B Hourihane, Murray DM (2nd Prize Young Investigators Award Winner)
- Inaugural INFANT research day, Cork, June 16th 2015 *Bayley Scales of Infant and Toddler Development: How does the 2yr old Irish population compare?* Ahearne CE, Hannon G, Kiely M, Kenny LK, J O'B Hourihane, Murray DM
- Irish Paediatric Association, Clarion Hotel, Cork, September 26th-27th 2014 *Touch-screen technology usage in toddlers – is there an app for that?* Ahearne C, Dilworth S, Rollings R, Murray S, Murray DM
- Irish Perinatal Society Annual Meeting, Davenport Hotel, D2, June 19th-20 2014 *1H-NMR measurement of cord glycerol succinate predicts severe encephalopathy and death in neonatal hypoxic-ischaemic encephalopathy.* C Ahearne, NM Denihan, BH Walsh, SN Reinke, BD Sykes, LC Kenny, DI Broadhurst, GB Boylan, DM Murray

## Poster Symposium

- Pediatric Academic Societies Annual Meeting, San Diego, April 25th-28th 2015. *1H-NMR Measurement of cord glycerol succinate and ketones predicts severe encephalopathy and death in neonatal hypoxic-ischaemic encephalopathy.* Ahearne CE, Denihan NM , Walsh BH, Reinke SN, Sykes BD, Kenny LC, Broadhurst DI, Boylan GB, Murray DM
- European Academy of Paediatric Societies, Barcelona, October 17th-21st 2014. *1H-NMR Measurement of Cord Glycerol Succinate Predicts Severe Encephalopathy and Death in Neonatal Hypoxic-Ischaemic Encephalopathy* Ahearne CE, Denihan NM , Walsh BH, Reinke SN, Sykes BD, Kenny LC, Broadhurst DI, Boylan GB, Murray DM

## Poster Presentation

- The 9th International Conference Brain Monitoring and Neuroprotection, Fota, Cork, Oct 1st-3rd 2015 *Early continuous multichannel EEG in a case of molybdenum cofactor deficiency* Power C, Ahearne C, McSweeney N, Murphy B, Boylan G
- The 9th International Conference Brain Monitoring and Neuroprotection, Fota, Cork, Oct 1st-3rd 2015 *Examining the validity of a digitalized assessment of executive functioning for toddlers: the babyscreen app* Wrigley C, Ahearne C, Murphy R, Murray D
- The 9th International Conference Brain Monitoring and Neuroprotection, Fota, Cork, Oct 1st-3rd 2015 *Bayley Scales of Infant and Toddler Development (Editon 3): How does the 2 year-old Irish population compare?* Ahearne C, Hannon G, Kiely M, Kenny L, Hourihane J, Murray D

- The 9th International Conference Brain Monitoring and Neuroprotection, Fota, Cork, Oct 1st-3rd 2015 *High electrographic seizure burden in neonatal hypoxic-ischemic encephalopathy is associated with abnormal outcome at 23-48 months.* Kharoshankaya L Stevenson N, Livingstone V, Murray D, Ahearne C, Murphy B, Boylan G
- The 9th International Conference Brain Monitoring and Neuroprotection, Fota, Cork, Oct 1st-3rd 2015 *Heart rate variability in infants with hypoxic-ischemic encephalopathy: correlation with 2-year neurodevelopmental outcome* Goulding R, Stevenson N, Ahearne C, Filan P, Boylan G
- Inaugural INFANT research day, Cork, June 16th 2015 *Examining the validity of a digitalized assessment of executive functioning for toddlers: the babyscreen app* Wrigley C, Ahearne C, Murphy R, Murray D (poster prize winner)
- Irish Paediatric Association Annual Meeting, Limerick, May 8th-9th 2015 *Bayley Scales of Infant and Toddler Development (Editon 3): How does the 2 year-old Irish population compare?* Ahearne C, Hannon G, Kiely M, Kenny L, Hourihane J, Murray D (poster prize winner)
- Pediatric Academic Societies Annual Meeting, San Diego, April 25-28th 2015. *Touch-screen technology usage in toddlers: is there an app for that?* C Ahearne, S Dilworth, R Rollings, DM Murray
- European Academy of Paediatric Societies, Barcelona, October 17th-21st 2014. *Heart rate variability in full-term neonates with hypoxic ischaemic encephalopathy.* Goulding RM, Ahearne CE, Stevenson NJ, Murray DM, Boylan GB.

# Abstract

## Introduction

Approximately 10 million or 8% of all babies will require resuscitation at birth each year. Perinatal asphyxia remains a significant cause of death and disability worldwide. Since the advent of therapeutic hypothermia, outcomes have improved for eligible infants. However, currently available tools are limited in their ability to predict neurodevelopmental outcome in the window required for initiation of cooling. Cooling itself alters the predictive value of some of our more reliable prognostic tools such as clinical assessment and electroencephalography (EEG). Robust prognostic markers available shortly after birth are urgently required to inform clinical care planning for affected infants.

Outside of the neonatal period, appropriate assessment of neurodevelopment in early childhood is important for the identification of those with deficits, to allow intervention and arrange support. While motor and language deficits are often revealed in the preschool period, cognitive difficulties can go undetected until later childhood. Conversely due to the demand on motor and language abilities often required during assessments of cognitive abilities, children with delays in these domains do not achieve scores that accurately reflect their cognition. An evidence-based understanding of cognitive development in children is still lacking. This is especially true where a perinatal injury has altered a child's developmental trajectory.

With this in mind, the aim of this thesis was to improve our ability to predict and measure neurodevelopmental outcome with particular reference to high-risk infants with perinatal asphyxia. This is achieved by testing the ability of promising novel

biomarkers, measured in umbilical cord blood, to predict neurodevelopmental outcome in early childhood. Furthermore, currently used measures of neurodevelopmental outcome in early childhood are examined for their utility and a novel touch-screen measure of cognitive development is proposed.

## Methodology

Four distinct cohorts were utilised in the course of this thesis. The initial aim of this thesis was completed with a carefully defined cohort of children with perinatal asphyxia and hypoxic-ischaemic encephalopathy (HIE) recruited from September 2009 to June 2011. The umbilical cord blood of these infants was collected at birth and was subsequently analysed using untargeted or semi-targeted techniques from proteomics, metabolomics and transcriptomics. Those markers identified as promising for prediction of HIE severity, as defined by 24 hour clinical assessment and EEG, were included for analysis of ability to predict childhood neurodevelopmental outcome. Outcome was measured based on performance in the Bayley Scales of Infant and Toddler Development (Edition 3) at three years. Biomarkers were compared to currently available biochemical and clinical markers.

A retrospective cohort was analysed to examine the performance of a low-risk local cohort on the Bayley-3 at two years and this was compared to available standardised scores.

As part of the development of a novel cognitive assessment tool, an initial survey of touch-screen usage in toddlers was carried out. A parental questionnaire was administered to an opportunistic sample over a five month period, May to September 2014, in in-patient and out-patient settings. Parents

were asked to report prevalence and quality of touch-screen usage in their toddlers.

The subsequent phase of the tool development was pilot testing of the "Babyscreen App". This was performed on a similarly defined cohort of infants with perinatal asphyxia as described above, as well as a contemporary control group, prospectively recruited from March 2013 to June 2015. The Babyscreen App was administered alongside the Bayley-3 and other parental reports of development and behaviour at age 18 months to 2 years.

## Results

IL-6 and IL-16 were previously identified as promising inflammatory proteins for prediction of HIE severity. 33 infants had early cord blood measurements of these proteins and neurodevelopmental outcome at three years. IL-16 was able to predict a severe outcome with an area under the ROC curve of 0.83 ($p$=0.01). Levels ≥ 514 pg/mL predicted a severe outcome with a sensitivity of 83% and a specificity of 81%. IL-6 was not found to predict neurodevelopmental outcome at three years.

Glial fibrillary acidic protein (GFAP) has been identified as a potential biomarker for HIE in post-natal samples. 169 infants (83 controls, 56 perinatal asphyxia, 30 HIE) had GFAP measured in umbilical cord blood at birth. In our cohort, GFAP was not found to be elevated in infants with HIE ($p$=0.57), nor was it correlated with neurodevelopmental outcome ($p$=0.92).

Human microRNA, miR-374a, has been found to be down-regulated in infants with HIE. Downstream factors of this microRNA were investigated for ability to predict HIE severity and outcome. 177 infants (88 controls, 56 perinatal asphyxia, 28 HIE) were included in analysis. ACVR2B was discovered to be

elevated in severe HIE compared to other groups ($p$<0.05). While there was a trend to increased ACVR2B in infants with an abnormal outcome, numbers with such an outcome were too low to determine significance.

Looking at promising metabolites for the prediction of outcome in HIE, two models were previously identified. The first was derived from combined direct infusion and liquid chromatography mass spectrometry based on alterations of decenoyl-L-carnitine, 3,5-tetradecadiencarnitine, PC ae C38:0, phenylalanine and proline (model A), and the second was derived from $^{1}$H-NMR spectroscopy based alterations of succinate, glycerol, 3-hydroxybutyrate and O-phosphocholine (model B). Model A measurements and neurodevelopmental outcome were available in 36 infants. This model could predict abnormal outcome with an area under ROC curve of 0.77, $p$<0.01. Thirty-one infants had both metabolomic analysis under Model B and neurodevelopmental outcome at 36-42 months. The metabolite score was robust to predict both severe outcome (area under ROC curve of 0.92, $p$<0.01) and intact survival (0.80, $p$=0.01).

In the retrospective analysis of a low-risk cohort of two-year old children for performance on the Bayley-3, 240 children were identified for inclusion. Language and fine motor scores were significantly higher compared to U.S standardised norms, 109 ± 13 v. 100 ± 15, $p$<0.001, and 11.5 ± 2 v. 10 ± 3, $p$<0.001 respectively.

For the examination of touch-screen usage in toddlers, 82 questionnaires were completed by parents of typically developing children aged 12 to 36 months. 71% of toddlers included had access to touch-screen devices for a median (IQR) of 15 (9-26) minutes per day. By 24 months the majority of

children were able to swipe, unlock and actively look for touch-screen features.

In testing a novel touch-screen based cognitive assessment, 95 children underwent administration of the Babyscreen App and the Bayley-3. Significant medium sized correlations occurred between various measures of app performance (including total tasks completed, average speed, accuracy and efficiency) and cognitive composite scores on the Bayley-3. Combined measures of overall app performance could predict cognitive scores less than 90 (1SD below the mean of our cohort) with an area under the ROC curve of 0.69 (0.55-0.83), $p$=0.02.

Previous touchscreen experience affected accuracy and efficiency but not other measures of app performance. Pre-existing behaviour traits as measured by parental report on the Child Behavioural Checklist were not associated with app performance. Correlations of app performance measures with motor and language composite scores were less than correlations with cognitive scores thereby suggesting reduced reliance on these other domains with use of the app.

## Conclusion

This thesis has shown that novel biomarkers measured in umbilical cord blood at birth can predict neurodevelopmental outcome. These promising proteins and metabolites can outperform currently available biochemical and clinical diagnostic and prognostic tools of HIE. I have also shown that a widely used neurodevelopmental assessment of early childhood has certain limitations for research and clinical utility including that of regional variation in scores. Lastly, this thesis has proposed a novel cognitive assessment using a touch screen platform. This is based on the prevalence and ability of children

to functionally use touch-screen devices as demonstrated in the survey of touch-screen usage in toddlers. Pilot testing of this novel cognitive assessment demonstrated concurrent validity against cognitive scores on the Bayley-3. Future work will encompass validation of these novel predictive biomarkers as well as testing of construct validity and predictive validity of our novel touch-screen cognitive assessment.

## Aims of Thesis

This thesis has three main aims:

1. To assess the ability of umbilical cord blood biomarkers to predict neurodevelopmental outcome following perinatal asphyxia
2. To critically evaluate the current methods available for outcome assessment in early childhood
3. To develop a novel tool for cognitive assessment of preschool age children

Hypoxic-ischaemic encephalopathy (HIE) has long been identified as a risk factor for abnormal neurodevelopment in children. However, our ability to prognosticate in the neonatal period has lacked sensitivity and specificity. As our understanding of the pathophysiological mechanisms of perinatal injury increase, so does our appreciation of the scope of developmental implications that it can have. This is especially true for deficits in cognitive skills which often don't become evident until later childhood when they have already begun to impact academic progress. Assessing neurodevelopmental outcome in early childhood is extremely challenging. Specifically, there is a paucity of tools available for assessing cognitive ability in the preschool years. Those that are available rely heavily on motor and verbal skills in order to complete the test. Deficits in these other domains can co-exist in children with HIE. This has hindered the assessment of specific cognitive abilities in these children.

Therefore, for neonatologists, there is a need for better tools to more accurately discuss longer-term prognosis with parents in the neonatal period. And, for paediatricians, there is a need for greater scrutiny of the current available methods of neurodevelopmental assessment to ensure we are appropriately assessing these high-risk children. There is a pressing need for a

cognitive assessment tool suitable for preschool children that will help identify those in need of intervention and educational supports.

The three aims of this thesis will be investigated as follows:

1. To assess the ability of umbilical cord blood biomarkers to predict neurodevelopmental outcome following perinatal asphyxia
    - Promising biomarkers measured in umbilical cord blood at birth will be tested for ability to predict neurodevelopmental outcome at 3 years in a cohort of infants with perinatal asphyxia and HIE
    - Previously identified markers from across functional biological levels; proteomics, metabolomics and transciptomics, will be examined
2. To critically evaluate the current methods available for outcome assessment in early childhood
    - The Bayley Scales of Infant and Toddler Development (Edition 3) will be examined in a retrospective cohort of low-risk Irish 2-year olds and performance compared to U.S and U.K standardised norms
3. To develop a novel tool for cognitive assessment of preschool age children
    - A touch-screen format will be proposed as a platform for cognitive assessment of toddlers
    - A survey of touch-screen usage and skills required for interaction in a contemporary Irish toddler cohort will be presented
    - Prototype development and testing will be described
    - The novel application will be pilot tested in a cohort of low-risk toddlers and performance compared to scores on the cognitive subscale of the Bayley-3

- A scoring system for the application will be described and the ability of this scoring system to predict abnormal cognitive performance will be tested
- The application will also be tested in a subgroup of infants with a diagnosis of HIE

## Scope of Thesis

Despite ongoing improvements in obstetric and neonatal care, perinatal asphyxia still accounts for a significant burden of neonatal morbidity and mortality worldwide (1, 2). The incidence of neonatal encephalopathy is 1.6 per 1000 births in countries with low neonatal mortality rates, rising to 12.1 per 1000 births in countries with higher mortality rates (1). Prior to the advent of therapeutic hypothermia for the treatment of hypoxic-ischaemic encephalopathy, nearly 40% of survivors would be left with some neurodevelopmental impairment (1). Moderate cooling in infants with moderate and severe encephalopathy has improved rates of survival with normal IQ at 6-7 years (RR=1.31) but concerns remain about the high incidence of cognitive deficits detected in later childhood (3-5).

Predicting and measuring neurodevelopmental outcome in children with perinatal asphyxia has been fraught with difficulties. The introduction of therapeutic hypothermia has created additional challenges in the prediction of childhood outcome in hypoxic ischaemic encephalopathy (HIE) from early markers, such as clinical examination and electroencephalography (EEG) (6, 7). Novel indicators of injury severity available in the perinatal period are increasingly under investigation and may have value in the prediction of later outcome in perinatal asphyxia. The first aim of this thesis is to assess the ability of promising umbilical cord blood biomarkers to predict childhood neurodevelopmental outcome (Chapters two and three).

As we continue to study the efficacy of emerging perinatal blood based and physiological biomarkers, as well as the effects of new treatments, we need to pay close attention to how neurodevelopmental outcome is measured. Various measures of short and longer term outcome are available but unfortunately no

perfect test exists (8). All current assessment techniques have inherent limitations. One of the most common standardised neurodevelopmental assessments used in clinical practice and in research is the Bayley Scales of Infant and Toddler Development. In Chapter four I will explore some of the salient points regarding the use of this assessment as an outcome measure. This will address aim two of this thesis. In particular I will look at how a two-year old Irish population of low-risk children perform using this measure.

There is increasing focus on the need for useful early measures of developmental function. Factors such as growing difficulty in obtaining research funding which allows long term follow-up to occur within the funding term, as well as the desire for early identification of developmental problems to allow intervention prior to school attendance, have led researchers to search for improved methods of assessment. Motor and language difficulties are identifiable in the infant and early childhood period respectively, due to the high demands on these abilities in these years. Meanwhile the cognitive impacts of an injury or intervention are often not detected until middle or late childhood when the demands of schooling reveal difficulties.

The more subtle neurocognitive impacts of perinatal hypoxia-ischaemia are increasingly being recognised (4, 9, 10). Recently, we have seen significant progress in the understanding of normal cognitive development in children. The process by which a perinatal injury affects this development remains to be elucidated. However, this research has been somewhat limited by the lack of early tests for some of the key cognitive abilities such as working memory, attention and processing speed. These abilities contribute to the child's ability to deal with a new learning challenge. An early assessment of cognitive abilities would therefore optimise follow-up in clinical practice and research as well as contribute to greater understanding of the

impact of hypoxic-ischaemic injury on childhood cognitive development. In Chapters five and six I will outline the development of a novel tool for this purpose completing the final aim of this thesis.

# 1.0 Introduction

*Publications arising from this chapter:*

*"Short and Long Term Prognosis in perinatal asphyxia: An update"*

*Ahearne C, Boylan G, Murray D.*

*World Journal of Clinical Pediatrics 2016 February 8; 5 (1): 67-74*

Perinatal brain injury caused by hypoxic-ischaemic injury remains a major cause of neonatal death and disability globally. Asphyxia accounts for 23% of neonatal deaths worldwide and represents a major area of focus in the Millennium Development Goals fight to reduce neonatal mortality (2). The advent of therapeutic hypothermia as a therapy for infants with moderate and severe encephalopathy has had a major impact on childhood outcomes following HIE (3, 11, 12). While the benefits of this intervention have primarily been felt in the developed world, attempts have been made to introduce cooling safely into low resource settings (13-16). Despite these significant advancements, many questions remain about the pathophysiology, optimal management and effects of injury in perinatal asphyxia. One of the key issues of importance to parents, clinicians and researchers is the long term prognosis of children following a hypoxic-ischaemic injury.

In this section I will give an overview of the important issues which must be addressed to deal with this question and summarise the literature to date focussing on outcome prediction and assessment in perinatal asphyxia and HIE.

## *1.1 Perinatal asphyxia and the pathophysiology of injury*

Perinatal asphyxia describes the interruption of blood flow or gas exchange to and from the fetus in the perinatal period (17). This may be due to prolonged partial asphyxia, sudden sub-total asphyxia, secondary to a sentinel event, or a combination of both (18). Hypoxic-ischaemic injury to the brain and vital organs may result if the perinatal asphyxia is of a sufficient degree, or prolonged, beyond the ability of the fetus to compensate (19-21).

Approximately 20 per 1000 deliveries will require significant resuscitation, with biochemical and clinical evidence of perinatal asphyxia (22). In countries with low neonatal mortality rates, 1.6 per 1000 of these infants will go on to develop signs of evolving encephalopathy consistent with hypoxic-ischaemic encephalopathy (HIE) though there is still a substantial burden of death and disability as a result (1). *(Figure 1.1.)*

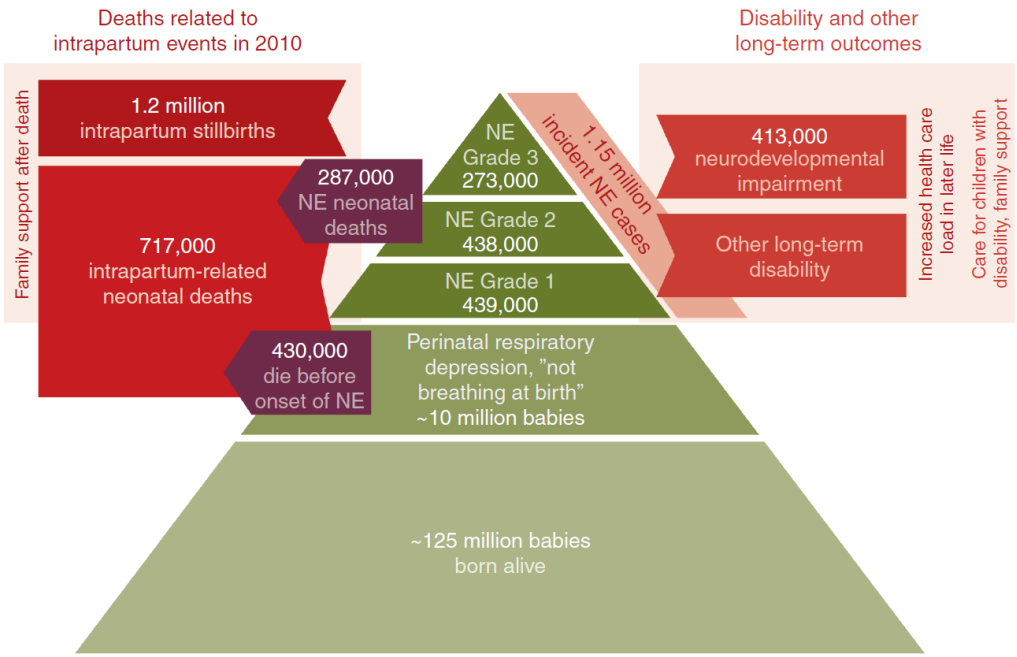



*Figure 1.1. Summary of deaths and disability outcomes that are intrapartum related based on 125 million births worldwide in 2010.*

Reproduced from Lee A et al., 2013 (1).

Approximately 80% of brain injury following neonatal encephalopathy and evidence of perinatal asphyxia is directly attributable to an acute phenomenon (23). The most common mechanism of injury is asphyxia due to circulatory compromise (18). Some interruption of placental or umbilical integrity e.g. abruption, cord accident, or clotting of placental arteries leads to diminished exchange of oxygen and carbon dioxide and disruption of glucose supply. This initial insult triggers a cascade of cellular events starting with the rapid depletion of ATP and leading to either cell death, by necrosis or apoptosis, or to survival. The complex interplay of energy failure, excitotoxicity, oxidative stress and inflammation has not been fully elucidated but below I will give an overview of the generally accepted mechanisms as we understand them.

The immature brain can withstand longer periods of energy deprivation than the adult brain. Despite this, it is vulnerable, once that critical threshold is reached, due to its developing state. The excitatory state required for development of synaptic connections and brain plasticity causes it to be more at risk of excitotoxic damage (18).

It is known that while some neuronal cell damage occurs during the initial insult or “primary” phase of injury, this is followed by a period of at least partial recovery. A “latent” phase then occurs during which functional activity in the brain remains suppressed but energy supply has been somewhat restored. A more severe injury will later trigger a secondary phase of deterioration (24, 25). *(Figure 1.2.)*

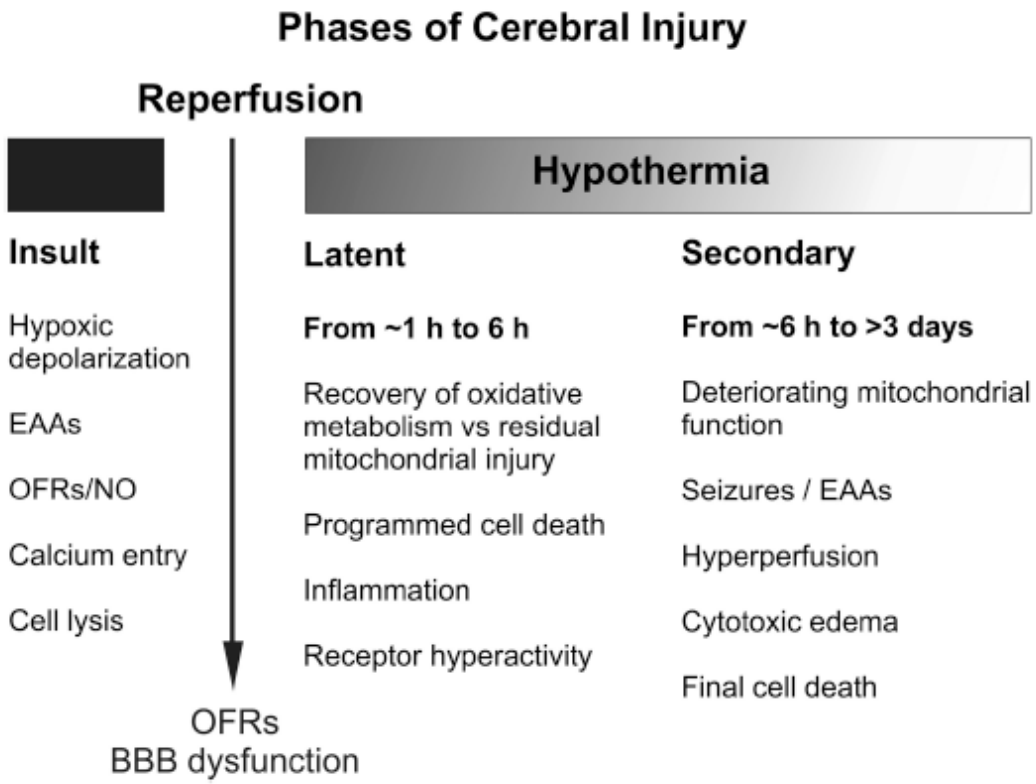



*Figure 1.2. Diagram illustrating pathological phases of cerebral injury after severe hypoxia-ischaemia.*

OFRs, oxygen free radicals; BBB, blood brain barrier; EAAs, excitatory amino acids; NO, nitric oxide. Reproduced from Wassink et al. 2014 (25)

Damage therefore evolves over hours to days after the initial insult. The mechanisms of injury will be further described in subsequent sections. It is this “latent” phase prior to secondary injury that creates a window for intervention in the time after the infant’s birth. This window provides the rationale for postnatal treatments such as therapeutic hypothermia.

The alterations to the cellular metabolic processes initiated by hypoxic-ischemic injury can occur with or without overall disruption of brain function. Those of a severity to cause dysfunction, which will then manifest as clinical alterations to

the neonates' neurological examination, become the clinical diagnosis of hypoxic-ischaemic encephalopathy (HIE). *(Figure 1.3.)*

HIE must be differentiated from other causes of neonatal encephalopathy, such as sepsis, meningitis or metabolic encephalopathy (17, 26, 27).

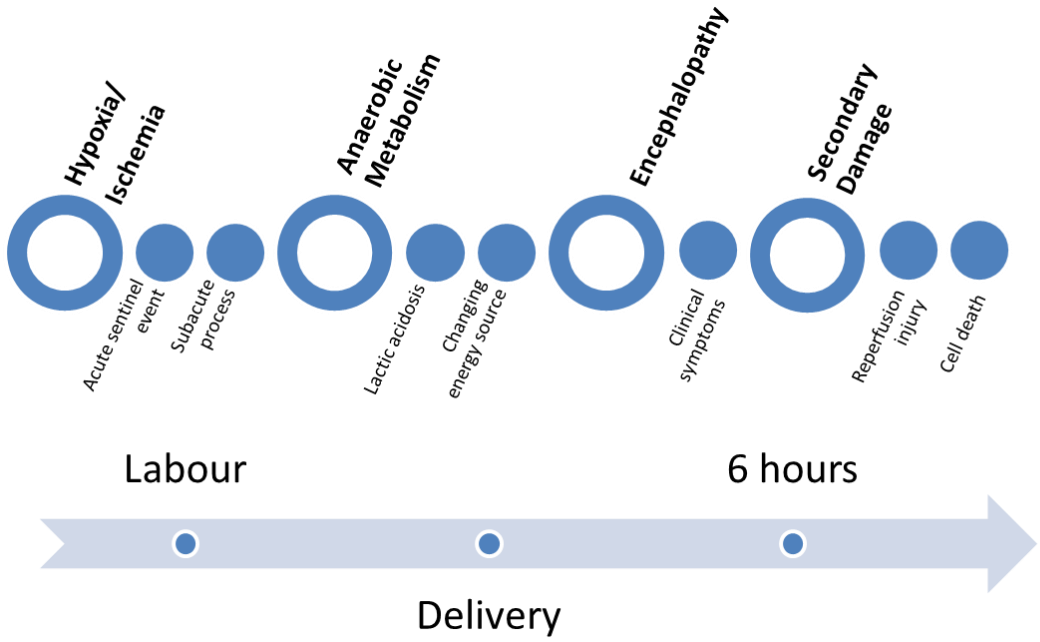



*Figure 1.3. Timeline of injury in hypoxic-ischaemic encephalopathy*

Original diagram.

### 1.1.1 Biochemical and Physiological mechanisms

Altered biochemical and physiological processes following hypoxic-ischaemic injury underpin the structural and clinical manifestations of HIE. Oxygen deprivation to the brain in the perinatal period can occur via two mechanisms, hypoxemia (with reduced oxygen content in the blood circulating to the brain), and/or ischaemia (with decreased quantity of the circulating blood required to delivery oxygen) (22). It is this loss of oxygen

supply along with the associated diminished glucose supply that triggers the consequent neurological injury (17). The hypoxic-ischemic insult causes a degree of immediate neuronal loss, and a delayed secondary impairment of energy metabolism and reperfusion injury (24, 25). The earliest changes are a decrease in brain glycogen, an elevation in lactate and a decrease in phosphocreatine (PCr), the principle storage form of high-energy phosphate in the brain (28, 29). This occurs as a result of the switch to an anaerobic state in an attempt to generate ATP. This appears to protect grey matter initially, but the process then becomes deleterious (17). Anaerobic glycolysis is an inefficient mechanism of ATP production and glucose demands quickly exceed supplies. Blood glucose becomes distinctly less indicative of brain glucose in this state (30). Ischaemic effects, through circulatory disruption, augment lactate accumulation and tissue acidosis over and above that of isolated hypoxia due to reductions in lactate clearance and buffering (31). ATP-dependent pump failure, particularly of the sodium/potassium ($Na^+/K^+$) pump which functions to maintain cellular homeostasis, leads to intracellular accumulation of $Na^+$, the osmotic passage of water and subsequent cytotoxic oedema and potentially cell death (32). *(Figure 1.4.)*

Following termination of the primary insult, restoration of blood flow, and hence oxygen, to damaged tissues occurs. During this reperfusion period pH normalises and aerobic metabolism is re-established (33). There is known to be a period of recovery with high-energy phosphate levels returning to baseline within about two to three hours (29, 34). This is followed by a secondary phase of energy failure. There is evidence however that loss of neuronal proteins precedes secondary energy failure in a rat model of hypoxic-ischaemic injury (35). This has been taken to suggest that secondary energy depletion is a consequence rather than a cause of cell damage (17).

Much of what we know about the pathophysiology of HIE is derived from animal studies of hypoxic-ischaemic models, such as the unilateral carotid ligation in the seven day old rat pup of the Vannucci model, rather than models of asphyxia where respiratory or placental gas exchange is disrupted (36). Where applied, these asphyxia models have shown the additional effects of hypercapnia on brain injury. Some of these effects include a beneficial influence on cerebral blood flow maintenance and oxygen dissociation profiles, but also a potentially damaging effect of worsening intracellular acidosis and hence tissue injury (31, 37, 38).

Energy failure is not the only mechanism of neuronal injury in HIE. In the immediate aftermath of the initial insult various mechanisms contribute to the pathway to cell death. These include energy depletion, accumulation of extracellular excitatory amino acids (particularly glutamate), increase in cytosolic calcium ($Ca^{2+}$), and generation of free radicals (17).

Neuronal cell death can follow a hypoxic-ischaemic insult through a necrotic or apoptotic pathway. Necrotic death typically occurs after intense, often relatively brief, insults, whereas apoptotic death typically occurs after less intense longer-acting insults (39-41). The mechanisms governing these processes are intricate and not fully understood. Factors other than the insult characteristics, such as developmental state of the cell, may affect the cell's fate. Apoptotic and necrotic states appear to be part of a continuum of cell death endpoints with hybrid cells between these extremes also evident. (17, 42)

Increased extracellular glutamate results from excessive pre-synaptic glutamate release and failure of glutamate re-uptake mechanisms (18). These mechanisms are reliant on the ATP-dependent re-uptake which has failed following the hypoxic-ischaemic insult and hypoxia-induced membrane depolarisation

*(Figure 1.4.)*(17). The excitotoxic effect of this accumulated glutamate is to contribute to acute cell swelling by activating receptor mediated $Na^+/K^+$ channels and to aggravate intracellular $Ca^{2+}$ accumulation. An early over-expression of glutamate receptors in the human hippocampus, cerebral cortex, basal ganglia and thalami accounts for their particular vulnerability in hypoxic-ischaemic injury (43).

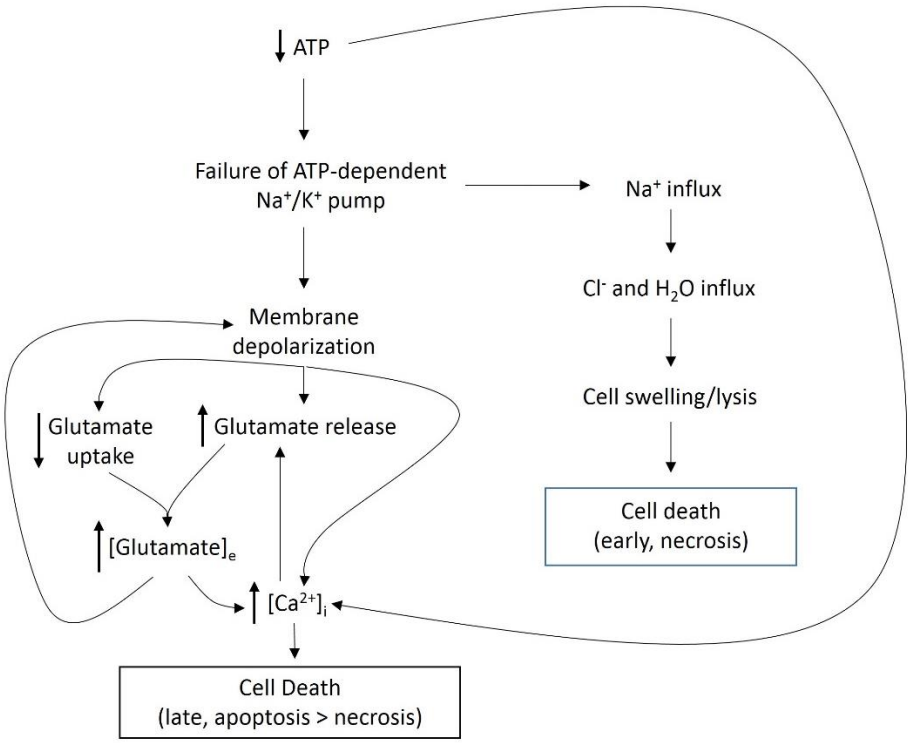



*Figure 1.4. Relation between energy depletion and cell death*

Adapted from Volpe 5th edition, 2008 (17).

Increased intracellular $Ca^{2+}$ can be attributed to failure of $Ca^{2+}$-pumping mechanisms and activation of NMDA and AMPA types of glutamate receptors (18, 32, 44). Intracellular calcium triggers multiple enzymatic processes responsible for the destruction of the cellular membrane, cytoskeleton and nucleus.

It also causes further neurotransmitter release through receptor activation, contributes to the generation of free radicals and aggravates ATP deficiency.

More severe brain injury has been associated with higher levels of free radical production (45). The mitochondrial electron transfer chain is one of the main sources of free radicals in hypoxia-ischaemia (46). Oxygen deprivation prevents the complete passage of electrons to cytochrome c oxidase and leads to the accumulation of the superoxide anion (47). Increased intra-cytosolic calcium leads to free radicals through four mechanisms; the action of cyclooxygenase and lipoxygenase on arachidonic acid due to the activation of phospholipase A2, the action of xanthine oxidase on hypoxanthine and xanthine, the auto-oxidation of catecholamines and the action of nitric oxide synthase leading to peroxinitrite formation (32). There is also evidence that early reactive microglia at injury sites are potent sources of free radicals (17, 48). Free radicals are responsible for peroxidation of polyunsaturated fatty acids of membrane phospholipids, damage to DNA and proteins containing unsaturated or sulfhydryl groups and activation of pro-apoptotic genes (25). The immature brain is particularly at risk from oxidative stress due to deficient antioxidant mechanisms and pro-oxidant characteristics such as high levels of polyunsaturated fatty acids in neuronal membranes and a relatively high concentration of non-protein-bound iron (49-51).

Inflammatory mechanisms also appear to play a role in the final common biochemical pathway to neuronal death (52). There is activation of microglia in the first hours after a hypoxic-ischaemic insult with resultant increase in neurotoxic substances such as excitatory amino acids, free radicals and cytokines (48). Microglial activation is followed by neutrophil accumulation in brain blood vessels (53). Neutrophils may cause injury by adhering to endothelium and obstructing blood flow, or by

releasing reactive oxygen species mediating vascular injury, but this process has yet to be fully elucidated (17). Inflammatory mechanisms provoked by prior exposure to molecular products of infection (e.g. lipopolysaccharide), seem to potentiate the effect of hypoxic-ischaemic insults leading to more severe brain injury (17, 54-57).

Some of the biochemical effects of hypoxia and ischaemia i.e. ionic pump failure, neurotransmitter redistribution and membrane alterations can predispose the brain to seizure activity. Seizures may in turn contribute to delayed neuronal loss (43, 58, 59).

Asphyxial injury also has a physiological impact on systemic and particularly cerebral blood flow (60, 61). The initial circulatory response to perinatal asphyxia is the redistribution of cardiac output to support cerebral perfusion with an increase in cerebral blood flow and loss of cerebral vascular autoregulation (62). More prolonged asphyxia is associated with reduced cardiac output leading to hypotension and reduced cerebral blood flow (17, 63).

In recent years, a third phase of injury, persistent following the subsidence of the secondary mechanisms of cell death, has been proposed. This ‘tertiary brain injury’ is characterised by persistent active processes that prevent regeneration of cerebral tissue or may exacerbate brain damage and continue for months or years after the triggering injury (64, 65). These mechanisms may include sensitisation to inflammation or injury, increased seizure susceptibility, impaired oligodendrocyte maturation and myelination and persistent inflammation and gliosis (66). Long after perinatal injury, neuropathological evidence can remain in the form of myelin deficits, reduced plasticity and altered cell number. By targeting the longstanding inflammation and epigenetic modifications at the heart of this tertiary brain

injury, new neuro-protective therapies may be developed with efficacy and influence beyond the current 6-hour window (64, 66).

### 1.1.2 Neuropathological Features of HIE

The predominant neuropathological injury observed in the setting of hypoxia-ischaemia is that of ‘selective neuronal necrosis’ (67). The term refers to necrosis of neurons in a characteristic, though often widespread, distribution. Three major patterns of neuronal injury are described in term infants subjected to hypoxic-ischaemic injury (17, 43). The pattern is dictated by the severity and duration of the insult. ‘Diffuse’ neuronal injury occurs following very severe and very prolonged injury. A ‘cerebral cortical-deep nuclear’ pattern is seen following moderate to severe insults of relatively long duration and a ‘deep nuclear-brain-stem’ pattern is observed in severe, relatively abrupt insults. Throughout the brain, neurons in the CA1 region of the hippocampus (Sommer’s sector), deeper layers of the cerebral cortex, putamen, thalamus and cerebellar Purkinje cells are most frequently injured by hypoxic-ischaemic insult (43, 68). Watershed regions of the cerebral cortex and areas deep in the sulci are particularly vulnerable to ischaemia (43).

The reason for selective vulnerability in neuronal groups appears to be due to several factors. Firstly, because of the loss of cerebrovascular autoregulation following injury, cerebral blood flow becomes more passively dependent on arterial blood pressure (62). Where systemic hypotension exists, cerebral ischaemia occurs particularly in watershed areas. Secondly, areas undergoing rapid neuronal differentiation at the time of the injury, such as the brain stem and hippocampus, maybe particularly susceptible to variations in perfusion due to

regional vascular supply (69, 70). Thirdly, regional metabolic factors, such as the high metabolic rate and energy use of the deep grey matter, could also predispose these areas to damage (71). Fourthly, regional variation in the distribution of glutamate receptors may render certain areas prone to injury. The basal ganglia, and the inferior olive nucleus and basis pontis of the brainstem demonstrate transient increased density of glutamate receptors in the perinatal and infant periods (17, 72).

These patterns of neuronal damage are also reflected in neuroimaging in the postnatal period. Infants with a history of an acute sentinel event are more likely to demonstrate basal ganglia-thalamic (BGT) injuries on magnetic resonance imaging (MRI). This is often accompanied by abnormalities in the appearance of the posterior limb of internal capsule (PLIC). More severe cases demonstrate brainstem changes (73). Cerebellar vulnerability is also evident in serial MRI. Le Strange et al., 2004, showed significant reduction in cerebellar growth in the first year of life in infants with severe BGT lesions (74).

## *1.2 Outcome in perinatal asphyxia*

### 1.2.1 Effects of intervention on outcome in perinatal asphyxia

Based on the substantial evidence of multiple randomized controlled trials, therapeutic hypothermia has become the standard of care for infants with moderate and severe hypoxic-ischaemic encephalopathy in the majority of neonatal units where the necessary resources are available (10-12, 104, 105). Indications for treatment vary somewhat between centres but usually involve some combination of biochemical and clinical evidence of perinatal asphyxia with overt clinical manifestations of encephalopathy often based on the recruitment criteria of the larger trials of therapeutic hypothermia (104, 105).

Unfortunately, using the current standard clinical markers outlined above, it is estimated that we currently mis-classify approximately 15-20% of infants as mild, or no encephalopathy and these infants are therefore not offered therapeutic hypothermia, worsening their long term prognosis (106). A recent study showed that a substantial proportion of those with mild encephalopathy who do not qualify for cooling go on to have brain injury on subsequent MRI (75). Hence the question of who should be cooled continues to be asked (76).

In the post-cooling era we have therefore been left with a heterogenous group of treated and untreated infants when we look across the spectrum of children affected by hypoxia-ischaemia at birth.

The majority of data concerning the diverse range of outcomes that have been observed following perinatal injury, were based on cohorts without cooling. In the subsequent sections data quoted will refer to non-cooled cohorts unless otherwise stated.

### 1.2.2 Outcome in perinatal asphyxia

The majority of infants who require significant resuscitation at birth recover quickly and have no signs of encephalopathy. These children had been felt, in general to have a normal outcome and function in line with their peers academically (5). For this reason, at present, neuro-protective intervention has been reserved for infants with moderate or severe HIE as outlined above. However, several large population based studies now suggest that the outcome in children with perinatal asphyxia without clinical encephalopathy is not completely normal. Odd et al. demonstrated an increased risk of low IQ at eight years in this group compared to a control group (77). This is concerning due to the potential risk of a huge burden of more subtle disability.

Outcomes in perinatal asphyxia are summarised in *Table 1.1*.

### 1.2.3 Short term outcome in HIE

For those infants who develop HIE, the most commonly used grading system remains the modified Sarnat score, dividing the infants into mild, moderate or severe depending on their clinical signs(78). The approximate breakdown tends to be mild (39%), moderate (39%) and severe (22%) (1). The management and outcome varies significantly with grade of HIE.

Of those with moderate HIE, approximately one third will develop clinical and electrographic seizures in the neonatal period (79). These seizures will usually commence between 18 and 20 hours following delivery and will last from minutes to hours (80). Following the cessation of seizures, encephalopathy will gradually improve to the point where oral feeding can recommence and care normalise. Both seizure burden and the time to achieve full oral feeding are useful in predicting the long term outcome of the infant (79).

The overall death rate in neonatal encephalopathy of all grades is 9.9% in developed countries but this rises acutely to 30% among those who qualify for cooling and precipitously to 76.8% when we consider severe encephalopathy alone. (1, 3).

### 1.2.4 Long term outcome in HIE

Prior to the cooling era approximately 26.4% of infants with neonatal encephalopathy survived with moderate to severe neurodevelopmental impairment and a further 14% survived with mild impairment (1). Reported rates of cerebral palsy following neonatal encephalopathy vary but are generally around 10-13% among survivors of moderate to severe encephalopathy (81, 82). The risk is increased threefold where there is a history of neonatal seizures (81). Dyskinetic CP and spastic quadriplegia are the most common subtypes with 80% of dyskinetic CP attributable to perinatal hypoxia-ischaemia at term (82). Sensory disruption is also increased following hypoxic-ischaemic injuries. Rates of hearing loss are reported to be as high as 17.1% in those with other persistent neurological deficits (83). Up to 41% of infants have an abnormality of some element of visual function in the first year of life following a diagnosis of neonatal encephalopathy, and, where this is associated with moderate to severe basal ganglia changes and severe white matter changes on MRI, this rises to 100% (84).

Therapeutic hypothermia has improved the outlook for infants with moderate to severe HIE, with increased likelihood of survival with normal IQ (RR=1.31) and improved survival without neurological abnormalities (RR=1.6) following therapeutic hypothermia at follow-up at six-seven years of life (3).

It is important to note that learning deficits may be present with or without motor or sensory dysfunction. Impairments in episodic memory associated with reduced hippocampal volume has been found in children following perinatal hypoxic-ischaemic injury but without associated neurological deficits (85). Robertson and Finer showed a reduction in school readiness scores as well as attention scores and increases in symptoms of explosiveness and irritability at five and half years in survivors of moderate encephalopathy without other disability (86). Marlow et al. also demonstrated memory and attention/executive function impairments in the severe encephalopathy group and increased special educational needs and lower achievement on national curriculum attainment scores in both moderate and severe groups at seven years (4). Odd et al. showed infants with encephalopathy had lower working memory, reading accuracy and comprehension scores and increased requirement for educational support (OR=6.24) between eight and eleven years (5). A Swedish population based study examining the long term outcome following moderate encephalopathy has shown that in late adolescence the rates of disability are even higher, with 30% having CP, and 70% of those without CP having cognitive disability which interfered with their daily life (9).

Neonatal encephalopathy has also been associated with increased behavioural difficulties. Those children with a history of moderate and severe encephalopathy have a significant increase in parent and teacher reported hyperactivity (4). There is also a reported increase in autistic spectrum disorders in these children by five years (RR=5.9) (87). Adverse perinatal events are also associated with an increased risk of psychotic symptoms including schizophrenia (88, 89)

The longer we follow these children the more evident it becomes that perinatal asphyxia and HIE have significant long term non-motor effects on these children.

| | | |
|---|---|---|
| **Short-term** | | |
| | Death | |
| | HIE | |
| | Neonatal seizures | |
| **Long-term** | | |
| | Motor | Cerebral Palsy |
| | Sensory | Hearing loss |
| | | Visual impairment |
| | Cognitive | Episodic and Working Memory |
| | | Attention |
| | Educational | Increased support requirements |
| | | Lower test scores |
| | Behavioural | Attention |
| | | Explosiveness |
| | | Irritability |
| | Neuropsychiatric | Psychotic symptoms |
| | Neurodevelopmental | Autistic Spectrum Disorders |

*Table 1.1: Outcomes in perinatal asphyxia.* A summary table of outcomes with increased incidence following hypoxic-ischaemic encephalopathy

## *1.3 Prediction of outcome in perinatal asphyxia*

While hypoxic-ischaemic mechanisms of injury can be suggested by a known perinatal insult, if identified, e.g. placental abruption or umbilical cord accident, biochemical evidence of metabolic acidosis, depressed Apgar scores, typical clinical signs and imaging findings, it can be very difficult to make this differentiation quickly after birth (90). However, as approximately 50 – 80% of neonatal encephalopathy can be attributed to hypoxia-ischaemia and given the potential benefit of treatment, our ability to identify those infants suffering from hypoxic-ischaemic injury is becoming increasingly important (17, 23, 91, 92). Current and novel markers for prediction of outcome are described below and summarised in *Table 1.2*.

### 1.3.1 Current Markers for Prediction of Outcome

The advent of therapeutic hypothermia as a neuro-protective treatment for those with moderate and severe encephalopathy has improved prognosis (3, 11). Hypothermia is, however, a time sensitive intervention, with a very narrow therapeutic window and must be instigated within 6 hours or ideally sooner following delivery to be effective (93). There is a need to promptly identify those who will benefit from current and emerging neuro-protective therapies to guide appropriate application of resources and permit prognostication. So the challenge has become the identification of those infants with signs of perinatal asphyxia who will develop moderate or severe HIE in the coming hours. Currently available indicators such as blood biochemistry, clinical examination and electrophysiology have limitations and their predictive power has been affected by the interceding intervention of therapeutic hypothermia yet they still remain the basis for prognostication in the critical first postnatal hours.

#### 1.3.1.1 Foetal and Maternal Factors

Certain Foetal and maternal factors have been reported to increase risk of perinatal distress. Hayes et al. found that presence of higher grade meconium, growth restriction, large head circumference, oligohydramnios, male sex, maternal pyrexia and increased uterine activity were associated with an increased likelihood of poor Foetal tolerance of labour and subsequent development of HIE (94). Badawi et al. found that the risk of moderate or severe neonatal encephalopathy increased with maternal age but decreased with increasing parity (95). Lower socioeconomic status based on maternal occupation as well as an absence of private health insurance, a family history of seizures and neurological disease, and infertility treatment also increased odds. Antenatal factors increasing risk of NE included maternal thyroid disease, severe pre-eclampsia and moderate to severe bleeding (95). The same group also published on the intra-partum risk factors for moderate to severe NE, which included maternal pyrexia, persistent occipito-posterior position, induction of labour and emergency operative delivery (96).

Certain pathological placental features have also been associated with a poorer outcome following delivery. Another study by Hayes et al. found that meconium phagocytosis, haemorrhage, raised placental to birthweight ratio and/or markers of infection/inflammation were independently associated with grade of NE and children with a poorer long-term outcome, i.e. death or low score on neurodevelopmental assessments, had a higher incidence of a combination of these features (97). Mir et al. found that diffuse chronic villitis increased the odds of an abnormal neurodevelopmental outcome, despite hypothermia, by a factor of nine (98). Nasiell et al. also associated placental

abnormalities with the risk of severe encephalopathy and identified velamentous or marginal cord insertion and histological abruption as significant factors (99).

#### 1.3.1.2 Cardiotocograph

Cardiotocography is widely used for the assessment of Foetal wellbeing in labour. In Ireland, based on a survey of national use in 2002, all maternity units had access to electronic Foetal monitoring (EFM) (100). 96% performed an admission CTG on all women and 36% routinely used continuous EFM in women without risk factors during labour. The interpretation of foetal heart monitoring requires an in depth understanding of the underlying physiological mechanisms. While a normal CTG pattern is reassuring for Foetal health, many infants with normal postnatal course will have abnormal features on EFM (101) Pathological patterns of decelerations and bradycardia are poorly predictive of the infant's condition at delivery but remain an impetus for obstetric intervention (19, 102-104). Characteristics associated with an increased risk of cerebral palsy include multiple late decelerations and decreased beat-to-beat variability but the false positive rate has been reported at 99.8% (105). The addition of ST analysis as an adjunct to CTG monitoring has been shown to provide more accurate information about Foetal distress (106). However there are concerns about human error affecting effective usage (107). A large scale randomised-controlled trial of computerised CTG interpretation with intelligent decision support is underway that aims to determine the effect of this system on poor neonatal outcomes (108).

#### 1.3.1.3 Acid-base balance

A disturbance in acid-base balance is one of the earliest and easily measured objective signs of Foetal distress. The degree of acidosis is measured by scalp or cord pH, with acidosis being used to determine the need for intervention (109). A pH of <7.00 gives a 50% chance of abnormal outcome, however it's positive predictive value for significant encephalopathy is low (110). This prediction might be improved by focusing on metabolic acidosis, and in particular lactate level. However, several large trials have shown that lactate monitoring during labour does not improve our ability to detect or prevent adverse labour outcomes compared to pH monitoring alone and may in fact increase rates of instrumental deliveries unnecessarily (111, 112).

#### 1.3.1.4 Apgar score

Almost all infants are born through the eyes of Virginia Apgar, with midwives worldwide using her clinical score to describe the condition of the infant at birth and the response to resuscitation. However, Apgar scores suffer from poor sensitivity and specificity, as 80% of those with an Apgar score of ≤7 at 5 min will have a normal outcome (110). Often felt to be useful at extremes, one in five babies with an Apgar score of 0 at 10 min will survive to school age without moderate or severe disability (113). A further difficulty is the subjective nature of the Apgar score, which leads to high levels of inter-observer variability. Subjective real time clinical scores have been shown to overestimate Apgar scores by a median value of 2.4 compared to later video enhanced estimation (114). Attempts have been made to improve on the conventional Apgar score with Expanded and Combined versions that take aspects of neonatal resuscitation into account (115, 116). In particular the Combined Apgar score at five minutes of life has shown some promise in the prediction of perinatal asphyxia (sensitivity 97% and specificity 99%) and

HIE, though it cannot distinguish severity of HIE, and long term outcome data to date has been unavailable (117).

#### 1.3.1.5 Clinical examination

The neurological examination of a neonate is a clinical skill learnt through experience and exposure. Standardised scores have been developed, and widely used in an attempt to improve inter-observer reliability.(118-120) However, the examination of a sick neonate is hampered by the need for sedative medications, anti-convulsants and intubation. Previous work by our group has shown that the best prediction of outcome is achieved by the examination at discharge. Examination on the first postnatal day, even using a standardised method, has poor prediction of outcome at two years (121). More recent studies have shown that therapeutic hypothermia interferes with our ability to accurately estimate the neurological state of the infant (6). An overview of neonatal assessment methodologies is covered in Section 1.4.1.

#### 1.3.1.6 Electrophysiological monitoring

Continuous electroencephalography (cEEG) and amplitude integrated EEG (aEEG) are important tools for assessment and prognostication in HIE. Both tools have been shown to offer excellent predictive ability as early as three to six hours following delivery (122). A normal EEG soon after birth gives 100% normal outcome at two years (123). However, an abnormal EEG, or aEEG is less helpful. The PPV of a severely abnormal aEEG for death or disability at six hours is 0.63 when assessed by voltage and 0.59 when the aEEG is assessed by pattern. These values drop slightly but not significantly in cooled infants (124). A recent study showed that, in the era of cooling, persistence of abnormalities on EEG at 36 and 48 hours was

predictive of abnormal outcome (125). In experienced hands EEG can be an excellent adjunct to clinical decision making. However in cases where therapeutic hypothermia is applied, its prognostic utility is pushed to later in the neonatal course.

Due to limitations in access to resources and expertise, one or two channel aEEG is more commonly used in neonatal units than cEEG. Hence aEEG was an inclusion criteria in the TOBY Study, a major trial of therapeutic hypothermia (126). Despite its perceived ease of use, aEEG is highly user dependent and many neonatologists lack confidence in their own abilities (127-129).

There is a lack of consensus in clinical practice over the appropriate grading and classification of neonatal cEEG (130). Numerous such systems have been proposed derived from the seminal works in neonatal EEG (123, 131-133). Features of cEEG used to classify neonatal cEEG include the amplitude, frequency, symmetry, synchrony and sleep-wake cycle, as well as transients and seizures in abnormal cases (134, 135). In this thesis, cEEG was applied as described in Lloyd et al, 2015 (136). It was then graded according to the previously described Murray et al, 2009, with its established predictive validity (123).

#### 1.3.1.7 Neuroimaging

Neuroimaging and particularly magnetic resonance imaging (MRI) has come into widespread use in the developed world to aid in prognosis of cases of perinatal asphyxia. Specific patterns of injury can be associated with particular long-term neurological deficits. The BGT pattern discussed previously is associated with more severe disability due to dyskinetic cerebral palsy. While infants with a ‘watershed’ injury pattern, associated with a more prolonged partial asphyxia and often with a history of hypotension, infection and hypoglycaemia, more

often demonstrate a normal early outcome but later development can be affected by suboptimal head growth, behavioural problems, language delay, epilepsy, reduced IQ and poorer visuospatial skills.(137) Specific findings on MRI, such as abnormal signal intensity of the posterior limb of the internal capsule (PLIC), have been shown to be highly predictive of severe adverse sequelae.(73)

Several studies have shown that more subtle measurements on MRI can help predict outcome. Liauw et al 2009 found that low apparent diffusion coefficient (ADC) values in normal-appearing basal ganglia and brainstem correlated with outcome (138). Alderliesten et al 2011 found that combining MR imaging score with ADC measurements for the basal ganglia and thalami improved prediction for abnormal outcome compared to MR imaging alone (139). A later paper by the same group showed that low ADC values in the posterior part of the corpus callosum were associated with abnormal outcomes but that this effect appeared to be attenuated somewhat in a cooled cohort (140).

Cooling appears to reduce the frequency and severity of BGT abnormalities compared to non-cooled infants but does not affect the predictive ability of an abnormal MRI for a poor neurodevelopmental outcome (141). Rollins et al. looked more closely at the outcome for infants who had a normal, or minimally abnormal, MRI following cooling and found that out of 52 such patients 40% had an abnormal neurodevelopmental outcome at 2 years and urged caution in prognosticating based on a relatively normal MRI (142).

### 1.3.2 Novel markers for the prediction of outcome

There is increasing interest in the possibility of developing more accurate, early and reliable biomarkers for predicting long term outcome. These bio- and physiomarkers may take the form of

physiological monitoring, neurophysiological, radiological, or biochemical parameters. In fact the ideal marker may be a combination of multiple indices.

Reduced heart rate variability (HRV) has shown potential to predict the evolution of moderate to severe encephalopathy and abnormal outcomes (143-145).

Improvements in magnetic resonance imaging (MRI) has improved our ability to delineate patterns of injury radiologically and thereby, aid in prognosis (137). Piglet models of phosphorous-MRS profiles within the first two hours post-injury can predict the evolution of injury severity (146).

Blood biomarkers have also shown promise to predict injury severity and outcome. Although no blood biomarker has entered into routine clinical use, there are a number which have shown promise based on pilot work in small cohorts. Protein markers, such as UCH-L1, IL-6 and IL-16 and Activin A have been shown to be altered significantly in cord blood taken at birth in cases of hypoxic-ischaemic injury (147-149). In addition, GFAP and S100B have been shown to be raised slightly later, reaching a peak at 24 hours (147, 150). Animal and, more recently, human studies have shown significant alterations in the metabolomics profile in infants with HIE (151-153). Transcriptomics has also begun to show promise in differentiating infants with perinatal asphyxia and HIE (154). There is even some evidence that circulating microRNAs in maternal blood may be able to detect evidence of hypoxia in the intrapartum period (155). Other bodily fluids such as urine and CSF have also been the subject of biomarker discovery work (156). A previous meta-analysis by Ramaswamy et al. in this area reported cerebrospinal fluid neuron-specific enolase and IL-1β to be potential markers of abnormal outcome in survivors (157).

This list of novel biomarkers is by no means exhaustive but gives an indication of the proactive research on-going in this growing field. In the future one or a combination of these markers may help to offer early, rapid and reliable identification of infants suitable for neuro-protective intervention and may also provide further insight into the complex biochemical responses of the body to hypoxic-ischaemic injury.

| *Predictors of Outcome* | *Pros* | *Cons* |
|---|---|---|
| Standard | | |
| *Acid-Base Balance* | Widely available test, can be measured early by scalp and cord sampling | Cannot differentiate degree of severity of injury, invasive testing |
| *pH* | Responds early to HI | Low PPV for abnormal outcome |
| *Lactate* | Better reflects metabolic mechanism | No advantage over pH |
| *Apgar Score* | Quick assessment of neonatal condition at birth, non-invasive | High inter-observer variability, poor predictor of long-term outcome |
| *Clinical Examination* | Non-invasive, good to track changes in clinical state as injury evolves, predictive at discharge | Requires clinical experience, affected by intubation and medications and hypothermia, poor predictor of long-term outcome |
| *EEG/aEEG* | early predictive value if normal, value of subclinical seizure detection, non-invasive | Requires resources, equipment to apply, clinical expertise to interpret |
| Novel | | |
| *HRV* | Differentiates severity of HIE, non-invasive | Requires specialist equipment |
| *MRI/MRS* | Specific patterns of injury aid prognosis, early changes apparent | Requires specialist equipment, requires transfer of sick infant to MRI machine/department, requires infant to remain still for |

| | | |
|---|---|---|
| | | prolonged periods |
| *Biomarkers* | Very promising in pilot studies | None validated for clinical use |
| *Serum* | Reflects systemic biochemical state | Mixed markers from cerebral and other organ dysfunction, only small volumes available, invasive testing |
| *Cord blood* | Large volumes possible, available early | Mixture of Foetal and placental blood |
| *CSF* | Reflects cerebral markers | Very difficult to sample |
| *Urine* | Relatively easy to sample but dependent on urine output | Affected by significant renal disease |
| | | |
| *Proteomics* | Relatively stable and easy to test | Requires specialist equipment, response to injury may be delayed |
| *Metabolomics* | Rapidly responsive to changes in biochemical state | Requires specialist equipment, highly sensitive to environmental factors |
| *Transcriptomics* | Involved in critical processes of cell cycle and cell death, very stable | Requires specialist equipment, most markers are completely novel and difficult to identify, they may also regulate multiple pathways |

*Table 1.2: Predictors of Outcome.* The current standard tools and the novel emerging techniques to predict outcome in perinatal

asphyxia are outlined with their respective advantages and disadvantages. HI: hypoxia-ischaemia, EEG: electroencephalograph, aEEG: amplitude-integrated electroencephalograph, HRV: heart rate variability, MRI: magnetic resonance imaging, MRS: magnetic resonance spectroscopy, HIE: hypoxic-ischaemic encephalopathy, CSF: cerebrospinal fluid, PPV: positive predictive value

## *1.4 Clinical Assessment of HIE*

### 1.4.1 HIE and the use of terminology

Neonatal encephalopathy (NE) is "a clinically defined syndrome of disturbed neurological function in the earliest days after birth in the term infant, manifested by difficulty with initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness and often seizures" (27). This definition is however broad and includes non-asphyxial causes of encephalopathy such as metabolic, developmental and established structural brain abnormalities.

Considerable debate surrounds the use of the terminology of 'neonatal encephalopathy' (NE) and 'hypoxic-ischaemic encephalopathy' (HIE) particularly in the clinical sphere (90, 91). Experimental studies in animal models are able to carefully model injury by controlling the type and timing of injury through hypoxic (such as reduced oxygen exposure), hypoxic-ischaemic (such as unilateral carotid ligation) and asphyxial (such as placental disruption) methodologies. In practice, HIE is a subset of NE (96). However, using clinical criteria alone, we are often unable to identify a specific aetiology for the encephalopathic state of an individual neonate. The proportion of NE attributable to an asphyxial cause has not been easy to quantify especially as a number of confounding factors can alter or exaggerate the infant's clinical presentation (26,27,158). Evidence from animal models of specific patterns of neuropathology, biochemical alteration, electrographic and neuroimaging changes in a well-defined hypoxic-ischaemic injury allows for correlation with clinical findings (90). With this in mind, the term hypoxic-ischaemic encephalopathy will be used where relevant in this thesis. All cases described as such were rigorously defined based on detailed prospective data collection of neonatal course, structured clinical exam, EEG

findings and long term follow-up to exclude other causes of neonatal encephalopathy such as sepsis, metabolic, genetic etc. As such every effort has been made to ensure that HIE is the appropriate diagnosis.

The following section will focus on reviewing the various standardised forms of neonatal assessment that have been applied to the physical examination of infants following presumed hypoxic-ischaemic injury.

### 1.4.2 Neonatal assessment

Clinical assessment of the neurological condition following Foetal distress has only emerged in a standardised form in recent decades. It has however earned great interest as to whether a reliable prognosis can be determined at an early stage. Acutely we need an accurate early predictor of severity and likely outcome to determine the usefulness of commencing interventions such as therapeutic hypothermia within the critical six hour window before secondary neuronal damage. We can see in clinical practice that parental concerns at this time in their infant's life naturally centre around survival in the first instance but then proceeds to apprehension about what the developmental outcome will be, both motor and cognitive, so ideally early assessment should also provide longer term prognostic information.

Neurological examination of the neonate has been an evolving science. While observations by Andre-Thomas in Paris in 1960 highlighted the conspicuousness of neurological signs originating from brainstem activity in the term neonate, Sarnat switched the focus to those neurological signs that reflect the upper cortical control systems on the basis that the main

location of brain damage in the neonate is actually the cerebral hemispheres (78, 159). As a consequence of these clinic-physiological correlates, more emphasis is put on signs that depend on upper structures such as passive and active tone in the axis, alertness tested by visual fixation and pursuit, and cranial signs linked to the volume increase of cerebral hemispheres at the expense of signs depending on brainstem function, such as primary reflexes and passive tone in limb flexor muscles (160).

To date a single neonatal neurological assessment has not been shown to correlate well with outcome especially since the advent of neuro-protective intervention. Rather serial measurements and time to recovery seem to be more helpful when it comes to prognosis as discussed below. The ideal neonatal neurological assessment in the context of the infant with perinatal asphyxia must be quick, easily reproducible among different assessors, applicable to an infant who is ventilated and sedated, and easily tracks changes in findings and timing.

#### 1.4.2.1 Early Neonatal Assessments

Earlier in the evolution of the standardised neonatal neurological assessment, Brazelton and Prechtl created methods which advanced the study but also required considerable clinical expertise and were quite involved and time inefficient. In 1973 Brazelton developed a method based on the interaction of the infant and the caretaker looking at behavioural items such as responses to auditory, visual, tactile and painful stimuli and at measures of irritability and consolability (161).

In 1977, Prechtl developed an examination highlighting that many of the neurological signs were very dependent on the behavioural state of the new-born at the time of examination. He

also developed a method for assessing the “general movements” of an infant proving that an assessment of motility can be a useful diagnostic and prognostic tool to be used in conjunction with a more structured neurological examination (162, 163). Burger et al 2009 performed a systematic review looking at the predictive validity of Prechtl’s general movements and found evidence from 15 studies of a high relationship (sensitivity ≥ 92%; specificity ≥ 82%; $p<0.01$) between this neonatal assessment and neurodevelopmental outcome at 12 to 24 months (164).

#### 1.4.2.2 Dubowitz

The Dubowitz examination originally compiled in 1980 and updated in 1998 attempted to address some of the problems of earlier tests in order to develop an assessment that was suitable for routine use, could be performed quickly and was reproducible among relatively inexperienced assessors (165, 166). It was also more suited to infants who were ventilated and undergoing intensive care. Record forms with diagrams were developed and so allow the examination to be repeated at different stages of care and by different staff. It includes an assessment of state and an option to record asymmetry. It differentiates items to be performed when the infant is in the quiet/sleep state and in fully awake states and can be adapted in its sequence to the state of the baby. Many items are also age dependent thus allowing for its adaptation to the preterm infant. It has established common clinical patterns of signs for intraventricular haemorrhage, periventricular leukomalacia, cerebral infarction and hypoxic-ischemic encephalopathy (120, 165). The Dubowitz examination, now often referred to as the Hammersmith Infant Neurological Examination (HINE), has allowed clinical examination to be related to MRI outcomes in

HIE (167). Infants with a normal MRI or minimal changes tend to have only minor tone abnormalities after the first week of life. Infants who have diffuse white matter changes but spared basal ganglia in the first week were hypotonic, had some difficulty in sucking and showed diminished alertness. They tended to recover by the end of the neonatal period generally only showing mild abnormalities of movements and head and limb tone. In contrast, infants with basal ganglia lesions always showed persistent and diffuse neurological abnormalities, including abnormalities of tone, movements, feeding and alertness (120, 162).

#### 1.4.2.3 Sarnat classification

In 1976, Sarnat and Sarnat reported three distinct clinical "stages" of post anoxic encephalopathy in a cohort of 21 neonates. These states were differentiated under the headings of level of consciousness, neuromuscular control (including muscle tone and posture), reflexes, autonomic function, seizures and EEG findings. Stage one was characterised by hyper alertness, distal flexion, weak suck but otherwise generally intact reflexes, generalised sympathetic overdrive, absence of seizures and generally normal EEG findings. Stage two manifests as lethargy, mild hypotonia, weakened reflexes, parasympathetic overdrive, seizures and abnormal EEG features such as low voltage activity and loss of sleep-wake cycling. The most severe stage three involves stupor, flaccidity, predominantly absent reflexes, depressed autonomic system and an EEG with increasing isoelectric phases. They also observed that persistence of more severe stages correlated with a poorer outcome. This observation has been repeatedly confirmed in later studies (78).

While the original Sarnat staging system was based on the observations of a small cohort, the use of this staging system or

its modified score has been replicated in multiple studies over many decades (168). Its validity has been established by its application in both research and clinical practice, though the best timing of its use and how often it should be reassessed is subject to debate (78).

The Sarnat scoring system appears to be most useful at the extremes of mild and severe states but our ability to predict outcome in those with a moderate Sarnat grade is less clear, with approximately 40% of infants having difficulties in later life (121).

#### 1.4.2.4 Thompson

The Thompson scoring system was developed at Groote Schuur Hospital NICU, South Africa, specifically for the early evaluation of infants with perinatal asphyxia (119). It was based on the Sarnat scoring system but purposely simplified to make it accessible for services in the developing world. The scoring system looks at 9 parameters; tone, level of consciousness, presence of seizures, posture, Moro, grasp, suck, respiratory status and fontanelle. Each sign is scored from 0 to 2 or 0 to 3. The maximum possible score is 22.

When compared with outcome at one year, defined as clinical evidence of cerebral palsy or a Griffiths Scales of Mental Development general quotient less than 70, the score was found to be predictive of outcome. The best correlation was with peak score; a peak score of 15 or higher had a positive predictive value of 92% and a negative predictive value of 82% for abnormal outcome, with a sensitivity and specificity of 71% and 96%, respectively (119). Even the short-term value of the Thompson score was supported by Horn, 2013 (169). They found that a Thompson score greater than six at three to five postnatal hours predicted an abnormal six hour aEEG with a sensitivity of

100% and a specificity of 67%. The same score at this time-point also predicted moderate-severe encephalopathy within 72 hours after birth (sensitivity 90% and specificity 92%). This highlights the potential of this scoring system to be useful for selecting candidates for therapeutic hypothermia while still within that critical six hour window after birth (169). The same group also highlighted the potential of the score to select those infants who will have a poor outcome despite cooling. They found that 75% of cooled infants who had a Thompson score of greater than 15 at three to five hours had a severely abnormal aEEG or death at 48 hours versus 18% of infants with a Thompson score less than 16 ($p$=0.004) (170).

#### 1.4.2.5 Amiel-Tison

Amiel-Tison in 1978 defined the degrees of abnormality on neonatal examination of HIE in a pre-cooling population as follows;

- Mild abnormality was consistent with hyper-excitability and mild abnormalities of tone. Responsiveness is normal with primary reflexes intact. There is an absence of seizures and a varying duration of symptoms.
- Moderate abnormality adds disturbance of responsiveness and primary reflexes to altered tone. This is associated with a progressive central nervous system (CNS) depression which increases to lethargy or a light coma within the first few days. Return to normal in the first week is exceptional.
- Severe abnormality is characterised by deep coma and repetitive seizures (118).

This system classified some infants as severe that would have been considered moderate in the Sarnat staging system. The 1978

definitions were updated in 1986 to provide a new categorisation system that separated three stages of neonatal signs and symptoms and subdivided each into two levels of severity (160). Stage one was characterised by hyperexcitability and mild abnormalities of tone. The subdivision of severity is the length of time the symptoms are present with a cut-off point of seven days based on observations that when infants' symptoms are resolved within seven days most had a normal outcome. Stage two was defined as a deepening CNS depression as previous and the subdivision in severity was made between infants that did and did not have seizures. They noted a tendency for those with seizures to have somewhat more CNS depression and a further decrease in primitive reflexes. Stage three was characterised by deep coma. This stage contrasted with the Sarnat severe stage by being associated with repetitive seizures or status epilepticus which was often difficult to control. The severity subdivision was made between the presence or absence of brainstem signs, particularly the oculo-vestibular reflex as other reflexes may be affected if the child is on ventilator support. This system allowed the infant to be scored from nought to six (normal to severely abnormal) in the following manner: 0 = normal, 1 = stage Ia, 2 = stage Ib, 3 = stage IIa, 4 = stage IIb, 5 = stage IIIa, 6 = stage IIIb.

The Amiel-Tison neurological assessment at term (ATNAT) has been developed to provide a framework for observing the development of cortical control in infants at term. It is quickly and easily administered, examines forebrain function and has been shown to predict the occurrence of CP after birth asphyxia at 12 to 15 months (171, 172). The central tenet of the ATNAT is that the stage of maturation of the two motor control systems (subcorticospinal and corticospinal) can be clinically evaluated in the term infant (173). Murray et al. correlated early neurological examination in HIE with neurodevelopmental

outcome and found the later the neurological examination is performed the greater its predictive ability e.g ATNAT on day 3 ($r=0.46$, $p<0.001$). However, better correlations were achieved with examination at discharge (a non-structured clinical exam) and Sarnat score with 24 month outcomes (121).

The Amiel-Tison neurologic assessment aimed to improve clinical accuracy by a simplified scoring system. It focused on the most meaningful items that reflect damage of cortical control pathways. It also facilitated overview of clinical progression in the term infant by clustering signs and symptoms under the following headings: cranial assessment, neurosensory function and spontaneous motor activity during the assessment, passive muscle tone, axial motor activity, primitive reflexes, palate and tongue, adaptedness to manipulations during the assessment, feeding autonomy, medical status at the time of assessment, unfavourable circumstances at the time of examination and complementary investigations along with a method for synthesising data for the term neonate or the preterm infant around 40 weeks corrected (160).

## *1.5 Assessment of Outcome in perinatal asphyxia*

Measurement of neurodevelopmental outcome in clinical and research environments presents a number of challenges. However, as evidenced above, outcome is our marker for interpreting the effectiveness of any intervention applied in the neonatal period. The choice of assessment depends hugely on the question that needs to be answered. Conventionally neurodevelopmental outcome is measured at approximately two years of age following perinatal risk factors. The gold standard is a standardised administered neurodevelopmental test that covers motor, language and cognitive domains. Assessment at this age can be lengthy and challenging. Few assessments have been standardised and validated in late infancy/early childhood but the most commonly used are the Bayley and Griffiths Scales (8).

### 1.5.1 Bayley Scales and Griffiths Scales

The Bayley Scales of Infant and Toddler Development, now in its third edition (BSID-III), has become the more popular of the two administered assessments (174). A description of the structure, development and challenges of this assessment can be found in Chapter four. Here I will give an overview of the alternative assessment option for this age group, the Griffiths Scales.

The Griffiths Scales consists of two sets of scales: one for children aged nought to two years and another for children aged two to eight years. Within the nought to two years scales a profile is obtained from the administration of five subscales examining locomotor, personal-social, language, eye-and-hand coordination and performance. Subscales are scored separately and sub-quotients can be calculated for each, or a total

developmental quotient with a mean of 100, standard deviation of 13, can be derived. The Griffiths Baby Scales was originally developed in 1954 but underwent revision in 1996 (175). This was designed for infants up to two years, while the extended scales was introduced in 1970, for children from two to eight years of age. This lack of continuity of norms for administration around two years of life makes it less attractive for clinical and research programmes that conduct their follow-up assessments at this time-point. The Griffiths Scale has had mixed success for prediction of later performance. Bowen et al. looked at Griffiths' performance at one and three years and compared with IQ at five years in a cohort of extremely low birth weight infants. Score at one year was poorly correlated with five year intelligence but Griffiths score at three years correlated quite well (176). Barnett et al. looked at the predictive value of the Griffiths in a cohort of infants with neonatal encephalopathy and found that a poor score at one and/or two years was a good predictor of impairment at school age but a normal score in the early years could not preclude later neurological, perceptual-motor, or cognitive abnormalities (177).

Griffiths III, the eagerly awaited re-standardisation of the Griffiths assessment, was announced in 2016 (178, 179). This latest version has undergone extensive redesign and re-standardisation. It will cater for administration to children from birth to six years without the previous separation of norms around two years. There will be five administered subscales; foundations of learning, language and communication, eye and hand coordination, personal/social-emotional and gross motor. Publication is expected later in 2016.

## *1.6 Cognitive Development in Children*

Interest in the cognitive development of children is a relatively recent phenomenon. The last century saw a surge in psychological theory concerning the processes by which children develop intellectually. These theories have had a significant impact on childhood education and parenting. However, it is only since the advancement of molecular genetics, functional neuroimaging and electrophysiological techniques that those theories have started to be linked to the neurobiology of the typically developing and the abnormal brain. The emerging results from this work have multidisciplinary applications in elucidating disease processes, identifying options for therapy both in neuroscience and in clinical psychology and giving insight into how each of us process the world around us.

In this section I will discuss childhood cognitive development from a number of perspectives. These perspectives will cross between the realms of developmental psychology, neuropsychology, neuroscience, medicine and genetics. I will start with introducing some of the 'domain-general' theories i.e. formed under the belief that all the changes in mental abilities that happen during a child's cognitive development are happening because of the same underlying process. These include constructivism and social constructivism. This compares with the 'domain-specific' approach where different mental abilities are thought to develop independently of one another. This will be discussed with a brief look at nativism and the 'theory of mind'. I will then introduce a more recent theory, 'neuroconstructivism', which attempts to navigate between these two groups by advocating a more domain-general start point which through development leads to domain-specificity in the adult brain.

I will then discuss structural and functional brain development and its relation to cognitive development and finally look at some of the evidence from molecular genetics that will influence how we approach learning abilities and disabilities.

### 1.6.1 Constructivism

#### 1.6.1.1 Piaget's Theory of Cognitive Development

One of the first psychologists to suggest that children think differently to adults was Jean Piaget (180), in his work with children from the 1920s to 1980s. This involved extensive observations of children's approaches to new learning situations. He held a constructivist approach to learning theory that was predicated on the idea that learning occurs through a child's experience of the world becoming internalised. Intellectual development was seen as a process of active exploration of the world where the child then constructs a mental representation of reality based on what is discovered by these explorations. These mental representations are called 'schema'. This was a significant departure from previous notions that saw the child as a black slate who absorbed knowledge as it was presented (181). This was the type of approach taken by the behaviourists, such as Pavlov and Skinner, who believed that the learner was passive and all behaviour could be explained as a response to external stimuli without reference to internal mental states or the ability to modify the effect through positive and negative reinforcement (182). Piaget was one of the first to emphasise the importance of play to learning. He believed that we are instinctively driven to learn to escape the 'disequilibrium' that occurs when we meet a new situation we do not understand.

While 'schema' are the mental structures we create to store all the information related to one aspect of the world, 'operations' are the way we understand the rules by which the world operates

(180). Piaget developed a staged theory of development based around the maturing of these operations:

- Sensorimotor stage – 0 to 2 years
- Preoperational stage – 2 to 7 years
- Concrete operational stage – 7 to 11 years
- Formal operational stage – 11 years+

Very young children have no operations at all and are hence pre-operational. Based on his observations and psychological experiments Piaget identified certain consistent 'errors of logic' that children possess at different developmental stages due to limits on operations available to them. The following errors of logic are evident in the sensorimotor and preoperational stage of development:

- Object Permanence – the idea that up to a certain age children believe that when an object is no longer visible it no longer exists and will not look for it. Piaget suspected children developed object permanence around eight months but subsequent experiments by Freeman et al., 1980, showed that environment affected results and Baillargeon and DeVos, 1991, showed, using a different experimental format, that some understanding of object permanence can be present from three months of age (183, 184)
- Egocentrism – difficulty understanding that other people's perception of the world can be different to their own. One of Piaget's seminal experiments was the "three mountains experiment" which asked children to sit in front of a model of three mountains and select from a collection of photos what view of the scene was held by a doll sitting at a different angle to the model. Piaget found that children below seven years tended to select a photo which showed their own perspective of the scene (180, 185). However, Donaldson, 1978, showed that children as young as three

and a half years were capable of appreciating another's point of view by developing an alternative experimental model – the policeman task. She felt that Piaget's experiment did not make "human sense" and was too abstract to engage the children (186).

- Conservation – the understanding that objects remain the same even when their appearance changes. Younger children tended to believe, for example, that there were more coins when a fixed number of coins were spread out and more liquid when a liquid was poured from a short wide container to a tall narrow container despite having observed all the changes being performed (180). McGarrigle, 1974, however, identified that the children's response to this experiment was strongly affected by the conditions in which they were asked. When a 'naughty teddy' rather than the experimenter altered the coins etc., the children were much more likely to respond with the correct answer (187). Displaying the early ability of children to respond to what they think the assessor requires of them.

#### 1.6.1.2 Case's Theory of Cognitive Development

Robbie Case, 1985, developed a theory of cognitive development based on information processing theory (188). He viewed cognitive development as dependent on the development of the brain and saw the brain as analogous to a computer. He saw children as developing intellectually by solving problems and their ability to do so was limited by information processing capacity. He proposed that cognitive and neural changes lead to greater ability to process information. This was achieved through myelination, growth of 'mental space' i.e. expanding working memory with maturity and 'automaticity', the concept

that with practice some problem-solving strategies become automatic (180).

Case saw children as having four instincts which together produce cognitive development:

- Instinct to explore
- Instinct to solve problems
- Instinct to imitate others
- Instinct to understand and co-operate with others.

Case, like Piaget, also proposed a staged theory of development where children transitioned to successive stages when their mental space reached a certain level. The idea of improved information processing with age has been supported by Kail, 1991, who found improved processing time with age on a number tasks irrespective of culture or the nature of the task (189). Case is seen to have developed Piaget's ideas and is often referred to as 'neo-Piagetian'.

### 1.6.2 Social Constructivism

#### 1.6.2.1 Vygotsky's theory of Cognitive Development

Vygotsky and Bruner (discussed below) emphasised culture, social interaction and language in cognitive development (180). Vygotsky agreed with Piaget that cognitive development takes place in stages characterised by different styles of thinking. However, he placed a strong emphasis on social interaction during learning and the culture in which a child grows up. He saw children as being born with basic mental functions such as the ability to perceive the outside world and focus attention on particular objects. Thinking and problem solving are higher mental functions and seen as the 'tools' of the culture and are transmitted to children by the older members of the culture. Experiences with other people gradually become internalised and

form the child's internal representation of the world. Vygotsky believed that children could never develop formal operational thinking without the help of others, the difference between what a child can learn on its own and what it can potentially learn through interaction with others is called the 'zone of proximal development' (ZPD) (180, 182). Vygotsky also placed more emphasis on the importance of language in cognitive development. He felt that while early on the sole function of language is communication, it later becomes internalised and used as a tool for thinking. Criticisms of Vygotsky come mainly from his emphasis on the role of culture in thinking and learning. If culture had such an importance we might expect different cultures to have more radically different approaches to thinking than they do. Some support for Vygotsky's views on the influence of culture comes from Luria and Yudovich, 1971, who looked at the thinking styles of the Uzbecki people of central of central Asia and found that when a culture changes, the thinking of the culture also changes (190). Vygotsky also controversially suggested that cultures that do not have formal schooling do not develop the ability for abstract thinking implying that some cultures are better at thinking than others. Successors of Vygotsky tend to moderate this by suggesting that different cultures have qualitatively rather than quantitatively different thinking tools (180).

#### 1.6.2.2 Bruner's theory of cognitive development

Bruner is a more recent theorist than Piaget and Vygotsky and has applied and adapted aspects of both (180). Bruner rejected the idea of developmental stages, an idea though prominent in earlier theories failed to be fully supported by later experimentation. He preferred to look at the form in which information is kept in the mind at different ages, termed 'modes

of representation'. Bruner's proposed modes of representation were:

- Enactive representation – the first to appear in children's minds. Thinking in the first year is based entirely on physical or motor actions. This representation form persists in adulthood as 'muscle memory'
- Iconic representation – information is kept in the mind as pictures or mental images. This form of representation appears at one year of age and must be present for drawing to be possible but does not aid problem-solving. Bruner and Kenney, 1966, developed an experiment using a grid of ordered, differently sized glasses and asked children to copy the order, a reproduction task, and to reverse the order, a transposition task. While children relying on iconic representation could perform the reproduction task they could not perform the transposition task (191)
- Symbolic representation – means that language and other symbolic forms such as numbers and music can now be used for thinking. This allows the child to categorise things and start thinking logically. Bruner believed that symbolic thought became possible when the child had achieved a certain level of mastery of language.

Bruner also built on Vygotsky's idea of the ZPD and proposed the concept of 'scaffolding' which emphasises the importance of the social dimension of learning. It is defined as the wide range of activities through which the adult, or more experienced peer assists the learner to achieve goals that would otherwise be beyond them (192).

### 1.6.3 Theory of Mind

The abrupt appearance of a 'theory of mind' around four years of age is put forward as evidence of a domain-specific view of

childhood cognitive development. The ‘theory of mind’ is an understanding of what other people believe, think and know and does appear to develop independently of general cognitive thinking. Wimmer and Perner, 1983, developed an experiment where children are presented with the story of a boy called Maxi who has left his chocolate in a green container and gone out. While he is away his mother takes some chocolate and puts the rest in the blue container and the children are then asked where will Maxi look for his chocolate on his return (193). Few three year olds could understand that Maxi does not know that his mother moved the chocolate and therefore would look where he had left it, in the green container, but the majority of four year olds could understand that Maxi’s knowledge is different from theirs and answered the question correctly. This experiment was repeated by Avis and Harris, 1991, with children in a remote part of Cameroon and their results were age consistent (194).

Leslie, 1994, suggested that the sudden onset of theory of mind at four years old occurs because a particular module of the brain suddenly becomes active (195). This modular theory of the brain is popular but does not explain how aspects of the child’s social environment can have a profound effect on theory of mind. Studies have shown that theory of mind is superior in children who have secure attachments and whose primary carers display high levels of sensitivity (196). Astington, 1998, suggested a theory that perhaps this is due to children internalising a theory of mind during their early interactions (197).

The case of seemingly impaired theory of mind in autism is also taken to offer evidence of domain specific cognitive development (198). Some autistic children can develop other mental abilities to a high level while demonstrating an impaired theory of mind. This would not be expected unless the theory of mind developed independently.

### 1.6.4 Nativism

Nativism argues for an innate specification of dissociated modules in the human brain. Annette Karmiloff-Smith, 2009, though not a proponent of nativism, outlines the four major arguments in favour of this nativist approach (199). Firstly, it draws from evidence in adults of predictable patterns of deficit following specific localised brain injury. Secondly, it is evolutionarily logical that the human brain has been required to develop perfectly adapted independently functioning modules to provide us with a 'Swiss army knife' of skills. Thirdly, some of the very early skills demonstrated by young children have been promoted as evidence of innately specified, core knowledge (200). Fourthly, the existence of genetic disorders that show deficits in some cognitive abilities but 'normal' scores in others illustrates the dissociation of these domains. This has led to a language in clinical practice of describing the affected domain as 'impaired' and the apparently unaffected domain as 'intact' or 'relatively intact'.

Elizabeth Spelke, 1998, supported nativist theory and was particularly interested in cognitive development in infancy. She argued that there is evidence to suggest that some skills, such as object representation, are present before infants are physically capable of effectively acting on objects directly (201). This would contradict constructivist theories which require experience and exploration of the world in order to build mental representations. Spelke specifically advocated for a view of human cognition based on four systems of core knowledge for representing; objects, actions, numbers and space, and a possible fifth for representing social partners (202).

A key influence of the nativism argument was however to open up the dialogue to more investigative research particularly in the

infant (201). Prior to this, such study was summarily dismissed as the intuition of the day assumed that cognitive ability prior to world exploration was impossible. This view has been generally dispelled by various experiments in the infant period including the work on object permanence by Baillargeon and Davis mentioned previously (183).

### 1.6.5 Neuroconstructivism

Neuroconstructivism began to emerge in the late 1990s with a view to moving away from the developmental psychology approach of describing children's abilities at specific ages towards an understanding of the underlying neural mechanisms that make these developmental trajectories possible. The approach aims to focus on the factors that influence the emergence of mental representations now referred to as 'neural activation patterns' (203). Neuroconstructivism attempts to integrate different views of brain and cognitive development including:

1. Probabilistic epigenesis which emphasises the interactions between experience and gene expression
2. Neural constructivism which focuses on the experience-dependent elaboration of small-scale neural structures
3. The 'interactive specialisation' view of brain development which stresses the role of interactions between different brain regions in functional brain development
4. Embodiment views that highlight the role of the body in cognitive development
5. The constructivist (Piagetian) approach to cognitive development with its focus on the proactive acquisition of knowledge, and

## 6. Approaches focusing on the role of the social environment for the developing child

Neuroconstructivism draws greatly from anatomical, neuroscientific and genetic evidence and integrates it with constructivist psychological theory. It proposes that a small number of domain relevant learning algorithms 'jump-start' the infant brain (204). The normal initial infant cortex is highly interconnected and it is only with time and the processing of different types of inputs that the child brain becomes increasingly specialised for function (205). Modules then emerge from a gradual and complex process of modularisation to produce the functionally more domain specific adult-type brain (206).

The impact of this is that a tiny impairment early on can have a cascading effect on subsequent acquisition of seemingly unrelated skills. While the deficit could permeate all parts of the cortex some parts of the brain might be more seriously affected than others dependent on the interaction between different algorithms and different structures in the environmental input. This might imply that domains previously determined to be 'intact' are unlikely to be completely unaffected by a developmental pathology (204). Grice et al., 2003, presents some evidence from work with children with William's syndrome that demonstrate this might be the case (207). In an experiment looking at EEG derived event-related potentials (ERP) in response to face processing and object processing, in this case a car, patients with William's syndrome were found to process faces and cars in the same way whereas healthy controls processed faces very differently. This difference would be unexpected considering that people with William's syndrome usually perform in the normal range on face processing tasks. This highlights that scores 'in the normal range' can mask differing cognitive and brain processes and offers a countering

argument for one of the rationale behind nativism. [For further information on the use of ERP in neuropsychology see Sur et al. (208)] Recently this work has been extended to show that face processing is already atypical in infancy, with infants with William's syndrome, unlike those in a control group only being sensitive to featural changes but not configural changes.(209). This provides further reinforcement against a distinct module for this skill.

Thomas, 2005, highlighted two unanswered problems with neuroconstructivism (210). Firstly, a clearer picture is needed of the initial domain relevancies that predate a particular domain and of the nature of the process that eventually delivers domain-specific functional structures. Secondly, construction of developmental trajectories from infancy to adulthood, needed to prove certain aspects of the theory, is hampered by various methodological issues, not least the lack of appropriate cognitive tests that can be used across this age range.

### 1.6.6 Structural and Functional Brain Changes in Cognitive Development

Unfortunately very little is yet understood about structural brain development in childhood and less about its relation to cognitive development. Until quite recently our knowledge relied on limited evidence from post-mortem analysis and clinical imaging of children to give insight into how the brain developed in childhood. Of course, this tended to give us more information on the pathological brain processes rather than typical healthy development (204). Recent progress in the development of sophisticated neuroimaging and neurophysiological measurements has expanded the methodological options for studying the developing brain without the need for highly invasive procedures or unnecessary radiation dosage (211).

The perinatal period of brain development, from 24th week gestation to the time of birth, is a period characterised by neuronal maturation (212). Around the time of birth most neurons have migrated to their appropriate locations within the cerebral cortex, hippocampus, cerebellum and other regions of the brain. Some neurogenesis continues in the first postnatal year in the hippocampus, olfactory bulb, and cerebellum. New granule cells are produced throughout adulthood in the dentate gyrus. The dendritic branching of neurons and the number of synaptic connections greatly increase in this time. Dendritic pruning and synapse elimination follows this period of overproduction (204). (*Figure 1.5*)

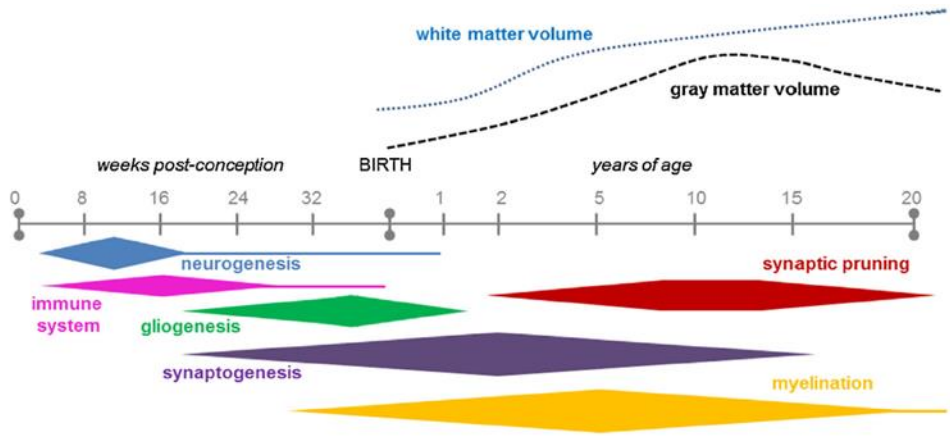



*Figure 1.5: The time course of key processes of brain development for 20 weeks gestation to 20 years.* Reproduced from Semple et al, 2013 (213).

Huttenlocher, 1979, was one of the first to study the time-course for synaptic development and pruning in the human brain (214). He found that unlike previous studies in primates, human synaptogenesis does not occur simultaneously across different regions of the brain. In the visual cortex synaptic overproduction reaches a maximum at the 4th postnatal month,

synapse elimination then continues to preschool age. The prefrontal cortex however peaks at three to four years of life and substantial decline does not occur until mid-to-late adolescence (215, 216). PET studies of glucose metabolism suggest that maturation of local metabolic rates closely parallel the time-course of overproduction and subsequent pruning of synapses. The prefrontal cortex shows a relatively prolonged maturation compared to the visual cortex (217).

Another important process evident in structural brain development is myelination. Myelination in the CNS is carried out by oligodendrocytes and is a very slow process. The presence of myelin has been noted in the spinal cord at the end of the first trimester and proceeds caudo-rostrally (218). In the CNS, afferent tracts become myelinated earlier than motor pathways. The vestibule-spinal tracts become myelinated at the end of the second trimester, whereas the pyramidal tracts begin at the end of the third trimester and are not completed until about two years. Cortical association fibres are the last to become myelinated. At birth the human brain is rather immature with regard to the extent of myelination. The rate of deposition of myelin is greatest during the first two postnatal years. Age-related changes in white matter myelination continue during childhood and adolescence (219). It is important to note, for structural studies, that the appearance of myelin on MRI lags one month behind the histological findings (220).

Casey et al., 2000, outlined some of the MRI studies to date that have attempted to examine postnatal brain development and to link with cognitive development (221). Some of the most consistent findings regarding brain maturation come from volumetric MRI studies and identify:

- A lack of any significant change in cerebral volume after five years of age (222, 223)

- A significant decrease in cortical grey matter after 12 years (224)
- An increase in cerebral white matter throughout childhood and young adulthood (225-227)

Regionally, as discussed above, the prefrontal cortex appears to be one of the last brain regions to mature. This has made skills attributable to this region a target for study in order to examine the relationship between structural development and cognitive development.

The first fMRI study looking at a typically developing paediatric population examined prefrontal activation in children aged 9-11 years performing a working memory task (228). The results demonstrated reliable activation in the dorsolateral prefrontal cortex and the anterior cingulate cortex and reflected findings in previous adult studies, although the percentage change was higher in children. This study was limited in size to six children and used a working memory task designed for adults, which further limited the extension of this testing to younger age groups.. The same authors performed an fMRI study where adults and children (7 - 12 years) performed a task of attention and inhibition and found reliable, and similarly localised, activation in the prefrontal cortex. Again, the volume of activation was significantly higher in children (229). They also identified that certain levels of activation in particular locations correlated with behavioural performance. While they have pioneered the use of this technology for exploring development, the studies are limited in their generalizability. They do not give an indication of developmental trajectory over time, are limited due to the range of appropriate tasks available and the cooperation of younger children to undergo the process.

EEG has also been used to begin to give us insights into how the brain develops in line with cognitive changes. Hudspeth and

Pribram, 1990, gave some insight into the early use of EEG for this purpose (230). By quantifying changes in the EEG frequency spectrum over time (QEEG) of 561 normally-developing children aged 1 - 21 years across multiple regions of the cranium they found that the brain exhibited five periods of increased maturation rates interspersed with periods of plateau. They suggested that the timing of these peaks of change corresponded to or slightly preceded the observed stages of development as described by Piaget. Interestingly they found that maturation patterns were synchronised across all brain regions in the first decade of life but then began to show regionally independent variations. The fifth stage occurred between 18 - 21 years and was almost exclusively apparent in the frontal regions. These post-pubertal peaks almost follow a posterior to anterior sequence, mirroring the known anatomical caudorostal progression of maturation mentioned above. This finding however, of synchronised maturation followed by later asynchronous maturation, may provide further support for the neuroconstructivist theory, indicating that it is only with time that the child's brain becomes increasingly specialised and localised in function. The strength of this work is that it seeks to determine the trajectory of development rather than making interpretations over a limited age range.

### 1.6.7 Molecular genetics and cognitive development

In the great debate examining the relative influence of genetics and the environment on the individual differences between our cognitive abilities, twin studies have contributed greatly to the discussion. The overwhelming, if frustrating, conclusion we have been left with is that both contribute significantly, if varyingly, to the individual differences when examining a single trait (204).

Through candidate gene studies many associations with cognitive ability have been made though few have been replicated (231, 232). The following are examples of single genes linked to intelligence or specific abilities that have been identified. Chorney et al., 1998, has linked IGFR2 on chromosome 6 to account for 2% of the variance in intelligence (233). Bermann and Noble, 1995, identified the D2 Dopamine Receptor gene (DRD2) as associated with spatial abilities but not general intelligence (234, 235). Henderson et al., 1995, noted that the Apolipoprotein E (APOE) gene appears to be associated with memory and speed of processing (236). APOE allelic expression has also been implicated in response to traumatic brain injury in the paediatric population (237). Although multiple monogenic causes of conditions associated with developmental delay e.g. PKU, Trisomy 21 etc. have been identified, none have given us insight into the genetic basis for the far more common developmental delay seen in otherwise healthy children (232). It now seems more likely that most of what we refer to as 'learning disability' is more accurately represented as the low end of the normal distribution of learning ability and caused by the same DNA variants (232).

More recently the genetic loci associated with cognitive ability have been determined using multivariate genetic research rather than previous univariate analysis (204). Multivariate genetic analysis focuses on the covariance between two or more traits and estimates the extent to which genetic factors that affect one trait also affect another trait. High genetic correlation, i.e. whether the same genes affect both measured traits, has been found between reading, language and mathematical abilities. These high genetic correlations indicate that there is substantial genetic overlap between different types of learning abilities and disabilities. This has led to these being referred to as 'generalist genes' (238). Most of the multivariate analysis has tested

general ability, the concept of intelligence, or *g* due to its high heritability. A review by Deary et al., 2006, concludes that "the genes for individual differences in ability are often general in effect: that is just as *g* is associated with diverse cognitive and biological functions, the genes underlying differences in *g* themselves might affect brain systems, rather than being specific for one or just a few cognitive modules" (231). They suggest that to progress the field in the understanding of diverse cognitive abilities, initially studies should focus on the examination of general ability.

Two genetic concepts are key to understanding the basis and possible implications of research to date:

- Pleiotropy – a gene affects many traits
- Polygenicity – many genes affect a trait

Together these concepts suggest that genetic input into brain structure is general not modular, consistent with domain-general or neuroconstructivist theory of cognitive development.

The support for polygenicity in cognitive abilities is based on the quantitative distribution of these skills rather than the dichotomous distribution seen in Mendelian single-gene disorders (232). This is now generally accepted to be the case with many common disorders. One of the consequences of polygenicity is that each individual gene will only have a small effect. To emphasise the contrast with single gene disorders, these multiple genetic variants of small effect are called quantitative trait loci (QTLs) (204, 232). The use of QTL terminology also has conceptual implications as it does not limit exploration to purely the coding sections of DNA.

Gene expression mapping through microarray analysis studies has allowed us to see pleiotropy in action and has provided some early support for the generalist gene hypothesis (238). Kovas

and Plomin, 2006, suggested three possible mechanisms by which generalist genes may work pleiotropically in the brain *(Figure 1.6)* (238). The first that genes would work specifically on one location within the brain but generally at a cognitive level seems most unlikely as evidence to date seems to support the widespread expression of candidate genes in multiple locations. The second is that genes act at multiple brain structures and functions but that each of these only affects a specific cognitive process. The third mechanism is that genes affect multiple structures and functions, which in turn affect multiple cognitive processes. The brain processes that could mediate either the second or third mechanism include genetically induced effects on neural plasticity, dendritic complexity, myelinisation and speed of nerve conduction (204). This links in with the neurobiological maturation outlined previously.

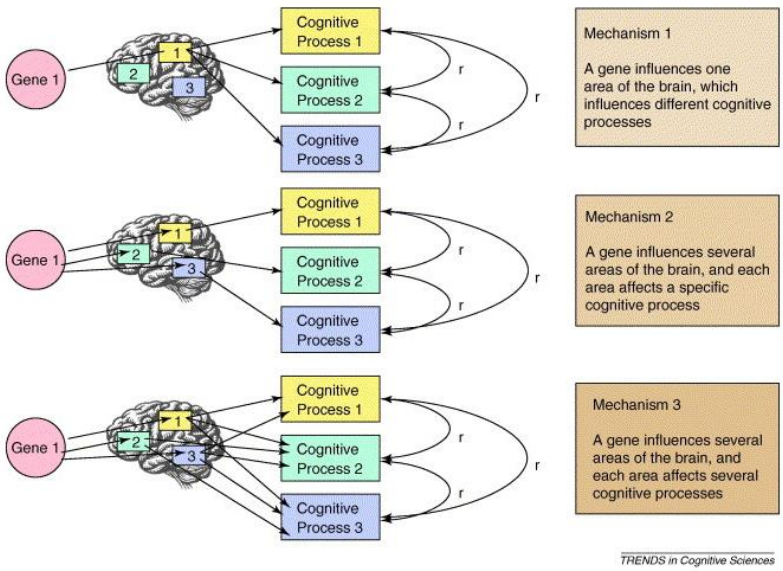



*Figure 1.6: Proposed mechanism of action of generalist genes on the brain.* Reproduced from Kovas 2006 (238)

Plomin and colleagues established the IQ QTL project to identify some of the genes responsible for the heritability of intelligence (239). They measured allelic frequency in or near genes likely to be relevant to neural functioning in using pooled DNA of children aged 6 - 12 years of age comparing those with high and low IQ. Initial work was promising, identifying allelic frequency differences between the high and low IQ groups in a new HLA marker and a new brain-expressed triplet repeat marker (CTGB33). However, progress has been extremely slow, perhaps related to effect sizes of QTLs being even smaller than previously expected (232).

Future research may require large genome-wide studies, which have their own challenges. The first genome-wide linkage study for intelligence by Posthuma et al., 2005, found QTLs for IQ on chromosome two and six closely related to loci known to be linked to defined developmental conditions (240). Advancement of genetic techniques using microarray, genome-wide analysis and DNA pooling as well as the increasing awareness of the influence of epigenetic factors further opens the up the field of molecular genetics to contribute to our understanding of cognitive development. We are, however, still far from understanding the complex influence that QTLs have on the developmental trajectory of different cognitive abilities as this will require looking at downstream targets and linking into the neurobiological maturation of the brain (204).

### 1.6.8 Clinical application

From a clinical perspective, it is important to have a fundamental understanding of the typically developing brain to give better comprehension of the way different pathologies can impact this process. It is clear that cognitive development

involves multilevel complexities which must be approached from multiple disciplines. Developmental delay is most suitably managed from a multidisciplinary approach and it is vital that clinicians can beneficially contribute to the dialogue.

So much of our practice is based on theories and 'evidence' derived for imperfect testing procedures and interpretations made in the light of advocacy of whichever theory seems most plausible to the experimenter. Many previous studies in this area are biased towards one or other side of the debate. As clinicians, we need to be able to critically analyse this work and interpret the empirical evidence now made possible through technological advancement.

The growing work in this area moves these previously disparate disciplines closer together towards an evidence based approach to understanding childhood learning ability and hence disability. While there is still substantial work left to be done, cross-disciplinary collaboration will be vital in progressing the field out of which clinicians will gain an improved understanding of how we can better help our patients.

## *1.7 Summary*

The above sections form the knowledge base required to progress the aims of this thesis. A thorough understanding of the pathophysiology of injury in perinatal asphyxia, the known outcome in these high-risk infants and the current state of the art for outcome prediction must be established in order to develop new tools for prognostication in perinatal asphyxia. Further discussion of the biological plausibility of the presented promising biomarkers is discussed in Chapters 2 and 3. Likewise an understanding of current assessment tools and normal cognitive development in children is necessary for the

development of a novel tool for cognitive assessment. Further discussion of the limitations of currently available tools for neurodevelopmental assessment is included in Chapters 4 and 5. Rationale for design of the proposed novel cognitive assessment tool is discussed in Chapters 5 and 6.

# 2.0 Cord Blood Proteins and Neurodevelopmental Outcome in Perinatal Asphyxia

*Publications arising from this chapter:*

*"Cord Blood IL-16 is Associated with 3-Year Neurodevelopmental Outcome in Perinatal Asphyxia and Hypoxic-Ischaemic Encephalopathy"*

*Caroline E Ahearne, Ruby Y Chang, Brian H Walsh, Geraldine B Boylan, Deirdre M Murray*

*Developmental Neuroscience. 2017. (In Press)*

*and*

*"Glial Fibrillary Acidic Protein is not an early marker of injury in perinatal asphyxia and hypoxic ischaemic encephalopathy"*

*Looney A-M, Ahearne C, Boylan GB, Murray DM.*

*Frontiers in Neurology. 2015; Dec 21;6:624.*

*and*

*Downstream mRNA target analysis in neonatal hypoxic-ischaemic encephalopathy identifies novel marker of severe injury: a proof of concept paper.*

*AM. Looney, CE. Ahearne, B. Hallberg, GB. Boylan, DM. Murray*

*Molecular Neurobiology. 2016 Dec 12 (Epub ahead of print)*

The following work was carried out as part of the BiHIVE Study (Biomarkers for Hypoxic-Ischaemic Encephalopathy). The overarching aim of this study has been to identify promising biomarkers measured in umbilical cord blood at birth for the prediction of injury severity in infants with perinatal asphyxia.

The study was designed to have multiple phases starting with biomarker discovery, continuing to biomarker validation and development of a point of care device for biomarker measurement at the cot-side. A panel of biomarkers sensitive and specific for the prediction of severity of encephalopathy in infants with perinatal asphyxia would support clinical decision making and guide management decisions in the care of these infants.

The biomarker discovery phase (BiHIVE 1) followed recruitment of infants from 2009 to 2011 in Cork University Maternity Hospital, Cork, Ireland. Samples of umbilical cord blood collected from these infants were examined, using a systems biology approach across functional levels, for potentially useful biomarkers in line with the aims of the study. Promising biomarkers that were shown to be predictive of clinical or electrographic grade of encephalopathy were then tested for their ability to predict neurodevelopmental outcome in early childhood.

This chapter presents the results of that work including biomarkers from the proteomic and transciptomic functional levels. Chapter three will present the results of biomarkers from the metabolome for ability to predict neurodevelopmental outcome.

## *2.1 IL-6 and IL-16 for the prediction of neurodevelopmental outcome in perinatal asphyxia*

### 2.1.1 Abstract

Activation of the inflammatory pathway is increasingly recognized as an important mechanism of injury following neonatal asphyxia and encephalopathy. This process may contribute to a poor prognosis in some cases, despite therapeutic hypothermia. Our group has previously identified raised interleukin-6 and interleukin-16, measured in umbilical cord blood at birth, to be predictive of grade of hypoxic-ischaemic encephalopathy.

Our aim in this study was to examine the ability of these cytokines to predict three-year neurodevelopmental outcome in the same cohort.As part of a prospective, longitudinal cohort study set in a single tertiary maternity unit, term infants with biochemical and clinical evidence of perinatal asphyxia were recruited at birth. Umbilical cord blood was collected and analysed for interleukin-6 and interleukin-16 using a Luminex assay. Neurodevelopmental outcome of these infants was assessed at three years using the Bayley Scales of Infant and Toddler Development (Edition 3).

Early cord blood measurement of IL-6 and IL-16 and long term outcome was available in 33/69 infants. Median (IQR) IL-16 differentiated infants with a severely abnormal outcome (n=6) compared to all others (n=27), [646 (466-1085) pg/mL vs. 383.5 (284-494) pg/mL, $p$=0.012].

IL-16 levels were able to predict a severe outcome with an AUROC (CI) of 0.827 (0.628-1.000), *p*=0.014. Levels ≥514pg/mL predicted a severe outcome with a sensitivity of 83% and a specificity of 81%. IL-16 also outperformed other routine biochemical markers available at birth for the prediction of severe outcome.

Apgar scores at 1 and 10 minutes were also predictive of a severe outcome, p=0.022 and 0.036 respectively. A combination of IL-16 with these clinical markers did not improve predictive value but IL-16 combined with EEG increased the AUROC. Interleukin-6 did not show any association with three-year outcome.

This is the first report studying the association of interleukin 16 measured at birth with long-term outcome in a cohort of neonates with perinatal asphyxia. Interleukin 16 may be an early biomarker of severe injury and aid in long term prognostication in infants with hypoxic-ischaemic encephalopathy.

### 2.1.2 Introduction

Perinatal asphyxia is one of the most common causes of neonatal mortality and morbidity with 20 per 1000 deliveries requiring significant resuscitation (22). In countries with low neonatal mortality rates, 1.6 per 1000 of these will go on to develop hypoxic-ischaemic encephalopathy (HIE) (1). On a global level, HIE causes nearly one million neonatal deaths each year (2). In countries with access to the resource, therapeutic hypothermia has significantly improved mortality and neurodevelopmental outcomes in infants with moderate and severe HIE (3, 11).

Currently, standard management for HIE is to commence therapeutic hypothermia within the first 6 hours of birth. An early biomarker to guide diagnosis and prognostication in these infants could support clinical decision-making in this crucial timeframe.

The role of inflammation in neonatal hypoxic-ischaemic injury has long been recognized, though incompletely defined. Experimental models of perinatal injury have consistently shown evidence of microglial activation in response to hypoxia-ischaemia followed by the release of diverse inflammatory mediators including cytokines (17). The effect of this inflammatory response is potentially either advantageous or deleterious and debate continues on the effect of this balance in many forms of CNS injury (241). Raised inflammatory mediators, including interleukin-6 (IL-6), measured in postnatal blood and CSF samples, as well as on magnetic resonance spectroscopy, have previously been shown to be associated with adverse outcomes (242-244). Inflammation and infectious sensitisation appears to increase injury, and may also reduce response to therapeutic hypothermia (245).

Work previously published by our group, following analysis of multiple candidate analytes, reported two cytokines measured in umbilical cord blood, interleukin-6 (IL-6) and interleukin-16 (IL-16), which were significantly increased in infants with subsequent moderate to severe clinical and electroencephalographic (EEG) grades of encephalopathy. The combination of 10-min Apgar and IL-16 was able to predict abnormal EEG better than other markers utilized in current practice (148). Raised IL-6 in postnatal samples had been previously described in HIE to be associated with more severe injury and poor long-term outcome (243, 246).

Prior to this work, levels of IL-16 in neonatal hypoxia-ischaemia had not been described. However, IL-16 alterations had been identified in other pathologies of the nervous system, specifically multiple sclerosis, autoimmune encephalomyelitis, cerebral infarctions, spinal cord injuries and astrocytic brain tumours (241, 247-249). The role of IL-16 remains poorly understood. No study to date has examined the relationship between cord blood levels of IL-16 or IL-6 and long-term neurodevelopmental outcome following HIE.

The primary aim of this study was to explore the association between the levels of cord blood inflammatory proteins IL-16 and IL-6 collected at birth and neurodevelopmental outcome at 3 years in the same cohort of infants with perinatal asphyxia and hypoxic ischaemic encephalopathy.

### 2.1.3 Materials and Methods

Recruitment took place from September 2009 to June 2011 in a single maternity hospital and was designed to recruit all grades of HIE; mild, moderate and severe. Infants of ≥ 36 weeks gestation were recruited if they met any of the following risk factors for perinatal asphyxia: umbilical cord pH <7.1, five minute Apgar score ≤6 or the need for intubation or cardiopulmonary resuscitation at delivery. Presence and grade of HIE was determined by modified Sarnat score, assigned at 24 hours after birth by a dedicated research fellow (B.H.W). Written informed parental consent was obtained for inclusion. The Clinical Research Ethics committee of the Cork Teaching Hospitals provided ethical approval.

In clinically identified cases of neonatal encephalopathy infants underwent multichannel video-electroencephalogram (EEG) monitoring as previously described (250). The entire video-EEG was reviewed by an experienced neonatal neurophysiologist (G.B.B). One hour epochs of the background EEG were graded at 6 hours and 24 hours after birth where available. An EEG grade was assigned at these time-points, based on a previously described classification system (123, 251) and designated as: (0) normal i.e. sleep cycles present on continuous background; (1) mildly abnormal, i.e. continuous, mild asymmetry but disrupted sleep cycles; (2) moderately abnormal, i.e. excessive discontinuity or presence of seizures and (3) severely abnormal, suppressed/isoelectric tracing and/or high seizure burden/status epilepticus.

Demographic and clinical details were collected prospectively. These included perinatal measures routinely used in the evaluation of infants with perinatal asphyxia such as Apgar scores at 1, 5 and 10 minutes, as well as pH, lactate and base deficit measured on first postnatal sampling. Therapeutic hypothermia (TH) was commenced at the discretion of the supervising clinician on duty based on compliance with the Total Body Hypothermia Study (TOBY) criteria (126) within the first six hours of life.

Infants meeting the above criteria had umbilical cord blood drawn at birth and processed according to strict standardised operating procedures. The plasma was then frozen at -80°C within 3 hours of birth. IL-6 and IL-16 were measured using Luminex technology (Rules Based Medicine, Austin, TX). Further information regarding sample processing and analysis is available from Walsh et al. (148)

Developmental outcome was assessed at 36-42 months of age using the Bayley Scales of Infant and Toddler Development (Edition 3) [BSID-III]. A research fellow blinded to the clinical history, EEG findings and cord blood cytokine levels measured at birth of the patients performed all BSID-III assessments (C.E.A).Neurodevelopmental outcome severity was defined a priori. Three-year outcome was designated as normal if the child scored >85 in all three BSID-III subscales; cognitive, language and motor. A mildly abnormal outcome was determined if one or two subscales scored ≤85 but >70, and a moderately abnormal outcome was determined if all three subscales scored ≤85 but >70. Infants were deemed to have a severely abnormal outcome if they scored ≤ 70 in all subscales or suffered death, dyskinetic or spastic quadriplegic cerebral palsy or autism. (1, 87, 252)

Statistical analysis was performed using IBM SPSS Statistic 22.0. Patient demographic information is presented as median (interquartile range) and n/n (percentage) as appropriate. Differences between groups were examined using Kruskal-Wallis and Mann-Whitney U as appropriate. Correlation of data was performed using the Pearson correlation coefficient or the Spearman rho (ρ) correlation coefficient as appropriate.

The ability of the cord blood proteins to predict a severe outcome at three years was assessed using Receiver Operating Characteristic (ROC) curve analysis. Biomarker utility was determined using the area under ROC curve (AUC), and best cut-off was determined using the coordinates of the curve. Sensitivity and specificity for a given cut-off are reported. A *p*-value $<0.05$ was considered significant. Binomial logistical regression was used to examine combinations of significant markers for association with outcome and the predicted probabilities resulting from the model were also assessed for ability to predict a severe outcome upon analysis of the ROC curve.

### 2.1.4 Results

Umbilical cord blood analysis and neurodevelopmental outcome were available for 33 infants out of 69 recruited at birth. There were 22 male and 11 females, the median (IQR) gestational age at birth was 40.4 (39.6-41.2) weeks and the median (IQR) birth-weight was 3.49 (3.12-4.09) kg. Of these, 20/33 (60%) infants were given a clinical diagnosis of HIE in the neonatal period (11 mild, 4 moderate and 5 severe) while the remaining 13/33 (40%) were infants with clinical or biochemical signs of perinatal asphyxia at birth who recovered quickly with no clinical signs of encephalopathy.

A total of 7/33 (21%) infants, four with moderate HIE and three with severe HIE, were treated with therapeutic hypothermia. Of the two severe HIE cases that were not treated, one died before initiation of cooling and the other developed symptoms outside the therapeutic window for cooling. Two of the seven cooled infants died in the neonatal period. See demographics in *Table 2.1*.

At the three-year follow-up, 23/33 (70%) had a normal outcome, 1/33 (3%) had a mildly abnormal outcome, 3/33 (9%) had a moderately abnormal outcome and 6/33 (18%) had a severely abnormal outcome *(Table 2.2)*. Those with a normal outcome included 12 infants with perinatal asphyxia, six with mild encephalopathy, four with moderate encephalopathy, and one with severe encephalopathy according to modified Sarnat score at 24 hours.

The one child with a mildly abnormal outcome at three years had suffered a mild clinical encephalopathy. All three children with moderately abnormal outcome had a mild clinical encephalopathy in the postnatal period. The six children with a severely abnormal outcome included one infant with perinatal asphyxia, one with mild encephalopathy and four with severe encephalopathy. Those with a severely abnormal outcome included three neonatal deaths, one child who developed dyskinetic CP and two children with an autism diagnosis. All of the surviving cooled infants had normal neurodevelopmental outcome at follow-up.

| | **Non-Severe Outcome (n=27) Median (IQR)** | **Severe Outcome (n=6) Median (IQR)** | ***p*-value*** |
|---|---|---|---|
| **Gestational Age (weeks)** | 40.4 (39.2-41.1) | 40.8 (39.7-41.4) | 0.372 |
| **Gender (Male/Female)** | 17/10 | 5/1 | 0.637 |
| **Birth Weight (kg)** | 3.5 (3.2-4.1) | 3.4 (3.1-3.7) | 0.569 |

*Table 2.1: Demographic details of the cohort.* * *p*-value derived from Mann-Whitney U, chi-squared or Fisher's Exact test as appropriate

| OUTCOME | Normal (n=23) | Mild (n=1) | Moderate (n=3) | Severe (n=6) |
|---|---|---|---|---|
| **Sarnat Score** | | | | |
| *Perinatal Asphyxia* | 12 | 0 | 0 | 1 |
| *Mild* | 6 | 1 | 3 | 1 |
| *Moderate* | 4 | 0 | 0 | 0 |
| *Severe* | 1 | 0 | 0 | 4 |
| **EEG 24h (n=18)** | | | | |
| *Normal* | 4 | 0 | 0 | 0 |
| *Mild* | 3 | 1 | 2 | 0 |
| *Moderate* | 2 | 0 | 1 | 0 |
| *Severe* | 2 | 0 | 0 | 3 |
| ***IL-6 median (IQR) pg/ml*** | ***11 (5-24)*** | ***15 (N/A)*** | ***8 (7-8)*** | ***31 (5-255)*** |
| ***IL-16 median (IQR) pg/ml*** | ***403 (284-517)*** | ***381 (N/A)*** | ***304 (139-304)*** | ***646 (466-1086)*** |

*Table 2.2: Clinical data of cohort by detailed outcome severity.*

#### 2.1.4.1 IL-16

While median (IQR) IL-16, measured in cord blood at birth, failed to differentiate across all four outcome groups as outlined above, p=0.063 *(Table 2.2),* it was able to differentiate infants with a severely abnormal outcome compared to all others; 646 (466-1085)pg/mL v. 383.5 (284-494)pg/mL, *p*=0.012 *(Figure 2.1)*. IL-16 levels were able to predict a severe outcome with an AUROC (CI) of 0.827 (0.628-1.000), *p*=0.014. Levels ≥514pg/mL predicted a severe outcome with a sensitivity of 83% and a specificity of 81%.

Other perinatal and neonatal markers were tested for the prediction of severe outcome in this cohort *(Table 2.3)*. Biochemical markers of pH, lactate and base deficit, measured on first postnatal blood sampling did not differentiate between outcome groups. Apgar scores at 1 and 10 minutes of life were significant for prediction of a severe outcome, p=0.022 and p=0.036 respectively.

Among early neonatal tools used to assess and prognosticate in perinatal asphyxia, modified Sarnat score assigned at 24 hours of life and EEG graded at 6 and 24 hours of life all performed well for the prediction of severe outcome at three years with EEG at both time-points having a AUC ≥0.9, p=0.033 and 0.008 respectively. Each significant variable was combined with IL-16 pairwise to attempt to identify an improved predictive model. Combining IL-16 and Apgar score at 10 minutes of life, the combination shown to be most promising in our previous work, did not improve the AUC for predicting severe three-year outcome, AUC (CI) of 0.819 (0.593-1.000), *p*=0.017. This is likely due to the high degree of correlation between them, $R^2$=0.38, *p*<0.01. Other combinations are shown in *Table 2.3*.

Combining IL-16 with EEG grade at 6 or 24 hours of life improved the AUC (CI) to 0.978 (0.913-1.000), $p$=0.011 and 1.000 (1.0-1.0), $p$=0.008, respectively.

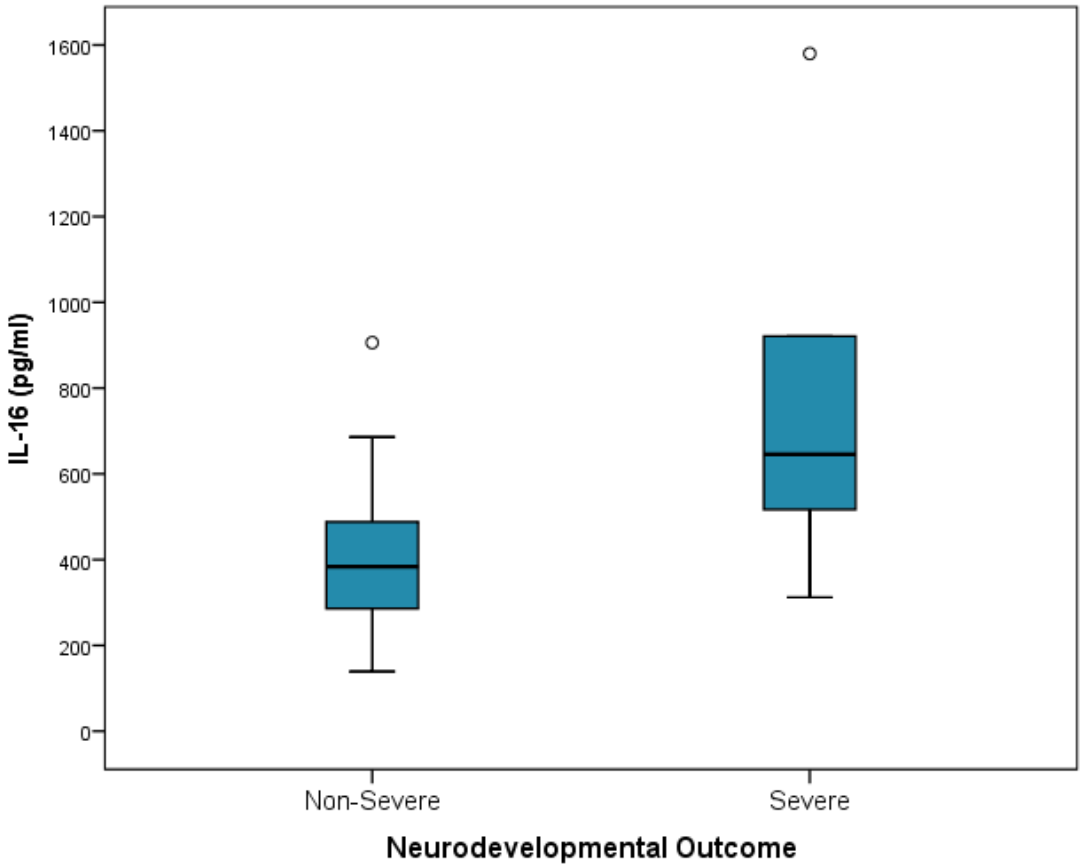



*Figure 2.1: Boxplot representing IL-16 levels (pg/mL) of infants with non-severe neurodevelopmental outcome compared to those with a severely abnormal outcome.*

| | Non-Severe | Severe | | Severe Outcome | |
|---|---|---|---|---|---|
| | (n=27) | (n= 6) | *p*-value | AUC (95%CI) | *p*-value |
| **IL-6 (pg/ml)** | 9.3 (5.5-19.0) | 31 (5.2-255.8) | 0.222 | 0.667 (0.388-0.945) | 0.208 |
| **IL-16 (pg/ml)** | 384 (284-494) | 646 (466-1086) | 0.012* | 0.827 (0.628-1.000) | 0.014* |
| **Biochemical Markers** | | | | | |
| **pH** | 7.04 (7.00-7.24) | 6.97 (6.61-7.19) | 0.429 | 0.636 (0.265-1.000) | 0.394 |
| **Lactate** | 8.2 (5.4-11.3) | 14.5 (10.6-21.8) | 0.067 | 0.803 (0.597-1.000) | 0.062 |
| **Base deficit** | 11.2 (8.9-13.6) | 12.7 (10.4-19.0) | 0.308 | 0.641 (0.391-1.000) | 0.288 |
| **Clinical Markers** | | | | | |
| **Apgar 1 min** | 4 (2-6) | 1 (0-3) | 0.021* | 0.802 (0.572-1.000) | 0.022* |
| **Apgar 5 min** | 6 (4-8) | 4 (0-7) | 0.109 | 0.713 (0.480-0.946) | 0.107 |
| **Apgar 10 min** | 9 (7-10) | 4 (0-9) | 0.035* | 0.780 (0.536-1.000) | 0.036* |
| **Sarnat Score** | - | - | - | 0.796 (0.551-1.000) | 0.025* |
| **EEG 6h** | - | - | - | 0.900 (0.754-1.000) | 0.033* |
| **EEG 24h** | - | - | - | 0.933 (0.815-1.000) | 0.008* |

| | | |
|---|---|---|
| **IL-16+Apgar 1** | 0.814 (0.554-1.000) | 0.018* |
| **IL-16+Apgar 10** | 0.819 (0.593-1.000) | 0.017* |
| **IL-16+Sarnat** | 0.827 (0.596-1.000) | 0.014* |
| **IL-16+EEG 6h** | 0.978 (0.913-1.000) | 0.011* |
| **IL-16+EEG 24h** | 1.000 (1.000-1.000) | 0.008* |

*Table 2.3: Median (IQR) values for clinical and biochemical makers available at delivery across outcome groups, with across group p-value from Mann-Whitney U testing, area under the ROC curve (95% CI), and p-value for ability of marker to predict severe outcome.* pH, lactate and base deficit were measured on first postnatal blood sampling. AUC= area under the receiver operator characteristic curve. * indicates *p*-value<0.05

Among surviving children who attended and could perform the BSID-III, n=27, levels of IL-16 were not found to correlate with scores in the individual subscales (Cognitive ρ=0.241, *p*=0.236, Language ρ=-0.016, *p*=0.944, Motor ρ=0.108, *p*=0.625).

#### 2.1.4.2 IL-6

IL-6 was unable to differentiate between infants with differing severity of neurodevelopmental outcome, *p*=0.520, and did not correlate with individual subscale composite scores (cognitive ρ=0.270, *p*=0.173, language ρ=0.221, *p*=0.299, motor ρ=0.018, *p*=0.935).

### 2.1.5 Discussion

Our analysis has shown an association between raised cord blood IL-16 and severely abnormal neurodevelopmental outcome at three years in a cohort of infants with perinatal asphyxia and HIE. These results must be carefully interpreted in the light of 50% follow up available and considerable overlap in IL-16 levels between infants with normal, mild and moderate outcomes. Due to the low numbers in the mild and moderate outcome groups we are limited in our ability to speculate reasons for this overlap.

Combination of IL-16 with other predictive clinical markers; Apgar and 1 and 10 minutes as well as EEG at 6 and 24 hours, showed equivalent or improved predictive value. These additional measures may in the future form part of a clinical decision algorithm for management of infants with perinatal asphyxia, however the subjective nature of Apgar scoring and the demands on resources of providing and interpreting continuous multichannel EEG may have bearing on this.

IL-16 is an unusual cytokine, which appears to have a crucial role in the CNS inflammatory response to injury. It is a glycoprotein of 56 kDA relative molecular mass and is mapped to chromosome position 15q26.1, a position distinct from all other cytokines (253). It was previously referred to as lymphocyte chemoattractant factor (LCF), reflecting its important inflammatory actions. These actions are both proinflammatory and immunomodulatory. IL-16 is produced in a variety of immune cells, including CD4+, CD8+ cells, monocytes and microglial cells as the inactive pro-IL16, which is then cleaved by caspase 3 from the active C-terminal (248).

Recently IL-16 has also been discovered to occur in a pre-formed state intracellularly in neutrophils. In normal circumstances, IL-16 becomes activated in a caspase-dependent manner during apoptosis. However, Roth et al. have shown that during pathological processes such as infection and auto-immunity, IL-16 may be activated as part of the cascade of necrotic cell death (254).

Active IL-16 has previously been shown to have a role in CD4+ cell recruitment and activation particularly in asthma and autoimmune conditions (255), but also following CNS injury, in particular following cerebral infarction (248). It is a potent chemoattractant and also has a regulatory function in cytokine induction, including IL-6, TNF-α and IL-1β (256). IL-16 is produced by microglia in response to CNS injury and in turn activates microglial migration to the site of injury and repair (257).

Following ischaemic infarction it reacts with CD4+ receptors and activates T-cell migration, through up-regulation of receptors such as T-cell CD25 and MHC Class 2 molecules. The neuronal variant, neuronal IL-16 (NIL-16), is indistinguishable from uncleaved IL-16 and has been found to be expressed in cerebellar granular cells and hippocampal neurons. NIL-16 may be intrinsic to neuronal growth and survival mechanisms (247, 258, 259).

These areas of the brain are also among those most sensitive to hypoxia (74, 82, 260). In studies of rat spinal cord injuries, IL-16 appears to accumulate significantly in cells within and adjacent to the lesion as well as in local microvasculature for several days after injury and it is therefore postulated to have a role in secondary damage (241). In the relapsing form of multiple sclerosis, IL-16 has come under close scrutiny for its possible role in neuroinflammation and axonal damage in this condition (247).

Similar findings have been described looking at a role for IL-16 in cerebral infarctions and in astrocytic brain tumours (248, 249). Along with its function in inflammatory cell activation and induction of proinflammatory cytokine release, IL-16 has also been associated with induction of increased intracellular calcium or inositol-(1, 4, 5)-triphosphate and translocation of protein kinase C, all of which can lead to neuronal ischaemic cell death (241).

There is, therefore, strong evidence of the involvement of IL-16 in the CNS response to injury and the results of this study are suggestive of a place for IL-16 in the pathophysiology of neonatal hypoxic-ischaemic injury though further investigation is required.

Based on the results of the current study IL-16 appears to be a biomarker worthy of further study for the prediction of short and long-term outcome following a hypoxic ischaemic insult. Raised levels are associated with more severe EEG findings in the neonatal period and more severe neurodevelopmental outcome at three years.

The lack of association between cord IL-6 and outcome in the present study may be related to the time-course of IL-6 response to injury. Previous studies have shown an association between postnatal IL-6 levels and injury severity but not with cord levels (246). Therefore, it may be that measurement at the time of birth is too early, and a later rise may be more helpful and more predictive of longer term outcome. Further discussion of IL-6 is available in our previous work by Walsh et al. 2013 (148).

Unfortunately, low numbers limit the interpretation of these results. These findings require validation in an alternate prospective cohort. The effect of treatment, where implemented, may be modulating outcome in these children and interferes with our full understanding of the meaning of a raised IL-16 level. However, this reflects the true clinical situation in a hospital with the resources to undertake such a study. Despite these results there is still much left to be learned about the role of IL-16 in these infants particularly about how severity of injury impacts levels. Additional investigation in animal models may help to further elucidate this process.

In this work we have shown, for the first time, the potential of an inflammatory protein measured in cord blood at birth to predict neurodevelopmental outcome at three years. With the development of a point-of-care testing platform, IL-16 levels could be measured quickly and contribute to clinical decision making within the crucial six hour time-frame.

## *2.2 Glial Fibrillary Acidic Protein is not an early marker of injury in perinatal asphyxia and hypoxic ischaemic encephalopathy*

### 2.2.1 Abstract

Brain specific glial fibrillary acidic protein (GFAP) has been suggested as a potential biomarker for hypoxic ischaemic encephalopathy (HIE) in newborns (147, 261). Previous studies have shown increased levels in postnatal blood samples. However, its ability to guide therapeutic intervention in HIE is unknown. Therapeutic hypothermia for HIE must be initiated within six hours of birth, therefore a clinically useful marker of injury would have to be available immediately following delivery.

The goal of our study was to examine the ability of GFAP to predict grade of encephalopathy and neurological outcome when measured in umbilical cord blood. Infants with suspected perinatal asphyxia (PA) and HIE were enrolled in a single, tertiary maternity hospital, where umbilical cord blood (UCB) was drawn, processed and bio-banked at birth. Expression levels of GFAP were measured by ELISA. In total 169 infants (83 controls, 56 PA, 30 HIE) were included in the study. GFAP levels were not increased in UCB of case infants (PA/HIE) when compared to healthy controls or when divided into specific grades of HIE. Additionally, no correlation was found between UCB levels of GFAP and neurodevelopmental outcome at 36 months.

### 2.2.2 Introduction

Perinatal asphyxia (PA) occurs when there is a disruption of oxygen delivery or blood supply to the foetus around time of birth. PA, when severe, leads to hypoxic-ischaemic encephalopathy (HIE) in the neonatal period. HIE remains one of the leading clinical challenges faced in the neonatal period and the greatest cause of acquired brain injury in term infants. The ability to optimise outcomes in neonatal HIE early and accurate prediction of the degree of encephalopathy is vital. Neuro-protective therapies must be commenced prior to the development of secondary brain injury, giving a narrow therapeutic window of less than six hours after delivery (126).

Approximately 20 per 1000 live births will require significant resuscitation at birth, and 10% of these infants will go on to have moderate to severe encephalopathy. Using currently available assessment methods it is estimated that 20% of infants with significant hypoxic injury are clinically misclassified in the first hours of life and do not receive hypothermia (262). Thus there is a critical need for improved biomarkers for prediction of grade of HIE and outcome (263).

Glial fibrillary acidic protein (GFAP), a monomeric intermediate filament protein predominant in astrocytes is known to display increased expression following acute brain injury or CNS degeneration (264). Previous studies, primarily carried out in adults have reported elevated levels of GFAP in traumatic brain injury, specifically related to focal mass lesions (265). Its use as a biomarker of HIE has previously been suggested, but the ability to differentiate between PA and HIE at birth has not been examined (147).

Furthermore, little work has been carried out to determine the use of GFAP as a marker of moderate/severe HIE specifically to guide therapeutic intervention. In order to improve our ability to identify infants who will benefit from therapeutic hypothermia in time for effective intervention, any clinically useful biomarker will need to be reliably altered at or soon after birth.

Therefore our aim was to examine the use of GFAP as an early clinical marker of HIE severity in full term neonates by determining expression in umbilical cord blood (UCB) samples from healthy control infants, infants with perinatal asphyxia and infants with HIE. We also wished to examine correlation of UCB GFAP with neurological outcome at 36 months.

### 2.2.3 Materials and Methods

#### 2.2.3.1 Study Population

The BiHIVE Study (Biomarkers of Hypoxic Ischaemic Encephalopathy), including all recruitment, consenting and sample processing procedures was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Through this study, any infant with suspected perinatal asphyxia or hypoxic ischaemic encephalopathy in Cork University Maternity Hospital (CUMH), Ireland born between May 2009 and June 2011, was recruited as previously described (154). Briefly, infants > 36 weeks gestational age were identified under strict enrolment criteria: cord pH<7.1 and/or Apgar score ≤ 6 at 5 minutes of life and/or requiring intubation or CPR at birth.

Once infants were stable, parents were approached and informed about the study, written consent was obtained for each infant. All clinical and demographic information deemed relevant to the study was then recorded prospectively. Healthy matched controls were recruited through the BASELINE Study (www.baselinestudy.net), a longitudinal birth cohort study based in Cork. Controls were matched for sex, gestation, birth-weight and gender and all had uncomplicated deliveries.

#### 2.2.3.2 Cord Blood Sampling

Umbilical cord blood samples were collected immediately after delivery for all infants in this study and processed within three hours following strict laboratory SOPs by a dedicated research team who were available 24 hours a day. Samples were stored at -80$^0$C in a monitored storage facility until analysis.

#### 2.2.3.3 Neonatal Assessment

All infants with suspected hypoxic ischaemic encephalopathy received continuous multi-channel EEG monitoring. Our protocol for EEG monitoring has been previously described (148). Clinical grade of encephalopathy was assigned by a dedicated research fellow (BW) using the modified Sarnat score. Additionally, grade was further confirmed using EEG analysis with an experienced neonatal electroencephalographer (GB) who reviewed all EEG data. Therapeutic hypothermia was commenced at the discretion of clinicians blinded to the study data in all infants deemed to have moderate or severe encephalopathy using the TOBY registry treatment criteria and protocol (126).

#### 2.2.3.4 GFAP Analysis

GFAP analysis was carried out by Banyan Biomarkers Inc., Alachua, Florida, on umbilical cord blood serum samples using Banyan Biomarker's proprietary sandwich Enzyme-Linked Immunosorbent Assays (ELISA) specific to GFAP. Detection levels for the Banyan Assay range from 0.03ng/ml to 50ng/ml. All samples were run in duplicate and inter/intra assay variability's of <10% were reported.

#### 2.2.3.5 Neurodevelopment Outcome

Where possible, developmental outcome was assessed at 36 months of age using the Bayley Scales of Infant and Toddler Development (Ed. III) [BSID-III] or the Ages and Stages Questionnaire (Ed. III) [ASQ 3] when the BSID-III was not possible due to reasons such as family relocation, etc. The Ages and Stages Questionnaire (ASQ 3) is a parent-completed developmental screening questionnaire.

It consists of 30 developmental items organised into five areas: Communication, Gross Motor, Fine Motor, Problem Solving and Personal-Social. Questionnaires for each child's appropriate age interval were administered.

All BSID-III assessments were performed by a dedicated research fellow blinded to the clinical history of the patients (CA). For infants who underwent the assessment using the BSID-III, outcome was determined using the composite scores of the three administered subscales; cognitive, language and motor. Infants were deemed to have an abnormal outcome if they scored ≤ 85 in two or more subscales or suffered neonatal death, cerebral palsy or autism. For infants who were assessed with ASQ3 only, results were considered abnormal if scores indicated the need for further assessment, falling > 2 SD below the standardised mean, was advised in more than one area.

#### 2.2.3.6 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (SPSS Inc., USA). Results were calculated using student t-tests, Mann-Whitney and Kruskal-Wallis tests, as appropriate.

### 2.2.4 Results

#### 2.2.4.1 Study Population

In total 169 infants were included in this study; 83 controls and 86 cases. Of the 86 cases, 56 were classified as perinatal asphyxia without HIE, 21 with mild HIE, five with moderate HIE and four with severe HIE, according to both the modified Sarnat assessment and EEG classification. Population demographics for the entire cohort are shown in *Table 2.4*.

#### 2.2.4.2 GFAP Expression

On analysis of ELISA results, there was no statistically significant difference in serum GFAP levels between case and control infants within this study ($p$=0.287). Similarly, when case infants were grouped as PA without HIE (n=56) and infants with HIE (n=30), no difference was observed ($p$=0.566, *Figure 2.2*). Additionally, when grade of HIE was specifically analysed, no difference was observed between UCB levels of GFAP in mild, moderate or severe HIE (0.199±0.095ng/ml vs 0.216±0.087ng/ml vs 0.168±0.258ng/ml, $p$=0.931). Finally, when grouped as infants who would be deemed eligible for therapeutic hypothermia (n=9) vs. those who would not (n=160), again no increase was observed ($p$=0.919, *Figure 2.3*).

#### 2.2.4.3 Neurodevelopmental Outcome at 36 months of age

In the total study population, outcome at 36 months was available in 116/169 (69%) infants (61 (73%) controls, 32 PA (57%) and 23 (77%) HIE). Of these, 70 infants underwent outcome assessment using the Bayley Scales of Infant and Toddler Development (Ed. III).

The remaining 46 infants underwent assessment using the Ages and Stages Questionnaire (ASQ 3). Further breakdown of grade of HIE and outcome is available in *Table 2.5*. When classed as infants with a normal outcome vs. infants with an abnormal outcome at 36 months, no discernible elevation in UCB GFAP levels was observed (*Figure 2.4)*.

| | ***Control*** | ***Perinatal Asphyxia*** | ***HIE*** |
|---|---|---|---|
| | **n=83** | **n=56** | **n=30** |
| ***Gestation (wk+day)*** | 40+3 (1+1) | 40+3 (2+1) | 40+3 (2+5) |
| ***Birth Weight (g)*** | 3518 (447.4) | 3596 (533.8) | 3495 (516.5) |
| ***Gender (M/F)*** | 48/35 | 35/21 | 20/10 |
| ***Mode of Delivery*** | | | |
| ***SVD*** | 31 (37%) | 18 (32%) | 6 (20%) |
| ***Instrumental*** | 36 (43%) | 28 (50%) | 15 (50%) |
| ***Elective Caesarean Section*** | 4 (5%) | …. | …. |
| ***Emergency Caesarean Section*** | 12 (15%) | 10 (18%) | 9 (30%) |
| ***1 min Apgar**** | 9 (9-9) | 5 (3-7) | 3 (1-5) |
| ***5 min Apgar**** | 10 (9-10) | 8 (6-9) | 5 (3-7) |
| ***Cord pH**** | 7.21 (7.15-7.26) | 7.04 (6.99-7.09) | 6.99 (6.91-7.08) |

*Table 2.4: Comparison of population demographics* for entire study cohort (n=169). Infants separated into controls, infants with perinatal asphyxia and infants with hypoxic-ischaemic encephalopathy (HIE). Data expressed as Mean (Standard deviation) or Median (Interquartile rage) where appropriate. ** Represents a p-value of <0.001 between groups calculated using Kruskal-Wallis or Mann Whitney U tests. M, male; F, female; SVD, spontaneous vaginal delivery*

| | ***Control*** | ***PA*** | ***Mild HIE*** | ***Mod/Sev HIE*** |
|---|---|---|---|---|
| ***Outcome*** | ***n=61*** | ***n=32*** | ***n=15*** | ***n=8*** |
| ***Normal*** | 60 | 30 | 9 | 4 |
| ***Abnormal*** | 1 | 2 | 6 | 4* |
| ***GFAP ng/ml*** | 0.20 (0.04-0.55) | 0.22 (0.04-0.14) | 0.20 (0.08-0.36) | 0.23 (0.05-0.63) |

*Table 2.5: Comparison of neurodevelopmental follow-up at 36 months of age*, primarily using the Bayley Scales of Infant and Toddler Development (Ed. III) (n=70) or the Ages and Stages Questionnaire (Ed. III) (n=46). No alteration in GFAP levels was detected in the umbilical cord blood from healthy control infants, infants with perinatal asphyxia (PA), infants with mild HIE or infants with moderate or severe (Mod/Sev) HIE. GFAP levels (ng/ml) are expressed as Mean (Range) * *Outcome defined by clinical diagnosis of CP or death*

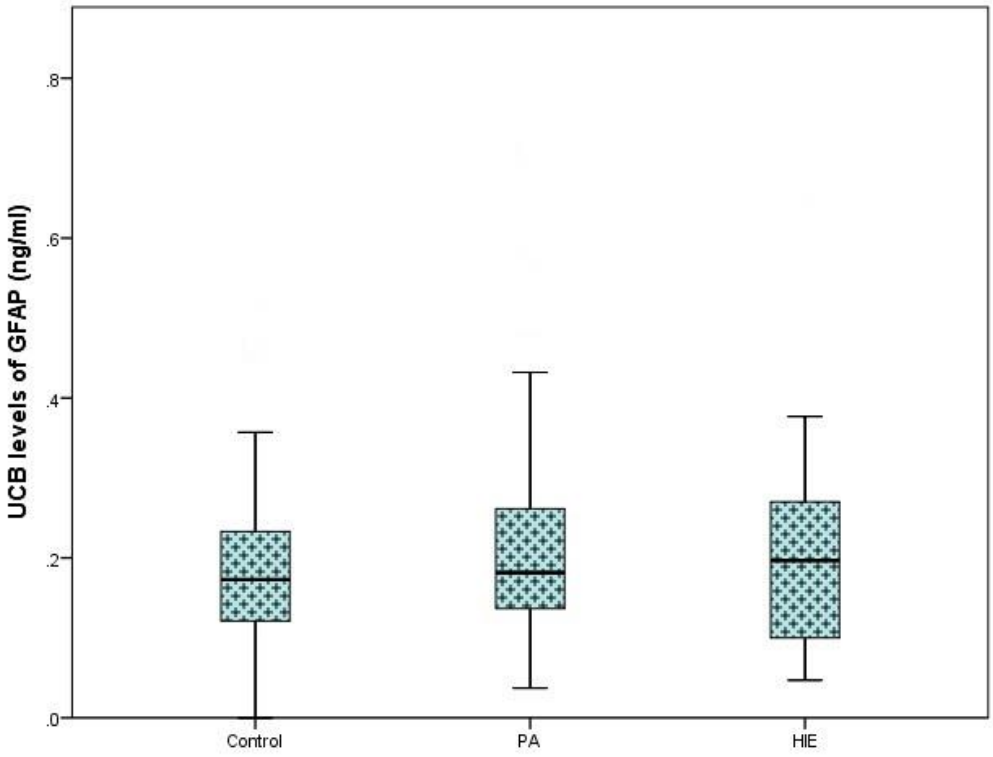



*Figure 2.2: Boxplot representing umbilical cord blood (UCB) levels of GFAP (ng/ml) following commercial ELISA analysis.* Infants grouped as healthy controls (n=83), infants with perinatal asphyxia (PA) without HIE (n=56) and infants with clinical and electrographically confirmed HIE (n=30). No significant alteration was detected between groups ($p$=0.566)

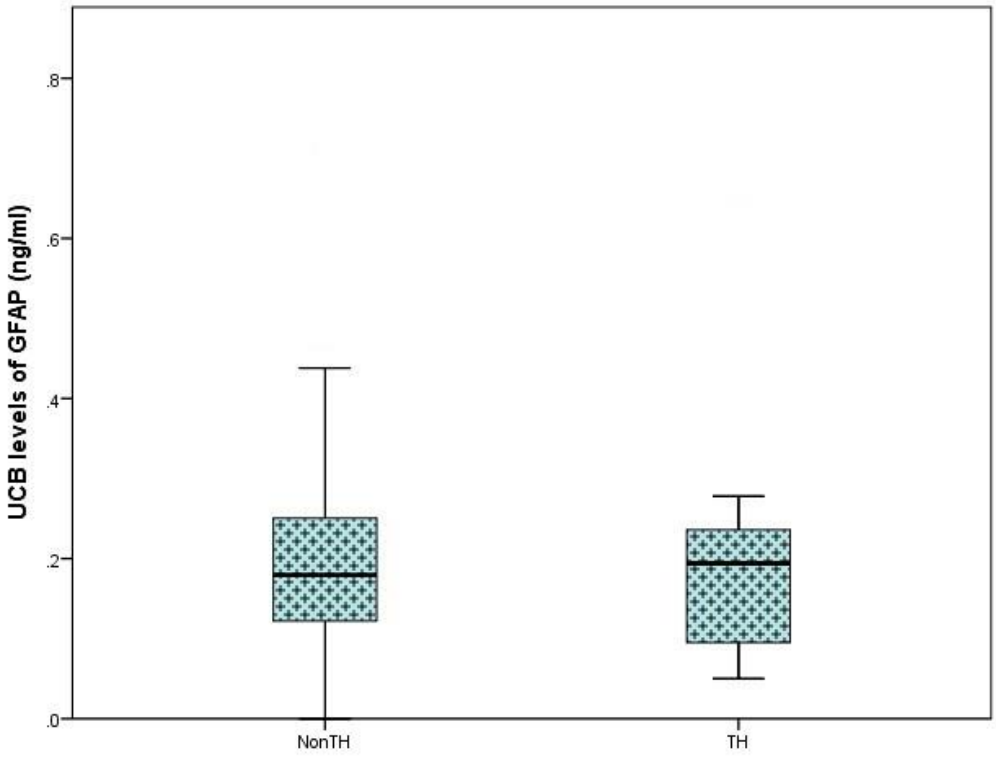



*Figure 2.3: Boxplot representing further analysis of umbilical cord blood (UCB) levels of GFAP (ng/ml) in infants which would be deemed eligible for therapeutic hypothermia* (TH; moderate and severe hypoxic ischaemic encephalopathy, n=9) and infants who would not meet the eligible criteria (NonTH; controls, perinatal asphyxia and mild hypoxic ischaemic encephalopathy, n=160). No significant elevation in GFAP levels was observed ($p$=0.919)

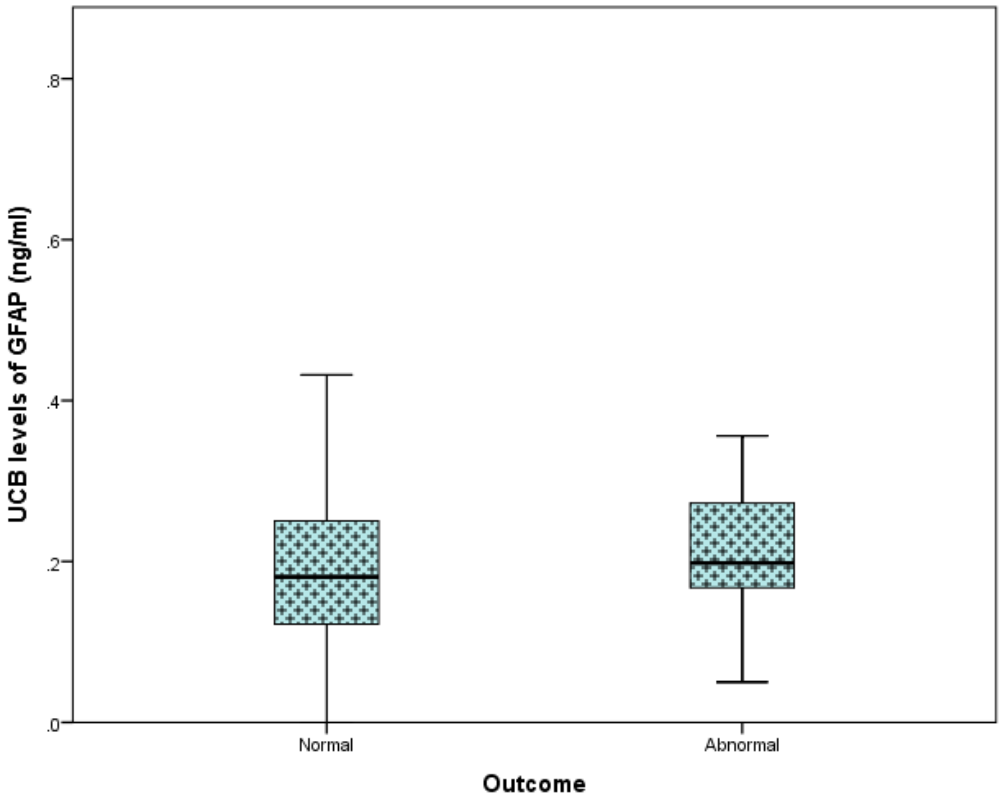



*Figure 2.4: Comparison of umbilical cord blood (UCB) levels of GFAP (ng/ml) from infants with a normal outcome at 24-36 months (n=103) compared to those with an abnormal outcome (n=13)*, presented as a box plot. A significant difference was not detected between groups ($p$=0.919)

### 2.2.5 Discussion

Increased levels of GFAP have been shown to be useful biomarkers of adult brain pathology (264, 266-271). In spite of this, investigations into its use in the neonatal field have been somewhat limited, with only a small number of publications suggesting GFAP as a biomarker in HIE with the ability to distinguish between grades of HIE (147, 261, 272-274). While these studies provided promising results, they also cited major limitations with regard to sample size and sample availability throughout. Postnatal blood samples were predominately used, with few studies determining levels in umbilical cord blood, which is arguably the earliest possible source for a biomarker of HIE. However, as HIE is known to encompass a two-stage evolving mechanism of injury, the time at which GFAP levels most represent degree of injury must be established.

In this study, our goal was to add to the current knowledge available for this promising biomarker specifically in relation to HIE. We aimed to validate the use of GFAP as a marker of HIE severity specifically and to overcome the previously cited limitations, mainly through the use of a larger, carefully defined population cohort and through the use of UCB as our sample source. All our samples are collected and processed in an operational biobank following strict SOPs. Additionally, UCB represents the earliest possible blood sample available after delivery. Although it is not taken directly from the infant, it provides a snapshot of an infant's circulation at the time of delivery and can therefore offer a unique insight into the neonatal condition.

This sample is relatively easy to obtain, does not involve an invasive procedure to an already stressed new-born and could potentially classify infants depending on degree of injury, contributing to rapid and reliable therapeutic decision-making.

A large library of previously published research focused on GFAP and CNS damage in general is available, however, a recent review of neonatal biomarkers by Bersani et al. (275) called for further work to investigate the validity of this protein with a particular focus on outcome. We discovered no significant alterations in GFAP expression between our case and control groups or between different grades of HIE. Additionally, we found no correlation of GFAP levels with outcome at 36 months of age.

Our findings initially seem to contradict previously published research regarding the potential use of GFAP as an early biomarker of moderate/severe HIE. However, most studies to date have focussed on post-natal samples, with significant elevation seen after 6-12 hours (147, 274). More recently, a study focussed on mixed cord samples in a historical cohort has shown no difference in serum GFAP levels between controls and infants with moderate-severe HIE (276). This is in keeping with previous work in adult TBI where peak levels occur at 12 hours post-injury.

This is the first prospective, carefully defined cohort study to focus on GFAP in UCB. Whilst there is good evidence that GFAP may be a useful marker of HIE severity in samples taken after 6 postnatal hours, we have not shown altered levels in UCB samples. This may limit the usefulness of GFAP as a useful biomarker to guide therapeutic intervention.

### 2.2.6 Conclusion

Serum GFAP is not altered in the UCB samples of infants with perinatal asphyxia, with or without clinical and electrographic HIE, compared to normal controls. GFAP therefore may not be the ideal early biomarker to predict eligibility for therapeutic intervention.

## *2.3 Downstream mRNA target analysis in neonatal hypoxic-ischaemic encephalopathy identifies novel marker of severe injury: a proof of concept paper.*

### 2.3.1 Abstract

#### 2.3.1.1 Background and Objective

Human microRNA miR-374a is downregulated in the umbilical cord blood (UCB) of infants with hypoxic ischaemic encephalopathy (HIE). The downstream targets of this microRNA (miRNA) are unclear, but one putative target is the activin-A receptor type IIb (ACVR2B). ACVR2B is required for activin-A function and previous reports have shown alterations of activin-A levels in neonatal HIE. Our aim was to investigate the expression of the potential downstream targets of miR-374a; activin-A and ACVR2B, at birth in a cohort of full term infants with perinatal asphyxia only (PA), and those with PA who developed clinical and electrographic HIE.

#### 2.3.1.2 Method

UCB was drawn and processed immediately after delivery. Levels of serum activin-A were measured using ELISA. mRNA levels of ACVR2B in whole blood were quantified using qRT-PCR. Outcome was assessed at three years of age using standardised developmental assessment.

#### 2.3.1.3 Results

In total 177 infants were enrolled; 88 healthy controls, 56 PA, 28 HIE. A statistically significant elevation of median (IQR) ACVR2B was detected in infants with severe HIE compared to moderate/mild HIE, PA and control groups (3.3(2.94-3.67) vs 0.91(0.55-1.21) vs 0.88(0.57-1.38) vs 0.84(0.74-1.24), *p*-values=0.04, 0.027, 0.025 respectively). Although serum activin-

A levels were elevated in infants with severe HIE, this elevation did not reach significance.

#### 2.3.1.4 Conclusion

ACVR2B may be a potential novel marker of HIE severity. This is the first study to examine the relationship between activin-A, its receptor AVCR2B and upstream miRNA miR-374a in cohort of carefully categorised and phenotyped infants. We have shown that miRNA analysis, combined with downstream target exploration may yield novel biomarkers for the prediction of HIE severity.

### 2.3.2 Introduction

Hypoxic ischaemic encephalopathy (HIE) remains one of the leading causes of neonatal mortality globally, resulting in an estimated one million deaths each year, with the same number of children surviving with long term disability (2). Early, rapid detection of infants at risk of hypoxic ischaemic brain injury immediately after delivery may lead to targeted therapeutic intervention, which in turn could reduce morbidity in this vulnerable population. Commencement of therapeutic hypothermia within six hours of birth, the only proven treatment for moderate and severe HIE (126), relies on swift and accurate identification of infants that are most at risk of brain injury. Although many potential markers have been studied, no early reliable biomarker of hypoxic brain injury has been validated for clinical use.

We have reported the downregulation of the microRNA (miRNA), hsa-miR-374a (miR-374a) in the umbilical cord blood of infants with HIE (154). As miRNAs can regulate target messengerRNA (mRNA) expression through suppression or degradation (277), examination of miRNA:mRNA relationships may provide novel insights into the pathology of conditions such as HIE. Using online databases (microRNA, TargetScan and miRBase), we investigated potential downstream targets of miR-374a. One target for miR-374a, repeatedly identified, is the activin-A receptor type IIb (ACVR2B), one of the primary receptors required for the functional activation of activin-A (278).

Activin-A, a member of the transforming growth factor (TGF-β ) superfamily, has been reported to be increased in the CSF, arterial cord blood and urine of infants with moderate and severe HIE (149, 279, 280). In addition, increases in activin subunits and receptor mRNAs following hypoxic ischaemic injury have

been reported in a Wister rat model of unilateral hypoxic ischaemic injury (281, 282), supporting the theory that activin-A is released into the bloodstream early after brain injury (283). No previous study has examined both ACVR2B and activin-A in human samples following hypoxic-ischaemic injury. Due to its likely role as a downstream target of miR-374a (currently the only miRNA reported to be downregulated in neonatal HIE), we wished to increase our understanding of this biological pathway.

To further elucidate the role of miR-374a in the pathophysiology of hypoxic ischaemic brain injury, we aimed to examine both activin-A levels and the expression of ACVR2B mRNA at birth in a cohort of infants with PA and HIE. We also aimed to correlate the levels of these protein, mRNA and miRNA markers with neurodevelopmental outcome at three years.

### 2.3.3 Methods

#### 2.3.3.1 Study Population and Sampling

This study is part of the BiHiVE Study (Biomarkers for Hypoxic Ischaemic Encephalopathy) which has received ethical approval from the Clinical Research and Ethics Committee of the Cork Teaching Hospitals. Specific details regarding recruitment, consent, clinical and electrographic assessment and cord blood sampling have been previously described (154, 284) but are outlines below.

Recruitment began in May 2009 in Cork University Maternity Hospital, Ireland following strict recruitment criteria, and concluded in June 2011. Infants were deemed eligible for the study if they were born at 36 weeks gestation or greater, and had one or more of the following: cord pH <7.1, Apgar score ≤6 at 5 minutes and/or required intubation or cardiac pulmonary resuscitation at birth. Infants were excluded from recruitment if the gestational age was <36 weeks at birth or if there were co-existing morbidities such as neonatal stroke, sepsis, metabolic encephalopathy or CNS malformation. Written informed consent was obtained for all infants recruited. Healthy control infants were recruited through a contemporaneous birth-cohort study (The Cork BASELINE Birth Cohort Study www.baselinestudy.net).

All infants with clinically suspected HIE had a standardised method of newborn neurological assessment (Amiel-Tison Neurological Assessment at Term) (160) and multi-channel EEG monitoring which commenced within the first 24hrs of life as per our previously described protocol (148).

Grade of encephalopathy was assigned using a modified Sarnat score at 24hrs after birth and confirmed by visual EEG analysis by an expert in neonatal EEG (GB). Our EEG grading system has also been previously reported (123). When a discrepancy occurred between clinical and electrographic grading of HIE, EEG was used to assign the HIE grade. Infants who required resuscitation at birth, met entry criteria, but did not subsequently develop clinical encephalopathy were defined as perinatal asphyxia (PA) without HIE.

#### 2.3.3.2 Umbilical cord blood samples

At birth, whole blood and serum samples were obtained from the umbilical cord of each infant, processed and stored within three hours from delivery of the placenta. These samples were collected and processed under strict standard operating procedures (SOPs). 3ml of cord blood was placed into TempusTM Blood RNA tubes (Applied Biosystems, Foster City, CA) and biobanked at -80$^0$C. Once collected and allowed to clot, serum samples were centrifuged at 2,400xg for 10 min at 4$^0$C followed by a second spin at 3000xg for 10 min to remove all red blood cells, before being stored at -80$^0$C.

#### 2.3.3.3 Activin-A Expression

To determine the concentration of activin-A in serum UCB blood samples, a commercially available DuoSet® (R&D Systems, Oxon, UK) was used according to manufacturer's instructions. The range of the assay was 125-8000 pg/ml and inter/intra assay variability's of <10% were observed.

#### 2.3.3.4 ACVR2B mRNA expression analysis

Total RNA was isolated from the whole blood in the Tempus system using the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit as per the manufacturer's instructions (Ambion, Life Technologies, Austin, Tx). First strand cDNA synthesis was then carried out utilizing the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies, Paisley, UK) according to manufacturer's instructions. ACVR2B mRNA expression was analysed using the TaqMan® Gene Expression Assay (Applied Biosystems, Life Technologies, Paisley, UK), using 18s as a reference gene, on a Rotor Gene 6000 (Corbett Life Sciences, Qiagen, Hilden, Germany). CT values were recorded for all samples and alterations in expression were analysed using the $2^{-\Delta\Delta Ct}$ method (285).

#### 2.3.3.5 Neurodevelopmental Outcome

Developmental outcome was assessed at 36 months of age using the Bayley Scales of Infant and Toddler Development (Ed. III) [BSID-III] or the Ages and Stages Questionnaire (Ed. III) [ASQ 3] when the BSID-III was not possible. All BSID-III assessments within this study were performed by a dedicated research fellow (CA) blinded to the clinical history of the patients. Composite scores of the three subscales; cognitive, language and motor were determined. If an infant was unable to attend for BSID-III assessment parents were asked to complete the Ages and Stages Questionnaire (Ed. III) [ASQ 3].

The Ages and Stages Questionnaire (ASQ3) is a validated, parent-completed developmental screening questionnaire. It consists of 30 developmental items organised into five areas: Communication, Gross Motor, Fine Motor, Problem Solving and Personal-Social. Questionnaires for each child's appropriate age interval were administered.

Infants were divided into two groups for analysis; a normal or abnormal outcome. Infants were assigned to the abnormal outcome group is they suffered neonatal death, cerebral palsy, autism or significant developmental delay. Infants were deemed to have significant developmental delay if they scored ≤ 85 in two or more subscales of the BSID-III. For those children who were assessed using ASQ alone, results were considered abnormal if scores indicated the need for further assessment, falling > 2 SD below the standardised mean in two or more areas.

#### 2.3.3.6 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (SPSS Inc., USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were run to establish if data was normally distributed. Following this, all parametric data was analysed using student t-tests or one way ANOVA followed by Tukey-b post hoc tests. Results are reported as mean (standard deviation). All non-parametric data was analysed using Mann-Whitney and Kruskal-Wallis tests, results are reported as median (IQR) or median [Min-Max]. Spearman's rank correlation coefficients were calculated to test the relationship between miR-374a and ACVR2B. Statistical significance was set at $<0.05$.

### 2.3.4 Results

#### 2.3.4.1 Study Population

In total 172 infants were included in this study; 88 controls and 84 cases. Of the 84 cases, 56 were classified as perinatal asphyxia without HIE (PA) and 28 with HIE (17 mild, 6 moderate HIE and 5 with severe HIE). Population demographics for the entire cohort are shown in *Table 2.6*. Of the 172 infants in the study, 154 had measurable serum levels of activin-A and 39 infants had RNA available for ACVR2B mRNA measurement. 22 infants had appropriate samples (serum and RNA) available for measurement of both activin-A and ACVR2B. Infant numbers for each analysis group are discussed in detail below.

#### 2.3.4.2 Activin A Expression

On analysis of ELISA results, 154 of the 172 infants recruited (90%), had measurable levels of activin-A, the remaining 10% were excluded as either no serum samples were available for analysis (11 controls and 5 cases ) or on analysis, measured levels were deemed outside of the ELISA assay range (>125pg/ml, 2 cases). No statistically significant difference in serum activin-A levels were observed between case (n=77) and control infants (77) within this study ($p$=0.684). Similarly, when case infants were grouped as PA without HIE (n=52) and infants with HIE (n=25), no difference was observed ($p$=0.621). An increase in activin-A expression was observed within the severe HIE group. However, this did not reach statistical significance, possibly due to the small sample size (n=3); (*Figure 2.5, Table 2.7*). An optimum cut-off value of ≥0.66ng/L in UCB was previously reported to identify infants with moderate and severe HIE with 93% sensitivity and 96% specificity (6). On testing this cut-off value within our cohort, a sensitivity and specificity of 44% and 62% were achieved respectively.

| | Control (C) | Perinatal Asphyxia (PA) | HIE (H) |
|---|---|---|---|
| | **n=88** | **n=56** | **n=28** |
| **Gestation (wks+d)** | 40+2 (0+1) | 40+3 (0+2) | 40+6 (0+3) |
| **Birth Weight (g)** | 3527 (403.8) | 3582 (562.7) | 3606 (602.1) |
| **Gender (M/F)** | 54/34 | 35/21 | 18/10 |
| **Mode of Delivery** | | | |
| **SVD** | 38 (43%) | 19 (34%) | 6 (21%) |
| **Instrumental Delivery** | 32 (36%) | 28 (50%) | 15 (54%) |
| **Elective Caesarean Section** | 7 (8%) | …. | …. |
| **Emergency Caesarean Section** | 11 (13%) | 9 (16%) | 7 (25%) |
| **1 min Apgar*** | 9 (9-9) | 5 (3-7) | 3 (1-5) |
| **5 min Apgar*** | 10 (9-10) | 8 (7-9) | 6 (3-7) |
| **Cord pH*** | 7.21 (7.14-7.26) | 7.03 (6.98-7.08) | 6.96 (6.91-7.09) |

** Represents a p-value of <0.001 between groups calculated using Kruskal-Wallis or Mann Whitney U tests. M, male; F, female; SVD, spontaneous vaginal delivery.*

*Table 2.6: Total Population Demographics. Data expressed as Mean (Standard Deviation) or Median (IQR)*

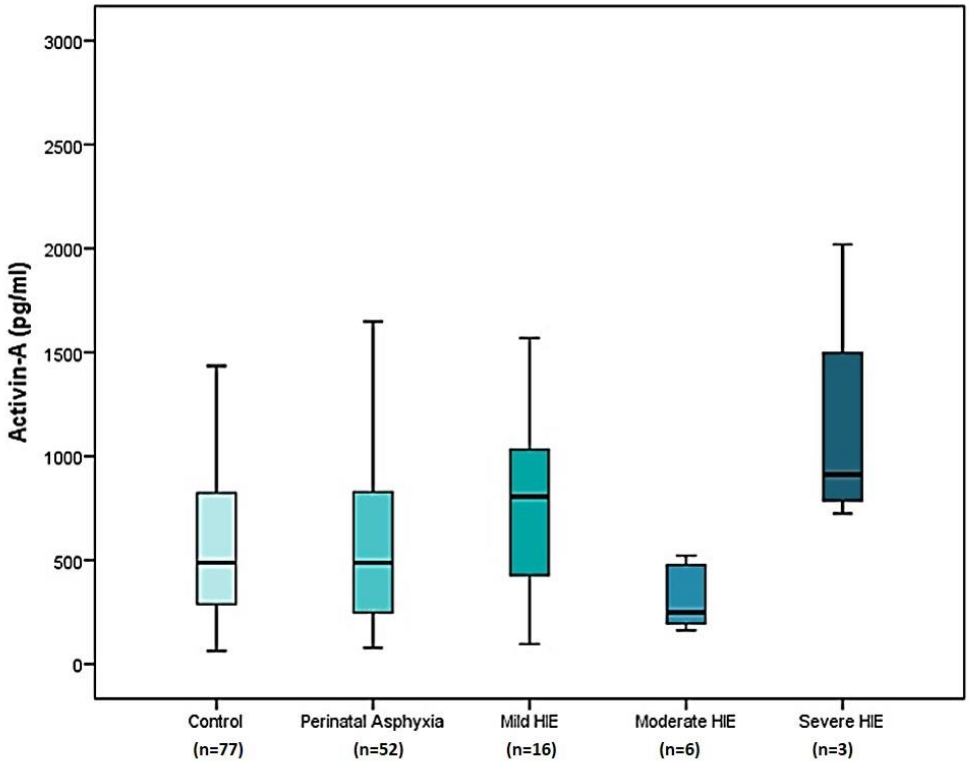



*Figure 2.5: Boxplot representing serum levels of activin-A (pg/ml) in healthy controls , infants with perinatal asphyxia (PA) without hypoxic ischaemic encephalopathy (HIE) and infants with confirmed mild HIE , moderate HIE and severe HIE.*

### 2.3.4.3 ACVR2B Expression

Umbilical whole blood samples suitable for mRNA analysis were available for 39 infants recruited to the study (13 control, 16 PA five mild, two moderate and three severe HIE). Analysis of qRT-PCR revealed an elevation of the ACVR2B expression in the umbilical cord blood of infants with severe HIE compared to all other groups (*Table 2.7*). Statistically significant elevations in expression were observed specifically between the control and severe HIE group ($p$=0.027) and between the perinatal asphyxia and severe HIE group ($p$=0.025) and between the mild/moderate and severe HIE group ($p$=0.04, *Figure 2.6*).

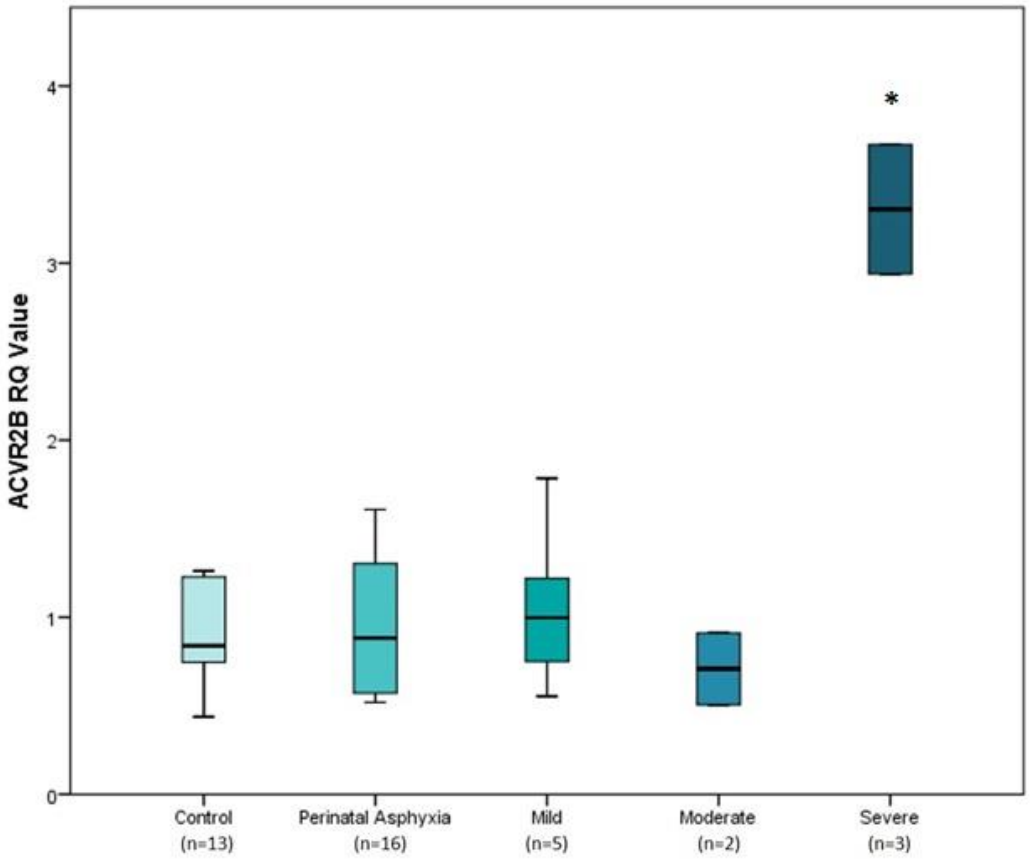



*Figure 2.6: Boxplot representing relative quantification (RQ) values of ACVR2B in umbilical cord whole blood in healthy controls (n=13), infants with perinatal asphyxia (PA) without hypoxic ischaemic encephalopathy (HIE) (n=16) and infants with confirmed mild HIE (n=5), moderate HIE (n=2) and severe HIE (n=3).* A significant increase in ACVR2B levels were observed between the control and severe HIE group (p=0.04) and between the PA and severe HIE group (p=0.03). * *represents a statistically significant difference of <0.05 between control and severe HIE group, between PA and severe HIE group and between moderate/mild HIE and severe HIE group.*

| ***Group*** | | ***Activin-A*** | | ***ACVR2B*** |
|---|---|---|---|---|
| | | ***pg/ml (n=154)*** | | ***RQ (n=39)*** |
| ***Control*** | ***n=77*** | 494.6 (279.8-838.1) | ***n=13*** | 0.838 (0.74-1.24) |
| ***PA*** | ***n=52*** | 516.3 (257.3-871.3) | ***n=16*** | 0.883 (0.57-1.38) |
| ***Mild/Moderate HIE*** | ***n=22*** | 604.5 (236,6-962.1) | ***n=7*** | 0.910 (0.55-1.21) |
| ***Severe HIE*** | ***n=3*** | 846.3 (724.6-2019.4] | ***n=3*** | 3.304 (2.94-3.67) |
| | | **Mann Whitney U Tests** | | **Mann Whitney U Tests** |
| | | **(p=)** | | **(p=)** |
| | ***(n vs. n)*** | | ***(n vs. n)*** | |
| ***Control vs. PA*** | ***77 vs. 52*** | 0.939 | ***13 vs. 16*** | 0.878 |
| ***Control vs. Mild/Moderate HIE*** | ***77 vs. 22*** | 0.626 | ***13 vs. 7*** | 0.874 |
| ***Control vs. Severe HIE**** | ***77 vs. 3*** | 0.071 | ***13 vs. 3*** | **0.027*** |
| ***PA vs. Mild/Moderate HIE*** | ***52 vs. 22*** | 0.716 | ***16 vs. 7*** | 0.947 |
| ***PA vs. Severe HIE**** | ***52 vs. 3*** | 0.088 | ***16 vs. 3*** | **0.025*** |
| ***Mild/ Moderate HIE vs. Severe HIE**** | ***22 vs. 3*** | 0.190 | ***7 vs. 3*** | **0.040*** |

*Table 2.7: Summary of all activin-A and ACVR2B comparisons between groups.* Data represented as Median (IQR) or Median [min-max] * *represents a statistically significant difference of <0.05 of ACBR2B expression*

#### 2.3.4.4 ACVR2B and miR-374a Correlation

Umbilical cord blood miR-374a levels for a number of infants within this cohort have previously been reported (154). Of the 39 infants with ACVR2B results in this study, 36 infants (13 control, 14 PA and 9 HIE) had previously reported miR-374a levels available for investigation. Spearman rank correlation analysis found a weak inverse correlation between levels of miR-374a and ACVR2B (r=-0.281, $p$=0.09).

#### 2.3.4.5 ACVR2B and Activin-A Correlation

Measurable activin-A and ACVR2B levels were available for 21 infants (2 control, 11 PA, 8 HIE). On analysis, a positive correlation was observed between levels of ACVR2B and activin-A (r=0.416, $p$=0.05), with both the mRNA and the protein increasing with level of HIE severity.

#### 2.3.4.6 Outcome at three years of age

Of the 172 infants in this study, four (severe HIE) died within the perinatal period and 101 (60%) underwent long-term follow-up at three years of age. Although all efforts were made to retain participants in the study, a number of families were non-contactable at the time of neurodevelopmental follow-up, resulting in a loss of numbers within our control and PA groups. 62 infants attended BSID-III appointments; 24 controls, 23 perinatal asphyxia, 10 mild HIE, four moderate HIE and one severe HIE, while a further 38 ASQ assessments were available. This 38 included 29 controls, seven perinatal asphyxia and two

mild HIE infants. Collectively, 53/88 controls (60%), 30/56 PA (54%), 21/28 HIE (75%) outcomes at three years were available.

90 of the 154 infants (58%) who had measurable activin-A levels received long term follow-up at three years of age; 45 control infants, 27 infants with PA only, 11 mild HIE, four moderate HIE and three infants with severe HIE, representing retention rates of 58%, 52% and 72% for controls, PA and HIE groups respectively. Of the 45 control infants, 44 had a normal developmental outcome. Of the infants with PA, 3/27 infants were found to have an abnormal outcome at three years of age. Finally of the 18 infants with HIE, 7/18 had an abnormal outcome at three years, including two deaths. All nine infants with abnormal outcomes underwent BSID-III assessments. Infants with an abnormal outcome (including those who died) had elevated levels of cord blood activin-A compared to those infants within the normal group, however, this elevation did not reach significance ($p$=0.15). Furthermore, as a substantial overlap of expression levels exist between groups, we failed to create an optimum cut-off value using ROC curve analysis (best attempt; 432pg/ml which achieved an AUC of 0.592, a sensitivity of 64%, specificity of 46%, positive predictive value of 14% and negative predictive value of 90%).

Of the infants who had reportable ACVR2B mRNA levels, two infants (severe HIE) died within the perinatal period and 20 (54%) had neurodevelopmental follow-up at three years of age; 19/22 with a normal outcome, and 3/22 with abnormal outcome. Although a trend to increased ACVR2B mRNA was observed in infants with abnormal outcome, the numbers in the abnormal group were too small to estimate statistical significance.

### 2.3.5 Discussion

We have shown that a combination of biomarker discovery techniques including transcriptomics and the investigation of downstream targets of miRNAs may provide candidate biomarkers in neonatal HIE. This is the first study to examine the relationship between activin-A, its receptor AVCR2B and upstream miRNA miR-374a in the same group of carefully categorised and phenotyped infants. We have observed a significant elevation of ACVR2B mRNA expression in infants with severe HIE compared to healthy controls, infants with PA or mild to moderate HIE. Additionally, we have attempted to validate the predictive efficacy of umbilical cord blood activin-A levels to determine grade of encephalopathy and subsequent neurodevelopmental outcome at three years of age. We have shown a greater increase in the expression of ACVR2B in umbilical cord blood compared to the binding protein activin-A.

The field of miRNA research continues to grow (286-288). As tiny non-coding RNA molecules which are structurally stable in the circulatory system (289) miRNA, potentially coupled with their downstream targets, have the potential to improve and even reform our current methods for classifying neonatal hypoxic ischaemic brain injury. Our recent work has identified a specific miRNA signature for neonatal PA and HIE in umbilical cord blood. Of the 70 miRNA found to be differentially expressed; the most promising was miR-374a (154). Very little is known about the function of miR-374a and as a result, it is unclear if alterations of miR-374a levels are the “cause or effect” of hypoxic ischaemic injury.

Online databases such as miRBase, TargetScan, and microRNA offer large repositories of miRNA and their predicted targets (290-292). Using specific algorithms, each database offers lists of potential targets for a chosen miRNA. Utilizing these

resources, we identified ACVR2B as a potential downstream target of miR-374a. The potential binding sites for miR-374a and ACVR2B are displayed in *Figure 2.7*. For this miRNA, target interaction with ACVR2B has yet to undergo functional confirmation. This confirmation will require expression manipulation and subsequent analysis of cultures cells using whole genome microarray analysis or by utilising newly reported miRNA-reporter gene assays as outlined by Carroll et al. (293). Our preliminary data in human umbilical cord blood taken immediately after birth is the first clinical evidence of the biological plausibility of this target pathway. We chose to first examine the clinical evidence in a carefully recruited cohort and hence a bedside-back-to-bench approach.

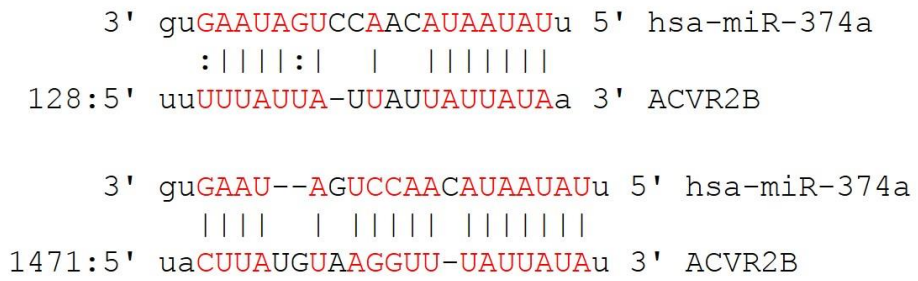



*Figure 2.7: Hsa-miR-374a / ACVR2B Alignment*

ACVR2B is a type 2 activin receptor known to bind activins and other TGF-β superfamily members. Functionally, these receptors are believed to be involved in the signal transduction pathway (294). While the majority of currently available literature on ACVR2B is focused on receptor interaction with myostatin and its role in muscle development and degeneration (295), it has also been revealed as a modulator of programmed neuronal cell death (296). The biological function of activin-A is activated

through binding with its receptor complexes and has been shown to have three to four times higher attraction to ACVR2B over ACVR2A (297). Upregulation of activin-A and upregulation of its preferred receptor may therefore occur in tandem, in response to hypoxic ischaemic injury.

The extensive expression of activin subunits and receptors has resulted in their association with a plethora of different mechanistic pathways and conditions, including HIE (298-300). In 1996, Lai et al. described increases in activin subunit and receptor mRNAs following hypoxic ischaemic injury, which were further exacerbated by subsequent stroke, in a unilateral model of hypoxic ischaemic injury in Wister rats (281). These observations were later confirmed by Wu et al. 1999, who additionally examined the neuro-protective potential of activin-A infusions following injury (282). Elevated levels of activin-A have also been reported in conditions involving other forms of neuronal damage, including intraventricular haemorrhage, paediatric open heart surgery and traumatic brain injury (283, 301) further supporting the belief that elevated levels of activin-A are detectable in the bloodstream following neuronal cell death.

The functional mechanism and pathways associated with miR-374a have yet to be revealed, however, the potential coupling of miR-374a and ACVR2B may play a role in the CNS response to hypoxic ischaemic injury. As small non-coding RNA, miRNA are believed to exert their functional power by regulating downstream mRNA expression through semi-complimentary binding which will either block translation of the mRNA or reduce the stability of the mRNA, and in this way mark it for degradation (288). However, it remains unclear if negative feedback loops exist within this system where mRNA or protein levels also exert an upstream effect to regulate miRNA expression. Nevertheless, it is generally believed that when a

specific miRNA is downregulated, expression levels of the downstream targets may be elevated due to a lack of repression. In this case, as we have shown, miR-374a is downregulated in infants with PA and HIE, with a corresponding elevation of ACVR2B mRNA and activin-A observed. Although a weak correlation has been found between miR-374a, ACVR2B and activin-A expression in this cohort, we acknowledge further validation using 3'-UTR binding luciferase assays are required to confirm the downstream binding of miR-374a with ACRV2B.

While the exact relationship between miR-374a and ACVR2B remains to be confirmed, we have observed a weak negative correlation between levels of miR-374a and ACVR2B when comparing levels within the same cohort of infants. Promisingly, we have shown a significant increase in ACVR2B mRNA levels in infants with severe HIE compared to other grades, non-encephalopathic PA and control infants, suggesting that ACVR2B analysis has potential as a method for the identification of infants with severe HIE at the time of birth.

Elevations of activin-A levels in biological fluids following perinatal asphyxia and HIE have been previously documented. Florio et al. have shown increased activin-A levels in a combined moderate and severe group of infants with PA (279, 280). Previous animal work with graded injury demonstrated increased activin-A expression in severe but not moderate HI injury (281). Although we have observed an increase in activin-A in cord blood samples from infants with severe HIE, we failed to replicate the results reported by the previously mentioned groups in our moderate graded infants. Our data also serves as a reminder that grouping moderate and severe HIE together may be misleading as the profile of infants with severe HIE can be biochemically very different to those with moderate HIE.

No previous study has investigated activin-A levels as a predictor of long term neurodevelopmental outcome. We observed an upregulation of activin A in the umbilical cord blood of infants who subsequently went on to have an abnormal outcome at three years of age. This group included one healthy control infant, three infants with perinatal asphyxia and five infants with mild HIE, none of which fit the current criteria for therapeutic intervention. In many cohorts, those with mild HIE or perinatal asphyxia are not included. It is possible that these infants have unrecognised injury detectable only by raised activin-A levels which may be largely attributable to HIE. Recently, Gagne-Loranger et al. observed that 40% of newborns with an initial diagnosis of mild HIE developed subsequent brain injury, particularly when they were not cooled (75).

Activin-A and its receptors have been reported to be key orchestrators of the immune system in response to inflammation by functioning as either a pro-inflammatory or anti-inflammatory agents depending on the activation state of the cell (302). Interestingly, activin-A has been shown to have an anti-inflammatory role in microglial cells by reducing the production of pro-inflammatory cytokines (303) such as Il-6, Il-8 and others which have previously been reported as increased following hypoxic ischaemic injury (304). Therefore, the increased levels of activin-A observed may have a neuro-protective effect. Our findings support previously reported animal and in vitro work which dubbed activin-A a potential “neuronal rescue agent” following hypoxic ischaemic injury and seizures, and highlighted the role of activin-A in neuronal survival and neuroprotection (282, 305-307). In particular, Wu et al. and Lai et al. have demonstrated increased neuronal survival in the hippocampus and dorsolateral striatum after direct intra-cerebro-ventricular infusions of activin-A after injury in an established rat model of HIE (281, 282). As activin-A expression increases

due to increased excitatory neurotransmitter release and injury, this increase may be viewed as an endogenous attempt to restrict the extent of neuronal loss, potentially through manipulation of neuro-active peptide expression (308) and facilitate recovery after HI injury.

While the findings reported here are promising, the limitations of this study must also be noted. As with most HIE studies the sample size, particularly in individual grades of HIE is very small. This number was further reduced within specific subsets i.e. activin-A, ACVR2B and outcome analysis. This reduction can primarily be attributed to the availability of whole blood RNA-preserved samples compared to serum samples and unavailability in 25% of cases for neurodevelopmental follow-up at three years of age. To ensure maximum retention of infants, the study used two different assessment techniques, one a screening parental questionnaire (the ASQ) and a direct assessment using BSID-III. Both have been validated for use in this age group. Although these techniques are not directly comparable, the ASQ has been shown to correlate well with BSID-III, and has an excellent negative predictive value meaning that we are unlikely to have missed significant disability (309). No abnormal outcome was defined solely by an ASQ assessment, allowing us to be confident of our outcome categorisation.

However, our study also has a number of strengths. For this study, we have used a contemporaneous cohort of in-born infants, recruited under strict enrolment criteria within a tertiary hospital and research centre. Case infants had continuous multichannel EEG monitoring and therapeutic hypothermia where deemed necessary and all laboratory processing and analysis was carried out using strict SOPs by experienced researchers. We are one of the only studies to include neurodevelopmental follow-up in both our control and PA infants, a factor which is usually

excluded. Also, while our overall retention rate is 60%, the rate within out HIE group specifically is 75%. To our knowledge this is one of the largest, most well-defined studies of HIE biomarkers and neurodevelopmental outcome to date and will add to the collective knowledge surrounding HIE injury. Importantly, we have shown that biomarker discovery using transcriptomic profiling and downstream target analysis can then be used to identify new potential mRNA or protein markers of disease severity in neonatal HIE.

### 2.3.6 Conclusion

We have shown that both activin-A and its preferred receptor, ACVR2B, are elevated in severe neonatal HIE at the time of birth. This adds to our knowledge of the potential downstream targets of miR-374a and supports further study of this biomarker pathway.

# 3.0 Cord Blood Metabolites and Neurodevelopmental Outcome in Perinatal Asphyxia

*Publications arising from this chapter:*

*"Early Cord Metabolite Index and Outcome in Perinatal Asphyxia and Hypoxic-Ischaemic Encephalopathy"*

*Ahearne CE, Denihan NM, Walsh BH, Reinke SN, Kenny LC, Boylan GB, Broadhurst DI, Murray DM.*

*Neonatology. 2016 Aug 3; 110 (4): 296-302*

## *3.1 Early Cord Metabolite Index and Outcome in Perinatal Asphyxia and Hypoxic-Ischaemic Encephalopathy*

### 3.1.1 Abstract

#### 3.1.1.1 Background

Two metabolite models measured in umbilical cord blood, the first derived from combined direct infusion and liquid chromatography mass spectrometry based on alterations of decenoyl-L-carnitine, 3,5-tetradecadiencarnitine, PC ae C38:0, phenylalanine and proline (model A), and the second derived from $^{1}$H-NMR spectroscopy based alterations of succinate, glycerol, 3-hydroxybutyrate and O-phosphocholine (model B) have shown potential for the prediction of hypoxic-ischaemic encephalopathy (HIE) severity.

#### 3.1.1.2 Objectives

To evaluate whether these metabolite indices can predict three-year neurodevelopmental outcome in infants with perinatal asphyxia and HIE, compared with current standard biochemical and clinical markers.

#### 3.1.1.3 Methods

From September 2009 to June 2011 infants at risk of perinatal asphyxia were recruited in a single maternity hospital. Cord blood was drawn and biobanked at delivery. Neonates were monitored for development of encephalopathy both clinically and electrographically. Neurodevelopmental outcome was assessed at 36-42 months using the Bayley Scales of Infant and Toddler Development Ed. III (BSID-III). Death, cerebral palsy and autism were also considered as abnormal end-points.

#### 3.1.1.4 Results

Model A measurements and neurodevelopmental outcome were available in 36 infants. Model A scores correlated significantly with outcome ($\rho^2$=0.18, $p$<0.01). This model could predict abnormal outcome with an area under ROC curve of 0.77, $p$<0.01.

Thirty-one infants had both metabolomic analysis under Model B and neurodevelopmental outcome at 36-42 months. The metabolite score significantly correlated with outcome ($\rho^2$=0.30 $p$<0.01), being robust to predict both severe outcome (area under ROC curve of 0.92, $p$<0.01) and intact survival (0.80, $p$=0.01).

There was no correlation between index scores and performance in the individual BSID-III subscales (cognitive, language, motor).

#### 3.1.1.5 Conclusion

These metabolite indices outperformed other standard biochemical markers at birth for prediction of outcome at three years. While further validation is required, these metabolite models could be used to provide important prognostic information to assist management planning in infants with perinatal asphyxia and HIE.

### 3.1.2 Introduction

Hypoxic-ischaemic injury in the perinatal period remains an important cause of neonatal encephalopathy and subsequent death and disability in children worldwide (1). The ability to predict severity in a timely manner is becoming increasingly important. Currently available assessment tools in hypoxic ischaemic encephalopathy (HIE) suffer from fundamental flaws that limit their usefulness (310). Foetal heart monitoring, Apgar scores, perinatal pH and lactate, lack sufficient sensitivity and specificity for the prediction of outcome (19, 102, 105, 110, 113, 311). The value of clinical and electrographic assessment is dependent on availability of resources and expertise and remains difficult to interpret due to the evolving nature of the underlying injury (130, 169, 170, 262). An early, robust, user-independent test that could aid prognostication is urgently needed.

Metabolomic analysis of umbilical cord blood is becoming an increasingly popular investigative field in maternal-neonatal medicine (312). Metabolomics can provide a phenotypic biochemical fingerprint by measuring complex interactions and concentration changes of multiple low molecular weight metabolites (313), while cord blood provides a potent source of early metabolic information. In HIE, metabolomics has the unique potential to detect rapid biochemical pathway alterations in response to the hypoxic-ischaemic environment (153). These changes may provide useful biomarkers to predict severity or quantify the hypoxic-ischaemic insult.

Using metabolomics, we have previously described alterations in the cord blood metabolite profile of infants with perinatal asphyxia and HIE (152, 250). Linear regression analysis revealed two separate metabolite models, the first based on alterations of decenoyl-L-carnitine, 3,5-tetradecadiencarnitine, PC ae C38:0,phenylalanine and proline (model A), the second, a

metabolite index, based on alterations of succinate, glycerol, 3-hydroxybutyrate and O-phosphocholine (model B). These models were promising for prediction of HIE severity, the first predicting HIE from all other outcomes (perinatal asphyxia and controls) with an area under the curve (AUC) of 0.92, the second predicting severe HIE with an AUC of 0.95. The long-term significance of these altered metabolite fingerprints is unknown. Therefore, the aim of this study is to correlate these metabolite models with three-year neurodevelopmental outcome in the same cohort. We also compare the performance of the best performing model against other standard biochemical, physiological and clinical markers available in the early neonatal period.

### 3.1.3 Method

#### 3.1.3.1 Patient selection

Recruitment took place from September 2009 to June 2011 in a single maternity hospital with 9000 deliveries per annum. Infants of ≥ 36 weeks gestation were recruited if they met any of the following risk factors for perinatal asphyxia: umbilical cord pH <7.1, 5 minute Apgar score ≤ 6 or the need for intubation or cardiopulmonary resuscitation at delivery. Eligibility required the infant to be in-born to allow the collection of umbilical cord blood, which was bio-banked within three hours of birth for metabolomic analysis. Written informed parental consent was obtained for inclusion. The Clinical Research Ethics committee of the Cork Teaching Hospitals provided ethical approval.

All consented cases had demographic and clinical details collected prospectively including Apgar scores at 1 and 5 minutes, degree of resuscitation as well as pH, lactate and base deficit measurements from arterial and venous cord sampling, and first postnatal sampling. Degree of resuscitation was classed as (i) none required, (ii) intermittent positive pressure ventilation >1min, (iii) intubation, (iv) CPR or (v) adrenaline given.

Cases were divided into (i) infants with clinical or biochemical signs of perinatal asphyxia at birth but who recovered quickly with no clinical signs of encephalopathy, or (ii) infants who developed an evolving clinical and electrographic encephalopathy consistent with HIE. HIE severity was determined by a modified Sarnat score assigned at 24 hours of life by a dedicated research fellow (B.H.W).

All cases underwent multichannel video-EEG monitoring as described (250). The entire video-EEG was reviewed by an experienced neonatal neurophysiologist (G.B.B). One hour epochs of the background EEG were graded at 6 hours and 24

hours after birth where available. An EEG grade was assigned at these time-points, based on a previously described classification system (123, 251), and designated as: (0) normal, sleep cycles present on continuous background; (1) mildly abnormal, e.g. continuous but abnormalities of sleep cycles; (2) moderately abnormal, e.g. discontinuity or presence of seizures and (3/4) severely abnormal, suppressed/isoelectric tracing and/or high seizure burden/status epilepticus.

Therapeutic hypothermia (TH) was commenced at the discretion of the supervising clinician on duty based on compliance with the TOBY criteria (126) within the first six hours of life.

#### 3.1.3.2 Three-year neurodevelopmental outcome

All eligible cases were invited for neurodevelopmental assessment between 36 to 42 months of age. The Bayley Scales of Infant and Toddler Development Edition III (BSID-III) was administered by a research fellow (C.E.A), blinded to the clinical background of the infants. Three-year outcome was designated as "normal" if the child scored >85 in the three BSID-III subscales; cognitive, language and motor. A mildly abnormal outcome was determined if one or two subscales scored ≤85 but >70, and a moderately abnormal outcome was determined if all three subscales scored ≤85 but >70. Due to low numbers, the mild and moderate outcome groups were combined into a "mild/moderate" group. A "severe" outcome included death, dyskinetic or spastic quadriplegic cerebral palsy (CP) or ≤70 in all BSID-III subscales.

#### 3.1.3.3 Metabolomic analysis

We have previously described the cord blood metabolomic profile in this cohort of infants with perinatal asphyxia and HIE

using combined targeted direct infusion (DI-) and liquid chromatography (LC-) tandem mass spectrometry (MS/MS) assay (AbsolutIDQ p180 kit, Biocrates Life Sciences AG, Innsbruck, Austria) (model A) and proton nuclear magnetic resonance ($^{1}$H-NMR) spectroscopy (model B). A detailed description of the metabolite extraction, spectra acquisition, metabolite identification and quantification is provided in this text (152, 250).

#### 3.1.3.4 Statistical analysis

Patient demographic information is presented as mean (standard deviation), median (interquartile range) and n (percentage) as appropriate.

##### 3.1.3.4.1 Model A

Concentrations of five metabolites were combined into a single biomarker using a simplified version of a previously reported metabolite predictive model (152):

$$y = \frac{proline \times 3{,}5 - tetradecadiencarnitine \times phenylalanine}{decenoyl - l - carnitine \times PC\ ae\ C38{:}0}$$

##### 3.1.3.4.2 Model B

Concentrations of four metabolites were combined into a simplified metabolite index derived from a previously published regression model predictive of HIE (250):

$$y = \frac{succinate \times glycerol}{3 - hydroxybutyrate \times O - phosphocholine}$$

Summary statistics for index scores, y, and other standard biochemical and clinical markers across each outcome group (normal, mild/moderate and severe) are reported. Differences between groups are examined using Kruskal-Wallis, Mann-Whitney U and chi-squared testing as appropriate. Correlation of non-parametric data is performed using Spearman's rho ($\rho$) correlation coefficient and reported as $\rho^2$.

The ability of the metabolite combination and other markers to predict a normal or severe outcome at three years was assessed using Receiver Operating characteristic (ROC) curve analysis. Index utility was determined using the area under ROC curve and best cut-off was determined using the coordinates of the curve. Sensitivity and specificity for a given cut-off are reported.

For examining the relationship between BSID-III subscale composite scores and metabolite index scores, metabolite scores were log transformed to normalise data and correlated with composite scores using Pearson's R. A $p$-value $<0.05$ was considered significant. All statistical analysis was performed using IBM SPSS statistics 21.0.

### 3.1.4 Results

#### 3.1.4.1 Study Population

##### 3.1.4.1.1 Model A

DI and LC-MS/MS metabolomic analysis was performed in 71 infants. Five infant samples failed metabolomic analysis and two were retrospectively excluded due to alternative diagnoses; one infant had a spinal cord injury and the other an inborn error of metabolism, potentially affecting their metabolomic profile at birth and/or outcome. Three-year outcome was available in 36/64 children, see *Table 3.1* for demographic information. Twenty-one of these 36 children were diagnosed with HIE based on a 24 hour Sarnat Score (12 mild, 3 moderate and 6 severe), the remaining 15 were part of the perinatal asphyxia group.

Following neurodevelopment assessment with the BSID-III, 23 children had a normal outcome and six children had a mild/moderately abnormal outcome. Five children with a normal outcome underwent TH, (one mild HIE, three moderate HIE and one severe HIE, defined by clinical Sarnat score). Seven children had a severe outcome. Four died during the neonatal period (one infant did not survive to initiation of therapy), and one developed dyskinetic cerebral palsy, microcephaly and epilepsy. This child did not receive therapeutic hypothermia as they were identified beyond the six hour treatment window. All five infants had severe HIE in the neonatal period. Two further infants were diagnosed with severe autism, one of whom had perinatal asphyxia at birth, the other mild HIE.

##### 3.1.4.1.2 Model B

For $^{1}$H-NMR spectroscopy, twelve infant samples from the cohort were excluded due to insufficient sample volume therefore metabolomic analysis was performed in 59 infants. As

above, two infants were retrospectively excluded due to alternative diagnoses. Outcome was available on 31/57 (54%) children, see *Table 3.1* for demographic information. Nineteen of these children were diagnosed with HIE based on a 24 hour Sarnat Score (10 mild, 3 moderate and 6 severe), the remaining 12 were part of the perinatal asphyxia group.

Using the BSID-III, 20 children were determined as having a normal outcome and six children as having a mild/moderately abnormal outcome. Four children with a normal outcome underwent TH, (three with a clinical grade of moderate HIE and one with severe HIE). All five children with a severe outcome were severely encephalopathic, four died during the neonatal period, and one developed dyskinetic cerebral palsy, microcephaly and epilepsy. There were no late deaths in the cohort and no infant with severe HIE had a BSID-III assessment.

*Table 3.1: Demographic details of the cohort*

| | **Model A** | | | | **Model B** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Normal** (n=23) | **Mild/Moderate** (n=6) | **Severe** (n=7) | ***p*-value** | **Normal** (n=20) | **Mild/Moderate** (n=6) | **Severe** (n=5) | ***p*-value** |
| **Gestational Age** (wks) | 40.6 (39.5-41.2) | 40.3 (38.1-40.8) | 41.0 (40.0-41.4) | 0.458† | 41.1 (40.2-41.2) | 40.3 (38.1-40.8) | 41.1 (40.2-41.4) | 0.209† |
| **Gender** (Male/Female) | 12/11 | 5/1 | 6/1 | 0.150†† | 11/9 | 5/1 | 4/1 | 0.326†† |
| **Birth Weight** (g) | 3630 (3170-4130) | 2955 (2842-3900) | 3430 (3220-4230) | 0.336† | 3860 (3390-4137) | 2955 (2843-3900) | 3430 (3285-4345) | 0.172† |
| **Sarnat Score** | | | | | | | | |
| PA/Mild/Mod/Severe | 14/5/3/1 | 0/6/0/0 | 1/1/0/5 | | 12/4/3/1 | 0/6/0/0 | 0/0/0/5 | |
| **Therapeutic hypothermia** | 5 | 0 | 3 | | 4 | 0 | 3 | |

†=Kruskal Wallis, ††=chi-squared, PA = perinatal asphyxia

### 3.1.4.2 Prediction of Outcome

#### 3.1.4.2.1 Model A

The median (IQR) metabolite model score significantly correlates with outcome ($\rho^2$=0.18, p=0.009) and significantly increases ($p$=0.032) as outcome deteriorates from normal 2240.6 (1909.6-3066.5) to mild/moderately abnormal 3252.1 (2808.3-4596.5) to severely abnormal 4067.5 (2393.8-5448.6) see *Figure 3.1.* Inspection of pairwise comparisons revealed the median metabolite model score for normal outcome was significantly different compared to the mild/moderate outcome group ($p$=0.043, $q$=0.128) and severe outcome group ($p$=0.039, $q$=0.117), though not when adjusted for false discovery. However, the model score could not differentiate between a mild/moderate and severe outcome ($p$=0.945, $q$=1.000).

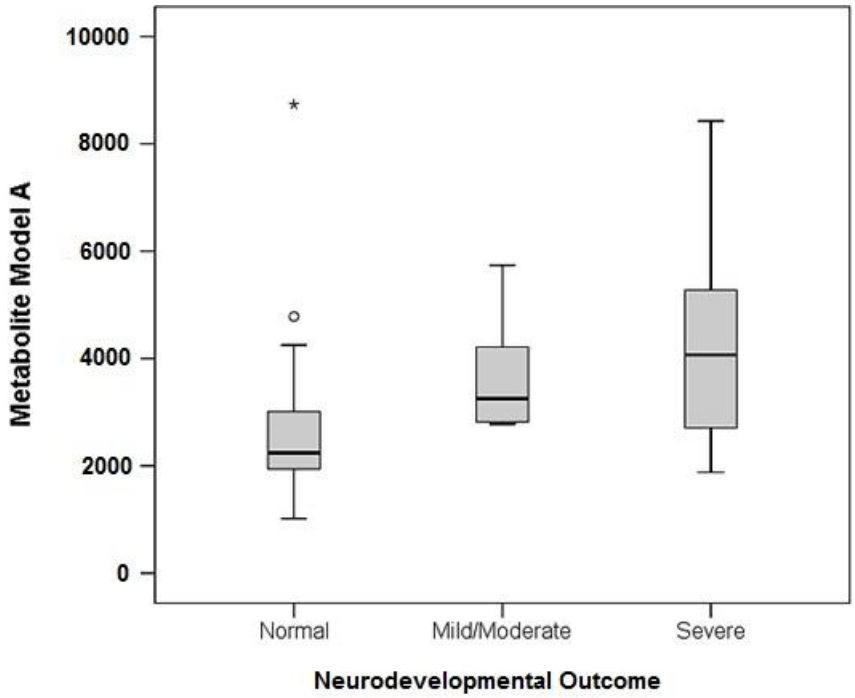



*Figure 3.1: Boxplot of metabolite model A across outcome groups*

This model may provide utility in predicting an abnormal outcome, therefore mild/moderate and severe outcome were grouped together (abnormal outcome group n=13) and compared to normal outcome (n=23). As expected the metabolite model was statistically altered between these two groups $p$=0.008. The model could predict an abnormal outcome with an AUROC of 0.77, $p$=0.009, see *Table 3.2 and 3.3*. Examination of the ROC curve revealed a score cut-off of y ≥ 2661.2 predicts an abnormal outcome with a sensitivity of 85% and a specificity of 65%.

The UCB metabolite model score had a stronger correlation with outcome group (ρ=0.429, $p$=0.009) than any of the standard markers assessed, see *Table 3.2*. Of the standard markers assessed, the Apgar scores at 1 and 5 minutes, and degree of resuscitation correlated with outcome group, and each significantly predicted an abnormal outcome. No correlation existed between outcome group and pH, lactate or base deficit (BD), either from cord sampling or first postnatal sample.

Focusing on surviving children for whom BSID-III was performed, i.e. normal (n=23) and mild/moderate (n=6) groups, the median (IQR) model score was significantly different between groups $p$=0.036 (2240.6 (1909.6-3066.5) *vs.* 3252.1 (2808.3-4596.5). However, when agreement between individual children's log transformed metabolite model scores and the composite scores of each BSID-III subscale were assessed, no significant correlation between the model scores, and the cognitive (R 0.055, $p$=0.777), language (R -0.208, $p$= 0.308) or motor (R 0.138, $p$= 0.511) composite scores was found (*Table 3.4 and Figure 3.2*).

*Table 3.2: Model A; Median (IQR) values for a selection of clinical and biochemical makers available at delivery across outcome groups.*

| | Normal | Mild/Moderate | Severe | | Correlation | | Abnormal Outcome[#] | |
|---|---|---|---|---|---|---|---|---|
| | (n=23) | (n=6) | (n= 7) | *p*-value[†] | $\rho^{2}$[††] | *p*-value | AUROC (95%CI) | *p*-value |
| **Metabolite Model A** | 2240.6 (1909.6-3066.5) | 3252.1 (2808.3-4596.5) | 4067.5 (2393.8-5448.6) | 0.032 | 0.18 | 0.009 | 0.77 (0.61, 0.93) | 0.009 |
| **Biochemical Markers** | | | | | | | | |
| **pH** | 7.13 (7.04-7.25) | 7.04 (6.94-7.14) | 7.12 (6.67-7.18) | 0.426 | 0.05 | 0.261 | 0.65 (0.43, 0.87) | 0.209 |
| **Lactate** | 9.0 (6.0-12.6) | 10.7 (7.1-12.0) | 11.0 (10.5-20.5) | 0.337 | 0.07 | 0.207 | 0.63 (0.40. 0.85) | 0.292 |
| **Base deficit** | 9.8 (4.4-14.1) | 9.1 (6.4-12.4) | 19.0 (10.1-22.5) | 0.135 | 0.08 | 0.164 | 0.64 (0.42, 0.86) | 0.233 |
| **Clinical Markers** | | | | | | | | |
| **Apgar 1 min** | 5 (3-6) | 3 (2-6) | 0 (0-2) | 0.018 | 0.19 | 0.008 | 0.73 (0.55, 0.91) | 0.024 |
| **Apgar 5 min** | 8 (5-9) | 5 (4-7) | 3 (0-7) | 0.020 | 0.22 | 0.004 | 0.77 (0.61, 0.93) | 0.008 |
| **Resuscitation** | | - | - | - | 0.19 | 0.009 | 0.74 (0.56, 0.92) | 0.022 |

Base deficit, pH and lactate were measured on admission to the NICU. † Kruskal-Wallis p-value for the difference across outcome group.

††Spearman's rho squared ($\rho^{2}$) correlation against outcome groups. [#]AUROC; area under the receiver operator curve and associated *p*-value testing the ability of each marker to predict severe and normal outcome.

*Table 3.3: Model A Median (IQR) values across outcome groups and predictive ability of the model.*

| | Metabolite model A | | |
|---|---|---|---|
| **Outcome group** | **Median (IQR)** | **AUROC (95%CI) †** | **p-value†** |
| **Normal (n=23)** | 2240.6 (1909.6-3066.5) | 0.766 (0.607 0.925) | 0.009 |
| **Mild/Moderate (n=6)** | 3252.1 (2808.3-4596.5) | 0.711 (0.541, 0.882) | 0.107 |
| **Severe (n=7)** | 4067.5 (2393.8-5448.6) | 0.704 (0.481, 0.982) | 0.097 |
| **Abnormal (Mild/Moderate/Severe) (n=13)** | 3368.2 (2798.1-5273.6) | 0.766 (0.607 0.925) | 0.009 |

AUROC; area under the receiver operator curve and associated *p*-value testing the ability of each marker to predict outcome group.

*Table 3.4: Model A; Median (IQR) values for cognitive, language and motor composite scores across outcome groups.*

| | **Normal Outcome (n=23) Median (IQR)** | **Mild/Moderate Outcome (n=6) Median (IQR)** | | **Correlation** | |
|---|---|---|---|---|---|
| | | | **p-value†** | **Pearson's R** | **p-value††** |
| **Cognitive Composite** | 95 (95-100) | 88 (84-96) | 0.013 | 0.055 | 0.777 |
| **Language Composite** | 109 (103-112) | 88 (79-103) | 0.010 | -0.208 | 0.308 |
| **Motor Composite** | 97 (91-103) | 85 (79-85) | <0.001 | 0.138 | 0.511 |

† *p*-value for between group differences calculated with Mann-Whitney U testing.

†† *p*-value for correlation between composite scores and log transformed metabolite model scores.

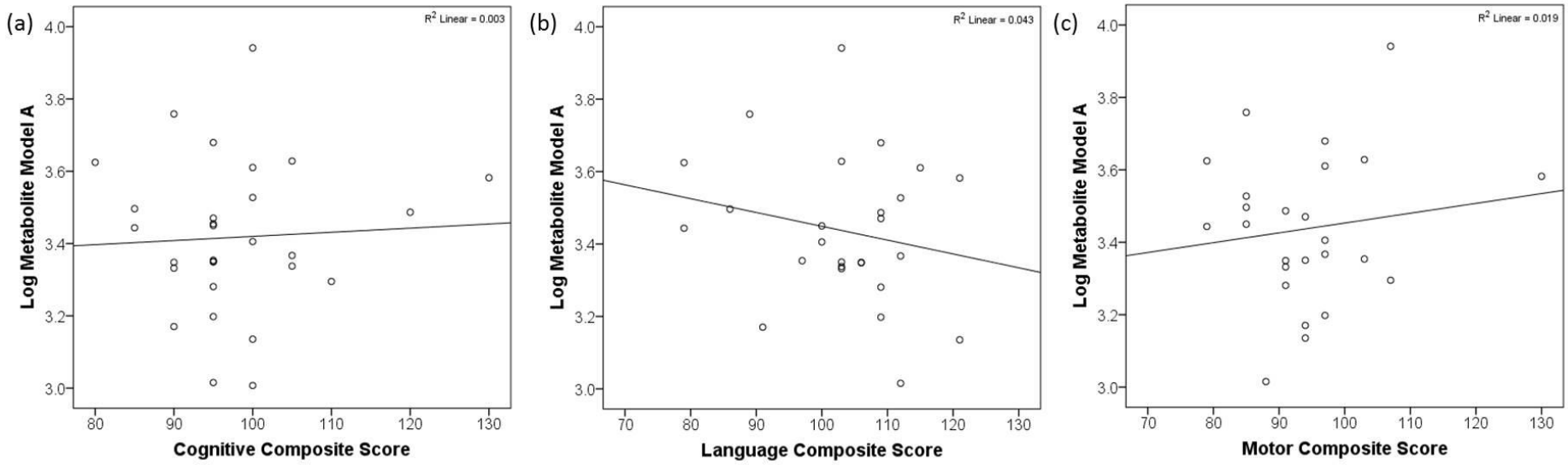



*Figure 3.1: Correlation between BSID-III composite scores, (a) cognitive, (b) language and (c) motor, and metabolite model A scores.*

3.1.4.2.2 Model B

The cord blood metabolite index derived from $^{1}$H-NMR spectroscopy had a stronger correlation with outcome group ($\rho^2$=0.30, $p$<0.01) than any of the standard markers assessed at birth, and predicted both a normal and a severe outcome with an area under the ROC curve of 0.80 ($p$=0.01) and 0.92 ($p$<0.01) respectively, see *Table 3.5*. Apgar scores at 1 and 5 minutes, and degree of resuscitation correlated with outcome group, each significantly predicting normal and severe outcome. There was no correlation between outcome group and pH, lactate or base deficit (BD), either from cord sampling or first postnatal sample. Similarly, none of these standard biochemical markers predicted normal outcome and only BD drawn from the first post-natal sample predicted severe outcome.

Unsurprisingly, both the modified Sarnat score and the continuous multichannel EEG's grade of encephalopathy, were strong predictors for both severe and normal outcomes. Encouragingly, the cord blood metabolite index, drawn hours before either the Sarnat score or the EEG was performed, had comparable predictive abilities to these well validated tools (*Table 3.6*).

Based on the coordinates of the ROC curve for the metabolite index, a score cut-off of y>2.4 predicted severe outcome with a sensitivity of 80% and a specificity of 100%. This index cut-off would have detected at birth all of those infants who subsequently died. It would have missed the child who had later developing symptoms and a poor outcome. Meanwhile an index score <0.13 predicts intact survival with a sensitivity of 65% and specificity of 91%.

*Table 3.5. Model B: Median (IQR) values for a selection of clinical and biochemical makers available at delivery across outcome groups* with across group p-value from Kruskal-Wallis testing, Spearman's rho squared ($\rho^2$) correlation against outcome groups, and area under the ROC curve (95% CI), and p-value for ability of marker to predict severe and normal outcome.

| | **Normal** | **Mild/Moderate** | **Severe** | | **Correlation** | | **Severe Outcome** | | **Normal Outcome** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (n=20) | (n=6) | (n= 5) | *p*-value | Spearman's $\rho^2$ | *p*-value | AUC (95%CI) | *p*-value | AUC (95%CI) | *p*-value |
| **Metabolite Model B** | 0.09 (0.05-0.16) | 0.15 (0.11-0.32) | 4.62 (2.18-10.69) | 0.01 | 0.30 | <0.01 | 0.92 (0.76-1) | <0.01 | 0.80 (0.64-0.97) | 0.01 |
| **Biochemical Markers** | | | | | | | | | | |
| **pH** | 7.07 (7.02-7.25) | 7.04 (6.94-7.14) | 6.95 (6.61-7.17) | 0.50 | 0.06 | 0.23 | 0.63 (0.25-1) | 0.42 | 0.65 (0.41-0.89) | 0.24 |
| **Lactate** | 8.6 (6.2-12.6) | 10.7 (7.1-12.0) | 14.5 (10.6-21.8) | 0.26 | 0.10 | 0.16 | 0.77 (0.53-1) | 0.11 | 0.65 (0.41-0.89) | 0.26 |
| **Base deficit** | 10.1 (5.7-17.1) | 9.1 (6.4-12.4) | 19.9 (13.6-23.3) | 0.05 | 0.11 | 0.13 | 0.89 (0.73-1) | 0.02 | 0.63 (0.39-0.87) | 0.32 |
| **Clinical Markers** | | | | | | | | | | |
| **Apgar 1 min** | 5 (3-7) | 3 (2-6) | 0 (0-1) | 0.01 | 0.26 | <0.01 | 0.94 (0.85-1) | 0.01 | 0.76 (0.58-0.95) | 0.02 |
| **Apgar 5 min** | 8 (5-9) | 5 (4-7) | 0 (0-5) | 0.02 | 0.25 | <0.01 | 0.86 (0.67-1) | 0.01 | 0.78 (0.61-0.95) | 0.01 |
| **Resuscitation** | - | - | - | - | 0.25 | 0.01 | 0.88 (0.69-1) | 0.01 | 0.77 (0.58-0.96) | 0.02 |

Base deficit, pH and lactate were measured on admission to the NICU. AUC = Area under the curve obtained with ROC analysis.

*Table 3.6. Model B: Correlation against outcome groups*, using Spearman's rho (ρ), and predictive ability of Sarnat score at 24 hours of life and continuous EEG grade at 6 and 24 hours of life for normal or severe outcome by area under the ROC curve.

| **Clinical Marker** | **Correlation** | | **Severe Outcome** | | **Normal Outcome** | |
|---|---|---|---|---|---|---|
| | Spearman's $\rho^2$ | *p*-value | AUC (95%CI) | *p*-value | AUC (95%CI) | *p*-value |
| **Metabolite Model B** | 0.30 | <0.01 | 0.92 (0.76-1) | 0.01 | 0.8 (0.64-0.97) | 0.01 |
| **Sarnat Score (24h)** | 0.41 | <0.01 | 0.98 (0.94-1) | <0.01 | 0.83 (0.68-0.97) | <0.01 |
| **EEG (6h)** | 0.42 | <0.01 | 0.96 (0.9-1) | <0.01 | 0.84 (0.7-0.98) | <0.01 |
| **EEG (24h)** | 0.56 | <0.01 | 1 (1-1) | <0.01 | 0.89 (0.78-1) | <0.01 |

AUC = Area under the curve obtained with ROC analysis.

Focusing on surviving children for whom BSID-III was performed, i.e. normal (n=20) and mild/moderate (n=6) groups, there was no difference in the median index (IQR) of infants with a mild/moderate outcome compared with normal outcome (0.15 (0.11-0.32) *vs.* 0.09 (0.05-0.16), $p$=0.18). The correlation between individual children's metabolite index scores, and the composite scores for the subscales of the BSID-III was assessed. This showed no significant correlation between the log transformed metabolite index scores, and the cognitive ($R^2$ 0.04, $p$= 0.32), language ($R^2$ 0.08, $p$= 0.18) or motor ($R^2$ 0.03, $p$= 0.46) composite scores (*Figure 3.3 and Table 3.7*).

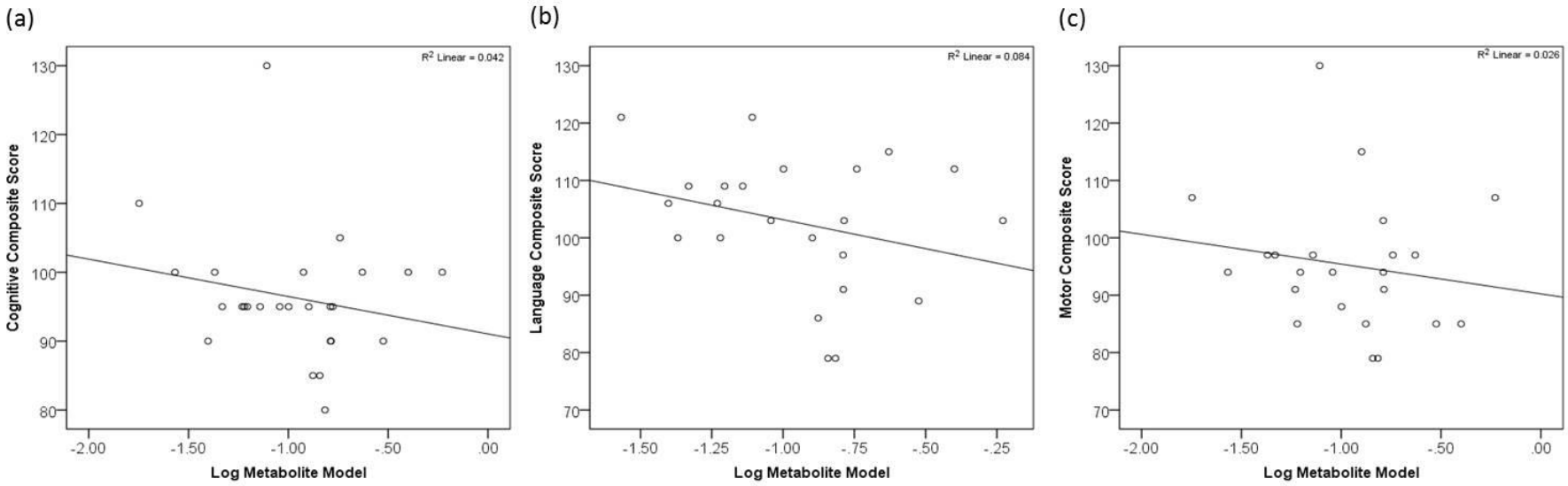



*Figure 3.3: Model B: Correlation between BSID-III composite scores and metabolite index B scores.* X-axis: a) cognitive, b) language and c) motor composite scores; Y-axis: Log transformed metabolite index

*Table 3.7: Model B: Median (IQR) values for cognitive, language and motor composite scores across outcome groups* and p-value for between group differences calculated with Mann-Whitney U testing. Pearson's R value and p-value are shown for correlation between composite scores and log transformed metabolite index B scores.

| | **Normal Outcome** | **Mild/Moderate Outcome** | | **Correlation** | |
|---|---|---|---|---|---|
| | Median (IQR) | Median (IQR) | p-value | Pearson's $R^2$ | p-value |
| **Cognitive Composite** | 95 (95-100) | 88 (84-96) | 0.02 | 0.04 | 0.32 |
| **Language Composite** | 106 (102-112) | 88 (79-103) | 0.01 | 0.08 | 0.18 |
| **Motor Composite** | 97 (94-105) | 85 (79-85) | <0.01 | 0.03 | 0.46 |

### 3.1.5 Discussion

In this cohort, we have examined the ability of two novel metabolite models to predict three-year neurodevelopmental outcome in a cohort of infants with perinatal asphyxia and HIE. We compared this to the ability of commonly used biochemical, physiological and clinical markers routinely measured in the perinatal and early neonatal period to predict outcome.

In terms of tools available at delivery, model A scores calculated from endogenous cord blood metabolites measured at birth performed well at predicting an abnormal outcome, as did 1 and 5 minute Apgar and degree of resuscitation. However, when the best cut-off score was chosen, ($y \geq 2661.2$) the model suffered from relatively low specificity, misclassifying several normal outcomes.

Model B scores performed well, as did 1 and 5 minute Apgar scores, for the prediction of both severe outcome and intact survival. Severe outcome was however predominated by infants who subsequently died and all infants with an index above the proposed cut-off of 2.4did not survive. Indeed, the only child to survive with a severe outcome was not predicted by this marker. This child had delayed evolution of encephalopathic symptoms perhaps related to the timing of injury and failed to meet cooling criteria within the first six hours of life. This marker may therefore have greater value in objectively identifying those at higher risk of death and may help to inform difficult decisions regarding redirection of care.

The biochemical markers routinely measured at delivery did not perform well at predicting intact survival with normal neurodevelopment in childhood. The metabolite models outperformed currently available markers in this regard. Indeed, for model B, all four children (three moderate HIE, one severe HIE) who received therapeutic hypothermia and had a normal

outcome also had a low metabolite index score. However, we cannot distinguish between the ability of the low metabolite score to predict those with a low risk of injury at birth, or to predict those with a good chance of responding well to therapeutic hypothermia. As it is not ethical to examine the score in a non-cooled cohort this question may be difficult to answer.

The metabolite index, whilst not ideal, showed superior predictive ability compared with all other biochemical markers currently available at delivery. These markers are in active use as part of clinical decision making and are central to the criteria for initiation of therapeutic hypothermia (126, 314). Even the stalwart Apgar score has certain disadvantages. Apgar scores are generally found to be most useful at extremes but, despite this, one in five infants with an Apgar score of 0 at 10 minutes of life will survive to school age without moderate or severe disability (113). In our cohort, even an Apgar score ≥ 6 at 5 minutes could only predict a normal outcome with a sensitivity of 65% and a specificity of 72%. Apgar scores also suffer from high inter-observer variability (114).

As expected, Sarnat score and continuous EEG grading performed at 24 hours of age correlated highly with outcome groups. However, while these tools are crucial to evaluation of and longer term prognostication in hypoxic-ischaemic injury, EEG analysis, in particular, requires significant expertise to utilise to its potential which may not always be available and both tools provide prognostic information at a later time point compared to cord blood analysis. An objective early user-independent biomarker would be useful for clinicians, particularly in situations necessitating transfer of patients for therapeutic hypothermia.

Metabolite combinations measured at birth could potentially support clinical decision making and contribute to a management algorithm for perinatal asphyxia alongside clinical markers mentioned above and EEG findings. As technology advances the ability to rapidly measure a combination of metabolites in a point of care device in the labour ward or NICU is a realistic possibility. Already there are bedside devices in use that measure similar small molecules such as glucose, lactate, cholesterol and ketones.

The biological plausibility of the index's metabolites strengthens our confidence in its predictive potential. For Model A, the involvement of the five metabolites discussed here in the pathophysiology of perinatal asphyxia and subsequent neurological injury has been further explicated in our previous work (152). With regards to Model B, hypoxia-ischaemia leads to alterations in cellular energy pathway molecules, such as glycerol and succinate, via mitochondrial dysfunction and subsequent disruption of the tricarboxylic acid cycle (151). Altered o-phosphocholine has been described in animal models of hypoxia-ischaemia (315). It is both an anabolic and catabolic metabolite of cell membrane metabolism and may indicate, along with glycerol, cellular membrane breakdown (316). The brain's ability to upregulate the metabolism of ketone bodies, in order to provide an alternative energy source, is an important means of cerebral adaptation in early life (317). 3-hydroxybutyrate's contribution to the model may indicate defective ketone production, complete consumption of ketone supply or inadequate prior adaptation to ketone metabolism, causing the secondary energy failure to become insurmountable and result in long-term damage. Additional information is available in Reinke et al (250).

The novelty of this study relies on the uniquely recruited cohort of term infants, for whom perinatal asphyxia and HIE have been

carefully defined both clinically and electrographically, alongside biospecimens that were meticulously biobanked and long term neurodevelopmental assessment. A scarce number of biomarker studies have been able to correlate biomarker findings in early life with infant outcome.

The present study had some limitations. Difficulties with study participant retention as well as the low rates of hypoxic-ischaemic injury in the high-income countries where biomarker research is possible, present significant ongoing challenges to successful biomarker discovery and validation (1, 8). As a result, the number of infants with abnormal outcomes in this cohort is low thus limiting interpretation of these results. Furthermore, treatment with therapeutic hypothermia complicates these studies by confounding our ability to accurately grade outcome. Pre-clinical validation of this metabolite index in a larger, independent cohort of HIE is essential, however this will require a multi-centre approach.

To date, this work has been focused on biomarker discovery. The highly dynamic nature of the metabolome is constantly affected by genetic oversight and environmental influence. Thus, metabolite concentrations will change rapidly over time. Studies in animal models of hypoxia-ischaemia will be helpful in identifying the timed detection of the metabolite alteration. Tracking metabolic changes through post-natal sampling over time will also help establish the pattern of change and speed to recovery of the metabolites. Furthermore, we need to unearth the long-term implications of this early metabolic dysfunction and its potential to lead to ongoing or tertiary brain injury (64, 66).

### 3.1.6 Conclusion

In conclusion, within the limitations of this study, we have demonstrated the potential of metabolite index scores measured at birth to objectively identify infants at high risk of death in the case of a raised score and those with the possibility of a normal outcome if the score is low. Model scores derived from metabolites measured at birth have the potential, in conjunction with important markers such as Sarnat staging and EEG, to provide a user-independent test that could guide critical management decisions in the early newborn period.

# 4.0 Regional Variation in the Bayley-3: a low risk healthy Irish population at 2 years

## *4.1 Abstract*


### 4.1.1 Objective

To investigate the performance of a low-risk healthy Irish population on the Bayley Scales of Infant and Toddler Development (Edition 3), given previous evidence of regional variation.

### 4.1.2 Design

A nested cohort study within the population based birth cohort study, the BASELINE Study, of low-risk singletons attending for developmental assessment at 2 years of age.

### 4.1.3 Setting

A single, tertiary maternity hospital in Cork, Ireland, with 9000 deliveries per annum.

### 4.1.4 Patients

240 children, known to be low risk healthy infants were included in analysis. Exclusions included a history of intra-uterine growth restriction, prematurity, hypoxic-ischaemic encephalopathy or congenital anomalies.

### 4.1.5 Main Outcome Measures

Children were assessed with the Bayley Scales of Infant and Toddler Development (Edition 3) at 2 years of age. Comparisons were made with U.S. and available U.K. normative data.

### 4.1.6 Results

Language and fine motor scores (mean±SD) were significantly higher compared to U.S standardized normative values, 109±13 v. 100±15, $p<0.001$ and 11.5±2 v. 10±3, $p<0.001$ respectively. This showed medium to large positive effect sizes. When compared with U.K. data, language and fine motor skills remained significantly higher 109±13 v. 102±17, $p<0.001$ and

11.5±2 v. 10.9±3, $p<0.001$, while cognitive scores were reduced 9.83±2 v. 10.7±3, $p<0.001$.

### 4.1.7 Conclusion

There is a need for regional population norms to assist clinical interpretation and use of scores. For research purposes this work also emphasises the importance of a contemporary control population.

## *4.2 Introduction*

The Bayley Scales of Infant and Toddler Development is a widely used, individually administered assessment of developmental functioning. The Bayley Scales have been used both in clinical practice and in research to identify infants at risk of developmental delay and to provide information for intervention planning.

The test is designed for infants and toddlers aged 16 days to 42 months and 15 days of life. It consists of 3 administered subscales (made up of 5 subtests); cognitive, language (receptive communication and expressive communication) and motor (fine and gross motor). The initial assessment was developed in 1969, but it has since been revised in 1993 and, most recently, in 2006 when Edition 3 (BSID-III) was introduced (174, 318, 319). For the BSID-III new normative data was produced, based on a U.S sample gathered from January 2004 to October 2004. 1700 children were included and stratified to demographically reflect the 2000 U.S census in terms of age, sex, parent education level, race/ethnicity and geographic region. 17 age bands were established with 100 participants in each. Age bands for younger ages were narrower shifting greater numbers into the normative sample of the first year of life. The sample for the BSID-III also included a proportion of children from special group studies i.e. Down Syndrome, Cerebral Palsy, Pervasive Developmental Delay, premature birth and language impairment (approx. 10% of the sample) in order to “improve representativeness”. Percentiles from raw scores were converted to standardised scores (Mean ± SD), scaled scores (10±3) and composite score (100±15) (320).

Since its introduction, the correct interpretation of the BSID-III has been under scrutiny. The BSID-III simplified administration procedures and instructions, and took particular care to include special group studies. Beneficially there is evidence of improved

predictive validity for the updated test (321). However, concerns have arisen about a number of aspects of score interpretation and clinical application. There has been considerable controversy about potential underestimation of developmental delay using the BSID-III and, hence, score inflation (322, 323) whether due to the mixed norms (inclusion of a 10% at-risk population) in the normative data, or previous underestimation of neurodevelopmental abilities by earlier editions of the assessment (324-326).

This has led to various propositions on how to reconcile these findings whether it be to impose an algorithm to compare Bayley II and III scores or to alter the cut-offs used to quantitatively identify those at risk of developmental delay (324, 327).

These issues with the BSID-III have implications for both clinical and research spheres. In a clinical context it makes interpretation of a child's scores difficult in terms of assigning degree of developmental delay, requirements for further investigation and management planning. For research it impacts on longitudinal comparability. If the Bayley II and III are not directly comparable, then changes in outcome over time or due to interceding intervention effect are harder to demonstrate. For example, if neurodevelopmental outcomes improve in a cohort of ex-premature infants over a number of years the question arises as to whether this is due to intervening changes in neonatal care or simply due to a change in the test edition used.

There is also a potential impact of environmental, cultural and hence regional variations on scores (328). This has been explored in a range of populations worldwide and has yielded an incongruous picture suggesting significant regional differences in performance across the infant and toddlers years using the BSID-III (328-334). See *Table 4.1*. These findings have

significant implications for interpretation of results within a given locality.

| Author | Region | Cohort | Age | Significant Differences | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Cognitive | Receptive Language | Expressive Language | Fine Motor | Gross Motor |
| **Bayley 2010** | UK and Ireland | 221 | 12 months and 24 months | ↑ | ↔ | ↔ | ↑ | ↓ |
| **Chinta 2014** | Australia | 156 | 3 years | ↑ | ↑ | ↑ | ↑ | ↔ |
| **Cromwell 2014*** | Malawi | 167 | 10 and 14 weeks, then 6, 9, 12, 15, 18, 24, and 30 months of age | ↑/↓ | ↑/↓ | ↑/↓ | ↑/↓ | ↑/↓ |
| **Krogh 2012**** | Denmark | 45 | 4,7,10 and 13 months | ↔/↑ | ↓ | ↓/↔ | ↑/↔ | ↓/↔ |
| **McGuinness 2012** | Northern Ireland | 655 | 2 to 3 years | ↑ | ↑ | ↑ | ↑ | ↓ |
| **Steenis 2015***** | Netherlands | 1912 | 14 days to 42 months 14 days | ↓ | ↓ | ↓ | ↓ | ↓ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Yu 2013** | Taiwan | 47 | 6, 12, 18 and 24 months | ↑/↔ | ↓ | ↑/↔ |

*Table 4.1: Summary of literature regarding geographical differences in Bayley Scales of Infant and Toddler Development (Edition 3) scores in healthy cohorts*

↑ = significantly higher scores; ↓ = significantly lower scores; ↔ = no significant difference

*Cromwell et al found higher scores in the first months of life and lower scores thereafter across all subscales in their cohort hence ↑/↓

**Krogh et al found slightly different findings across the ages of testing in their cohort, overall trends are shown here

***Steenis et al found scores fluctuated across ages of testing, trend shown here based on results across all ages

## *4.3 Aim*

Our aim was to establish if score discrepancies also existed in our region by examining the normative data for a cohort of low-risk healthy 2 year olds in a contemporary Irish population.

## *4.4 Methods*

### 4.4.1 Participants

From 2008 to 2011 all primigravida attending a large maternity unit with nearly 9000 deliveries per annum in Cork, Ireland were prospectively invited to participate in the first longitudinal study of pregnancy and early childhood in Ireland, the BASELINE Study. Written informed parental consent was obtained for inclusion. The Clinical Research Ethics committee of the Cork Teaching Hospitals provided ethical approval (ECM 5(9) 01/07/2008).

Children were followed up at 0, 2, 6, 12, 24 and 60 months and assessed for various measures of growth, nutrition and allergy and developmental progress. The full description of the Birth Cohort Study protocol has been previously published (335).

As part of a nested cohort study, a representative sample of healthy, low-risk singletons were invited for neurodevelopmental assessment with the BSID-III at 2 years. These infants were selected from the total birth cohort. Exclusion criteria included intra-uterine growth restriction (IUGR) based on a customized birth centile <10%, a history of hypoxic-ischaemic encephalopathy (HIE), prematurity or congenital anomalies.

Additional analysis was performed including children invited for formal neurodevelopmental assessment with the BSID-III after demonstrating abnormal results on parental questionnaires used for developmental and behavioural screening. These included the

Ages and Stages Questionnaire Edition 3 (336) and the Child Behaviour Checklist for ages 1½-5 (337).

### 4.4.2 Procedure and Analysis

Neurodevelopmental assessment was carried out using the Bayley Scales of Infant and Toddler Development (Edition 3) [BSID-III], using the original American English-language version. The test was administered by two dedicated research psychologists trained in administration and scoring of the BSID-III and blinded to the clinical history of the child.

A retrospective analysis was performed of the nested subgroup of the total population based birth cohort study. Statistical analysis consisted of comparing mean scaled scores for each subtest to the U.S standardised norms of the BSID-III using t-tests. This analysis was repeated following the inclusion of the group of children tested following abnormal screener results as described above for all children with completed assessments. The same method was used to compare scaled scores from our cohort to U.K means sourced from the Bayley-III UK and Ireland supplement (333). Effect size in both cases was calculated using Cohen's D. Results are presented as mean (±SD), mean difference (95% confidence interval). A $p$-value of $<0.05$ was considered significant. Patient demographic information is presented above as mean (standard deviation), median (range) and n (percentage) as appropriate. All statistical analysis was performed using IBM SPSS statistics 21.0.

## *4.5 Results*

### 4.5.1 Study Population

In total, 2183 infants were recruited to the BASELINE Study. The cohort was predominantly Caucasian at 98%, with a high level of maternal education. Overall 55% of mothers reported a University education and the mean maternal age at the time of birth was 30.9 years ± 4.7 years. After exclusions, 244 low-risk infants attended for developmental assessment. There were 4 further exclusions of children who had attended for developmental assessment but failed to complete any of the subtests, for example, due to non-compliant behaviour or intercurrent illness etc. The remaining 240 children were then included for analysis and the record forms of their assessments reviewed. 198 children completed all 5 subtests of the BSID-III and a further 42 were incomplete but had completed at least 1 subtest and were included in the analysis of that subtest. (*Figure 4.1*)

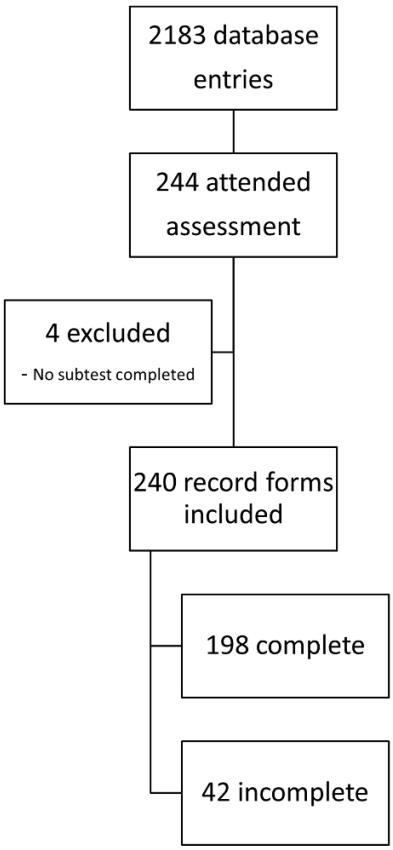



*Figure 4.1: Patient flow diagram*

Of the 240 assessments included, 121 children were male (50.4%) and 119 female (49.6%). The median (range) age at assessment was 27 months and 5 days (24 months 13 days – 32 months 28 days). Mean (±SD) gestational age at birth was 40 weeks and 1 day (± 1 week and 2 days) and mean (±SD) birth weight was 3613g (±445g).

### 4.5.2 Comparison with U.S. normative data

Compared with U.S norms, the scaled scores of our cohort, with a mean (±SD), for receptive and expressive communication were significantly higher ($p$<0.001), 11.2 (±2.2) and 11.8 (±2.8) respectively, compared to normative values of 10 (±3). The effect size was measured by Cohen's D at +0.47 and +0.61

respectively. This had an overall effect on the composite score for the language subscale, 109 (± 13.2) v. 100 (±15), $p<0.001$. Therefore, Language composite scores were 9 points or 0.6 SD higher than the standardised mean. The scaled score for the fine motor subtest was also significantly raised compared to normative data, 11.5 (±2.3) v. 10 (±3), effect size +0.56. This also led to significantly increased motor composite score, 106 (±11.3) v. 100 (±15), $p<0.001$, despite a lack of significant change in the gross motor scaled score, 10.3 (±2.6), $p=0.151$. Motor composites were therefore 0.4 SD above the standardised mean. No significant difference in the cognitive subscale scores was evident comparing this cohort to standardised values, 99 (±10.5) v. 100 (±15), $p=0.214$. (See *Table 4.2* and *Figure 4.2*)

Interestingly only 3% of the cohort scored ≤85 (i.e. 1SD below the mean) in the language subscale and only 2% in the motor subscale compared with 9% of the cognitive subscale. At the same time 26% of the cohort scored ≥115 in the language subscale while 14% scored ≥1SD above the mean in the motor subscale and only 6% in the cognitive subscale.

| Subtest Score | N | Mean (SD) | Mean Difference (95% CI) | p-value | Effect size Cohen's D (US) |
|---|---|---|---|---|---|
| Cognitive Scaled Score | 234 | 9.83 (2.1) | -0.171 (-0.44, 0.10) | 0.214 | -0.07 |
| Receptive Language Scaled Score | 214 | 11.24 (2.2) | 1.238 (0.95, 1.53) | <0.001 | +0.47 |
| Expressive Language Scaled Score | 209 | 11.77 (2.8) | 1.766 (1.38, 2.15) | <0.001 | +0.61 |
| Fine Motor Scaled Score | 230 | 11.50 (2.3) | 1.496 (1.19, 1.8) | <0.001 | +0.56 |
| Gross Motor Scaled Score | 216 | 10.25 (2.5) | 0.250 (-0.09, 0.59) | 0.151 | +0.09 |

*Table 4.2: Mean, mean difference, p-value and Cohen's D when comparing results with BSID-III US normative data.* Scaled scores were compared with a test score of 10 (SD=3).

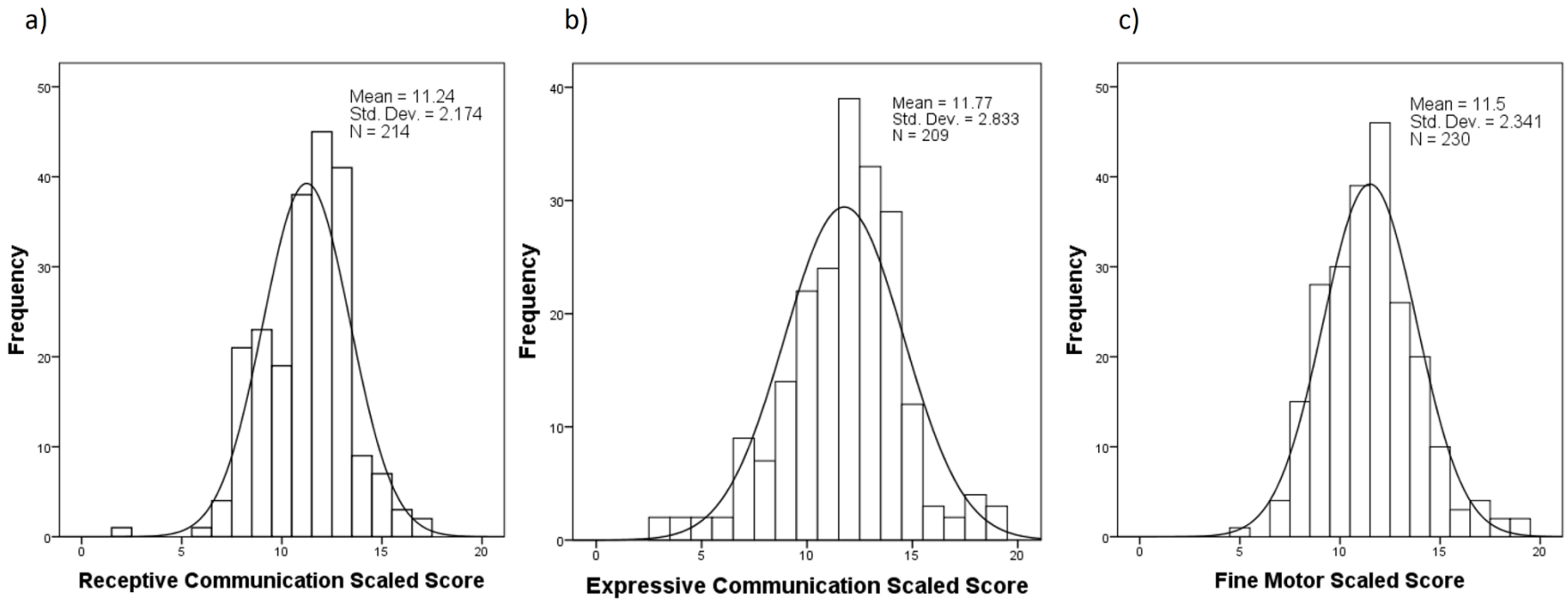



*Figure 4.2: Histogram of Receptive Language (a) and Expressive Language (b) and Fine Motor scaled scores*

### 4.5.3 Comparison with U.S. normative data after inclusion of a clinical sub-group

The inclusion of children from the special group studies, making up 10% of the cohort, in the U.S normative data could potentially affect the comparability of these 2 cohorts. Unfortunately, we were unable to include an equivalent group of high-risk children. However, we did have a small cohort of children who underwent BSID-III assessments following abnormal results on parental questionnaires. 44 children with abnormal screeners attended for developmental assessment. 28 children completed all 5 administered subscales and a further 16 children had incomplete assessments. Only completed assessments were included in this analysis i.e. 198 from the low-risk groups and 28 from the abnormal screeners group.

When the BSID-III results from the children in the abnormal screeners and low-risk groups were compared, composite scores were significantly lower in the abnormal screeners group across all 3 subscales; cognitive: 88 (±17) v. 100 (±10), $p<0.001$, language: 84 (±20) v. 110 (±13), $p<0.001$, motor: 91 (±19) v. 106 (±11), $p=0.005$.

The groups were then combined i.e. 226 children including 12.3% “clinical” population and compared with the U.S normative data. Even including this sub-group of at-risk children, language and fine motor subtest scores remained higher than U.S. scores; receptive language: 10.8 (±2.8) v. 10 (±3), expressive language: 11.3 (±3.3) v. 10 (±3), fine motor: 11.3 (±2.6) v. 10 (±3). Though effect sizes by Cohen’s D were smaller, +0.28, +0.41 and +0.48, respectively, following the inclusion of the clinical group, results remained significant, $p<0.001$ for each.

### 4.5.4 Comparison with U.K data

Although full normative data is not available, our results were also compared with the available U.K mean values. Scaled scores for all subtests showed significant variation with language and motor subtest being above the U.K means, whilst cognitive scores were lower, $p<0.001$. (*Table 4.3*).

### 4.5.5 Comparison between assessors

As the BSID-III assessments were carried out by two different assessors over the three-year time-period (assessor A carried out for first 84 and assessor B carried out the subsequent 156) we compared each of these subgroups to each other and to the U.S norms to confirm consistency with the above findings. Assessors' differed significantly from each other in the scores assigned for gross motor and cognitive assessment. However, for both assessors there was a consistent overall increase in receptive language, expressive language and fine motor subtests compared to U.S. norms. (*Table 4.4*).

| Subtest Score | N | Mean (SD) | UK Mean (SD)* | Mean Difference (95% CI) | p-value | Effect size Cohen's D (UK) |
|---|---|---|---|---|---|---|
| Cognitive Scaled Score | 234 | 9.83 (2.1) | 10.72 (2.72) | -0.891 (-1.16, -0.62) | <0.001 | -0.37 |
| Receptive Language Scaled Score | 214 | 11.24 (2.2) | 10.29 (2.89) | 0.948 (0.66, 1.24) | <0.001 | +0.37 |
| Expressive Language Scaled Score | 209 | 11.77 (2.8) | 10.27 (2.79) | 1.496 (1.11, 1.88) | <0.001 | +0.54 |
| Fine Motor Scaled Score | 230 | 11.50 (2.3) | 10.93 (2.51) | 0.566 (0.26, 0.87) | <0.001 | +0.24 |
| Gross Motor Scaled Score | 216 | 10.25 (2.5) | 9.12 (2.83) | 1.130 (0.79, 1.47) | <0.001 | +0.42 |

*Table 4.3: Mean, mean difference, p-value and Cohen's D when comparing results with BSID-III UK normative data* (Note: a full set of UK norms for children of different ages is not available).

* = Source (333)

| Subscale | Assessor A n=84 | | | Assessor B n=156 | | | Difference Between Assessors | |
|---|---|---|---|---|---|---|---|---|
| | Mean (SD) | Mean Difference* (95% CI) | p-value | Mean (SD) | Mean Difference* (95% CI) | p-value | Mean Difference (95% CI) | p-value |
| **Cognitive** | 10.73 (2.6) | +0.735 (0.18,1.29) | 0.010 | 9.33 (1.6) | -0.669 (-0.93,-0.41) | <0.001 | +1.404 (0.867, 1.940) | <0.001 |
| **Receptive Communication** | 11.69 (2.0) | +1.691 (1.24,2.14) | <0.001 | 10.96 (2.2) | +0.962 (0.58,1.34) | <0.001 | +0.729 (0.132, 1.32) | 0.017 |
| **Expressive Communication** | 12.25 (2.8) | +2.247 (1.62,2.87) | <0.001 | 11.46 (2.8) | +1.461 (0.97,1.95) | <0.001 | +0.786 (-0.002, 1.574) | 0.050 |
| **Fine Motor** | 11.53 (2.6) | +1.530 (0.96,2.10) | <0.001 | 11.48 (2.2) | +1.476 (1.12,1.83) | <0.001 | +0.054 (-0.581, 0.689) | 0.874 |
| **Gross Motor** | 11.50 (3.0) | +1.500 (0.83,2.17) | <0.001 | 9.51 (1.9) | -0.485 (-0.80,-0.17) | 0.003 | 1.985 (1.328, 2.642) | <0.001 |

*Table 4.4: Comparison of both assessors with BSID-III U.S normative data and with each other.* *Mean difference of each assessor to the U.S. norms where mean is compared to a test score of 10 (SD=3).

## *4.6 Discussion*

In this work we have demonstrated further evidence of regional difference in BSID-III performance. Population differences in BSID-III performance have been previously recognised. Chinta et al compared BSID-III scores in an Australian population at 3 years with the original American standardised norms and found significantly higher results in their cohort in all subtests excepting gross motor skills (328). Yu et al described and compared performance in the BSID-II and BSID-III in term and preterm infants in a Taiwanese population (329). Though they did not directly compare BSID-III with the standardised norms and their term group was smaller in size, their results show a trend to lower language composite scores (especially in younger age groups) and higher motor composite scores. Cromwell et al demonstrated the variation in the scores of Malawian children compared to US norms throughout development from 10 weeks of life to 30 months of age finding significant differences (330). In Europe, Danish infants in the first year of life showed differences from U.S. norms particularly in receptive language where scores were significantly lower at all time-points postulated to be attributable to differences in language difficulty (332). Recently a comprehensive comparison of newly developed Dutch normative data, using the adapted Bayley-III-NL test, with US norms by Steenis et al also highlighted the clinically relevant differences across age groups (331).

Even within the relatively small geographic region of the U.K and Ireland there are variations in performance. The Bayley-III UK and Ireland Supplement reported a sample of 221 children aged around 12 and 24 months and found higher means in cognitive and fine motor subtests of the BSID-III while gross motor scores were lower than the U.S findings (333). An unpublished report from Queen's University Belfast looked at BSID-III performance in nearly 650 children between 2 and 3

years old from across Northern Ireland and found a similar profile of variations though quite significant increases in language and fine motor skills, particularly receptive language (334). Interestingly all 3 investigations in this region have some similarities. They all found fine motor skills in particular to be increased in a 2 year old population compared to the U.S and both studies from the island of Ireland found language to be significantly increased. However receptive language had the greatest increase in Northern Ireland while expressive language showed a more pronounced increase in our work in which children from the most southern province in Ireland were tested (334).

This suggestion of regional differences in the neurodevelopmental trajectories of infants and toddlers makes clinical interpretation of BSID-III results on a case by case basis more difficult without being able to refer to contemporary regional normative data.

Two years of life is a common time-point for follow-up of high-risk groups for clinical and research purposes (8) and at this age our healthy comparison cohort has shown differing performance in language and fine motor skills with small to medium effect sizes. This may be affected by factors unique to our group. Our birth cohort is made up of a population with highly educated mothers. This may influence neurodevelopmental outcomes, though the relationship is known to be quite complex (338). This does however reflect the general Irish population where 62% of women will attend some form of tertiary education (339) likely related to high state-level funding and support. Therefore, within the Republic of Ireland, widespread higher education is not particularly exceptional. Our cohort were also highly motivated to participate in research and to attend multiple follow-up appointments over several years. It is likely that there is an element of volunteer bias in the data of participants.

The differing results from disparate geographical regions, even within Europe, may represent cultural differences in parenting behaviours as well as environmental impacts on opportunities for learning and development. Ireland as an island nation and shaped by its climate and history may, over many generations, have promoted indoor activities, interpersonal communication in all its forms, crafts and art to its children which may account for particular skills in these areas. However, it must be remembered that we have captured only a single time-point, though a clinically very relevant one, in the trajectory of early child development in Ireland and the results profile and pattern may in fact look very different at another age. Therefore further normative data from throughout the infant and toddler period would be useful to assist interpretation.

Another possible explanation of the results is the potential effect of the mixed norms used in standardisation of the BSID-III i.e. the inclusion of children at risk of developmental delay. While we were unable to include an exactly comparable group we did offer analysis on a cohort that included 12.3% of children who had been identified as at-risk based on screening questionnaires completed by the caregiver. When the scores of this mixed cohort were compared with U.S. norms, language and fine motor skills remained significantly higher. This is supported by the Dutch data by Steenis et al (331), which did include a representative sample from special group studies, and demonstrated that regional differences can persist despite this consideration.

The influence of the Flynn effect, the observed rise in IQ scores with time, has been proposed to account for some of the score inflation seen in some studies. The Flynn effect is generally considered to correspond to a rise in standard score points of 3 per decade (340). A technical report on the BSID-III rebuts these suggestions as the Bayley scales are developmental assessment

rather than an intelligence measure the Flynn effect may not be applicable (341). The BSID-III normative data derives from children tested in 2004 and our cohort was tested from 2011 to 2013, which would allow a Flynn effect of 2.1, for those in the first year of testing, to 2.7 for those in the last year of testing. This could account partially but not completely for the increases we have described and the variations might be expected to be more equivalent across subtests.(342).

Whatever the reason for these differences in scores, their existence creates considerable difficulty for clinical interpretation and for their application in research. In an Irish population it appears we may be at risk of underestimating developmental delay if relying on U.S standardised scores. Of course this needs to be interpreted in the light of different sampling frameworks used for U.S. and Irish data collection. Ideally to support this we could go on to assess the clinical needs of an Irish population in these areas or attempt to corroborate with later performance but this is beyond the scope of the current study. Regional restandardisation of the Bayley assessment may be necessary to reliably interpret a child's scores in a clinical context. While this is unlikely to be feasible for many jurisdictions, the application of alternative cut-offs for characterising developmental delay on a regional basis may be needed. However this still requires a level of local normative data that can be difficult and costly to collect.

In research, this work highlights the value and importance of having a contemporary comparison group in longitudinal studies subjected to identical neurodevelopmental outcome measures as case group participants. The variations between the two assessors found in this work further reinforces this need. Little is known about the inter-rater variability of the BSID-III. One Italian study shown high correlation between two assessors, but while scoring was performed independently, administration

depended on each other. (343) Differences may arise despite equivalent training and test administration due the nature of psychometric testing. While neurodevelopmental assessment of all contemporary control populations adds cost and burden to researchers it is necessary to ensure meaningful interpretation of results.

The strengths of this study include the comparable numbers of children tested in this age group to those of the same age tested in the U.S. normative data as well as the prospective and meticulous data collection within this birth cohort study that allowed the identification of those with a low-risk neonatal course.

The limitations include that these results are based on a convenience sample of Irish children that was not designed from the outset to provide a representative sample of the Irish population. We are also unable to provide a comparable sample from special group studies to study the effect this would have on our results. It is also beyond the scope of this study to examine how results might vary with age in a similar population. Finally the results are based on the work of two assessors who performed assessments in two different size groups during overlapping but slightly different time frames. Reassuringly both assessors scored most children in the normal range. For research, studies involving more than one assessor would ideally attempt to negate assessor-based differences in testing and scoring by using a method similar to Deroma et al or to use video-recording of sessions to allow joint review and discrepancy checks.

## *4.7 Conclusion*

In conclusion, this study has shown higher scores in Language and Fine Motor skills in a cohort of Irish 2-year-olds compared to U.S. normative data. This adds to the evidence of regional variation in BSID-III scores and suggests the need for alternate cut-offs on a regional basis for the clinical interpretation of a child's results and emphasises the importance of contemporary control groups in longitudinal research studies.

# 5.0 Touch Screen Usage in Toddlers: a feasibility study for a novel assessment tool

*Publications arising from this chapter:*

*"Touch-screen Technology Usage in Toddlers"*

*Ahearne C, Dilworth S, Rollings R, Livingstone V, Murray D.*

*Arch Dis Child 2016; 101:181-183.*

When we consider the specific deficits now understood to be part of the cognitive development of high risk infants, and the inherent challenges in assessing the pre-school age group, it is clear that a novel, early and reliable assessment tool for cognitive ability would benefit both identification of developmental difficulties and early intervention planning.

In this chapter, I will explain the rationale for the development of a touch-screen cognitive assessment for toddlers particularly following perinatal asphyxia and describe findings of a parental questionnaire into touch-screen usage in toddlers. The results of this questionnaire point to the feasibility of testing toddlers using a touch-screen platform.

## *5.1 Background*

It is almost universally accepted that in clinical practice no single assessment tool is suitable for all situations (8, 344). Multiple factors must be considered in determining the appropriate choice (345, 346). For inclusion in clinical or research follow-up, an assessment tool must be appropriate to the age of the cohort in terms of both administration and standardisation. The tool should be acceptable across the given age-range of children being assessed and capable of showing differences in abilities. As discussed in Chapter four, the population used to standardise a test can have major bearing on the validity of its application in a different cohort. Age profile, geographic area, cultural appropriateness and learning opportunities can affect the representativeness of normative ranges (328). Without appropriate normative data any comparison with a novel cohort would be less robust. Orton et al, 2015, found a 34% discrepancy when comparing rates of delay against a local normative cohort rather than the U.S. data using the BSID-III (347).

Psychometric considerations are key to assessment choice but also to result interpretation. Alyward, 2009, described developmental testing as a "moving target" due to the influence of the Flynn effect over time and repeated re-standardisation of tests (345). Other psychometric factors to be taken into account include test reliability; the extent to which a test yields the same results when administered repeatedly, and test validity; the extent to which a test measures what it purports to measure. These are summarised by Johnson and Marlow, 2006 (348).

In the longitudinal follow-up of at risk infants, it is necessary to account for the specific aetiology of the pathology that puts this child at risk. First of all, it is important to distinguish between developmental disorders and acquired brain injury (204). Brain injuries that are acquired in childhood such as stroke, neoplasm or cerebral infection will affect the developmental trajectory of a child in a different manner to a developmental disorder secondary to a genetic abnormality or early brain malformation. Brain development is a dynamic process in the postnatal period up to adolescence (349, 350). This allows time for brain plasticity to act to redistribute or preserve function in cases of more focal acquired brain injury. It has long been known that children with an early localised injury have less severe outcomes than adults with comparable lesions (351, 352). This is not the case for developmental disorders where damage results from in-built errors in brain formation from the outset. At this early stage neurogenesis itself is likely to be disrupted, not just the later synaptogenesis and synaptic pruning (213). If we are to consider a neuroconstructivist theory of cognitive development we could assume that the earlier the deviation away from the typical trajectory the more varied and insidious the resultant developmental profile (353). Perinatal asphyxia, while an acquired injury, does, however, seem to have more in common

with developmental disorders, likely due to the timing of injury. This has important implications for prognosis and therapy (204).

The aetiology of the injury will also guide test choice based on what is known about outcome in that particular condition. Test choice will have to fit the predicted deficit that needs to be excluded or included. Conditions that have implications for other facets of development such as language and motor function will also affect how practically the cognitive domain can be assessed.

It is also vital to consider why a test is being performed. Most tests will tell you very little in isolation and for clinical purposes there can be more value in a test if it is repeated over a time period to establish a trajectory (204). If a test is being performed for prognostication, the predictive validity of the test must be known and appropriate for purpose. A test that is predictive for IQ might not be able to give you information on how the child will perform at school as IQ has been shown to have poor correlation with academic success or job performance (180, 354). It is also important to remember that other factors including personality traits such as conscientiousness may have a more significant influence on academic performance than IQ (355).

### 5.1.1 Perinatal Brain Injury and Cognitive Outcome

Up until recently cognitive outcomes after neonatal encephalopathy were considered only to occur in the context of an associated motor deficit, however, there is now mounting evidence that this is not the case (260, 356). Several studies involving longer term follow-up have also revealed more subtle cognitive, behavioural and intellectual deficits that were previously unrecognised. In 2008, Lindstrom et al. published a follow-up study of 15-19 year olds who had suffered moderate

encephalopathy in the neonatal period which showed that 81% suffered cognitive effects with and without CP (9). Marlow et al. followed-up a cohort of children with moderate and severe encephalopathy to seven years old. In survivors of severe encephalopathy without motor disability, scores in multiple cognitive domains were significantly decreased compared with peers. In the moderate encephalopathy group, certain domains including language, sensorimotor, narrative memory and sentence repetition were also significantly below peers. Functionally both groups were found to have more frequent special education needs with lower achievement on national curriculum attainment targets (4). Participants in the NICHD hypothermia trial were found, at six to seven years, to have IQ scores more than one standard deviation below the mean in around 40% of children without CP. Interestingly, 20% of children with a normal IQ and 28% of those with IQ scores of 70 to 84 had received special educational support or had been held back at school (10). Again this emphasises how disconnected functional academic performance can be from IQ scores. This is likely due to deficits in specific cognitive abilities, rather than general abilities, affecting learning needs.

Undoubtedly, in the past, the more serious potential outcomes of death and major disability from hypoxic-ischaemic injury were the primary focus of concern and a child surviving relatively intact was, understandably, seen as a positive outcome. However, the advent of therapeutic hypothermia has had considerable impact on improving rates of major disability (3, 126). This has broadened interest into the more subtle impairments which nonetheless can have an effect on the child's academic attainment, working life and even social independence. Azzopardi et al. 2014, included multiple psychometric assessments in the follow-up of a six to seven year old population of children with moderate to severe encephalopathy

who were randomised between cooling and non-cooling groups. However, they did not show significant differences in the results of most of these assessments between the cooled and non-cooled children (3). The impact and mechanism of hypoxic-ischaemic injury on the cognitive development of the developing brain is still poorly understood. It is even less clear how therapeutic hypothermia interacts with this process and what effect it has.

Other high-risk groups have also been found to have specific patterns of cognitive deficit and learning difficulties. Prematurity appears to have an effect not only on overall IQ but more specifically on selective and sustained attention, and shift/inhibition skills (357). Mulder at al. 2010, published findings of a follow-up of premature infants at 9-10 years that showed that processing speed and working memory were predictive of academic attainment in this group (358). Infants with Foetal growth restriction were also been found to suffer cognitive deficits particularly in short-term memory (359).

Often these cognitive deficits have only become apparent as performance demands increase with age and progression through the school system. However, the opinion that other measures of cognitive functioning should now be included in the follow-up of high-risk infants is gaining ground (344).

### 5.1.2 Rationale for development of a new cognitive assessment

There are few tests of cognitive functioning generally available that are appropriate to the toddler age group. This can be a particularly challenging time-point for assessment as administration can be lengthy and the strange environment and unfamiliar assessor can be intimidating for the child (8). Yet, this is a significant time-point for developmental follow-up as it permits timely identification of delay to allow intervention prior to school attendance. Evidence regarding the efficacy of early

intervention has been disappointingly mixed. A report by Herskind et al, 2015, examined this issue with reference to cerebral palsy (360). They argue that due to the substantial evidence for neuroplasticity and the "sensitive period" in childhood that allows for increased receptiveness to environmental stimuli, infants ought to benefit from early intervention. However, evidence to support a positive influence has been lacking. They highlight certain limitations that may have hindered these studies. These include difficulties identifying those most at risk of disabilities, identifying them early enough and matching appropriate evaluation to appropriate intervention. These challenges are as applicable to cognitive deficits as to motor ones. The biomarkers discussed earlier in this thesis and the novel cognitive assessment described in the next chapter may help alleviate these problems and allow further study of this topic. A recent Cochrane review, 2015, did show a positive influence of early intervention on the motor and cognitive skills of ex-preterm infants with the benefit on cognitive abilities persisting to preschool years (361). However evidence of ongoing benefit is lacking. Similarly specific data regarding the efficacy of early intervention in HIE is not available.

Of course there are other reasons why longer term follow up should be carried out. These include resource planning for specific deficits identified, including appropriate school choice and extra assistance as described by Doyle et al, 2014 (346). Benefits extend not just to the patient but also the family as well as the medical and research community. Johnson and Marlow, 2006, also discuss the benefits of longer term neurodevelopmental outcome with specific reference to preterm infants (348). These include those discussed above as well contributions to service audit, monitoring outcome of perinatal

care and interventions, and to inform ethical debate regarding babies at borderline viability.

At present our ability to assess cognitive development in early childhood is limited. The two major modes of assessment of any domain are through parental questionnaire and/or administered assessment. Outcome measures may also be differentiated into those acting as screening tools and those providing a developmental assessment. However, Johnson and Marlow argue that screening tools have little utility in an inherently at-risk population and are poor at detecting subtle deficits (348). Therefore, robust, validated assessments are required.

Few parental questionnaires have dealt with cognitive development across the infant and toddler age range. In fact until recently none were specified for testing cognition and in fact only dealt with certain areas. The Ages and Stages Questionnaire (ASQ) is used primarily as a screening tool between the ages of 4 months and 5 years to establish risk of developmental delay (336). It covers 5 developmental domains including a section on problem-solving. Several studies have looked at the validity of the ASQ by comparing against an administered developmental assessment, the Bayley Scales (309, 362, 363). While some have shown moderate overall correlation, Gollenberg et al., 2010, looked specifically at the correlation between the problem-solving domain of the ASQ and Bayley-2 scores and failed to find a significant result (362).

The other two parental questionnaires that cover this period are the Child Development Inventory (CDI) and the Infant Development Inventory (IDI) (364, 365). Both were developed by Ireton. The CDI is a lengthy undertaking of 270 items for assessing development of social, self-help, motor, language, letter and number skills as well as behaviour problems in children between 15 months and 5 years. Data from the

normative sample of 568 children showed the test's ability to identify children in special education and demonstrated small to medium correlations across the domains to preschool reading achievement (366). The CDI has also been shown to correlate well with other assessments including the Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS) and the Bayley Scales of Infant Development, 2nd Edition (BSID-II) (367). This test is however very long and does not specifically examine cognitive deficits. The IDI asks parents to describe their infant in terms of current behaviours (364). It only covers infants up to 21 months and has yet to be validated against other standardised tests.

The Parent Report of Children's Abilities (PARCA) assesses non-verbal cognitive ability in 2 year old children (368). A recent update was tested for validity against the Bayley-2 in a sample of ex-preterm infants and showed good correlation with MDI scores (369). While this assessment may provide an inexpensive way to assess cognitive deficit, it is only for use at 2 years of age.

Baker et al., 2013, presented the development of a novel parental report for cognitive assessment, the Cognitive Developmental Questionnaire (CDQ) (370). This questionnaire is designed for children from 10 to 24 months and comprises a parentally administered "Games" section and a reported "Questionnaire" section. Pilot testing showed a roughly linear correlation with age and strong positive correlations with raw Bayley-2 MDI scores. This is promising data but requires further validation.

Parental reports are a relatively cheap and easy method of assessing cognitive development. They negate the need for training a third party in administration and scoring and save time bringing parents and toddlers into a clinic. They are also completed by a parent who knows the child best and has seen

them over a period of time rather than just a snapshot seen by an assessor. However they are troubled by recall and response bias and an inherent variability that makes their use as an ultimate tool limited (348).

There are several administered assessments that have some element of cognitive assessment. These are summarised in *Table 5.1*. I will give an overview of some of the more commonly used of these still in current practice.

Infant cognitive assessment instruments.

| Assessment instrument | Age range | Domains of assessment |
|---|---|---|
| Battelle Developmental Inventory (Newborg, Stock, & Wnek, 1984) | 1 month to 8 years | Cognitive, personal social, adaptive, motor and communication |
| Bayley Scales of Infant Development BSID-II (Bayley, 1969, 1993); Bayley Scales of Infant and Toddler Development BSID-III (Bayley, 2005) | 1–42 months | Cognitive, communication, motor and behavior |
| Cattell Infant Intelligence Test (Cattell, 1940) | 2–30 months | Cognitive |
| Clinical Adaptive Test/Clinical Linguistic Auditory Milestone Scale CAT/CLAMS (Accardo & Capute, 1996) | Birth to 36 months | Language, problem solving and visual-motor skills |
| Cognitive Abilities Scale CAS-2 Infant Form (Bradley-Johnson & Johnson, 2001) | 3 months to 24 months | Exploration of objects, communication with others and initiation and imitation |
| Denver Developmental Screening Test-Denver II (Franenburg, Didds, Fandal, Kazuk, & Cohrs, 1975) | Birth to 6 years | Language, gross motor, fine motor-adaptive, personal-social and behavior |
| Griffiths Developmental Schedule (Griffiths, 1996) | 1–60 months | Locomotor, hearing and speech, eye and hand co-ordination, performance, practical reasoning and personal-social |
| Gesell Developmental Schedules (Knobloch, Stevens, & Malone, 1980) | 1 week to 36 months | Adaptive, gross motor, fine motor, language and personal-social |
| Infant Psychological Development Scale (Uzgiris & Hunt, 1975) | 2 weeks to 2 years | Object permanence, use of objects as means, learning and foresight, development of schemata, development of an understanding of causality, conception of objects in space, vocal imitation and gestural imitation |
| Infant-Toddler Developmental Assessment IDA (Provence, Erikson, Vater, & Palmeri, 1995 | Birth to 36 months | Gross motor, fine motor, language/communication, relationship to peers, emotions and feeling states and coping behavior |
| The Mullen Scales of Early Learning (Mullen, 1995) | Birth to 68 months | Gross motor, visual reception, fine motor, expressive language and receptive language |

*Table 5.1: Infant cognitive assessment.* Reproduced from Baker et al. (2013) (370).

The Mullen Scales of Early Learning (MSEL) provides a measure of fine motor, visual reception, receptive language, expressive language and gross motor skills of children from birth to 68 months (371). An Early Learning Composite (ELC) standardised score can be obtained as a measure of global cognitive functioning. Moderate correlations are reported with the original Bayley Scales of Infant Development (318). Caudle et al., 2014, looked at the nonverbal cognitive development of children with cochlear implants. They examined the predictive validity of the MSEL for later assessment with the Leiter International Performance Scales-Revised (LIPS-R) and found moderate correlations between the MSEL Visual Reception score and the LIPS-R fluid reasoning score (372). The MSEL has been tested on a range of high-risk children and is being increasingly used in studies of children with autism spectrum disorders (373). While the test is able to identify children with developmental delay in these groups there is little evidence of a differential profile that can distinguish the disorders from each other (374). Also the MSEL standardisation, carried out 15 and 23 years ago, is somewhat outdated (348).

The Battelle Developmental Inventory, recently re-standardised to a second edition, (BDI-2) is used to assess developmental skills in children from birth to 8 years (375). The test uses a combination of observation and informant report to assess five domains including personal/social, adaptive, motor, communication and cognitive. The five domains can be summed to yield a total developmental quotient. The second edition has improved psychometric properties compared to the original with acceptable reliability, internal consistency and basic validity (375). Matson et al., 2010, looked at the ability of the BDI-2 to differentiate developmental profiles of children with Down Syndrome, Global Developmental Delay (GDD) and Prematurity (376). They noted that at 2-years the test showed those with

GDD and Down Syndrome had significantly more motor problems than those who were premature, while those with GDD had significant deficits in personal-social skills compared to the other two groups. Differentiation based on cognitive skills was not apparent. Studies regarding the predictive validity of the BDI-2 for later cognitive performance are lacking.

The Griffiths Scales, as previously discussed in Chapter 1, is also used for assessment in early childhood but does not have a dedicated subscale for cognitive assessment (175). The predictive validity of this test for later cognitive performance has also been somewhat limited especially at younger age ranges (176, 177). Griffiths III, with its dedicated subscale of Foundations of Learning, may change this but psychometric data has yet to be released and studies of predictive validity are unlikely to be available for several years (178, 179).

The most commonly used assessment following a high risk perinatal injury is the Bayley Scales of Infant and Toddler development (174, 318, 319). Unfortunately, the Bayley has been shown to be quite poor at detecting mild to moderate cognitive deficits particularly at younger ages and is poorly predictive of later measures of cognitive function (321, 377). Similar to other tests previously discussed, the Bayley Scales rely heavily on motor and language skills in order to perform well in the cognitive section. Cognitive tasks require the child to be able to manipulate small objects such as pegs and puzzle pieces and they require the child to understand specific verbal instructions. In testing children with physical or language impairments this has to date required deviations from the standard practice due to the their influence on relative performance (174). The Bayley-3 administration manual advises that modifications put the test outside of the purview of the standardisation sample and recommends using “your professional judgement” to decide how these modifications impact test scores.

None of the tests described above, other than perhaps the visual reception subtest of the MSEL, have been specifically designed to test nonverbal cognition. Moreover, none of these assessments have been designed to detect the specific cognitive deficits known to be affected in perinatal brain injury. Therefore, a focused language-independent cognitive assessment capable of testing the disease-relevant cognitive domains would aid early identification and guide intervention in high-risk infants.

### 5.1.3 Conceptual development of a novel cognitive assessment

As described above few assessment tools are designed for cognitive assessment in the toddler age group. Even fewer are focused on specific cognitive constructs. Executive function refers to a complex cognitive construct encompassing the whole set of processes underlying controlled, goal-directed responses to novel or difficult situations (204).

O'Reilly and Munakata, 2000, identified three different kinds of processing performed by neural networks in the brain;

- The slow learning of overlapping distributed representations of the environment, performed by the posterior cortex
- Active maintenance by the prefrontal cortex of limited amounts of information over short time intervals, to enable problem solving
- Rapid acquisition of unique conjunctions of novel information by the hippocampus and related structures.

It is this second type of processing related to executive functions, with particular reference to working memory, and fluid intelligence that we wished to examine with a novel, touchscreen based, language independent, cognitive assessment (378, 379). Fluid intelligence describes novel problem solving

abilities. This stands opposed to crystallised intelligence which refers to the ability to use learned knowledge (380). Many of the above-mentioned tests include tasks that test crystallised intelligence such as counting, knowledge of colours etc. which can be dependent on available experience and exposure. In describing theories of adult intelligence Cattell explains that crystallised intelligence is a product of fluid intelligence augmented by time invested in scholarly pursuit with an influence of interest and memory *(Figure 5.1)* (380, 381). Therefore pre-existing fluid intelligence is required for later knowledge. The above processing functions are key to managing the novel learning challenges that will be faced by young children during their academic development. They also encompass those cognitive abilities that appear to be at risk following perinatal injury.

These neural networks, however, likely represent the adult brain state in learning. Therefore the next question is, what is the evidence that these skills would be measurable in young children?

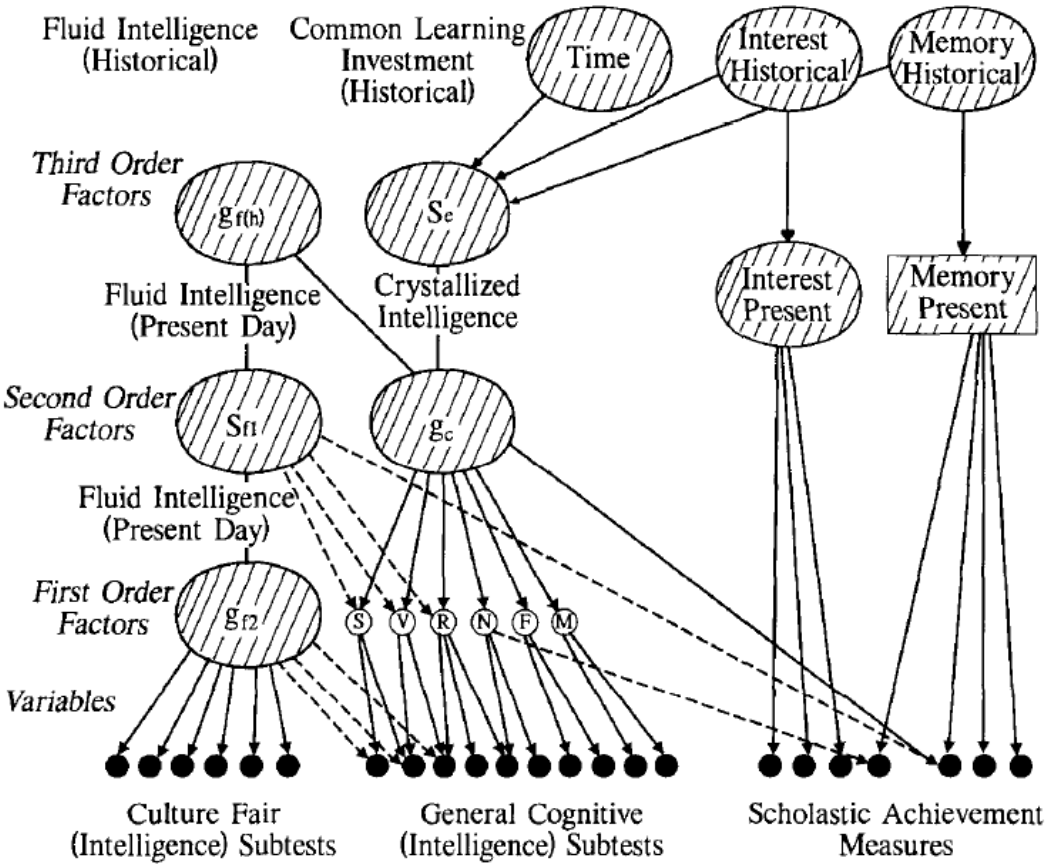



*Figure 5.1: Hypothesised causal relationship of fluid intelligence, crystallised intelligence and adult intelligence.* Reproduced from Ackerman et al, 1996, based on Cattell's theories (381).

Rose et al, 2004, described the dimensions of cognition in infants at 7 months following a battery of tasks and found evidence of 3 dimensions accounting for 37% of the variance in factor analysis. These were determined to be an **attention related factor, a speed factor and a memory factor** (382). Mulder et al., 2014, summarised the main components of executive function to be (i) **working memory**, defined as the ability to hold information in memory while performing mental operations on this information; (ii) **inhibitory control**, defined as the ability to suppress automatized and predominant

responses; and shifting, or the ability to change cognitive set in order to switch between different tasks (383). There remains, however, considerable debate among neuropsychologists as to whether there is a one or multiple factor solution to executive functionality in children. It has been suggested that there may be elements of unity and diversity to these cognitive processes (384).

Finally, in order to ensure that a new cognitive assessment tool could display predictive validity, there would need to be support for the continuity and predictive ability of early specific cognitive abilities for later performance. Fagan et al, 2007, found that information processing ability, defined by a test of selective attention, in children aged 6 to 12 months was predictive of adult IQ and academic achievement (385). Following a battery of tasks testing **attention, processing speed, memory and representational competence** at 5 time points from 7 months, Rose et al, 2012, demonstrated that these abilities showed continuity at different stages of development and were predictive of IQ at 11 years (386). This implies that testing these domains can provide important prognostic information from a very early age.

### 5.1.4 Touch-screen platform for neuropsychological assessment

Computerisation of neuropsychological assessments has been in progress for decades. Although while widely used for scoring, computerized administration has lagged behind (387). In the paediatric population computerization has been applied to the assessment of attention in Attention-Deficit-Hyperactivity Disorder (ADHD) where precision, and repetition, limit human administration. Even the touch-screen format has been used to assess children as young as four years in the form of the Cambridge Neuropsychological Testing Automated Battery

(CANTAB) (387-389). This touch-screen assessment battery was initially developed for the diagnosis of adults with dementia but has shown considerable promise in differentiating certain patterns of neuropathology in the paediatric population. Luciana and Nelson, 2002, administered six subtests of the CANTAB to 400 typically developing children between the ages of four and twelve years (390). In terms of administration, children from age five on were able to follow instructions to complete the assessment and participant attrition only occurred in more difficult tasks from ages five to eight. Four year olds had distinctly more administration errors and were often not able to get a response from the screen on first attempt. Thus screen sensitivity was determined to be an important consideration. This study also found no difference in performance in children for whom English was not their first language. They determined that the CANTAB could be used to assess intact function in those with weak verbal skills. Green et al, 2009, used the CANTAB in a cohort of eight to 15 year olds with a history of Foetal alcohol syndrome and successfully found differences in multiple executive function domains compared to age-matched controls (389). Very recently Semmelmann et al, 2016, published a study testing selected experimental paradigms that tested a variety of cognitive skills in children aged one to ten years (391). They found that children from aged two onwards were able to engage with these tasks and respond allowing data regarding accuracy and reaction time to be collected.

The potential advantages of the use of digital technology for assessment are numerous. The portability of modern touch-screen tablets would allow easy transport and use by a health care professional in a variety of environments, not dependent on transporting a large kit and having appropriate furniture available. Touch-screen technology allows an almost infinite potential for progression through increasing complexities of

tasks. Children would be able to directly interact with the screen and cue the next level of difficulty which would only be limited be the programming of the application. Tests could theoretically be extended across any age group, which would help in future functional studies of cognitive development where availability of appropriate tests has hindered understanding of developmental trajectories. Under appropriate data protection measures each test carried out on a touch screen device could be remotely uploaded to a central server either for the assessors' own data collection or more usefully for the continual updating of normative values over time and geographic regions and to build disease specific profiles for score feedback in special groups.

Touch-screens also give the possibility to more accurately record reaction times. As noted by Semmelmann et al, 2016, previously with traditional computer-based tests the younger child would often need to look to the keyboard or mouse and then back to the monitor while inputting their response (391). This introduces a substantial source of error in calculating response times. In looking at response times in their own study they did comment on an important design consideration for touch-screen tests. They found that the more complicated the action response required, i.e. dragging and dropping versus tapping, the higher the potential difference in reaction times between ages. This advantage of touch-screen technology could allow for less subjectivity and easier analysis.

Touch-screen technology also has the potential to increase the purity of cognitive assessment by reduce the confounding influence of motor skills, language ability and social influences. In her review of the use of CANTAB in children, Monica Luciana discusses some of these factors (387). She also comments that conversely this aim for purity can affect the ecological validity of the testing.

These potential benefits led to the proposal of a touch-screen platform as the basis of a novel cognitive assessment.

The first step in the development of this novel touch-screen based cognitive assessment is to determine the prevalence of touch-screen usage and the functional abilities of children for touch-screen interaction at a young age. This question was answered using a parental questionnaire. In the following section the methodology and results of this study will be outlined.

## *5.2 Touch-Screen Usage in Toddlers*

### 5.2.1 Abstract

#### 5.2.1.1 Objective

To establish the prevalence and patterns of use of touch-screen technologies in the toddler population.

#### 5.2.1.2 Design

Parental questionnaires were completed for children aged 12 months to three years examining access to touch-screen devices and ability to perform common forms of interaction with touch-screen technologies.

#### 5.2.1.3 Results

Out of the 82 questionnaires completed on typically developing children, 71% of toddlers had access to touch-screen devices for a median (IQR) of 15 minutes per day (9.375-26.25). By parental report, 24 months was the median age of ability to swipe (IQR: 19.5-30.5), unlock (IQR: 20.5 – 31.5) and active looking for touch-screen features (IQR: 22-30.5). 25 (21-31.25) months was the median age of ability to identify and use specific touch screen features. 32.8% of toddlers could perform all four skills.

#### 5.2.1.4 Conclusion

From two years of age toddlers have the ability to interact purposefully with touch-screen devices and demonstrate a variety of common skills required to utilise touch-screen technology.

### 5.2.2 Introduction

Touch-screen phones and tablets are increasingly available to children. Yet little is known about how this technology is being used by children and what effect this might have (392). In 1999, the American Academy of Pediatrics (AAP) made recommendations discouraging the use of media in those under two years of age (393). This was based on increasing awareness of the risks posed by violence, sexual content and advertising in media to children. More specifically, in the toddler age group, concerns centered around displacing other developmentally crucial interpersonal interactions and play (394). Early television watching has been associated with later attentional problems, sleep disruption and even immediate impact on executive functioning linked with the pacing of viewed media (395-397). Therefore, in 2011, a policy statement reaffirming the AAP's guidelines was released (394) which repeated existing health concerns and also highlighted that, despite parental beliefs, toddlers do not gain much educational value from watching television, no matter what the content.

All of these apprehensions were, however, based on passive forms of technology prior to the widespread introduction of touch-screen formats (392). The interactivity of touch-screen media could provide a different experience to the developing brain of the toddler. In a recent report, Cristia et al. addressed touch-screen usage in a cohort of French 5 – 40 month olds, looking at preferred activities on such devices and type of interactive gestures used and found widespread usage at approximately 76% with purposeful interaction, particularly tapping, to be occurring in this age group (398). As a further basis to answer these questions our aim was to ascertain the current usage prevalence in our cohort and to quantify the types of interactions toddlers have on a day-to-day basis with currently available portable touch-screen devices.

### 5.2.3 Method

Parents of children aged 12 months to three years were asked to complete a parental questionnaire over a five month period from May to September 2014. A survey method was chosen to give an indication of touch-screen usage in the child's typical daily environment. Recruitment took place in a university hospital in both in-patient and out-patient settings where a sample of parents of toddlers were approached by medical staff and asked to participate. Sample size was calculated based on national census data for this age group and was estimated at 96 children (399). Ethical approval was received from the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Informed consent was obtained from each parent or guardian (Appendix A). The novel parental questionnaire examined the exposure and access the child had to touch-screen technology, length of usage per day and types of interactions with the screen i.e. ability to unlock the screen, swipe through pages or images and ability to recognise and interact with specific touch-screen features such as application icons for games (See Appendix A.3). Parents were also asked if they had downloaded any games or applications specifically for the child's use. They were not asked to specify type. Parental occupations were used to determine socio-economic status (SES) using the UK National Statistics Socio-economic classification (NS-SEC) three class version (400).

Toddlers at high risk of or under investigation for developmental delay were excluded.

Responses were analysed using frequency analysis and are presented as median (interquartile range) and n/n (%) as appropriate. Spearman's correlation coefficient (ρ) was used to examine if time spent of touch-screen devices differed with age.

Mann-Whitney testing was used to compare if ability to interact with the screen changed with age. All data analysis was carried out using IBM SPSS statistics 22.

### 5.2.4 Results

Questionnaires were completed for 91 infants. Parents of five further infants were approached but declined participation. These children were due for imminent discharge or were felt by their parents to be too unwell (response rate 95%). Nine were excluded due to existing developmental concerns.

Of the 82 remaining, 47/82 (57%) were male. SES was reported for only 46/82 (56%) of the cohort; of those 11/46 (24%) were NS-SEC 1, 17/46 (37%) NS-NEC 2 and 18/46 (39%) NS-NEC 3. The median (IQR) age of toddlers at the time of questionnaire was 24 (20-30) months.

67/82 (82%), of parents reported owning a touch-screen device. 58/67 (87%) of those owning a touch-screen device gave their child the device to play with for a cumulative time, median (IQR), of 15 (9-26) minutes per day. Usage time did not correlate significantly with age ($\rho = 0.079$, $p = 0.572$). 36/58 (62%) had downloaded applications specifically for their child's use. 53/58 (91%) of parents who gave their child a touch-screen device reported that their child could swipe across the screen. 29/58 (50%) reported their child was able to unlock a touch screen and 37/58 (64%) felt their child actively looked for touch screen features. The median age of performing these three skills was 24 months (IQR of individual items displayed in *Table 5.2*). While ability to swipe and active looking for touch-screen features varied significantly with age, $p = 0.03$ and 0.04 respectively, ability to unlock the screen did not, $p = 0.106$. 42/58 (72%) felt their child was able to specifically identify and use touch screen features with a median (IQR) age of 25 (21-31) months and this skill significantly improved with age, $p=0.002$. Of note 19/58 (33%) of the toddlers could perform all four skills at a median (IQR) age of 29 (24-32) months.

*Table 5.2: Ability to interact with screen showing median age (in months) and interquartile range for four skills: ability to swipe across screen, ability to unlock screen, ability to actively look for touch-screen features and ability to identify and use specific touch-screen features. Difference in age of skill attainment calculated by Mann-Whitney U testing and p-values are displayed.*

| Skill | | Median | IQR | p-value |
|---|---|---|---|---|
| **Swipe** | Yes | 24 | 19.5, 30.5 | *0.03* |
| | No | 20 | 14.5, 22 | |
| **Unlock** | Yes | 24 | 20.5, 31.5 | *0.106* |
| | No | 23 | 18, 28.5 | |
| **Active Looking** | Yes | 24 | 22, 30.5 | *0.04* |
| | No | 21 | 15, 26.5 | |
| **Identify and Use** | Yes | 25 | 21, 31.25 | *0. 002* |
| | No | 18 | 13.5, 24 | |

### 5.2.5 Discussion

We have shown that touch-screen technology usage in this population is widespread, although based on subjective parental report, with inherent recall and response bias. Children as young as 12 months are able to use such devices and by 24 months have developed an array of skills that would allow them to interact purposefully with a touchscreen. The work presented here is limited by a preponderance of male infants of responders and insufficient numbers to explore the effect of gender, socio-economic status and neurodevelopment on touch-screen interaction. Also, the in-patient/out-patient status and reason for admission or attendance of the child was not recorded. There is the possibility that a hospital based sample, even if typically developing, could differ in their touch-screen usage from a wider community sample. The content children are interacting with then becomes the bigger question. Touch-screen devices can be used as portable media players making them no different from television and worryingly Cristia et al. found photo and video viewing to be the most common activities performed on touch-screen devices (398). Many applications designed for infants and toddlers already exist but there is no regulation of their quality, educational value or even safety. Some of the issues that arise with passive watching of television still apply; exposure to unsuitable materials, and/or visually fast-paced content and displacement of other developmentally important activities. Touch-screen platforms, when used to their strengths, offer many features which differentiate them from other forms of media and offer the potential for more positive effects (392). Interactive touch-screen applications offer a level of engagement not previously experienced with other forms of media and more akin to traditional play. They can also adapt to an individual child's level allowing for increasing complexity and providing positive feedback for a task achieved. The impact of this

widespread use of touch-screen technologies on child development has yet to be fully evaluated but a study by Bedford et al., 2016, has shown that earlier touch-screen use, specifically scrolling of the screen, was associated with earlier fine motor achievement (401). There is a need for further study on the effects of early touch-screen usage on other developmental domains as well as behavioural and social functioning.

This opens up the potential application of these devices for both assessment of development and early intervention in high risk children. As shown here a touch-screen testing platform could be feasible and acceptable for these purposes in the toddler age group. However, further prospective testing is required in a variety of populations both typically developing and those at risk of developmental delay to explore the trajectory of development of touch-screen skills and the effects of pathology on this process.

# 6.0 Methodology and Testing of a Novel Touch-Screen Cognitive Assessment

*Contributors to this chapter:*

Caroline Ahearne, Research fellow (C.A)

Conal Wrigley, Research psychologist (C.W)

Emma Hennessy, Research psychologist (E.H)

Sinead Dilworth, Medical student (S.D)

Catherine O'Connor, Clinical psychologist (C.O'C)

Neil Marlow, Professor of Neonatology (N.M)

Michelle de Haan, Paediatric Neuropsychologist (M. de H)

Raegan Murphy, Applied psychologist (R.M)

Steven Burgess, Hello Games (S.B)

Sean Murray, Managing Director, Hello Games (S.M)

Deirdre Murray, PI (D.M)

## *6.1 Methodology of developing a touch-screen assessment*

### 6.1.1 Aims of the Babyscreen App

Incorporating the evidence from previous sections, a novel cognitive assessment, referred to as the Babyscreen App, was developed with the following key aims:

- To perform targeted cognitive assessment using touch-screen technology at an early age

- To perform focused testing of key neuropsychological constructs:
  - Attention
  - Working memory
  - Processing speed

- To examine a child's response to a novel learning exercise

- To reduce the influence of language and motor skills on performance

Long-term goals of the Babyscreen App would be:

- To remove the need for an assessor to be present in the room thereby reducing subjectivity of assessment. Previous evidence from developmental psychology by McGarrigle, 1974, has shown how experimenter/assessor influence can profoundly affect the performance and behaviour of young children (187)

- To be capable of detecting subtle or early indicators of cognitive difficulties

- To be predictive of later intellectual and learning ability to identify those as risk requiring intervention

- To be used potentially to monitor effects of intervention and to track developmental trajectories

- Ultimately the Babyscreen App would be developed into a validated product that could provide a much needed tool for neuropsychometry in pre-school children.

### 6.1.2 Prototype Development

Following concept development and refinement by myself (C.A), and project PI (D.M), initial consultation with expert advisors occurred. This input included a clinician with considerable experience in longitudinal follow up of infants (N.M), a clinical psychologist (C.O'C), a neuropsychologist (M.de H) and experts in the computer gaming industry with considerable success in developing interactive touch-screen games for children (Hello Games). Then, in partnership with Hello Games, we began developing a prototype for a tablet based touch-screen assessment. The team at Hello Games included S.M, managing director, who contributed technical expertise and oversight, and S.B, who carried out technical development and provided day-to-day technical support.

#### 6.1.2.1 Feasibility

Early decisions pertaining to feasibility were made C.A with input and support from S.M and D.M. Having established the widespread usage of touch-screen technologies in toddlers in the survey study (C.A and S.D) described in Chapter 5, we began to

consider how the user interacts physically with a touch-screen device (*Figure 6.1*).

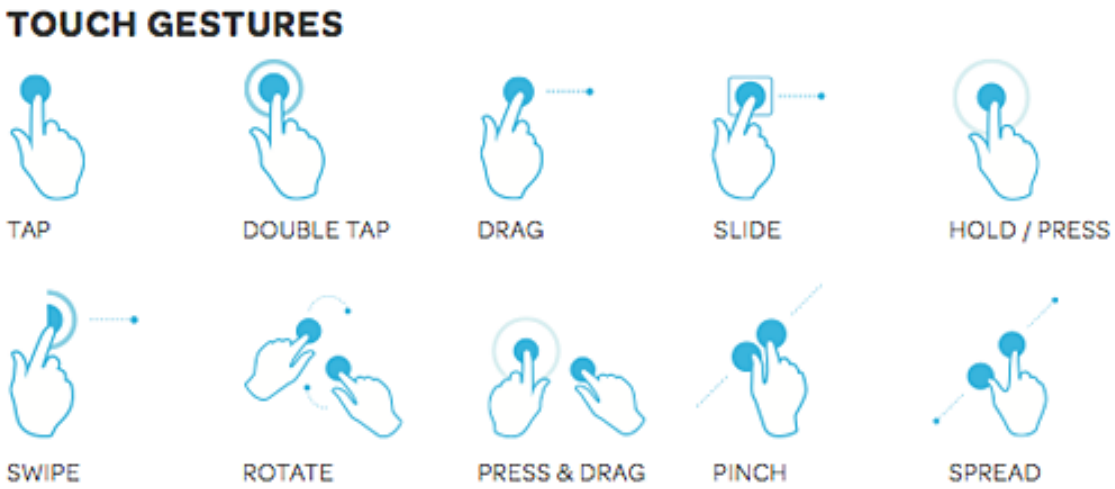



*Figure 6.1: Touch-screen gestures.* Adapted from Kale, 2016 (402)

A reasonable progression in terms of skill complexity is:

- Touch
    - Using hand, fingertips and isolated extended index finger
- Tap, hold/press
- Swipe
- Hold and drag
- Spread/zoom – fingers moving in synchrony but in opposite directions

Aziz et al, in a conference paper at the Science and Information conference in 2013 presented their study of 37 children aged two to four years (403). The children were recorded playing with different applications demanding varying touch gestures. They found that all two year olds could tap and drag/slide, all three

year olds could tap, drag/slide and drag & drop, while four year olds were able to successfully use these gestures as well as free rotate, pinch, flick and spread. The purpose of this presentation was to optimise app design for this age group but it is also relevant for testing.

For our purposes, it was decided to limit the requirement for more complex touch-screen gestures in the prototype and to progress the complexity slowly through the tasks. Firstly, touch, and then to incorporate some requirement to swipe and drag. In considering the touch gesture, the need to ensure some degree of intention to the child's gesture was considered. Therefore, the touch requirement was made into a brief hold. This speaks to the challenges of screen sensitivity for participant engagement discussed in section 5.1.4.

This functionality would be best served by a multi-touch capable surface. At this stage the Apple iPad 1 was selected as the pilot platform for the prototype.

While some motor interaction with the screen is at present unavoidable this represents a vastly reduced requirement of complex fine motor abilities to achieve a cognitive task.

For the purposes of initial testing, prototype tasks were designed to suit children from 12 months to three years. Though children as young as 12 months would not be tested on the application, consideration was given to testing children of 18 months who might have deficits in the testing domains.

One of the other considerations, was the feasibility of one of the other goals of this assessment, that of removing the assessor from the room. It was determined for initial testing that an assessor would be required to administer the app transition between screens when appropriate and terminate the test. When further data has been collected further automation could be

introduced to reduce the need for assessor presence towards zero.

Though the intention of the Babyscreen App is to specifically focus on the abilities of attention, working memory and processing speeds we are aware that there may be benefits to the assessment of other cognitive and developmental domains. Likewise, these may be tested using a touch-screen format. Possible evolutions, additions and adaptations of the app are discussed later in section 6.2.5.2 on future work.

#### 6.1.2.2 Early Task Development

Early task development was conceived by C.A, S.M and D.M. In order to meet our goals of testing using a novel learning experience, it was decided to develop tasks using basic geometric shapes. There would be an initial introductory phase of familiarisation to allow the child, particularly those with no or minimal prior touch screen exposure, to become aware of the cause and effect nature of touching the screen and getting a response. This was achieved through a 'rule learning' demonstration. It was decided that the goal object to achieve each task would be to press and hold a star. However, the star would require a progressively complicated approach to access. Initially, it would be the solitary target available and would then appear in the presence of distractor stars, which would later be hidden and finally it would be only accessible through triggering another object.

Tasks were developed by C.A to have face validity for testing the three key neuropsychological constructs outlined above: attention, working memory and processing speed. A story board format was used to optimise the order and progression of tasks.

An initial series of 19 tasks were chosen to commence prototype testing. (Please see section 6.1.3 for figures of app tasks)

#### 6.1.2.3 Acceptability

Acceptability of the app prototype to toddler age children was determined over a five month prototype testing phase occurring simultaneously with the survey study outlined in Chapter 5. Parents of children aged 12 months to three years were approached for participation (S.D). Informed consent was obtained for observation of the child interacting with the app prototype and for video-recording for review (see Appendix A). Ethical approval was provided by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. Following each week of testing S.D fed back to C.A and D.M, videos were reviewed and areas for improvement were identified and discussed.

During this prototype testing phase we focused on the qualitative response of the children to the app tasks, particularly the technical aspects. Children were observed for emotional response to tasks, behavioural responses to tasks i.e. if children were repeatedly making the same error on a particular task and general interest and engagement.

During this phase no standardised cut-offs were imposed on the children's performance. Children were allowed to proceed with each task to completion or visual demonstration requirement. The requirement for a visual demo would be determined by the child's behaviour. Examples included persistent looking from the device to the guardian or assessor, frustration with device, putting down the device indicating loss of interest, or giving a verbal response to offer of help where the child was capable. Verbal encouragement could be given by parent and assessor but parents were asked not to actively assist their child in

completing the task. The administration of the test would be discontinued only if the child became upset or persistently refused to engage and was not automatically after a particular number of failed tasks, i.e. no discontinuation rule was imposed.

51 children were observed interacting with the Babyscreen App prototype.

Quantitative data cannot be provided for this phase of development as tasks were modified on a regular basis in terms of both content and quality. This was a qualitative phase of development.

#### 6.1.2.4 App iteration

Throughout the above prototype testing phase there was an ongoing, fortnightly, feedback and iteration process with our collaborators at Hello Games (C.A, D.M, S.B, S.M). This iteration cycle of short testing phase, feedback, modification and repeat is a widely used process in computer game development (404).

Some of the most significant or challenging modifications that arose during the iteration process included:

- Timing of hold – this concerned finding the optimal time requirement for the child to hold down the star in order to elicit a response. There was a difficult balance to be sought between ensuring a hold was intentional and reducing frustration as result of non-responsiveness. As well as modifying response time we also incorporated a building colour and noise through touch and opted to permit multiple quick touches within the radius of star as well as a longer hold to provoke a response
- Introduction of face features – due to initial issues with engagement, the introduction of facial features to the

geometric shapes was tested. This included eyes that blinked and a basic smiling mouth shape. Child engagement improved dramatically with these additions. There may be concern that children with face processing difficulties may be disadvantaged though not specifically detected by this alteration (209, 405, 406). We attempted to negate this by introducing a rule change task that shifted face features to the non-target object, *see task nine below in task descriptions*. Further tasks could also be added to examine face processing skills specifically, *see future work*.

- Positive reinforcement on successful completion of task- between tasks visual stimulation of balloons rising up through a transition screen, and positive music was introduced to improve engagement and motivation. For more complex tasks, in-task noises were introduced to promote interaction.
- Modification of task contents – during iteration the length of assessment was deemed too long, though generally less than 10 minutes, this was evident by frequent early termination of assessment due to loss of interest on video review. Where possible tasks were removed if deemed to be repetitious or modified to add variation. This was based on face validity of constructs included.

#### 6.1.2.5 Final evaluation and SOP development

Following app iteration it was apparent that many children required a demonstration to indicate how a task was performed in order to achieve it. It was determined that after a given timeframe which allowed for a first attempt, children could be offered a demonstration in a “demo mode” which would be triggered by the assessor. Thereby, the demonstration by the

examiner would not be counted as part of the child's overall attempt. After a demonstration the child could then make a second attempt to complete the task. If they failed to do so after a further timeframe the assessor could trigger the application to proceed to the next task. If the app is progressed manually to the next task there is no transitionary screen offering positive reinforcement as outlined above.

The timeframes for the first and second attempt allowances were determined by video review (C.A). 10 videos of children performing the app were available where all tasks were attempted and all attempts were visible to the camera. For each task the time to completion on each attempt was noted, as well as time to distraction, i.e. the app was pushed away or parent/assessor was asked/gestured to help. Time frames to the nearest five seconds were determined between the upper range of task completion times and the lower range of distraction times.

Prior on the onset of the pilot testing phase, a standardised operating procedure was developed for testing (C.A). This would allow all pilot phase testing, and testing contributing to normative data collection to be carried out in a uniform manner to reduce the influence of potentially biasing factors.

The following instructions were provided for testing:

<u>Environment:</u>

- Quiet room
- Minimum outside noise
- Temperature regulated (23 degrees on auto setting of air conditioning or equivalent)
- Window (If present) closed with blinds shut
- Ambient electric lighting

- Appropriate sized table (see pictures), single child appropriate chair with one parent/guardian sitting adjacent and assessor sitting opposite
    - Table specifications: Community Playthings Multitable (56 x 112 cm) with medium adjustable legs set to lowest level (approx. 40cm) D243
    - Chair specifications: Community Playthings 2 x low teacher chair J432, 1 x 2-3yo 21cm chair J708

*Figure 6.2: Testing Environment*. Original photograph. Photo credit: Caroline Ahearne

- One Child, one parent/guardian and assessor only present in room
- No other toys present

- iPAD to be placed flat on table with double-sided tape/adhesive to secure iPAD to table
- Child must be sitting on own chair facing the table with both hands free

Testing:

- Set volume to approx. 70% of max
- Enable “guided access” to restrict child from closing down application
    - This prevents the child using the home button to navigate away from the testing application
- Enter demographic information
- Note Unique Study ID generated for linking with video and other testing records
    - An anonymised study ID unique to each device is generated with each new test
    - A unique device ID allows for data retrieval
- Proceed to Screen 1

iPad signals at 10s for visual demonstration (corners light up multi-coloured), assessor saying “Let me show you”, activates “demo-mode” by pressing diagonal corners (see Figure 6.3), demonstration completed with device in “demo-mode” and task repeats

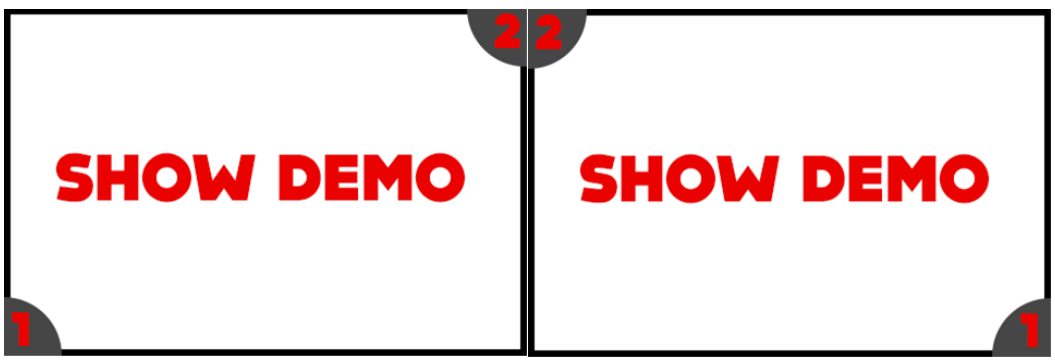


*Figure 6.3: Technique to trigger app demonstration.* Original Diagram. Credit: Steve Burgess (Hello Games)

After further signal at 10s (corners light up blue), proceed to next task if task not completed by pressing two adjacent corners (see Figure 6.4)

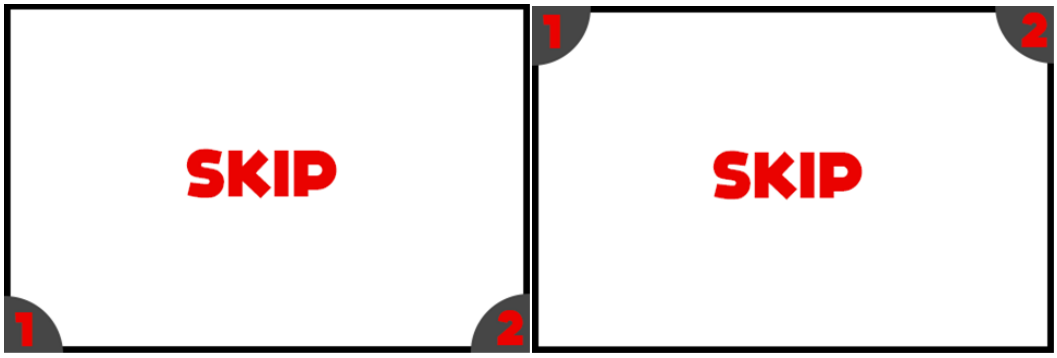



*Figure 6.4: Technique to trigger screen skip*. Original Diagram. Credit: Steve Burgess (Hello Games)

- Proceed to subsequent tasks

Demonstration after 20s signal if task not completed saying “Let me show you”, activate “demo-mode” as above, visual demonstration carried out in “demo-mode”, task repeats

After 20s further proceed to next task if not completed by pressing two adjacent corners, (See Figure 6.4)

- End test if child unhappy or distressed in any way by continuing by pressing all four corners simultaneously (see Figure 6.5)
- Cut-offs are not being imposed during the validation phase


*Figure 6.5: Technique to trigger app to quit.* Original Diagram. Credit: Steve Burgess (Hello Games)

- Non-directive encouragement and praise allowed to motivate as required:
  - "That was a difficult one, let's try the next one"
  - "Well done, good job"
- When not performing a visual demonstration or progressing to next task, avoid any directive gestures at the screen that might provide hints etc.

For each test an output excel file was generated on completion. This could be accessed directly from the iPad via iTunes or could be accessed from a central server followed data upload over Wi-Fi. Available output parameters were as follows:

- Was test quit? (y/n)
- 1st Attempt Interval (ms)
- 1st attempt completed? (y/n)
- 1st attempt skipped? (y/n)
- 1st attempt time (ms)
- 1st attempt demo? (y/n)
- 1st attempt delay to demo (ms)
- 1st attempt selection bias
- 2nd attempt Interval (ms)

- 2nd attempt completed? (y/n)
- 2nd attempt skipped (y/n)
- 2nd attempt time (ms)
- 2nd attempt selection bias

### 6.1.3 Task Descriptions

Descriptions of tasks included in pilot testing including a priori consensus construct labelling, as agreed by C.A and C.W, are as follows:


*Figure 6.6: Babyscreen Task 1.*

**Task 1**

**Description:** A gold star with a smiling face is presented on the iPad screen and the objective is to interact with the star by touch until it disappears. The task provides a rule learning task for the participant.

**Construct:** Attention


*Figure 6.7: Babyscreen Task 2*

**Task 2**

**Description:** Two gold stars are presented on screen and the objective is to interact with both by touch until they disappear from the screen. Task two confirms rule comprehension, increasing the number of interactive stimuli.

**Construct:** Processing speed, attention, short-term memory


*Figure 6.8: Babyscreen Task 3*

**Task 3**

**Description:** One gold target star with two plain blue stars without smiling faces are presented. The blue stars serve as distractors for the participant. The objective is to interact with the gold star by touch until it disappears from the screen.

**Construct:** Selective attention, working memory, processing speed


*Figure 6.9: Babyscreen Task 4*

**Task 4**

**Description:** Nine stars are presented in a 3x3 grid, eight of which are plain blue star distractors and one gold target star. The objective is to locate the correct target star and interact with the gold star until it disappears from the screen. The purpose of this task is to identify if the participant can selectively attend to the correct target star and inhibit from interacting with the plain blue target stars as achieved in the prior task but with increasing distractors.

**Construct:** Selective attention, working memory, processing speed

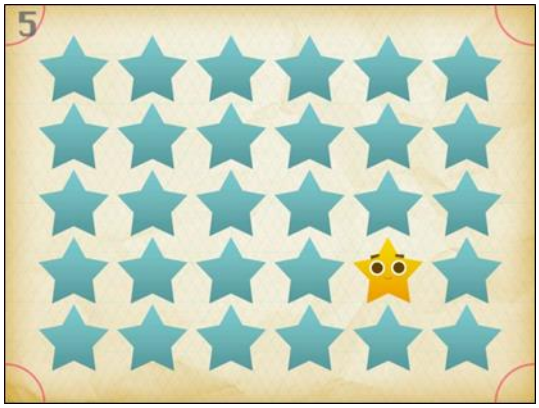



*Figure 6.10: Babyscreen Task 5*

**Task 5**

**Description:** 29 blue distractor stars and one target gold star are presented in a 6x5 grid formation. The objective is to interact with the target gold star by touch until it disappears from the screen. As above with again increasing distractors.

**Construct:** Selective attention, working memory, processing speed


*Figure 6.11: Babyscreen Task 6*

**Task 6**

**Description:** 30 stars are presented in a 6x5 grid, 29 of which are gold stars, one is a blue target star. Prior to this task, correct responses required interaction with the gold target star. The purpose of this task is rule reversal in order to measure constructs of ability in shifting attention. The objective is to

interact with the blue target star by touch until it disappears from the screen.

**Construct:** Rule change, shift, processing speed

*Figure 6.12: Babyscreen Task 7*

**Task 7**

**Description:** 30 multi-coloured stars are presented in a 6x5 grid of random assembly, one of which is a target gold star. The purpose is to switch the previous rule reversal and include a further level of learning through numerous coloured distractors. The task required the participant to shift their attention from a previously learned rule and selectively attend to the correct response item. The objective of the task is to interact with the gold target star until it disappears from the screen.

**Construct:** Attention, shift, processing speed

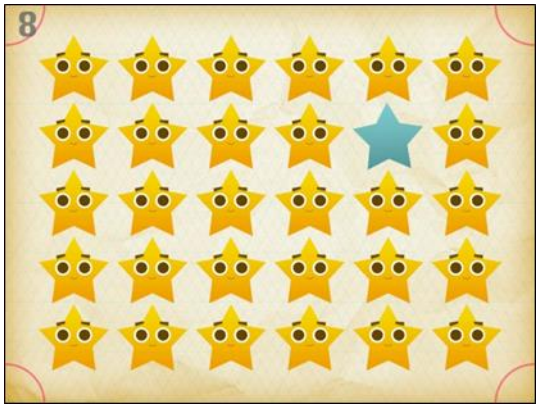



*Figure 6.13: Babyscreen Task 8*

**Task 8**

**Description:** 30 stars are presented in a 6x5 grid, one of which is a blue target star. The purpose is a rule reversal from a target gold star to a target blue star to measure participant's abilities in shifting attention from previously considered correct items. There is a further dimension to the challenge in that the face features are now on the non-target stars. The objective is to interact with the target blue star by touch until it disappears from the screen.

**Construct:** Rule change, shift, processing speed


*Figure 6.14: Babyscreen Task 9*

**Task 9**

**Description:** 30 stars are presented in a grid, one of which is a blue target star. The task requires the participant to remember the correct response from the previous task and illicit a correct response from memory, while inhibiting from interacting with gold stars. The objective is to interact with the target blue star by touch until it disappears from the screen.

**Construct:** Working memory, processing speed


*Figure 6.15: Babyscreen Task 10*

**Task 10**

**Description:** One target gold star is presented on the right hand of the screen. Two cups fall from the top of the screen. The cup on the left is blue and the cup on the right is red. The red cup on the right is now hiding the star. The objective is to interact with the red cup covering the star by lifting it above the target gold star. Once done, this will remove the red cup and the objective is then to interact with the target gold star by touch until it

disappears from the screen. The purpose of this task is to see if the participant can correctly retrieve the target star from the correct cup.

**Construct:** Working memory, object permanence

*Figure 6.16: Babyscreen Task 11*

**Task 11**

**Description:** One target gold star is presented on the left hand of the screen. Two cups fall from the top of the screen. The cup on the left is blue and the cup on the right is red. The blue cup on the left is now hiding the star. The objective is to interact with the blue cup covering the star by lifting it above the target gold star. Once done, this will remove the blue cup. The objective is then to interact with the target gold star by touch until it disappears from the screen. The purpose of which is to see if the participant correctly remembers the procedure from the prior task and shift their attention to the new object location while inhibiting from interacting with the incorrect cup.

**Construct:** Working memory, object permanence

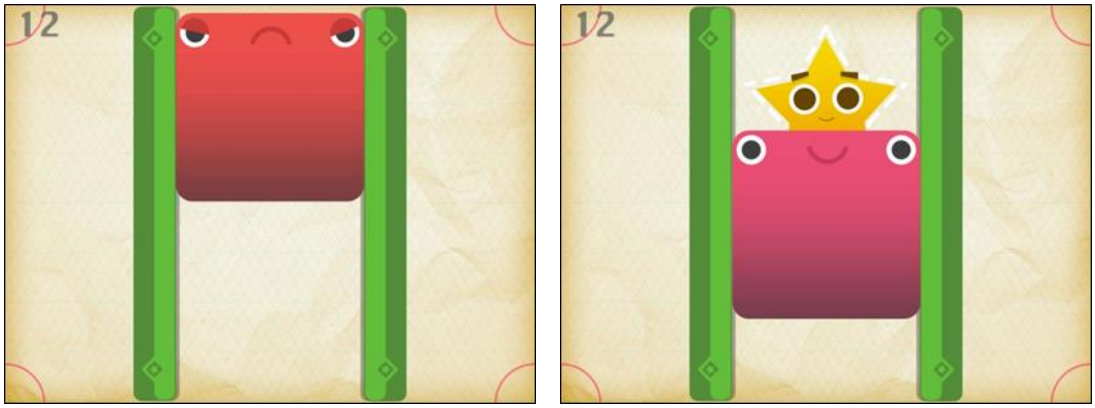



*Figure 6.17: Babyscreen Task 12*

**Task 12**

**Description:** A target gold star is presented between two green posts. A red box appears on the screen between the two green posts and vertically inclines until it covers the target gold star. Once the target gold star is covered, the red box visually portrays a sad face. The box can be moved both upwards and downwards between the green posts. If the red box is moved downwards to a certain point, the target gold star is revealed. The red box displays a happy face and changes to a lighter shade of red. Once uncovered, the objective is to interact with the target gold star by touch until it disappears from the screen. The purpose of which is to identify if the participant remembers where the star is hidden and if they can correctly locate the star and complete the task.

**Construct:** Attention, object permanence

*Figure 6.18: Babyscreen Task 13*

**Task 13**

**Description:** A target gold star is presented on the left hand side screen. A blue cup falls from the top of the screen and covers the gold star. The blue cup moves from the left hand side of the screen to the right hand side. A red cup now falls from the top left hand side of the screen and is placed next to the blue cup. The purpose of which is to identify if the child has learned that the target gold star has now shifted places with the blue cup from the left hand side of the screen to the right hand side of the screen. The objective is to lift the blue cup, reveal the target gold star and interact with the target gold star by touch until it disappears from the screen.

**Construct:** Working memory, processing speed

*Figure 6.19: Babyscreen Task 14*

**Task 14**

**Description:** A target gold star is presented on the left hand side of the screen. The objective is to interact with the target gold star by touch until it disappears from the screen. A target gold star is presented between two green posts on the right hand side of the screen. A red box appears on the screen between the two green posts and vertically inclines until it covers the target gold star. Once the target gold star is covered, the red box visually portrays a sad face. The box can be moved upwards and downwards between the green posts. Once the red box is interacted with by touch, the eyes of the red box shift to a straight line. If the red box is moved downwards to a certain point, the target gold star is revealed. The red box displays a happy face and changes to a lighter shade of red. Once uncovered, the objective is to interact with the target gold star by touch until it disappears from the screen. The purpose of which is to identify if both tasks, previously learned, can be recalled from memory.

**Construct:** Working memory, processing speed


*Figure 6.20: Babyscreen Task 15*

**Task 15**

**Description:** A blue button with a smiling face is presented. An outline of a star is presented across from the blue button. Once the blue button is pressed, the colour changes to a lighter blue and the facial expression changes. Once the blue button is held, a target gold star is presented within the outline grid. The purpose of which is for the participant to hold the blue button and simultaneously interact with the target gold star. The objective is to hold the blue button while interacting with the target gold star by touch until it disappears from the screen. The task requires the participant to use both hands in bilateral integration, which is a novel experience in the assessment. The purpose of which is to identify if the participant can successfully multi-task the procedure of both hands to complete the task.

**Construct:** Planning, processing speed


*Figure 6.21: Babyscreen Task 16*

**Task 16**

**Description:** Two round blue buttons are presented on screen with one on the lower left-hand side and one on the higher right-hand side of the screen. On the lower right-hand side and higher left-hand side of the screen, there are outlines of stars. Once a blue button is pressed, the colour changes to a lighter blue and the facial expression changes and a target gold star appears. The participant is required to hold the blue button and

simultaneously interact with the target gold star. The participant must remember the procedure of the previous task and elicit the correct hand movements to successfully complete both tasks. The objective is to hold each blue button, while interacting with the target gold stars by touch, until they disappear from the screen.

**Construct:** Working memory, planning, processing speed

*Figure 6.22: Babyscreen Task 17*

**Task 17**

**Description:** A target gold star is presented between two green posts on the left hand side of the screen. A red box appears between the two green posts and inclines until it covers the target gold star. Once covered, the red box visually portrays a sad face. The box can be moved both upwards and downwards between the green posts. If the red box is moved downwards to a certain point, the target gold star is revealed. The red box displays a happy face and changes to a lighter shade of red. Once uncovered, the objective is to interact with the target gold star by touch until it disappears from the screen. A blue button with a smiling face is presented on the higher right hand side of the screen. Underneath the blue button is an outline of a star. Once the blue button is held, a target gold star is presented within the outline grid. The objective is to hold the blue button

while interacting with the target gold star by touch until it disappears from the screen. The purpose of which is to identify if both tasks, previously learned, can be recalled from memory and completed.

**Construct**: Working memory, processing speed


*Figure 6.23: Babyscreen Task 18*

**Task 18**

**Description:** 30 stars are presented in a grid, one of which is a blue target star. The objective is to interact with the target blue star by touch until it disappears from the screen. The purpose of the final task is to identify if the participant can recall the rule reversal from previous tasks and correctly interact with the blue target star

**Construct:** Delayed memory, rule change, processing speed

## *6.2 Performance of the Babyscreen App in a pilot cohort*

Following prototype development and finalisation, formal pilot testing was commenced by C.A. During this phase no further modifications to the content or quality of the Babyscreen App could be introduced.

As part of an ongoing prospective case-control cohort study, the BiHIVE 2 study, the Babyscreen App was tested in a cohort of children aged 18 to 24 months alongside other measures of developmental and behavioural outcome.

### 6.2.1 Aims

The aims of this work were to establish the performance of a population of 18-24 month olds on the Babyscreen App and to correlate this performance with BSID-III results.

### 6.2.2 Methodology

The BiHIVE Study was established to identify a panel of biomarkers measured in umbilical cord blood at birth that could predict severity of hypoxic-ischaemic injury and neurodevelopmental outcome in a cohort of infants with signs of perinatal asphyxia at delivery. Promising biomarkers were to be identified using a multilevel approach with semi-targeted and, where possible, untargeted analysis in proteomics, metabolomics and transcriptomics. It is envisaged that an optimal panel of biomarkers determined by bioinformatic techniques would be developed into a point-of-care device to be used in the delivery suite. This would meaningfully contribute to the clinical decision making process, guiding management and prognostication.

Following the initial biomarker discovery phase, BiHIvE 1, a second prospectively recruited cohort was established for the purposes of biomarker validation, BiHIVE 2 (Validation of Biomarkers in Hypoxic-Ischaemic Encephalopathy).

The BiHIVE 2 cohort was recruited from March 2013 to June 2015 in two European sites, Cork University Maternity Hospital, Cork, Ireland and Karolinska Institutet and University Hospital, Karolinska, Sweden. Both sites represent large tertiary maternity hospitals with extensive experience in managing hypoxic-ischaemic encephalopathy including the use of therapeutic hypothermia.

Informed consent for umbilical cord blood analysis, data collection and neurodevelopmental assessment was sought from parents for participation. For the Cork site, ethical approval was provided by the Clinical Research Ethics committee of the Cork Teaching Hospitals.

Cases were identified for recruitment if they met the following criteria:

- Infants having a gestational age at birth of ≥36 weeks and any one of the following:
    - Apgar score ≤6 at 5 minutes of life
    - Cord pH < 7.1
    - Requiring intubation, CPR or IPPV > 10 minutes

If an infant met the recruitment criteria, the attending midwives drew umbilical cord blood, where technically possible, and filled four blood bottles provided in pre-prepared study packs which were stored in a designated research refrigerator on the labour ward. Midwives then contacted the research team via an on-call phone number available 24/7 to inform them that bloods had been collected. A designated researcher then attended to process the umbilical cord blood according to a standardised operating

procedure and place in freezer at -80°C within three hours of the infant's birth.

A contemporary control population was also recruited prospectively. These parents were approached early in labour to be part of the well babies group. Any delivery not meeting the above case criteria was eligible to be a control. Parents of infants who were initially consented as controls but then went on to develop perinatal asphyxia were then re-approached to consent as cases. Documentation for both groups is available in Appendix B.

Midwifery and obstetric staff were made aware of the study and requested to be involved through an extensive plan of promotional and educational activities. On-site promotional posters and reminders on noticeboards, neonatal resuscitaires and point-of-care blood gas processing devices were displayed. Ongoing education was provided to small groups within the delivery suite to capture rotating staff and to minimise interruption of patient care with regular on the ground reinforcement by research staff. Several feedback sessions at formal multidisciplinary teaching sessions were provided with updates on recruitment and results.

All cases and controls had demographic and clinical data collected during their in-patient stay. All infants were assessed neurologically using the Thompson score on day one, day two, day three of life and at discharge depending on length of in-patient stay. All infants requiring admission to the neonatal unit who developed hypoxic-ischaemic encephalopathy had a modified Sarnat score performed at 24 hours of life. EEG application and therapeutic hypothermia was provided based on clinical requirement as determined by the supervising consultant on duty.

C.A was responsible for patient recruitment, data collection, Sarnat staging, Thompson score administration and neurodevelopmental assessment. Responsibility for initial umbilical cord blood processing was shared between Dr Ann-Marie Looney, Dr Niamh Denihan and myself (C.A). EEG application was also provided on a 24/7 basis and during the period of BiHIVE 2 recruitment was performed by four dedicated research personnel, Mr Robert Goulding, Mr Rhodri Lloyd, Dr Luidmilla Kharoshankaya and myself (C.A).

In order to optimise patient retention for neurodevelopmental assessment, a retention plan was developed and administered for enrolled patients (C.A). This involved early discussion of neurodevelopmental assessment during the neonatal in-patient period. At this time we endeavoured to record details such as the child's name and optimal contact information for future communication. Parents also received a newsletter when the infants turned six months of age which reminded them of the study, linked them to our website and prompted them to update us with any change in their contact information. Multiple channels of contact for the study were provided, text, phone and email, and C.A responded to all contact made. When the infants turned one year of age they received a birthday card from the study team. Finally, just prior to 18 months of age, parents received an information leaflet regarding neurodevelopmental assessment which detailed its contents and informed the parent that they would soon be contacted by phone to invite them for the assessment and arrange a convenient appointment if they were agreeable (C.A). These documents can be found in Appendix C.

Neurodevelopmental outcome assessment was carried out between 18 and 24 months of age (C.A and E.H). Assessment consisted of the Cognitive, Language and Motor scales of the Bayley Scales of Infant and Toddler Development (Edition 3)

and the Babyscreen App. During app administered a behavioural observation record was completed for each child (See Appendix D). Parents were also asked to complete three questionnaires. Firstly, they were asked to complete a developmental screening questionnaire, designed by C.A, to establish any pre-existing developmental concerns from the parents, any current involvement in supportive services and any diagnoses already made that might affect the neurodevelopmental assessment interpretation (See Appendix E). This questionnaire was based on the known long-term complications that can arise in HIE. Secondly, they were asked to complete the social-emotional and adaptive behaviour questionnaire as part of the Bayley-3 and, thirdly, the Child Behavioural Checklist (1 ½ - 5 years). Parents were then provided with a report of their child's performance in the Bayley-3. Any concern for developmental delay detected during the session for children not already involved with services were referred onwards for clinical review.

### 6.2.3 Analysis

The performance of participants on neurodevelopmental outcome measures and the Babyscreen App is initially reported separately followed by comparison between the two. Lastly, performance of the at-risk group of children with HIE on the Babyscreen App is discussed.

Performance on the Babyscreen App was examined using several parameters. Up to 18 different tasks were presented to each child. Tasks were administered consecutively and in the same order as described above in section 6.1.3. If the child was not successful in the first 20 seconds of each task, a demonstration was provided and children were permitted a further 20 seconds to achieve the task. Therefore, children could achieve a task with or without a demonstration or could fail the task outright.

The time up to the demonstration is referred to as “attempt 1” and the time following the demonstration is referred to as “attempt 2”. Children who succeeded in completing the task prior to a demonstration were successful in attempt 1 and therefore had an attempt 2 time equal to zero.

For each task a time for attempt 1 and attempt 2, where applicable, was available in milliseconds. Time to completion was highly skewed, therefore data was log transformed to aid further analysis. For each task where distractors were present, i.e. objects visible on the screen that were not the target for task completion, the first target that the child interacted with was automatically noted in app output as ‘selection bias’. This applied to tasks 3, 4, 5, 6, 7, 8, 9, 10, 11, 13 and 18.

Individual tasks were excluded from analysis for the following reasons:

- Not available due to early discontinuation of test
- Not available due to technical fault i.e. app skipped a task unexpectedly or corners responded inappropriately to commands
- Child’s behaviour during task administration prevented appropriate assessment i.e. child became distracted and moved away from table during timing or task was inadvertently completed while child was distracted
- Parents gave overt hints or completed some or all of the task despite instructions

A scoring system was developed to allow performance in each task for each participant to be categorised for speed, accuracy and efficiency (C.A, C.W, R.M).

#### 6.2.3.1 Speed scores

For each participant the completion time for their successful attempt i.e. attempt 1 or attempt 2 was divided into tertiles. The first tertile represent the 'fast' group with the shortest completion times, the second tertile represent the 'medium' group and the third tertile represent the 'slow' group.

Speed scores were then assigned as per *Table 6.1* dependent on speed tertile and whether the task was successfully achieved in attempt 1 or attempt 2.

| **Group** | **1st Attempt** | **2nd Attempt** |
|---|---|---|
| **Fast** | 6 | 3 |
| **Medium** | 5 | 2 |
| **Slow** | 4 | 1 |

*Table 6.1: Speed score assignment*

Children who failed the task received a speed score of 0.

This led to a score distribution for each task as per *figure 6.24*.

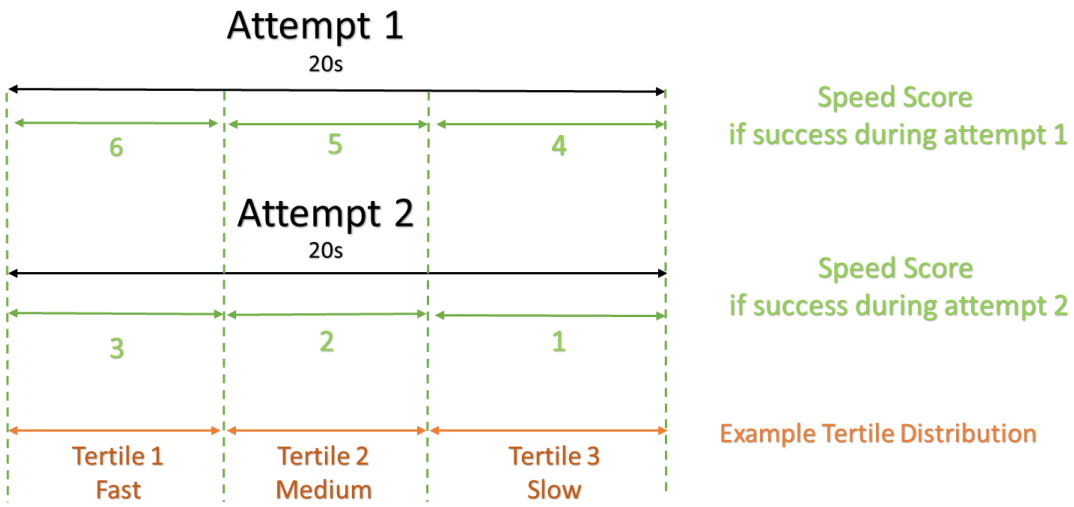


*Figure 6.24: Example Speed Score Distribution Graphic* (original diagram)

### 6.2.3.2 Accuracy scores

Accuracy scores were determined based on the selection bias of the participants' successful attempt. Therefore, if a child initially interacted with the correct target star/cup etc. in the first attempt and were successful a score of four was given. Other scores were assigned as per *Table 6.2*. Again, a failed attempt resulted in an accuracy score of 0. Accuracy scores were only available on tasks where distractors were evident i.e. tasks 3, 4, 5, 6, 7, 8, 9, 10, 11, 13 and 18.

| **Group** | **1st Attempt** | **2nd Attempt** |
|---|---|---|
| **Target Stimuli and Completed** | 4 | 2 |
| **Distractor Stimuli then Target Stimuli and Completed** | 3 | 1 |

*Table 6.2: Accuracy score assignment*

### 6.2.3.3 Efficiency Scores

Efficiency scores were based on speed tertile and accuracy in the participants' successful attempt and were assigned as per *Table 6.3*. As previously, a failed attempt resulted in a zero score. Efficiency scores were also only available on tasks where distractors were present.

| Group | 1st Attempt | 2nd Attempt |
|---|---|---|
| **Fast Correct** | 12 | 6 |
| **Medium Correct** | 11 | 5 |
| **Slow Correct** | 10 | 4 |
| **Fast Incorrect** | 9 | 3 |
| **Medium Incorrect** | 8 | 2 |
| **Slow Incorrect** | 7 | 1 |

*Table 6.3: Efficiency score assignment*

#### 6.2.3.4 Summative scores

A number of summative scores were assigned for overall app performance:

- Total tasks completed successfully (TTC)
- Total tasks completed successfully without requiring a visual demo (TTCWVD)
- Average speed score (combined speed score/number of tasks administered)
- Average Accuracy Score (combined accuracy score/number of distractor tasks administered)
- Average Efficiency Score (combined efficiency score/number of distractor tasks administered)

These scores were examined for internal correlations and confounding effects of participant age, previous touch-screen use and behavioural difficulties. Previous touch-screen use was determined by parental report and categorised as 1) none, 2) occasional, 3) two to three times per week and 4) daily.

Behavioural difficulties were determined by total problem score in the Child Behavioural Checklist for Ages 1 ½ to 5.

#### 6.2.3.5 Neurodevelopmental comparison analysis

Performance on individual tasks, as well as summative scores, was compared to Bayley-3 cognitive subscale scores. The ability of the summative scores to predict abnormal Bayley-3 cognitive performance was then examined. Analysis was also performed looking for the effects of language and motor skills on both cognitive subscale and Babyscreen App results, to test the app's aim of reducing the influence of language and motor ability on cognitive testing. A combined score of performance on the Babyscreen App was also derived and tested for correlation with cognitive scores and for predictive ability of abnormal cognitive performance. Each summative score for each child was rescaled to a 0 to 100 scale using the following formula:

*New score = [(original score –minimum possible score on original scale)/(max possible score on original scale – minimum possible score on original scale)]*100*

The combined score was derived by summing the five rescaled summative scores giving a resulting range of 0 to 500.

Finally the performance of a cohort of children diagnosed with hypoxic-ischaemic encephalopathy on the Babyscreen App was examined.

#### 6.2.3.6 Statistical analysis

Descriptive data is presented as n (%), mean (standard deviation), median (interquartile range) as appropriate. Differences between groups are reported using Students t-test, one way analysis of variance (ANOVA), Kruskal-Wallis, Mann-

Whitney U and chi-squared testing as appropriate. Correlations were performed using Pearson's R or Spearmann's correlation coefficient as appropriate. Ability of summative scores to predict abnormal Bayley-3 performance was examined using the area under the receiver operator curve (ROC). Coordinates of the curve were used to determine optimal cut-off points for summative scores. Testing of cut-offs in the special group study was performed by examining positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity and test agreement (Kappa). Results were considered significant with *p*-value >0.05. Statistical analysis was performed using IBM SPSS Statistic 22.0.

### 6.2.4 Results

#### 6.2.4.1 Neurodevelopmental Outcome

136 children attended for follow-up as part of the BiHIVE 2 study (neurodevelopmental follow-up ongoing). 58 children were recruited as controls at birth, 63 suffered perinatal asphyxia but had no evidence of encephalopathy at birth and 15 suffered hypoxic-ischaemic encephalopathy (HIE). Of the 15 with HIE in the neonatal period, 10 were diagnosed clinically with mild encephalopathy, four with moderate encephalopathy and one with severe encephalopathy. All five children with moderate or severe encephalopathy underwent therapeutic hypothermia after birth.

Overall, 74 infants were male and 62 were female. Demographic details are shown in *Table 6.4*.

116/136 (85%) of children had a developmental screening questionnaire completed for them. 12/116 (10%) of respondents reported having concerns about their child's development prior to attending their neurodevelopmental assessment. 3/116 (2.6%) of children had attended early intervention services prior to assessment. 4/116 (3.4%) had attended speech and language therapy, 6/116 (5.2%) had attended physiotherapy and 1/116 (0.9%) had attended occupational therapy. No child had attended a neurologist prior to assessment. There were no concerns about vision or hearing among parents in the cohort and no child had been diagnosed with cerebral palsy, epilepsy, autism spectrum disorder, attention deficit-hyperactivity disorder, microcephaly, dyspraxia or sensory processing disorder.

Median (IQR) age at follow-up was 20 (19-21) months. One child attended but failed to complete any of the three administered subscales of the BSID-III, cognitive, language and motor. A further 39 children failed to complete one or more subscale.

Across all cognitive subscales administered (n=135), 11% fell more than one standard deviation (SD) below the mean in composite scores (i.e. <85) and 4% scored more than one SD above the mean. In the language subscale (n=111) 9% scored <85 and 5% were >125, and in the motor subscale (n=112) 3% scored <85 and fewer than 1% scored in the superior range.

Median (IQR) scores in the subscales of the Bayley scales of Infant and Toddler Development (Edition 3) [BSID-III] are shown in *Table 6.5* and *Figure 6.25*. When scores were compared across all three clinical groups described above, significant differences were found in the cognitive, language and motor subscales with all $p$-values $\leq 0.01$.

However, differences between the administered subscales in control and perinatal asphyxia groups were not significant. Therefore, subsequent analysis of the performance of this cohort on the Babyscreen App looks at the control and perinatal asphyxia groups taken together, and the HIE group is examined separately as a high risk group study.

114/136 (84%) of children had the Child Behavioural Checklist (1 ½ - 5 years) completed for them. Reported scores in all scales demonstrated a right skewed distribution with very low rates of reporting in the borderline or clinical ranges. For scales related to problems with internalising behaviours i.e. emotional reactivity, anxiety/depression, somatic complaints and signs of withdrawal, only 1/114 (0.9%) respondents reported in the borderline range and 4/114 (3.6%) reported in the clinical range. For scales related to problems with externalising behaviours i.e. attention problems and aggressive behaviour, 4/114 (3.6%) reported in the borderline range and 2/114 (1.8%) reported in the clinical range. Median (IQR) scores for the total cohort across all reported scales are shown in *Table 6.6*. There was no significant difference in CBCL scores across clinical groups (i.e.

control, perinatal asphyxia and HIE) for internalising, externalising or total problems with *p*-values of 0.052, 0.575 and 0.743 respectively.

| | Control n = 58 | PA n=63 | HIE n=15 | p-value |
|---|---|---|---|---|
| **Sex (M/F)** | 31/27 | 34/29 | 9/6 | 0.90 |
| **MOD** | | | | <0.01* |
| ***SVD*** | 39 | 16 | 6 | |
| ***Forceps*** | 2 | 10 | 2 | |
| ***Ventouse*** | 10 | 29 | 5 | |
| ***EmCS*** | 7 | 8 | 2 | |
| | median (IQR) | median (IQR) | median (IQR) | |
| **GA (w+d)** | 39+2 (38+4-40+3) | 40+3 (39+3-41+2) | 40+4 (39+3-41+2) | 0.01* |
| **BW (kg)** | 3.6 (3.3-3.9) | 3.6 (3.2-3.9) | 3.5 (3.1-4.1) | 0.79 |
| **Cord pH** | 7.22 (7.16-7.30) | 7.04 (6.99-7.08) | 6.91 (6.85-7.05) | <0.01* |
| **Apgar 1 min** | 9 (9-9) | 6 (4-8) | 3 (1-3) | <0.01* |
| **Apgar 5 min** | 10 (10-10) | 9 (7-10) | 6 (4-6) | <0.01* |

*Table 6.4: Demographic details of cohort.* *=statistical significance, PA = perinatal asphyxia, HIE= hypoxic-ischaemic encephalopathy, M=male, F=female, MOD = method of delivery, SVD= spontaneous vaginal delivery, EmCS = emergency caesarean section, GA = gestational age (reported as weeks+days), BW= birthweight (reported in kilograms).

| | Controls median (IQR) n=58 | Perinatal Asphyxia median (IQR) n=63 | HIE median (IQR) n=15 | *p*-value* | *p*-value† |
|---|---|---|---|---|---|
| **Cognitive Composite** | 100 (93-110) | 105 (95-110) | 90 (90-100) | 0.80 | 0.01** |
| **Language Composite** | 103 (97-116) | 106 (100-112) | 93 (82-99) | 0.34 | <0.01** |
| **Motor Composite** | 103 (97-107) | 103 (99-110) | 94 (91-99) | 0.19 | 0.01** |
| **Social-Emotional Composite** | 105 (100-115) | 110 (105-125) | 120 (105-130) | 0.04** | 0.02** |
| **General Adaptive Composite** | 102 (91-108) | 101 (92-110) | 102 (92-110) | 0.98 | 0.99 |

*Table 6.5: Bayley Scales of Infant and Toddler Development (Edition 3) subscale composite scores for BiHIVE 2 cohort*

**p*-value for difference between scores of control and perinatal asphyxia groups (Mann Whitney U testing)

†*p*-value for difference across the three groups (Kruskal-Wallis)

**statistical significance $p<0.05$

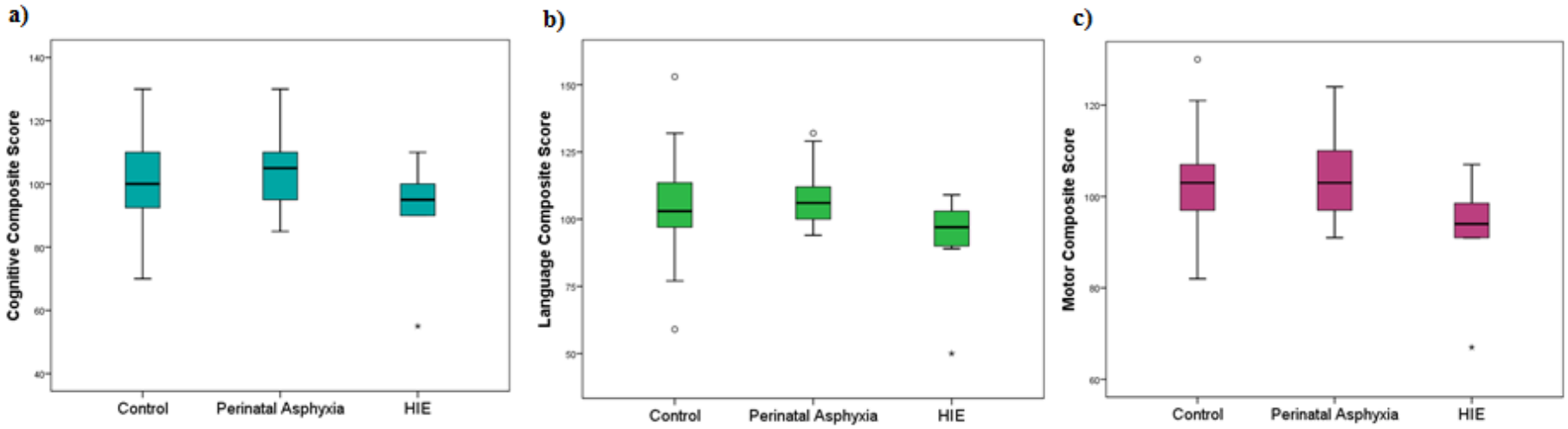



*Figure 6.25: Box-plot of cohort performance on the three administered subscales of the Bayley-3*, a) cognitive, b) language and c) motor

| CBCL Scale | Median | IQR | Normal Range |
|---|---|---|---|
| **Empirically Based Scales** | | | |
| **Emotionally Reactive** | 1 | 0-2 | ≤ 5 |
| **Anxious/Depressed** | 1 | 0-2 | ≤ 6 |
| **Somatic Complaints** | 1 | 0-2 | ≤ 4 |
| **Withdrawn** | 0 | 0-1 | ≤ 4 |
| **Sleep Problems** | 1 | 0-3 | ≤ 7 |
| **Attention Problems** | 1 | 0-2 | ≤ 5 |
| **Aggressive Behaviour** | 4 | 2-9 | ≤ 20 |
| **Internalising** | 3 | 1-6 | ≤ 13 |
| **Externalising** | 5 | 3-11 | ≤ 20 |
| **Total Problems** | 15 | 8-29 | ≤ 51 |
| **DSM-Oriented Scales** | | | |
| **Affective Problems** | 1 | 0-2 | ≤ 5 |
| **Anxiety Problems** | 2 | 0-3 | ≤ 7 |
| **Pervasive Developmental Problems** | 1 | 0-2 | ≤ 6 |
| **Attention Deficit Hyperactivity Problems** | 3 | 1-4 | ≤ 9 |
| **Oppositional Defiant Problems** | 1 | 0-3 | ≤ 7 |

*Table 6.6: CBCL Scores for total cohort*. Normal range is provided for reference, scores above these cut-offs lie in the borderline or clinical ranges. IQR = interquartile range; DSM = Diagnostic and Statistical Manual of Mental Disorders.

#### 6.2.4.2 App performance

121/136 (89%) of the cohort were eligible for Babyscreen App testing and inclusion in performance analysis. These were children who attended for developmental assessment and belonged to either the control or perinatal asphyxia group as assigned in the neonatal period, 58 and 63 children respectively. In 95/121 (79%) of attendances the Babyscreen App was administered and the data was included for analysis. Of the remaining 26/121 (21%) not included; in 6/121 (5%) of cases the app was unavailable for administration, in 5/121 (4%) the child's behaviour prevented administration of any tasks, and in 15/121 (12%) the app output file did not save to the device and could not be retrieved for analysis. Of the 5 children that failed to comply due to behaviour, 3/5 were 19 months, the median cognitive score of this subgroup was lower than the whole group average at 95 (77.5-105). Also 3/5 of the group also failed to complete at least 1 subscale of the Bayley in conjunction with failure to comply with the Babyscreen App. No link with behavioural attributes, as per CBCL scores, and failure to comply could be identified. These factors may contribute in part to non-compliance but observation of a larger cohort would be of benefit for future development.

For this cohort of 95 children, 1710 tasks (95 x 18) could potentially be administered and 1256/1710 (73%) of tasks were administered. Tasks may not be administered and therefore not included in analysis due to reasons as outlined in section 6.2.3.

The majority of children completed most administered tasks successfully without requiring a demonstration. For descriptive statistics regarding task completion see *Table 6.7*. The need for a visual demo and the failure rate increased from Task 8 onwards. Failure rates peaked in tasks 15 to 17. (*Figure 6.26*)

Median (IQR) time to completion was maximal in Task 10 with 34.2 (19.8-44.4) seconds required by children to complete the task successfully. This was due to the higher levels of demonstration requirement. Tasks 15-17 also had low levels of success without demonstration but had high levels of failure, reducing the number of children completing the task on which to measure completion time.

| | N | Completed | | Failed | Time to Completion (attempt 1 + attempt 2) | |
|---|---|---|---|---|---|---|
| | | without demo | with demo | | Median (s) | IQR (s) |
| **Task 1** | 95 | 69 (73%) | 17 (18%) | 9 (9%) | 8.4 | 3.4-19.1 |
| **Task 2** | 92 | 83 (90%) | 3 (3%) | 6 (7%) | 7.6 | 4.3-13.9 |
| **Task 3** | 86 | 82 (95%) | 1 (1%) | 3 (4%) | 3.3 | 1.7-6.1 |
| **Task 4** | 82 | 79 (96%) | 2 (2%) | 1 (1%) | 3.3 | 1.6-9.4 |
| **Task 5** | 83 | 73 (88%) | 5 (6%) | 5 (6%) | 3.6 | 1.8-7.8 |
| **Task 6** | 82 | 68 (83%) | 11 (13%) | 3 (4%) | 5.0 | 2.5-11.4 |
| **Task 7** | 82 | 76 (93%) | 4 (5%) | 2 (2%) | 3.0 | 1.5-6.5 |
| **Task 8** | 68 | 35 (51%) | 14 (21%) | 19 (28%) | 12.2 | 6.4-25.8 |
| **Task 9** | 76 | 46 (61%) | 17 (22%) | 13 (17%) | 8.6 | 2.6-24.7 |
| **Task 10** | 74 | 21 (28%) | 30 (41%) | 23 (31%) | 34.2 | 19.8-44.4 |
| **Task 11** | 71 | 37 (52%) | 16 (23%) | 18 (25%) | 15.7 | 6.8-32.8 |
| **Task 12** | 70 | 50 (71%) | 14 (20%) | 6 (9%) | 11.0 | 6.4-28.7 |
| **Task 13** | 61 | 48 (79%) | 4 (6%) | 9 (15%) | 9.5 | 5.1-16.0 |
| **Task 14** | 59 | 47 (80%) | 5 (8%) | 7 (12%) | 11.2 | 7.2-26.7 |
| **Task 15** | 55 | 8 (15%) | 11 (20%) | 36 (65%) | 28.6 | 19.7-37.5 |
| **Task 16** | 40 | 13 (33%) | 3 (7%) | 24 (60%) | 26.6 | 19.8-44.1 |
| **Task 17** | 37 | 13 (35%) | 6 (16%) | 18 (49%) | 23.7 | 14.3-42.5 |
| **Task 18** | 43 | 36 (84%) | 3 (7%) | 4 (9%) | 7.1 | 2.9-13.0 |

*Table 6.7: Babyscreen App performance: descriptive statistics on task completion*

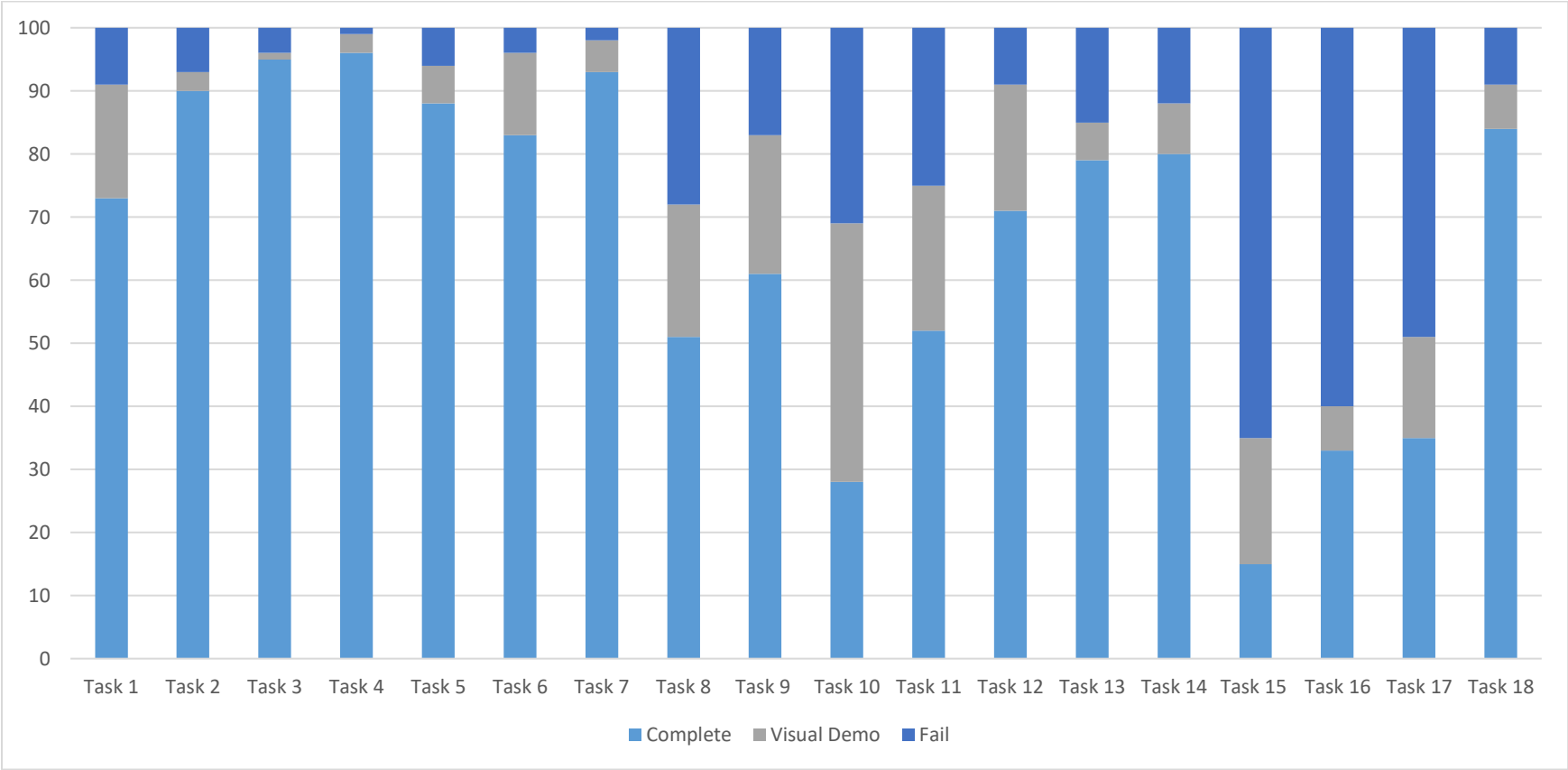



*Figure 6.26: Bar chart representing percentage completion, completion with visual demo and failure in each of Babyscreen App tasks administered*

Speed scores (0-6) were assigned for all tasks administered. The distribution of speed scores assigned for this cohort are shown in *Table 6.8*. Accuracy scores (0-4) were assigned for children performing tasks 3-11, 13 and 18 (i.e. those where distractors were present), and distributed as per *Table 6.9*. Finally, efficiency scores (0-12) were assigned for all distractor tasks administered and distributed as per *Table 6.10*.

Summative scores, as outlined in section 6.2.3.4, were assigned to each child to reflect their overall performance on the Babyscreen App. There were medium to large positive internal correlations between the summative scores; total tasks completed, total tasks completed without visual demo, average speed score, average accuracy score and average efficiency score, $p<0.01$ *(Table 6.11)*.

App performance was examined in the light of age across the cohort. Testing could be performed between 18 and 24 months but 112/121 (93%) of the cohort tested fell between 19 and 22 months. Time to completion of any successful attempt did not differ by age at testing in any task except for Task 14, $p=0.016$. In terms of summative scores, total tasks completed correlated with age, Spearman's $\rho=0.35$, $p<0.01$, as did total tasks completed without a demonstration, Spearman's $\rho=0.30$, $p<0.01$. Therefore, older children were able to succeed in more tasks than younger children and did so without requiring as many visual demos. The remaining summative scores did not change with age. Average speed, accuracy and efficiency did not appear to be affected by age in this cohort, $\rho=0.12$ ($p=0.24$), $\rho=0.14$ ($p=0.19$) and $\rho=0.14$ ($p=0.19$) respectively.

App performance was also tested for the effects of previous touchscreen usage to rule out confounding influence. Higher levels of previous touchscreen exposure were not associated with overall performance from the point of view of total tasks

completed or speed, $\rho$=0.04 ($p$=0.67) and $\rho$=-0.06 ($p$=0.55) respectively but did influence accuracy and hence efficiency, $\rho$=0.27 ($p$=0.01) and $\rho$=0.23 ($p$=0.03) respectively.

The effect of the child's behaviour on app performance was examined using the total problems score on the child behavioural checklist as a surrogate marker. Total problems showed no correlation with any of the summative scores measured. *(Table 6.11)*

| | N | Speed Score | | | | | | | Median |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | |
| **Task 1** | 95 | 9 (9%) | 11 (12%) | 3 (3%) | 3 (3%) | 17 (18%) | 34 (36%) | 18 (19%) | 5 |
| **Task 2** | 92 | 6 (7%) | 2 (2%) | 1 (1%) | | 30 (33%) | 34 (37%) | 19 (21%) | 5 |
| **Task 3** | 86 | 3 (4%) | 1 (1%) | | | 26 (30%) | 33 (38%) | 23 (27%) | 5 |
| **Task 4** | 82 | 1 (1%) | 2 (2%) | | | 30 (37%) | 23 (28%) | 26 (32%) | 5 |
| **Task 5** | 83 | 5 (6%) | 2 (2%) | 1 (1%) | 2 (2%) | 30 (36%) | 24 (29%) | 19 (23%) | 5 |
| **Task 6** | 82 | 3 (4%) | 6 (7%) | 4 (5%) | 1 (1%) | 21 (26%) | 24 (29%) | 23 (28%) | 5 |
| **Task 7** | 82 | 2 (2%) | 3 (4%) | | 1 (1%) | 31 (38%) | 23 (28%) | 22 (27%) | 5 |
| **Task 8** | 68 | 19 (28%) | 3 (4%) | 4 (6%) | 7 (10%) | 13 (19%) | 16 (24%) | 6 (9%) | 4 |
| **Task 9** | 76 | 13 (17%) | 7 (9%) | 5 (7%) | 5 (7%) | 20 (26%) | 12 (16%) | 14 (18%) | 4 |
| **Task 10** | 74 | 23 (31%) | 11 (15%) | 14 (19%) | 5 (7%) | 6 (8%) | 8 (11%) | 7 (10%) | 2 |
| **Task 11** | 71 | 18 (25%) | 7 (10%) | 5 (7%) | 4 (6%) | 13 (18%) | 14 (20%) | 10 (14%) | 4 |
| **Task 12** | 70 | 6 (9%) | 8 (11%) | 3 (4%) | 3 (4%) | 15 (21%) | 22 (31%) | 13 (18%) | 5 |
| **Task 13** | 61 | 9 (15%) | 1 (2%) | 1 (2%) | 2 (3%) | 26 (43%) | 6 (10%) | 16 (26%) | 4 |
| **Task 14** | 59 | 7 (12%) | 3 (5%) | 2 (3%) | | 18 (31%) | 17 (29%) | 12 (20%) | 4 |
| **Task 15** | 55 | 36 (65%) | 3 (6%) | 5 (9%) | 3 (6%) | 4 (7%) | 3 (6%) | 1 (2%) | 0 |
| **Task 16** | 40 | 24 (60%) | | 2 (5%) | 1 (3%) | 7 (18%) | 3 (8%) | 3 (8%) | 0 |
| **Task 17** | 37 | 18 (49%) | 2 (5%) | 4 (11%) | | 6 (16%) | 4 (11%) | 3 (8%) | 1 |
| **Task 18** | 43 | 4 (9%) | 2 (5%) | 1 (2%) | | 11 (26%) | 13 (30%) | 12 (28%) | 5 |

*Table 6.8: Speed Scores assigned for each task* (as per section 6.2.3.1).

| | N | Accuracy Score | | | | | Median |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | |
| **Task 3** | 86 | 3 (4%) | 1 (4%) | | 16 (19%) | 66 (77%) | 4 |
| **Task 4** | 82 | 1 (1%) | | 2 (2%) | 36 (44%) | 43 (52%) | 4 |
| **Task 5** | 83 | 5 (6%) | 3 (4%) | 2 (2%) | 38 (46%) | 35 (42%) | 3 |
| **Task 6** | 82 | 3 (4%) | 9 (11%) | 2 (2%) | 41 (50%) | 27 (33%) | 3 |
| **Task 7** | 82 | 2 (2%) | 3 (4%) | 1 (1%) | 42 (51%) | 34 (42%) | 3 |
| **Task 8** | 68 | 19 (28%) | 11 (16%) | 3 (4%) | 32 (47%) | 3 (4%) | 3 |
| **Task 9** | 76 | 13 (17%) | 11 (15%) | 6 (8%) | 31 (41%) | 15 (20%) | 3 |
| **Task 10** | 74 | 23 (31%) | 16 (22%) | 14 (19%) | 10 (14%) | 11 (15%) | 1 |
| **Task 11** | 71 | 18 (25%) | 4 (25%) | 12 (17%) | 13 (18%) | 24 (34%) | 3 |
| **Task 13** | 61 | 9 (15%) | 2 (3%) | 2 (3%) | 21 (34%) | 27 (44%) | 3 |
| **Task 18** | 43 | 4 (9%) | 3 (7%) | | 22 (51%) | 14 (33%) | 3 |

*Table 6.9: Accuracy Scores assigned for each task* (as per section 6.2.3.2).

| | N | Efficiency Score | | | | | | | | | | | | | Median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | |
| **Task 3** | 86 | 3 (4%) | 1 (1%) | | | | | | 7 (8%) | 7 (8%) | 2 (2%) | 19 (22%) | 26 (30%) | 21 (24%) | 11 |
| **Task 4** | 82 | 1 (1%) | | | | 2 (2%) | | | 20 (24%) | 11 (13%) | 5 (6%) | 10 (12%) | 12 (15%) | 21 (26%) | 10 |
| **Task 5** | 83 | 5 (6%) | | 1 (1%) | 2 (2%) | 2 (2%) | | | 17 (21%) | 13 (16%) | 8 (10%) | 13 (16%) | 11 (13%) | 11 (13%) | 9 |
| **Task 6** | 82 | 3 (4%) | 5 (6%) | 3 (4%) | 1 (1%) | 1 (1%) | 1 (1%) | | 12 (15%) | 18 (22%) | 11 (13%) | 9 (11%) | 6 (7%) | 12 (15%) | 8 |
| **Task 7** | 82 | 2 (2%) | 2 (2%) | | 1 (1%) | 1 (1%) | | | 22 (27%) | 13 (16%) | 7 (9%) | 9 (11%) | 10 (12%) | 15 (18%) | 9 |
| **Task 8** | 68 | 19 (28%) | 3 (4%) | 4 (6%) | 4 (6%) | | | 3 (4%) | 13 (19%) | 16 (24%) | 3 (4%) | | | 3 (4%) | 7 |
| **Task 9** | 76 | 13 (17%) | 5 (7%) | 3 (4%) | 3 (4%) | 2 (3%) | 2 (3%) | 2 (3%) | 19 (25%) | 7 (9%) | 5 (7%) | 1 (1%) | 5 (7%) | 9 (12%) | 7 |
| **Task 10** | 74 | 23 (31%) | 7 (10%) | 8 (11%) | 1 (1%) | 4 (5%) | 6 (8%) | 4 (5%) | 3 (4%) | 4 (5%) | 3 (4%) | 3 (4%) | 4 (5%) | 4 (5%) | 2 |
| **Task 11** | 71 | 18 (25%) | 2 (3%) | 2 (3%) | | 5 (7%) | 3 (4%) | 4 (6%) | 5 (7%) | 8 (11%) | | 8 (11%) | 6 (9%) | 10 (14%) | 7 |
| **Task 13** | 61 | 9 (15%) | 1 (2%) | 1 (2%) | | | | 2 (3%) | 15 (25%) | 3 (5%) | 3 (5%) | 11 (18%) | 3 (5%) | 13 (21%) | 8 |
| **Task 18** | 43 | 4 (9%) | 2 (5%) | 1 (2%) | | | | | 11 (26%) | 8 (19%) | 3 (7%) | | 5 (12%) | 9 (21%) | 8 |

*Table 6.10: Efficiency Scores assigned for each task* (as per section 6.2.3.3).

| | Median (IQR) | TTC | TTCWVD | Ave speed score | Ave accuracy score | Ave efficiency score | Age | Previous touchscreen use | CBCL total problems |
|---|---|---|---|---|---|---|---|---|---|
| **Total tasks Completed (TTC)** | 12 (8-14) | - | | | | | | | |
| **Total tasks completed without visual demo (TTCWVD)** | 10 (7-13) | 0.93** | - | | | | | | |
| **Average speed score** | 3.5 (3-4) | 0.67** | 0.75** | - | | | | | |
| **Average accuracy score** | 2.8 (2.2-3.1) | 0.64** | 0.70** | 0.67** | - | | | | |
| **Average efficiency score** | 7.4 (5.7-8.6) | 0.64** | 0.72** | 0.73** | 0.97** | - | | | |
| **Age (months)** | 20 (19-21) | 0.35** | 0.30** | 0.12 | 0.14 | 0.14 | - | | |
| **Previous touchscreen use** | - | 0.04 | 0.14 | -0.06 | 0.27* | 0.23* | 0.06 | - | |
| **CBCL total problems** | 14.5 (7.8-29.3) | 0.02 | 0.14 | 0.04 | 0.02 | 0.03 | 0.08 | 0.03 | - |

*Table 6.11: Correlation matrix showing correlations between summative scores, previous touchscreen usage and total problem scores of the Child Behavioural Checklist.* *$p<0.05$, **$p<0.01$ TTC=total tasks completed, range (0-18), TTCWVD= total tasks completed without requiring a visual demonstration, range (0-18), CBCL=child behavioural checklist

#### 6.2.4.3 Comparison of Babyscreen App performance and Bayley-3 Cognitive Scores

Performance of the cohort of children on the Babyscreen App was compared with their contemporary performance in the cognitive subscale of the Bayley Scales of Infant and Toddler Development (Edition 3). Performance on individual tasks was examined for relationship to cognitive scores. Cognitive scores differed significantly between children who successfully completed and children who failed, on tasks 1, 2, 7, 9 and 10 *(Table 6.12)*. Amongst children who succeeded in each task, cognitive scores differed significantly between children who required a demonstration to complete the task and those that did not in tasks 1, 6 and 11 *(Table 6.13)*.

Speed scores, accuracy scores and efficiency score for each task were correlated against cognitive scores from the Bayley-3 (*Table 6.14*). Small to medium positive correlations were found between some of the app tasks and cognitive scores. Significant correlations were found between speed scores in tasks 1, 10 and 11 and cognitive scores. Tasks with distractors were also assigned accuracy and efficiency scores. Accuracy scores significantly correlated with cognitive scores on tasks 6, 10 and 11 while efficiency scores correlated significantly on tasks 6, 10 and 13.

| | N | Task completed | | *p*-value |
|---|---|---|---|---|
| | | Yes<br>Median (IQR) | No<br>Median (IQR) | |
| **Task 1** | 95 | 105 (95-115) | 100 (85-102) | 0.019* |
| **Task 2** | 92 | 105 (100-115) | 90 (81-106) | 0.038* |
| **Task 3** | 86 | 105 (100-115) | 100 (90-100) | 0.929 |
| **Task 4** | 82 | 105 (98-110) | 105 (105-105) | 0.902 |
| **Task 5** | 83 | 105 (100-115) | 110 (93-113) | 0.963 |
| **Task 6** | 82 | 105 (100-115) | 95 (85-95) | 0.063 |
| **Task 7** | 82 | 105 (100-114) | 78 (70-78) | 0.004* |
| **Task 8** | 68 | 100 (98-115) | 105 (100-115) | 0.354 |
| **Task 9** | 76 | 105 (100-115) | 95 (90-105) | 0.044* |
| **Task 10** | 74 | 105 (100-115) | 100 (90-105) | 0.003* |
| **Task 11** | 71 | 105 (100-115) | 100 (90-111) | 0.160 |
| **Task 12** | 70 | 105 (100-110) | 108 (98-119) | 0.532 |
| **Task 13** | 61 | 105 (100-115) | 105 (90-110) | 0.233 |
| **Task 14** | 59 | 105 (100-115) | 100 (95-110) | 0.375 |
| **Task 15** | 55 | 105 (95-115) | 105 (100-110) | 0.857 |
| **Task 16** | 40 | 103 (100-110) | 103 (100-114) | 0.692 |
| **Task 17** | 37 | 105 (100-115) | 100 (99-110) | 0.169 |
| **Task 18** | 43 | 105 (100-115) | 105 (105-109) | 0.856 |

*Table 6.12: Median (Interquartile Range) cognitive scores on Bayley-3 of children who succeeded and failed in each Babyscreen App task*, *p*-value represents between group differences on Mann Whitney U testing.
*$p<0.05$

| | N | Demo Required | | *p*-value |
|---|---|---|---|---|
| | | Yes<br>Median (IQR) | No<br>Median (IQR) | |
| **Task 1** | 86 | 100 (90-105) | 105 (100-115) | 0.005* |
| **Task 2** | 86 | 105 (105-105) | 105 (100-115) | 0.294 |
| **Task 3** | 83 | 105 (105-105) | 105 (99-115) | 0.940 |
| **Task 4** | 81 | 100 (90-100) | 105 (100-110) | 0.672 |
| **Task 5** | 78 | 105 (105-115) | 105 (98-115) | 0.308 |
| **Task 6** | 79 | 95 (90-105) | 105 (100-115) | 0.005* |
| **Task 7** | 80 | 103 (86-119) | 105 (100-110) | 0.840 |
| **Task 8** | 49 | 100 (90-119) | 105 (100-115) | 0.430 |
| **Task 9** | 63 | 105 (95-115) | 105 (100-115) | 0.925 |
| **Task 10** | 51 | 108 (100-115) | 105 (100-118) | 0.923 |
| **Task 11** | 53 | 100 (93-109) | 110 (100-115) | 0.035* |
| **Task 12** | 64 | 103 (90-110) | 105 (100-115) | 0.141 |
| **Task 13** | 52 | 108 (100-126) | 105 (100-115) | 0.679 |
| **Task 14** | 52 | 100 (98-113) | 105 (100-115) | 0.546 |
| **Task 15** | 19 | 105 (95-110) | 110 (85-119) | 0.545 |
| **Task 16** | 16 | 100 (85-100) | 105 (100-113) | 0.146 |
| **Task 17** | 19 | 100 (88-123) | 110 (103-115) | 0.467 |
| **Task 18** | 39 | 115 (90-115) | 103 (100-115) | 1.000 |

*Table 6.13: Median (Interquartile Range) cognitive scores on Bayley-3 of children who required a demonstration and those who did not in order to complete in each Babyscreen App task*, *p*-value represents between group differences on Mann Whitney U testing. *$p<0.05$

| | N | Speed Score | Accuracy Score | Efficiency Score |
|---|---|---|---|---|
| **Task 1** | 95 | **0.238** | | |
| **Task 2** | 92 | 0.196 | | |
| **Task 3** | 86 | 0.022 | 0.011 | 0.034 |
| **Task 4** | 82 | 0.135 | 0.031 | 0.082 |
| **Task 5** | 83 | 0.066 | -0.013 | 0.045 |
| **Task 6** | 82 | 0.164 | **0.308** | **0.248** |
| **Task 7** | 82 | 0.122 | 0.174 | 0.166 |
| **Task 8** | 68 | 0.008 | -0.014 | 0.013 |
| **Task 9** | 76 | 0.148 | 0.155 | 0.158 |
| **Task 10** | 74 | **0.324** | **0.320** | **0.333** |
| **Task 11** | 71 | **0.236** | **0.237** | 0.216 |
| **Task 12** | 70 | 0.125 | | |
| **Task 13** | 61 | 0.228 | 0.206 | **0.257** |
| **Task 14** | 59 | 0.088 | | |
| **Task 15** | 55 | 0.007 | | |
| **Task 16** | 40 | 0.026 | | |
| **Task 17** | 37 | 0.307 | | |
| **Task 18** | 43 | 0.101 | 0.058 | 0.095 |

*Table 6.14: Correlations between speed scores, accuracy scores and efficiency scores against cognitive scores.* Coefficients highlighted in bold correspond to $p$-value<0.05.

Summative scores of app performance were correlated with cognitive composite scores. Significant medium sized positive correlations were evident between each of the summative scores and cognitive composite scores *(Figure 6.27 and Table 6.15)*. As two of the summative score, total tasks completed and total tasks completed without visual demo, were affected by the age of the child, partial correlations controlling for age were performed. Correlations with cognitive scores remained significant despite age being controlled.

One of the aims during development of the Babyscreen App was to reduce the demand of language skills on performance in a cognitive assessment. While overall performance in language abilities and cognitive abilities are always likely to be somewhat correlated, many cognitive assessments including the cognitive section of the Bayley rely heavily on verbal instructions and hence verbal comprehension to complete the tasks successfully. In fact in our cohort cognitive composite scores correlated significantly with language composite scores, $\rho=0.523$ ($p<0.001$). When language composite scores were correlated with each of the five summative scored of performance on the Babyscreen App, correlations were smaller *(Table 6.16)*. Median (IQR) language composite scores were 103 (97-121). In fact only the number of total tasks successfully completed and average speed score correlated significantly with language composite scores.

The median (IQR) motor composite score of the cohort was 103 (98-110). Motor composite scores correlated significantly with cognitive composite scores in this cohort, $\rho=0.564$ ($p<0.001$). However, when motor composite scores were correlated with summative performance scores on the Babyscreen App, correlations were again smaller only reaching significance in total number of tasks completed successfully, total number of

tasks completed without requiring a demonstration and average speed score *(Table 6.16)*.

The ability of performance on the app to predict abnormal cognitive composite scores on the Bayley-3 was examined. When the ability of each summative score to differentiate children with cognitive composite scores above 85 (1 SD below the standardised mean) from those with scores below was tested, no significant difference was detected *(Table 6.17)*. Performance in Babyscreen App summative scores was hence unable to predict scores less than 85. However, when an alternative cut-off of 90 on cognitive composite scores was used, performance in each of the five summative scores was capable of predicting cognitive performance below this cut-off *(Table 6.17)*. As the mean (SD) score in the cognitive subscale of the Bayley-3 was 102 (±12), this alternative cut-off corresponds to 1SD below the mean in our pilot cohort. This cut-off has also been proposed in the literature (324).

By converting each summative score for each child to a 0-100 scale, a combined score of overall app performance was derived. This combined score could range from 0 to 500 based on a sum of the rescaled summative scores. Median (IQR) combined score for the cohort was 314 (256-368). Combined scores correlated with cognitive composite scores but did not differ substantially from correlations derived from each individual summative score, $\rho$=0.302 ($p$=0.003). The combined score was able to predict cognitive composite scores less than 90 with an area under the ROC of 0.69 (0.55-0.83), $p$=0.02.

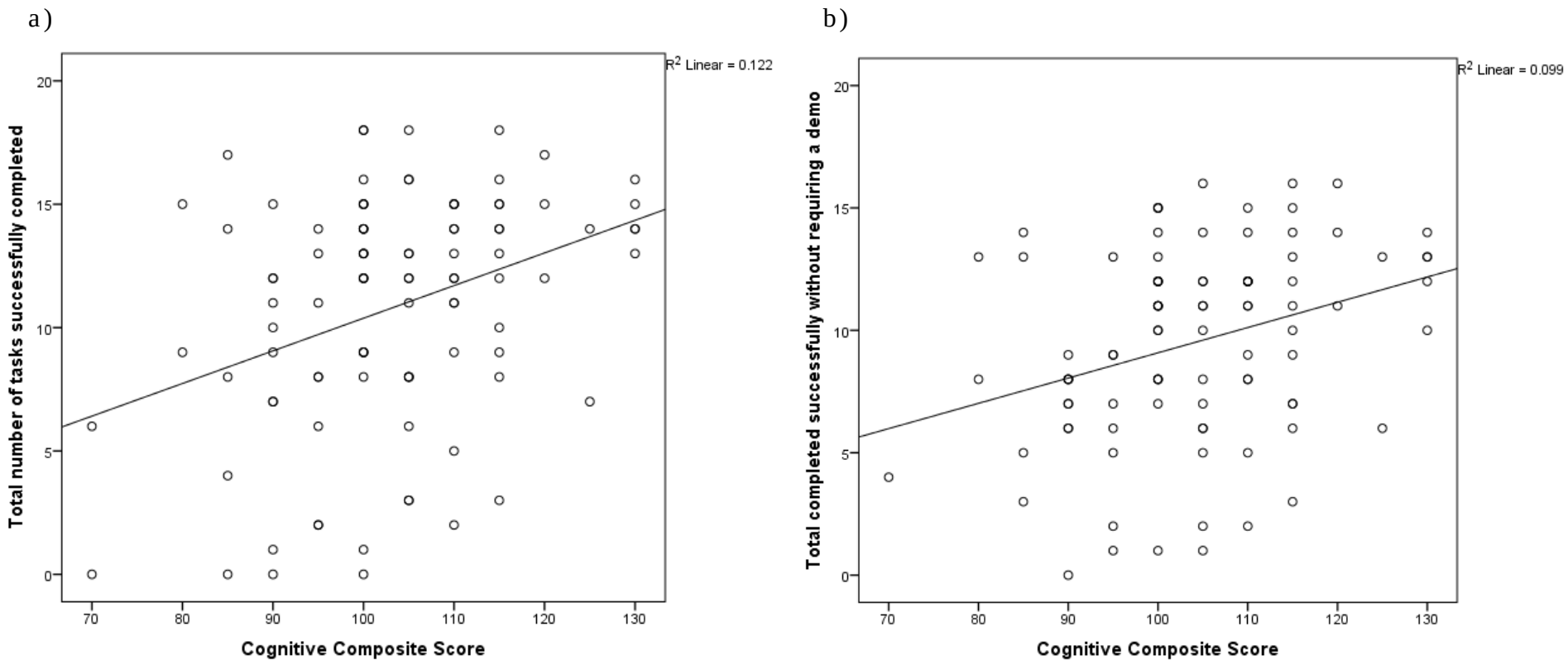



*Figure 6.27: Scatterplots of a) total number of tasks completed successfully and b) total number of tasks completed without visual demo* on the y-axis and cognitive composite scores on the x-axis

| | Median | IQR | Spearmann's rho (*p*-value) | Pearson's R (*p*-value) | Partial correlation* (*p*-value) | R against age (*p*-value) |
|---|---|---|---|---|---|---|
| **Total Tasks Completed** | 12 | 8-14 | 0.323 (0.001) | 0.350 (<0.001) | 0.351 (<0.001) | 0.348 (<0.001) |
| **Total tasks completed without demo** | 10 | 7-12.5 | 0.308 (0.003) | 0.315 (0.002) | 0.313 (0.002) | 0.285 (0.006) |
| **Average Speed Score** | 3.5 | 3.0-4.1 | 0.373 (<0.001) | 0.434 (<0.001) | 0.431 (<0.001) | 0.143 (0.162) |
| **Average Accuracy Score** | 2.8 | 2.2-3.1 | 0.321 (0.002) | 0.255 (0.014) | 0.250 (0.016) | 0.113 (0.282) |
| **Average Efficiency Score** | 7.4 | 5.7-8.6 | 0.338 (0.001) | 0.268 (0.009) | 0.264 (0.011) | 0.111 (0.289) |

*Table 6.15: Correlation of app scores against the cognitive composite scores of the BSID-III.* *partial correlation controlling for age

| | Correlation with Language Composite Scores | | Correlation with Motor Composite Scores | |
|---|---|---|---|---|
| | **Spearmann's ρ** | ***p*-value** | **Spearmann's ρ** | ***p*-value** |
| **Total Tasks Completed** | 0.224 | 0.048* | 0.222 | 0.046* |
| **Total tasks completed without demo** | 0.212 | 0.070 | 0.236 | 0.036* |
| **Average Speed Score** | 0.273 | 0.016* | 0.260 | 0.019* |
| **Average Accuracy Score** | 0.150 | 0.203 | 0.019 | 0.094 |
| **Average Efficiency Score** | 0.136 | 0.246 | 0.214 | 0.058 |

*Table 6.16: Correlations between language composite scores, motor composite scores and the summative scores of performance on the Babyscreen App.* $*p<0.05$

| | *Cut-off<85* | | | | *Cut-off <90* | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mann-Whitney *p*-value** | **AUROC** | **CI** | ***p*-value** | **Mann-Whitney *p*-value** | **AUROC** | **CI** | ***p*-value** |
| **Total tasks** | 0.174 | 0.638 | 0.41-0.87 | 0.175 | 0.014* | 0.681 | 0.54-0.82 | 0.015* |
| **Total tasks w/o demo** | 0.624 | 0.556 | 0.30-0.81 | 0.626 | 0.020* | 0.685 | 0.54-0.83 | 0.020* |
| **Average Speed Score** | 0.177 | 0.637 | 0.42-0.85 | 0.177 | 0.002* | 0.734 | 0.61-0.86 | 0.002* |
| **Average Accuracy Score** | 0.156 | 0.652 | 0.44-0.87 | 0.156 | 0.003* | 0.738 | 0.61-0.87 | 0.003* |
| **Average Efficiency Score** | 0.179 | 0.644 | 0.43-0.86 | 0.179 | 0.005* | 0.726 | 0.59-0.87 | 0.005* |

*Table 6.17: Mann Whitney p-value indicates the ability of each of the summative scores to differentiate children with cognitive scores below and above and the cut-off.* Ability of summative scores to predict abnormal Bayley cog scores is shown with AUROC, CI and *p*-value. AUROC = area under the receiver operator curve, CI = confidence interval

#### 6.2.4.4 High Risk Group Study – Hypoxic-Ischaemic Encephalopathy

While there was a significant between-group difference in Bayley-3 composite scores in all three administered subscales detected when comparing the HIE group to the perinatal asphyxia and control group (see *Table 6.5*), there was no significant differences in the HIE group for app performance on overall measures (*Table 6.18*).

| | **Pilot Group Median (IQR) n=121** | **HIE Group Median (IQR) n=15** | **Mann-whitney *p*-value** |
|---|---|---|---|
| **Total Tasks Completed** | 12 (8-14) | 13 (7-14) | 0.774 |
| **Total tasks completed without demo** | 10 (7-13) | 9 (6-10) | 0.214 |
| **Average speed score** | 3.5 (3.0-4.1) | 3.3 (2.6-3.6) | 0.087 |
| **Average accuracy score** | 2.8 (2.2-3.1) | 2.5 (2.4-2.9) | 0.559 |
| **Average efficiency score** | 7.4 (5.7-8.6) | 6.6 (5.9-8.2) | 0.620 |
| **Age (months)** | 20 (19-21) | 21 (19-22) | 0.153 |

*Table 6.18: Comparison of summative scores in the pilot group (made of control and perinatal asphyxia cohorts) versus the hypoxic-ischaemic encephalopathy group*

In the HIE group, overall app performance measures failed to correlate with Bayley-3 cognitive composite scores and cut-off

points identified in the pilot group for these measures did not significantly predict abnormal scores less than 90 (*Table 6.19*).

| | Cut-off* | PPV | NPV | Sensitivity | Specificity | Fisher's Exact | Kappa (*p*-value) |
|---|---|---|---|---|---|---|---|
| **Total tasks completed (n=12)** | <12 | 80% | 86% | 80% | 86% | 0.07 | 0.66 (0.02) |
| **Total tasks completed without demo (n=12)** | <10 | 50% | 75% | 80% | 43% | 0.58 | 0.21 (0.41) |
| **Average speed score (n=12)** | <3.34 | 43% | 60% | 60% | 43% | 1.00 | 0.03 (0.92) |
| **Average accuracy score (n=9)** | <2.61 | 40% | 50% | 50% | 40% | 1.00 | -0.10 (0.76) |
| **Average efficiency score (n=9)** | <6.80 | 50% | 66% | 75% | 40% | 1.00 | 0.14 (0.64) |

*Table 6.19: Utility testing of cut-offs in app performance summative scores for ability to predict cognitive scores less than 90 in the HIE group.* *Cut-offs derived from coordinates on the ROC in the pilot cohort. PPV= positive predictive value, NPV = negative predictive value.

### 6.2.5 Discussion

This work has shown the performance of a novel cognitive assessment in a cohort of toddlers using a touch-screen platform. This pilot cohort of low-risk 18-24 month olds were tested using the Babyscreen App and the Bayley Scales of Infant and Toddler Development (Edition 3). We have shown that summative scores derived from app performance correlate with performance on the cognitive subscale of the Bayley-3. We have also shown that compared to the cognitive subscale of the Bayley-3, the Babyscreen App correlates less with language and motor performance. This supports the hypothesis that the Babyscreen App will be able to reduce reliance on language and motor skills for the specific assessment of cognition. Finally, we have shown the ability of performance on the Babyscreen App to predict low scores less than 90 on the cognitive subscale of the Bayley-3. Altogether this gives an indication of the potential of the Babyscreen App as a tool for cognitive assessment in toddlers.

This prospective study with detailed data collection regarding the neonatal course of the participants is the first to develop and test a touch-screen cognitive assessment in this age group. It is part of an ongoing development process to produce a validated and standardised tool that would aid psychologists and paediatricians in the early detection of disorders of cognition in children.

#### 6.2.5.1 Strengths and Limitations

The strengths of the Babyscreen App include its portability and ease of administration. The Bayley-3 requires a two day training course in administration and scoring, the use of a kit contained in a briefcase and handwritten scoring on a record form. Administration for the complete assessment is age and performance dependent, but takes approximately 90 minutes. The

Babyscreen App requires access to an iPAD which automatically produces a results output. The scoring of the assessment also has the potential to be further automated. It requires minimal input from an assessor and takes approximately 10 minutes to administer.

The Babyscreen App has also been shown, in our work, to be less reliant on language and motor skills than the Bayley cognitive scale. This opens up the opportunity for assessment of children with mild to moderate specific language or motor deficits for the specific assessment of cognition.

It also appears that, in this cohort, performance on the app is not affected by previous touchscreen experience. A test of cognitive functioning that was confounded by the need for prior skills in the platform would unfairly disadvantage the minority of children who had not been exposed to this media. Therefore, this is a promising finding. However, while we know frequency of previous usage, we do not have details of the content of the touchscreen media the children were already exposed to. Children who are merely shown videos on a touchscreen device may be reported to have frequent exposure but may not have developed the skills required to interact with the screen and hence may have biased this result. Further study will be required to confirm this finding.

Among our participants, parentally reported behaviour also did not appear to affect app performance. It had been suspected that children who suffered various behavioural variants such as being withdrawn or aggressive may have also been disadvantaged in the app performance. While some children were unable to comply with app administration due to poor attention or defiance, overall this did not appear to correlate with reported behavioural difficulties. However, as behaviour was dependent on parental report there is an inherent bias that must be taken

into consideration. Also, our interaction with the children is based on one brief window in time. This is unlikely to give us an accurate representation of the children's overall compliance. Overall, a cohort with more clearly defined behavioural difficulties, such as ADHD, may perform differently.

The ability of the Babyscreen App to correlate with the cognitive subscale of the Bayley is an important indicator of its utility for cognitive assessment. However, this is just the first step in testing its function. An ideal cognitive assessment for toddlers should improve on the Bayley-3, which is predominantly a measure of developmental progress and move towards a robust neuro-psychometric test of cognitive development.

The limitations of this work include the technical challenges of the use of a new technology. During the pilot phase, several glitches such as task skipping, non-functioning commands and loss of output files emerged. These technical difficulties resulted in lost data and affected result interpretation. These faults require resolution and optimisation prior to further testing.

There was also a small subgroup who did not comply with testing. Participation may be improved with in-app changes such as more attractive graphics and optimising the positive reinforcement measures. There may also be options for improving the testing environment to make it more comfortable and conducive to the task. Even practical measures such as ensuring testing occurs at an optimal time of day, when the child is not tired or hungry, may enhance compliance.

Due to the limited age range of the cohort tested we did not observe age related differences in individual task completion times or in summative scores except for total tasks completed and total tasks completed without visual demo. The Babyscreen

App requires testing in a larger cohort over a broader age range in order to fully explore for age related differences and to determine floor and ceiling boundaries in performance.

The summative scores of performance on the Babyscreen App were unable to predict scores on the cognitive subscale of the Bayley-3 less than 85 (1 standard deviation below the reference mean). This is likely due to the low numbers in the cohort who scored below this cut-off. Alternatively this may be too low for our cohort. All summative scores were able to significantly predict a cognitive composite score less than 90. This score was tested as it has been suggested as an alternative cut-off for monitoring developmental delay due to the noted discrepancies in scoring between the Bayley-2 and the Bayley-3 (324). Testing in a broader cohort of abilities would be required to determine if the app is able to predict more abnormal scores.

The Babyscreen App was unable to differentiate cognitive performance in the high risk group study of a cohort of infants with hypoxic-ischaemic encephalopathy at birth, though total tasks completed less than 12 showed good agreement, Kappa=0.66, for abnormal cognitive scores between both groups. There are a number of potential explanations for this. Firstly, this small group is unlikely to be representative of the broad spectrum of outcomes in children with HIE. These children also represent a heterogeneous group predominated by mild encephalopathy with only one infant with severe HIE. There is also a mix of treating and untreated cases with only those with moderate and severe encephalopathy receiving therapeutic hypothermia. The alternative possibility is that the trajectory of cognitive development is so altered in children following hypoxic-ischaemic injury that cut-offs determined from a well-baby group may not apply. Testing in a larger group with carefully documented severity and treatment over a period of

time in childhood would be necessary to further investigate these issues.

#### 6.2.5.2 Future Work

The Babyscreen App represents a quantum leap forward in cognitive assessment of the toddler age group. It moves neuropsychological testing towards a more progressive, socially-acceptable format, offering a new dimension to engaging children in this age group.

The next step is to proceed with further validation of the neuropsychological constructs represented by the tasks. This will likely lead to task refinement and development to further reduce the need for an assessor to be present. After this, normative values will have to be established in larger population studies and a standardised scoring mechanism. From a technological point-of-view test output will have to be streamlined to give a standardised score for users to draw interpretations from. Data could also be continually uploaded to central servers to provide up to date normative information on a regional, age-related or disease specific basis. Predictiveness of the Babyscreen App will also need to be established for later academic skills and cognitive ability. The Babyscreen App also requires further examination in alternate special groups of high-risk infants such as premature infants which is already underway. The app will also be developed as a commercial product for dissemination among psychologists and paediatricians.

There is great potential to adapt this app for multiple users; for psychologists as a more formal assessment with detailed output, or in a simplified version as a basic screening tool that would be downloadable. Tasks could be adapted to test using progressive complexity for an extended age range applicability. Special tests

could be added to screen for specific developmental or behavioural conditions such as response inhibition in ADHD and face processing for ASD (406, 407).

Other possible evolutions include adaptations for children with specific disabilities. The field of human-computer interaction has expanded rapidly in recent decades and has had huge implications for people with a variety of disabilities. For example, alternative sensory feedback such as vibrations or enhanced visual reinforcement could be included for children with hearing impairment to replace sound as encouragement. Sensory substitution such as this has been previously proposed using a vast array of methods as outline by Visell, 2009 (408). For children with visual impairments, previous work that could be applied to cognitive assessment includes the use of tactile gamepads and audio cues that allow children to play a memory game by Raisamo et al, 2007, and the use of 3D sound-interactive environments to pose problems solving tasks by Sanchez et al, 2006 (409, 410).

Even children with more severe motor disabilities could eventually be assessed with triggered assistive technologies. These include mouse responses triggered by isolated body part movement, eye movement or even blinking which have been used with success in adults (411-413).

### 6.2.6 Conclusion

The Babyscreen App represents a promising tool for cognitive assessment in toddlers. This novel test has shown promise for testing of cognitive abilities by showing correlation with the cognitive subscale of the Bayley-3 and for prediction of lower scores. It has also shown decreased reliance on language and motor abilities for cognitive assessment, a novel progression in this age group. While further testing, and likely further

development, is required to establish the Babyscreen App as a useful tool for this purpose, this pilot work represents the first step in the pursuit of this much needed goal.

# 7.0 Discussion

## *7.1 Summary of Main Findings*

The purpose of this thesis was to contribute both knowledge, and novel tools, for the prediction and assessment of neurodevelopmental outcomes in children with perinatal asphyxia at birth.

The first aim, to improve our ability to predict outcome in these high-risk infants, was achieved by testing the ability of previously identified biomarkers of HIE severity to predict neurodevelopmental outcome at three years. These biomarkers were identified based on their biological plausibility in the context of our latest understanding of the pathophysiology of perinatal asphyxia as outlined in section 1.1.1. Beyond this, semi-targeted and untargeted techniques at the frontiers of biomarker discovery were used to identify novel markers from across the functional biological levels including proteomics, metabolomics and transcriptomics. Interleukin-16, activin-A receptor type IIb and two metabolite models have been shown here to significantly predict neurodevelopmental outcome and to surpass many of the markers currently used in clinical practice to evaluate and prognosticate in these infants. Also finding a lack of association between GFAP and outcome, where previous research has found positive results at later time-points, gives us possible clues to the timing of biochemical responses to injury.

The second aim of this thesis was to examine the limitations of currently used methods of neurodevelopmental assessment. This was accomplished by looking at the performance of a low-risk Irish population in the Bayley-3. The Bayley Scales of Infant and Toddler Development (Edition 3) is the most commonly used standardised tool of neurodevelopmental assessment used in this

jurisdiction at present. It is most frequently used for follow-up of high risk groups in both clinical and research settings (326). Standardised, administered developmental assessments are currently the gold standard for follow-up in the toddler age group, as discussed in section 5.1.2. They lack the bias of parental reports and can, when children are tested on serial occasions, track response to interventions and aid resource planning. They do, however, have their challenges. The Bayley-3 in particular has suffered from questions due to discrepancies in re-standardisation, it's predictive validity and also from concerns regarding regional variation in scores, covered in section 4.2. It was this latter concern that prompted this study. On analysis of a retrospective cohort of children, who formed part of a birth cohort study, significantly higher scores in language and fine motor skills were found compared to U.S. norms. This is likely to have implications for paediatricians and researchers alike when interpreting results of assessments. Results must be taken in context of the geographic region, the testing timeframe, as well as the more subjective influence of the assessor.

Lastly, this thesis aimed to improve the state of the art for the cognitive assessment of toddlers. Our understanding of the cognitive development of children is still evolving. The developmental theories of Piaget, Vygotsky and Bruner, discussed in section 1.6.1 and 1.6.2, still form the basis of childhood educational techniques today. These theories are predominantly based on simple observation. Progress has been made with the use of functional MRI and a vast array of neurophysiological tests, but gaps in our knowledge still exist. One of the reasons for this is a lack of appropriate tests. This is especially true for children with a perinatal risk factor such as HIE where the trajectory of their cognitive development has been altered by injury leading to specific cognitive deficits as

described in section 5.1.1 (414). Brain development has been affected at a peak time of synaptogenesis, altering further progression with ill-defined consequences. There has been a paucity of tools available to assess cognitive development in preschool age children. One of the few is the cognitive subscale of the Bayley-3. Previous versions have unfortunately shown poor predictive ability for later IQ. This, however, has been improved in the latest edition. Bode et al found good predictive power of the Bayley-3 in a U.S cohort of pre-terms (321). Spencer-Smith et al found poor sensitivity for prediction of delay on the Differential Ability Scales (DAS) when using U.S. norms, but this improved when delay was classified according to local normative data (415).

This thesis presents the development of a novel tool for assessment of non-verbal cognition in toddlers that addresses some of the weaknesses of the Bayley-3 assessment. Primarily it updates the format of the assessment to a touch-screen platform. This speaks to the contemporary changes in the nature of childhood play with children increasingly becoming digital natives. It also brings with it certain practical advantages. These include portability, adaptability, and ease of data collection, analysis and storage. This novel cognitive assessment employs aspects of various theories of cognitive development in childhood; from the error of logic identified by Piaget i.e. object permanence, incorporated into tasks where the target object is hidden, to the social learning dimension of Vygotsky and Bruner shown in the visual demonstration offered by the assessor. The summative scores discussed could be used as markers of the domain-general view of cognitive development, while closer examination of individual, or subgroups, of tasks could be used to assess domain-specific constructs.

The pilot testing of this novel tool, the Babyscreen App, showed correlation between measures of performance on the app and

scores of the cognitive scale of the Bayley-3. It also showed decreased reliance on language and motor abilities to succeed in the app compared with the Bayley-3. This could allow children with specific motor or language deficits to have their cognitive abilities assessed without being substantially disadvantaged by delays in other areas.

## *7.2 Strengths*

The strengths of this thesis may be described in terms of the relevant sections. Chapters two and three are derived from work related to the BiHIVE 1 Study (Biomarkers in Hypoxic Ischaemic Encephalopathy – biomarker discovery phase). The BiHIVE Study has been a uniquely designed cohort study. It consisted of a prospectively recruited neonatal group with clearly defined inclusion criteria. Umbilical cord blood was collected and processed according strict standardised operating procedures and was stored in a fully monitored bio-banking facility. Biomarker discovery was performed across functional biological levels through primarily untargeted or semi-targeted approaches. All participants had comprehensive data collected. HIE was diagnosed by modified Sarnat score by a dedicated research fellow at 24 hours and was confirmed by analysis of EEG recordings. All recruited infants were also followed up with standardised administered neurodevelopmental assessment. This comprehensive study design has not been equalled in the literature.

Chapter four, looking at the performance of a low-risk Irish population in the Bayley-3, was based on retrospective analysis. However meticulous data collection during the conduct of the study allowed certain strengths. Recruitment had taken place at birth and each recruit had extensive information collected regarding perinatal course, as well as at multiple time-points

after birth. This allowed for very specific inclusion and exclusion criteria and selection of children without any risk factors typically associated with developmental delay. The original record forms were reviewed for and scoring double-checked to ensure accuracy.

Chapter five presented the results of a survey of touch-screen usage in toddlers. Prior to this work there was limited evidence in the literature around the prevalence and quality of usage of touch-screen technology by toddlers. This is a topic of great general interest to parents and paediatricians alike, as touch-screen technology becomes more ubiquitous in children's environments, and becomes part of their entertainment and learning. The novelty of this work will help form the basis for further study of the impact of these technologies as well as their potential.

Chapter six presents the pilot testing of a novel touch-screen based cognitive assessment, the Babyscreen App. The strengths of this work includes its prospective design, concurrent validity testing against the current standard measure of cognitive ability in this age group, the Bayley-3 cognitive subscale, and its examination of behaviour and previous touch-screen exposure as confounding factors. This application is completely novel and fills a specific niche requirement in the follow-up of high-risk children. During the design process there has been input from experts in the field of neurodevelopment follow-up in high risk infants. This has opened up opportunities for further testing in alternate cohorts in different regions with a variety of risk factors.

## *7.3 Limitations*

The limitations of the analysis presented in Chapter two and three chiefly involve difficulties with patient retention for neurodevelopmental outcome and low numbers of abnormal outcomes. Patient retention was challenging due to the time interval between recruitment and neurodevelopmental follow up. This time lag resulted in difficulty contacting parents as contact details were out of date as well as interval emigration, and to attrition of interest in the study. This led to the development of a retention plan instituted during the recruitment of our validation cohort. The details of this are described in Chapter six. This improved retention in the BiHIVE 2 cohort by approximately 20-30%. Exact figures are not available as follow-up in the BiHIVE 2 Study is still ongoing. The low number of abnormal outcomes also had significant impacts on our ability to draw more wide-ranging conclusions from the work. However, for our patients this is, of course, a positive finding and likely represents improvements in obstetric and neonatal care. Of course this is not the case outside of tertiary units and especially in the developing world where such care is not available. While research of this nature may only be feasible at present in centres such as those included here, the impacts of this work will have a wide benefit in the developed world.

In the a priori definitions of developmental delay used for classifying neurodevelopmental outcome in this work, an autism diagnosis was used as a severe outcome measure. This was due to the increased risk of this neuro-behavioural disorder associated with hypoxic-ischaemic encephalopathy (87). Autistic Spectrum Disorder (ASD) is however multifactorial in its aetiology and the increased rates of diagnosis in HIE may be partially attributable to the male preponderance of this cohort (94, 416). In our study, a number of participants without a diagnosis of HIE later had a diagnosis of ASD. Of course, in this

instance there are factors other than birth injury at play. However, because it would be impossible to untangle the causal factors in children with a diagnosis of HIE and ASD, all children with ASD were treated equally. For the work described here the inclusion or exclusion of these participants did not materially alter our findings, but it is important to keep in mind that for larger cohorts/future work the use of ASD as an outcome measure may affect overall results.

The limitations in Chapter four include the retrospective design. This meant it was not possible plan comparison measures of performance such as use of other assessments of the developmental domains or follow-up assessment of IQ or academic performance in order to verify the findings of the comparison between Irish and U.S. performance on the Bayley-3. It was also not possible to examine performance of children at different ages thereby establishing a developmental trajectory. This would have been valuable to see how geographical variation might affect development over time. The other challenge, which is consistent across all developmental assessments, is that of inter-observer variability, or more accurately inter-assessor variability. Our study had two distinct assessors who were similarly trained and who observed each other performing approximately 10 hours of assessments to maximise comparability. However differences in mean scores occurred between assessors. This may be due to inherent differences in the cohort examined or due to subjective differences in the assessors themselves. These differences could hypothetically be due to the assessors' ability to make the child at ease in the room, to draw them out, idiosyncrasies in task administration or observational skills in scoring tasks. These can be limited to a certain extent and other studies have used video-taping review to ensure equivalent interpretation and scoring where multiple assessors are used (343). While there are benefits to using a

single assessor to ensure comparability across a cohort, this does not necessarily improve comparison against standardised scores.

In Chapter five, where touch-screen usage in toddlers was derived from a parental questionnaire, limitations were primarily based on survey style of the study. An observed performance using a touch-screen tool might have given more accurate information regarding the range of skills utilised. A closer assessment of the content of touch-screen applications that the children were exposed to would also have been valuable. Perhaps this could be done by asking parents to complete a diary over a pre-determined time period.

Chapter six, the pilot testing of the Babyscreen App, was limited in the first instance by technical difficulties. This is to be expected in the testing of any new technology but especially given the novelty of the application and the nature of the cohort being tested. However, this led to a loss of task data which had to be taken into account in how the analysis was approached. Similarly small numbers of children succeeding in tasks after a visual demonstration meant that the distribution of these speeds could not be used to provide cut-offs for performance. This also had to be taken into account in analysis. Reliance on parental report of previous touch-screen usage and behaviour also limits the conclusions we can draw about these potential confounders. This is however only pilot testing of this novel application and it promisingly showed some concurrent validity with the Bayley-3 cognitive scale. Further testing is required and already underway.

## *7.4 Impact of Thesis*

This thesis contributes new dimensions to our ability to prognosticate for high-risk infants in the perinatal period and to

evaluate the cognitive consequences for these children at a much earlier age than previously thought possible. A range of novel biomarkers measurable at birth have been shown here to predict neurodevelopmental outcome in early childhood. Several outperform currently available biochemical markers at birth and precede the more robust prognostic information that can be gleaned from EEG and serial clinical assessment. These new markers have the potential to substantially influence clinical care pathways and decision making algorithms in the acute and ongoing management in infants with perinatal asphyxia.

*Figure 7.1* shows the views and downloads of one of the articles published as part of this work. This original research entitled "Glial Fibrillary Acidic Protein Is Not an Early Marker of Injury in Perinatal Asphyxia and Hypoxic-Ischemic Encephalopathy" has also been cited recently in Free Radical Biology in Medicine in an article by Chafer-Pericas et al which looks at novel peroxidation biomarkers in umbilical cord blood in HIE (417). This highlights the impact of our work, which has already informed and furthered the search for biomarkers in this rapidly expanding area of research.

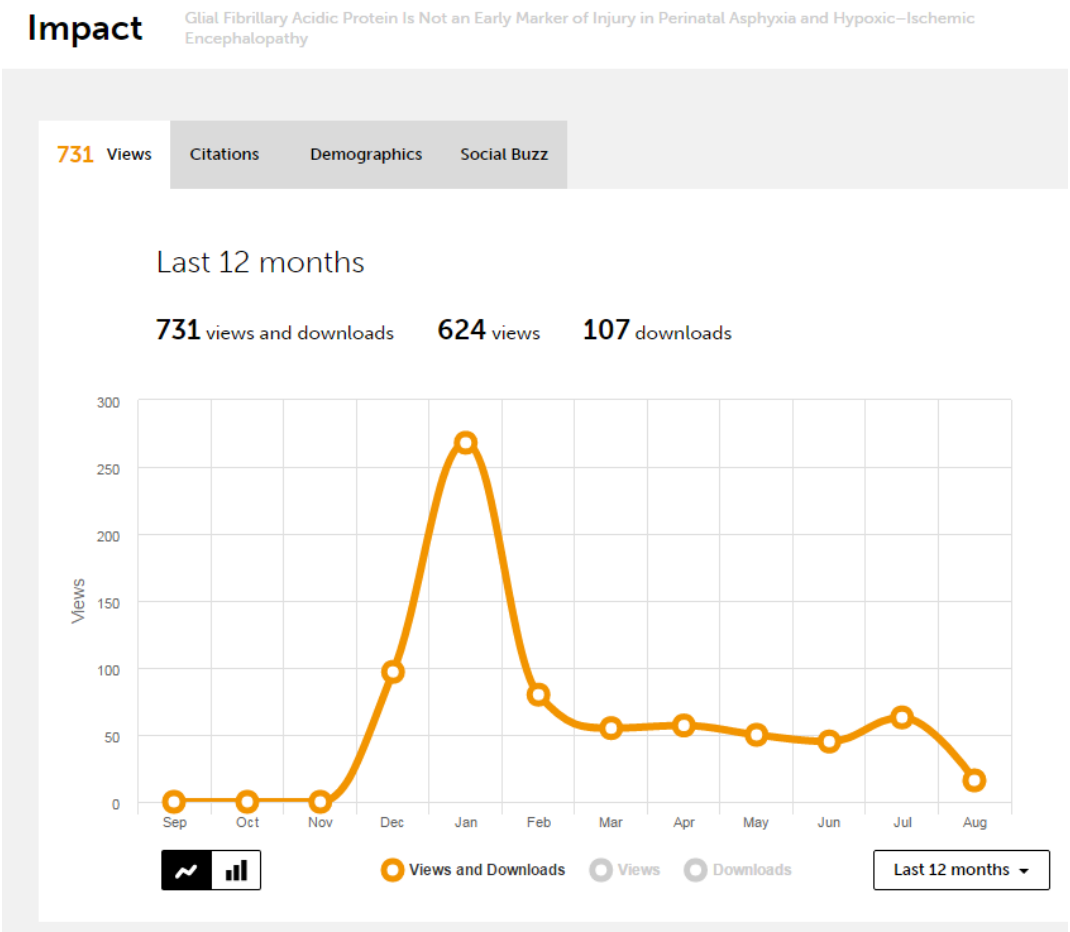



*Figure 7.1: Graphic of views and downloads*. Available from Frontiers in Neurology (418)

This thesis has also presented pertinent information on the Bayley-3 neurodevelopmental assessment in an Irish population. The findings of this work will inform interpretation of results of this assessment, frequently used in clinical and research setting, by paediatricians and other health care professionals.

Finally this thesis described the development and pilot testing of a novel cognitive assessment for toddlers using a touch-screen platform. As part of the groundwork for the development of this tool, market research into the prevalence and quality of touch-screen usage by toddlers was performed and results published. This preliminary study has undoubtedly been the most impactful aspect of this thesis to date. After acceptance for publication,

this article was selected for press release and was presented to the public through general and social media. This public dissemination can be measured through online data mining groups such as Altmetric. This article received an Altmetric score of 145 which puts it in the top 5% of all research outputs scored (*Figure 7.2*). This article was also selected for attention by the training bodies of healthcare professionals. It was cited in the clinical digest of Nursing Standard, an official publication of the Royal College of Nursing (419). The Royal College of Paediatrics and Child Health (RCPCH) in the UK also presented the findings of this study and commented on the implications. Responding to the study, Prof Russell Viner, Officer for Health Promotion for the Royal College of Paediatrics and Child Health, said:

"There is evidence that suggests excessive time spent in front of any screen – whether that's a television, iPad or mobile phone – promotes obesity and interferes with normal sleep patterns in children. However our children are digital natives in a way no previous generation has been before, so it's unclear whether the use of these new devices improves cognition and coordination for children under the age of two. Therefore more research is needed on the benefits and harms of different types of screen use. Only then can we really be sure what screen time recommendations we should put in place for young children."(420)

In more unexpected quarters, this work is having impacts in changing the future of play for pre-schoolers. This paper has been cited by the Preschool Front End Team at LEGO who are presenting at the 14th Participatory Design Conference in Denmark on their ongoing work in designing for play in this age group (421).

More recently this work has been cited by researchers as part of an article series being promoted under the research topic “Touch screen tablets touching children's lives” by Frontiers in Psychology (401, 422).

The topic of touch-screen usage in toddlers has proved to be of substantial public and academic interest. Parents are looking for guidance on safety of exposure considering previous recommendations to avoid media at this age (394). Paediatricians are equally looking for help and information on health implications. Researchers are looking for ways to use this tool, which commands so much interest from toddlers, to assess learning, development and cognition. Towards the later aim this thesis has been able to add another step. The development and pilot testing of a novel application to test cognitive development has been described here. This touch-screen assessment, the Babyscreen App, has been shown here to correlate well with the cognitive subscale of the Bayley-3 and to be able to predict lower scores in a cohort of low-risk children. Though there is further work to be done to fully validate this test, it has great potential to influence the study and assessment of cognitive development in children.

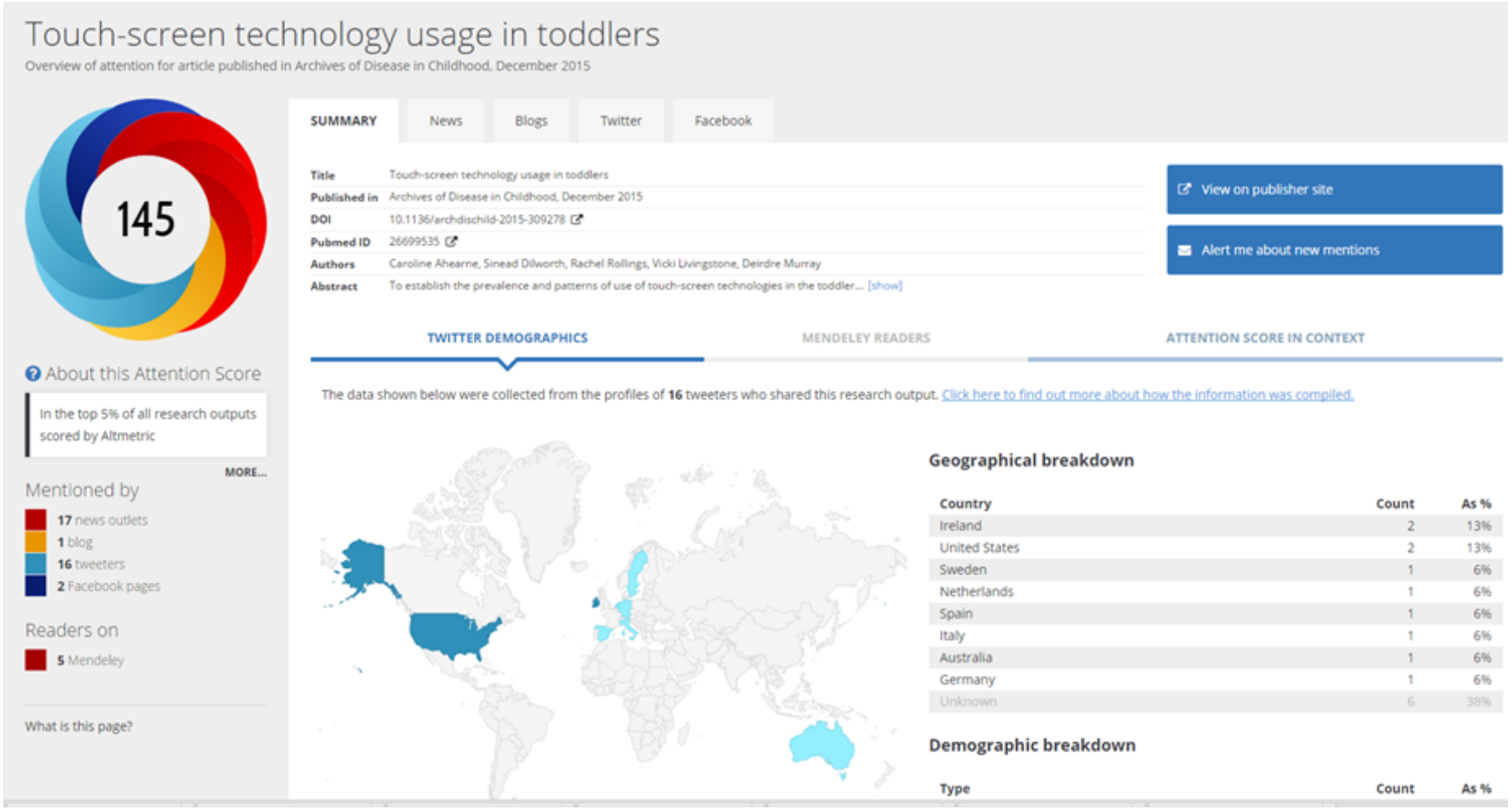



*Figure 7.2 Altmetric data output*. Available from Altmetric (423).

## *7.5 Future Work*

Many elements of this thesis form parts of active ongoing and planned research. Analysis of umbilical cord blood from the validation cohort of BiHIVE 2 is progressing to validate the promising biomarkers presented in Chapters 2 and 3. Neurodevelopmental outcome assessment is also ongoing for this cohort. At the end of this process large scale bio-informatic analysis will be used to identify the optimal combination of such biomarkers that would contribute to a useful point-of-care test to predict severity and outcome among infants that suffer perinatal asphyxia and birth. Such a test could inform clinical care and management planning in this high-risk group. It could also open up opportunities to test these biomarkers in lower resource settings which may be more likely to benefit from rapid detection of high-risk infants to facilitate transfer etc.

In Chapter 4 regional variation in Bayley-3 scores was examined. It is vital for clinicians and researchers alike to have a thorough understanding of the strengths and limitations of developmental assessments in current use. As previously outlined, testing in the toddler age group is extremely challenging but important. Optimal test choice and interpretation will undoubtedly benefit clinical outcomes, research accuracy and critical analysis. There is further work to be done to elucidate regional variations in test scores by testing across age groups, ideally with serial measurements over time, and to look at the concurrent and predictive validity of these differences. There is also a need to develop improved methods of standardising test administration, thereby reducing inter-assessor variability, as discussed in section 4.6.

Further testing of the Babyscreen App is also in progress. While the development of this app is still in its infancy it has

enormous potential for the future. The psychometric constructs being tested by the app are under investigation in a cohort of typically developing 2 to 3 year olds. This will establish the neuropsychometric validity of the tests. Further examination of the scoring system is also required. Due to the low numbers of children succeeding in the second attempt it was not possible to examine the distribution of these speeds. Testing in a larger cohort is necessary to correct this. The performances of an expanded cohort will be compared to other tests of specific cognitive skills. The app is also planned for inclusion in the follow-up of other research studies to tests its efficacy in larger groups of high-risk infants including HIE and prematurity. Further testing in children with specific motor and language deficits will be important to establish the app's utility in specifically testing cognition in these circumstances. Later follow-up of these children is also required to establish the predictive validity of this test for childhood intelligence and academic achievement. This will also allow follow up of some of the more subtle elements of the test. It would be possible to differentiate the children who succeed in their first attempt at a task, a 'trial-and-error' approach, from children who consistently require a visual demonstration for success, a 'social learning' approach. I would hypothesise that these two groups may demonstrate different learning types in later childhood. The social learners, who fit well with Vygotsky's 'zone of proximal development' or Bruner's 'scaffolding', may thrive in teacher-led environments, while the trial and error group may display more independent learning. It will also be interesting to look at the speed scores of children across broader age ranges. I would suspect that there is a certain time-point when speed scores become more reflective of actual internal processing speed. This is because motivations appear to change over time. An older child can be instructed to try to achieve a task as fast as possible, especially if spurred on by the promise of a reward. A

younger child, despite having a correct answer, may pause to seek approval from their guardian, or assess the perceived threat of the assessor before making their move. A way to overcome this would be to assess processing speed by visual tracking technologies with time to eye contact with the target object compared with time to physical response. This issue of test response is highlighted in the work by McGarrigle, who showed that experimental design, and specifically the influence of the experimenter, appears to alter childrens' responses (187). This work also prompted one of the other planned future developments of the app, the elimination of the assessor entirely. While certain standardisations, i.e. of the environment etc, would still be necessary for formal testing, it is conceivable that the app would be able to run without the need for an assessor present. Other future evolutions and adaptations of the app are discussed in detail in section 6.2.5.2. The app also has the potential to contribute to our understanding of cognitive development in both typically and atypically developing children. Despite the recent progress in neuroimaging and neurophysiological techniques for investigating cognition, progress has been limited by a lack of appropriate tests in the toddler age group. This was elaborated on in sections 1.6.5. and 1.6.6. This has been a stumbling block to supporters of various theories of cognitive development, particularly the more recent neuroconstructivism (210).Tests to stimulate specific cognitive constructs across early childhood, such as the Babyscreen app, could allow such inquiry and permit tracking of developmental trajectories across time. While we are still a long way from population based standardisation and dissemination of the Babyscreen app, it is exciting to consider the possibilities for this novel test on a modern platform that fulfils such a gap in our clinical and academic requirements.

# 8.0 Bibliography


1. Lee AC, Kozuki N, Blencowe H, Vos T, Bahalim A, Darmstadt GL, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatric research. 2013;74 Suppl 1:50-72.
2. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? Lancet. 2005;365(9462):891-900.
3. Azzopardi D, Strohm B, Marlow N, Brocklehurst P, Deierl A, Eddama O, et al. Effects of hypothermia for perinatal asphyxia on childhood outcomes. The New England journal of medicine. 2014;371(2):140-9.
4. Marlow N, Rose AS, Rands CE, Draper ES. Neuropsychological and educational problems at school age associated with neonatal encephalopathy. Archives of disease in childhood Foetal and neonatal edition. 2005;90(5):F380-7.
5. Odd DE, Whitelaw A, Gunnell D, Lewis G. The association between birth condition and neuropsychological functioning and educational attainment at school age: a cohort study. Archives of disease in childhood. 2011;96(1):30-7.
6. Gunn AJ, Wyatt JS, Whitelaw A, Barks J, Azzopardi D, Ballard R, et al. Therapeutic hypothermia changes the prognostic value of clinical evaluation of neonatal encephalopathy. The Journal of pediatrics. 2008;152(1):55-8, 8.e1.
7. Thoresen M, Hellstrom-Westas L, Liu X, de Vries LS. Effect of hypothermia on amplitude-integrated electroencephalogram in infants with asphyxia. Pediatrics. 2010;126(1):e131-9.
8. Marlow N. Measuring neurodevelopmental outcome in neonatal trials: a continuing and increasing challenge. Archives of disease in childhood Foetal and neonatal edition. 2013;98(6):F554-8.
9. Lindstrom K, Hallberg B, Blennow M, Wolff K, Fernell E, Westgren M. Moderate neonatal encephalopathy: pre- and perinatal risk factors and long-term outcome. Acta obstetricia et gynecologica Scandinavica. 2008;87(5):503-9.
10. Pappas A, Shankaran S, McDonald SA, Vohr BR, Hintz SR, Ehrenkranz RA, et al. Cognitive outcomes after neonatal encephalopathy. Pediatrics. 2015;135(3):e624-34.
11. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. The Cochrane database of systematic reviews. 2013;1:Cd003311.
12. Guillet R, Edwards AD, Thoresen M, Ferriero DM, Gluckman PD, Whitelaw A, et al. Seven- to eight-year follow-up of the CoolCap trial of head cooling for neonatal encephalopathy. Pediatric research. 2012;71(2):205-9.

13. Robertson NJ, Hagmann CF, Acolet D, Allen E, Nyombi N, Elbourne D, et al. Pilot randomized trial of therapeutic hypothermia with serial cranial ultrasound and 18-22 month follow-up for neonatal encephalopathy in a low resource hospital setting in Uganda: study protocol. Trials. 2011;12:138.
14. Robertson NJ, Iwata O. Bench to bedside strategies for optimizing neuroprotection following perinatal hypoxia-ischaemia in high and low resource settings. Early human development. 2007;83(12):801-11.
15. Thayyil S, Bhutta ZA, Ramji S, Costello AM, Robertson NJ. Global application of therapeutic hypothermia to treat perinatal asphyxial encephalopathy. International health. 2010;2(2):79-81.
16. Thomas N, Chakrapani Y, Rebekah G, Kareti K, Devasahayam S. Phase changing material: an alternative method for cooling babies with hypoxic ischaemic encephalopathy. Neonatology. 2015;107(4):266-70.
17. Volpe JJ. Neurology of the Newborn. 5 ed. Philadelphia: Saunders; 2008.
18. Fatemi A, Wilson MA, Johnston MV. Hypoxic-ischemic encephalopathy in the term infant. Clinics in perinatology. 2009;36(4):835-58, vii.
19. Ugwumadu A. Understanding cardiotocographic patterns associated with intrapartum Foetal hypoxia and neurologic injury. Best practice & research Clinical obstetrics & gynaecology. 2013;27(4):509-36.
20. Low JA. Determining the contribution of asphyxia to brain damage in the neonate. The journal of obstetrics and gynaecology research. 2004;30(4):276-86.
21. Parer JT. Effects of Foetal asphyxia on brain cell structure and function: limits of tolerance. Comparative biochemistry and physiology Part A, Molecular & integrative physiology. 1998;119(3):711-6.
22. Vannucci RC. Hypoxic-ischaemic encephalopathy. Am J Perinat. 2000;17(3):113-20.
23. Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. The Lancet. 2003;361(9359):736-42.
24. Gunn AJ, Thoresen M. Hypothermic neuroprotection. NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics. 2006;3(2):154-69.
25. Wassink G, Gunn ER, Drury PP, Bennet L, Gunn AJ. The mechanisms and treatment of asphyxial encephalopathy. Frontiers in neuroscience. 2014;8:40.
26. Edwards AD, Nelson KB. Neonatal encephalopathies: Time to reconsider the cause of encephalopathies. British Medical Journal. 1998;317(7172):1537-8.

27. Nelson KB, Leviton A. How much of neonatal encephalopathy is due to birth asphyxia? American journal of diseases of children (1960). 1991;145(11):1325-31.
28. Wyatt JS, Edwards AD, Azzopardi D, Reynolds EO. Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic-ischaemic brain injury. Archives of disease in childhood. 1989;64(7 Spec No):953-63.
29. Azzopardi D. Prognosis of newborn infants with hypoxic-ischaemic brain injury assessed by phosphorous magnetic resonance spectroscopy. Ped Res. 1989;25(5):445-51.
30. Holowach-Thurston J, Hauhart RE, Jones EM, Ikossi MG, Pierce RW. Decrease in brain glucose in anoxia in spite of elevated plasma glucose levels. Pediatric research. 1973;7(8):691-5.
31. Siesjö BK, Plum F. Pathophysiology of anoxic brain damage. Biology of brain dysfunction: Springer; 1973. p. 319-72.
32. Mishra OP, Delivoria-Papadopoulos M. Cellular mechanisms of hypoxic injury in the developing brain. Brain research bulletin. 1999;48(3):233-8.
33. Penrice J, Lorek A, Cady EB, Amess PN, Wylezinska M, Cooper CE, et al. Proton magnetic resonance spectroscopy of the brain during acute hypoxia-ischemia and delayed cerebral energy failure in the newborn piglet. Pediatric research. 1997;41(6):795-802.
34. Barrett RD, Bennet L, Davidson J, Dean JM, George S, Emerald BS, et al. Destruction and reconstruction: hypoxia and the developing brain. Birth defects research Part C, Embryo today : reviews. 2007;81(3):163-76.
35. Vannucci RC, Towfighi J, Vannucci SJ. Secondary energy failure after cerebral hypoxia-ischemia in the immature rat. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2004;24(10):1090-7.
36. Rice JE, 3rd, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Annals of neurology. 1981;9(2):131-41.
37. Vannucci RC, Connor JR, Mauger DT, Palmer C, Smith MB, Towfighi J, et al. Rat model of perinatal hypoxic-ischemic brain damage. Journal of neuroscience research. 1999;55(2):158-63.
38. Vannucci RC, Towfighi J, Heitjan DF, Brucklacher RM. Carbon dioxide protects the perinatal brain from hypoxic-ischemic damage: an experimental study in the immature rat. Pediatrics. 1995;95(6):868-74.
39. Edwards AD, Yue X, Cox P, Hope PL, Azzopardi DV, Squier MV, et al. Apoptosis in the brains of infants suffering intrauterine cerebral injury. Pediatric research. 1997;42(5):684-9.

40. Taylor DL, Edwards AD, Mehmet H. Oxidative metabolism, apoptosis and perinatal brain injury. Brain pathology (Zurich, Switzerland). 1999;9(1):93-117.
41. Yue X, Mehmet H, Penrice J, Cooper C, Cady E, Wyatt JS, et al. Apoptosis and necrosis in the newborn piglet brain following transient cerebral hypoxia-ischaemia. Neuropathol Appl Neurobiol. 1997;23(1):16-25.
42. Northington FJ, Ferriero DM, Graham EM, Traystman RJ, Martin LJ. Early Neurodegeneration after Hypoxia-Ischemia in Neonatal Rat Is Necrosis while Delayed Neuronal Death Is Apoptosis. Neurobiology of disease. 2001;8(2):207-19.
43. Inder TE, Volpe JJ. Mechanisms of perinatal brain injury. Seminars in Neonatology. 2000;5(1):3-16.
44. Bickler PE, Gallego SM, Hansen BM. Developmental changes in intracellular calcium regulation in rat cerebral cortex during hypoxia. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 1993;13(5):811-9.
45. Tan S, Zhou F, Nielsen VG, Wang Z, Gladson CL, Parks DA. Increased injury following intermittent Foetal hypoxia-reoxygenation is associated with increased free radical production in Foetal rabbit brain. Journal of neuropathology and experimental neurology. 1999;58(9):972-81.
46. Poyton RO, Ball KA, Castello PR. Mitochondrial generation of free radicals and hypoxic signaling. Trends in Endocrinology & Metabolism. 2009;20(7):332-40.
47. Vannucci RC. Experimental biology of cerebral hypoxia-ischemia: relation to perinatal brain damage. Pediatric research. 1990;27(4 Pt 1):317-26.
48. Deng YY, Lu J, Ling EA, Kaur C. Role of microglia in the process of inflammation in the hypoxic developing brain. Frontiers in bioscience (Scholar edition). 2011;3:884-900.
49. McQuillen PS, Ferriero DM. Selective vulnerability in the developing central nervous system. Pediatric neurology. 2004;30(4):227-35.
50. Vexler ZS, Ferriero DM. Molecular and biochemical mechanisms of perinatal brain injury. Seminars in Neonatology. 2001;6(2):99-108.
51. Dorrepaal CA, Berger HM, Benders MJ, van Zoeren-Grobben D, Van de Bor M, Van Bel F. Nonprotein-bound iron in postasphyxial reperfusion injury of the newborn. Pediatrics. 1996;98(5):883-9.
52. Hagberg H, Gressens P, Mallard C. Inflammation during Foetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. Annals of neurology. 2012;71(4):444-57.
53. Hudome S, Palmer C, Roberts RL, Mauger D, Housman C, Towfighi J. The role of neutrophils in the production of hypoxic-ischemic brain injury in the neonatal rat. Pediatric research. 1997;41(5):607-16.

54. Brochu ME, Girard S, Lavoie K, Sebire G. Developmental regulation of the neuroinflammatory responses to LPS and/or hypoxia-ischemia between preterm and term neonates: An experimental study. Journal of neuroinflammation. 2011;8:55.
55. Wang X, Hagberg H, Nie C, Zhu C, Ikeda T, Mallard C. Dual role of intrauterine immune challenge on neonatal and adult brain vulnerability to hypoxia-ischemia. Journal of neuropathology and experimental neurology. 2007;66(6):552-61.
56. Wang X, Stridh L, Li W, Dean J, Elmgren A, Gan L, et al. Lipopolysaccharide sensitizes neonatal hypoxic-ischemic brain injury in a MyD88-dependent manner. Journal of immunology (Baltimore, Md : 1950). 2009;183(11):7471-7.
57. Eklind S, Mallard C, Leverin AL, Gilland E, Blomgren K, Mattsby-Baltzer I, et al. Bacterial endotoxin sensitizes the immature brain to hypoxic--ischaemic injury. The European journal of neuroscience. 2001;13(6):1101-6.
58. Ingvar M. Cerebral blood flow and metabolic rate during seizures. Relationship to epileptic brain damage. Annals of the New York Academy of Sciences. 1986;462:194-206.
59. Pereira de Vasconcelos A, Ferrandon A, Nehlig A. Local cerebral blood flow during lithium-pilocarpine seizures in the developing and adult rat: role of coupling between blood flow and metabolism in the genesis of neuronal damage. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2002;22(2):196-205.
60. Behrman RE, Lees MH, Peterson EN, De Lannoy CW, Seeds AE. Distribution of the circulation in the normal and asphyxiated Foetal primate. American journal of obstetrics and gynecology. 1970;108(6):956-69.
61. Rudolph AM. The Foetal circulation and its response to stress. Journal of developmental physiology. 1984;6(1):11-9.
62. Lou HC, Lassen NA, Tweed WA, Johnson G, Jones M, Palahniuk RJ. Pressure passive cerebral blood flow and breakdown of the blood-brain barrier in experimental Foetal asphyxia. Acta paediatrica Scandinavica. 1979;68(1):57-63.
63. Rosenberg AA. Regulation of cerebral blood flow after asphyxia in neonatal lambs. Stroke; a journal of cerebral circulation. 1988;19(2):239-44.
64. Fleiss B, Gressens P. Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy? The Lancet Neurology. 2012;11(6):556-66.
65. Dammann O. Persistent neuro-inflammation in cerebral palsy: a therapeutic window of opportunity? Acta paediatrica. 2007;96(1):6-7.
66. Thornton C, Rousset CI, Kichev A, Miyakuni Y, Vontell R, Baburamani AA, et al. Molecular mechanisms of neonatal brain injury. Neurology research international. 2012;2012:506320.

67. Scott RJ, Hegyi L. Cell death in perinatal hypoxic–ischaemic brain injury. Neuropathology and Applied Neurobiology. 1997;23(4):307-14.
68. Rivkin MJ. Hypoxic-ischemic brain injury in the term newborn. Neuropathology, clinical aspects, and neuroimaging. Clinics in perinatology. 1997;24(3):607-25.
69. Mallard EC, Waldvogel HJ, Williams CE, Faull RL, Gluckman PD. Repeated asphyxia causes loss of striatal projection neurons in the Foetal sheep brain. Neuroscience. 1995;65(3):827-36.
70. Mallard EC, Williams CE, Gunn AJ, Gunning MI, Gluckman PD. Frequent episodes of brief ischemia sensitize the Foetal sheep brain to neuronal loss and induce striatal injury. Pediatric research. 1993;33(1):61-5.
71. Nelson C, Silverstein FS. Acute disruption of cytochrome oxidase activity in brain in a perinatal rat stroke model. Pediatric research. 1994;36(1 Pt 1):12-9.
72. Silverstein FS, Torke L, Barks J, Johnston MV. Hypoxia-ischemia produces focal disruption of glutamate receptors in developing brain. Brain research. 1987;431(1):33-9.
73. Rutherford M, Malamateniou C, McGuinness A, Allsop J, Biarge MM, Counsell S. Magnetic resonance imaging in hypoxic-ischaemic encephalopathy. Early human development. 2010;86(6):351-60.
74. Le Strange E, Saeed N, Cowan FM, Edwards AD, Rutherford MA. MR imaging quantification of cerebellar growth following hypoxic-ischemic injury to the neonatal brain. AJNR American journal of neuroradiology. 2004;25(3):463-8.
75. Gagne-Loranger M, Sheppard M, Ali N, Saint-Martin C, Wintermark P. Newborns Referred for Therapeutic Hypothermia: Association between Initial Degree of Encephalopathy and Severity of Brain Injury (What About the Newborns with Mild Encephalopathy on Admission?). American journal of perinatology. 2016;33(2):195-202.
76. Thoresen M. Who should we cool after perinatal asphyxia? Seminars in Foetal & neonatal medicine. 2015;20(2):66-71.
77. Odd D, Lewis G, Whitelaw A, Gunnell D. Resusctiation at birth and cognition at 8 years of age: a cohort study. Lancet. 2009;373:1615-22.
78. Sarnat HB, Sarnat MS. Neonatal encephalopathy following Foetal distress: A clinical and electrographic study. Arch Neurol. 1976;33:696-705.
79. Glass HC. Clinical neonatal seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. The Journal of pediatrics. 2009;155:318-23.
80. Filan P, Boylan GB, Chorley G, Davies A, Fox GF, Pressler R, et al. The relationship between the onset of electrographic seizure activity after birth and the time of cerebral injury in utero. BJOG : an international journal of obstetrics and gynaecology. 2005;112(4):504-7.

81. Dixon G, Badawi N, Kurinczuk JJ, Keogh JM, Silburn SR, Zubrick SR, et al. Early Developmental Outcomes After Newborn Encephalopathy. Pediatrics. 2002;109(1):26-33.
82. Rennie JM, Hagmann CF, Robertson NJ. Outcome after intrapartum hypoxic ischaemia at term. Seminars in Foetal & neonatal medicine. 2007;12(5):398-407.
83. Jiang ZD. Long-term effect of perinatal and postnatal asphyxia on developing human auditory brainstem responses: peripheral hearing loss. International Journal of Pediatric Otorhinolaryngology. 1995;33:225-38.
84. Mercuri E. Visual function at school age in children with neonatal encephalopathy and low Apgar scores. Archives of Disease in Childhood - Foetal and Neonatal Edition. 2004;89(3):F258-F62.
85. Gadian DG, Aicardi J, Watkins KE, Porter DA, Mishkin M, Vargha-Khadem F. Developmental amnesia associated with early hypoxic-ischaemic injury. Brain. 2000;123(499-507).
86. Robertson CM, Finer NN. Educational readiness of survivors of neonatal encephalopathy assoc with birth asphyxia at term. Dev Behav Ped. 1988;9(5):298-306.
87. Badawi N. Autism following a history of newborn encephalopathy: more than a coincidence? Developmental medicine and child neurology. 2006;48:85-9.
88. Zammit S, Odd D, Horwood J, Thompson A, Thomas K, Menezes P, et al. Investigating whether adverse prenatal and perinatal events are associated with non-clinical psychotic symptoms at age 12 years in the ALSPAC birth cohort. Psychological medicine. 2009;39(9):1457-67.
89. de Haan M, Wyatt JS, Roth S, Vargha-Khadem F, Gadian DG, Mishkin M. Brain and cognitive-behavioural development after asphyxia at term birth. Dev Sci. 2006;9(4):350-8.
90. Volpe JJ. Neonatal encephalopathy: an inadequate term for hypoxic-ischemic encephalopathy. Annals of neurology. 2012;72(2):156-66.
91. Dammann O, Ferriero D, Gressens P. Neonatal encephalopathy or hypoxic-ischemic encephalopathy? Appropriate terminology matters. Pediatric research. 2011;70(1):1-2.
92. Shah DK, Lavery S, Doyle LW, Wong C, McDougall P, Inder TE. Use of 2-channel bedside electroencephalogram monitoring in term-born encephalopathic infants related to cerebral injury defined by magnetic resonance imaging. Pediatrics. 2006;118(1):47-55.
93. Thoresen M, Tooley J, Liu X, Jary S, Fleming P, Luyt K, et al. Time is brain: starting therapeutic hypothermia within three hours after birth improves motor outcome in asphyxiated newborns. Neonatology. 2013;104(3):228-33.
94. Hayes BC, McGarvey C, Mulvany S, Kennedy J, Geary MP, Matthews TG, et al. A case-control study of hypoxic-ischemic encephalopathy in newborn infants at >36 weeks gestation.

American journal of obstetrics and gynecology. 2013;209(1):29 e1- e19.
95. Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, et al. Antepartum risk factors for newborn encephalopathy: The Western Australian case-control study. British Medical Journal. 1998;317(7172):1549-53.
96. Badawi N, Kurinczuk JJ, Keogh JM, Alessandri LM, et al. Intrapartum risk factors for newborn encephalopathy: The Western Australian case-control study. British Medical Journal. 1998;317(7172):1554-8.
97. Hayes BC. The placenta in infants > 36 weeks gestation with neonatal encephalopathy:a case control study. Archives of disease in childhood Foetal and neonatal edition. 2013;98:F233-F40.
98. Mir IN, Johnson-Welch SF, Nelson DB, Brown LS, Rosenfeld CR, Chalak LF. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental outcomes following hypothermia. American journal of obstetrics and gynecology. 2015;213(6):849.e1-7.
99. Nasiell J, Papadogiannakis N, Lof E, Elofsson F, Hallberg B. Hypoxic ischemic encephalopathy in newborns linked to placental and umbilical cord abnormalities. The journal of maternal-Foetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2015:1-6.
100. Devane D, Lalor J, Bonnar J. The use of intrapartum electronic Foetal heart rate monitoring: a national survey. Irish medical journal. 2007;100(2):360-2.
101. Spencer JA, Badawi N, Burton P, Keogh J, Pemberton P, Stanley F. The intrapartum CTG prior to neonatal encephalopathy at term: a case-control study. BJOG: An International Journal of Obstetrics & Gynaecology. 1997;104(1):25-8.
102. Ugwumadu A. Are we (mis)guided by current guidelines on intrapartum Foetal heart rate monitoring? Case for a more physiological approach to interpretation. BJOG : an international journal of obstetrics and gynaecology. 2014.
103. Holzmann M, Wretler S, Cnattingius S, Nordstrom L. Cardiotocography patterns and risk of intrapartum Foetal acidemia. Journal of perinatal medicine. 2015;43(4):473-9.
104. Graham EM, Adami RR, McKenney SL, Jennings JM, Burd I, Witter FR. Diagnostic accuracy of Foetal heart rate monitoring in the identification of neonatal encephalopathy. Obstetrics and gynecology. 2014;124(3):507-13.
105. Nelson KB, Dambrosia JM, Ting TY, Grether JK. Uncertain value of electronic Foetal monitoring in predicting cerebral palsy. The New England journal of medicine. 1996;334(10):613-8.

106. Luttkus AK, Noren H, Stupin JH, Blad S, Arulkumaran S, Erkkola R, et al. Foetal scalp pH and ST analysis of the Foetal ECG as an adjunct to CTG. A multi-center, observational study. Journal of perinatal medicine. 2004;32(6):486-94.
107. Doria V, Papageorghiou AT, Gustafsson A, Ugwumadu A, Farrer K, Arulkumaran S. Review of the first 1502 cases of ECG-ST waveform analysis during labour in a teaching hospital. BJOG : an international journal of obstetrics and gynaecology. 2007;114(10):1202-7.
108. Brocklehurst P. A study of an intelligent system to support decision making in the management of labour using the cardiotocograph - the INFANT study protocol. BMC pregnancy and childbirth. 2016;16:10.
109. van den Berg PP, Nelen WLDM, Jongsma HW, Nijland R, Kollée LAA, Nijhuis JG, et al. Neonatal complications in newborns with an umbilical artery pH <7.00. American journal of obstetrics and gynecology. 1996;175(5):1152-7.
110. Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. Bmj. 1988;297(6640):24-7.
111. Rorbye C, Perslev A, Nickelsen C. Lactate versus pH levels in Foetal scalp blood during labor - using the Lactate Scout System. The journal of maternal-Foetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2015:1-5.
112. East CE, Leader LR, Sheehan P, Henshall NE, Colditz PB, Lau R. Intrapartum Foetal scalp lactate sampling for Foetal assessment in the presence of a non-reassuring Foetal heart rate trace. The Cochrane database of systematic reviews. 2015;5:Cd006174.
113. Natarajan G. Apgar scores at 10 min and outcomes at 6-7years following hypoxic-ischaemic encephalopathy. Archives of disease in childhood Foetal and neonatal edition. 2013;98(6):F473-9.
114. O'Donnell CP, Kamlin CO, Davis PG, Carlin JB, Morley CJ. Interobserver variability of the 5-minute Apgar score. The Journal of pediatrics. 2006;149(4):486-9.
115. ACOG Committee Opinion. Number 333, May 2006 (replaces No. 174, July 1996): The Apgar score. Obstetrics and gynecology. 2006;107(5):1209-12.
116. Rudiger M, Braun N, Aranda J, Aguar M, Bergert R, Bystricka A, et al. Neonatal assessment in the delivery room--Trial to Evaluate a Specified Type of Apgar (TEST-Apgar). BMC pediatrics. 2015;15:18.
117. Dalili H, Nili F, Sheikh M, Hardani AK, Shariat M, Nayeri F. Comparison of the four proposed Apgar scoring systems in the assessment of birth asphyxia and adverse early neurologic outcomes. PloS one. 2015;10(3):e0122116.

118. Amiel-Tison C. A method for neurological evaluation within the first year of life: experience with full-term newborn infants with birth injury. Ciba Found Symp. 1978;59:107-37.
119. Thompson CM. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. Acta paediatrica. 1997;86:757-61.
120. Dubowitz L, Ricciw D, Mercuri E. The Dubowitz neurological examination of the full-term newborn. Mental retardation and developmental disabilities research reviews. 2005;11(1):52-60.
121. Murray DM, Bala P, O'Connor CM, Ryan CA, Connolly S, Boylan GB. The predictive value of early neurological examination in neonatal hypoxic-ischaemic encephalopathy and neurodevelopmental outcome at 24 months. Developmental medicine and child neurology. 2010;52(2):e55-9.
122. Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. Archives of disease in childhood Foetal and neonatal edition. 1999;81(1):F19-23.
123. Murray DM, Boylan GB, Ryan CA, Connolly S. Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. Pediatrics. 2009;124(3):e459-67.
124. Azzopardi D. Predictive value of amplitude integrated EEG in infants with hypoxic-ischaemic encephalopathy: data from a randomised trial of theraputic hypothermia. Archives of disease in childhood Foetal and neonatal edition. 2014;99(1):F80-2.
125. Weeke LC, Boylan GB, Pressler RM, Hallberg B, Blennow M, Toet MC, et al. Role of EEG background activity, seizure burden and MRI in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischaemic encephalopathy in the era of therapeutic hypothermia. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2016;20(6):855-64.
126. Azzopardi D, Strohm B, Edwards AD. Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Eng J Med. 2009;361:1349-58.
127. Rennie JM, Chorley G, Boylan GB, Pressler R, Nguyen Y, Hooper R. Non-expert use of the cerebral function monitor for neonatal seizure detection. Archives of disease in childhood Foetal and neonatal edition. 2004;89(1):F37-40.
128. Shellhaas RA, Soaita AI, Clancy RR. Sensitivity of amplitude-integrated electroencephalography for neonatal seizure detection. Pediatrics. 2007;120(4):770-7.
129. Boylan G, Burgoyne L, Moore C, O'Flaherty B, Rennie J. An international survey of EEG use in the neonatal intensive care unit. Acta paediatrica. 2010;99(8):1150-5.
130. Walsh BH, Murray DM, Boylan GB. The use of conventional EEG for the assessment of hypoxic ischaemic encephalopathy in the newborn: a review. Clinical

neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2011;122(7):1284-94.
131. Andre M, Lamblin MD, d'Allest AM, Curzi-Dascalova L, Moussalli-Salefranque F, T SNT, et al. Electroencephalography in premature and full-term infants. Developmental features and glossary. Neurophysiologie clinique = Clinical neurophysiology. 2010;40(2):59-124.
132. Selton D, Andre M. Prognosis of hypoxic-ischaemic encephalopathy in full-term newborns--value of neonatal electroencephalography. Neuropediatrics. 1997;28(5):276-80.
133. van Lieshout HB, Jacobs JW, Rotteveel JJ, Geven W, v't Hof M. The prognostic value of the EEG in asphyxiated newborns. Acta neurologica Scandinavica. 1995;91(3):203-7.
134. 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. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2009;120(6):1046-53.
135. Boylan G, Murray D, Rennie J. The normal EEG and aEEG. Neonatal cerebral investigation: Cambridge University Press Cambridge; 2008. p. 83-91.
136. Lloyd R, Goulding R, Filan P, Boylan G. Overcoming the practical challenges of electroencephalography for very preterm infants in the neonatal intensive care unit. Acta paediatrica. 2015;104(2):152-7.
137. de Vries LS, Groenendaal F. Patterns of neonatal hypoxic-ischaemic brain injury. Neuroradiology. 2010;52(6):555-66.
138. Liauw L, van Wezel-Meijler G, Veen S, van Buchem MA, van der Grond J. Do apparent diffusion coefficient measurements predict outcome in children with neonatal hypoxic-ischemic encephalopathy? AJNR American journal of neuroradiology. 2009;30(2):264-70.
139. Alderliesten T, de Vries LS, Benders MJ, Koopman C, Groenendaal F. MR imaging and outcome of term neonates with perinatal asphyxia: value of diffusion-weighted MR imaging and (1)H MR spectroscopy. Radiology. 2011;261(1):235-42.
140. Alderliesten T, de Vries LS, Khalil Y, van Haastert IC, Benders MJ, Koopman-Esseboom C, et al. Therapeutic hypothermia modifies perinatal asphyxia-induced changes of the corpus callosum and outcome in neonates. Journal of perinatology : official journal of the California Perinatal Association. 2015;10(4):e0123230.
141. Rutherford M, Ramenghi LA, Edwards AD, Brocklehurst P, Halliday H, Levene M, et al. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. The Lancet Neurology. 2010;9(1):39-45.
142. Rollins N, Booth T, Morriss MC, Sanchez P, Heyne R, Chalak L. Predictive value of neonatal MRI showing no or minor

degrees of brain injury after hypothermia. Pediatric neurology. 2014;50(5):447-51.
143. Vergales BD, Zanelli SA, Matsumoto JA, Goodkin HP, Lake DE, Moorman JR, et al. Depressed heart rate variability is associated with abnormal EEG, MRI, and death in neonates with hypoxic ischemic encephalopathy. American journal of perinatology. 2014;31(10):855-62.
144. Goulding RM, Stevenson NJ, Murray DM, Livingstone V, Filan PM, Boylan GB. Heart rate variability in hypoxic ischemic encephalopathy: correlation with EEG grade and 2-y neurodevelopmental outcome. Pediatric research. 2015;77(5):681-7.
145. Goulding RM, Stevenson NJ, Murray DM, Livingstone V, Filan PM, Boylan GB. Heart rate variability in hypoxic ischemic encephalopathy during therapeutic hypothermia. Pediatric research. 2017;81(4):609-15.
146. Cady EB, Iwata O, Bainbridge A, Wyatt JS, Robertson NJ. Phosphorus magnetic resonance spectroscopy 2 h after perinatal cerebral hypoxia-ischemia prognosticates outcome in the newborn piglet. Journal of neurochemistry. 2008;107(4):1027-35.
147. Chalak LF, Sanchez PJ, Adams-Huet B, Laptook AR, Heyne RJ, Rosenfeld CR. Biomarkers for severity of neonatal hypoxic-ischemic encephalopathy and outcomes in newborns receiving hypothermia therapy. The Journal of pediatrics. 2014;164(3):468-74.e1.
148. Walsh BH, Boylan GB, Livingstone V, Kenny LC, Dempsey EM, Murray DM. Cord blood proteins and multichannel-electroencephalography in hypoxic-ischemic encephalopathy. Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2013;14(6):621-30.
149. Florio P, Frigiola A, Battista R, Abdalla Ael H, Gazzolo D, Galleri L, et al. Activin A in asphyxiated full-term newborns with hypoxic ischemic encephalopathy. Frontiers in bioscience (Elite edition). 2010;2:36-42.
150. Gazzolo D, Abella R, Marinoni E, di Iorio R, Li Volti G, Galvano F, et al. New markers of neonatal neurology. The journal of maternal-Foetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2009;22 Suppl 3:57-61.
151. Solberg R, Enot D, Deigner HP, Koal T, Scholl-Burgi S, Saugstad OD, et al. Metabolomic analyses of plasma reveals new insights into asphyxia and resuscitation in pigs. PloS one. 2010;5(3):e9606.
152. Walsh BH, Broadhurst DI, Mandal R, Wishart DS, Boylan GB, Kenny LC, et al. The metabolomic profile of umbilical cord

blood in neonatal hypoxic ischaemic encephalopathy. PloS one. 2012;7(12):e50520.
153. Denihan NM, Boylan GB, Murray DM. Metabolomic profiling in perinatal asphyxia: a promising new field. BioMed research international. 2015;2015:254076.
154. Looney AM, Walsh BH, Moloney G, Grenham S, Fagan A, O'Keeffe GW, et al. Downregulation of Umbilical Cord Blood Levels of miR-374a in Neonatal Hypoxic Ischemic Encephalopathy. The Journal of pediatrics. 2015;167(2):269-73.e2.
155. Whitehead CL, Teh WT, Walker SP, Leung C, Larmour L, Tong S. Circulating MicroRNAs in maternal blood as potential biomarkers for Foetal hypoxia in-utero. PloS one. 2013;8(11):e78487.
156. Lv H, Wang Q, Wu S, Yang L, Ren P, Yang Y, et al. Neonatal hypoxic ischemic encephalopathy-related biomarkers in serum and cerebrospinal fluid. Clinica chimica acta; international journal of clinical chemistry. 2015;450:282-97.
157. Ramaswamy V, Horton J, Vandermeer B, Buscemi N, Miller S, Yager J. Systematic review of biomarkers of brain injury in term neonatal encephalopathy. Pediatric neurology. 2009;40(3):215-26.
158. Leviton A. Why the term neonatal encephalopathy should be preferred over neonatal hypoxic-ischemic encephalopathy. American journal of obstetrics and gynecology. 2013;208(3):176-80.
159. Andre T, Chesni Y, Autgaerden. [Some points of neurologic semiology in the newborn and young infant; exploration of various afferences; reaction to digital and palmar stimuli, rhythm, inhibition of reflexes, static and locomotor aptitude of the upper limbs, affect and affectivity]. La Presse medicale. 1954;62(3):41-4.
160. Amiel-Tison C. Update of the amiel-tison neurologic assessment for the term neonate or at 40 weeks corrected age. Pediatric neurology. 2002;27(3):196-212.
161. Brazelton TB. Neonatal behavioural assessment scale. London: Spastics International Medical Publication/W. Heinemann Med. Books; 1973.
162. Mercuri E, Dubowitz L. Neurological examination of the newborn. Current Paediatrics. 1999;9:42-50.
163. Einspieler C, Prechtl HF. Prechtl's assessment of general movements: a diagnostic tool for the functional assessment of the young nervous system. Mental retardation and developmental disabilities research reviews. 2005;11(1):61-7.
164. Burger M, Louw QA. The predictive validity of general movements--a systematic review. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2009;13(5):408-20.

165. Dubowitz L, Dubowitz V, Palmer P, Verghote M. A new approach to the neurological assessment of the preterm and full-term newborn infant. Brain & development. 1980;2(1):3-14.
166. Dubowitz L, Mercuri E, Dubowitz V. An optimality score for the neurologic examination of the term newborn. The Journal of pediatrics. 1998;133(3):406-16.
167. Haataja L, Mercuri E, Guzzetta A, Rutherford M, Counsell S, Flavia Frisone M, et al. Neurologic examination in infants with hypoxic-ischemic encephalopathy at age 9 to 14 months: use of optimality scores and correlation with magnetic resonance imaging findings. The Journal of pediatrics. 2001;138(3):332-7.
168. Levene ML, Kornberg J, Williams TH. The incidence and severity of post-asphyxial encephalopathy in full-term infants. Early human development. 1985;11(1):21-6.
169. Horn AR. Early clinical signs in neonates with hypoxic-ischaemic encephalopathy predict an abnormal amplitude-integrated electroencephalogram at age 6 hours. BMC pediatrics. 2013;13:52.
170. Horn AR, Swingler GH, Myer L, Linley LL, Chandrasekaran M, Robertson NJ. Early clinical predictors of a severely abnormal amplitude-integrated electroencephalogram at 48 hours in cooled neonates. Acta paediatrica. 2013;102(8):e378-84.
171. Amess PN. Early brain proton magnetic resonance spectroscopy and neonatal neurology related to neurodevelopmental outcome at 1 year in term infants after presumed hypoxic-ischaemic brain injury. Developmental medicine and child neurology. 1999;41:436-45.
172. Paro-Panjan D, Neubauer D, Kodric J, Bratanic B. Amiel-Tison Neurological Assessment at term age: clinical application, correlation with other methods, and outcome at 12 to 15 months. Developmental medicine and child neurology. 2005;47(1):19-26.
173. Paro-Panjan D, Sustersic B, Neubauer D. Comparison of two methods of neurologic assessment in infants. Pediatric neurology. 2005;33(5):317-24.
174. Bayley N. Bayley Scales of Infant and Toddler Development. 3rd Ed. San Antonio, TX: PsychCorp; 2006.
175. Griffiths R. The Griffiths mental development scales from birth to two years, manual, the 1996 revision. Henley: Association for Research in Infant and Child Development, Test Agency; 1996.
176. Bowen JR, Gibson FL, Leslie GI, Arnold JD, Ma PJ, Starte DR. Predictive value of the Griffiths assessment in extremely low birthweight infants. Journal of paediatrics and child health. 1996;32(1):25-30.
177. Barnett AL, Guzzetta A, Mercuri E, Henderson SE, Haataja L, Cowan F, et al. Can the Griffiths scales predict neuromotor and perceptual-motor impairment in term infants with neonatal encephalopathy? Archives of disease in childhood. 2004;89(7):637-43.

178. Gee M, Gee K. Evolution of the Griffiths: from GMDS to Griffiths III [Web article]. 2016 [
179. Stroud L, Green E, McAlinden P, Bloomfield S. G598(P) Child development and its assessment: revising the griffiths mental development scales. Archives of disease in childhood. 2016;101(Suppl 1):A356.
180. Jarvis M, Chandler E. Angles on child psychology: Nelson Thornes; 2001.
181. Kay D, Kibble J. Learning theories 101: application to everyday teaching and scholarship. Advances in physiology education. 2016;40(1):17-25.
182. Flood E. Child Development: Gill & MacMillan; 2010.
183. Baillargeon R, DeVos J. Object permanence in young infants: further evidence. Child development. 1991;62(6):1227-46.
184. Freeman NH, Lloyd S, Sinha C. Infant search tasks reveal early concepts of containment and canonical usage of objects. Cognition. 1980;8(3):243-62.
185. Piaget J, Inhelder B. The child's concept of space: Routledge & Paul; 1956.
186. Donaldson M. Children's minds. London: Fontana; 1978.
187. McGarrigle J, Donaldson M. Conservation accidents. Cognition. 1974;3(4):341-50.
188. Case R. Intellectual development: Birth to adulthood: Academic Pr; 1985.
189. Kail R. Processing time declines exponentially during childhood and adolescence. Developmental psychology. 1991;27(2):259.
190. Luria AR, Yudovich FI. Speech and the Development of Mental Processes in the Child. 1971.
191. Bruner J, Kenney H. The development of the concepts of order and proportion in children. Studies in Cognitive Growth New York: Wiley. 1966.
192. Smith P, Cowie H, Blades M. Understanding Children's Development. Oxford: Blackwell; 1998.
193. Wimmer H, Perner J. Beliefs about beliefs: Representation and constraining function of wrong beliefs in young children's understanding of deception. Cognition. 1983;13(1):103-28.
194. Avis J, Harris PL. Belief-desire reasoning among Baka children: evidence for a universal conception of mind. Child development. 1991;62(3):460-7.
195. Leslie AM. Pretending and believing: Issues in the theory of ToMM. Cognition. 1994;50(1-3):211-38.
196. Symons DK, Clark SE. A Longitudinal Study of Mother-Child Relationships and Theory of Mind in the Preschool Period. Social Development. 2000;9(1):3-23.
197. Wilde Astington J. Theory of mind, Humpty Dumpty, and the icebox. Human Development. 1998;41(1):30-9.
198. Baron-Cohen S, Leslie AM, Frith U. Does the autistic child have a "theory of mind"? Cognition. 1985;21(1):37-46.

199. Karmiloff-Smith A. Nativism versus neuroconstructivism: Rethinking the study of developmental disorders. Developmental psychology. 2009;45(1):56-63.
200. Pinker S. The language instinct: The new science of language and mind: Penguin UK; 1995.
201. Spelke ES. Nativism, empiricism, and the origins of knowledge. Infant Behavior and Development. 1998;21(2):181-200.
202. Spelke ES, Kinzler KD. Core knowledge. Dev Sci. 2007;10(1):89-96.
203. Westermann G, Mareschal D, Johnson MH, Sirois S, Spratling MW, Thomas MSC. Neuroconstructivism. Developmental Science. 2007;10(1):75-83.
204. Reed J, Warner-Rogers J. Child neuropsychology: concepts, theory, and practice: John Wiley & Sons; 2009.
205. Johnson MH. Functional brain development in humans. Nature Reviews Neuroscience. 2001;2(7):475-83.
206. Karmiloff-Smith A. Beyond modularity: A developmental approach to cognitive science. Cambridge, MA: MIT Press; 1992.
207. Grice SJ, de Haan M, Halit H, Johnson MH, Csibra G, Grant J, et al. ERP abnormalities of illusory contour perception in Williams syndrome. NeuroReport. 2003;14(14):1773-7.
208. Sur S, Sinha VK. Event-related potential: An overview. Industrial psychiatry journal. 2009;18(1):70-3.
209. D'Souza D, Cole V, Farran EK, Brown JH, Humphreys K, Howard J, et al. Face processing in Williams syndrome is already atypical in infancy. Frontiers in psychology. 2015;6:760.
210. Thomas MS. Characterising Compensation:(Commentary on Ullman and Pierpont,"Specific Language Impairment is not Specific to Language: The Procedural Deficit Hypothesis"). Cortex. 2005;41(3):434-42.
211. Casey BJ, De Haan M. Introduction: new methods in developmental science. Developmental Science. 2002;5(3):265-7.
212. Marin-Padilla M. Origin, formation, and prenatal maturation of the human cerebral cortex: an overview. Journal of craniofacial genetics and developmental biology. 1989;10(2):137-46.
213. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. Progress in neurobiology. 2013;106-107:1-16.
214. Huttenlocher PR. Synaptic density in human frontal cortex—developmental changes and effects of aging. Brain research. 1979;163(2):195-205.
215. Huttenlocher PR. Morphometric study of human cerebral cortex development. Neuropsychologia. 1990;28(6):517-27.
216. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. Journal of comparative Neurology. 1997;387(2):167-78.

217. Chugani HT, Phelps ME, Mazziotta JC. Positron emission tomography study of human brain functional development. Annals of neurology. 1987;22(4):487-97.
218. Yakovlev PI, Lecours AR. The myelogenetic cycles of regional maturation of the brain. Regional development of the brain in early life. 1967:3-70.
219. Paus T, Collins D, Evans A, Leonard G, Pike B, Zijdenbos A. Maturation of white matter in the human brain: a review of magnetic resonance studies. Brain research bulletin. 2001;54(3):255-66.
220. Van der Knaap M, Valk J. Magnetic Resonance of Myelin. Myelination, and Myelin Disorders, Berlin Heldelberg New York: Springer-Verlag. 1995:1-19.
221. Casey BJ, Giedd JN, Thomas KM. Structural and functional brain development and its relation to cognitive development. Biological Psychology. 2000;54(1–3):241-57.
222. Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey B, Kozuch PL, et al. Quantitative magnetic resonance imaging of human brain development: ages 4–18. Cerebral cortex. 1996;6(4):551-9.
223. Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB. Brain development, gender and IQ in children. Brain. 1996;119(5):1763-74.
224. Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, et al. Brain development during childhood and adolescence: a longitudinal MRI study. Nature neuroscience. 1999;2(10):861-3.
225. Jernigan TL, Zisook S, Heaton RK, Moranville JT, Hesselink JR, Braff DL. Magnetic resonance imaging abnormalities in lenticular nuclei and cerebral cortex in schizophrenia. Archives of General Psychiatry. 1991;48(10):881-90.
226. Pfefferbaum A, Mathalon DH, Sullivan EV, Rawles JM, Zipursky RB, Lim KO. A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. Archives of neurology. 1994;51(9):874-87.
227. Caviness V, Kennedy D, Richelme C, Rademacher J, Filipek P. The human brain age 7–11 years: a volumetric analysis based on magnetic resonance images. Cerebral cortex. 1996;6(5):726-36.
228. Casey B, Cohen JD, Jezzard P, Turner R, Noll DC, Trainor RJ, et al. Activation of prefrontal cortex in children during a nonspatial working memory task with functional MRI. Neuroimage. 1995;2(3):221-9.
229. Casey B, Trainor RJ, Orendi JL, Schubert AB, Nystrom LE, Giedd JN, et al. A developmental functional MRI study of prefrontal activation during performance of a go-no-go task. Journal of cognitive neuroscience. 1997;9(6):835-47.

230. Hudspeth WJ, Pribram KH. Stages of brain and cognitive maturation. Journal of Educational Psychology. 1990;82(4):881-4.
231. Deary IJ, Spinath FM, Bates TC. Genetics of intelligence. European journal of human genetics : EJHG. 2006;14(6):690-700.
232. Plomin R, Kennedy JKJ, Craig IW. The quest for quantitative trait loci associated with intelligence. Intelligence. 2006;34(6):513-26.
233. Chorney M, Chorney K, Seese N, Owen M, Daniels J, McGuffin P, et al. A quantitative trait locus associated with cognitive ability in children. Psychological science. 1998;9(3):159-66.
234. Berman SM, Noble EP. Reduced visuospatial performance in children with the D2 dopamine receptor A1 allele. Behavior genetics. 1995;25(1):45-58.
235. Petrill SA, Plomin R, McClearn GE, Smith DL, Vignetti S, Chorney MJ, et al. No association between general cognitive ability and the A1 allele of the D2 dopamine receptor gene. Behavior genetics. 1997;27(1):29-31.
236. Henderson A, Jorm A, Korten A, Christensen H, Jacomb P, Easteal S, et al. Apolipoprotein E allele∈ 4, dementia, and cognitive decline in a population sample. The Lancet. 1995;346(8987):1387-90.
237. Kurowski B, Martin LJ, Wade SL. Genetics and outcomes after traumatic brain injury (TBI): what do we know about pediatric TBI? Journal of pediatric rehabilitation medicine. 2012;5(3):217-31.
238. Kovas Y, Plomin R. Generalist genes: implications for the cognitive sciences. Trends in cognitive sciences. 2006;10(5):198-203.
239. Plomin R, McClearn GE, Smith DL, Vignetti S, Chorney MJ, Chorney K, et al. DNA markers associated with high versus low IQ: the IQ Quantitative Trait Loci (QTL) Project. Behavior genetics. 1994;24(2):107-18.
240. Posthuma D, Luciano M, Geus EJCd, Wright MJ, Slagboom PE, Montgomery GW, et al. A Genomewide Scan for Intelligence Identifies Quantitative Trait Loci on 2q and 6p. The American Journal of Human Genetics. 2005;77(2):318-26.
241. Mueller CA, Schluesener HJ, Conrad S, Pietsch T, Schwab JM. Spinal cord injury-induced expression of the immune-regulatory chemokine interleukin-16 caused by activated microglia/macrophages and CD8+ cells. Journal of neurosurgery Spine. 2006;4(3):233-40.
242. Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ, et al. Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. Pediatric research. 2004;56(6):960-6.

243. Foster-Barber A, Dickens B, Ferriero DM. Human perinatal asphyxia: correlation of neonatal cytokines with MRI and outcome. Developmental neuroscience. 2001;23(3):213-8.
244. Martin-Ancel A, Garcia-Alix A, Pascual-Salcedo D, Cabanas F, Valcarce M, Quero J. Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. Pediatrics. 1997;100(5):789-94.
245. Osredkar D, Thoresen M, Maes E, Flatebo T, Elstad M, Sabir H. Hypothermia is not neuro-protective after infection-sensitized neonatal hypoxic-ischemic brain injury. Resuscitation. 2014;85(4):567-72.
246. Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. Pediatrics. 2002;110(4):673-80.
247. Skundric DS, Cruikshank WW, Drulovic J. Role of IL-16 in CD4+ T cell-mediated regulation of relapsing multiple sclerosis. Journal of neuroinflammation. 2015;12:78.
248. Schwab JM. Human focal cerebral infarctions induce differential lesional interleukin-16 (IL-16) expression confined to infitrating granulocytes, CD8+ T-lymphocytes and activated microglia/macrophages. Journal of Neuroimmunology. 2001;114:232-41.
249. Liebrich M, Guo LH, Schluesener HJ, Schwab JM, Dietz K, Will BE, et al. Expression of interleukin-16 by tumor-associated macrophages/activated microglia in high-grade astrocytic brain tumors. Archivum immunologiae et therapiae experimentalis. 2007;55(1):41-7.
250. Reinke SN, Walsh BH, Boylan GB, Sykes BD, Kenny LC, Murray DM, et al. 1H NMR derived metabolomic profile of neonatal asphyxia in umbilical cord serum: implications for hypoxic ischemic encephalopathy. Journal of proteome research. 2013;12(9):4230-9.
251. Pressler R, Boylan GB, Morton M, Binnie CD, Rennie JM. Early serial EEG in hypoxic-ischaemic encephalopathy. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2001;112:31-7.
252. Badawi N, Felix JF, Kurinczuk JJ, Dixon G, Watson L, Keogh JM, et al. Cerebral palsy following term newborn encephalopathy: a population-based study. Developmental medicine and child neurology. 2005;47(5):293-8.
253. Muhlhahn P, Zweckstetter M, Georgescu J, Ciosto C, Renner C, Lanzendorfer M, et al. Structure of interleukin 16 resembles a PDZ domain with an occluded peptide binding site. Nature structural biology. 1998;5(8):682-6.
254. Roth S, Agthe M, Eickhoff S, Möller S, Karsten CM, Borregaard N, et al. Secondary necrotic neutrophils release interleukin-16C and macrophage migration inhibitory factor from stores in the cytosol. Cell Death Discovery. 2015;1:15056.

255. Cruikshank WW. Interleukin 16. J Leukoc Biol. 2000;67:757-66.
256. Mathy NL. Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. Immunology. 2000;100:63-9.
257. Croq F, Vizioli J, Tuzova M, Tahtouh M, Sautiere PE, Van Camp C, et al. A homologous form of human interleukin 16 is implicated in microglia recruitment following nervous system injury in leech Hirudo medicinalis. Glia. 2010;58(14):1649-62.
258. Fenster CP, Chisnell HK, Fry CR, Fenster SD. The role of CD4-dependent signaling in interleukin-16 induced c-Fos expression and facilitation of neurite outgrowth in cerebellar granule neurons. Neuroscience letters. 2010;485(3):212-6.
259. Kurschner C, Yuzaki M. Neuronal interleukin-16 (NIL-16): a dual function PDZ domain protein. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1999;19(18):7770-80.
260. Gonzalez FF, Miller SP. Does perinatal asphyxia impair cognitive function without cerebral palsy? Archives of disease in childhood Foetal and neonatal edition. 2006;91(6):F454-9.
261. Douglas-Escobar M. A pilot study of novel biomarkers in neonates with Hypoxic-Ischaemic Encephalopathy. Ped Res. 2010;68:531-6.
262. DuPont TL, Chalak LF, Morriss MC, Burchfield PJ, Christie L, Sanchez PJ. Short-term outcomes of newborns with perinatal acidemia who are not eligible for systemic hypothermia therapy. The Journal of pediatrics. 2013;162(1):35-41.
263. Ferriero DM, Bonifacio SL. The search continues for the elusive biomarkers of neonatal brain injury. The Journal of pediatrics. 2014;164(3):438-40.
264. Middeldorp J, Hol EM. GFAP in health and disease. Progress in neurobiology. 2011;93(3):421-43.
265. Mondello S, Papa L, Buki A, Bullock MR, Czeiter E, Tortella FC, et al. Neuronal and glial markers are differently associated with computed tomography findings and outcome in patients with severe traumatic brain injury: a case control study. Critical care (London, England). 2011;15(3):R156.
266. Brophy GM, Mondello S, Papa L, Robicsek SA, Gabrielli A, Tepas J, 3rd, et al. Biokinetic analysis of ubiquitin C-terminal hydrolase-L1 (UCH-L1) in severe traumatic brain injury patient biofluids. Journal of neurotrauma. 2011;28(6):861-70.
267. Kaneko T, Kasaoka S, Miyauchi T, Fujita M, Oda Y, Tsuruta R, et al. Serum glial fibrillary acidic protein as a predictive biomarker of neurological outcome after cardiac arrest. Resuscitation. 2009;80(7):790-4.
268. Mondello S, Palmio J, Streeter J, Hayes RL, Peltola J, Jeromin A. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is increased in cerebrospinal fluid and plasma of patients after epileptic seizure. BMC neurology. 2012;12:85.

269. Nylen K, Ost M, Csajbok LZ, Nilsson I, Blennow K, Nellgard B, et al. Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. Journal of the neurological sciences. 2006;240(1-2):85-91.
270. Papa L, Akinyi L, Liu MC, Pineda JA, Tepas JJ, 3rd, Oli MW, et al. Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. Critical care medicine. 2010;38(1):138-44.
271. Setsuie R, Wada K. The functions of UCH-L1 and its relation to neurodegenerative diseases. Neurochemistry international. 2007;51(2-4):105-11.
272. Blennow M, Hagberg H, Rosengren L. Glial fibrillary acidic protein in the cerebrospinal fluid: a possible indicator of prognosis in full-term asphyxiated newborn infants? Pediatric research. 1995;37(3):260-4.
273. Burtrum D, Silverstein FS. Hypoxic-ischemic brain injury stimulates glial fibrillary acidic protein mRNA and protein expression in neonatal rats. Experimental neurology. 1994;126(1):112-8.
274. Ennen CS, Huisman TA, Savage WJ, Northington FJ, Jennings JM, Everett AD, et al. Glial fibrillary acidic protein as a biomarker for neonatal hypoxic-ischemic encephalopathy treated with whole-body cooling. American journal of obstetrics and gynecology. 2011;205(3):251.e1-7.
275. Bersani I, Auriti C, Ronchetti MP, Prencipe G, Gazzolo D, Dotta A. Use of early biomarkers in neonatal brain damage and sepsis: state of the art and future perspectives. BioMed research international. 2015;2015:253520.
276. Zaigham M, Lundberg F, Hayes R, Unden J, Olofsson P. Umbilical cord blood concentrations of ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) and glial fibrillary acidic protein (GFAP) in neonates developing hypoxic-ischemic encephalopathy. The journal of maternal-Foetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2016;29(11):1822-8.
277. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-97.
278. Xia Y, Schneyer AL. The biology of activin: recent advances in structure, regulation and function. The Journal of endocrinology. 2009;202(1):1-12.
279. Florio P, Luisi S, Bruschettini M, Grutzfeld D, Dobrzanska A, Bruschettini P, et al. Cerebrospinal fluid activin a measurement in asphyxiated full-term newborns predicts hypoxic ischemic encephalopathy. Clinical chemistry. 2004;50(12):2386-9.
280. Florio P, Luisi S, Moataza B, Torricelli M, Iman I, Hala M, et al. High urinary concentrations of activin A in asphyxiated

full-term newborns with moderate or severe hypoxic ischemic encephalopathy. Clinical chemistry. 2007;53(3):520-2.
281. Lai M, Sirimanne E, Williams CE, Gluckman PD. Sequential patterns of inhibin subunit gene expression following hypoxic-ischemic injury in the rat brain. Neuroscience. 1996;70(4):1013-24.
282. Wu DD, Lai M, Hughes PE, Sirimanne E, Gluckman PD, Williams CE. Expression of the activin axis and neuronal rescue effects of recombinant activin A following hypoxic-ischemic brain injury in the infant rat. Brain research. 1999;835(2):369-78.
283. Phillips DJ, Nguyen P, Adamides AA, Bye N, Rosenfeld JV, Kossmann T, et al. Activin a release into cerebrospinal fluid in a subset of patients with severe traumatic brain injury. Journal of neurotrauma. 2006;23(9):1283-94.
284. Looney A-M, Ahearne C, Boylan GB, Murray DM. Glial Fibrillary Acidic Protein is not an early marker of injury in perinatal asphyxia and hypoxic ischaemic encephalopathy. Frontiers in Neurology. 2015;6:264.
285. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nature protocols. 2008;3(6):1101-8.
286. Cheng L, Doecke JD, Sharples RA, Villemagne VL, Fowler CJ, Rembach A, et al. Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. Molecular psychiatry. 2015;20(10):1188-96.
287. Etheridge A, Lee I, Hood L, Galas D, Wang K. Extracellular microRNA: a new source of biomarkers. Mutation research. 2011;717(1-2):85-90.
288. Mishra PJ. MicroRNAs as promising biomarkers in cancer diagnostics. Biomarker research. 2014;2:19.
289. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(30):10513-8.
290. Betel D, Wilson M, Gabow A, Marks DS, Sander C. The microRNA.org resource: targets and expression. Nucleic acids research. 2008;36(Database issue):D149-53.
291. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic acids research. 2014;42(Database issue):D68-73.
292. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;120(1):15-20.
293. Carroll AP, Tooney PA, Cairns MJ. Design and interpretation of microRNA-reporter gene activity. Analytical biochemistry. 2013;437(2):164-71.

294. Lee SJ, Reed LA, Davies MV, Girgenrath S, Goad ME, Tomkinson KN, et al. Regulation of muscle growth by multiple ligands signaling through activin type II receptors. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(50):18117-22.
295. Gao F, Kishida T, Ejima A, Gojo S, Mazda O. Myostatin acts as an autocrine/paracrine negative regulator in myoblast differentiation from human induced pluripotent stem cells. Biochemical and biophysical research communications. 2013;431(2):309-14.
296. Koszinowski S, Buss K, Kaehlcke K, Krieglstein K. Signaling via the transcriptionally regulated activin receptor 2B is a novel mediator of neuronal cell death during chicken ciliary ganglion development. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience. 2015;41:98-104.
297. Thulluru HK, Michel OJ, Oudejans CB, van Dijk M. ACVR2A promoter polymorphism rs1424954 in the Activin-A signaling pathway in trophoblasts. Placenta. 2015;36(4):345-9.
298. Luisi S, Florio P, Reis FM, Petraglia F. Expression and secretion of activin A: possible physiological and clinical implications. European journal of endocrinology / European Federation of Endocrine Societies. 2001;145(3):225-36.
299. Phillips DJ, Jones KL, Scheerlinck JY, Hedger MP, de Kretser DM. Evidence for activin A and follistatin involvement in the systemic inflammatory response. Molecular and cellular endocrinology. 2001;180(1-2):155-62.
300. Tessier C, Prigent-Tessier A, Bao L, Telleria CM, Ferguson-Gottschall S, Gibori GB, et al. Decidual activin: its role in the apoptotic process and its regulation by prolactin. Biology of reproduction. 2003;68(5):1687-94.
301. Florio P, Abella RF, de la Torre T, Giamberti A, Luisi S, Butera G, et al. Perioperative activin A concentrations as a predictive marker of neurologic abnormalities in children after open heart surgery. Clinical chemistry. 2007;53(5):982-5.
302. Aleman-Muench GR, Soldevila G. When versatility matters: activins/inhibins as key regulators of immunity. Immunology and cell biology. 2012;90(2):137-48.
303. Sugama S, Takenouchi T, Kitani H, Fujita M, Hashimoto M. Activin as an anti-inflammatory cytokine produced by microglia. J Neuroimmunol. 2007;192(1-2):31-9.
304. Jenkins DD, Rollins LG, Perkel JK, Wagner CL, Katikaneni LP, Bass WT, et al. Serum cytokines in a clinical trial of hypothermia for neonatal hypoxic-ischemic encephalopathy. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2012;32(10):1888-96.
305. Iwahori Y, Saito H, Torii K, Nishiyama N. Activin exerts a neurotrophic effect on cultured hippocampal neurons. Brain research. 1997;760(1-2):52-8.

306. Krieglstein K, Suter-Crazzolara C, Fischer WH, Unsicker K. TGF-beta superfamily members promote survival of midbrain dopaminergic neurons and protect them against MPP+ toxicity. The EMBO journal. 1995;14(4):736-42.
307. Schubert D, Kimura H, LaCorbiere M, Vaughan J, Karr D, Fischer WH. Activin is a nerve cell survival molecule. Nature. 1990;344(6269):868-70.
308. Fann MJ, Patterson PH. Neuropoietic cytokines and activin A differentially regulate the phenotype of cultured sympathetic neurons. Proceedings of the National Academy of Sciences of the United States of America. 1994;91(1):43-7.
309. Schonhaut L, Armijo I, Schonstedt M, Alvarez J, Cordero M. Validity of the ages and stages questionnaires in term and preterm infants. Pediatrics. 2013;131(5):e1468-74.
310. Murray DM, Ryan CA, Boylan GB, Fitzgerald AP, Connolly S. Prediction of seizures in asphyxiated neonates: correlation with continuous video-electroencephalographic monitoring. Pediatrics. 2006;118(1):41-6.
311. Murray DM, Boylan GB, Fitzgerald AP, Ryan CA, Murphy BP, Connolly S. Persistent lactic acidosis in neonatal hypoxic-ischaemic encephalopathy correlates with EEG grade and electrographic seizure burden. Archives of disease in childhood Foetal and neonatal edition. 2008;93(3):F183-6.
312. Fanos V, Atzori L, Makarenko K, Melis GB, Ferrazzi E. Metabolomics application in maternal-Foetal medicine. Biomed Res Int. 2013;2013:720514.
313. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chemical Society reviews. 2011;40(1):387-426.
314. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. The Lancet. 2005;365(9460):663-70.
315. Liu J, Sheldon RA, Segal MR, Kelly MJ, Pelton JG, Ferriero DM, et al. 1H nuclear magnetic resonance brain metabolomics in neonatal mice after hypoxia-ischemia distinguished normothermic recovery from mild hypothermia recoveries. Pediatric research. 2013;74(2):170-9.
316. Farooqui AA, Horrocks LA, Farooqui T. Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chemistry and physics of lipids. 2000;106(1):1-29.
317. Prins ML. Cerebral metabolic adaptation and ketone metabolism after brain injury. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2008;28(1):1-16.
318. Bayley N. Bayley Scales of Infant Development. New York, NY: The Psychological Society; 1969.

319. Bayley N. Bayley Scales of Infant Development. 2nd Edition. San Antonio, TX: The Psychological Corporation; 1993.
320. Bayley N. Bayley Scales of Infant and Toddler Development - Third Edition: Technical Manual. San Antonio, TX: PsychCorp; 2006.
321. Bode MM, D'Eugenio DB, Mettelman BB, Gross SJ. Predictive validity of the Bayley, Third Edition at 2 years for intelligence quotient at 4 years in preterm infants. Journal of developmental and behavioral pediatrics : JDBP. 2014;35(9):570-5.
322. Anderson PJ. Underestimation of developmental delay by the new bayley-III scale. Arch Pediatr Adolesc Med. 2010;164(4):352-6.
323. Vohr BR, Stephens BE, Higgins RD, Bann CM, Hintz SR, Das A, et al. Are outcomes of extremely preterm infants improving? Impact of Bayley assessment on outcomes. The Journal of pediatrics. 2012;161(2):222-8 e3.
324. Aylward GP. Continuing issues with the Bayley-III: where to go from here. Journal of developmental and behavioral pediatrics : JDBP. 2013;34(9):697-701.
325. Jary S, Whitelaw A, Walloe L, Thoresen M. Comparison of Bayley-2 and Bayley-3 scores at 18 months in term infants following neonatal encephalopathy and theraputic hypothermia. Developmental medicine and child neurology. 2013;55(11):1053-9.
326. Moore T, Johnson S, Haider S, Hennessy E, Marlow N. Relationship between test scores using the second and third editions of the Bayley Scales in extremely preterm children. The Journal of pediatrics. 2012;160(4):553-8.
327. Johnson S, Moore T, Marlow N. Using the Bayley-III to assess neurodevelopmental delay: which cut-off should be used? Pediatric research. 2014;75(5):670-4.
328. Chinta S, Walker K, Halliday R, Loughran-Fowlds A, Badawi N. A comparison of the performance of healthy Australian 3-year-olds with the standardised norms of the Bayley Scales of Infant and Toddler Development (version-III). Archives of disease in childhood. 2014;99(7):621-4.
329. Yu Y-T, Hsieh W-S, Hsu C-H, Chen L-C, Lee W-T, Chiu N-C, et al. A psychometric study of the Bayley Scales of Infant and Toddler Development – 3rd Edition for term and preterm Taiwanese infants. Research in Developmental Disabilities. 2013;34(11):3875-83.
330. Cromwell EA, Dube Q, Cole SR, Chirambo C, Dow AE, Heyderman RS, et al. Validity of US norms for the Bayley Scales of Infant Development-III in Malawian children. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2014;18(2):223-30.
331. Steenis LJ, Verhoeven M, Hessen DJ, van Baar AL. Performance of Dutch Children on the Bayley III: A Comparison Study of US and Dutch Norms. PloS one. 2015;10(8):e0132871.

332. Krogh MT, Væver MS, Harder S, Køppe S. Cultural differences in infant development during the first year: A study of Danish infants assessed by the Bayley-III and compared to the American norms. European Journal of Developmental Psychology. 2012;9(6):730-6.
333. Bayley N. Bayley scale of Infant and Toddler Development (3rd Edition). UK and Ireland Supplement Manual. London: Pearson Assessment; 2010.
334. McGuinness C, Connolly P, Eakin A, Miller S. The Developmental Status of 2-3 year old Children entering Group-Based Settings in Northern Ireland. Belfast: Centre for Effective Education, Queen's University Belfast; 2012.
335. O'Donovan SM, Murray DM, Hourihane JO, Kenny LC, Irvine AD, Kiely M. Cohort profile: The Cork BASELINE Birth Cohort Study: Babies after SCOPE: Evaluating the Longitudinal Impact on Neurological and Nutritional Endpoints. International journal of epidemiology. 2015;44(3):764-75.
336. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. Journal of pediatric psychology. 1997;22(3):313-28.
337. Achenbach TM, Rescorla LA. Manual for the ASEBA preschool forms & profiles: An integrated system of multi-informant assessment; Child behavior checklist for ages 1 1/2-5; Language development survey; Caregiver-teacher report form: University of Vermont; 2000.
338. Ronfani L, Vecchi Brumatti L, Mariuz M, Tognin V, Bin M, Ferluga V, et al. The Complex Interaction between Home Environment, Socioeconomic Status, Maternal IQ and Early Child Neurocognitive Development: A Multivariate Analysis of Data Collected in a Newborn Cohort Study. PloS one. 2015;10(5):e0127052.
339. CSO. CSO Education Statistics 2007 [Available from: http://www.cso.ie/en/studentscorner/education/educationstatistics/.
340. Sundet JM, Barlaug DG, Torjussen TM. The end of the Flynn effect?: A study of secular trends in mean intelligence test scores of Norwegian conscripts during half a century. Intelligence. 2004;32(4):349-62.
341. PearsonEducation. Factors Contributing to Differences Between Bayley-III and BSID-II Scores (Bayley-III Technical Report No.2) San Antonio: Pearson Education; 2008 [cited 2015 28/08/2015]. Available from: http://images.pearsonassessments.com/images/tmrs/tmrs_rg/BayleyIII_TechRep.pdf?WT.mc_id=TMRS_Bayley_III_Technical_Report_2.
342. Robertson CM, Hendson L, Biggs WS, Acton BV. Application of the Flynn effect for the Bayley III Scales. Arch Pediatr Adolesc Med. 2010;164(11):1072-3; author reply 3.

343. Deroma L, Bin M, Tognin V, Rosolen V, Valent F, Barbone F, et al. [Interrater reliability of the Bayley III test in the Italian Northern-Adriatic Cohort II]. Epidemiologia e prevenzione. 2013;37(4-5):297-302.
344. Aylward GP. Cognitive and neuropsychological outcomes: more than IQ scores. Mental retardation and developmental disabilities research reviews. 2002;8(4):234-40.
345. Aylward GP. Developmental screening and assessment: what are we thinking? Journal of developmental and behavioral pediatrics : JDBP. 2009;30(2):169-73.
346. Doyle LW, Anderson PJ, Battin M, Bowen JR, Brown N, Callanan C, et al. Long term follow up of high risk children: who, why and how? BMC pediatrics. 2014;14:279.
347. Orton JL, McGinley JL, Fox LM, Spittle AJ. Challenges of neurodevelopmental follow-up for extremely preterm infants at two years. Early human development. 2015;91(12):689-94.
348. Johnson S, Marlow N. Developmental screen or developmental testing? Early human development. 2006;82(3):173-83.
349. Stiles J, Paul B, Hesselink J. Spatial cognitive development following early focal brain injury: Evidence for adaptive change in brain and cognition. PROCESSES OF CHANGE IN BRAIN AND COGNITIVE DEVELOPMENT: ATTENTION AND PERFORMANCE XXI. 2006:535-61.
350. Blakemore SJ, Choudhury S. Development of the adolescent brain: implications for executive function and social cognition. Journal of child psychology and psychiatry. 2006;47(3-4):296-312.
351. Basser LS. Hemiplegia of early onset and the faculty of speech with special reference to the effects of hemispherectomy. Brain. 1962;85:427-60.
352. Lenneberg EH, Chomsky N, Marx O. Biological foundations of language: Wiley New York; 1967.
353. Karmiloff-Smith A. Preaching to the Converted? From Constructivism to Neuroconstructivism. Child development perspectives. 2009;3(2):99-102.
354. Côté JE, Levine CG. Attitude versus Aptitude Is Intelligence or Motivation More Important for Positive Higher-Educational Outcomes? Journal of Adolescent Research. 2000;15(1):58-80.
355. Chamorro-Premuzic T, Furnham A. Personality predicts academic performance: Evidence from two longitudinal university samples. Journal of Research in Personality. 2003;37(4):319-38.
356. Armstrong-Wells J, Bernard TJ, Boada R, Manco-Johnson M. Neurocognitive outcomes following neonatal encephalopathy. NeuroRehabilitation. 2010;26(1):27-33.
357. Mulder H, Pitchford NJ, Hagger MS, Marlow N. Development of executive function and attention in preterm

children: a systematic review. Developmental neuropsychology. 2009;34(4):393-421.
358. Mulder H, Pitchford NJ, Marlow N. Processing speed and working memory underlie academic attainment in very preterm children. Archives of disease in childhood Foetal and neonatal edition. 2010;95(4):F267-72.
359. Walker DM, Marlow N. Neurocognitive outcome following Foetal growth restriction. Archives of disease in childhood Foetal and neonatal edition. 2008;93(4):F322-5.
360. Herskind A, Greisen G, Nielsen JB. Early identification and intervention in cerebral palsy. Developmental medicine and child neurology. 2015;57(1):29-36.
361. Spittle A, Orton J, Anderson PJ, Boyd R, Doyle LW. Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants. The Cochrane database of systematic reviews. 2015(11):Cd005495.
362. Gollenberg AL, Lynch CD, Jackson LW, McGuinness BM, Msall ME. Concurrent validity of the parent-completed Ages and Stages Questionnaires, 2nd Ed. with the Bayley Scales of Infant Development II in a low-risk sample. Child: care, health and development. 2010;36(4):485-90.
363. Veldhuizen S, Clinton J, Rodriguez C, Wade TJ, Cairney J. Concurrent validity of the Ages And Stages Questionnaires and Bayley Developmental Scales in a general population sample. Academic pediatrics. 2015;15(2):231-7.
364. Ireton H. Infant Development Inventory. Minneapolis: Behavior Science Systems Inc.; 1994.
365. Ireton H. Child Developmental Inventory. Minneapolis: Behavior Science Systems Inc; 1998.
366. Ireton H, Glascoe FP. Assessin Children's Development Using Parents' Reports The Child Development Inventory. Clinical Pediatrics. 1995;34(5):248-55.
367. Doig KB, Macias MM, Saylor CF, Craver JR, Ingram PE. The Child Development Inventory: A developmental outcome measure for follow-up of the high-risk infant. The Journal of pediatrics. 1999;135(3):358-62.
368. Saudino KJ, Dale PS, Oliver B, Petrill SA, Richardson V, Rutter M, et al. The validity of parent-based assessment of the cognitive abilities of 2-year-olds. Br J Dev Psychol. 1998;16:349-63.
369. Johnson S, Wolke D, Marlow N. Developmental assessment of preterm infants at 2 years: validity of parent reports. Developmental Medicine & Child Neurology. 2008;50(1):58-62.
370. Baker M, Schafer G, Alcock KJ, Bartlett S. A parentally administered cognitive development assessment for children from 10 to 24 months. Infant behavior & development. 2013;36(2):279-87.
371. Mullen E. Mullen Scales of Early Learning. Los Angeles (CA): Western Psychological Services; 1995.

372. Caudle SE, Katzenstein JM, Oghalai JS, Lin J, Caudle DD. Nonverbal cognitive development in children with cochlear implants: relationship between the Mullen Scales of Early Learning and later performance on the Leiter International Performance Scales-Revised. Assessment. 2014;21(1):119-28.
373. Akshoomoff N. Use of the Mullen Scales of Early Learning for the assessment of young children with Autism Spectrum Disorders. Child neuropsychology : a journal on normal and abnormal development in childhood and adolescence. 2006;12(4-5):269-77.
374. Burns TG, King TZ, Spencer KS. Mullen scales of early learning: the utility in assessing children diagnosed with autism spectrum disorders, cerebral palsy, and epilepsy. Applied neuropsychology Child. 2013;2(1):33-42.
375. Newborg J. Battelle developmental inventory - second edition. Itasca (IL): Riverside; 2005.
376. Matson JL, Hess JA, Sipes M, Horovitz M. Developmental profiles from the Battelle developmental inventory: a comparison of toddlers diagnosed with Down Syndrome, global developmental delay and premature birth. Developmental neurorehabilitation. 2010;13(4):234-8.
377. Hack M, Taylor HG, Drotar D, Schluchter M, Cartar L, Wilson-Costello D, et al. Poor predictive validity of the Bayley Scales of Infant Development for cognitive function of extremely low birth weight children at school age. Pediatrics. 2005;116(2):333-41.
378. Pennington BF. Diagnosing learning disorders: A neuropsychological framework: Guilford Press; 2008.
379. O'Reilly RC, Munakata Y. Computational explorations in cognitive neuroscience: Understanding the mind by simulating the brain: MIT press; 2000.
380. Cattell RB. Abilities: Their structure, growth, and action. 1971.
381. Ackerman PL. A theory of adult intellectual development: Process, personality, interests, and knowledge. Intelligence. 1996;22(2):227-57.
382. Rose S. Dimensions of cognition in infancy. Intelligence. 2004;32(3):245-62.
383. Mulder H, Hoofs H, Verhagen J, van der Veen I, Leseman PP. Psychometric properties and convergent and predictive validity of an executive function test battery for two-year-olds. Frontiers in psychology. 2014;5:733.
384. Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD. The unity and diversity of executive functions and their contributions to complex “frontal lobe” tasks: A latent variable analysis. Cognitive psychology. 2000;41(1):49-100.
385. Fagan JF, Holland CR, Wheeler K. The prediction, from infancy, of adult IQ and achievement. Intelligence. 2007;35(3):225-31.

386. Rose SA, Feldman JF, Jankowski JJ, Van Rossem R. Information Processing from Infancy to 11 Years: Continuities and Prediction of IQ. Intelligence. 2012;40(5):445-57.
387. Luciana M. Practitioner review: computerized assessment of neuropsychological function in children: clinical and research applications of the Cambridge Neuropsychological Testing Automated Battery (CANTAB). Journal of child psychology and psychiatry, and allied disciplines. 2003;44(5):649-63.
388. Canini M, Battista P. Computerized neuropsychological assessment in aging: testing efficacy and clinical ecology of different interfaces. Computational and mathematical methods in medicine. 2014;2014:804723.
389. Green CR, Mihic AM, Nikkel SM, Stade BC, Rasmussen C, Munoz DP, et al. Executive function deficits in children with Foetal alcohol spectrum disorders (FASD) measured using the Cambridge Neuropsychological Tests Automated Battery (CANTAB). Journal of child psychology and psychiatry, and allied disciplines. 2009;50(6):688-97.
390. Luciana M, Nelson CA. Assessment of neuropsychological function through use of the Cambridge Neuropsychological Testing Automated Battery: performance in 4-to 12-year-old children. Developmental neuropsychology. 2002;22(3):595-624.
391. Semmelmann K, Nordt M, Sommer K, Röhnke R, Mount L, Prüfer H, et al. U Can Touch This: How Tablets Can Be Used to Study Cognitive Development. Frontiers in psychology. 2016;7(1021).
392. Christakis DA. Interactive media use at younger than the age of 2 years: time to rethink the American Academy of Pediatrics guideline? JAMA pediatrics. 2014;168(5):399-400.
393. Education CoP. Media Education. Pediatrics. 1999;104(2):341-3.
394. Communications Co, Media. Media Use by Children Younger Than 2 Years. Pediatrics. 2011;128(5):1040-5.
395. Christakis DA, Zimmerman FJ, DiGiuseppe DL, McCarty CA. Early Television Exposure and Subsequent Attentional Problems in Children. Pediatrics. 2004;113(4):708-13.
396. Garrison MM, Liekweg K, Christakis DA. Media Use and Child Sleep: The Impact of Content, Timing, and Environment. Pediatrics. 2011;128(1):29-35.
397. Lillard AS, Peterson J. The immediate impact of different types of television on young children's executive function. Pediatrics. 2011;128(4):644-9.
398. Cristia A, Seidl A. Parental Reports on Touch Screen Use in Early Childhood. PloS one. 2015;10(6):e0128338.
399. CSO. Central Statistics Office [Available from: http://www.cso.ie/en/.
400. ONS. Office for National Statistics licensed under the Open Government Licence v.3.0. [Available from: http://www.ons.gov.uk/ons/index.html.

401. Bedford R, Saez de Urabain IR, Cheung CHM, Karmiloff-Smith A, Smith TJ. Toddlers' Fine Motor Milestone Achievement Is Associated with Early Touchscreen Scrolling. Frontiers in psychology. 2016;7(1108).
402. Kale G. Which browser is best for touchscreen in Windows? : Winaero; 2016 [Available from: http://winaero.com/blog/which-browser-is-best-for-touchscreen-in-windows/.
403. Aziz NAA, Batmaz F, Stone R, Chung PWH, editors. Selection of touch gestures for children's applications. Science and Information Conference (SAI), 2013; 2013: IEEE.
404. Techopedia. Iterative Game Design 2016 [Available from: https://www.techopedia.com/definition/27045/iterative-game-design.
405. Tye C, Mercure E, Ashwood KL, Azadi B, Asherson P, Johnson MH, et al. Neurophysiological responses to faces and gaze direction differentiate children with ASD, ADHD and ASD+ADHD. Developmental cognitive neuroscience. 2013;5:71-85.
406. Nomi JS, Uddin LQ. Face processing in autism spectrum disorders: From brain regions to brain networks. Neuropsychologia. 2015;71:201-16.
407. Wodka EL, Mahone EM, Blankner JG, Larson JC, Fotedar S, Denckla MB, et al. Evidence that response inhibition is a primary deficit in ADHD. Journal of clinical and experimental neuropsychology. 2007;29(4):345-56.
408. Visell Y. Tactile sensory substitution: Models for enaction in HCI. Interacting with Computers. 2009;21(1–2):38-53.
409. Raisamo R, Patomäki S, Hasu M, Pasto V. Design and evaluation of a tactile memory game for visually impaired children. Interacting with Computers. 2007;19(2):196-205.
410. Sánchez J, Sáenz M. 3D sound interactive environments for blind children problem solving skills. Behaviour & Information Technology. 2006;25(4):367-78.
411. Jacob R, Karn KS. Eye tracking in human-computer interaction and usability research: Ready to deliver the promises. Mind. 2003;2(3):4.
412. Chau M, Betke M. Real time eye tracking and blink detection with usb cameras. Boston University Computer Science Department; 2005.
413. Betke M, Gips J, Fleming P. The camera mouse: visual tracking of body features to provide computer access for people with severe disabilities. IEEE Transactions on neural systems and Rehabilitation Engineering. 2002;10(1):1-10.
414. Annaz D, Karmiloff-Smith A, Thomas M, Reed J, Warner Rogers J. Child neuropsychology: Concepts, theory and practice. Wiley-Blackwell Chichester, UK; 2008.
415. Spencer-Smith MM, Spittle AJ, Lee KJ, Doyle LW, Anderson PJ. Bayley-III Cognitive and Language Scales in Preterm Children. Pediatrics. 2015;135(5):e1258-65.

416. Lai MC, Lombardo MV, Baron-Cohen S. Autism. Lancet. 2014;383(9920):896-910.
417. Chafer-Pericas C, Cernada M, Rahkonen L, Stefanovic V, Andersson S, Vento M. Preliminary case control study to establish the correlation between novel peroxidation biomarkers in cord serum and the severity of hypoxic ischemic encephalopathy. Free radical biology & medicine. 2016;97:244-9.
418. Media F. Frontiers in Neurology Neuropediatrics 2015 [Available from: http://journal.frontiersin.org/article/10.3389/fneur.2015.00264/full#.
419. RCN. Many toddlers are adept at mastering the use of smartphones and tablets. Nursing Standard. 2016;30(20):14.
420. RCPCH. RCPCH responds to screen time study published in ADC 2016 [Available from: http://www.rcpch.ac.uk/news/rcpch-responds-screen-time-study-published-adc.
421. Caglio A, Lethin S, Hashemian Y, editors. Let's play!: designing for preschool children. Proceedings of the 14th Participatory Design Conference: Short Papers, Interactive Exhibitions, Workshops-Volume 2; 2016: ACM.
422. Zack E, Barr R. The Role of Interactional Quality in Learning from Touch Screens during Infancy: Context Matters. Frontiers in psychology. 2016;7(1264).
423. Altmetric. Technology Usage in Toddlers 2015 [Available from: https://www.altmetric.com/details/4915013.

# 9.0 Appendices

## A. Babyscreen App Questionnaire and Prototype Testing Documents

### A.1 Participant Information Sheet

ucc
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

CUH
Ospidéal Ollscoil Chorcaí
Cork University Hospital

**Research Subject Information Sheet**

**Introduction**

You have been invited to participate in a research project to look at how well children aged 18 months to 2 ½ years play with touch screen devices. This will allow us to determine whether it would be possible to develop a touch screen program that could be used by doctors to measure the progress of young children.

**Nature of the Research Project**

We are asking children aged 18 months to 2 ½ years to play with a touch screen for a few minutes to measure the age at which a child could be assessed using a touch screen programme.

We are also asking you to complete a brief questionnaire. This can be done by filling out the form and handing it immediately back to the co-investigator. The questionnaire should take no more than 5-10 minutes to complete. Some biographical information will be collected as a part of the questionnaire, including parents/legal guardian name and age. To reduce the potential risk for breach of confidentiality, all information collected will be stored securely, and the confidentiality of the subjects involved will be prioritized and respected at all times. Information gathered will never be shared with anyone not involved in the above stated research project, and will not be used in any way for further follow up.

Your participation in this research project is voluntary. You may withdraw from participating in the research project at any time.

If you have any questions or concerns about your involvement in this project, please contact the research co-investigator Rachel Rollings, by email r.rollings@umail.ucc.ie or by phone at 0873625660.

## A.2 Participant Consent Form

Consent form Version 3

**CONSENT BY RESEARCH SUBJECT FOR PARTICIPATION IN RESEARCH PROJECT**

Title of Research Project: Baby Screen Project

Chief Investigator: Dr. Deirdre Murray, Senior Lecturer/Consultant Paediatrician
Department of Paediatrics and Child Health, University College Cork
Clinical Investigations Unit, Cork University Hospital, Wilton, Cork
Co-Investigator: Rachel Rollings, r.rollings@umail.ucc.ie
Sinead Dilworth S.DILWORTH1@ucig.ie
Caroline Ahearne cahearne@ucc.ie

You are being asked to participate in a research study. This consent form gives detailed information about the research study in order to allow you to make an informed decision about whether you are willing to participate in this study. All the risks and benefits associated with this research project will be discussed with you so that you may address any concerns or questions. This process is known as informed consent. Once you understand the study, you will be asked to sign this form if you wish to participate.

**NATURE AND DURATION OF PROCEDURE(S):**

The purpose of this research project is to establish whether children, aged 12 months to 3 years, are capable of playing with touch screen technology and whether they can appropriately manipulate and interact with touch screen games or programs. This will allow us to determine whether a new assessment tool to measure children's intellectual function could be developed on, for example, an iPad. A questionnaire will be handed out to parents/legal guardians asking about their child's experiences with touch screen items. The questionnaire should take no more than 10-15 minutes. Minimal information about parents/legal guardians' age and name, and the child's age, will be collected. All attempts to keep this information confidential and secure will be undertaken to minimize the risk of breach of confidentiality. Your child will also be given a touch screen to play with for a few minutes to observe their level of interaction and ability to play with a touch screen program or game. This interaction will be video-recorded to allow us to better assess how your child plays with the touch screen. All attempts will be made to avoid including identifying features such as faces in the video.

**AGREEMENT TO CONSENT**

The research project has been fully explained to me. I have had the opportunity to ask questions concerning any and all aspects of the research project and any related matters or issues of concern. I am aware that my participation is voluntary and that I may withdraw my consent at any time.

I, the undersigned, hereby consent to participate as a subject in the above described research project conducted at Cork University Hospital or Mercy University Hospital. I have received a copy of this consent form and research subject information sheet for my records. I understand that if I have any questions concerning this research, I can contact the chief investigator or co-investigators listed above.

**Signed (Parent or Legal Guardian):** _____

**Witnessed:** _____

## A.3 Touch-screen usage questionnaire

ucc

Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

CUH

mercy
UNIVERSITY HOSPITAL

**Baby Screen Project**

**Questionnaire**

Please complete the following questionnaire:

Parent/Legal Guardian 1: mother/father Age:_____ Occupation:

Parent/Legal Guardian 2: mother/father Age:_____ Occupation:

Child's Age: _____ circle: Male Female

Please circle as appropriate:

1. Do you own an iPad/iPhone? YES NO

2. Do you give your child a phone/iPad/other technology to play with? YES NO

3. Is the technology they play with touch screen? YES NO

4. Does your child have specific games or apps downloaded for them? YES NO

5. Number of minutes per day your child spends playing with a touch screen (approx.)?

6. Can your child unlock the touch screen item? YES NO

7. Can they swipe across the touch screen (i.e. swipe through pictures)? YES NO

8. Are they able to specifically identify and use touch screen features (i.e. touch screen icons, know which buttons to tap)? YES NO

9. Do you believe your child actively looks for the touch screen features (i.e. look specifically for their game icon)? YES NO

10. Do you believe your child randomly touches the screen and doesn't understand the features available? YES NO

## B. BiHIVE 2 Patient Information and Consent Forms

### B.1 Case Consent Form




**BiHIVE 2 STUDY**

**The Investigation and Validation of Predictive Biomarkers in**

**Hypoxic-Ischaemic Encephalopathy**

## Information and Consent form

### Information and consent leaflet:

**Introduction**

We would ask you to read the information leaflet given to you again now, and give you the opportunity to ask any questions you may have.

**Do I have to take part in this study?** It is entirely your choice. If you agree we will ask you to sign a consent form, and you will be given a copy of this to keep. You are free to change your mind and withdraw your baby from the study at any time, without giving a reason. The care that you or your baby receive now or in the future will not be affected in any way by your decision whether or not to take part.

**What will happen to my baby if I agree to take part?** Because your baby has needed resuscitation at birth, blood has been drawn from the umbilical cord and placenta after the baby was born. This is part of routine care in our hospital and most other modern maternity hospitals. This blood is used to measure acid levels in the blood. We have also drawn extra blood and stored it in 3-4 extra tubes. If you agree to take part in the study this extra blood will be analysed at a later period to measure levels of other chemicals in the blood. This blood is otherwise discarded and if you do not wish to take part in the study we will discard this stored blood immediately. We are collecting these samples because we hope to identify which chemicals and proteins can best predict how babies cope with stressful deliveries and resuscitation. We are looking for markers in the blood which can predict which infants may have suffered significant stress and who may need treatment in the first few hours after birth. Most importantly we want to improve our ability to predict a baby's development over the first few years of life.

We would also like to see if these chemicals change over the first few days of life. Therefore if your baby is having a blood test as part of their routine care, we will take an extra 1.5 milliliters of blood on the first, second and third day of life or if your baby develops seizures (convulsions). This is unlikely, but if it happens seizures can also affect a baby's develop and this is important for us to study. We will only take the sample if your baby requires a blood test or intravenous line for other reasons as part of their medical care.

After your baby is born, we will be able to gather information about your baby's birth, and treatment from your maternity notes and the baby's notes.

To accurately measure any effect on your baby's brain we wish to record an EEG (electroencephalograph) and other vital signs such as heart rate, breathing rate and oxygen saturation levels. This is part of routine monitoring in our hospital and carrying out in all babies who have signs of brain irritation or injury. This is usually carried out for up to 24 hours. However as part of the study we may ask you to allow us to monitor your baby's brain waves for longer, so that we can gather extra information.

Lastly as part of the study we will ask to meet you and your baby again between 18 months and 2 years of age to monitor their growth and development. This will involve short questionnaires and a one hour developmental assessment.

Ospideal Maithreachais na h'Ollscoile Corcaigh
Cork University Maternity Hospital

HRB Health Research Board

UCC

## BiHIVE INFORMED CONSENT FORM

**Principal Investigator**: Dr. Deirdre Murray **Phone Number**: +353 21 4901271

**EEG ID Number**_____ **Patient ID Number for this study**_____

**Y/N**

- I have read the information leaflet about this research and have been given a copy to keep. The information has been fully explained to me and I have been able to ask questions. I understand why the research is being done and any risks involved. ☐
- I agree to donate a sample of my baby's umbilical cord blood for this research project. I understand that giving a sample for this research is voluntary and that I am free to withdraw my approval at any time without my medical treatment being affected. ☐
- I agree to donate a sample of DNA from my baby's umbilical cord blood for storage and research related to the project ☐
- I agree to donate a sample of my baby's blood for this research project on day 1, 2 or 3 of life, or if my baby develops seizures. I understand that giving a sample for this research is voluntary and that I am free to withdraw my approval at any time without my medical treatment being affected. ☐
- I agree to allow my baby to take part in the follow up visits required at 18-24 months of age ☐
- I give permission for research personnel to look at my baby's medical records to obtain information about my baby's progress after birth, and the records of my labour and delivery. I have been assured that this information will be kept confidential ☐
- I agree to donate a sample that can be used now or in the future for commercial collaborative research to develop screening tests for brain injury in children. I understand that I will not benefit financially if this research leads to a development of a new treatment or medical test. ☐
- I give permission for my baby's sample and information collected about my baby to be stored for possible future research related to this study *without my further consent being required* and subject to approval by a research ethics committee. ☐

| Role | Print Name | Signature | Date |
|---|---|---|---|
| Parent/legal guardian 1 | | | |
| Parent/legal guardian 2 | | | |
| Investigator | | | |
| Witness | | | |

| Insert Hospital Addressogram Label Here | **Local Principal Investigator: Dr Deirdre Murray** |
|---|---|

B.2 Case Patient Information Leaflet

# BiHiVE 2 study

## The Investigation and Validation of Predictive Biomarkers in Hypoxic-ischaemic Encephalopathy

## Information Leaflet for Parents

### What is the BiHiVE study?

Some baby's require resuscitation when they don't cry or move after delivery. This may occur when a baby has had a difficult or stressful time during labour, or before delivery. Sometimes this can even lead to long term injury to the baby. The injury which we worry about the most is newborn brain injury. This type of brain injury is called hypoxic-ischaemic encephalopathy or HIE. As your baby has required resuscitation they may need careful monitoring for the next few days. We have no good way of knowing whether your baby has had a significant injury, and usually can't tell for sure until at least 24 hours after birth. The BiHiVE study hopes to find chemical and protein markers

in the blood which can tell us very soon after birth which babies have suffered significant injury so that we can intervene and improve their outcome. At the moment we have no good reliable early marker which can give us this information straight after birth. We hope that in the future a simple blood test at delivery will be able to give doctors and parents more information within hours of a baby's birth.

Because your baby has needed resuscitation at birth, blood has been drawn from the umbilical cord and placenta after the baby was born. This is the standard care in our hospital and many other modern maternity hospitals. This blood is used to measure acid levels in the blood. We have also drawn extra blood and stored it in 3-4 tubes. If you wish your baby to take part in the study we will store this blood to use for research into newborn brain injury. If you do not wish to take part in the study these samples will be destroyed.

If your baby is admitted to Neonatal Intensive Care Unit, and, if they are having a blood test as part of their routine care, we will ask for an extra 1.5 milliliters of blood on the first, second and third day of life. We will not disturb your baby an extra time for this sample, and if your baby does not require a blood test for other reasons, we will not take this sample. These samples will be frozen and stored for analysis.

Our current best way of looking at those who might be at risk of brain injury is to carry out a recording of their brain waves using an EEG machine. This is routinely done on the neonatal unit. We want to be able to compare what is currently our best method, to the simpler blood test which we hope to develop, and therefore all babies in the study will also have an EEG if they have signs of brain irritation (HIE).

In order to help babies who have difficulties after birth, it is also important for us to study babies who have had normal, uneventful deliveries. These babies act as controls so that we can know that the changes we find in the unwell babies are genuine. If your baby is a control infant, blood is taken from the umbilical cord and stored at birth. We will follow up your baby with an examination the day after birth. This takes about 5 minutes and will not upset your baby in any way. It can be carried out when they are still asleep in many cases. In addition we would like to contact you at 2 years of age to look at the growth and development of your child. The researchers will be very clear with you to reassure you that your baby is a control, or well baby.

## What will the study involve?

If you are happy for the research team to follow your baby after they are born, the extra samples of blood taken from your baby's umbilical cord will be frozen, stored and analyzed later when we have collected enough samples. An additional 1.5 milliliters of

blood will be taken if the baby is having other blood samples on day 1, 2 and 3, these will be analyzed once enough samples are collected. We will look at many different chemicals and proteins which may change in babies with significant brain injury. We will compare the levels to those of babies who did not need resuscitation. Your baby's progress after birth will be recorded. If your baby needs any extra medical care the details of this care will be collected from your baby's medical notes. This information will be anonymous, and will be identified only using a study number, not your baby's name or address.

We will also ask you to meet us again at 24 months of age so that we can record the progress and development of your baby. You will be contacted and an appointment will be made for a follow up assessment at a time convenient for you and your family. This will happen in the Cork University Hospital. In these follow up appointments, your baby's development will be assessed and discussed with you. Your baby will not need any further samples to be taken after the initial sample from the placenta for this study.

## What are my options?

Being part of the BiHiVE study is completely voluntary. You or your child can withdraw from this study at any stage. Not being part of this study will not affect any part of the medical care of you or your baby.
The BiHiVE study will be managed by the staff of the Department of Paediatrics and Child Health, University College Cork. We will publish our results in international medical journals. Once a year we will organize a meeting to tell families about how the study is progressing.

## Where can I get more information?

If you have any questions regarding the BiHiVE study, please contact the research coordinators

Dr Deirdre Murray
Dept of Paediatrics and Child Health
Clinical Investigations Unit,
Cork University Hospital
Tel: +353 21 4901271
Fax: +353 21 434 5217
Email: d.murray@ucc.ie

## B.3 Control Consent Form

Ospideal Maithreachais na h'Ollscoile Corcaigh
Cork University Maternity Hospital

HRB Health Research Board

ucc

**BiHIVE INFORMATION LEAFLET AND CONSENT**

# BiHIVE 2 STUDY

**The Investigation and Validation of Predictive Biomarkers in Hypoxic-Ischaemic Encephalopathy**

## Consent Form Control Group (Well babies)

### Information and consent leaflet:

**Introduction**

We would ask you to read the information leaflet given to you again now, and give you the opportunity to ask any questions you may have.

**Do I have to take part in this study?** It is entirely your choice. If you agree we will ask you to sign a consent form, and you will be given a copy of this to keep. You are free to change your mind and withdraw your baby from the study at any time, without giving a reason. The care that you or your baby receive now or in the future will not be affected in any way by your decision whether or not to take part.

**What will happen to my baby if I agree to take part?** In order to study differences in normal healthy births and those that needed resuscitation at birth we need to study a healthy control group. Your baby had an uneventful normal delivery and this is why we are asking your permission to include your baby as a **control** (well baby) in this study. We are asking if you would allow us to draw blood from the umbilical cord and placenta after your baby is born. This blood is used to measure acid levels in the blood. We store it and it will be analysed at a later period to measure levels of other chemicals in the blood. We are collecting these samples because we hope to identify which chemicals and proteins can best predict how babies cope with stressful deliveries and resuscitation. We will be looking at normal levels in the control group and comparing them to the study group of resuscitated babies. We are looking for markers in the blood which can predict which infants may have suffered significant stress and who may need treatment in the first few hours after birth. Most importantly we want to improve our ability to predict a baby's development over the first few years of life.

After your baby is born, we will be able to gather information about your baby's birth, and treatment from your maternity notes and the baby's notes.

Lastly as part of the study we will ask to meet you and your baby again between 18 months and 2 years of age to monitor their growth and development. This will involve short questionnaires and a one hour developmental assessment.

Ospideal Maithreachais na h'Ollscoile Corcaigh
Cork University Maternity Hospital

HRB Health Research Board

UCC

## BiHIVE INFORMED CONSENT FORM

**Principal Investigator**: Dr. Deirdre Murray **Phone Number**: +353 21 4901271

**EEG ID Number**_____ **Patient ID Number for this study**_____

| | Y/N |
|---|---|
| • I have read the information leaflet about this research and have been given a copy to keep. The information has been fully explained to me and I have been able to ask questions. I understand why the research is being done and any risks involved. | ☐ |
| • I agree to donate a sample of my baby's umbilical cord blood for this research project. I understand that giving a sample for this research is voluntary and that I am free to withdraw my approval at any time without my medical treatment being affected. | ☐ |
| • I agree to donate a sample of DNA from my baby's umbilical cord blood for storage and research related to the project. | ☐ |
| • I agree to allow my baby to take part in the follow up visits required at 18-24 months of age | ☐ |
| • I give permission for research personnel to look at my baby's medical records to obtain information about my baby's progress after birth, and the records of my labour and delivery. I have been assured that this information will be kept confidential | ☐ |
| • I agree to donate a sample that can be used now or in the future for commercial collaborative research to develop screening tests for brain injury in children. I understand that I will not benefit financially if this research leads to a development of a new treatment or medical test. | ☐ |
| • I give permission for my baby's sample and information collected about my baby to be stored for possible future research related to this study *without my further consent being required* and subject to approval by a research ethics committee. | ☐ |

| Role | Print Name | Signature | Date |
|---|---|---|---|
| Parent/legal guardian 1 | | | |
| Parent/legal guardian 2 | | | |
| Investigator | | | |
| Witness | | | |

| Insert Hospital Addressogram Label Here | **Local Principal Investigator: Dr Deirdre Murray** |
|---|---|

B.4 Control Patient Information Leaflet


# BiHiVE 2 study

## The Investigation and Validation of Predictive Biomarkers in Hypoxic-ischaemic Encephalopathy

## Information Leaflet for Parents Control Group (well babies)

## What is the BiHiVE study?

The BiHiVE study is a research study currently taking place in your hospital looking at infants who have needed resuscitation after birth. This happens in about 2% of deliveries. Some of these infants may be at risk of long term problems and need intervention in the first few days of life. At the moment we have no good reliable early marker which can give us this information straight after birth. Through this study we hope to find chemical and protein markers in the umbilical cord blood which can tell us very soon after birth which babies have suffered significant injury so that we can intervene and improve their outcome. We hope that in the future a simple blood test at delivery will be able to give doctors and parents more information within hours of a baby's birth.

In order to help babies who have difficulties after birth, it is also important for us to study babies who have had normal, uneventful deliveries. These babies act as **controls (well babies)** so that we compare them to infants who are unwell. We are asking for your baby to be a control infant for the study. If your baby is a control infant, blood is taken from the umbilical cord and stored at birth. We will follow up your baby with an examination the day after birth. This takes about 5 minutes and will not upset your baby in any way. It can be carried out when they are still asleep in many cases. In addition we would like to contact you at 2 years of age to look at the growth and development of your child. The researchers will be very clear with you to reassure you that your baby is a control (well baby).

## What will the study involve?

If you are happy for the research team to follow your baby after they are born, the extra samples of blood taken from your baby's umbilical cord will be frozen, stored and analyzed later when we have collected enough samples. We will look at many different chemicals and proteins and we will compare their levels to those of babies who needed resuscitation. Your baby's progress after birth will be recorded. If your baby needs any extra medical care the details of this care will be collected from your baby's medical notes. This information will be anonymous, and will be identified only using a study number, not your baby's name or address.
We will also ask you to meet us again at 24 months of age so that we can record the progress and development of your baby. You will be contacted and an appointment will be made for a follow up assessment at a time convenient for you and your family. This

will happen in the Cork University Hospital. In these follow up appointments, your baby's development will be assessed and discussed with you.

## What are my options?

Being part of the BiHiVE study is completely voluntary. You or your child can withdraw from this study at any stage. Not being part of this study will not affect any part of the medical care of you or your baby.
The BiHiVE study will be managed by the staff of the Department of Paediatrics and Child Health, University College Cork. We will publish our results in international medical journals. Once a year we will organize a meeting to tell families about how the study is progressing.

## Where can I get more information?

If you have any questions regarding the BiHiVE study, please contact the research coordinator.

Dr Deirdre Murray
Dept of Paediatrics and Child Health
Clinical Investigations Unit,
Cork University Hospital
Tel: +353 21 4901271
Fax: +353 21 434 5217
Email: d.murray@ucc.ie

## C. Patient Neurodevelopment Retention Documents

### C.1 Birthday Card

**HAPPY 1ST BIRTHDAY**

**FROM THE BIHIVE TEAM**

C.2 Newsletter

**HELLO BABY_____**

**(So sorry if your name is missing we didn't catch it on your birthday)**

## We are buzzing to hear how you are doing.

We are the BiHIVE Study Team:





1. Dr Deirdre Murray- Principal Investigator
2. Dr Caroline Ahearne- Research Fellow who will contact you for follow up appointments
3. Jean Conway- Project Manager
4. Ann Marie Looney- Phd in Biomarkers
5. Niamh Denihan- Phd in Biomarkers

We would like to keep you as informed as possible with the progress of the BiHIVE Study you are involved with at Cork University Maternity Hospital. Up to date news on the progress of the BiHIVE study is available on our website. This includes recruitment numbers, contact information, published papers and presentations on breakthroughs being made by our team. None of this would be possible without YOU!

LINK TO THE BiHIVE WEBSITE: http://www.medscinet.net/BIHIVE/

On behalf of the team I want to thank you very much for your continued participation. It is important for us to keep in touch with you; can you please let us know your baby's name (if we have not recorded it above) or if your contact information has changed (address or phone numbers). You can text/phone us at 086-3893430 or send us an e-mail at BiHIVE@ucc.ie

# The BiHIVE Study

## *Information Leaflet for Parents about Developmental Assessment*

Dear parent,

At birth you and your baby were part of an important research study called **the BiHiVE study**. This study is looking closely at babies who required extra help or resuscitation after birth. As part of this study umbilical cord blood was stored and is now being examined to look for early markers of distress and the need for resuscitation and intensive care after birth. In particular we are working hard to find ways of predicting the small number of babies who have significant brain injury after birth. As part of this research it is important that we find out how your child is doing now, several years on. It is just as important for us to see babies who are doing very well as it is to see those who now have difficulties. We have now been funded by the Health Research Board to continue our research and contact all of our parents and children again to arrange follow up appointments. We would be extremely grateful if you would consider continuing in the study and attending for a developmental assessment with your child.

*If you are happy to come in for a detailed developmental assessment you will be contacted by telephone to make an appointment. The appointment will last for approximately an hour and a half. We will contact you by telephone over the next few months to make sure that you are happy to take part and to arrange an appointment.*

***What is a developmental assessment?***

We are interested in how your child's thinking skills, language skills and movement skills are developing. The developmental assessment will be administered by a research psychologist **or BiHiVE research doctor specialising in paediatrics.** They will give your child different tasks to do to see how his/her development is progressing.

One of these tasks will be based on a touchscreen tablet and will involve a brief game which your child will be asked to play with for 5-10 minutes. This is a new test which we hope in the future will be used to test how young children think, learn and remember. They will also ask your permission to video record your child's neurodevelopmental assessment session. This will help with the interpretation of your child's assessment when their scores are being reviewed. The videos will be stored anonymously, and will not have any identifiable details. They will be saved securely according to ethical and data protection guidelines in the neonatal brain research server. We hope that this will be an interesting opportunity for you to find out about your child's development and to see how s/he tackles different tasks and problems.

This type of assessment is helpful in deciding if your child's development is progressing appropriately or if your child needs further help or assessment in certain areas. If your child does need further help, we will discuss this with you after the assessment and arrange follow up assessments if required.
It is important to remember that this assessment is being carried out as part of a research study and is not an alternative to a clinical assessment.

***Further developments in the BiHiVE study:***

The BiHiVE study has identified chemical markers at birth which can help to predict long term outcome. In addition, over the last few years there is new evidence suggesting that some of these chemical markers may be altered for many years following an early brain insult, and may affect their long term development. If your baby did have signs of brain irritation at birth we are now beginning to look at chemical markers at 2 years of age in these children. When you

return for your assessment we would like to take the opportunity to discuss this new research with you and ask whether you would like to take part. If you do wish to take part it would involve your child donating a blood sample of 6 mls, (about a teaspoon) which would be stored and tested at a later date. Some will be stored as serum, and some as DNA.

Both this addition test, and the video-recording is entirely optional and you are not under obligation to take part. We will ask you to sign a new consent form to cover this blood test and the video recording of your child's assessment when you visit us in the Children's Discovery Centre.

***A reminder about your options…***

Being part of **the BiHiVE study** is completely voluntary. You or your child can withdraw from **the BiHiVE study** at any stage. Not being part of **the BiHiVE study** will not affect any part of the medical care of you or your baby.

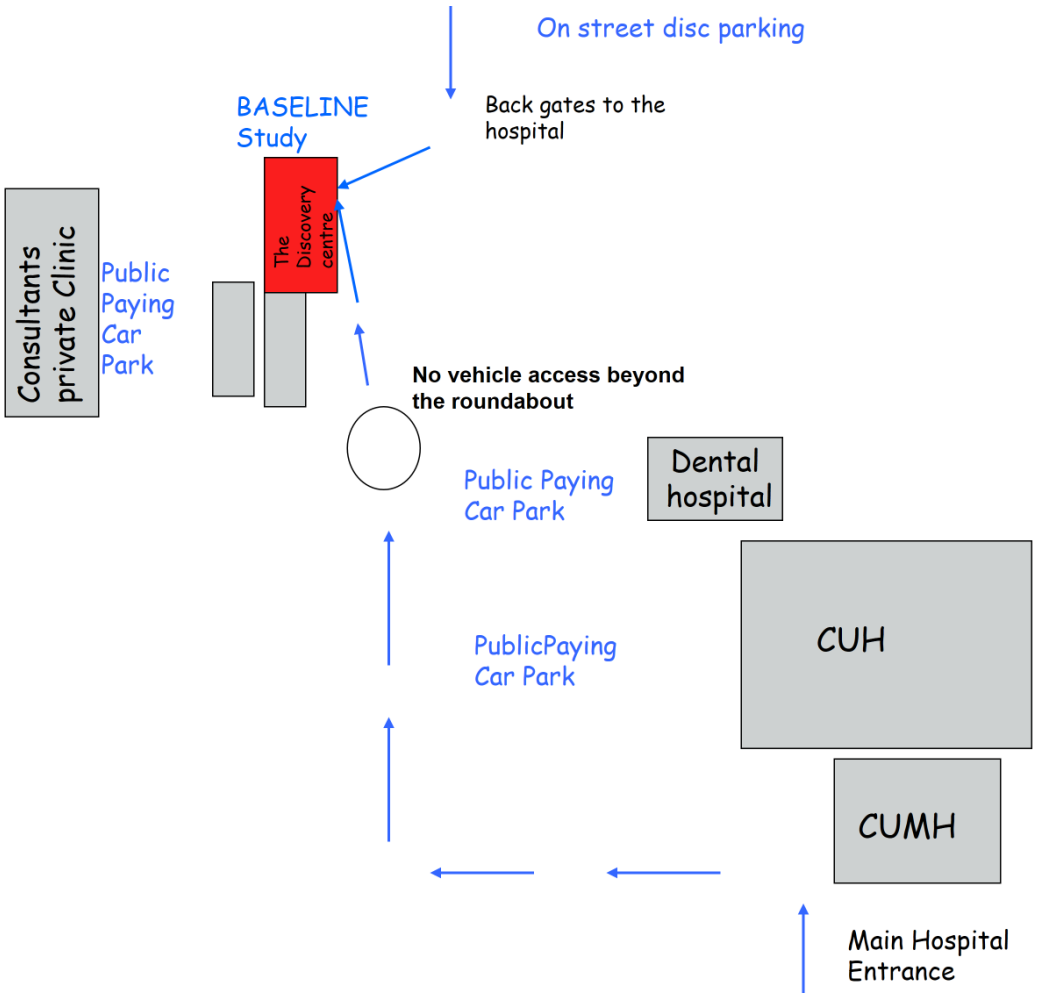
***Contact Details:***


Dr Caroline Ahearne
Research fellow
Neonatal Brain Research Group,
Cork University Maternity Hospital,
Wilton, Co. Cork
Email: cahearne@ucc.ie

Dr Deirdre Murray
Dept of Paediatrics and Child Health,
Clinical Investigations Unit,
Cork University Hospital
Wilton, Co. Cork
Email: d.murray@ucc.ie

BiHiVE telephone: 0863893430
(available 9am – 5pm, Mon to Fri)
BiHIVE email: BiHIVE@ucc.ie

## C.4 Map to Discovery Centre



# The BiHIVE Study


## *Your Appointment For Developmental Assessment*

Dear parent,

At birth you and your baby were part of an important research study called **the BiHiVE study**. This study is looking closely at babies who required extra help or resuscitation after birth. As part of this study umbilical cord blood was stored and is now being examined to look for early markers of distress and the need for resuscitation and intensive care after birth. In particular we are working hard to find ways of predicting the small number of babies who have significant brain injury after birth. As part of this research it is important that we find out how your child is doing now, several years on. It is just as important for us to see babies who are doing very well as it is to see those who now have difficulties. We have now been funded by the Health Research Board to continue our research and contact all of our parents and children again to arrange follow up appointments.

Thank you for agreeing to participate in the follow-up developmental assessment for the BiHiVE study. We are very grateful for your involvement. This assessment is carried out using the Bayley Scales of Infant and Toddler Development (3rd Edition). The appointment will take approximately an hour and a half.

Your appointment will take place at the Discovery Centre (please find a map enclosed) on _____ at _____.

We have also enclosed a developmental screening questionnaire and the Bayley Social-Emotional and Adaptive Behaviour Questionnaire and would be very grateful if you could complete this and bring it with you to your appointment.

***What is a developmental assessment?***

We are interested in how your child's thinking skills, language skills and movement skills are developing. The developmental assessment will be administered by a research psychologist **or BiHiVE research doctor specialising in paediatrics.** They will give your child different tasks to do to see how his/her development is progressing. This is an interesting opportunity for you to find out about your child's development and to see how s/he tackles different tasks and problems.

This type of assessment is helpful in deciding if your child's development is progressing appropriately or if s/he child needs further help or assessment in certain areas. If your child does need further help, we will discuss this with you after the assessment. You will be sent a written report of the results after the assessment.
It is important to remember that this assessment is being carried out as part of a research study and is not an alternative to a clinical assessment.

***A reminder about your options…***

Being part of **the BiHiVE study** is completely voluntary. You or your child can withdraw from **the BiHiVE study** at any stage. Not being part of **the BiHiVE study** will not affect any part of the medical care of you or your child.

***Where can I get more information?***

BiHiVE telephone: 0863893430 (available 9am – 5pm, Mon to Fri)
BiHIVE email: BiHIVE@ucc.ie

If you have any questions regarding the follow-up developmental assessment please contact:
Dr Caroline Ahearne
Research fellow
Neonatal Brain Research Group, Cork University Maternity Hospital.
Email: cahearne@ucc.ie
Tel: 0857107179

If you have any questions regarding **the BiHiVE study** in general please contact the research coordinators
Dr Deirdre Murray
Dept of Paediatrics and Child Health, Clinical Investigations Unit, Cork University Hospital. Email: d.murray@ucc.ie

## D. Babyscreen App Behaviour Observation Record

Demographic Info
Study ID:
Sex:
Age:
Previous Touch Screen Use:
Average Time Per Day (minutes):
Appropriate Development:

| Task ID | Was test quit? (y/n) | Hint Given (y/n) | Behavioural Observations |
|---|---|---|---|
| Screen 1 Single star | | | |
| Screen 2 2 stars | | | |
| Screen 3 Star in 3 grid | | | |
| Screen 4 Star in 9 grid | | | |
| Screen 5 Star in 30 grid | | | |
| Screen 6 Rule reversal 30 grid | | | |
| Screen 7 Multi-coloured 30 grid | | | |
| Screen 8 Rule reversal faces 30 grid | | | |
| Screen 9 Rule reversal faces 30 grid | | | |
| Screen 10 Right cup cover | | | |
| Screen 11 Left cup cover | | | |
| Screen 12 Vertical box slide star reveal | | | |
| Screen 13 Cup Cover- Visible displacement to right | | | |
| Screen 14 Combo star plus vertical box slide | | | |
| Screen 15 Blue button | | | |
| Screen 16 2 blue buttons | | | |
| Screen 17 Combo vertical box slide and blue button | | | |
| Screen 18 Delayed Rule reversal faces 30 grid | | | |

## E. BiHIVE 2 Developmental Questionnaire



### BiHIVE Developmental Screening Questionnaire

*This questionnaire is used as a screening tool as part of our follow-up with the BiHIVE study to highlight any developmental concerns or medical issues that could have an effect on the result of your child's assessment. When you attend for your child's assessment we ask that you bring it with you. Should you have any questions or concerns we can discuss it at your appointment or you can contact us using the contact details below. Thank you for your time and support of the BiHIVE study.*

*Please tick appropriate response:*

| | | Yes | No |
|---|---|---|---|
| **1.** | Have you any concerns about your child's development? | ☐ | ☐ |

If yes, please detail below:

_____

_____

_____

| | | Yes | No |
|---|---|---|---|
| **2.** | Is your child attending/been referred to any of the following services: | | |
| | a. Early Intervention Services | ☐ | ☐ |
| | i. Enable Ireland, ☐ | | |
| | ii. COPE foundation, ☐ | | |
| | iii. Brothers of Charity ☐ | | |
| | iv. Other: _____ | | |
| | ▪ If yes, name of Doctor:_____ | | |
| | b. Speech and Language Therapy | ☐ | ☐ |
| | c. Physiotherapy | ☐ | ☐ |
| | d. Occupational Therapy | ☐ | ☐ |

**3.** Is your child attending/been referred to a Neurologist? ☐ ☐

- If yes, name of Doctor:_____

**4.** Does your child have any problems with their vision? ☐ ☐

**5.** Does your child have any problems with their hearing? ☐ ☐

**6.** Has your child been diagnosed with any of the following medical conditions:

a. Cerebral Palsy ☐ ☐

b. Epilepsy/Seizures (including absences and infantile spasms) ☐ ☐

c. Autism/Autistic Spectrum Disorder ☐ ☐

d. Attention-Deficit Hyperactivity Disorder ☐ ☐

e. Microcephaly/Small Head Size ☐ ☐

f. Developmental Coordination Disorder/Dyspraxia ☐ ☐

g. Sensory Processing Disorder ☐ ☐

**7.** *If* your child has been diagnosed with cerebral palsy:

a. Do they walk independently without mobility assist device?

| All of the time | Most of the time | Some of the time | Rarely | Never |
|---|---|---|---|---|
| | | | | |

b. Do they sit independently and maintain balance?

| All of the time | Most of the time | Some of the time | Rarely | Never |
|---|---|---|---|---|
| | | | | |

c. Do they use a mobility assist device?

| Never | For walking | For standing | For sitting | For all movement |
|---|---|---|---|---|
| | | | | |

**8.** If there any other issues you would like to discuss or bring to our attention please outline below:

_____

_____

_____

_____

_____

Looking forward to seeing you at your assessment.

Kind Regards,

Caroline Ahearne

The BiHIVE team

**Contact details:**

Dr Caroline Ahearne
Research fellow
Neonatal Brain Research Group,
Cork University Maternity Hospital,
Wilton, Co. Cork
Email: cahearne@ucc.ie

Dr Deirdre Murray
Dept of Paediatrics and Child Health,
Clinical Investigations Unit,
Cork University Hospital
Wilton, Co. Cork
Email: d.murray@ucc.ie

BiHiVE telephone: 0863893430
(available 9am – 5pm, Mon to Fri)

BiHIVE email: BiHIVE@ucc.ie