Early postnatal cranial ultrasonography linear measures to predict neurodevelopment at 2 years in infants born at <30 weeks' gestation without major brain injury on sequential imaging

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Abstract

Background: Infants born very preterm – <32 weeks' gestational age (GA) – are at risk of long-term adverse neurodevelopment related to perinatal brain injury and aberrations in brain growth and maturation. Neuroimaging within the newborn period can aid clinicians to identify high-risk infants for developmental surveillance and early intervention therapy. Cranial ultrasonography (cUS) is the most frequently used neuroimaging modality for preterm infants because it is widely available, portable, and repeatable. Whilst early and sequential cUS can reliably detect most major preterm brain injury, it remains less sensitive than brain magnetic resonance imaging (MRI) for the more prevalent, diffuse white matter injury associated with very preterm birth and adverse neurodevelopment. Declining rates of major preterm brain injury further diminishes the sensitivity of cUS to predict neurodevelopmental outcomes. In the absence of major brain injury, and where brain MRI is not readily accessible, there is a need to improve the prognostic utility of cUS, not least to better understand why some very preterm infants without major brain injury seen on cUS later develop motor and cognitive impairments. Sequential cUS, from the first weeks after birth and up to term-equivalent age (TEA), affords an opportunity to explore the prognostic utility of early postnatal brain growth as a potential marker of neurodevelopment in very preterm infants.

Objectives: In my thesis, I aimed to explore the utility of early postnatal cUS linear measures of brain size and brain growth to predict neurodevelopment at 2 years in infants born at <30 weeks' GA and without major brain injury. My first study aimed: (1) to assess the reproducibility of linear measures of brain tissue and fluid spaces made from cUS performed as part of routine clinical care; (2) to evaluate early postnatal brain growth using sequential cUS linear measures made from birth and up to TEA; and (3) to explore perinatal predictors of early postnatal brain growth. My second and third studies aimed to examine the associations of early postnatal cUS linear measures with (4) neurobehaviour at TEA and (5) neurodevelopment at 2 years, respectively.

Methods: Participants comprised 144 infants born at <30 weeks' GA, and 201 term-born controls, at a single centre between January 2011 and December 2013. Infants with major brain injury seen on cUS, or congenital or chromosomal abnormalities known to affect neurodevelopment, were excluded. Linear measures of brain tissue and fluid spaces were made from cUS performed as part of routine clinical care from the first weeks after birth and up to TEA. Perinatal data were collected prospectively. Neurobehaviour was assessed at TEA using Prechtl's Qualitative Assessment of General Movements (GMs) and the Hammersmith Neonatal Neurological Examination (HNNE). Neurodevelopment was assessed at 2 years using the cognitive, language, and motor scales on the Bayley Scales of Infant and Toddler Development, 3rd Edition, and risk of adverse neurodevelopment at 2 years was defined by a composite of either: cerebral palsy, any developmental delay, deafness, or blindness. Assessors were blinded to the clinical course of participants.

Results: 429 scans were assessed for 144 infants. Several linear measures showed excellent reproducibility. All measures of brain tissue increased with postnatal age, except for the biparietal diameter (BPD) which decreased within the first postnatal week and increased thereafter. GA ≥28 weeks at birth was associated with slower growth of the BPD and ventricular width compared with GA <28 weeks. Postnatal corticosteroid administration was associated with slower growth of the corpus callosum length (CCL), transcerebellar diameter (TCD) and vermis height. Sepsis and necrotising enterocolitis were associated with slower growth of the TCD. Larger brain sizes, assessed by linear measures of the BPD at 1-week and 2-months, and CCL at 1-month, were associated with lower risk of a suboptimal HNNE and abnormal GMs at TEA, respectively. Faster positive growth rates of the CCL and TCD between birth and 1-month were related to lower risk of abnormal GMs at TEA. A larger measure of the right anterior horn width (AHW) at 1-month, and faster positive rates of increase of the left and right AHW between 1- and 2-months, were related to lower risk of abnormal GMs and a suboptimal HNNE at TEA, respectively. Larger measures of brain tissue at 1-week, 1-month, and 2-months were related to higher cognitive and language scores, and larger measures of brain tissue at 2-months were related to lower risk of adverse neurodevelopment. Faster growth of the TCD between 1-month and 2-months was related to a higher cognitive score and lower risk of adverse neurodevelopment. Unexpectedly, larger measures of fluid spaces at 1-

month and 2-months were related to higher language and motor scores, larger measures of fluid spaces at 1-week and 1-month were related to lower risk of adverse neurodevelopment, and a faster increase of the interhemispheric distance between 1-week and 1-month was related to a higher language score and lower risk of adverse neurodevelopment.

Conclusions: Early postnatal brain growth in infants born at <30 weeks' GA can be evaluated using sequential linear measures made from routine cUS, and is associated with perinatal predictors of long-term development. Linear measures of larger brain tissue from the first weeks after birth are related to lower risk of atypical neurobehaviour at TEA and better cognitive and language development at 2 years. My thesis provides evidence to support associations of early postnatal cUS linear measures of brain size and brain growth with neurodevelopment at 2 years in infants born <30 weeks' GA and free of major brain injury. The relationships between larger fluid spaces and better neurodevelopment warrant further exploration.

Presentations

Perinatal Society of Australia and New Zealand Annual Meeting. *Associations of Early Postnatal Brain Growth with Neurodevelopment at 2 Years in Infants Born <30 Weeks' Gestational Age.* Sydney, Australia, 2020.

Joint European Neonatal Societies, 2nd Congress. *Perinatal Associations of Early Postnatal Brain Growth in Infants Born <30 Weeks' Gestational Age.* Venice, Italy 2017.

Perinatal Society of Australia and New Zealand Annual Meeting. *Associations of Early Postnatal Brain Growth with Neurobehaviour at Term-Equivalent Age in Infants Born <30 Weeks' Gestational Age.* Townsville, Australia, 2016.

Pediatric Academic Societies Annual Meeting. *Associations of Early Postnatal Brain Growth with Neurobehaviour at Term-Equivalent Age in Infants Born <30 Weeks' Gestational Age.* Baltimore, United States of America, 2016.

Research Week, The Royal Women's Hospital, Melbourne. *Associations of Early Postnatal Brain Growth with Neurobehaviour at Term-Equivalent Age in Infants Born <30 Weeks' Gestational Age.* Melbourne, Australia, 2016.

Declaration

This is to certify that:

i. the thesis comprises only my original work towards the PhD,
ii. due acknowledgements have been made in the text to all other material used, and
iii. the thesis is fewer than 100,000 words in length, exclusive of tables, references, and appendices.

My thesis is part of a larger prospective cohort study named VIBeS-2, conceptualised by a team of investigators led by Professor Alicia Spittle. I acknowledge the combined efforts of VIBeS-2 study investigators, collaborators, and coordinators in the recruitment of participants, data collection, and assessments. Cranial ultrasonography was not included as part of a research protocol in VIBeS-2, apart from clinical reporting of major brain injury that was collated in the dataset. I contributed to greater than 75% to the published work (Chapter 5) and manuscripts prepared for publication (Chapters 6 and 7) included in my thesis, including conceptualisation, data collection, analysis, and writing and editing of the manuscripts.

Rocco Cuzzilla

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To my partner, David Mackin, thank you for prioritising our relationship. Your unconditional love and understanding have provided me with relative perspective on all matter of things, not least our sense of self and purpose.

I set out on this path of study to learn from the newborns we provide care for, and to probe deeply the questions their parents ask by their cot. This thesis, and my day-to-day work, are because of each and every one of you, past, present, and future.

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Abbreviations

AHW	Anterior Horn Width
Bayley-III	Bayley Scales of Infant and Toddler Development, 3rd Edition
BPD	Biparietal Diameter
BW	Birth Weight
CCL	Corpus Callosum Length
cPVL	Cystic Periventricular Leukomalacia
cUS	Cranial Ultrasonography
DCD	Developmental Coordination Disorder
GA	Gestational Age
GMFCS	Gross Motor Functional Classification System
GMH	Germinal Matrix Haemorrhage
GMH-IVH	Germinal Matrix Haemorrhage-Intraventricular Haemorrhage
GMs	General Movements
HNNE	Hammersmith Neonatal Neurological Examination
ICC	Intraclass Correlation Coefficient
IHD	Interhemispheric Distance
MRI	Magnetic Resonance Imaging
NEC	Necrotising Enterocolitis
NICU	Neonatal Intensive Care Unit
PNA	Postnatal Age
PHVD	Post-haemorrhagic Ventricular Dilatation
PVHI	Periventricular Haemorrhagic Infarction
PVL	Periventricular Leukomalacia
TCD	Transcerebellar Diameter
TEA	Term-Equivalent Age
VH	Vermis Height
VI	Ventricular Index
VIBeS-2	Victorian Infant Brain Study-2
VW	Ventricular Width

Problem Statement

Infants who are born very preterm, that is, less than 32 weeks' gestational age (GA), are at considerably higher risk of long-term adverse neurodevelopmental outcomes compared with infants born at term. Approximately 50% of children born very preterm demonstrate cognitive and behavioural deficits at school age, and up to 40% have motor impairments, including cerebral palsy.[1-3] Consequently, there is a clinical imperative to identify high-risk infants within the neonatal period to ensure they receive follow-up for developmental surveillance beyond the neonatal intensive care unit (NICU) and, where necessary, are referred to early intervention programs which have the potential to optimise their functional outcomes. Moreover, focusing on high-risk infants within the early postnatal period provides the earliest opportunity for therapeutic intervention, coinciding with critical processes in their brain development. Neonatal neuroimaging is one of several clinical applications that can be used by clinicians to identify high-risk infants. Typical patterns of brain injury, and alterations in brain growth and maturation, seen on neonatal neuroimaging are related to many of the neurodevelopmental sequelae of very preterm birth.[4] Cranial ultrasonography (cUS), the most frequently used neuroimaging modality for preterm infants, can reliably detect most major brain injuries associated with preterm birth and long-term motor and cognitive impairments.[5] However, the majority of very preterm children with neurosensory deficits do not have findings of overt focal pathology on neonatal cUS, diminishing the sensitivity of cUS for the prediction of neurodevelopmental outcomes following very preterm birth. In the absence of major brain injury, the prognostic utility of early postnatal brain growth following very preterm birth has not been fully explored. My thesis aimed to explore the utility of early postnatal brain growth, assessed by linear measures made from sequential cUS, to predict neurodevelopmental outcomes at 2 years' corrected age for children born very preterm.

Chapter 1

Introduction

Chapter Overview

In this introductory chapter, I present what is currently known on the public health issue of very preterm birth, and the burden of neurodevelopmental sequelae observed in surviving children who were born very preterm. Then, I discuss the clinical relevance of early identification of very preterm infants at risk of adverse neurodevelopment, with regard to focusing on high-risk infants for ongoing developmental surveillance and timely referral to early intervention programs. Following this, I describe the most commonly used clinical tools that help clinicians to identify high-risk infants within the neonatal period, with particular focus on perinatal risk factors, neurobehaviour, and neuroimaging.

1.61 Very Preterm Birth

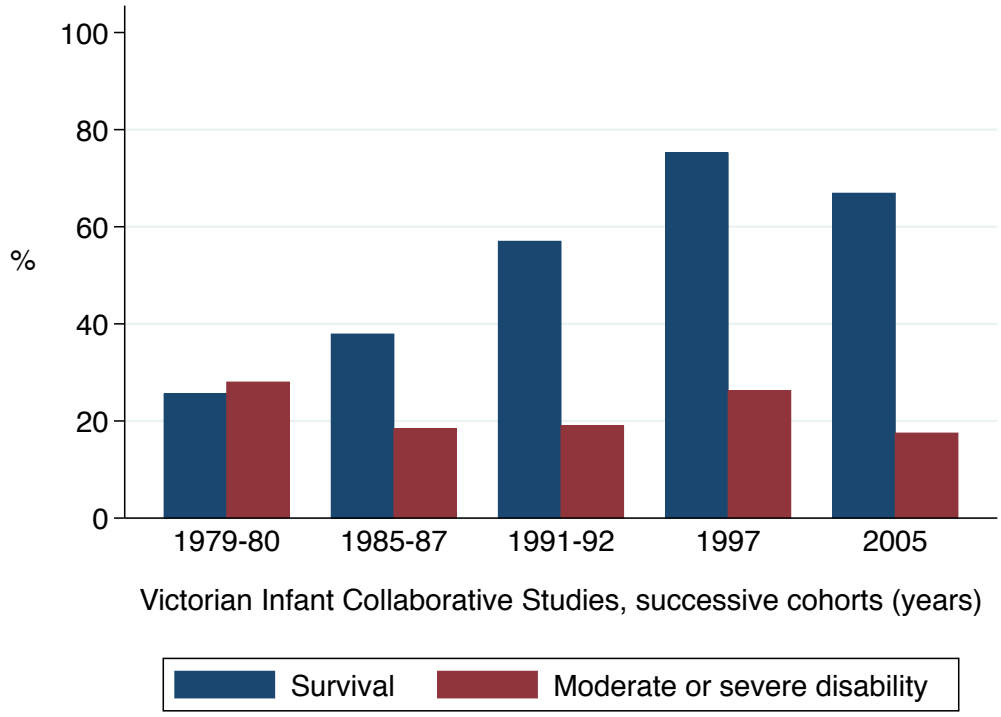
Preterm birth, defined by delivery at less than 37 weeks' GA, accounts for approximately 9% of all live births in Australia, and in comparable healthcare settings, and is the most common cause of neonatal mortality and morbidity throughout the developed world.[6][7][8][9] The causes and indications for preterm birth are many and varied, frequently occurring in the setting of spontaneous onset of labour, and include maternal or fetal complications, such as chorioamnionitis, preeclampsia, fetal growth restriction, cervical insufficiency, and multiple pregnancy.[10] Advanced maternal age and socio-economic deprivation contribute to higher rates of preterm birth.[11-13] Despite evidence-based interventions to prevent preterm birth, including the administration of progesterone and tocolytics, and increased surveillance of high-risk pregnant women, the incidence of preterm birth has increased in Australia over recent decades, from 6.8% in 1991 to 8.8% in 2017.[14][15][16][17]

Infants who are born very preterm, that is, less than 32 weeks' GA, account for approximately 1% of all live births in Australia, and up to one-third of all admissions to the NICU.[18] Although a relatively small proportion of all births, very preterm birth is associated with a disproportionately higher risk of neonatal mortality and morbidity, and a substantial burden on healthcare resources.[19, 20] Advances in perinatal care in recent decades have led to improved rates of survival of very preterm infants, most notably associated with the introduction of antenatal corticosteroids (1980s) and exogenous surfactant therapy (1990s), and the willingness to provide intensive care, especially for infants born at the threshold of viability.[19] Infants who are born very preterm now have survival rates in excess of 90% in high income countries, with their risk of neonatal mortality significantly diminishing with increasing GA and weight at birth.[21-24]

1.2 Neurodevelopmental Sequelae of Very Preterm Birth

Although their survival has improved considerably over recent decades, infants who are born very preterm remain at significant risk of adverse neurodevelopmental outcomes. Figure 1.1 describes the rates of survival and of moderate-to-severe neurodevelopmental disability in survivors at 2 years within geographic cohorts of liveborn infants who were extremely preterm, that is, less than 28 weeks' GA, and/or extremely low birth weight, that is, less than 1000 g, in Victoria, Australia, across the last four decades. Moderate-to-severe neurodevelopmental disability was defined by the presence of one or more of: moderate or severe cerebral palsy, moderate or severe cognitive or language delay, deafness, or blindness.[25] The prevalence of moderate-to-severe neurodevelopmental disability at 2 years' corrected age has remained relatively unchanged across this period, affecting approximately 1 in 5 survivors of very preterm birth, compared with 1 in 50 normal birth weight term controls.[25] Consequently, the absolute numbers of surviving preterm infants with adverse neurodevelopmental outcomes has increased over time, presenting an important public health issue for clinicians and healthcare policy makers alike that extends beyond the NICU.[25]

Figure 1.1 Survival and moderate-to-severe neurodevelopmental disability to 2 years in infants born extremely low birth weight[26]



1.2.1 Cognitive Impairment and Behavioural Problems

The predominant neurosensory impairments associated with very preterm birth are cognitive and behavioural in nature, with up to 50% of children born very preterm demonstrating cognitive deficits and behavioural difficulties at school age, compared with approximately 10-20% of children born at term.[1, 27] Specifically, children born very preterm are at greater risk of cognitive and language delays, lower performance on a range of higher-order executive functions, including working memory, cognitive flexibility, and inhibition, poorer academic performance, and neurobehavioural impairments compared with term-born controls.[1, 25, 28] In a cohort of extremely preterm (<28 weeks' GA) and/or extremely low birth weight (<1000 g) infants assessed at 2 years, cognitive and language composite test scores on a standardised assessment tool were found to be approximately two-thirds and one standard deviation, respectively, below the mean for normal birth weight term

controls, conferring a clinically important difference in cognitive functioning between these groups.[25]

1.2.2 Cerebral Palsy and Other Motor Deficits

Up to 40% of children born very preterm demonstrate motor deficits at school age, and approximately 10% have a diagnosis of cerebral palsy.[2, 3] Cerebral palsy, a non-progressive neurologic condition defined by abnormal tone, posture, and movement, and often limiting activity, occurs as a result of a permanent injury to the motor pathway of the developing brain.[29] An accurate diagnosis of cerebral palsy can be made as early as 6 months' corrected age for prematurity, mostly in moderate and severe cases, optimising opportunities for early intervention at a critical time in brain development.[30] Preterm birth is an independent risk factor for cerebral palsy; in Australia, 43% of all children diagnosed with cerebral palsy are born preterm.[31] The risk of cerebral palsy is inversely proportional to GA at birth, with a recent meta-analysis reporting a prevalence of 14.6% in children who were born between 22 and 27 weeks' GA, 6.2% for those born between 28 and 31 weeks' GA, 0.7% between 32 and 36 weeks' GA, and 0.11% at term.[32]

Severity of cerebral palsy may be classified with reference to the Gross Motor Function Classification System (GMFCS), a widely used functional classification system comprising 5 levels of activity limitation for persons with cerebral palsy.[33] Mild cerebral palsy relates to independent ambulation (GMFCS Level I), moderate cerebral palsy describes ambulation with assistance (GMFCS Levels II and III), and severe cerebral palsy refers to non-ambulation (GMFCS Levels IV and V).[34] Most preterm children with cerebral palsy are ultimately able to ambulate, with or without assistance (GMFCS Levels I-III). A recent systematic review of eight studies reporting the severity of cerebral palsy in preterm children found 31.4% with mild, 23.3% moderate, 28.4% severe, and a further 17% with an unclassified severity of cerebral palsy.[3] Moreover, in a pooled analysis of nine studies including preterm children, the predominant pattern of cerebral palsy was diplegia (41.5%), followed by hemiplegia (28%), quadriplegia (10.4%), monoplegia (0.2%), and a further 20% unspecified.[35]

Developmental coordination disorder (DCD) is a more common cause of motor impairment than cerebral palsy, affecting up to 40% of children born preterm.[2, 36] DCD is a neurodevelopmental disorder defined by a marked impairment of motor coordination and learning of motor skills, not otherwise attributable to concomitant neurosensory deficits such as cerebral palsy, intellectual impairment, or vision impairment.[37] Children with DCD often have difficulties performing tasks, such as handwriting or throwing a ball, and subsequently have lower rates of participation in physical activity and higher risk of social isolation and behavioural problems.[38]

1.2.3 Hearing and Vision Impairments

Infants born very preterm are more likely to develop hearing and vision impairments compared with term-born infants. Hearing impairment, necessitating the use of a hearing amplification device, is observed in approximately 2-3% of children born very preterm, and 0.5% of children born at term.[25, 39] Vision impairment, defined by visual acuity <6/60 in the better eye, and mostly attributed to retinopathy of prematurity, occurs in 1-2% of children born very preterm.[39] Although neurosensory impairments are clinically-important outcomes, the absolute numbers of children born preterm who develop deafness or blindness are relatively small compared with the preponderance of those with cognitive and/or motor deficits.

1.3 Predicting Neurodevelopmental Outcomes of Very Preterm Infants within the Neonatal Period

The ability to identify very preterm infants within the newborn period who are at high risk of long-term adverse neurodevelopment is of vital importance to these infants, their families, and their treating clinicians. This is especially pertinent to the follow-up of high-risk infants beyond the neonatal nursery in healthcare systems with limited resources. During the course of their developmental surveillance, the detection of neurosensory deficits in children born very preterm allows for timely referral to early intervention services with the potential to optimise functional

outcomes.[40] The early postnatal period also provides an opportunity for therapeutic intervention during a critical time in brain maturation and development. To this end, several pharmacological agents, including melatonin and erythropoietin, are currently being investigated as neonatal therapies with the aim to improve long-term neurodevelopmental outcomes.[41-43]

Improving the accuracy of prognostication of neurodevelopmental outcomes for the individual infant also affords the clinician with an opportunity to adapt their counselling of families. Antenatal counselling of expectant parents of very preterm infants necessarily relies on population-level data derived from longitudinal cohort studies, and is mostly based on GA at birth.[25, 27, 44] Whilst no single clinical assessment of a very preterm infant within the neonatal period provides absolute certainty of a future neurosensory impairment, clinicians may use information learned from the infant's perinatal course, including history, examination, and investigations, to hone their assessment of the infant's likelihood of later developmental impairment. In a recent study, Cheong et al demonstrated an association between cumulative risk of long-term adverse neurodevelopment in very preterm children at 8 years, and four perinatal variables, namely intraventricular haemorrhage (IVH), cystic periventricular leukomalacia (cPVL), postnatal corticosteroids, and surgery within the neonatal period.[45] The absence of these risk factors confers a more favourable outcome for the infant, with a significantly lower risk of long-term moderate to severe motor or cognitive deficits into childhood than may have been otherwise conveyed by clinicians during their antenatal and early postnatal counselling of families of very preterm infants.[45]

Underscoring the importance of prognostication is knowledge that the uncertainty of outcomes inherent with very preterm birth is a source of considerable stress for families. Parents of very preterm children are more likely to report symptoms of depression and anxiety following the birth of their child than parents of term-born children.[46] Providing families with greater certainty of neurodevelopmental outcomes for their infant, whether that be a higher or lower likelihood of motor or cognitive impairment, may alleviate some of their anxiety.[47]

Several clinical tools, currently accessible to clinicians in the course of their routine care of the very preterm infant, may be employed by clinicians within the newborn period to help them identify infants at risk of adverse neurodevelopment. In this section, I briefly outline the more common of these clinical assessments, including perinatal risk factors, neonatal neurobehaviour, and neonatal neuroimaging.

1.3.1 Perinatal Risk Factors

Several variables, related to demographics and the perinatal clinical course of the very preterm infant, are associated with the risk of adverse neurodevelopment following very preterm birth. A multitude of studies reporting long-term neurologic and developmental outcomes of very preterm infants relate higher risk of cerebral palsy and other motor impairments, and neurobehavioural and cognitive deficits, with decreasing GA at birth, lower birth weight, and male sex.[48-50] Intra-uterine growth restriction in very preterm infants is also associated with higher risk of adverse neurodevelopment, notwithstanding the confounding problem with how growth restriction is defined in many of these studies; intra-uterine growth restriction and small for gestational age are often used interchangeably, despite potential differences in their underlying causes.[51] Intra-uterine growth restriction is presumed to have an underlying pathological cause, although not always determined, whereas small for gestational age may be constitutional or pathological.

Antenatal administration of corticosteroids to pregnant women at imminent risk of preterm birth is associated with a reduction in risk of respiratory distress syndrome, IVH, and necrotising enterocolitis (NEC), and improved survival rates of infants born very preterm.[52] There is less evidence for the association of antenatal corticosteroids with any difference in neurodevelopmental outcomes following very preterm birth, although in a systematic review and meta-analysis that specifically evaluated neurodevelopmental outcomes following a single course of antenatal corticosteroids administered to women in threatened preterm labour, Sotiriadis et al report an association between steroid exposure and lower risk of cerebral palsy, motor delay, and severe disability.[52-54] There remains some contention about the relationships between repeat courses of antenatal corticosteroids and long-term neurodevelopment.[55, 56] Magnesium sulphate administered to pregnant women at

risk of imminent preterm birth less than 30 weeks' GA is associated with a reduction in risk of cerebral palsy.[57]

Complications of preterm birth may confer a higher risk of adverse neurodevelopment for the very preterm infant, particularly pro-inflammatory states that are injurious to progenitor cells, such as pre-oligodendrocytes and immature neurons of the developing brain. Bronchopulmonary dysplasia, defined by the need for supplemental oxygen at 36 weeks' post-menstrual age, is a common sequela of very preterm birth, affecting up to 25% of infants born very preterm, with its incidence increasing with decreasing GA and weight at birth.[58] There is equivocal evidence for the role of bronchopulmonary dysplasia in the development of later motor and cognitive impairments in children born very preterm, as many of the risk factors associated with chronic lung disease in the post-surfactant era, such as prolonged mechanical ventilation and late-onset sepsis, are independently associated with higher risk of adverse neurodevelopment.[59-61] However, there is evidence to support the independent association between severe chronic lung disease and later cognitive impairment in extremely preterm infants.[60] Postnatal corticosteroids, a surrogate marker of severe bronchopulmonary dysplasia, are sometimes administered to mechanically ventilated infants to facilitate extubation, and to prevent or mitigate the severity of bronchopulmonary dysplasia, and are associated with an increased risk of cerebral palsy, motor impairment, and cognitive deficits.[62, 63] A recent meta-analysis evaluating the evidence for the role of chorioamnionitis in the development of cerebral palsy concluded that histologic chorioamnionitis, but not clinical chorioamnionitis, is a risk factor for cerebral palsy in preterm children.[64] Postnatal sepsis and NEC are associated with increased risk of motor and cognitive impairments in very preterm infants.[65-67]

Major brain injury, related to very preterm birth and its associated morbidities, is related to adverse neurodevelopmental outcomes in very preterm infants.[5, 68] The findings of overt, focal pathology on neonatal neuroimaging, such as IVH, cerebellar haemorrhage, and cPVL, and its correlation with later neurodevelopmental outcomes in children born very preterm, is discussed in my literature review presented in Chapter 2.

Recent evidence has shown that one or more of the following perinatal morbidities – major brain injury, including high-grade IVH and cPVL, postnatal corticosteroids, and surgery in the newborn period – confer the greatest risk of neurodevelopmental impairment in very preterm children, independently of many of the other perinatal risk factors, including, but not limited to, GA at birth, birth weight, and sex.[45] However, it remains that perinatal variables, including GA at birth, birth weight, and intra-uterine growth restriction, increase the likelihood of subsequent morbidities and interventions associated with risk of neurodevelopmental impairment, including bronchopulmonary dysplasia and postnatal corticosteroids, and NEC and surgery.

1.3.2 Neonatal Neurobehaviour

Conceptualised by Brazelton in the 1970s, neonatal neurobehaviour is a function of complex interactions between an infant's biology, behaviour, and their environment, and is a reflection of the infant's neurological integrity.[69] Brain development following very preterm birth is shaped by the infant's experience in the NICU, including varying exposure to perinatal morbidities and the quality of interactions with caregivers and the environment. Consequently, atypical patterns of neurobehaviour and motor functioning observed in some very preterm infants may be a manifestation of aberrant brain growth and maturation.

Neurobehavioural assessments of infants in the early postnatal period generally include an evaluation of neuromuscular and movement-related functions (tone, antigravity posture, reflexes, and spontaneous and elicited movement patterns), attention and orientation to stimuli (vision and hearing), and mental state (irritability and consolability).[70] The infant's behavioural state may influence the quality of neurobehaviour and motor functioning in other domains, including muscle tone and movement, and items drawing on the infant's attention. It is therefore important that neurobehavioural assessments are performed when the infant is in an appropriate behavioural state. Moreover, longitudinal assessments of very preterm infants within the early postnatal period provide a developmental trajectory, and therefore may afford clinicians and researchers alike insight into an individual infant's underlying

brain development, which may reflect reorganisation after brain injury or maturation following interventional therapy.

Prechtl's Qualitative Assessment of General Movements (GMs) and the Hammersmith Neonatal Neurological Examination (HNNE) are two of the more commonly used neurobehavioural assessment tools in the clinical care of very preterm infants. GMs and the HNNE have high discriminative and predictive validity when used to assess very preterm infants in the early postnatal period, helping clinicians to identify infants at risk of adverse neurodevelopment.[70]

1.3.2.1 Prechtl's Qualitative Assessment of General Movements

GMs assessment involves a structured observation of an infant's spontaneous movements with respect to their complexity, variability, and fluency.[71] In the early postnatal period following very preterm birth and up to 8 weeks' post-term, GMs are within the "writhing" period, and may be categorised as either normal or abnormal. Normal GMs within the writhing period are characterised by small to moderate amplitude and speed, and demonstrate an ellipsoid pattern giving them their writhing quality, whereas abnormal GMs are further qualified as either poor repertoire, cramped synchronised, or chaotic, depending on the degree to which the complexity, variability, and fluency of movements differ from normal. Poor repertoire movements are noted for their simplicity, lacking both complexity and variability, and appearing monotonous. Cramped synchronised movements appear rigid and are characterised by simultaneous contraction of all limb and trunk muscles. Chaotic movements are characterised by large amplitude movements of sudden onset, with marked lack of fluency. Between 6 and 20 weeks' post-term, GMs are within the "fidgety" period, and may be categorised as normal, abnormal, or absent. Normal fidgety movements are of small amplitude, moderate speed, and variable acceleration, and are continual in the awake infant, except when fussing or crying. Abnormal fidgety movements are exaggerated in amplitude, speed, and jerkiness, whereas absence of fidgety movements characterises infants who never demonstrate fidgety movements at any time during the fidgety period. GMs are assessed from video recordings of the infant of at least 3 minutes in duration, and in the appropriate behavioural state to assess their spontaneous movements; infants must be alert, but

not fussing, crying, or sucking on a pacifier, and their attention must not be distracted by any environmental stimuli. Assessors undergo training and pass examinations in basic and advanced courses administered by the General Movements Trust.

The nature and trajectory of GMs in the preterm period are related to long-term outcomes.[72, 73] Abnormal GMs are commonly observed in very preterm infants at term-equivalent age, with approximately 70% categorised as poor repertoire.[74] Abnormal GMs in the early postnatal period up to term-equivalent age correlate with worse neurodevelopmental outcomes at 1 year of age in infants born very preterm.[75] In general, normal GMs in the writhing period can provide reassurance of normal cognitive outcomes, but abnormal GMs are less specific for later cognitive deficits.[76, 77] Moreover, poor repertoire GMs that do not normalise by 8 weeks' post-term are related to a higher risk of an adverse cognitive outcome in very preterm children at school age.[78, 79] A recent systematic review evaluating the prognostic utility of GMs for cerebral palsy reported a sensitivity of 98% and specificity of 91%.[3] Specifically, the absence of fidgety movements within the fidgety period is highly predictive of cerebral palsy, with a sensitivity of up to 100% and specificity of up to 95%.

1.3.2.2 Hammersmith Neonatal Neurological Examination

The HNNE is a structured observational and physical assessment of an infant's neurological functioning across 6 subscales, including: tone and posture, tone patterns, reflexes, spontaneous movements, behaviour, and abnormal signs. There are 34 items across the 6 subscales, each of which is scored 0 or 1 referenced to criterion, with normative data for 224 low-risk term-born infants; the combination of scores across these items provides for a total score out of 34.[80-82] The concept of an optimal score is arbitrarily defined by a score greater than the 10th centile in term-born controls, such that a suboptimal score may reflect poorer neurological functioning, and, by speculation, less optimal brain development. Reference norms for the HNNE have been published for both preterm and term-born infants, with preterm infants demonstrating not only lower scores, but also a wider range of scores, at term-equivalent age compared with term-born infants.[80-84] The HNNE has good predictive utility for the development of adverse neurodevelopment in very preterm

infants.[85, 86] Moreover, the HNNE correlates well with abnormalities on conventional magnetic resonance imaging (MRI) of very preterm infants at 4 months' post-term.[87]

1.3.3 Neonatal Neuroimaging

Neuroimaging of the very preterm infant is routinely used in the early postnatal period to identify infants with perinatal brain injury and alterations in brain development that may discriminate infants who are at greater risk of long-term adverse neurodevelopmental outcomes. cUS and MRI are the most commonly used neuroimaging modalities for very preterm infants, providing clinicians with the ability to document the presence, timing, and evolution of brain injury and aberrant brain growth and maturation. The clinical utility of cUS and MRI for the prediction of long-term outcomes following very preterm birth is the subject of my literature review in Chapter 2.

1.4 Outline of Thesis

My thesis continues with a literature review of the current evidence for the clinical utility of neuroimaging in the neonatal period to predict neurodevelopment in very preterm infants. Following this, I present 3 studies (published, or submitted for publication) relating to the utility of early postnatal brain growth, assessed by linear measures made from sequential cUS, to predict neurodevelopmental outcomes in very preterm infants. I then discuss the findings of my studies in the context of what is already known in this field of research, highlight my contributions to this field of neonatal neurology, and draw conclusions that could inform clinical practice and future research.

Chapter 2:	*Literature Review: The Utility of Neonatal Neuroimaging to Predict Neurodevelopmental Outcomes in Very Preterm Infants*
Chapter 3:	*Aims and Hypotheses*
Chapter 4:	*General Methodology*
Chapter 5:	*Study 1: Reproducibility of Cranial Ultrasonography Linear Measures, Early Postnatal Brain Growth, and Associations with Perinatal Variables*
Chapter 6:	*Study 2: Associations of Neurobehaviour at Term-Equivalent Age with Early Postnatal Cranial Ultrasonography Linear Measures*
Chapter 7:	*Study 3: Associations of Neurodevelopment at 2 Years with Early Postnatal Cranial Ultrasonography Linear Measures*
Chapter 8:	*Discussion, Clinical Implications, Future Directions, and Conclusions*

Chapter 2

Literature Review: The Utility of Neonatal Neuroimaging to Predict Neurodevelopmental Outcomes in Very Preterm Infants

Chapter Overview

In this chapter, I evaluate the evidence for the clinical utility of neonatal neuroimaging, namely cUS and conventional brain MRI, to predict neurodevelopmental outcomes in infants born very preterm. I begin by describing the role of cUS, the most commonly used neuroimaging modality for preterm infants, in reliably detecting most major brain injury associated with very preterm birth and later adverse neurodevelopment. Following this, I discuss the limitations of cUS to predict neurodevelopmental outcomes in very preterm infants, particularly with regard to the "apparently normal" cUS free of major brain injury. I then compare the prognostic utility of cUS with brain MRI of the brain, with regard to the clinical application of conventional brain MRI in the NICU. Then, I identify a research gap, and formulate the overarching question guiding my thesis work, that is: *what is the utility of early postnatal brain size and growth, assessed using linear measures made from sequential cUS, to predict neurodevelopmental outcomes at 2 years of age in very preterm infants free of major brain injury seen on neonatal cUS?* Following this, I describe linear measures of brain tissue and fluid spaces made from fetal and neonatal sonography, and, where available, their relationships with neurodevelopmental outcomes in very preterm infants.

2.1 Cranial Ultrasonography

Since the introduction of cUS to neonatal intensive care in the late 1970s and throughout the 1980s, the application of cUS in research and clinical practice has contributed in great measure to the current understanding of the nature, timing, and evolution, as well as perinatal associations, of major brain lesions related to very preterm birth and long-term neurodevelopmental sequelae of surviving infants.[5, 88-93] Consequently, neonatal cUS has an important role in the care of very preterm infants, used by clinicians to identify infants at risk of adverse neurodevelopment, to provide more accurate prognostication about the outcomes of individual infants when counselling families, and, at a population level, to evaluate therapies and clinical practice in neonatal intensive care.

cUS is the most frequently used neuroimaging modality for preterm infants.[94] It is widely available, safe, inexpensive, portable, and easily repeatable. As a bedside investigation, cUS can be applied to even the smallest and sickest preterm infants from as early as their day of birth. Moreover, cUS is highly sensitive for the detection of major brain injury associated with preterm birth, including germinal matrix haemorrhage-intraventricular haemorrhage (GMH-IVH), periventricular haemorrhagic infarction (PVHI), post-haemorrhagic ventricular dilatation (PHVD), large cerebellar haemorrhage, and cPVL, but is less sensitive for perinatal arterial ischaemic stroke.[95-97]

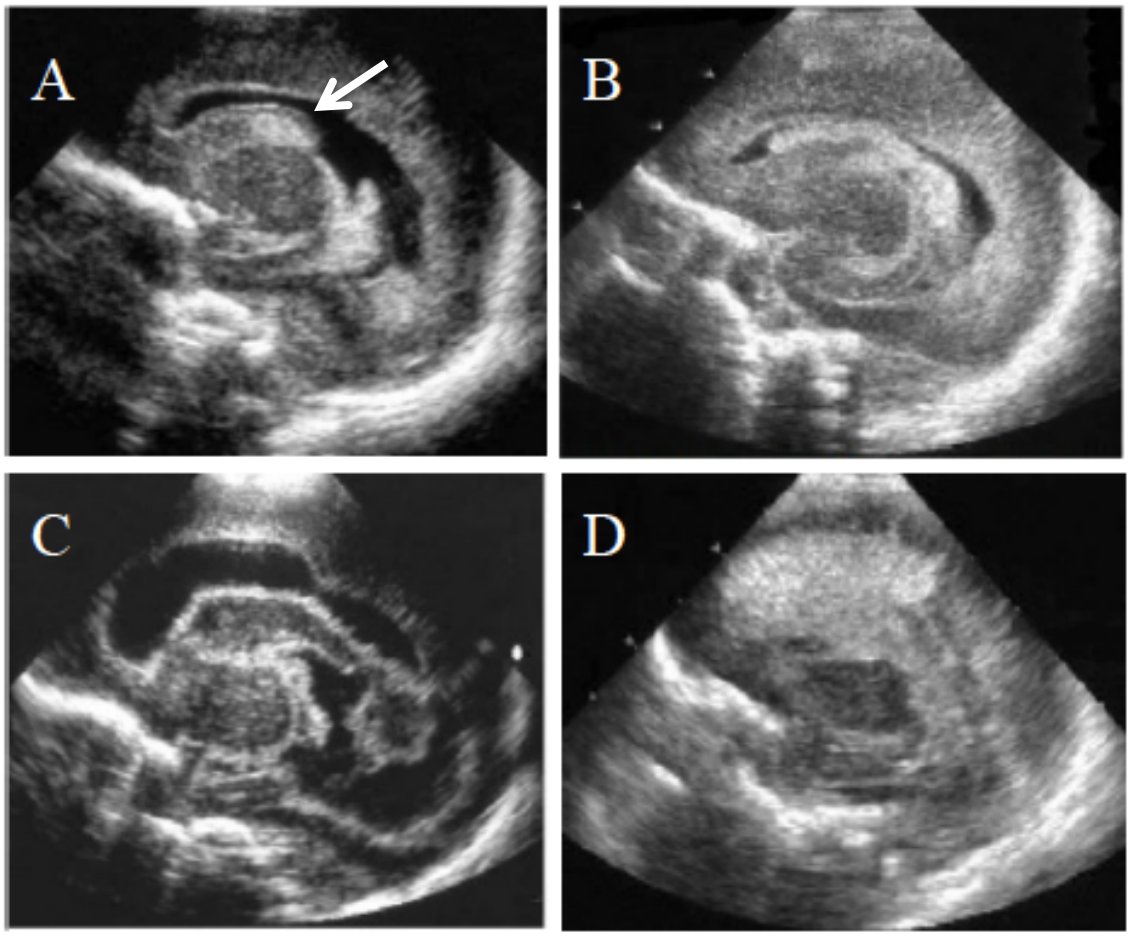
2.2 Major Preterm Brain Injury

2.2.1 Germinal Matrix Haemorrhage-Intraventricular Haemorrhage

In the late 1970s, Papile et al used computed tomography imaging and post-mortem examination to describe GMH-IVH in the preterm infant, leading to the Papile classification that is widely used to this day.[98] The Papile classification comprises 4 grades, increasing in severity from grade I through to grade IV (Figure 2.1). Grade I IVH, also referred to as a germinal matrix haemorrhage (GMH), pertains to a haemorrhage confined to the germinal matrix, a highly vascularised area of the developing brain consisting of progenitor neuronal and glial cells. The germinal matrix is located adjacent to the head of the caudate nucleus,

and extends along the margins of the lateral ventricles beneath the ventricular 17ubependymal. It is most active between 8 and 28 weeks' gestation, and undergoes almost complete involution between 32 and 36 weeks' gestation, with remnants persisting often near the caudate nucleus and posterior to the thalami near the origins of the optic radiations.[99] GMH is most often observed in the caudo-thalamic groove and, less commonly, in other areas of the germinal matrix. Grade II IVH describes a haemorrhage within the ventricular system, and can include haemorrhage isolated to the choroid plexus. Grade III IVH is defined by haemorrhage within the ventricular system accompanied by acute dilatation of the lateral ventricle; it is often, but not always, observed in the setting of a large IVH. Grade IV IVH, more recently referred to as a PVHI or haemorrhagic parenchymal infarction, describes a GMH-IVH associated with parenchymal involvement, with haemorrhage seen within the white matter adjacent to the ventricular margin.[99] In my thesis, I have chosen to use the term PVHI, rather than grade IV IVH, as I believe it more appropriately reflects the pathogenesis of PVHI as a complication of GMH-IVH (see section 2.2.1.1). Volpe proposes that PVHI occurs following obstruction of venous drainage by an ipsilateral GMH-IVH, rather than IVH breaching the ventricular wall into the periventricular parenchyma.[100]

Figure 2.1 Papile classification of germinal matrix haemorrhage-intraventricular haemorrhage



Cranial ultrasonography images through the anterior fontanelle in the parasagittal plane demonstrating the frontal, occipital, and temporal horns of the lateral ventricle.
A. Grade I IVH, or GMH (arrow). B. Grade II IVH. C. Grade III IVH. D. Grade IV IVH, or PVHI.
GMH=germinal matrix haemorrhage; IVH=intraventricular haemorrhage; PVHI=periventricular haemorrhagic infarction.

Advances in perinatal care over the last 40 years have been associated with a significant reduction in the rates of GMH-IVH in very preterm infants, from 50% in the 1980s, to approximately 15-25% in recent years.[99, 101, 102][103] However, the incidence is significantly greater in extremely preterm and extremely low birth weight infants, who are also more likely to have higher-grade IVH, that is grade III and PVHI, compared with more mature preterm infants.[104, 105] Approximately 4% of very preterm and very low birth weight infants will have a PVHI, with rates of up to 30% reported in infants born weighing <750 g.[5, 99, 104] In Australia and New Zealand, the rates of higher-grade IVH have steadily declined in recent decades, with an incidence of 4% reported for very preterm infants born in 2016 who survived to day 3; most PVHI occurred in infants born <26 weeks' gestation.[106] Differences in the reported rates of IVH (and other postnatal morbidities) between centres, and between neonatal networks, may reflect varying methodology in defining the denominator. Rates of IVH may be artificially diminished by the exclusion of all liveborn infants who died within the first days after birth, or by including infants who were not scanned within the first week after birth.

The propensity for GMH-IVH in the preterm population relates to the vulnerability of the germinal matrix vascular supply to perturbations in blood flow, particularly in the early hours and days following very preterm birth when cerebral autoregulation is undergoing physiologic adaptation.[107] Recent evidence also suggests that anomalous germinal matrix vasculature may contribute to causation of GMH-IVH.[108, 109] Consequently, most GMH-IVH occurs within the first 72 hours after birth, with up to 90% of all haemorrhages identified within the first 4 days.[99] The finding of GMH-IVH outside of this time-window, either antenatally or after day 4 from birth, should prompt consideration for further investigation, especially for thrombophilia as a cause of intracranial haemorrhage.[110]

Risk factors for the development of GMH-IVH in very preterm infants include lower gestation, lower birth weight, male sex, outborn status (born outside a tertiary neonatal centre), fetal distress, perinatal asphyxia, need for resuscitation at birth, low Apgar scores, metabolic acidosis, pneumothorax, sepsis, and chorioamnionitis.[111-113] Perinatal factors associated with a lower risk of GMH-IVH in this population include antenatal corticosteroids and magnesium sulphate administration, intrauterine growth restriction, birth by caesarean section, and early postnatal surfactant administration.[111, 113, 114]

As knowledge of the underlying mechanisms of GMH-IVH has evolved, the Papile classification has been adapted to better reflect the aetiology of GMH-IVH in the preterm infant.[99] The Volpe classification of GMH-IVH emerged in the late 1990s; it makes two important distinctions from the Papile classification.[115] Firstly, the definition of grades II and III IVH in the Volpe classification is dependent on the extent of the haemorrhage seen within the lateral ventricle in the parasagittal view; haemorrhage filling less than 50% of the ventricle defines a grade II IVH, and greater than 50% defines a grade III IVH, regardless of the presence of acute ventricular dilatation. The other point of difference, and perhaps the most notable for its departure from conventional thinking at the time, relates to the exclusion of grade IV IVH in the Volpe classification. Volpe conceptualised PVHI as a complication of IVH, rather than as an extension of the IVH into the adjacent periventricular white matter, as proposed by Papile.

2.2.1.1 Periventricular Haemorrhagic Infarction

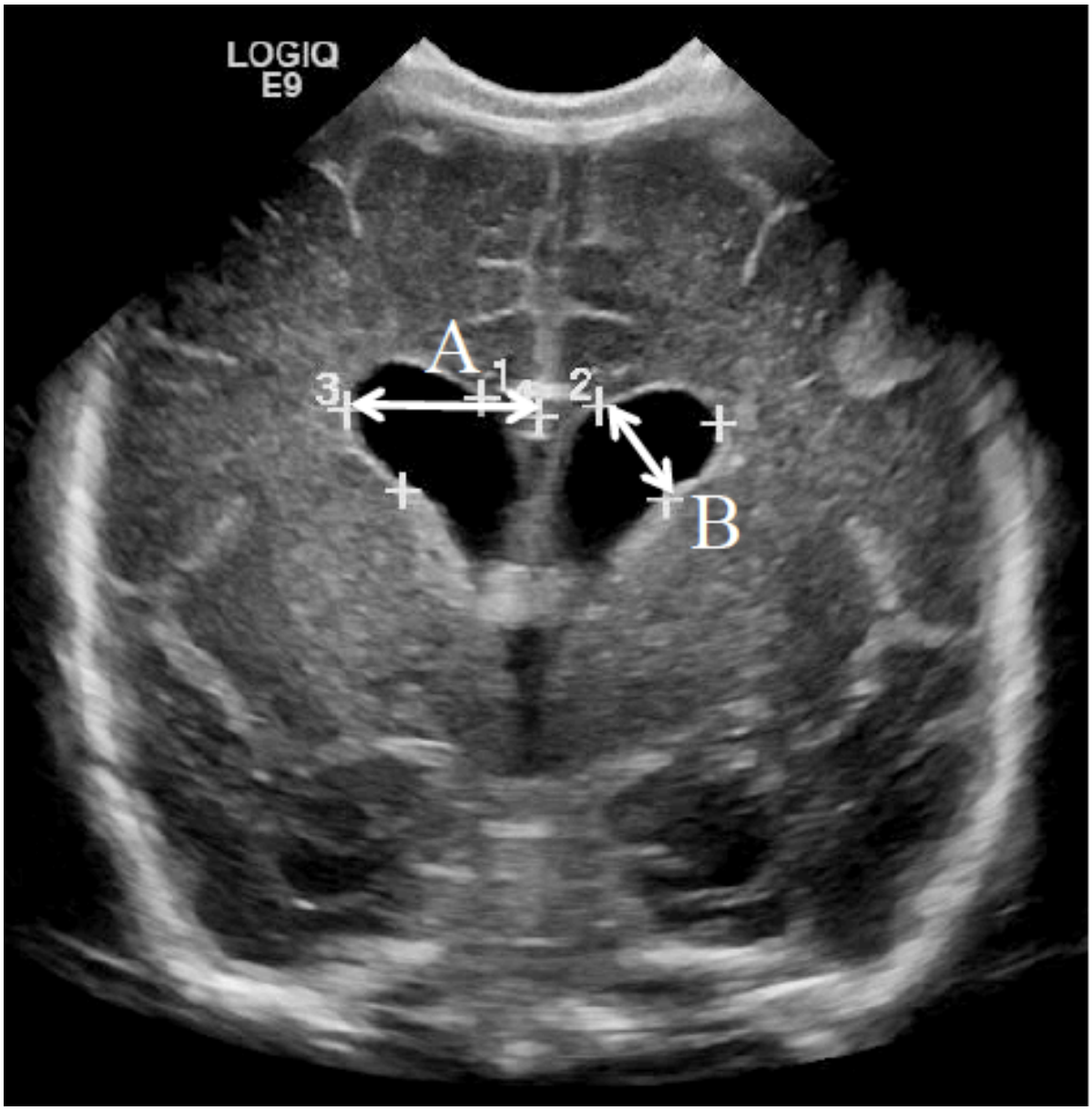
The pathophysiological basis of PVHI is haemorrhage into an infarcted area of periventricular white matter, usually caused by the GMH-IVH obstructing adjacent venous drainage from medullary veins that feed into the terminal vein.[116] Consequent venous congestion and ischaemia of the periventricular parenchyma lead to infarction and, subsequently, venous haemorrhage.[117] Although most PVHI is associated with a large IVH, it may occur in the setting of lower-grade GMH-IVH, including GMH. In fact, 15-20% of PVHIs are seen with an ipsilateral GMH alone, further reinforcing Volpe's classification of PVHI as a complication of GMH-IVH; PVHI need not occur in the setting of a large IVH.[99].

2.2.1.2 Post-Haemorrhagic Ventricular Dilatation

Up to 25% of IVH is complicated by progressive PHVD, also termed post-haemorrhagic hydrocephalus when more severe, whilst the acute dilatation that may occur soon after the onset of the IVH often resolves.[99] PHVD manifests from either obstruction of cerebrospinal fluid drainage (non-communicating hydrocephalus), most often at the level of the aqueduct of Sylvius, or sometimes the outflow from the 4th ventricle, or from impaired cerebrospinal fluid absorption (communicating hydrocephalus), perhaps as a consequence of ventriculitis, although the mechanism is not well understood.

PHVD can be assessed using linear measures of the lateral ventricles made from cUS. The most frequently used linear measures to assess for PHVD in clinical practice include the ventricular index (VI) and anterior horn width (AHW), as described in Figure 2.2.[118-120] PHVD is somewhat arbitrarily defined as either a VI >2 SD above the mean for normative values referenced by post-menstrual age at assessment, or an AHW >6 mm at any time.[118, 119] Measurement of the thalamo-occipital distance, the diagonal distance between the thalamic and occipital horn margins of the lateral ventricle in the parasagittal plane, also provides evidence of posterior ventricular enlargement, and is often underestimated but no less important to the assessment of PHVD.[121]

Figure 2.2 Linear measures of the lateral ventricles used in the management of post-haemorrhagic hydrocephalus



Cranial ultrasonography image through the anterior fontanelle in the coronal view at the level of the foramina of Monro.

A=ventricular distance (horizontal distance from the most lateral margin of the lateral ventricle to the falx);

B=anterior horn width (diagonal distance between the walls of the lateral ventricles).

2.2.2 Cerebellar Haemorrhage

Cerebellar haemorrhage is a recognised complication of very preterm birth, with an incidence of up to 10% in very preterm infants, and as high as 29% in infants born weighing <750 g.[122] MRI studies report higher rates of cerebellar injury in very preterm infants than cUS, mostly related to the findings of smaller punctate cerebellar haemorrhages on MRI that otherwise evade detection on cUS.[123] Use of supplementary acoustic windows, including the mastoid and posterior fontanelles, improves the sensitivity of cUS for detecting most posterior fossa lesions, including cerebellar haemorrhage.[124] However, despite optimal imaging of the posterior fossa, cUS remains limited to the detection of larger cerebellar haemorrhages, with only 6-9% of cerebellar haemorrhages in very preterm or very low birth weight infants detected by cUS.[96, 125-127]

The cause of cerebellar haemorrhage in very preterm infants remains unknown, however many of the perinatal risk factors for GMH-IVH in this population, particularly pertaining to cardiorespiratory morbidity, are also associated with the development of cerebellar haemorrhage.[122] The subpial germinal matrix present in the granular cell layer of the cerebellar hemispheres, most prominent at 24 weeks' gestation but mostly involute by 30 weeks' gestation, may be predisposed to the same risk of injury with perturbations in cerebral blood flow as documented in GMH-IVH.[122] Similarly, injury to vessels within the subependymal layer lining the roof of the fourth ventricle may lead to haemorrhage within the cerebellar vermis.[122] It is unsurprising then that cerebellar haemorrhage following very preterm birth is associated with supratentorial lesions, particularly GMH-IVH.[122]

2.2.3 Periventricular Leukomalacia

There are two main forms of PVL observed in the very preterm infant: cystic and diffuse (non-cystic). As cUS became increasingly applied in neonatal intensive care throughout the 1980s, reports began to appear documenting the timing, evolution, and perinatal associations of cPVL in very preterm infants.[128, 129] cPVL had previously been described at post-mortem examination as a "softening" of the cerebral white matter.[93] On cUS, PVL may appear echodense in the early phase, with echolucent cysts forming in the most severe cases between 2 and 4 weeks after the initial insult.[93, 130] cUS in the late-

1970s through to the early 1980s was performed using linear-array technology, limiting the ability of cUS to detect cysts within the brain, unless very large, until mechanical-sector scanners became available thereafter.[131]

In cPVL, the most common region of the brain affected is the trigone, but it is also seen in the frontal regions. In more severe cases, cPVL extends throughout the centrum semiovale to the subcortical deep white matter regions of the cerebral hemispheres.[93] Moreover, cUS evidence of echogenicity and echolucency seen in the cerebral white matter in both non-cystic and cystic forms of PVL is most often bilateral, symmetric, and seen adjacent to the lateral ventricles. This contrasts with the echogenicity seen in PVHI, which is unilateral in 65-75% of cases, and often asymmetric when bilateral, and has the appearance of a fan-shaped flare emanating from the lateral margin of the lateral ventricle.[99] It is important to highlight a practical challenge facing clinicians and researchers alike when reporting cUS findings of focal echogenic lesions, as PVHI and PVL may appear similar. Studies have reported lower rates of inter-observer agreement for hyperechoic lesions on cUS compared with other overt signs of brain injury, including cysts and ventriculomegaly.[132, 133] Frequent, sequential cUS to document the evolution of the lesion may provide further evidence of the underlying aetiology, and help guide the clinician to prognosticate outcomes.

de Vries et al have classified PVL into 4 grades based on cUS.[93] Grade I describes periventricular echogenicity persisting for ≥7 days. Grade II describes small periventricular cystic lesions confined to the fronto-parietal regions. Grade III describes more extensive periventricular cystic lesions through the fronto-parietal-occipital regions. Grade IV describes extensive periventricular cystic lesions extending to the subcortical region.

Diffuse PVL, with or without punctate white matter lesions, is the most common type and pattern of brain injury associated with very preterm birth, as elucidated by neonatal brain MRI in infants born very preterm.[134] Non-cystic and cystic forms of PVL may be manifestations of different pathological processes, with the non-cystic form resulting from apoptosis of oligodendrocyte precursors, specifically pre-oligodendrocytes, and the cystic form a consequence of cellular necrosis.[135] Putative mechanisms for both forms of PVL relate to the effects of inflammation, infection, and, less commonly, ischaemia, on biological processes in the developing brain.[136] The pro-inflammatory state, including free-radical production, excitotoxicity, and oxidative stress, leads to neuronal and glial cell injury.[135]

Consequently, PVL impairs brain growth, resulting in relative “brain atrophy”, or the encephalopathy of prematurity as conceptualised by Volpe, manifesting as reduced cerebral white and grey matter volumes, thinning of the corpus callosum, enlarged extra-axial space, and ventriculomegaly.[135] These findings are well described in brain MRI studies, demonstrating very preterm infants to have more cerebral white matter abnormalities and regional reductions in brain volumes at term-equivalent age compared with infants born at term.[4, 137]

2.3 Utility of Neonatal Neuroimaging to Predict Neurodevelopmental Outcomes in Very Preterm Infants

In an ever-expanding body of research exploring the prognostic utility of neonatal neuroimaging for infants who are born very preterm, there is strong evidence to support the association of adverse neurodevelopmental outcomes with perinatal brain injury and aberrant postnatal brain growth and maturation following very preterm birth.[4, 5, 138] Consequently, neuroimaging has an important role in the NICU where it is used by clinicians to screen infants for brain injury, to monitor the evolution of established intracranial pathology, and to identify infants at high risk of neurodevelopmental impairment who may stand most to benefit from ongoing surveillance and, where appropriate, early intervention therapies. The ability of neuroimaging to discriminate infants with regard to their risk of adverse neurodevelopment also affords clinicians infant-specific information which they may use when counselling families about the likelihood of whether or not their child may have later neurodevelopmental impairments. In some instances, the finding of major brain injury in an infant may portend a high risk of death or severe neurosensory or motor disability, aiding clinicians and families alike in their decision-making around the ongoing provision of intensive care. This section of my literature review focuses on the clinical application of neonatal cUS and MRI as prognostic tools to identify very preterm infants at high risk of adverse neurodevelopmental outcomes.

2.3.1 Neonatal Cranial Ultrasonography with Major Brain Injury

In a prospective cohort study of all infants born at <33 weeks' GA, and admitted to a single centre over a 9-year period from 1990 to 1999, de Vries et al reported on the predictive utility of cUS findings of major brain injury for cerebral palsy (Table 2.1).[5] Major brain injury was defined by high-grade IVH (grades III or IV), cPVL (grades II, III, or IV), focal arterial infarction, or persistent echogenicity in the basal ganglia. Of the 1636 infants included in the study, 189 (12%) had findings of major brain injury on neonatal cUS, and a further 319 (19%) had isolated grade I PVL (persistent echogenicity >7 days). Cerebral palsy was diagnosed in 76 (5%) of the 1460 surviving preterm children at a minimum age of 2 years, of whom 70 had abnormal findings on neonatal cUS, 58 of which were major brain lesions. With respect to the children with isolated findings of PVL, the severity of cerebral

palsy increased with increasing grade of PVL. All 12 children with grade I PVL developed a mild diplegia, and all 17 children with grade III cPVL were wheelchair-dependent with severe diplegia or quadriplegia. Of note, all 6 (9%) children diagnosed with cerebral palsy who had no abnormalities detected on neonatal cUS were able to walk independently.

Table 2.1 Abnormal neonatal cranial ultrasonography and outcomes in infants born <33 weeks' gestational age

Neonatal cUS brain injury (n=508)	Died, n (%) (n=85)	Survived, n (%) (n=423)	Cerebral palsy, n (%) (n=70)
IVH grade III, n=56	24 (43)	32 (57)	4 (12)
IVH grade III + cPVL, n=8	2 (25)	6 (75)	5 (83)
IVH grade IV, n=64	28 (44)	36 (56)	17 (47)
PVL grade I, n=319	16 (5)	303 (95)	12 (4)
cPVL grade II, n=20	3 (15)	17 (85)	10 (59)
cPVL grade III, n=29	11 (38)	18 (62)	17 (94)
Focal arterial infarct, n=12	0 (0)	12 (100)	5 (42)

Adapted from de Vries et al[5]. cPVL=cystic periventricular leukomalacia; cUS=cranial ultrasonography; IVH=intraventricular haemorrhage; n=number. No child had grade IV cystic periventricular leukomalacia. Excludes 6 children with cerebral palsy who did not have findings of major brain injury on their neonatal cranial ultrasonography (clinical details provided in text).

In de Vries et al's study, grade I PVL was the most common finding, seen in approximately 1 in 5 infants born <33 weeks' GA, of whom only 4% of survivors were diagnosed with cerebral palsy (all had mild spastic diplegia).[5] Increasing severity of PVL was associated with greater risk of death or cerebral palsy, the latter diagnosed in 59% and 94% of children with grades II and III PVL, respectively. Of the 120 infants with evidence of isolated grade III or IV IVH on neonatal cUS, 52 (43%) died, and 21/68 (31%) survivors were diagnosed with cerebral palsy. Unfortunately, the location of the PVHI or focal arterial infarct was not reported, but may have been pertinent to the prediction of unilateral cerebral palsy, as I discuss later. It is salient to note at this point that cerebral palsy does not account for the majority of neurodevelopmental impairments associated with children born preterm, but rather developmental delay.

In studies reporting the neurodevelopmental outcomes of very preterm infants, death and disability are invariably competing outcomes. There may be instances in the NICU where a decision is made by clinicians and families to provide palliative care to infants with confirmed major brain injury. It is unknown whether infants who have died following a decision to palliate within the neonatal period would have otherwise survived intact, that is without disability, despite their high risk of adverse neurodevelopment. de Vries et al do not specifically address this issue in their study, however I note that they report considerably lower rates of survival for infants with higher grades of PVL and IVH, and it is possible that the higher risk of adverse neurodevelopment in the setting of these pathologies may have influenced decision-making leading to the palliation of a number of these infants.[5] Of note, the survival rate of very preterm infants in de Vries et al's study – approximately 89% – is comparable to rates of survival in similar healthcare settings in recent years.[5, 21-24]

With consideration to the outcomes of surviving children in de Vries et al's study, major brain injury detected on neonatal cUS had 76% sensitivity and 95% specificity for the prediction of cerebral palsy.[5] However, the study reported a relatively low positive predictive value of 48%; that is, for very preterm children with major brain injury detected on neonatal cUS, only about half were diagnosed with cerebral palsy. In fact, most children (87.5%) with isolated grade III IVH, and those with grade IV IVH whose lesion was not in the motor pathway, did not develop cerebral palsy. A reasonable assumption can be made that lesions that do not disrupt the motor pathway are associated with a lower risk of motor impairment compared with those that do, irrespective of the size of the lesion.[139-141] However, another study included both the site and size of a grade IV IVH lesion in assessing its associated risk for later neurodevelopmental impairment.[99, 142] Devising a scoring system for the prediction of adverse neurodevelopment in the setting of a grade IV IVH, Bassan et al reported a higher risk for motor and cognitive deficits when lesions included 2 or more of 5 periventricular zones, were bilateral, or were associated with midline shift.[99, 142] Using this scoring system, a grade IV IVH satisfying all criteria prognosticates a certain motor deficit and a 70% risk of cognitive impairment, albeit in a small study of 30 preterm children assessed a 30 months' corrected age.[142] On the other hand, a unilateral grade IV IVH involving one periventricular zone, and not associated with midline shift, confers a 0-33% risk of an abnormal motor examination and a 14% risk of cognitive impairment.[142] However, this is likely to underestimate the risk of motor impairment where the lesion is located within the motor pathway – specifically within the precentral gyrus posterior to the

periventricular trigonal region – even without involving other zones, and without midline shift.[139, 140] A significant limitation of Bassan et al's scoring system is that it was devised in a small study of only 58 infants with a grade IV IVH, of whom 23 (40%) died within the NICU.[104] The prognostic utility Bassan et al's scoring system should be explored further in a larger study, and with particular consideration to the site of the lesion. However, there are potential challenges in replicating Bassan's study, including how the inception cohort is defined (GA at birth, denominator with respect to all liveborn infants or only survivors), timing and frequency of scans within the first week after birth to ascertain the extent of PVHI, findings of other brain lesions, and when and how children are assessed at follow-up.

The finding of PHVD on neonatal cUS, a recognised complication of IVH, significantly increases the risk of adverse neurodevelopment in very preterm infants, with neurosensory and motor deficits observed in 70-95% of survivors.[99] Infants who receive intervention for PHVD, whether it be temporising or definitive, are at greatest risk of adverse neurodevelopmental outcomes.[143] The drainage of cerebrospinal fluid in cases of progressive PHVD is intended to protect the brain from further injury, yet there is no widely-held consensus on the threshold for intervention. A recent study evaluated the neurodevelopmental outcomes of very preterm infants with PHVD, comparing an "early" or "late" approach to management, with or without definitive drainage by placement of a permanent shunt.[118] The "early" approach included intervention based on linear measures of the lateral ventricles exceeding normal; that is, a ventricular index >95th centile for post-menstrual age, or an anterior horn width >6 mm. The "late" approach included intervention when signs of raised intra-cranial pressure were present, and almost always included the immediate placement of a shunt. Management of PHVD with the "early" approach, even if placement of a permanent shunt ensued, was associated with better neurodevelopmental outcomes compared with the "late" approach.[118] This finding was unsurprising given that infants managed by a "late" approach had, by definition, signs of cerebral injury, including apnoea, seizures, altered conscious state, and abnormal eye movements.

The sensitivity of major brain injury detected on neonatal cUS for the prediction of cerebral palsy in de Vries et al's study is significantly higher than that reported by others.[4, 5, 144, 145] This may be explained by differences in the timing and frequency of cUS between studies, the varying expertise of the sonographers acquiring the images, and the

ability of reporters interpreting the scans to reliably detect major brain injury. In de Vries et al's study, as is common practice in many of the NICUs across the Netherlands, very preterm infants were routinely scanned with greater frequency than is common in Australia and the United States, including days 1, 2, and 3 after birth, then weekly until 32 weeks' post-menstrual age, thereafter fortnightly until term-equivalent age.[5, 94, 146] More frequent scanning from the early days after birth and up to term-equivalent age affords the potential to detect persistent echogenicities (grade I PVL) and cystic changes within the cerebral white matter, which, if observed, raises the sensitivity of cUS for the prediction of cerebral palsy, and potentially other neurosensory impairments.[5] I elaborate on the prognostic utility of the "apparently normal" cUS later in my literature review.

Cerebellar injury is an increasingly recognised complication of preterm birth, but its presence is often not reported in studies evaluating the prognostic utility of neonatal cUS.[4, 5, 122, 147] Reasons for this include the lack of targeted imaging of the cerebellum using supplementary acoustic windows to better image the posterior fossa, and the lack of sensitivity of cUS to detect more subtle cerebellar injury.[96] Cerebellar haemorrhage is associated with a high risk of adverse neurodevelopment, with up to 40% of very preterm infants with an isolated cerebellar haemorrhage seen on neonatal cUS developing later cognitive, language, and behavioural impairments, and almost 50% having a severe motor impairment, the latter being greater if associated with supratentorial parenchymal injury, such as grade IV IVH.[68] Haemorrhage within the cerebellar vermis is especially associated with a greater risk of later behavioural deficits and global developmental delay.[68] It is less certain what role smaller, punctate lesions within the cerebellum, observed by brain MRI but evading detection using cUS, have in mediating neurodevelopmental outcomes following very preterm birth.[148] Cerebellar haemorrhage seen on brain MRI that is too small to be detected by cUS is associated with a better neurodevelopmental outcome compared with larger cerebellar haemorrhage seen on cUS.[149] However, there is evidence to suggest that cerebellar injury to the developing brain has a deleterious effect on cerebral cortical development.[150] Disruption of cortico-thalamo-cerebellar connectivity in very preterm infants is associated with adverse neurodevelopmental and psychiatric outcomes.[151]

Whilst not considered to be a major brain injury, there is some evidence to support the association of isolated low-grade IVH, that is grades I and II IVH, with higher risk of neurosensory impairment in very preterm infants.[152] However, several other studies have

shown no significant difference in neurodevelopment between very preterm infants with low-grade IVH and no IVH.[132, 145, 147]

2.3.2 Neonatal Cranial Ultrasonography without Major Brain Injury: The "Apparently Normal" Scan

In the absence of major brain injury detected on neonatal cUS, what remains of the prognostic utility of the "apparently normal" cUS to predict neurodevelopmental outcomes in very preterm infants? In a prospective, geographical cohort study of 1473 extremely-low-birth weight infants with "apparently normal" cUS, Laptook et al assessed the prevalence of, and risk factors for, cerebral palsy and cognitive impairment (defined by a Mental Developmental Index <70 (<2 SD below the normative test mean) using the Bayley Scales of Infant Development-II) determined between 18 and 22 months' corrected age.[147] All infants had had an early (mean 6 days, SD 5; range 0-28) and a late (mean 47 days, SD 25; range 5-127) scan, all of which were assessed to be normal. A normal scan was defined by the absence of IVH or periventricular echodensity or echolucency, and normal ventricular size (assessed subjectively). In those infants with normal neonatal cUS, the prevalence of cerebral palsy was 9.4%, cognitive impairment 25.3%, and either cerebral palsy or cognitive impairment 29.2%. The finding of high rates of neurodevelopmental impairment and cerebral palsy in this study of preterm children with "apparently normal" neonatal cUS, which equates to a negative predictive value of 90.6%, contrasts with the study by de Vries et al which had reported a 99% negative predictive value for cerebral palsy in the setting of a neonatal cUS which showed no major brain injury or persistent periventricular echogenicity.[5] Only 6 (0.4%) surviving children diagnosed with cerebral palsy in de Vries et al's study had no overt focal brain injury seen on neonatal cUS, albeit in a cohort that included infants born <33 weeks' GA rather than only those of extremely-low-birth weight.[5] Of these 6 children, 2 had severe intra-uterine growth restriction, and 2 were each one of monozygotic twins. Extremely-low-birth weight and growth restricted infants are at high risk of brain injury and cerebral palsy.[132, 153, 154]

The increased frequency of cUS from the first days after birth and up to term-equivalent age in de Vries et al's study undoubtedly improved the detection of overt, focal pathology associated with later adverse neurodevelopment, and specifically cerebral palsy.[5] Moreover, de Vries et al showed that 29% of very preterm children diagnosed with cerebral

palsy had cysts first detected on cUS after 28 days' postnatal age (PNA), later than is commonly expected.[5] The nature of cPVL is that it evolves over time, with cysts usually appearing between 2 and 4 weeks, and occasionally up to 6 weeks, after an insult, before glial scarring occurs and the cysts coalesce with the lateral ventricle. It is likely that the earlier timing of, and less frequent, scanning of infants in Laptook et al's study contributed to their finding of a lower sensitivity of cUS for the prediction of cerebral palsy compared with de Vries et al's study, as cystic changes within the cerebral white matter may have been missed.[5, 147] Often, without having confirmed cystic changes on previous imaging, subtle white matter volume loss and ex-vacuo ventricular dilatation are the only signs of cysts ever having been present. Frequent cUS of very preterm infants affords the best opportunity to detect cysts and other brain injury, to more accurately time the onset of injury, and, most importantly, improve the sensitivity of this widely-used imaging modality to predict neurodevelopmental outcomes following very preterm birth. The absence of overt, focal brain pathology on frequent cUS performed from the first days after birth and up to term-equivalent age in very preterm infants can reassure clinicians and families alike that the likelihood of cerebral palsy is almost negligible.[5] This is a significantly lower risk than could otherwise be determined by an infant's perinatal history or neurobehaviour within the early postnatal period.

2.3.3 Neonatal Magnetic Resonance Imaging

Compared with predicting motor outcomes, there is less evidence to support the utility of cUS to predict cognitive and behavioural deficits following very preterm birth, despite these latter developmental impairments accounting for the greater burden of adverse neurodevelopmental outcomes in this population.[1] This may be because higher-order cognitive and behavioural functioning relies on complex and diffusely located neural pathways that connect multiple cortical and subcortical brain structures.[141, 155] Advanced brain MRI techniques, including diffusion tensor imaging and resting-state functional connectivity, are helping to elucidate white matter tracts that relate cognitive and motor impairments with very preterm birth.[156] Injury to these tracts is likely to be more diffusely distributed and often mild, evading detection by cUS. However, the application of advanced brain MRI techniques in clinical practice is hindered by expense, time-consumption, and the lack of expertise involved with complex post-acquisition processing. Moreover, many of the relationships of cerebral microstructure and neurodevelopmental outcomes following very

preterm birth are largely based on group analyses, and are not specific to assessing risk for the individual infant.

Nonetheless, the prognostic utility of conventional brain MRI, that is, the more clinically applicable T1 and T2 imaging, in very preterm infants has been evaluated in a multitude of studies.[4, 47, 139] Most of these studies use brain MRI to image the very preterm infant at term-equivalent age, when an assessment of brain injury, maturation, and development may be made.[4, 47, 139] For example, myelination of the posterior limbs of the internal capsules cannot be reliably seen on conventional T1 and T2 imaging until approximately 38 weeks' post-menstrual age. Motor deficits in preterm and term children with evidence of grade IV IVH involving the motor pathway, as seen on early cUS, can be reliably predicted by asymmetric myelination of the ipsilateral posterior limb of the internal capsule on brain MRI at term-equivalent age.[139]

Brain MRI is superior to cUS for detecting more subtle and diffuse cerebral white matter injury – including the non-cystic form of PVL – that constitutes the predominant form of preterm brain injury and accounts for the commoner cognitive, motor, and behavioural deficits found in the preterm population.[157] Brain MRI has shown preterm infants to have regional reductions in brain volumes and less mature microstructural organisation at term-equivalent age compared with term-born infants.[158] In turn, smaller brain volumes and alterations in brain maturation following preterm birth are related to the risk of adverse neurodevelopment in preterm children and adolescents.[137, 138, 159, 160]

In a prospective cohort study of 167 infants born <30 weeks' GA, Woodward et al compared brain MRI at term-equivalent age with early cUS up until around 6 weeks' PNA in predicting neurodevelopmental outcomes at 2 years.[4] Brain MRI was qualitatively assessed for evidence of white and grey matter injury, and impaired brain growth and maturation, using a scoring system. White matter was assessed using 5 items, each on a 3-point scale, including the presence and extent of: (1) white matter abnormalities; (2) cysts; (3) white matter volume; (4) ventriculomegaly; and (5) corpus callosal thinning. Grey matter was assessed using 3 items, each on a 3-point scale: (1) extra-axial space; (2) gyral maturity; and (3) grey matter abnormalities. More white and grey matter abnormalities, reductions in white matter volume, increased ventricular size, and increased extra-axial spaces were taken to reflect increasing severity of brain injury. The scoring system used in this study, based on

an assessment of brain injury, brain growth, and brain maturation on brain MRI at term-equivalent age, demonstrated greater sensitivity compared with early cUS to predict neurodevelopmental impairments in very preterm children. Specifically, the finding of moderate-to-severe white matter injury on MRI at term-equivalent age more sensitively predicted severe cognitive (41%) and motor (65%) deficits, and cerebral palsy (65%), in very preterm children at 2 years than the finding of major brain injury seen on early cUS imaging (15%, 18%, and 18%, respectively). However, Woodward et al's study should be interpreted with caution. Their study did not compare cUS contemporaneously with brain MRI, and their assessment of cUS was limited to the first 6 weeks after birth and to the findings of major brain injury alone, including grades III and IV IVH, and cPVL. de Vries et al have shown much improved sensitivity (76%) of cUS for the prediction of cerebral palsy when infants were imaged frequently from birth and up to term-equivalent age.[5]

Studies comparing contemporaneous cUS and brain MRI at term-equivalent age for the prediction of adverse neurodevelopment following very preterm birth have shown only modest benefit of brain MRI over cUS, albeit with greater financial outlay.[47, 161] However, it is likely that the timing of neuroimaging in very preterm infants at term-equivalent age underestimates the incidence of early postnatal brain injury and its contribution to prediction models of long-term neurodevelopmental outcomes.[162] Martinez-Biarge et al have shown that timing of neuroimaging is critical to ascertaining both the incidence and severity of non-haemorrhagic cerebral white matter injury.[162] Often, the only evidence of earlier, cerebral white matter injury in the very preterm infant at term-equivalent age is the presence of ventricular dilatation, presumed to be ex-vacuo in the absence of any evidence of earlier IVH. In their study of 82 infants born at <36 weeks' GA, in which infants had sequential imaging from birth and up to term-equivalent age with a combination of cUS and brain MRI, up to one-third of infants with cysts seen on early neuroimaging demonstrated smaller and/or less extensive cysts at term-equivalent age.[162]. Applying a novel scoring system, which combined sequential cUS and brain MRI within the early postnatal period up to term-equivalent age, and included the site of the lesion and degree of myelination of the posterior limb of the internal capsule, Martinez-Biarge et al demonstrated that the severity of cerebral white matter injury was associated with the presence and severity of cerebral palsy at 2 years (Spearman's rank correlation 0.88; $p<0.0001$).[163] Only 3 (9%) of the 34 children with mild white matter injury developed cerebral palsy, all of whom were walking, albeit delayed. In contrast, 31 (91%) of the 34

children with severe white matter injury developed cerebral palsy (mostly moderate to severe). Of the 3 remaining children with severe white matter injury who did not develop cerebral palsy, neuroimaging showed that their cysts did not involve the motor pathways, and myelination of the posterior limb of the internal capsule was complete or partially complete.

Although neonatal neuroimaging has an important role in identifying very preterm infants at risk for adverse neurodevelopment, the optimal modality and timing of neuroimaging is currently under debate.[123, 164] cUS can reliably detect most major brain injury, including GMH-IVH, cPVL, and larger cerebellar haemorrhage, as well as focal infarction in the deep grey matter, which is less common, providing cUS is performed at the appropriate time in relation to the timing of the insult.[123, 165] cUS is less sensitive than MRI for more subtle and diffuse lesions in the cerebral white matter, deep grey matter, and cortex, as well as punctate haemorrhages in the cerebellum as detected usually around term-equivalent age.[5, 96, 134, 144] However, the clinical application of brain MRI is somewhat restricted in the early postnatal period, as it may not be feasible or safe to move extremely small infants receiving intensive care to a scanner on a frequent schedule, its availability is limited by expertise and cost, and there are concerns about the use of sedation in very preterm infants, which is sometimes required to allow the appropriate sequences to be obtained.[123, 166] Moreover, very preterm infants who no longer need intensive care, but have not yet reached term-equivalent age, are routinely transferred to non-tertiary neonatal centres where neonatal brain MRI is often unavailable. In a prospective cohort of very-low-birth weight infants born <29 weeks' GA aiming to perform brain MRI at 30 weeks' post-menstrual age, only 52 (33%) of the 158 infants included in the study had their planned brain MRI at 30 weeks' post-menstrual age, with at least mild adverse events (mostly cardio-respiratory or temperature instability) noted in 26 (50%) of these infants within 24 hours following their brain MRI.[166] Of the 106 infants who did not have brain MRI performed at 30 weeks' post-menstrual age, 32 (20%) died before 30 weeks' post-menstrual age, 36 (23%) were transferred to another centre, and 38 (24%) were too unwell to be transported to the MRI.[166] As a bedside tool, cUS can be safely repeated in the smallest and sickest of preterm infants, and it is relatively inexpensive as a single-operator modality with negligible running costs, provided that the operator is appropriately experienced in handling sick preterm infants and is aware of the risk of infection. Whilst ultrasonography has the potential to lead to tissue injury through the production of heat, this is not a real risk given the short period of imaging required to perform a thorough scan using modern ultrasonography

technology appropriately set up for imaging the preterm infant. The ideal timing and combination of cUS and brain MRI for the prediction of neurodevelopmental outcomes in very preterm infants has yet to be determined. The prognostic utility of cUS, with or without findings of major brain injury, needs to be explored further, and brain MRI needs to allow individualised, rather than group, predictions of cognitive and behavioural outcomes.

The incidence of major brain injury following preterm birth has declined to relatively low rates in recent years, currently detected by cUS in fewer than 10% of very preterm infants in Australia and New Zealand.[167] Lower rates of major brain injury in very preterm infants may lower the sensitivity of cUS for the prediction of later neurosensory and motor impairments, given the preponderance of preterm infants with adverse neurodevelopment who do not have findings of major brain injury on their neonatal cUS. If cUS is to remain the most frequently used neuroimaging modality for preterm infants, there is a pressing need to improve its clinical utility in current cohorts for the prediction of long-term neurodevelopmental outcomes.

2.4 Neonatal Brain Biometry to Predict Neurodevelopmental Outcomes in Very Preterm Infants

Simple linear measures of brain tissue and fluid spaces made from brain MRI in very preterm infants at term-equivalent age are associated with poorer neurodevelopmental outcomes at 2 years.[159, 168] Using brain MRI in very preterm infants at term-equivalent age, Kidokoro et al extended the qualitative scoring system described by Woodward et al to include several linear measures of brain tissue and fluid spaces.[4, 169] Kidokoro et al also included a qualitative assessment of the deep nuclear grey matter, consistent with other studies that have shown these structures to be susceptible to injury following preterm birth.[123, 165] The inclusion of linear measures is largely based on the premise that preterm infants have been shown to have regional reductions in brain volumes at term-equivalent age, and that the severity of cerebral white matter injury, both cystic and non-cystic, is related to brain size.[4, 137, 160] The use of simple linear measures as surrogate markers of brain size, which may in turn relate to the influence of cerebral white matter injury on subsequent brain growth and maturation, has practical advantages over the measurement of brain volumes. The use of voxel-based morphometry in brain MRI to determine regional brain volumes has limited clinical application given the requirement for post-acquisition image processing and the requisite expertise. Important to their concurrent validity, simple linear measures made from brain MRI in very preterm infants at term-equivalent age correlate well with regional brain volumes.[159] Moreover, ventricular volumes assessed by brain MRI correlate well with simple linear measures of the ventricles made from cUS, as do simple linear measures made from brain MRI with those made from cUS.[170-172]

A recent study by Skiold et al comparing contemporaneous cUS and brain MRI in very preterm infants at term-equivalent age, for the prediction of neurodevelopment between 2 and 3 years, included the use of simple linear measures in a proposed cUS scoring system.[161] For comparison, this study used the established brain MRI scoring system validated by Woodward et al that is in common use today, which, of note, includes a qualitative, but not quantitative, assessment of brain injury, growth, and maturation.[4] Using linear measures to supplement a qualitative assessment of brain size and fluid spaces, Skiold et al showed cUS to be as sensitive as brain MRI to predict cerebral palsy (75%) and severe cognitive delay (100%).[161] Moreover, the negative predictive values of a normal or

mildly abnormal cUS at term-equivalent age for later normal neurologic status or cognitive outcome were 98% and 100%, respectively; one child with cerebral palsy had a normal cUS and brain MRI at term-equivalent age.[161] Until now, other studies comparing the use of contemporaneous cUS and brain MRI in very preterm infants at term-equivalent age for the prediction of later neurodevelopmental outcomes had largely focused on cUS assessment of brain injury (for example, cysts), and a limited qualitative assessment of brain size (for example, ventricuolomegaly), and reported, somewhat marginal, superior sensitivity of brain MRI over cUS.[47, 164] The use of simple linear measures made from cUS for the prediction of neurodevelopmental outcomes following very preterm birth warrants further research.

2.4.1 Cranial Ultrasonography Linear Measures of Postnatal Brain Growth Following Very Preterm Birth

Several studies have evaluated postnatal brain growth in preterm infants using linear measures made from cUS (Table 2.2).[173-175] Most of these studies are cross-sectional by design, with linear measures made once in individual infants, and mostly within the first days after birth, thereby reflecting the size of the brain tissue or fluid space in the immediate postnatal period. Therefore, most of the linear measures of "postnatal brain growth" reported for very preterm infants do not reflect *actual* postnatal brain growth because they are not made from sequential linear measures within individual infants from birth, and then longitudinally throughout the postnatal period, but rather from scans performed in the early postnatal days for infants born across the third trimester of gestation. Ipso facto, normative reference data for actual postnatal brain growth made from sequential cUS following very preterm birth are limited.[173-175] Anderson et al's studies were the first to report the use of sequential cUS linear measures to quantify postnatal brain growth, that is, change in the linear measure over time, with respect to PNA, in individual infants, reporting normative reference data for the corpus callosum length (CCL) and vermis height (VH) made in the sagittal plane through the anterior fontanelle.[174, 175] This was followed by a study by Roelants et al who published reference normative data for postnatal brain growth for the CCL and corpus callosum-fastigium length, albeit with respect to post-menstrual age, rather than PNA.[173]

Almost all of the studies reporting normative reference data for the various linear measures were performed in infants who were appropriately grown for gestation with respect to birth weight, and excluded infants who were known to have intracranial pathology, or congenital, chromosomal, metabolic, or infective conditions which may have influenced brain growth and maturation. A notable exception is Anderson et al's study, which included 8 infants with IVH (grade unstated) in their evaluation of the CCL and VH in 61 infants who received sequential cUS from birth and up to term-equivalent age, representing 13% of their study cohort.[175]

Table 2.2 Cranial ultrasonography linear measures of postnatal brain growth in preterm infants

Author, Year, Country	Sample	Linear measure (imaging plane)	Brain growth (mm/week)
Brain tissue			
Corpus callosum			
Roelants et al[173] 2016 The Netherlands	140 infants GA <29 weeks Longitudinal	Corpus callosum length (sagittal)	1.5 mm/week (24-28 weeks' PMA) 0.75 mm/week (28-32 weeks' PMA)
		Corpus callosum-fastigium length (sagittal)	1.13 mm/week (24- 32 weeks' PMA)
Anderson et al[175] 2006 New Zealand	61 infants GA <34 weeks Longitudinal	Corpus callosum length (sagittal)	Non-linear Slower growth between 2-6 weeks' PNA
Anderson et al[174] 2005 New Zealand	64 infants GA <34 weeks Longitudinal	Corpus callosum length (sagittal)	0.77-0.91 mm/week PNA
Cerebellum			
Graça et al[176] 2013 Portugal	80 infants GA <32 weeks Cross-sectional	Transcerebellar diameter (coronal; axial, mastoid fontanelle); vermis height (sagittal); vermis AP diameter (sagittal)	Growth not stated with respect to GA
Imamoglu et al[177] 2013 Turkey	321 infants, AGA (birth weight 10^{th}-90^{th} centile) GA 26-42 weeks Cross-sectional	Transcerebellar diameter (coronal, mastoid fontanelle)	2 mm/week PMA
		Vermis height (sagittal)	0.53 mm/week PMA
Pogliani et al[178] 2008 Italy	434 infants, AGA, singleton GA 25-42 weeks Cross-sectional	Vermis height (sagittal)	0.72 mm/week PMA
Zuccotti et al[179] 2008 Italy	85, SGA, singleton GA 27-41 weeks Cross-sectional	Vermis height (sagittal)	0.69 mm/week PMA
Anderson et al[175] 2006 New Zealand	61 infants GA <34 weeks Longitudinal	Vermis length (sagittal)	0.75 mm/week PNA
Davies et al[180] 2001 Australia	221 infants GA 23-32+6 weeks (to nearest day) Cross-sectional	Transcerebellar diameter (coronal, mastoid fontanelle)	GA 0.47 weeks/mm
Cuddihy et al[181] 1999 New Zealand	86 infants GA <32 weeks and birth weight <2000g Cross-sectional	Vermis diameter (sagittal)	1.31 mm/week PMA
Swaminathan et al[182] 1999 Australia	106 infants GA 23-32 weeks Cross-sectional	Transcerebellar diameter (coronal, mastoid fontanelle)	1.61 mm/week PMA

Author, Year, Country	Sample	Linear measure (imaging plane)	Brain growth (mm/week)
Fluid spaces			
Extra-axial space			
Armstrong et al[183] 2002 New Zealand	201 infants GA <37 weeks Cross-sectional	Subarachnoid space	0.20 mm/week PMA
Ventricles			
Sondhi et al [184] 2008 India	1483 infants GA 25-42 weeks Cross-sectional	Anterior horn width	0.056 mm/week (25-34 weeks' PMA) 0.175 mm/week (34-42 weeks' PMA)
		Thalamo-occipital distance	0.167 mm/week (25-31 weeks' PMA) 0.727 mm/week (31-42 weeks' PMA)
		3rd ventricle width	0.082 mm/week PMA
		4th ventricle	0.088 mm/week PMA
		Ventriculo-hemispheric ratio	0.28
Davies et al[120] 2000 Australia	120 infants GA <33 weeks Cross-sectional	Anterior horn width (coronal)	0.019 mm/week PMA
		Thalamo-occipital distance (parasagittal)	0.109 mm/week PMA
		3rd ventricle width (transverse)	0.033 mm/week PMA
		4th ventricle width (transverse)	0.14 mm/week PMA
		4th ventricle length (transverse)	0.017 mm/week PMA
Liao et al[185] 1986 Japan	540 infants (366 term-born infants, GA 37-40 weeks; 174 preterm infants, GA 24-36 weeks) Longitudinal (term infants until 6 days and preterm infants until 6 weeks' PNA)	Ventricular index	0.017 mm/week PMA
		Ventricular axis	0.012 mm/week PMA
		Ventricular depth (=anterior horn width)	No relationship with GA
Perry et al[186] 1985 Australia	533 infants GA >26 weeks Cross-sectional	Ventricular width (=anterior horn width)	No relationship with GA
Levene et al[119] 1981 United Kingdom	273 infants, including 50 term-born infants GA 27-42 weeks Cross-sectional	Ventricular index (coronal plane and axial)	0.24 mm/week PMA

Imaging through the anterior fontanelle, unless otherwise stated. AGA=appropriate for gestational age with respect to birth weight; AP=antero-posterior; GA=gestational age; PMA=post-menstrual age; PNA=postnatal age.

2.4.2 Relationships of Neonatal Cranial Ultrasonography Assessment of Brain Growth with Neurodevelopmental Outcomes in Very Preterm Infants

There is emerging evidence to support the utility of cUS assessment of neonatal brain growth in prediction models of neurodevelopmental outcome following very preterm birth (Table 2.3).[161, 174, 175, 187, 188] Qualitative assessments of intra-cranial fluid spaces on cUS were applied in 2 such studies to evaluate associations of brain size at term-equivalent age with later neurodevelopment in very preterm infants.[187, 188] Horsch et al qualitatively assessed cUS at term-equivalent age for evidence of "brain atrophy" in a cohort of 81 very preterm infants, only 1 of whom had major brain injury detected on earlier cUS (grade III IVH).[187] In 64 (79%) children followed at 3 years, they reported a relationship between brain atrophy at term-equivalent age, defined by enlarged extra-axial spaces, with or without ventriculomegaly, and poorer motor (Psychomotor Development Index 91.4 v 106.5; p=0.001), cognitive (Mental Developmental Index 91.8 v 101.9; p=0.02), and behavioural scores (Behaviour Rating Scale 41.1 v 66.4; p=0.01) using the Bayley Scales of Infant Development, 2nd Edition. Moreover, in a prospective cohort study of 93 infants born at <31 weeks' GA, Brouwer et al evaluated the relationships between brain injury and brain size, assessed by neonatal cUS and MRI, and later neurodevelopmental outcomes at 2 years.[188] Brain injury was assessed using sequential cUS from the first days after birth and up to term-equivalent age, and brain injury and brain size were assessed by both cUS and MRI at term-equivalent age. Neurodevelopment at 2 years was assessed using the fine and gross motor scaled scores and cognitive composite score of the Bayley Scales of Infant and Toddler Development, 3rd Edition, with normative test means (SD) of 15 (3) and 100 (15), respectively. With regard to cUS assessment of brain size at term-equivalent age, ex-vacuo ventriculomegaly was independently associated with poorer fine and gross motor outcomes (β -2.2; 95% CI -3.5, -0.8; p=0.002, and β -1.4; 95% CI -2.4, -0.5; p=0.003, respectively) and poorer cognitive outcome (β -9.2; 95% CI -14.6, -3.7; p=0.001), and enlarged extra-axial space was independently associated with poorer gross motor outcome (β -0.9; 95% CI -1.7, -0.1; p=0.033).[188] Of note, the addition of conventional brain MRI at term-equivalent age, using the Kidokoro scoring system to assess brain injury and brain growth (including several linear measures), to prediction models of later neurodevelopment, made little or no statistical difference to any of the relationships between sequential cUS, including cUS at term-equivalent age, and neurodevelopment at 2 years.[169, 188]

Interestingly, in the study by Brouwer et al, they reported no relationship between moderate or severe brain injury on early cUS with ex-vacuo ventriculomegaly or enlarged extra-axial spaces on cUS at term-equivalent age. An explanation for this may be the small number of infants (12; 12.9%) included in their study who had moderate or severe brain injury observed on early cUS, relative to those with ex-vacuo ventriculomegaly (18; 19.4%) or enlarged extra-axial space (41; 44.4%) on cUS at term-equivalent age.[188] However, evidence of ex-vacuo ventriculomegaly and enlarged extra-axial space is frequently observed on neuroimaging in very preterm infants at term-equivalent age, and likely reflects the influence of the more common, yet subtle, diffuse white matter injury related to very preterm birth, reliably seen by brain MRI but evading cUS, on subsequent brain growth and maturation,.[134, 144, 188] The qualitative assessment of ex-vacuo ventriculomegaly, enlarged extra-axial space, reductions in white matter volume, and poor gyral maturation in very preterm infants at term-equivalent age, even in the absence of major brain injury seen on earlier cUS, has been well documented by cUS studies, and these findings are related to the risk of later motor and cognitive impairments.[187, 189] Interestingly, there was no association between ex-vacuo ventricular dilatation and enlarged extra-axial spaces reported in the study by Brouwer, with no explanation provided in their discussion.[188]

However, the qualitative assessment of ex-vacuo ventriculomegaly, as distinguished from PVHD, may prove difficult without knowledge of a previous GMH-IVH, or the benefit of sequential cUS. Whilst there are several hallmark features of ex-vacuo ventriculomegaly, including prominent dilatation of the occipital horns, irregularity of the ventricular margins, and reductions in adjacent cerebral white matter and deep nuclear grey matter volumes, the assessment of ventricular dilatation remains subjective.

Several studies have reported relationships between neonatal brain size in very preterm infants, quantified using linear measures of brain tissue and fluid spaces made on cUS, and neurodevelopmental outcomes up to 2 years' corrected age (Table 2.3).[173-175, 190] In a prospective cohort study of 44 infants born at <30 weeks' GA free of major brain injury on cUS, Fox et al reported a correlation between ventricular size at 1-month PNA and poorer motor outcomes at 2 years' corrected age.[190] Some of the linear measures of the lateral ventricles applied in Fox et al's study of very preterm infants at 1-month PNA, namely the ventricular mid-body and anterior horn widths, were also used in a later study by Skiold et al to quantitatively assess ventriculomegaly in very preterm infants at term-equivalent age

(see Chapter 2, Section 2.4). In a prospective cohort study of 47 infants born at <32 weeks' GA, Anderson et al measured the CCL on sequential cUS from birth and up to term-equivalent age.[174] They reported that a shorter CCL at term-equivalent age was associated with poorer neurodevelopment outcomes at 2 years' corrected age. They also reported that the growth of the CCL from birth and up to term-equivalent age was about half of that expected when compared with reference normative data for fetuses within the third trimester (0.11-0.13 mm/day v 0.20-0.27 mm/day, respectively), although they did not show that growth of the CCL from birth and up to term-equivalent age was related to neurodevelopmental outcomes at 2 years. However, in a follow up study of the same cohort, Anderson et al evaluated the growth of the CCL within 3 time epochs with respect to PNA: birth to 2 weeks, 2 to 6 weeks, and 6 weeks to term-equivalent age.[175] They found that the growth of the CCL was impaired in most preterm infants by 6 weeks' PNA, independent of GA, and that a slower growth of the CCL between 2 and 6 weeks' PNA was associated with poorer motor outcomes, including cerebral palsy, at 2 years. They also found that the growth of the VH correlated well with the growth of the CCL; this finding suggest that the VH may also be a potential biometric marker of later neurodevelopment in very preterm infants.

Overall, the aforementioned studies exploring the utility of cUS linear measures of brain growth to predict neurodevelopment in very preterm infants included small sample sizes, and were heterogeneous in methodology with regard to neonatal cUS, including which linear measures were used, and at which time points they were made within the neonatal period (Table 2.3). One study explored the relationships of linear measures of brain size, albeit limited to ventricle size and the biparietal diameter, at an early time point, namely 1-month PNA, whereas all the other studies applied linear measures at the later time point of term-equivalent age.[190] Only 2 studies, using the same cohort of infants, explored the utility of sequential linear measures of brain growth from birth and up to term-equivalent age.[174, 175] Methodology with regards to neurodevelopmental outcomes was relatively homogeneous between studies, with all studies including neurodevelopment assessment by the Bayley Scales (2nd or 3rd Editions), and 3 of the 6 studies including neurologic evaluation for cerebral palsy. Four of the 6 studies had follow up rates <90%. Whilst there is evidence that cUS has a role in predicting neurodevelopment, further research using prospective cohorts, with large sample sizes and high follow-up rates is needed.

Table 2.3 Relationships of neonatal cranial ultrasonography assessment of brain growth with neurodevelopmental outcomes in very preterm infants

Author, Year, Country	Sample	cUS	Follow up	Outcome
Skiold et al[161] 2018 Sweden	84 infants GA <27 weeks	Term-equivalent age Qualitative and quantitative scoring system, including 6 linear measures Ventricular measures: frontal horn short (anterior horn width) and long axes; ventricular midbody Extra-axial spaces: sinu-cortical width, interhemispheric distance Corpus callosum thickness	64 infants (and 85 term-controls) (76% follow up) 30 months BSID-III	Moderate to severe cUS abnormalities at term-equivalent age related to cerebral palsy (sensitivity 75% and specificity 90%) and severe cognitive delay (sensitivity 90% and specificity 100%). Negative predictive values of normal or mildly abnormal cUS at term-equivalent age for normal neurological signs or cognitive outcome were 98% and 100%, respectively.
Brouwer et al[188] 2014 The Netherlands	93 infants GA <31 weeks Excluded: infants with congenital abnormalities, or born outside referral district	Term-equivalent age Qualitative assessment: extra-axial space and ventriculomegaly	93 infants (100% follow up) 2 years BSID-III	Ex-vacuo ventriculomegaly at term-equivalent age related to worse cognitive (composite score β -9.2; 95% CI -14.6, -3.7; p=0.001), worse fine motor (scaled score β -2.2; 95% CI -3.5, -0.8; p=0.002), and worse gross motor (scaled score β -1.4; 95% CI -2.4, -0.5; p=0.003) outcomes. Enlarged extra-axial space related to worse gross motor (β -0.9; 95% CI -1.7, -0.1; p=0.033) outcome.
Fox et al[190] 2014 Australia	44 infants GA <30 weeks Excluded: infants with IVH (grades III or IV) or cPVL on cUS	1-month postnatal age Ventricular measures: anterior horn width, ventricular index, ventricular transverse width, anterior horn height, ventricular midbody; Biparietal diameter	44 infants (100% follow up) 2 years BSID-III Neurologic examination	Larger ventricular measures related to poorer motor composite scores, for example: decrease in motor composite score of mean (SE) of 3.3 (1.2) and 3.5 (1.3) for every 1 mm increase in mid-body height, right and left, respectively (p≤0.01 for both).

Author, Year, Country	Sample	cUS	Follow up	Outcome
Anderson et al[175] 2006 New Zealand	61 infants GA <33 weeks and birth weight <1500 g	Sequential scans from birth and up to term-equivalent age Corpus callosal length	46 infants (75% follow up) 2 years BSID-II Neurologic examination	Slower growth of corpus callosum length between 2 and 6 weeks' postnatal age related to serious motor delay [mean (SD) 0.04 (0.02) v 0.11 (0.05) mm/day; p=0.05] and cerebral palsy [mean (SD) 0.07 (0.05) v 0.12 (0.05); p=0.02).
Anderson et al[174] 2005 New Zealand	64 infants GA <33 weeks and birth weight <1500 g	Sequential scans within first week after birth and at term-equivalent age Corpus callosal length	55 infants (86% follow up) 2 years BSID-II Neurologic examination	Longer CCL at term-equivalent age observed in infants with MDI 85-100 [mean (SD) 44.3 (3.0) mm] compared with MDI 70-85 [mean (SD) 40.2 (2.6) mm], p=0.003, and for those infants with PDI 85-100 [mean (SD) 44.2 (3.1) mm] compared with PDI 70-85 [mean (SD) 41.7 (3.1) mm], p=0.017.
Horsch et al[187] 2005 Germany	81 infants GA <32 weeks or birth weight <1500 g Excluded: infants with chromosomal or congenital anomalies, congenital infections, or metabolic disorders	Term-equivalent age Qualitative assessment of "brain atrophy": enlarged extra-axial space (subarachnoid and interhemispheric spaces) and poor gyral maturity, with or without ventriculomegaly	64 infants (79% follow up) 3 years BSID-II	Brain atrophy versus no brain atrophy: MDI 91.8 v 101.9 (p=0.02); PDI 91.4 v 106.5 (p=0.001); Behaviour Rating Scale 41.1 v 66.4 (p=0.01)

β=coefficient; BSID=Bayley Scales of Infant Development (II=2nd Edition; III=3rd Edition); cPVL=cystic periventricular leukomalacia; cUS=cranial ultrasonography; GA=gestational age; HINE=Hammersmith Infant Neurological Examination; IVH=intraventricular haemorrhage; MDI=Mental Developmental Index; PDI=Psychomotor Developmental Index; SD=standard deviation.

2.4.3 Ultrasonography Linear Measures of Fetal Brain Growth

Routine obstetric ultrasonography of the developing fetus throughout gestation includes several linear measures of various body structures, including intracranial brain tissue and fluid spaces, used in clinical practice to assess fetal maturity, and to screen fetuses for abnormal growth and development.[191, 192] Data describing "typical" fetal brain growth and development, particularly within the third trimester of gestation from 24 to 40 weeks' post-menstrual age, provide normative reference values that allow comparison with preterm infant postnatal brain growth and development.[193-208] Table 2.4 summarises available published data for linear measures of fetal brain growth, derived from brain tissue and fluid spaces, made from ultrasonography of the developing fetus throughout gestation. Methodologically, unless otherwise stated, the studies described in Table 2.4 have the following in common: (1) cross-sectional study by design, that is, linear measures were made from ultrasonography performed on fetuses at one time-point alone; (2) included apparently normal, or uncomplicated, pregnancies, where ultrasonography was indicated for the clinical assessment of fetal morphometry, well-being, or growth; (3) included apparently normal fetuses, with no fetal anomalies, including normal cerebral development; (4) gestational age was based on the known first day of the last menstrual cycle, and/or confirmed by early ultrasonography assessment by fetal biometry. There are some notable differences, and limitations, between studies reporting ultrasonography linear measures of fetal brain growth. Brain growth, that is change in linear measure over time with respect to post-menstrual age, is not always reported, but can often be inferred from the mean measurements of the linear measures made at each GA, with a range and/or 5^{th} and 95^{th} centiles of measurements reported for many of the linear measures. Studies have included a widely variable number of fetuses (from <100, to >24000), with many of the smaller studies including only very few fetuses, and therefore measurements, at any given time-point with respect to GA. Moreover, although most studies report to have included only "normal" fetuses, that is, fetuses without anomalies, no studies have reported on the use of other fetal measures relied on to exclude intracranial, chromosomal, or other anomalies, including MRI and molecular karyotyping, nor have they included postnatal assessments of the infant, including neurologic and neurodevelopmental assessments.

Table 2.4 Ultrasonography linear measures of fetal brain growth

Author, Year, Country	Sample	Linear measure (imaging plane)	Growth (mm/week PMA)
Brain tissue			
Cerebral hemispheres			
Kurmanavicius et al[193] 1999 Switzerland	6557 singleton fetuses GA 12 – 42 weeks	Biparietal diameter (transverse) Occipito-frontal diameter (transverse)	2.10 mm/week PMA (GA 23 – 42 weeks) 2.18 mm/week PMA (GA 23 – 42 weeks)
Corpus callosum			
Goldstein et al[194] 2011 Israel	252 AGA and 24 symmetric growth restricted (estimated fetal weight <10th centile) fetuses GA 16 – 36 weeks	Corpus callosum length (sagittal)	1.8 mm/week PMA (AGA fetuses) 1.6 mm/week PMA (growth restricted fetuses)
Zhang et al[195] 2009 China	622 singleton fetuses GA 16 – 39 weeks	Corpus callosum length (sagittal)	1.495 mm/week PMA
Achiron and Achiron[196] 2001 Israel	270 AGA fetuses GA 16 – 37 weeks	Corpus callosum length (sagittal) Corpus callosum thickness (coronal) Corpus callosum width (coronal)	1.92 mm/week PMA 0.085 mm/week PMA 0.225 mm/week PMA
Malinger and Zakut[197] 1993 Israel	101 singleton fetuses, excluding 50 fetuses with growth restriction GA18 – 42 weeks	Corpus callosum length (sagittal) Corpus collosum genu width (sagittal) Corpus callosum body width (sagittal) Corpus callosum splenium width (sagittal)	1.29 mm/week PMA 0.12 mm/week PMA 0.06 mm/week PMA 0.11 mm/week PMA
Cerebellum			
Chavez et al[198] 2007 United States	55 growth restricted (estimated fetal weight <10th centile) and 16 large-for-gestational age (estimated fetal weight >90th centile) fetuses GA 14 – 40 weeks	Transcerebellar diameter (transverse)	Similar to AGA fetuses, see Chavez et al 2004[200]
Chavez et al[199] 2006 United States	Twin pregnancies, including 250 monochorionic and 1028 dichorionic twin fetuses GA 14 – 38 weeks	Transcerebellar diameter (transverse)	Similar to AGA fetuses, see Chavez et al 2004[200]
Chavez et al[200] 2004 United States	2597 singleton fetuses GA 14 38 weeks	Transcerebellar diameter (transverse)	1.59 mm/week PMA (GA 23 – 38 weeks)
Chavez et al[201] 2003 United States	24026 singleton fetuses GA 14 – 38 weeks	Transcerebellar diameter (transverse)	1.59 mm/week PMA (GA 23 – 38 weeks)

Author, Year, Country	Sample	Linear measure (imaging plane)	Growth (mm/week PMA)
Zalel et al[202] 2002 Israel	256 singleton fetuses GA 18 – 38 weeks	Vermis width (axial) Vermis height (sagittal)	0.61 mm/week PMA 0.55 mm/week PMA
Malinger et al[203] 2001 Israel	101 singleton fetuses GA 21 – 39 weeks	Vermis antero-posterior diameter (sagittal) Vermis cranio-caudal diameter (sagittal)	0.7 mm/week PMA 0.7 mm/week PMA
Vinkesteijn et al[204] 2000 The Netherlands	360 singleton AGA (estimated fetal weight >10th centile) and 73 growth restricted (estimated fetal weight <5th centile) fetuses GA 17 – 34 and GA 24 - 34 weeks, respectively	Transcerebellar diameter (quasi-transverse)	1.89 mm/week PMA (GA 23 – 34 weeks)
Snijders et al[205] 1994 United Kingdom	103 small-for-gestational age infants, with growth restriction presumed to be from placental insufficiency GA 19 – 39 weeks	Transcerebellar diameter (?plane)	1.25 mm/week PMA (GA 20 – 40 weeks)
Hill et al[206] 1990 United States	675 AGA and 92 large-for-gestational age fetuses GA 14 – 42 weeks	Transcerebellar diameter (transverse)	1.65 mm/week PMA (GA 23 – 40 weeks)
Hata et al[207] 1989 Japan	116 singleton fetuses GA 17 – 40 weeks	Transcerebellar diameter (transverse)	1.44 mm/week PMA
Fluid spaces			
Extra-axial space			
Malinger et al[208] 2000 Israel	80 singleton fetuses GA 16 – 40 weeks	Sub-arachnoid space (coronal)	Sino-cortical width constant Cranio-cortical width increased between 20-28 weeks' PMA, thereafter decreased
Ventricles			
Cardoza et al[209] 1988 United States	100 singleton fetuses GA 14 – 38 weeks	Lateral ventricular atrium width (transverse)	Constant between 14 and 38 weeks' PMA
Denkhaus and Winsberg[210] 1979 Germany	178 fetuses GA 13 – 40 weeks	Bifrontal horn width (transverse)	0.48 mm/week PMA

AGA=appropriate for gestational age; GA=gestational age; PMA=post-menstrual age.

2.4.3.1 Ultrasonography Linear Measures of Fetal Brain Tissue

2.4.3.1.1 Corpus Callosum

The corpus callosum is a major white matter structure of the brain, forming the largest commissure of axons connecting the cortex of one cerebral hemisphere to the other.[196, 197] It is bordered superiorly by the interhemispheric fissure, inferiorly by cavum septum pellucidum, and laterally by the lateral ventricles. The corpus callosum begins its development in the 5th gestational week as a commissural plate within the lamina terminalis.[196] The earliest commissural axons can be identified from the 10th gestational week, with development progressing rostro-caudally to form the genu, followed by the splenium.[196] The morphology of the corpus callosum, in a form similar to that seen in the adult, can reliably be seen on ultrasound from the 16th gestational week provided appropriate imaging views can be obtained.[197] As a high-order cerebral structure that continues to develop relatively late in gestation, the corpus callosum provides a sensitive marker of fetal brain development and maturation. Consequently, aberrant corpus callosum appearance or growth can be a harbinger of other cerebral and fetal anomalies, as seen in partial or total agenesis of the corpus callosum.[211, 212]

Four studies have documented fetal growth of the corpus callosum, from as early as 16 weeks' post-menstrual age and up to term, using linear measures made on ultrasonography.[194-197] All studies measured corpus callosum length in the sagittal plane, measuring the rostro-caudal length from the most anterior margin of the genu to the most posterior margin of the splenium. Growth in the length of the corpus callosum ranged from 1.29 to 1.92 mm/week post-menstrual age, with a slower growth from approximately 21-23 weeks' GA compared with earlier. The wide range in growth rates reported between studies may be explained by differences in methodology. It is plausible that the slower rate of growth in corpus callosum length reported by Malinger and Zakut reflects the absence of data before 18 weeks' GA, and the smaller numbers of fetuses included in the lower GA groups, compared with the other studies.

Goldstein et al's study was the only study to include growth restricted fetuses, reporting that corpus callosum length was below the 25th centile in 77.3%, and below the 50th centile in 95.5%, of growth restricted fetuses (n=24), compared with appropriate-for-

gestational age fetuses (n=252), at any time-point between 16 and 36 weeks' post-menstrual age ($p<0.001$).[194] They also found that the growth of the corpus callosum length was slower in growth restricted fetuses compared with appropriate-for-gestational age fetuses, but the statistical significance of this finding was not stated (1.6 v 1.8 mm/week post-menstrual age; p value not stated).

2.4.3.1.2 Cerebellum

The cerebellum, comprised of the vermis and cerebellar hemispheres, is positioned in the posterior fossa. The vermis begins its development in the 9th week of gestation, with fusion of the rhombic lips superiorly in the midline extending inferiorly until closure of the vermis by the 15th week of gestation.[213] Some studies report that the postero-inferior aspect of the vermis can remain open until the 18th week of gestation; fetal ultrasonography assessment for posterior fossa abnormalities before this time may overestimate vermis pathology, including partial agenesis of the vermis.[213] Moreover, routine fetal ultrasonography in the axial plane can poorly characterise the posterior fossa, particularly as optimal imaging of the vermis requires angulation of the probe to a near sagittal plane to adequately image the superior and inferior lobes of the vermis.[203] This can give the appearance of an enlarged cisterna magna, or incomplete vermis, again leading to the overestimation of vermis pathology. Imaging in the mid-sagittal plane affords the opportunity to visualise the vermis, the 4th ventricle, and the cisterna magna in one plane, as described by Courchesne, but this view is often difficult to obtain in the fetus unless imaging is performed trans-vaginally.[214]

Two studies have documented fetal growth of the cerebellar vermis.[203, 213] Both studies applied different linear measures to assess growth of the vermis. Malinger et al measured the antero-posterior and cranio-caudal diameters of the vermis in the mid-sagittal plane using trans-vaginal ultrasonography, reporting growth rates of both linear measures to be 0.7 mm/week post-menstrual age.[203] Zalel et al, using a trans-abdominal approach, measured vermis width in the axial plane and vermis height in the mid-sagittal plane, reporting vermis growth to be 0.61 and 0.55 mm/week post-menstrual age, respectively.[213]

Transcerebellar diameter, measured by ultrasonography in the transverse plane, is the most reliable linear measure for estimating GA.[200] The transcerebellar diameter has been

shown in several studies to correlate with post-menstrual age, even in the presence of growth restriction, in small- and large-for-gestational age fetuses, and in multiple gestations.[198, 199, 204-206]

2.4.3.2 Ultrasonography Linear Measures of Fetal Fluid Spaces

Three studies have evaluated ultrasonography linear measures of fetal fluid spaces.[208, 209] Malinger et al measured sub-arachnoid space in the coronal plane.[208] Sino-cortical width remained constant (mean 3.0 mm, SD 0.9 mm), whereas the cranio-cortical width increased between 20 and 28 weeks' post-menstrual age, thereafter decreasing again. However, neither sino-cortical nor cranio-cortical width correlated with GA. Lateral ventricular atria width in Cardoza et al's study was reported to remain relatively stable (mean 7.6 mm, SD 0.6 mm) from 14 to 38 weeks' post-menstrual age, providing a useful normative reference value to define fetal ventriculomegaly, albeit arbitrarily, using a lateral ventricular atria width >10 mm at any time-point within the second and third trimesters of gestation.[209] Denkhaus and Winsberg reported a correlation between the measurement of the bifrontal horns and GA from 13 weeks' post-menstrual age (11 mm) and up to term (24 mm); this linear measure is the fetal equivalent of the combined left and right Levene indices measured postnatally to assess for PHVD.[119, 210]

2.5 Research Gap

cUS is the most frequently used neuroimaging modality for very preterm infants, but its clinical application to screening for overt, focal brain injuries may be limiting its ability to predict neurodevelopmental outcomes. Findings of major brain injury on neonatal cUS are highly specific for later motor, but not cognitive, outcomes, however, its positive predictive value for cerebral palsy is only fair at best, and likely much less so for non-motor outcomes. What then for the predictive utility of the apparently normal cUS? The use of frequent cUS, from the early days after birth and up to term-equivalent age, has been shown to improve the negative predictive value for cerebral palsy to 99%, but even then, the utility of this scanning regimen to predict the more common findings of cognitive deficits in children born very preterm is unknown. Sequential cUS affords the opportunity to evaluate early postnatal brain

growth as a potential marker of later neurodevelopment that has not been fully explored. There is evidence that several linear measures of brain size and fluid spaces made on cUS and MRI within the neonatal period are related to cognitive and motor impairments and developmental delays following very preterm birth. However, cUS studies that have explored the use of linear measures have been invariably small, or limited to few linear measures, and linear measures mostly made at term-equivalent age. There is a need to explore the utility of a broader range of linear measures of brain tissue and fluid spaces, made from sequential cUS from the first days after birth and up to term-equivalent age, to better understand the process of brain growth in the early postnatal period following very preterm birth, and to evaluate the utility of linear measures of early postnatal brain growth for the prediction of neurodevelopmental outcomes in children born very preterm and without major brain injury seen on neonatal cUS. Chapter 3 details the aims and hypothesis that I have explored in my thesis, with Chapter 4 detailing the methodology of how I have used sequential cUS performed as part of the routine clinical care of infants born at <30 weeks' GA, without major brain injury seen on neonatal cUS, to evaluate relationships with neurodevelopment at 2 years.

Chapter 3

Aims and Hypotheses

Chapter Overview

In this chapter, I detail the aims and hypotheses of my thesis, and provide only a brief description of the methods I undertake to answer each aim. Chapter 4 describes the general methodology of my thesis in greater detail, including study design, process for acquisition of cUS and linear measures, and developmental assessments within the NICU and at follow-up at 2 years. Chapters 5, 6, and 7 present the results of my thesis; Chapter 5 addresses the first 3 aims, Chapter 6 relates to aim 4, and Chapter 7 pertains to the final aim.

3.1 Aim 1

To assess the reproducibility of linear measures made from cUS performed as part of routine clinical care in infants born at <30 weeks' GA

3.1.1 Hypothesis

Simple linear measures of brain tissue and fluid spaces made from standard imaging planes on cUS will be highly reproducible.

3.1.2 Rationale

cUS linear measures should be simple to measure and reproducible if they are to be explored in research, and used in clinical practice, as potential markers of early postnatal brain growth and long-term neurodevelopment. Several linear measures of brain tissue and fluid spaces made from cUS are already used in the clinical care of the newborn infant. However, there are limited data for the reproducibility of a broader range of linear measures made from cUS performed as part of routine clinical care in infants born at <30 weeks' GA.

3.1.3 Methods

Linear measures of brain tissue and fluid spaces were chosen based on their potential clinical importance, ease of recognition on standard imaging planes, and well-defined anatomical landmarks. Intra- and inter-observer reproducibility of linear measures was assessed by the intra-class correlation coefficient and Bland-Altman plot analysis. Linear measures with excellent intra- and inter-observer reproducibility – defined by ICC >0.80 – were considered for use in the current study.

3.2 Aim 2

To evaluate early postnatal brain growth using linear measures made from cUS performed as part of routine clinical care in infants born at <30 weeks' GA and without major brain injury on neonatal cUS

3.2.1 Hypothesis

Simple and reproducible linear measures of brain tissue and fluid spaces made from cUS will be useful to quantify early postnatal brain growth in infants born at <30 weeks' GA free of major brain injury on neonatal cUS. Sequential linear measures of brain tissue and fluid spaces will increase with increasing PNA, with the absolute size and rates of change of the linear measures demonstrating variable rates of change across the early postnatal period.

3.2.2 Rationale

Infants who are born very preterm have smaller brain tissue volumes and larger fluid spaces on brain MRI at term-equivalent age compared with term-born infants. However, early postnatal brain growth following very preterm birth remains poorly understood. Linear measures of brain tissue and fluid spaces made from sequential cUS, performed from as early as birth and up to term-equivalent age, may provide important insights as to the usefulness of these linear measures to describe patterns of postnatal brain growth following very preterm birth.

3.2.3 Methods

Linear measures of brain tissue and fluid spaces were made from sequential cUS performed as part of routine clinical care. Brain size (mm) was assessed at various time-points with respect to PNA, including birth, 1-week, 1-month, 2-months, and at term-equivalent age. Brain growth (mm/week PNA) was assessed within

various time-epochs with respect to PNA, including from birth to 1-week, 1-week to 1-month, 1-month and up to term-equivalent age, and birth and up to term-equivalent age. Descriptive statistics were used to present data on brain size across time-points with respect to PNA. Mixed effects models were used for the evaluation of brain growth within time-epochs with respect to PNA to account for multiple measurements within individuals.

3.3 Aim 3

To examine the relationships between cUS linear measures of early postnatal brain growth and perinatal determinants of long-term neurodevelopment in infants born at <30 weeks' GA and without major brain injury on neonatal cUS

3.3.1 Hypothesis

cUS linear measures of early postnatal brain growth will be related to perinatal determinants of long-term neurodevelopment in infants born at <30 weeks' GA free of major brain injury on neonatal cUS.

3.3.2 Rationale

Long-term neurodevelopmental impairments observed in children born very preterm are related to perinatal brain injury and alterations in early postnatal brain growth and maturation. It is likely that the neurodevelopmental sequelae of preterm birth are mediated by the influence of one or more perinatal variables, including infant demographics and morbidities, on early postnatal brain growth and maturation.

3.3.3 Methods

Relationships between cUS linear measures of early postnatal brain growth, from as early as birth and up to term-equivalent age, and perinatal determinants of long-term neurodevelopment were examined separately using interaction terms in mixed effects models to account for multiple measurements within individuals. Perinatal variables examined included GA at birth, BW z score, sex, multiple gestation, antenatal corticosteroids, antenatal magnesium sulphate, chorioamnionitis, bronchopulmonary dysplasia, postnatal corticosteroids, sepsis and/or necrotising enterocolitis, neonatal surgery, and low-grade IVH, and were included individually as interaction terms in the mixed effects models.

3.4 Aim 4

To explore the relationships between cUS linear measures of early postnatal brain growth and neurobehaviour at term-equivalent age in infants born at <30 weeks' GA and without major brain injury on neonatal cUS

3.4.1 Hypothesis

Neurobehaviour at term-equivalent age will be related to cUS linear measures of early postnatal brain growth in infants born at <30 weeks' GA free of major brain injury on neonatal cUS.

3.4.2 Rationale

Neurobehavioural assessment of very preterm infants at term-equivalent age provides an opportunity within the neonatal period to identify infants at risk for later

neurodevelopmental impairments. Neonatal neurobehaviour reflects the neuronal integrity of the developing brain, and can be reliably evaluated using validated and standardised assessment tools. Infants who are born very preterm are more likely to demonstrate less mature neurobehaviour compared with term-born infants, even in the absence of overt focal pathology seen on their neonatal cUS.

3.4.3 Methods

Infant neurobehaviour at term-equivalent age was assessed using Prechtl's Qualitative Assessment of General Movements and the Hammersmith Neonatal Neurological Examination. Relationships between neurobehaviour at term-equivalent age and cUS linear measures of early postnatal brain growth, from as early as birth and up to term-equivalent age, were explored using logistic regression models, fitted using generalised estimating equations.

3.5 Aim 5

To explore the relationships between cUS linear measures of early postnatal brain growth and neurodevelopment at 2 years' corrected age in infants born at <30 weeks' GA and without major brain injury on neonatal cUS

3.5.1 Hypothesis

Neurodevelopment at 2 years will be related to cUS linear measures of early postnatal brain growth in infants born at <30 weeks' GA free of major brain injury on neonatal cUS.

3.5.2 Rationale

Very preterm infants are at risk of long-term adverse neurodevelopment related to perinatal brain injury and altered brain growth and maturation. cUS linear measures of early postnatal brain growth could be potential biomarkers of later motor and cognitive impairments, providing clinicians with an early opportunity to identify high risk infants who may benefit from ongoing developmental surveillance and, where appropriate, early intervention therapy.

3.5.3 Methods

Neurodevelopment at 2 years was assessed using the cognitive, language, and motor scales of the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) by assessors blinded to the clinical history. Relationships between neurodevelopment at 2 years and cUS linear measures of early postnatal brain growth, from as early as birth and up to term-equivalent age, were explored using linear and logistic regression models, fitted using generalised estimating equations.

Chapter 4

General Methodology

Chapter Overview

This chapter provides a detailed description of the general methodology I have used to address each of the aims of my thesis. It describes the study design, acquisition of cUS, developmental assessment tools, and statistical methods.

4.1 Study Design

The current study was nested within a prospective longitudinal observational cohort study of 149 infants born at <30 weeks' GA, and 201 term-born controls called VIBeS-2 (Victorian Infant Brain Studies). The VIBeS-2 Study aimed to explore the relationships between neonatal neuroimaging, neurobehaviour, and long-term neurodevelopment, and parental psychological well-being.[215] Infants were recruited from a single centre, The Royal Women's Hospital, Melbourne – one of 3 perinatal NICUs in Melbourne – between January 2011 and December 2013. The Royal Women's Hospital is a tertiary perinatal centre where there are approximately 7,500 live-births per year, including approximately 120 infants born at <30 weeks' GA.

4.1.1 Participants and Recruitment

To be eligible for inclusion, preterm participants had to be born at <30 weeks' GA, and term-born controls between 37 and <42 weeks' GA. GA was determined by either ultrasonography within the first trimester or, where this was unavailable, the timing of the last menstrual period. Infants were excluded if they met one or more of the following criteria:

- Suspected or confirmed congenital or chromosomal abnormalities known to affect long-term neurodevelopment;
- Assessed by the treating clinicians to be critically unwell and not expected to survive to discharge from the NICU; or
- Non-English speaking parents, as there was no funding available for interpreter services.

For this thesis, preterm participants who had evidence of major preterm brain injury seen on their neonatal cUS were also excluded to provide an inception cohort with apparently "normal" neonatal cUS. The rationale for this was to explore the prognostic utility of cUS linear measures, without the addition of major brain injury

seen on cUS. Major preterm brain injury was defined by one or more of the following overt focal pathologies seen on cUS performed as part of clinical care:

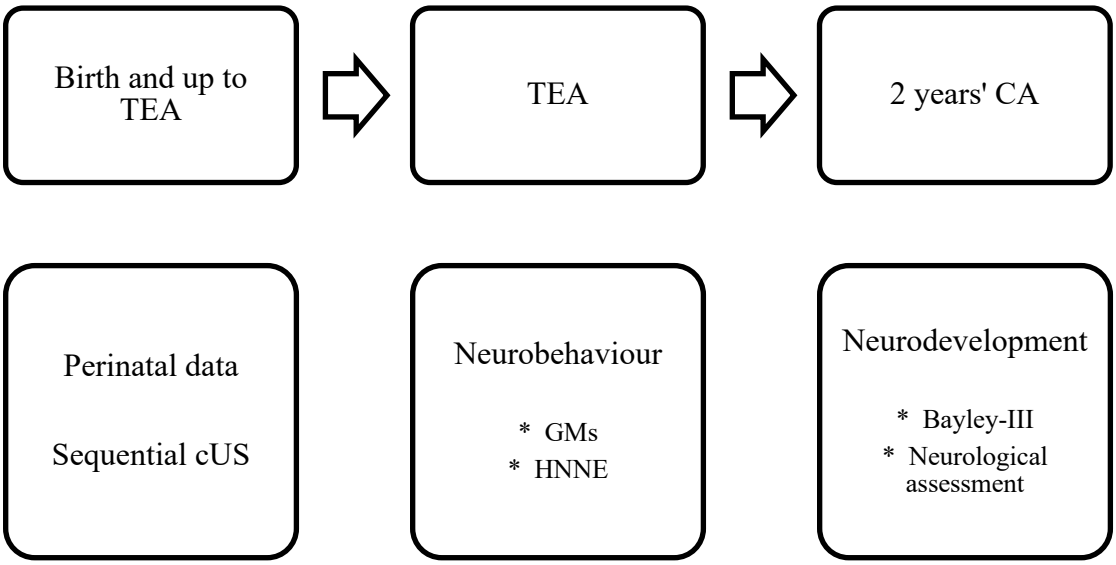
- High-grade IVH, defined by grades III or IV IVH according to the classification system described by Papile;[98]
- Post-haemorrhagic hydrocephalus, defined by Levene Index >95th centile plus 4 mm for post-menstrual age *and* anterior horn width >10 mm;[216]
- Cerebellar haemorrhage;
- Arterial ischaemic stroke; or
- Cystic PVL, defined by grades II, III, or IV according to the classification system described by de Vries.[93]

The study was approved by the Human Research Ethics Committees of the relevant institutions. Study coordinators approached the parents of all eligible infants as soon as practicable after their birth, and written informed consent was obtained from the parents of all participants.

4.1.2 Study Protocol and Timeline

The protocol and timeline of the current study is detailed in Figure 4.1. Preterm participants had sequential cUS performed as part of their clinical care, performed from as early as the first days after birth and up to term-equivalent age, if they were still inpatients at the Royal Women's Hospital. Term-born controls did not have cUS performed. Outcomes of interest in the current study included neurobehaviour at term-equivalent age and neurodevelopment at 2 years' corrected age.

Figure 4.1 Study protocol and timeline



Bayley-III=Bayley Scales of Infant and Toddler Development, 3rd Edition; CA=corrected age; cUS=cranial ultrasonography; GMs=Prechtl's Qualitative Assessment of General Movements; HNNE=Hammersmith Neonatal Neurological Examination; TEA=term-equivalent age.

4.2 Cranial Ultrasonography

4.2.1 Equipment and Personnel

cUS was performed using a General Electric Logiq 9 ultrasonography machine (GE Healthcare Technologies, Waukesha, Wisconsin, USA) and an 8 MHz broadband curvilinear transducer. The frequency, focal point, and depth of cUS were adjustable at time of imaging by the sonographer. Standard images were acquired through the anterior fontanelle (5 images in the coronal plane, and 5 images in the sagittal and parasagittal planes) and the mastoid fontanelle (1 image in the coronal plane). Additional imaging may have been performed in individual infants where the sonographer or clinicians had suspected or confirmed intracranial pathology, including the use of supplementary acoustic windows, including the posterior and temporal fontanelles, and the foramen of Magnum. Images were stored electronically

at the time of acquisition using Synapse (Fujifilm Medical Company, Tokyo, Japan), a patient archiving and communications system.

cUS was performed by neonatal paediatricians, and advanced neonatal paediatric trainees under the supervision of neonatal paediatricians, all of whom had received formal training in cUS under the auspices of the Australasian Society for Ultrasound in Medicine and had between 6 months and 20 years' experience in neonatal cUS. Scans were reviewed for brain injury by a neonatal paediatrician with formal training and accreditation in diagnostic ultrasonography and 20 years' experience in neonatal cUS.

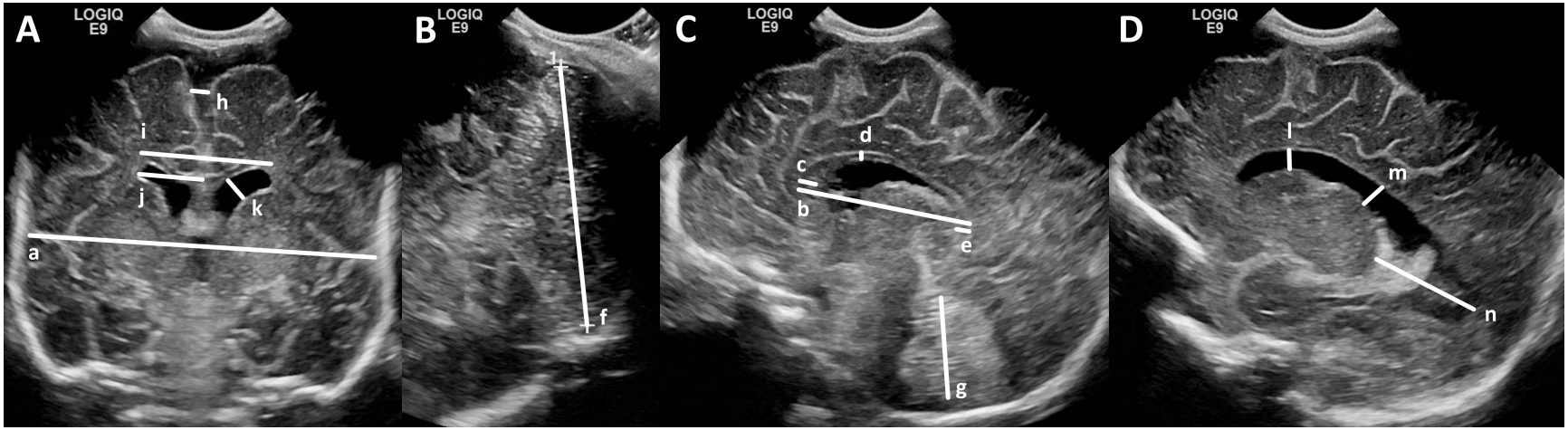
4.2.2 Protocol for Routine Cranial Ultrasonography

As per local protocol for the surveillance of preterm brain injury, infants born at <30 weeks' GA were scanned on, or around, 7, 28 and 60 days' PNA. Infants born at <28 weeks' GA were also scanned on, or around, day 1 PNA. Additional cUS was performed depending on clinical need, including at times where clinicians had suspected intracranial pathology based on the infant's disposition, for example, clinical deterioration, including cardiorespiratory compromise or seizures, or where more frequent scans were warranted to screen infants for complications of confirmed intracranial pathology, for example, post-haemorrhagic hydrocephalus in the setting of IVH.

4.2.3 Linear Measures Made from Cranial Ultrasonography

Nineteen linear measures of brain tissue and fluid spaces were explored in the current study on the basis of their potential clinical importance and apparent ease of measurement using readily identifiable anatomical landmarks on standard imaging planes (Figure 4.3 and Table 4.1). Selected linear measures were based upon those reported by others in research studies (see Chapter 2, Section 2.4), several of which are commonly used in clinical practice.[119, 121, 175, 177, 190, 217] For example, the Levene Index (also known as the Ventricular Index) and AHW are used to guide management of the infant with post-haemorrhagic hydrocephalus, and the TCD, if measured within the first days after birth, can be used to estimate GA.[119, 200, 216]

Figure 4.2 Linear measures made from cranial ultrasonography



A=anterior fontanelle, coronal plane, level of the foramina of Monro; B=mastoid fontanelle, coronal plane, posterior to the fourth ventricle; C=anterior fontanelle, sagittal midline plane; D=anterior fontanelle, parasagittal plane, frontal, occipital, and temporal horns of the lateral ventricle.

Linear Measures of Brain Tissue: (a) biparietal diameter; (b) corpus callosum length; (c) corpus callosum genu width; (d) corpus callosum body width; (e) corpus callosum splenium width; (f) transcerebellar diameter; (g) vermis height.
Linear Measures of Fluid Spaces: (h) interhemispheric distance; (i) ventricular width; (j) ventricular index; (k) anterior horn width; (l) anterior horn height; (m) ventricular midbody width; (n) thalamo-occipital distance.

Table 4.1 Linear measures made from cranial ultrasonography

Linear measure	Abbreviation	Anatomical landmarks
Brain tissue		
Biparietal diameter	BPD	Distance between the most lateral margins of the cerebral hemispheres at the level of the Sylvian fissures
Corpus callosum length	CCL	Distance between the most anterior and most posterior margins of the corpus callosum
Corpus callosum genu width	CCGW	Distance between the most anterior and most posterior margins of the genu of the corpus callosum
Corpus callosum body height	CCBH	Distance between the most superior and most inferior margins of the mid-body of the corpus callosum
Corpus callosum splenium width	CCSW	Distance between the most anterior and most posterior margins of the splenium of the corpus callosum
Transcerebellar diameter	TCD	Distance between the most lateral margins of the cerebellum
Vermis height	VH	Distance between the most antero-superior and postero-inferior margins of the cerebellar vermis
Fluid spaces		
Interhemispheric distance	IHD	Distance between the most medial margins of the most superior gyri
Anterior horn width, left and right	AHW	Diagonal distance between the most superior and most inferior margins of the lateral ventricle
Levene Index, left and right	LI	Distance between the most lateral margin of the lateral ventricle and the falx
Ventricular width	VW	Distance between the most lateral margins of the left and right lateral ventricles
Anterior horn height, left and right	AHH	Distance between the most superior and most inferior margins of the frontal horn of the lateral ventricle
Ventricular mid-body height, left and right	VMBH	Diagonal distance between the most superior and most inferior margins of the mid-body of the lateral ventricle
Thalamo-occipital distance, left and right	TOD	Diagonal distance between the most anterior and most posterior margins of the occipital horn of the lateral ventricle

All episodes of cUS performed for each preterm participant during their admission to the NICU were routinely archived on Synapse (Fujifilm Medical Company, Tokyo, Japan), providing the primary data source for cUS used in the current study. For each cUS episode, linear measures were made once only by myself (primary observer), using electronic callipers (millimetres, mm; to 2 decimal places) on the digitised images stored on Synapse (Fujifilm Medical Company, Tokyo, Japan), and viewed on a desktop computer screen. Digitised images were not manipulated, for example by using the zoom function, for the purpose of making measurements. For linear measures of brain tissue, the callipers were placed on the outer margins of the echoes, whereas for linear measures of fluid spaces, the callipers were placed on the inner margins of the echoes.

The series of images acquired for any given episode of cUS were reviewed for quality. Although in most cases the standard images needed to make all linear measures were acquired and stored, there were several instances where the quality of the digitised image was assessed to be inadequate, and the linear measures were not made. The reasons for this included:

- Image not acquired in the standard plane;
- Image not centred in the correct plane;
- Margins of the linear measure not clearly, or entirely, visible; or
- Image not stored.

4.3 Perinatal Variables

Perinatal variables, to be explored in relation to early postnatal linear measures made from cUS, were chosen *a priori* on the basis of their known associations with perinatal brain injury in infants who are born very preterm, and long-term neurodevelopmental outcomes following very preterm birth (Table 4.2) (see Chapter 1, Section 1.3.1).[45, 56, 57, 65]

Table 4.2 Perinatal variables

Perinatal variable	Comment
Gestational age at birth	Determined by first trimester ultrasonography where available, or by menstrual history
Birth weight	
Birth weight z score	Calculated using The British Growth Reference norms for gestational age at birth and sex[218]
Small-for-gestational age	Birth weight z score <-2 SD below the mean
Sex	Female or male
Multiple birth	Singleton or multiple birth
Chorioamnionitis	Confirmed by placental histology, available only in 74% of the cohort
Antenatal corticosteroids	Administered to the mother at any time prior to birth; given as any number of doses of betamethasone
Antenatal magnesium sulphate	Administered to the mother intrapartum for either maternal or fetal indications
Bronchopulmonary dysplasia	Defined by oxygen requirement at 36 weeks' post-menstrual age
Postnatal corticosteroids	Administered to the infant to facilitate extubation; given as a variable course of dexamethasone with respect to dose and duration, and may have included repeat courses
Sepsis, confirmed	Blood or cerebrospinal fluid culture-positive and use of antibiotics for ≥5 days
Necrotising enterocolitis	Stage ≥2 modified Bell's criteria[219]
Low-grade intraventricular haemorrhage, grades 1 or 2	Detected on cranial ultrasonography using Papile classification[98]

SD=standard deviation.

4.4 Neurologic and Neurodevelopmental Outcomes

4.4.1 Neurobehaviour at Term-Equivalent Age

Neonatal neurobehaviour reflects an infant's neurological integrity, and its assessment by standardised assessment tools has been shown to predict risk of long-term motor and cognitive outcomes for infants born very preterm (see Chapter 1,

Section 1.3.2).[70] In the VIBeS-2 Study, preterm participants were assessed at multiple time-points from the first weeks after birth and up to term-equivalent age, and term-controls were assessed at term-equivalent age, using the most common of the neonatal neurobehaviour assessment tools, including:

- Prechtl's Qualitative Assessment of General Movements (GMs); and
- Hammersmith Neonatal Neurological Examination (HNNE).

Both neurobehavioural assessments were used as they can provide different, but complementary, information about the neurological integrity of the infant, and therefore help the clinician to discriminate infants at high risk of adverse neurodevelopment, and one involves handling (HNNE) whereas the other may be performed by observation alone (GMs), affording greater opportunities for assessment.

For the current study, only the neurobehavioural assessments performed at term-equivalent age, that is, between ≥38 and <44 weeks' post-menstrual age, were used as a short-term neurologic outcome to interrogate their relationships with early postnatal brain growth from the early days after birth and up to term-equivalent age.

Neurobehavioural assessments were performed by an experienced, and appropriately certified, multidisciplinary team of developmental therapists, nursing, and medical professionals, blinded to the participants' clinical history, including cUS.

4.4.1.1 General Movements

In the current study, writhing GMs at term-equivalent age were categorised as either normal or abnormal, providing a dichotomous outcome variable. Abnormal GMs at term-equivalent age encompassed those infants with either poor repertoire, cramped synchronised, or chaotic GMs.[71] GMs were assessed from video recordings of the infant in the appropriate behavioural state to assess their spontaneous movements; this meant that the infant was alert, but not fussing or crying. Two observers independently assessed the infant's video recording of GMs. Using their GMs assessments of 80 infants included in the study, the two observers

demonstrated 90% inter-observer agreement, with a Cohen's kappa value of 0.75 based on the 8 instances where they had disagreed.[74] Where there was disagreement between the two observers, a third observer, with greater experience in assessing GMs, and who is a qualified GMs trainer with the GMs Trust, was the final arbiter of the infant's assessment category.

4.4.1.2 Hammersmith Neonatal Neurological Examination

In the current study, the HNNE total score at term-equivalent age was used as a categorical outcome variable by dichotomising the preterm participant cohort into "suboptimal" and "optimal" categories based on the $<10^{th}$ and $\geq 10^{th}$ centiles, respectively, for the total scores obtained within the contemporaneous cohort of 201 term-born controls.[84] This assumes that the HNNE total scores obtained in our population of term-born infants can be applied to assess the optimality of neurobehaviour for infants born very preterm. Studies have shown that preterm infants assessed at term-equivalent age demonstrate characteristic differences across several items of the HNNE compared with term-born infants.[80, 81] One of a pool of 6 observers assessed the HNNE of all participants, blinded to their clinical course, including cUS. The observers independently scored the HNNE immediately after their assessment.

4.4.2 Neurodevelopment at 2 Years

Preterm participants and term-controls underwent the following assessments at 2 years' corrected age, conducted by an experienced team of developmental therapists and paediatricians who were blinded to the participant's clinical history, including cUS:

- Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III);[220] and
- Neurological examination, specifically assessing for the presence and severity of cerebral palsy.

4.4.2.1 Bayley Scales of Infant and Toddler Development, Third Edition

The Bayley-III is a standardised assessment tool of a child's development within the following 3 domains:

- Cognition;
- Language: receptive and expressive; and
- Motor: fine and gross.

The scores obtained within each of the developmental domains were used as both a continuous and a categorical outcome variable; the former, by way of the composite scores, and the latter, by dichotomising the preterm participant cohort into "delay" and "no delay" categories based on <-1 SD and ≥-1 SD below the mean, respectively, of the composite scores obtained within the contemporaneous cohort of term-born controls. The reason for using our own reference normative values, rather than the test norm mean (SD) of 100 (15), is based on the finding of an underestimation of developmental delay in Australian children using reference test norms.[221, 222]

4.4.2.2 Neurological Examination

A neurological examination was performed on all children by one of several paediatricians, experienced in assessing for the presence and severity of cerebral palsy. Cerebral palsy was defined as a non-progressive neurological disorder caused by injury to the developing brain, characterised by abnormal tone, posture, and movement, and often leading to activity limitation.[223] Cerebral palsy can be reliably diagnosed after 18 months of age, corrected for prematurity.[3] The Gross Motor Functional Classification System (GMFCS), proposed by Palisano, was used to grade the severity of cerebral palsy as follows:[33]

- Level I: ambulant, independent walking;
- Level II: ambulant, independent walking, assistance with climbing stairs eg. holds rail;

- Level III: ambulant, independent walking with mobility apparatus, for example, with use of a K-frame;
- Level IV: ambulant, with wheelchair; and
- Level V: non-ambulant.

4.4.3 Participants with Adverse Neurodevelopment

Where participants were identified as having suspected or confirmed developmental delay, feedback was provided to their parents at the time of assessment, followed by a detailed written report. With parental consent, appropriate referrals were made to paediatricians and allied health professionals for ongoing management of the child and their parents.

4.5 Data Management

Study coordinators prospectively collected data for participating infants, including demographics and clinical details of their perinatal course. Data were entered into a password secured data program. Participants received a study number, allowing for the de-identification of their names from their data.

4.6 Statistical Analysis

Data were analysed using Stata, Version 13.1 (StatCorp, College Station, Texas, USA). A summary of the data analysis is outlined below, with more details provided in the subsequent chapters reporting the results of my thesis in manuscript format.

4.6.1 Aim 1

To assess the reproducibility of linear measures made from cUS performed as part of routine clinical care, 30 scans were randomly selected from the pool of scans

performed for preterm participants included in the current study. Linear measures were made by me on two occasions, at least one month apart, and on one occasion by another observer. Both observers are neonatal paediatricians, and had had 3 years' and 20 years' experience in performing cUS, respectively, at the time of making their observations. I provided instruction to the other observer on how to make the linear measures, as described in Figure 4.3 and Table 4.1. The scans were not anonymised, but both observers were blinded to the participant's clinical history.

Intra- and inter-observer reproducibility of linear measures was assessed by the intra-class correlation coefficient (ICC) and Bland-Altman plot analysis.[224, 225] Individual ICC analysis was performed using two-way mixed effects models to estimate the consistency of agreement within, and between, observers, where the linear measure was assumed to be a random effect, and the observer assumed to be a fixed effect. Intra- and inter-observer ICCs are reported for each of the 19 linear measures. The higher the ICC (range 0-1), the more consistent the agreement between measurements made by the same, or different, observers. In general, an ICC >0.80 is considered to be excellent, >0.60 to 0.80 good, >0.40 to 0.60 moderate, >0.20 to 0.40 fair, and ≤0.20 poor. Bland-Altman analysis was used to assess systematic bias, and to assess further the degree of agreement between measurements reported by the same, and different, observers.[225] Intra- and inter-observer mean differences and the 95% limits of agreement, reflecting 1.96 standard deviations above and below the mean, are reported for each of the 19 linear measures. Bland-Altman plots demonstrate the relationship between paired differences (y-axis) and the means of measurements (x-axis). Linear measures with excellent intra- and inter-observer reproducibility, assessed by having ICCs >0.80, were considered for use in the subsequent aims of the current study.

4.6.2 Aim 2

To evaluate early postnatal brain size and brain growth for infants born at <30 weeks' GA, and without major brain injury seen on neonatal cUS, linear measures were made from cUS performed from as early as birth and up to term-equivalent age. Eight linear measures with excellent intra- and inter-observer reproducibility – 4 of

brain tissues (the BPD, CCL, TCD, and VH) and 4 reflecting fluids spaces (the IHD, left and right AHW, and VW) – were used for these analyses.

Linear measures of brain size (mm) were assessed at various time-points with respect to PNA, including: birth (days 0-2, inclusive), 1-week (days 3-11, inclusive), 1-month (days 21-35, inclusive), 2-months (days 50-70, inclusive), and at term-equivalent (≥37 and <42 weeks' post-menstrual age). Linear measures were made from a single scan for each participant at each time-point, where available. Linear measures of brain growth (mm/week PNA) were assessed within various time-epochs with respect to PNA, including: from birth to 1-week (days 0-6, inclusive), from 1-week to 1-month (days 7-27, inclusive), from 1-month to term-equivalent age (day 27 to ≥37 and <42 weeks' post-menstrual age, inclusive), and from birth to term-equivalent age. Linear measures were made from all scans performed for each participant within each time-epoch, where available. Linear measures of brain growth (mm/week PNA), or the rate of change of the linear measure with respect to PNA, within any given time-epoch, were assessed using mixed effects linear regression fitted to all of the sequential measurements from all participants, with PNA as the time variable, and a random effect for participant fitted with an unstructured covariance matrix. The model included a random effect to account for multiple measurements for each participant, with a fixed effect of time. Time-points for brain size, and time-epochs for brain growth, were chosen to reflect the timing of routine cUS for preterm infants admitted to the unit during the study period. Analyses were repeated with adjustment for GA, BW z score, and sex, which were chosen *a priori* to be important determinants of brain growth and long-term neurodevelopment. BW z score was used rather than BW because of the collinear relationship between GA at birth and BW.

4.6.3 Aim 3

Associations between perinatal variables and brain growth with respect to PNA (fitting a single effect of time from birth up to term-equivalent age) were explored separately by including an interaction term between the perinatal variable and PNA in a mixed effects linear regression model. Continuous variables were dichotomised for this analysis: GA group (<28 weeks or 28-29 weeks) and BW z

score group (<-2 or ≥-2 standard deviations from the mean). Analyses were repeated with adjustment for GA, BW z score, and sex, with the exception that GA and BW z score were not adjusted for when exploring the effect of GA group and BW z score group, respectively.

4.6.4 Aim 4

Infant neurobehaviour at term-equivalent age was assessed using GMs and the HNNE. GMs were dichotomised as either normal or abnormal. The HNNE was scored out of 34 with a score of 0 or 1 being assigned to each item, to provide a total score. A suboptimal score of <28 was based on the <10th centile for the contemporaneously recruited cohort of term-born controls. The HNNE was then dichotomised as either optimal or suboptimal. Relationships between neurobehaviour at term-equivalent age and cUS linear measures of early postnatal brain size and brain growth, from as early as birth up to term-equivalent age, were explored separately using logistic regression, fitted using generalised estimating equations and are reported with robust (sandwich) estimates of standard errors to account for clustering because of multiple births within the same family. All analyses were adjusted for GA at birth, BW z score, and sex.

4.6.5 Aim 5

Neurodevelopment at 2 years' corrected age was assessed using the cognitive, language, and motor subtests of the Bayley-III, and neurological examination for cerebral palsy, deafness, and blindness.[226] Bayley-III composite scores for preterm participants were standardised using the contemporaneous cohort of term-born controls.[221] Primary outcomes were the cognitive, language, and motor scores on the Bayley-III. Secondary outcome was risk of adverse neurodevelopment, defined by a composite of either cerebral palsy, any developmental delay (Bayley-III composite score <-1 SD in any domain relative to term-born controls), deafness (required hearing aids or worse), or blindness (visual acuity <6/60 in the better eye).[221] Relationships of early postnatal cUS linear measures of early postnatal brain size and brain growth, from as early as birth up to term-equivalent age, with neurodevelopment at 2 years were explored using linear (for the continuous primary

outcomes) and logistic (for the categorical secondary outcome) regression models, fitted using generalised estimating equations and are reported with robust (sandwich) estimates of standard errors to account for clustering because of multiple births within the same family. All analyses were adjusted for GA at birth, BW z score, and sex.

4.7 Conclusion

This chapter outlines the overall methodology I have used to address each of the aims of my thesis. My thesis is nested within a larger prospective cohort study of 149 children born very preterm, and 201 term-born controls, to explore the prognostic utility of cUS linear measures of brain size and brain growth in infants born <30 weeks' GA and free of major brain injury on cUS. The strengths of the methodology include prospective enrolment of the inception cohort, with a large sample size, and blinded outcome assessments with a term-born control group. Detailed methodology for each aim is outlined in chapters 5, 6, and 7.

Chapter 5

Study 1: Reproducibility of Cranial Ultrasonography Linear Measures, Early Postnatal Brain Growth, and Associations with Perinatal Variables

Chapter Overview

This chapter is presented in manuscript form, as published in the American Journal of Neuroradiology (see Appendix 1).[227]

Study 1 encompasses the first three aims of my thesis, namely, the reproducibility of cUS linear measures, the evaluation of early postnatal brain size and brain growth using sequential cUS linear measures, and the exploration of associations between perinatal variables and early postnatal brain growth.

With respect to the reproducibility of cUS linear measures, this chapter reports the intra-class correlation coefficients of intra- and inter-observer measurements. Conclusions drawn from analysis of Bland-Altman plots of intra- and inter-observer measurements are presented in Appendix 2.

5.1 Abstract

5.1.1 Background

This study of infants born at <30 weeks' gestational age (GA) without major brain injury aimed to (1) assess the reproducibility of linear measures made from cranial ultrasonography (cUS), (2) evaluate brain growth using sequential cUS linear measures from birth to term-equivalent age, and (3) explore perinatal predictors of postnatal brain growth.

5.1.2 Methods

Participants comprised 144 infants born at <30 weeks' GA at a single center between January 2011 and December 2013. Infants with major brain injury on cUS or congenital or chromosomal abnormalities were excluded. Brain tissue and fluid spaces were measured from cUS performed as part of routine clinical care. Brain growth was assessed in three time epochs: <7, 7-27 and >27 days' postnatal age. Data were analyzed using intraclass correlation coefficients and mixed effects regression.

5.1.3 Results

429 scans were assessed for 144 infants. Several linear measures showed excellent reproducibility. All measures of brain tissue increased with postnatal age, except for the biparietal diameter (BPD) which decreased within the first postnatal week and increased thereafter. GA ≥28 weeks at birth was associated with slower growth of the BPD and ventricular width compared with GA <28 weeks. Postnatal corticosteroid administration was associated with slower growth of the corpus callosum length, transcerebellar diameter (TCD) and vermis height. Sepsis/necrotizing enterocolitis was associated with slower growth of the TCD.

5.1.4 Conclusions

Postnatal brain growth in infants born at <30 weeks' GA can be evaluated using sequential linear measures made from routine cUS, and is associated with perinatal predictors of long-term development.

5.2 Introduction

Preterm infants are at risk of long-term neurodevelopmental impairments related to perinatal brain injury and altered brain maturation.[4, 5] The optimal modality and timing of neuroimaging for identifying high-risk infants is under debate.[123] Whilst early and sequential cranial ultrasonography (cUS) can reliably be used to detect major brain injury, it is less sensitive than MRI for the more prevalent, diffuse white matter injury associated with preterm birth.[4, 144, 228] Nonetheless, cUS remains the most widely used neuroimaging modality for newborn infants because it is readily available, bedside and easily repeatable, and is sensitive enough for detecting most major pathology.

In the absence of overt brain injury and where MRI is not accessible, there is a need to improve the prognostic utility of cUS, not least to better understand why some preterm infants without major brain injury seen on cUS later develop motor and cognitive impairments.[4, 147] Most infants born preterm have at least one early and one later neonatal cUS scan affording an opportunity to quantify early brain growth as a potential marker of long-term development that has not been fully exploited.[94]

Linear measures of brain tissue and fluid spaces made from neonatal cUS have been associated with neurodevelopmental outcomes in preterm children.[175, 190] However, only two studies have evaluated early postnatal brain *growth* using sequential cUS.[173, 175] In a study of 140 infants born at <29 weeks' gestational age (GA), Roelants et al reported normative data for the growth of the corpus callosum length (CCL) and corpus callosum-fastigium length with respect to post-menstrual age (PMA), but they did not relate brain growth with postnatal age (PNA).[173] The study of 61 very-low-birth weight infants by Anderson et al found that slower growth of the CCL between 2 and 6 weeks' PNA was predictive of motor delay and cerebral palsy at 2 years; however this study included infants with major brain injury.[175]

To explore further the usefulness of cUS in assessing brain growth in preterm infants without major brain injury, we aimed to (1) assess the reproducibility of linear

measures of brain tissue and fluid spaces made from cUS, (2) evaluate brain growth with respect to PNA using sequential cUS linear measures from birth to term-equivalent age (TEA), and (3) explore the associations between perinatal variables and postnatal brain growth measured by cUS.

5.3 Materials and Methods

5.3.1 Study Participants

One hundred and forty-nine infants born at <30 weeks' GA at The Royal Women's Hospital, Melbourne, between January 2011 and December 2013 were recruited into a prospective longitudinal study of neuroimaging, neurobehavior and long-term development.[215] Infants with congenital or chromosomal anomalies known to affect neurodevelopment were excluded. For the current study, 5 infants with major preterm brain injury detected on cUS were also excluded, including infants with grades III or IV intraventricular hemorrhage (IVH), post-hemorrhagic ventricular dilatation and cystic periventricular leukomalacia, leaving 144 infants for analysis.[93, 98] The study received institutional approval from the hospital´s Human Research Ethics Committee, and written informed consent was obtained from the parents of all participants.

5.3.2 Cranial Ultrasonography

cUS was performed as part of routine clinical care using a General Electric Logiq 9 ultrasound machine and an 8 MHz broadband curvilinear transducer (GE Healthcare Technologies, Wisconsin, USA). Standard images were acquired through the anterior fontanel (5 images in different coronal planes, an image in the mid-sagittal plane, and 2 images in different parasagittal planes both on the left and right) and the mastoid fontanel (1 image in the coronal plane). As per local protocol for the surveillance of preterm brain injury, infants born at <30 weeks' GA were scanned on, or around, days 7, 28 and 60 PNA. Infants born at <28 weeks' GA were also scanned on, or around, day 1 PNA. Additional scans were performed depending on clinical

need. The current study included all neonatal scans performed from birth to TEA (<42 weeks' PMA).

Nineteen linear measures of brain tissue and fluid spaces were explored on the basis of potential clinical importance, ease of recognition of anatomical landmarks on standard imaging planes, and evaluation of their reproducibility (see Chapter 4, Figure 4.2). A neonatologist (RC) blinded to the clinical course of study participants, and with 4 years' experience in performing cUS, obtained measurements using electronic calipers on stored digital images (Synapse, Fujifilm Medical Company, Tokyo, Japan). Thirty scans (7%) were randomly selected to assess the reproducibility of the linear measures. Measurements were made by the same observer (RC) twice, at least one month apart, and once by another observer (SR) with 20 years' experience in performing cUS.

5.3.3 Perinatal Predictors of Long-Term Neurodevelopment

Perinatal variables were chosen *a priori* on the basis of their known associations with brain injury and long-term neurodevelopmental outcomes following preterm birth.[229] These included GA at birth (determined by first-trimester ultrasonography where available, or by menstrual history), birth weight standard deviation score (BW z-score), sex, multiple gestation, chorioamnionitis (confirmed by placental histology), antenatal corticosteroids (any number of doses of betamethasone) and magnesium sulfate (for maternal or fetal indications), bronchopulmonary dysplasia (defined as oxygen requirement at 36 weeks' PMA), postnatal corticosteroids, confirmed sepsis (blood or cerebrospinal fluid culture-positive and use of antibiotics for ≥5 days) and/or necrotising enterocolitis (NEC; defined by stage ≥2 modified Bell's criteria), and low-grade IVH (grades I or II) on cUS (defined by Papile's classification).[98, 230]

5.3.4 Data Analysis

Data were analysed using Stata Version 13.1 (StatCorp, Texas, USA). Intra- and inter-observer agreements were assessed by intraclass correlation coefficients (ICC). Linear measures with both intra- and inter-observer ICC >0.80 were

considered for inclusion in further analyses. Brain growth (or rate of change) with respect to PNA was assessed using mixed effects linear regression fitted to all of the sequential measurements from all individuals, with PNA as the time variable and a random effect for individual fitted with an unstructured covariance matrix. The model included a random effect to account for multiple measurements for each infant, with a fixed effect of time. Firstly, overall brain growth was assessed from birth to TEA using a single time variable. Brain growth was then assessed within three time epochs to reflect the timing of our routine cUS by fitting separate effects of time in the periods <7, 7-27 and >27 days' PNA. Analyses were repeated with adjustment for GA, BW z-score and sex. Associations between perinatal variables and brain growth with respect to PNA (fitting a single effect of time from birth to TEA) were explored separately by including an interaction between the perinatal variable and PNA in the mixed effects linear regression model. Continuous variables were dichotomized for this analysis: GA group (<28 weeks or 28-29 weeks) and BW z-score group (<-2 or ≥-2 standard deviations from the mean). Analyses were repeated with adjustment for GA, BW z-score and sex, with the exception that GA and BW z-score were not adjusted for when exploring the effect of GA group and BW z-score group, respectively. Measurements of the left and right anterior horn widths (AHW) had included the germinal layer hemorrhage when present. Consequently, all analyses involving the AHW were restricted to infants without an ipsilateral IVH in order to eliminate the influence of the germinal layer hemorrhage on the generalizability of results with respect to rates of change of the AHW with PNA.

5.4 Results

Characteristics for the 144 infants included in the current study are described in Table 5.1. Within the 144 infants, 429 scans were performed from birth to TEA. The mean number of scans per infant was 3 (range 1-8). For the 77 infants born at <28 weeks' GA, 291 scans were assessed (mean 3.8; range 1–8 per infant), and 138 scans for the 67 infants born at ≥28 weeks' GA (mean 2.1; range 1-6 per infant). Almost two-thirds (n=272; 63%) of the scans were performed at <28 days' PNA but the majority of infants had scans up to 33 weeks' PMA.

Table 5.1 Infant characteristics

Perinatal variable	Summary, n=144 infants
Gestational age, weeks [mean, (SD)]	27.7 (1.5)
Birth weight, g [mean, (SD)]	1017 (259)
Small for gestational age, birth weight <-2 SD – n (%)	16 (11)
Female – n (%)	75 (52)
Multiple birth – n (%)	64 (44)
Cesarean section – n (%)	106 (74)
Antenatal corticosteroids – n (%)	134 (93)
Antenatal magnesium sulfate – n (%)	103 (72)
Respiratory distress from birth – n (%)	141 (98)
Surfactant – n (%)	91 (63)
Duration of positive pressure ventilation, hours [median, (25^{th}–75^{th} centile)]	19 (0–96)
Duration of supplemental oxygen, hours [median, (25^{th}–75^{th} centile)]	19 (4–56)
Bronchopulmonary dysplasia[#] – n (%)	46 (32)
Postnatal corticosteroids – n (%)	18 (13)
Intraventricular hemorrhage, grades I or II – n (%)	25 (17)
Retinopathy of prematurity, any – n (%)	32 (22)
Sepsis, confirmed[##] – n (%)	20 (14)
Necrotising enterocolitis – n (%)	17 (12)
Sepsis, confirmed and/or necrotising enterocolitis – n (%)	31 (22)
Survived to discharge home – n (%)	139 (97)

n=number; SD=standard deviation; [#]defined as oxygen requirement at 36 weeks' post-menstrual age; [##]blood or cerebrospinal fluid culture-positive and use of antibiotics for ≥5 days.

5.4.1 Intra- and Inter-Observer Reproducibility

Intra- and inter-observer ICC are shown in Table 5.2. Eight linear measures, four of brain tissue [biparietal diameter (BPD), CCL, transcerebellar diameter (TCD) and vermis height (VH)] and four reflecting fluid spaces [interhemispheric distance (IHD), left and right AHW and ventricular width (VW)], had ICCs >0.8 both between and within observers, and were used to evaluate brain growth.

Table 5.2 Reproducibility of cranial ultrasonography linear measures

Linear measure	Intra-observer		Inter-observer	
	ICC	95% CI	ICC	95% CI
Brain tissue				
Biparietal diameter	0.98	0.95, 0.99	0.97	0.94, 0.99
Corpus callosum length	0.99	0.97, 0.99	0.98	0.95, 0.99
Corpus callosum genu width	0.78	0.53, 0.90	0.38	-0.04, 0.69
Corpus callosum body height	0.74	0.52, 0.87	0.58	0.27, 0.78
Corpus callosum splenium width	0.80	0.56, 0.92	0.21	-0.22, 0.58
Transcerebellar diameter	0.99	0.98, 0.99	0.98	0.96, 0.99
Vermis height	0.91	0.82, 0.96	0.81	0.62, 0.91
Fluid spaces				
Interhemispheric distance	0.98	0.96, 0.99	0.98	0.95, 0.99
Anterior horn width, left	0.94	0.87, 0.97	0.91	0.81, 0.96
Anterior horn width, right	0.96	0.90, 0.98	0.95	0.89, 0.98
Ventricular index, left	0.85	0.70, 0.93	0.85	0.69, 0.93
Ventricular index, right	0.63	0.32, 0.82	0.59	0.26, 0.80
Ventricular width	0.87	0.72, 0.94	0.89	0.76, 0.95
Anterior horn height, left	0.86	0.72, 0.94	0.76	0.53, 0.88
Anterior horn height, right	0.92	0.82, 0.96	0.78	0.55, 0.90
Ventricular mid-body height, left	0.73	0.49, 0.87	0.47	0.11, 0.72
Ventricular mid-body height, right	0.81	0.61, 0.91	0.52	0.18, 0.75
Thalamo-occipital distance, left	0.93	0.81, 0.97	0.80	0.52, 0.93
Thalamo-occipital distance, right	0.92	0.80, 0.97	0.62	0.22, 0.84

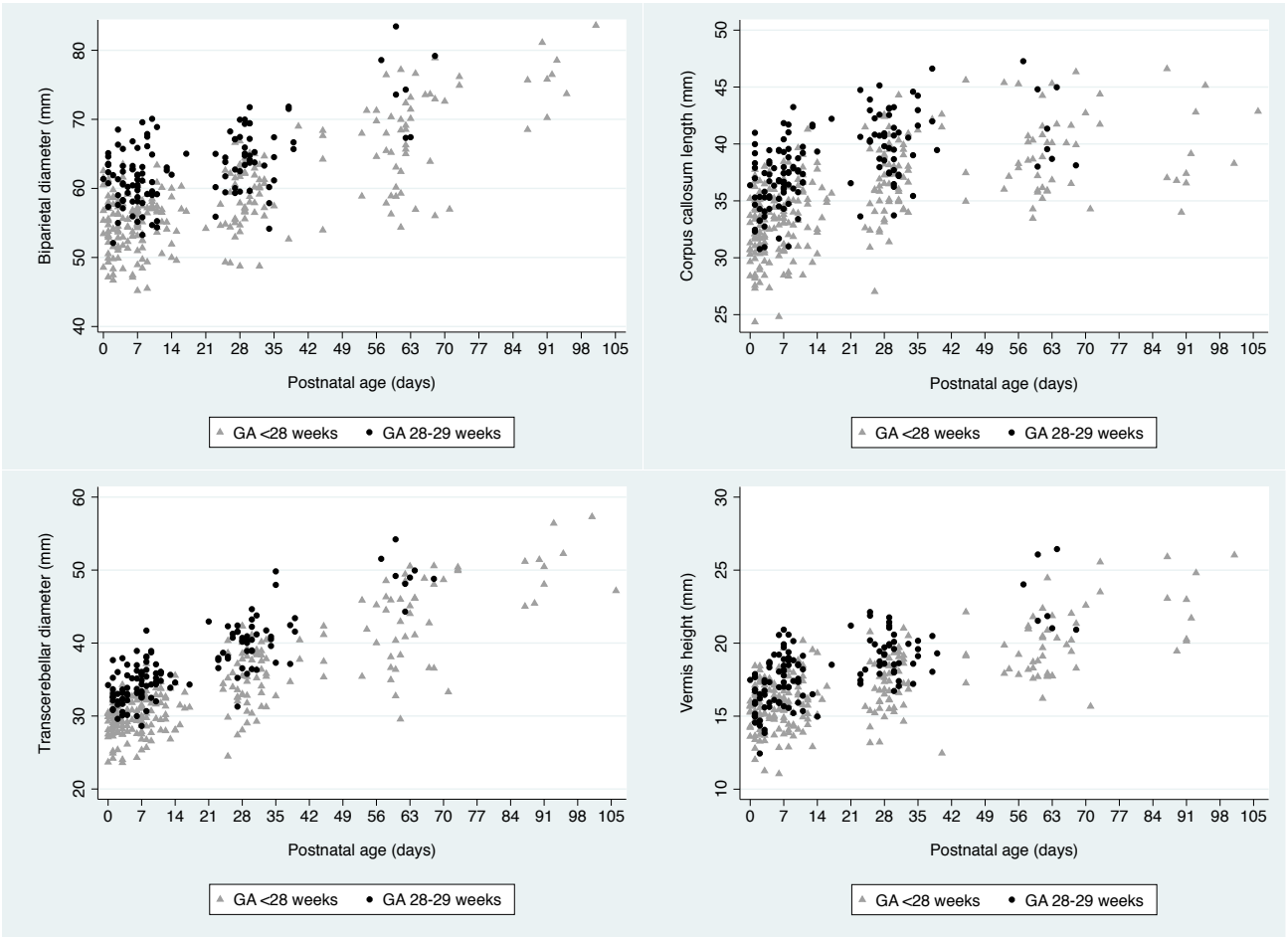
ICC=intraclass correlation coefficient; CI=confidence interval.

5.4.2 Brain Growth with Respect to Postnatal Age

There was evidence that all linear measures of brain tissue increased with PNA, before and after adjustment for GA, BW z-score and sex, overall and within the three time epochs, with the exception of the BPD which decreased within the first postnatal week (Figure 5.1, Table 5.3). Faster rates of growth of the BPD, TCD and VH were observed after 27 days' PNA compared with 7-27 days' PNA; however the rate of growth of the CCL more than halved after 27 days' PNA compared with 7-27 days' PNA (Figure 5.1, Table 5.3).

Figure 5.1 Cranial ultrasonography linear measures with respect to postnatal age

A. Brain tissue



B. Fluid spaces

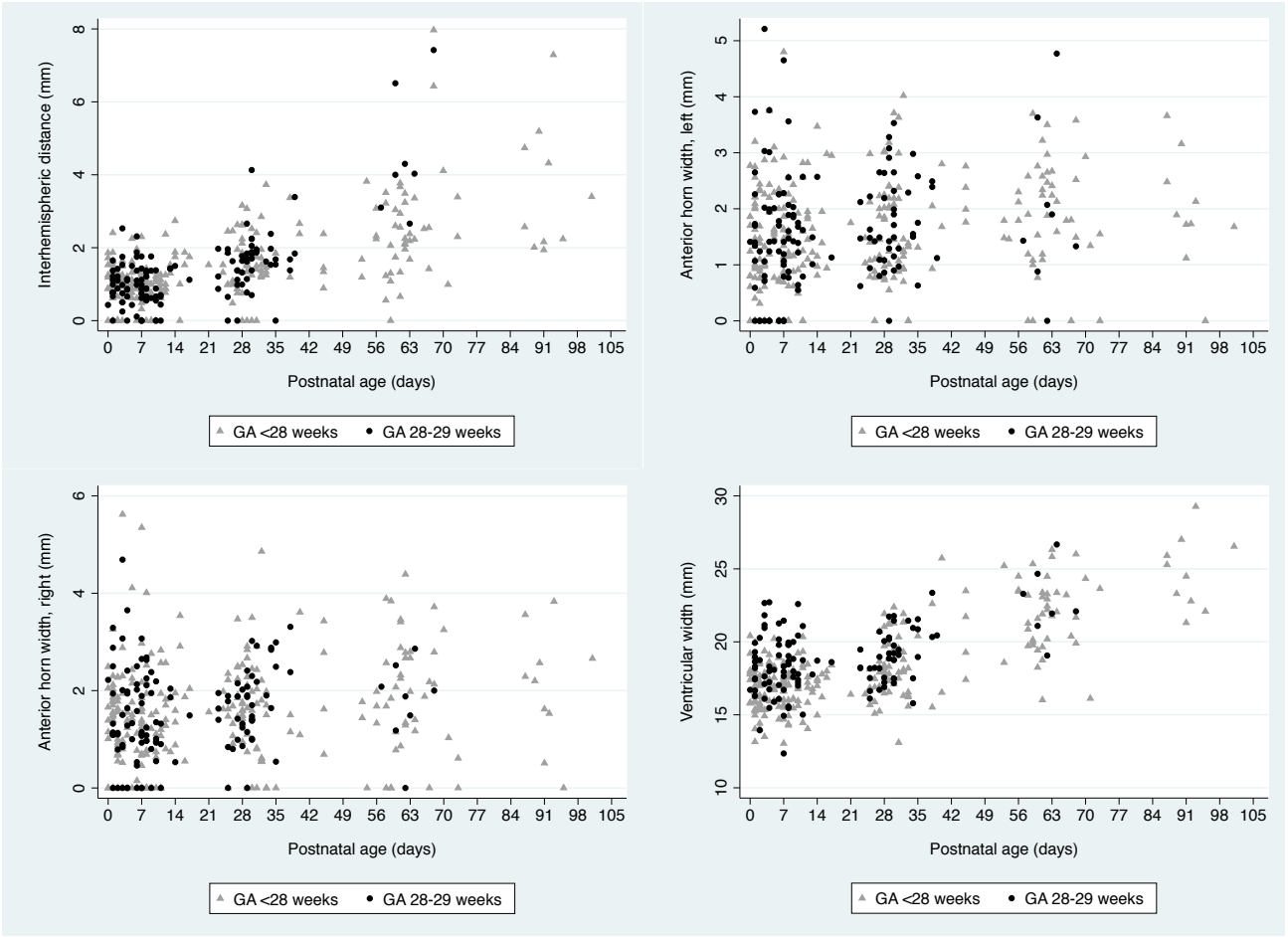


Table 5.3 Brain growth with postnatal age

A. Brain tissue

Postnatal age	Unadjusted growth (mm/week PNA)			Adjusted[#] growth (mm/week PNA)		
	β	95% CI	p value*	β	95% CI	p value*
Biparietal diameter						
Birth to TEA	1.50	1.37, 1.63	<0.001	1.54	1.41, 1.66	<0.001
<7 days	-0.36	-2.15, 1.43	0.69	-2.01	-3.66, -0.36	0.017
7-27 days	1.30	0.90, 1.70	<0.001	1.30	0.93, 1.66	<0.001
>27 days	1.98	1.69, 2.26	<0.001	2.17	1.94, 2.40	<0.001
Corpus callosum length						
Birth to TEA	0.91	0.84, 0.97	<0.001	0.89	0.83, 0.96	<0.001
<7 days	1.04	0.02, 2.06	0.045	0.99	0.06, 1.92	0.038
7-27 days	1.10	0.83, 1.36	<0.001	1.09	0.84, 1.33	<0.001
>27 days	0.45	0.33, 0.58	<0.001	0.50	0.38, 0.62	<0.001
Transcerebellar diameter						
Birth to TEA	1.81	1.72, 1.91	<0.001	1.76	1.66, 1.85	<0.001
<7 days	1.93	0.92, 2.95	<0.001	1.22	0.34, 2.11	0.007
7-27 days	1.73	1.35, 2.10	<0.001	1.52	1.13, 1.90	<0.001
>27 days	2.01	1.82, 2.19	<0.001	2.03	1.86, 2.20	<0.001
Vermis height						
Birth to TEA	0.60	0.54, 0.65	<0.001	0.59	0.54, 0.65	<0.001
<7 days	0.83	0.10, 1.55	0.026	0.52	-0.17, 1.22	0.141
7-27 days	0.51	0.28, 0.74	<0.001	0.46	0.26, 0.67	<0.001
>27 days	0.58	0.44, 0.73	<0.001	0.71	0.60, 0.83	<0.001

B. Fluid spaces

Postnatal age	Unadjusted growth (mm/week PNA)			Adjusted[#] growth (mm/week PNA)		
	β	95% CI	p value*	β	95% CI	p value*
Interhemispheric distance						
Birth to TEA	0.22	0.19, 0.26	<0.001	0.22	0.18, 0.26	<0.001
<7 days	-0.04	-0.32, 0.24	0.788	-0.04	-0.33, 0.24	0.773
7-27 days	0.19	0.10, 0.28	<0.001	0.19	0.10, 0.28	<0.001
>27 days	0.27	0.18, 0.36	<0.001	0.29	0.21, 0.36	<0.001
Anterior horn width, left^						
Birth to TEA	0.09	0.06, 0.13	<0.001	0.10	0.07, 0.14	<0.001
<7 days	0.42	-0.26, 1.09	0.229	0.53	-0.15, 1.20	0.125
7-27 days	0.11	0.01, 0.21	0.038	0.11	0.01, 0.21	0.034
>27 days	0.05	-0.02, 0.11	0.143	0.06	-0.002, 0.12	0.059
Anterior horn width, right^						
Birth to TEA	0.09	0.05, 0.12	<0.001	0.09	0.06, 0.13	<0.001
<7 days	-0.04	-0.37, 0.28	0.791	-0.04	-0.37, 0.28	0.788
7-27 days	0.20	0.08, 0.32	0.001	0.20	0.08, 0.32	0.001
>27 days	0.05	-0.02, 0.12	0.173	0.06	-0.01, 0.13	0.096
Ventricular width						
Birth to TEA	0.58	0.52, 0.65	<0.001	0.59	0.52, 0.65	<0.001
<7 days	1.05	-0.27, 2.37	0.118	0.57	-0.67, 1.82	0.368
7-27 days	0.27	0.07, 0.47	0.007	0.30	0.10, 0.49	0.003
>27 days	0.75	0.62, 0.88	<0.001	0.80	0.66, 0.93	<0.001

[#]Adjusted for gestational age, birth weight z-score and sex. ^Restricted to infants without an ipsilateral intraventricular hemorrhage. β=regression coefficient (change in the linear measure per week postnatal age); CI=confidence interval; PNA=postnatal age; TEA=term-equivalent age. *p value reflects strength of the evidence for an association between the linear measure and postnatal age.

Rates of change of linear measures of fluid spaces were variable within the first postnatal week, with little evidence of an association between linear measures and PNA (Figure 5.1, Table 5.3). The IHD and VW increased after the first postnatal week, with faster rates of change after 27 days' PNA (1.5 and 2.7 times, respectively) compared with 7-27 days' PNA. Although the rates of change for the AHW appeared to decrease with successive time epochs, there was little statistical evidence of an association between the AHW and PNA with the exception of the right AHW between 7-27 days' PNA.

5.4.3 Associations between Perinatal Variables and Postnatal Brain Growth

There was strong evidence of associations between many of the perinatal variables and brain growth with respect to PNA, which remained after adjustment for GA, BW z-score and sex (Table 5.4). Infants born at 28-29 weeks' GA showed slower growth of the BPD and VW than infants born at <28 weeks' GA. Postnatal corticosteroid administration was associated with slower growth of the CCL, TCD and VH, and bronchopulmonary dysplasia was associated with slower growth of the CCL and TCD. Infants with BW z-score <-2 SD below the mean showed slower growth of the CCL than infants with BW ≥-2 SD below the mean. Sepsis/NEC and antenatal corticosteroid administration were associated with slower growth of the TCD.

Table 5.4 Associations between perinatal variables and brain growth measured by cranial ultrasonography

Perinatal variable		Adjusted[#] growth (mm/week PNA)		Interaction term*
		β	95% CI	p value
Biparietal diameter				
GA	<28 weeks	1.88	1.71, 2.05	0.002
	≥28 weeks	1.29	0.96, 1.62	
Corpus callosum length				
BW z-score	≥-2 SD	0.91	0.84, 0.98	0.045
	<-2 SD	0.73	0.57, 0.89	
Postnatal	No	0.92	0.85, 1.00	0.046
corticosteroids	Yes	0.77	0.63, 0.90	
Bronchopulmonary	No	0.98	0.88, 1.07	0.01
dysplasia	Yes	0.81	0.73, 0.89	
Transcerebellar diameter				
Antenatal	No	2.26	1.81, 2.70	0.023
corticosteroids	Yes	1.73	1.63, 1.83	
Postnatal	No	1.84	1.74, 1.95	<0.001
corticosteroids	Yes	1.41	1.22, 1.61	
Bronchopulmonary	No	1.89	1.75, 2.02	0.008
dysplasia	Yes	1.63	1.49, 1.76	
Sepsis/NEC	No	1.82	1.71, 1.93	0.016
	Yes	1.55	1.36, 1.74	
Vermis height				
Postnatal	No	0.63	0.58, 0.69	0.011
corticosteroids	Yes	0.49	0.39, 0.59	
Ventricular width				
GA	<28 weeks	0.64	0.57, 0.71	0.004
	≥28 weeks	0.41	0.27, 0.55	

[#]Adjusted for gestational age (GA), birth weight z-score (BW z-score) and sex. SD=standard deviation; β=regression coefficient; CI=confidence interval; PNA=postnatal age; NEC=necrotizing enterocolitis. *p-value for the interaction between the perinatal variable and postnatal age.

5.5 Discussion

In this study of early postnatal brain growth in infants born at <30 weeks' GA without major brain injury, several linear measures of brain tissue and fluid spaces were reliably made from routine clinical cUS scans. Most linear measures of brain tissue increased with PNA but rates of change were more variable for the fluid spaces. Several perinatal variables related to long-term development were associated with postnatal brain growth. Bronchopulmonary dysplasia and postnatal corticosteroid administration were associated with slower growth of the CCL and TCD, and postnatal corticosteroid administration was also associated with slower growth of the VH. Sepsis/NEC was associated with slower growth of the TCD.

Measurements of brain growth for use in clinical practice should be simple to obtain and be reproducible. The cUS linear measures explored in this study had well-defined anatomical landmarks on standard imaging planes obtained during routine clinical care, and several showed excellent intra- and inter-observer reproducibility, as also shown by others.[173, 174, 190, 217]

Although cUS affords an opportunity to make sequential measurements from birth, there are limited published data relating to postnatal brain *growth* in preterm infants. To our knowledge, this study is the first to describe the growth of the BPD in the early postnatal period in preterm infants using cUS. Decreasing size of the BPD in the first postnatal week found in the current study may relate to a reduction in brain water in the immediate postnatal days rather than deformational changes in head shape.[231] Growth of the BPD after the first 27 days (2.17 mm/week PNA) approximates to expected fetal growth (2.10 mm/week PMA) as reported from cross-sectional data by Kurmanavicius et al.[193] However, relatively slower growth of the BPD was observed between 7 and 27 days (1.30 mm/week PNA) compared with >27 days after birth. Higher GA at birth was related to slower growth of the BPD from birth to TEA. Normative data for fetal growth of the BPD demonstrates a slowing of growth with increasing PMA.[193, 232] However, it is also plausible that the more mature infants in our cohort who remained in our unit for a longer period, and had more cUS scans, had a greater burden of morbidity affecting brain growth. We also

speculate that slower growth of the BPD in the more mature infants in the early postnatal period might also relate to extrinsic changes in head shape. However, infants born at <30 weeks' GA have smaller brains at TEA than term-born controls, and most are likely to have slower rates of brain growth immediately after birth than normally expected *in-utero*.[159, 232]

In the current study, CCL growth was relatively constant from birth to 27 days' PNA, but slowed by half thereafter. Similarly, Anderson et al showed that postnatal growth of the CCL in very-low-birth weight infants approximated fetal growth (1.4-1.89 mm/week PMA) within the first 2 postnatal weeks, but subsequently slowed to approximately half of that expected.[175, 196, 197] Moreover, the current study found that BW z-score <-2 SD was associated with slower CCL growth from birth to TEA, a finding consistent with Roelants et al.[173]

The TCD, measured in the fetus or the newborn in the first postnatal days is a reliable marker of GA, even with fetal growth restriction, small- and large-for-gestational age fetuses, and multiple gestation (1.25–1.89 mm/week PMA).[177, 180, 198, 199] Cross-sectional preterm MRI studies have reported cerebellar size, but our study is the first to report postnatal *growth* of the TCD in preterm infants using sequential cUS measurements.[232, 233] Similar to fetal growth patterns, TCD growth in the current study progressively increased between time epochs. Unlike the TCD, the VH increased with PNA at a relatively constant rate of change from birth to TEA, approximating fetal growth (0.55 mm/week PMA).[213]

Bronchopulmonary dysplasia and postnatal corticosteroids were associated with slower CCL and TCD growth with PNA, and postnatal corticosteroid administration was also related to slower growth of the VH. Tam et al reported a similar association between postnatal corticosteroid usage, a marker of severe bronchopulmonary dysplasia, and impaired cerebellar growth in very preterm infants using serial MRI between 32 weeks' PMA and TEA.[234] In another MRI study of very preterm infants scanned at TEA and 7 years, Thompson et al reported an association between postnatal corticosteroids and delayed maturation of the posterior corpus callosum, a region that develops later in gestation and contributes to its length.[235] In the current study, sepsis/NEC was related to slower growth of the

TCD. Sepsis/NEC has been associated with preterm white matter injury at TEA, but its relationship with brain growth and maturation in the early postnatal period remains unclear.[65] The relationship between antenatal corticosteroid exposure and slower TCD growth reported here is novel and needs to be replicated in future studies.

Liao et al and Levene et al reported serial measurements of the ventricular index in preterm infants from birth to 6 weeks and 6 months' PNA and found rates of change similar to those in the current study for the VW (the sum of left and right ventricular indices).[119, 185] We found that lower GA was related to a faster rate of change in the VW, possibly resulting from a greater burden of diffuse cerebral white matter injury in the less mature infants leading to greater *ex vacuo* dilatation. Rates of change for the AHW with respect to PNA were variable, and increased little after the first postnatal week, as reported in other longitudinal studies.[119, 184-186]

A major strength of the current study is its use of simple and reproducible linear measures obtained from standard cUS performed as part of routine clinical care. We use the mastoid fontanel for cerebellar imaging and TCD measurement, a reliable approach that is not technically difficult.[176] Our study however has several limitations. We used routine clinical scans timed to detect major preterm brain injury. There is therefore an inherent bias in our study cohort as the smaller, less mature infants were more likely to have had a greater burden of brain injury and have had a greater number of scans for monitoring the progression of any overt findings. It is also likely that these infants had more comorbidities and longer periods of hospitalization. In our neonatal unit, infants who no longer need tertiary-level care are transferred to non-tertiary hospitals from as early as 32 weeks' PMA. The more mature infants in our cohort, unless they were very sick, were likely to have had fewer scans limited to the early days and weeks after birth. However, the mixed effects model used allows for the fact that different infants had different numbers of scans.

The cohort used in the current study will have developmental assessments throughout childhood, which will allow the relationships between sequential cUS linear measures of early postnatal brain growth and neurodevelopment in later childhood to be determined in the future.

5.6 Conclusions

Brain growth in infants born at <30 weeks' GA can be assessed using simple and reproducible linear measures made on cUS. Several perinatal variables already shown to be related to long-term development, including GA, BW z-score, bronchopulmonary dysplasia, postnatal corticosteroids and sepsis/NEC, were associated with early postnatal brain growth. Further research is needed to evaluate the usefulness of brain growth, as evaluated using sequential cUS linear measures, as a marker of long-term developmental outcome.

Chapter 6

Study 2: Associations of Neurobehaviour at Term-Equivalent Age with Early Postnatal Cranial Ultrasonography Linear Measures

Chapter Overview

This chapter is presented in manuscript form, as prepared for submission to Early Human Development.

Study 2 relates to the fourth aim of my thesis, that is, to explore the associations between early postnatal cUS linear measures and neurobehaviour at TEA in infants born at <30 weeks' GA and free of major brain injury seen on cUS. Neurobehaviour at TEA was assessed using GMs and the HNNE, both commonly applied in research and clinical practice to discriminate infants with atypical development and to predict neurodevelopmental outcomes.

As part of the larger study's research protocol (VIBeS-2), preterm participants had neurobehavioural assessments performed weekly from the first weeks after birth and up to 32 weeks' post-menstrual age, thereafter fortnightly until TEA (38 to 44 weeks' post-menstrual age).[215] For the current study, I chose neurobehaviour at TEA as the outcome measure. Neurobehavioural assessment of the infant at this later time-point may reflect the influence of preterm birth, and its associated short-term morbidities, on the integrity of the developing brain within the newborn period.

Title Page

Relationships between Early Postnatal Cranial Ultrasonography Linear Measures and Neurobehaviour at Term-Equivalent Age in Infants Born <30 Weeks' Gestational Age and Without Major Brain Injury

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Abbreviations: AHW=anterior horn width; BPD=biparietal diameter; BW=birth weight; CCL=corpus callosum length; cUS=cranial ultrasonography; GA=gestational age; GMH-IVH=germinal matrix haemorrhage-intraventricular haemorrhage; IHD=interhemispheric distance; MRI=magnetic resonance imaging; PNA=postnatal age; PVL=periventricular leukomalacia; TCD=transcerebellar diameter; TEA=term-equivalent age; VH=vermis height; VW=ventricular width.

6.1 Abstract

6.1.1 Background

Neurobehaviour and neuroimaging at term-equivalent age (TEA) predict later development in very preterm infants. However, the relationship between early postnatal brain development assessed by cranial ultrasonography (cUS) and neurobehaviour at TEA remains unknown.

6.1.2 Objective

To explore the relationships between early postnatal cUS linear measures and neurobehaviour at TEA in infants born <30 weeks' gestational age (GA) and without major brain injury.

6.1.3 Materials and Methods

Prospective cohort study of 139 infants born at <30 weeks' GA and free of major brain injury on cUS. cUS was performed as part of clinical care from birth up to 2-months' postnatal age. Neurobehaviour was assessed at TEA using General Movements (GMs) (categorised as "normal" or "abnormal") and the Hammersmith Neonatal Neurological Examination (HNNE) (categorised as "suboptimal" or "optimal", referenced to the 10th centile for term-born controls). Data analysis included logistic regression.

6.1.4 Results

Abnormal GMs and a suboptimal HNNE were assessed for 80/115 (70%) and 52/106 (49%) preterm infants at TEA, respectively. Larger brain sizes, assessed by linear measures of the biparietal diameter at 1-week and 2-months, and corpus callosum length (CCL) at 1-month, were associated with lower risk of a suboptimal HNNE and abnormal GMs at TEA, respectively. Faster positive growth rates of the CCL and transcerebellar diameter between birth and 1-month were related to lower

risk of abnormal GMs at TEA. A larger measure of the right anterior horn width (AHW) at 1-month, and faster positive rates of increase of the left and right AHW between 1- and 2-months, were related to lower risk of abnormal GMs and a suboptimal HNNE at TEA, respectively.

6.1.5 Conclusion

cUS linear measures of larger brain tissue from the first weeks after birth are related to lower risk of atypical neurobehaviour at TEA in infants born at <30 weeks' GA. Further studies are warranted to explore the relationship of early postnatal cUS linear measures with long-term neurodevelopmental outcomes.

6.2 Introduction

Infants born very preterm at <32 weeks' gestational age (GA) are at risk of adverse neurodevelopment, with as many as 50% expressing cognitive and/or motor deficits at school-age.[1, 236] In the newborn period, clinicians rely on neuroimaging and neurobehavioural assessments to more accurately prognosticate outcomes for the very preterm infant, to better counsel families, and to plan follow-up for developmental surveillance after discharge from neonatal intensive care.[164, 237] Underscoring the importance of identifying the high-risk infant in the newborn period is the opportunity to initiate early intervention therapy during a critical window in brain development when developing neural pathways can be modulated with the potential to improve functional outcomes.[78]

Neuroimaging of the very preterm infant from the first days after birth and up to term-equivalent age (TEA) has elucidated typical patterns of brain injury, and regional aberrations in brain growth and maturation, associated with long-term motor, cognitive, and behavioural impairments.[4, 5, 235] Cranial ultrasonography (cUS), the most frequently used neuroimaging modality for preterm infants, can be used to reliably detect most major brain injury associated with preterm birth and adverse neurodevelopment, including germinal matrix haemorrhage-intraventricular haemorrhage (GMH-IVH), cystic periventricular leukomalacia (PVL), and larger cerebellar haemorrhage.[124, 146, 238] Early postnatal cUS findings of overt focal pathology, specifically grade IV GMH-IVH and cystic PVL, that disrupt the descending motor pathway are highly specific, but not sensitive, for cerebral palsy and other motor impairments.[5] Declining rates of high-grade GMH-IVH and cystic PVL associated with advances in perinatal care in recent decades have further diminished the sensitivity of cUS to predict outcomes for very preterm infants, evidenced by the poor positive predictive value of early postnatal cUS.[164] In the absence of major brain injury, the utility of cUS to discriminate infants at risk of adverse neurodevelopment remains limited.

Skiold et al recently published a novel cUS scoring system comparing the prognostic utility of contemporaneous cUS and brain magnetic resonance imaging

(MRI) for very preterm infants at TEA.[161] Using quantitative linear measures to supplement a qualitative assessment of brain injury, growth, and maturation, Skiold et al showed their cUS scoring system to be as sensitive as brain MRI to predict cerebral palsy (75%) and severe cognitive delay (100%).[4, 161] In their study, the negative predictive values of a normal or mildly abnormal cUS at TEA for later normal neurologic status or cognitive outcome were 98% and 100%, respectively.[161] Other studies comparing the predictive validity of contemporaneous cUS and brain MRI for very preterm infants at TEA have largely limited the application of cUS to an assessment of brain injury (for example, cysts) and qualitative markers of suboptimal brain growth (for example, ventricuolomegaly and enlarged extra-axial space), and have reported, somewhat marginal, superior sensitivity of brain MRI over cUS.[47, 164]

Sequential cUS linear measures of brain tissue and fluid spaces made from the early weeks after birth may provide insights to the timing of disrupted maturational processes following very preterm birth and improve the sensitivity of cUS to predict neurodevelopmental outcomes, even in the absence of major brain injury. cUS linear measures of the lateral ventricles at 1-month postnatal age (PNA) in a cohort of very preterm infants free of major brain injury have been shown to relate to poorer motor outcomes at 2 years.[190] We have previously shown that linear measures can be reliably made from cUS performed as part of routine clinical care for very preterm infants, and several of these measures were associated with perinatal determinants of later development, including GA at birth, birth weight (BW), postnatal corticosteroids, and sepsis and/or necrotising enterocolitis.[227]

The relationship between early postnatal cUS linear measures and functional outcomes following very preterm birth remains unknown. Neonatal neurobehaviour reflects the integrity of the developing brain, and atypical neurobehavior and motor functioning in very preterm infants have been shown to discriminate infants at risk of later adverse neurodevelopment.[70, 237] This study aimed to evaluate the relationships between early postnatal cUS linear measures and neurobehaviour at TEA in infants born at <30 weeks' GA and free of major brain injury seen on cUS. We hypothesised that early postnatal cUS linear measures of smaller brain size and larger fluid spaces would be associated with atypical neurobehaviour at TEA.

6.3 Materials and Methods

6.3.1 Study Participants

Between January 2011 and December 2013, 149 infants born at <30 weeks' GA, and 201 term-born controls, were recruited from The Royal Women's Hospital, Melbourne, into a prospective longitudinal study of neuroimaging, neurobehaviour, and long-term development.[215] Infants with congenital or chromosomal anomalies known to affect neurodevelopment were excluded. For the current study, 5 preterm participants with major brain injury detected by cUS (grades III or IV GMH-IVH [n=5], post-haemorrhagic ventricular dilatation [n=3], and/or cystic PVL [n=1]) were also excluded, as were a further 5 infants who died during the primary hospitalisation, leaving 139 preterm participants in the inception cohort.[93, 98] This study received institutional approval from the hospital's Human Research Ethics Committee, and written informed consent was obtained from the parents of all participants.

6.3.2 Cranial Ultrasonography Linear Measures

Linear measures were made from cUS performed as part of routine clinical care using a Logiq 9 Ultrasound System and an 8 MHz broadband curvilinear transducer (GE Healthcare, Milwaukee, Wisconsin). Eight linear measures – four of brain tissue [biparietal diameter (BPD), corpus callosum length (CCL), transcerebellar diameter (TCD), and vermis height (VH)] and four reflecting fluid spaces [interhemispheric distance (IHD), left and right anterior horn widths (AHWs), and ventricular width (VW)] – were chosen on the basis of their potential clinical importance, ease of recognition of anatomical landmarks on standard imaging planes, and excellent inter-observer agreement (see Chapter 4, Figure 4.2).[190, 227] The methodology and reliability for these cUS linear measures have been described previously.[227] Only cUS data from preterm participants were included, as term-born controls did not have cUS performed as part of their routine clinical care.

Linear measures of *brain size* in mm were made from a single scan for each participant at 3 time-points with respect to PNA, including 1-week (days 3-11,

inclusive), 1-month (days 21-35, inclusive), and 2-months (days 50-70, inclusive). Linear measures of *brain growth* in mm/week were made from all scans performed for each participant within 2 time-epochs with respect to PNA, including between birth and 1-month (days 0-35, inclusive), and between 1-month and 2-months (days 21-70, inclusive).

6.3.3 Neurobehaviour at Term-Equivalent Age

Neurobehaviour was assessed for preterm and term-born participants by one of several allied health professionals blinded to their clinical history, including cUS findings, using Prechtl's Qualitative Assessment of General Movements (GMs) and the Hammersmith Neonatal Neurological Examination (HNNE).[71, 82] GMs and the HNNE have demonstrated predictive validity in both preterm and term infants, and are frequently used in research and clinical practice to discriminate infants at risk of later adverse motor, cognitive, and behavioural outcomes.[3, 70, 237, 239] GMs and the HNNE are both referenced to standardised criterion, and normative values have been reported for low-risk term-born infants assessed by the HNNE.[82] Preterm participants were assessed sequentially from the first weeks after birth and up to TEA (between ≥38 and <44 weeks' post-menstrual age). In the current study, only the assessments performed at TEA were included to provide a functional outcome measure to correlate with early postnatal cUS linear measures.

6.3.3.1 Prechtl's Qualitative Assessment of General Movements

Assessment of GMs involves observation of an infant's spontaneous movements with respect to their complexity, variability, and fluency.[71] GMs have origins in the fetal period and persist up to 20 weeks' post-term, after which voluntary anti-gravity movements predominate.[71] In the early postnatal period following very preterm birth, and up to 8 weeks post-term, GMs are within the "preterm" and "writhing" periods, and may be categorised as either normal or abnormal with reference to post-menstrual age at assessment. Normal GMs within this time are characterised by small to moderate amplitude and speed, and demonstrate an ellipsoid pattern giving them their writhing quality. Abnormal GMs encompass the sub-categories of poor repertoire, cramped synchronised, or chaotic GMs, depending on

the degree to which the complexity, variability, and fluency of movements differ from normal. Poor repertoire movements are noted for their simplicity, lacking both complexity and variability, and appear monotonous. Cramped synchronised movements appear rigid and are characterised by simultaneous contraction of all limb and trunk muscles. Chaotic movements are characterised by large amplitude and sudden onset, with marked lack of fluency.

GMs were assessed independently by two observers from video recordings of the infant in an appropriate behavioural state, that is alert, but not fussing or crying. In a sample of 80 infants, the two observers demonstrated a 90% inter-observer agreement, with a Cohen's kappa value of 0.75.[74] When disagreement between the two observers occurred, a third observer, with greater experience in assessing GMs, and who is a qualified GMs trainer with the GMs Trust, was the final arbiter of the GMs assessment. In the current study, GMs were categorised as either "normal" or "abnormal" to provide a dichotomous outcome measure.

6.3.3.2 Hammersmith Neonatal Neurological Examination

The HNNE is a structured observational and physical assessment of an infant's behaviour and neurological functioning, including 34 items across 6 domains: tone and posture, tone patterns, reflexes, spontaneous movements, behaviour, and abnormal signs.[82] Each item is scored 0 or 1, referenced to normative data published for 224 low-risk term-born infants, and their sum contributes to an "optimality score" between 0 and 34.[80-82]

One of 6 observers independently assessed the HNNE of all participants, and then tabulated the optimality score. In the current study, the HNNE optimality score was used as a categorical outcome measure by dichotomising the preterm cohort into "suboptimal" and "optimal" categories using an optimality score cut-off of <28 and ≥ 28, respectively, referencing the 10^{th} centile for the term-born controls.[84]

6.3.4 Data Analysis

Data were analysed using STATA Version 13.1 (StatCorp, College Station, Texas). Relationships between early postnatal cUS linear measures and neurobehaviour at TEA were explored using logistic regression modelling fitted to generalised estimating equations. Firstly, we explored the relationships between linear measures of *brain size* and neurobehaviour at TEA. For each of the 3 time-points, linear measures were included separately in the model, with neurobehaviour as the dependent variable, and the linear measure (mm) and PNA at time of scan (days) as covariates. Secondly, we explored the relationships between linear measures of *brain growth* and neurobehaviour at TEA. Brain growth, that is, the rate of change of the linear measure with respect to PNA (mm/week PNA), was calculated for each linear measure for each participant using linear regression modelling within each of the 2 time-epochs. For each of the 2 time-epochs, the calculated brain growth was included separately in the model, with neurobehaviour as the dependent variable, and the calculated brain growth (mm/week) and time between the first and last scans within that time-epoch (days) as covariates. Relationships between linear measures and neurobehaviour at TEA were then adjusted for GA at birth, BW z score, and sex, known determinants of brain development and later neurodevelopment in very preterm infants.

6.4 Results

One hundred and thirty-seven (99%) of the 139 preterm participants included in the current study underwent neurobehavioural assessment at TEA. One infant was too unwell to be assessed, and another could not be assessed due to geographical distance. Perinatal characteristics of preterm participants are described in Table 6.1.

Table 6.1 Preterm participant characteristics

Perinatal variable	n=137
Gestational age, weeks [mean, (SD)]	27.8 (1.5)
Birth weight, g [mean, (SD)]	1025 (260)
Birth weight z score [mean, (SD)]	-0.46 (1.03)
Male – n (%)	66 (48)
Multiple gestation – n (%)	63 (46)
Chorioamnionitis – n (%)	37 (35)
Antenatal corticosteroids – n (%)	127 (93)
Antenatal magnesium sulphate – n (%)	99 (72)
Bronchopulmonary dysplasia – n (%)	43 (31)
Postnatal corticosteroids – n (%)	15 (11)
GMH-IVH, grades I or II – n (%)	23 (17)
Sepsis/NEC – n (%)	27 (20)

GMH-IVH=germinal matrix haemorrhage-intraventricular haemorrhage; n=number; NEC=necrotising enterocolitis.

6.4.1 General Movements at Term-Equivalent Age

One hundred and fifteen (84%) of the 137 preterm participants assessed at TEA had GMs that could be scored, representing 83% of the study cohort. The majority of GMs (n=80; 70%) were abnormal: 79 poor repertoire, 1 cramped synchronised. No GMs at TEA were categorised as chaotic. Table 6.2 describes the number of preterm participants and their GMs for each of the cUS time-points and time-epochs evaluated. Fewer infants were included at later time-points and time-epochs than earlier, reflecting the local practice of transferring infants to secondary

units when they no longer required intensive care, usually from 32 weeks' post-menstrual age.

Table 6.2 General Movements at term-equivalent age for preterm participants

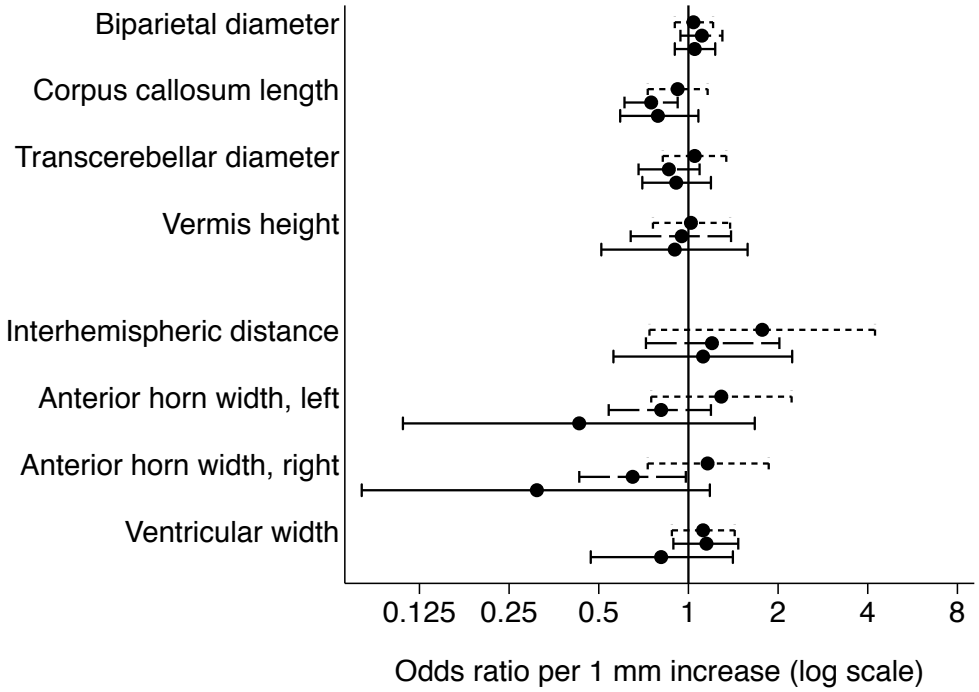
	Participants, n	General Movements, n (%)		
		Normal	Abnormal	
			Poor repertoire	Cramped synchronised
Brain size				
1-week	104	33 (32)	70 (67)	1 (1)
1-month	89	25 (28)	63 (71)	1 (1)
2-months	44	9 (21)	34 (77)	1 (2)
Brain growth				
Birth to 1-month	89	25 (28)	63 (71)	1 (1)
1- to 2-months	46	11 (24)	34 (74)	1 (2)

n=number.

6.4.1.1 Relationships of Early Postnatal Brain Size with General Movements at Term-Equivalent Age

The relationships between early postnatal cUS linear measures of brain size and the risk of abnormal GMs at TEA are described in Figure 6.1 and Tables 6.3-6.5. Lower risk of abnormal GMs at TEA was associated with larger measures of the CCL at 1-week, 1-month, and 2-months, BPD at 1-week, and TCD at 2-months. After adjustment for GA at birth, BW z score, and sex, only the relationship between risk of abnormal GMs at TEA and a larger CCL at 1-month persisted, however, a relationship between abnormal GMs at TEA and a larger right AHW at 1-month emerged.

Figure 6.1 Relationships of early postnatal brain size with risk for abnormal General Movements at term-equivalent age



Adjusted for gestational age at birth, birth weight z score, and sex. Data are presented as the odds ratio (and 95% confidence interval) for abnormal General Movements per 1 mm increase in linear measure. Legend: Dot=mean; Line=95th confidence interval; Short dash line=1-week postnatal age; Long dash line=1-month postnatal age; Solid line=2-months' postnatal age.

Table 6.3 Brain size at 1-week postnatal age and risk for abnormal General Movements at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	**0.91**	**0.84, 0.99**	**0.022**	1.04	0.9, 1.21	0.566
Corpus callosum length	**0.83**	**0.72, 0.95**	**0.006**	0.92	0.73, 1.16	0.485
Transcerebellar diameter	0.88	0.77, 1.01	0.06	1.05	0.82, 1.34	0.711
Vermis height	0.84	0.68, 1.05	0.135	1.02	0.76, 1.38	0.872
Fluid spaces						
Interhemispheric distance	1.65	0.68, 4.04	0.27	1.77	0.74, 4.23	0.201
Anterior horn width, left^	1.13	0.65, 1.97	0.664	1.29	0.75, 2.22	0.355
Anterior horn width, right^	1.05	0.66, 1.66	0.845	1.16	0.73, 1.86	0.523
Ventricular width	0.95	0.76, 1.19	0.682	1.12	0.88, 1.43	0.352

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

Table 6.4 Brain size at 1-month postnatal age and risk for abnormal General Movements at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	0.98	0.9, 1.06	0.557	1.11	0.94, 1.3	0.223
Corpus callosum length	**0.81**	**0.7, 0.92**	**0.001**	**0.75**	**0.61, 0.92**	**0.006**
Transcerebellar diameter	0.89	0.79, 1	0.054	0.86	0.68, 1.09	0.206
Vermis height	0.86	0.66, 1.12	0.258	0.95	0.64, 1.39	0.779
Fluid spaces						
Interhemispheric distance	1.15	0.69, 1.93	0.589	1.2	0.72, 2.02	0.483
Anterior horn width, left^	0.92	0.64, 1.33	0.66	0.81	0.54, 1.19	0.28
Anterior horn width, right^	0.73	0.47, 1.15	0.176	**0.65**	**0.43, 0.98**	**0.039**
Ventricular width	1.13	0.88, 1.44	0.344	1.15	0.89, 1.47	0.283

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

Table 6.5 Brain size at 2-months' postnatal age and risk for abnormal General Movements at term-equivalent age

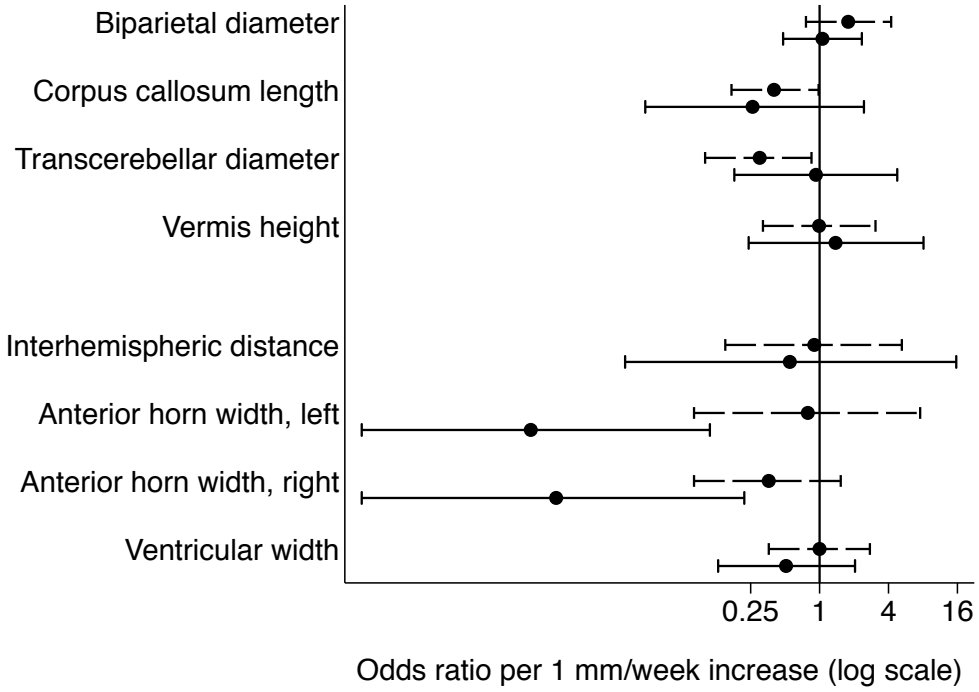
Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	0.98	0.91, 1.05	0.563	1.05	0.9, 1.23	0.504
Corpus callosum length	**0.75**	**0.61, 0.92**	**0.005**	0.79	0.59, 1.08	0.136
Transcerebellar diameter	**0.84**	**0.72, 0.98**	**0.023**	0.91	0.7, 1.19	0.505
Vermis height	0.75	0.53, 1.06	0.103	0.9	0.51, 1.58	0.702
Fluid spaces						
Interhemispheric distance	0.78	0.49, 1.25	0.299	1.12	0.56, 2.23	0.751
Anterior horn width, left^	0.4	0.11, 1.44	0.159	0.43	0.11, 1.67	0.221
Anterior horn width, right^	0.31	0.07, 1.4	0.127	0.31	0.08, 1.18	0.086
Ventricular width	0.83	0.58, 1.18	0.295	0.81	0.47, 1.41	0.46

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

6.4.1.2 Relationships of Early Postnatal Brain Growth with General Movements at Term-Equivalent Age

The relationships between early postnatal cUS linear measures of brain growth and the risk of abnormal GMs at TEA are described in Figure 6.2 and Tables 6.6-6.7. Lower risk of abnormal GMs at TEA was associated with faster positive growth rates of the TCD between birth and 1-month, and left AHW between 1- and 2-months, before and after adjustment for GA at birth, BW z score, and sex. Only after adjustment for GA at birth, BW z score, and sex, lower risk of abnormal GMs was related to faster positive growth rates of the CCL between birth and 1-month, and right AHW between 1- and 2-months.

Figure 6.2 Relationships of early postnatal brain growth with risk for abnormal General Movements at term-equivalent age



Adjusted for gestational age at birth, birth weight z score, and sex. Data are presented as the odds ratio (and 95% confidence interval) for abnormal General Movements per 1 mm/week increase in linear measure. Legend: Dot=mean; Line=95th confidence interval; Dash line=Birth to 1-month postnatal age; Solid line=1-month to 2-months' postnatal age.

Table 6.6 Brain growth between birth and 1-month postnatal age and risk for abnormal General Movements at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	2.05	0.74, 5.65	0.166	1.78	0.76, 4.22	0.186
Corpus callosum length	0.41	0.16, 1.01	0.053	**0.4**	**0.17, 0.98**	**0.045**
Transcerebellar diameter	**0.26**	**0.09, 0.74**	**0.011**	**0.3**	**0.1, 0.85**	**0.023**
Vermis height	1	0.33, 3.06	0.996	0.99	0.32, 3.08	0.991
Fluid spaces						
Interhemispheric distance	0.91	0.17, 4.86	0.912	0.9	0.15, 5.23	0.903
Anterior horn width, left^	0.48	0.07, 3.29	0.452	0.79	0.08, 7.56	0.84
Anterior horn width, right^	0.65	0.2, 2.13	0.478	0.36	0.08, 1.53	0.165
Ventricular width	1.21	0.44, 3.31	0.714	1	0.36, 2.75	0.997

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

Table 6.7 Brain growth between 1- and 2-months' postnatal age and risk for abnormal General Movements at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	0.78	0.38, 1.6	0.501	1.06	0.48, 2.34	0.889
Corpus callosum length	0.81	0.06, 10.1	0.867	0.26	0.03, 2.44	0.236
Transcerebellar diameter	0.79	0.23, 2.68	0.706	0.93	0.18, 4.77	0.929
Vermis height	0.81	0.14, 4.58	0.813	1.38	0.24, 8.09	0.718
Fluid spaces						
Interhemispheric distance	0.09	0.002, 3.58	0.202	0.55	0.02, 15.63	0.725
Anterior horn width, left^	**0.03**	**0.002, 0.42**	**0.009**	**0.003**	**0.0001, 0.11**	**0.002**
Anterior horn width, right^	0.06	0.001, 4.07	0.193	**0.005**	**0.0001, 0.22**	**0.007**
Ventricular width	0.24	0.03, 1.7	0.153	0.51	0.13, 2.04	0.34

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

6.4.2 Hammersmith Neonatal Neurological Examination at Term-Equivalent Age

One hundred and six (77%) of the 137 preterm participants assessed at TEA had complete data available from their HNNE assessment, representing 76% of the study cohort. The mean (SD) HNNE optimality scores for preterm infants and term-controls were 28.1 (3.4) and 31.6 (2.0), respectively, with a suboptimal HNNE determined *a priori* for 52 (49%) preterm infants.[84] Table 6.8 describes the number of participants and their HNNE for each of the cUS time-points and time-epochs evaulated. Again, fewer preterm infants were included at later time-points and time-epochs than earlier.

Table 6.8 Hammersmith Neonatal Neurological Examination at term-equivalent age for preterm participants

	Participants, n	Hammersmith Neonatal Neurological Examination	
		Total Score, mean (SD)	Suboptimal, n (%)
Brain size			
1-week	93	25.6 (3.9)	48 (52)
1-month	82	25.7 (3.9)	40 (49)
2-months	39	25.0 (4.3)	22 (56)
Brain growth			
Birth to 1-month	81	28.1 (3.4)	40 (49)
1- to 2-months	37	27.4 (3.7)	21 (57)

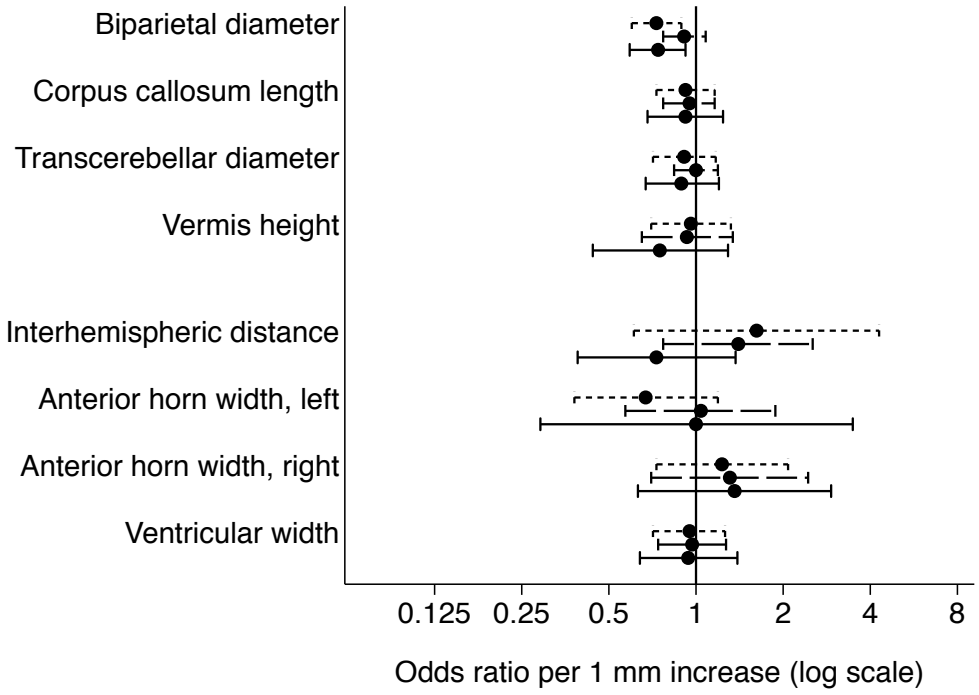
n=number.

6.4.2.1 Relationships of Early Postnatal Brain Size with the Hammersmith Neonatal Neurological Examination at Term-Equivalent Age

The relationships between early postnatal cUS linear measures of brain size and the risk of a suboptimal HNNE at TEA are described in Figures 6.3 and Tables 6.9-6.11. Lower risk of a suboptimal HNNE at TEA was associated with larger measures of the BPD at 1-week, 1-month, and 2-months, and CCL and TCD at 1-

week. After adjustment for GA at birth, BW z score, and sex, only the relationships with the BPD at 1-week and 2-months persisted. There was no evidence of any relationship between the HNNE and linear measures of fluid spaces at any time-point.

Figure 6.3 Relationships of early postnatal brain size with risk for suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age



Adjusted for gestational age at birth, birth weight z score, and sex. Data are presented as the odds ratio (and 95% confidence interval) for suboptimal Hammersmith Neonatal Neurological Examination per 1 mm increase in linear measure. Legend: Dot=mean; Line=95th confidence interval; Short dash line=1-week postnatal age; Long dash line=1-month postnatal age; Solid line=2-months' postnatal age.

Table 6.9 Brain size at 1-week postnatal age and risk for a suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	**0.82**	**0.72, 0.93**	**0.002**	**0.73**	**0.6, 0.89**	**0.002**
Corpus callosum length	**0.82**	**0.7, 0.96**	**0.015**	0.92	0.73, 1.16	0.467
Transcerebellar diameter	**0.86**	**0.74, 0.99**	**0.033**	0.91	0.71, 1.17	0.458
Vermis height	0.84	0.67, 1.06	0.151	0.96	0.7, 1.32	0.821
Fluid spaces						
Interhemispheric distance	1.42	0.53, 3.83	0.488	1.62	0.61, 4.29	0.332
Anterior horn width, left^	0.58	0.32, 1.08	0.084	0.67	0.38, 1.19	0.174
Anterior horn width, right^	1.07	0.63, 1.81	0.815	1.23	0.73, 2.08	0.446
Ventricular width	0.85	0.66, 1.1	0.222	0.95	0.71, 1.26	0.703

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

Table 6.10 Brain size at 1-month postnatal age and risk for a suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	**0.88**	**0.79, 0.98**	**0.017**	0.91	0.77, 1.08	0.296
Corpus callosum length	0.87	0.74, 1.01	0.072	0.95	0.77, 1.16	0.606
Transcerebellar diameter	0.93	0.84, 1.04	0.227	1	0.84, 1.19	0.992
Vermis height	0.83	0.64, 1.08	0.166	0.93	0.65, 1.34	0.714
Fluid spaces						
Interhemispheric distance	1.31	0.7, 2.42	0.396	1.4	0.77, 2.53	0.274
Anterior horn width, left^	0.94	0.59, 1.51	0.806	1.04	0.57, 1.88	0.902
Anterior horn width, right^	1.16	0.69, 1.93	0.579	1.31	0.7, 2.44	0.395
Ventricular width	0.92	0.74, 1.16	0.501	0.97	0.74, 1.27	0.843

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

Table 6.11 Brain size at 2-months' postnatal age and risk for a suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age

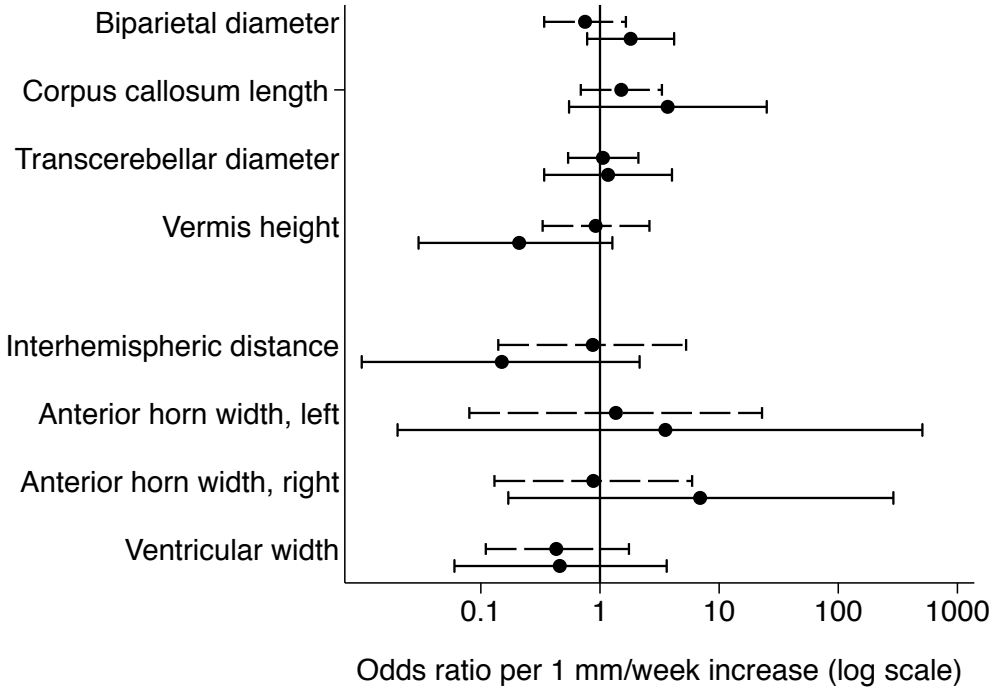
Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	**0.88**	**0.78, 0.99**	**0.028**	**0.74**	**0.59, 0.92**	**0.007**
Corpus callosum length	0.87	0.69, 1.09	0.227	0.92	0.68, 1.24	0.582
Transcerebellar diameter	0.88	0.76, 1.02	0.095	0.89	0.67, 1.2	0.444
Vermis height	0.78	0.58, 1.05	0.101	0.75	0.44, 1.29	0.305
Fluid spaces						
Interhemispheric distance	0.7	0.38, 1.28	0.244	0.73	0.39, 1.37	0.33
Anterior horn width, left^	0.72	0.32, 1.64	0.431	1	0.29, 3.48	0.995
Anterior horn width, right^	1.11	0.59, 2.06	0.752	1.36	0.63, 2.93	0.431
Ventricular width	0.89	0.66, 1.2	0.438	0.94	0.64, 1.39	0.76

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

6.4.2.2 Relationships of Early Postnatal Brain Growth with the Hammersmith Neonatal Neurological Examination at Term-Equivalent Age

The relationships between early postnatal cUS linear measures of brain growth and risk of a suboptimal HNNE at TEA are described in Figure 6.4 and Tables 6.12-6.13. Lower risk of a suboptimal HNNE was associated with a faster positive rate of increase of the IHD between 1- and 2-months. After adjustment for GA at birth, BW z score, and sex, there was no evidence to support any relationships between lower risk of a suboptimal HNNE at TEA and rates of change of linear measures of either brain tissue or fluid spaces within any time-epoch.

Figure 6.4 Relationships of early postnatal brain growth with risk for suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age



Adjusted for gestational age at birth, birth weight z score, and sex. Data are presented as the odds ratio (and 95% confidence interval) for suboptimal Hammersmith Neonatal Neurological Examination per 1 mm/week increase in linear measure. Legend: Dot=mean; Line=95th confidence interval; Dash line=Birth to 1-month postnatal age; Solid line=1-month to 2-months' postnatal age.

Table 6.12 Brain growth between birth and 1-month postnatal age and risk for a suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	0.71	0.33, 1.56	0.399	0.75	0.34, 1.65	0.474
Corpus callosum length	1.52	0.72, 3.18	0.272	1.51	0.69, 3.31	0.305
Transcerebellar diameter	0.98	0.51, 1.87	0.95	1.06	0.54, 2.1	0.867
Vermis height	0.91	0.36, 2.26	0.836	0.92	0.33, 2.6	0.88
Fluid spaces						
Interhemispheric distance	0.89	0.15, 5.23	0.894	0.87	0.14, 5.28	0.883
Anterior horn width, left^	1.44	0.12, 17.03	0.772	1.36	0.08, 22.91	0.83
Anterior horn width, right^	1.35	0.27, 6.83	0.714	0.88	0.13, 5.93	0.895
Ventricular width	0.53	0.16, 1.82	0.317	0.43	0.11, 1.75	0.241

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

Table 6.13 Brain growth between 1- and 2-months' postnatal age and risk for a suboptimal Hammersmith Neonatal Neurological Examination at term-equivalent age

Linear measure	Unadjusted			Adjusted[#]		
	OR	95% CI	p value	OR	95% CI	p value
Brain tissues						
Biparietal diameter	1.19	0.78, 1.79	0.419	1.81	0.78, 4.19	0.168
Corpus callosum length	2.75	0.61, 12.33	0.186	3.7	0.55, 25.1	0.18
Transcerebellar diameter	0.77	0.21, 2.76	0.684	1.17	0.34, 4.02	0.797
Vermis height	0.3	0.09, 1	0.05	0.21	0.03, 1.27	0.09
Fluid spaces						
Interhemispheric distance	**0.14**	**0.02, 0.97**	**0.046**	0.15	0.01, 2.15	0.163
Anterior horn width, left^	0.81	0.32, 2.04	0.661	3.54	0.02, 508.27	0.618
Anterior horn width, right^	3.51	0.28, 43.2	0.327	6.93	0.17, 290.14	0.31
Ventricular width	0.46	0.09, 2.37	0.352	0.46	0.06, 3.63	0.465

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. CI=confidence interval; OR=odds ratio.

6.5 Discussion

This study provides some evidence of a relationship between early postnatal cUS linear measures and neurobehaviour at TEA in infants born at <30 weeks' GA and free of major brain injury seen on cUS. Supporting our hypothesis, several linear measures of larger brain size in the first weeks and months after birth, including the BPD, CCL, and TCD, were associated with lower risk of abnormal GMs and a suboptimal HNNE at TEA, and faster positive growth rates of the CCL and TCD between birth and 1-month were related to lower risk of abnormal GMs at TEA. There was no evidence to support any association of larger measures of fluid spaces at any time-point and higher risk of atypical neurobehaviour at TEA. Contrary to our hypothesis, there was evidence to support a relationship between a larger right AHW at 1-month and lower risk of abnormal GMs at TEA, but not for other linear measures of fluid spaces. There was also evidence of a relationship of a faster positive rate of increase of the AHW and IHD between 1- and 2-months with lower risk of abnormal GMs and a suboptimal HNNE.

Atypical neonatal neurobehaviour has been associated with cUS findings of major brain injury in very preterm infants, including GMH-IVH and cystic PVL.[240, 241] However, many more preterm infants demonstrate atypical neurobehaviour in the newborn period than have cUS evidence of major brain injury. In the current study of very preterm infants free of major brain injury seen on cUS, approximately 70% of preterm participants assessed at TEA had abnormal GMs – the majority of which were categorised as poor repertoire – and 50% had a suboptimal HNNE. The integrity of the developing brain following preterm birth must therefore be adversely affected by mechanisms other than overt focal pathologies readily detected by cUS. Microstructural brain injury and perturbations in neuromaturational processes following preterm birth have been described using histopathological and advanced brain MRI techniques.[242-244] The magnitude of disruption to typical brain development is dependent on the timing of the insult(s) with respect to post-menstrual age (in this case, preterm birth and its morbidities), and manifest by variable growth and maturation of regional brain structures.[245]

In a study of the same cohort, with the inclusion of 3 infants with major brain injury seen on cUS, Olsen et al showed that sequential GMs performed from birth and up to TEA were related to markers of brain injury, growth, and maturation on brain MRI at TEA.[246] With respect to linear measures of brain size, they observed a relationship between a larger measure of the TCD at TEA and lower risk of abnormal GMs at TEA, but not with GMs at earlier time-points. The current study provides evidence to support a relationship between a faster positive growth of the TCD between birth and 1-month and lower risk of abnormal GMs at TEA, suggesting that insults to the developing cerebellum within the first weeks after preterm birth may be important determinants of cerebellar growth and neurobehavioural and motor functioning. In a study of very preterm infants, Spittle et al showed that abnormal GMs at 3 months' post-TEA – within the "fidgety" period – were related to cerebral white matter injury and a smaller measure of the TCD on brain MRI at TEA, providing further evidence of the importance of early postnatal cerebellar growth on neurobehaviour.[247, 248] Absence of fidgety GMs is highly specific for cerebral palsy, and, apart from sequential GMs, provide the best evidence for the predictive validity of GMs.[249, 250] Although the finding of abnormal GMs at TEA is not in itself highly predictive of later adverse neurodevelopment, the associations of a larger measure of the CCL at 1-month, and faster growth of the CCL and TCD between birth and 1-month, with lower risk for abnormal GMs at TEA suggests that these linear measures should be explored further as early markers of long-term development.

There was evidence in the current study to support associations of a larger measure of the BPD at 1-week and 2-months with lower risk of a suboptimal HNNE at TEA. The HNNE assesses a broad range of an infant's neurological functioning, including spontaneous movement and antigravity postures, elicited reflexes and responses to environmental stimuli, and behavioural modulation. It is therefore unsurprising that single linear measures of brain tissue or fluid spaces are, for the most part, unrelated to this complex assessment of an infant's biology and behaviour, and their interaction to the environment.

Olsen et al reported an association between a larger measure of the IHD on brain MRI at TEA and abnormal GMs from the early weeks after birth and up to

TEA.[246] However, in the current study, there was no evidence of any relationship between larger fluid spaces and greater risk of atypical neurobehaviour at TEA. The reasons for this difference could be explained by the time-point at which suboptimal brain growth influences the absolute size of the IHD, that is, enlargement of extra-axial spaces may occur later and be more reliably assessed at TEA than earlier, or that measurements of the IHD between the two neuroimaging modalities do not compare.

A major strength of this study includes the use of simple and reproducible linear measures made from cUS performed as part of routine clinical care, using standard imaging planes with easily identifiable anatomical landmarks, supporting the generalisability of these linear measures in clinical practice. The study also recruited one of the largest cohorts of very preterm infants, most of whom were free of major brain injury seen on cUS but otherwise had similar rates of perinatal morbidities that are observed in comparable healthcare settings, affording an opportunity to interrogate the influence of very preterm birth on early postnatal brain growth unencumbered by major brain injury. Participants recruited to the larger study (VIBeS-2) are also being followed into childhood, permitting further studies to explore the relationships between early postnatal cUS linear measures with later neurodevelopmental outcomes.

There are, however, several limitations of our study. Although the majority of the infants had scans until approximately 33 weeks' post-menstrual age, the more mature infants in the cohort were less likely to have had linear measures beyond the first month after birth as it is standard practice in this tertiary unit to transfer infants to non-tertiary units from as early as 32 weeks' post-menstrual age, if they no longer require intensive care. The smaller number of infants who had scans after the first postnatal month were therefore more likely to have been less mature at birth, or to have had a greater burden of morbidities that led to the need for ongoing tertiary-level care, diminishing the power of the study to explore associations between linear measures and neurobehaviour. Another limitation of the study includes the high proportion of preterm participants with abnormal GMs, particularly given that the study excluded infants with major brain injury seen on cUS.

The current study was not designed to provide absolute values for any linear measure that may help clinicians to identify infants at risk of adverse neurodevelopment. We propose a future study, specifically designed for research purposes, in which infants are scanned frequently from the early days after birth and up to TEA, to provide normative reference data for postnatal brain growth in very preterm infants using sequential cUS linear measures. Using these data, there is the potential to evaluate the prognostic utility of linear measures with regard to absolute size, which may better discriminate high-risk infants. Furthermore, linear measures of whole and regional brain growth, reflecting cerebral and cerebellar brain size and ventricular and extra-axial fluid spaces, could be included with markers of brain injury and brain maturation in the validation of a scoring system using sequential cUS from birth and up to TEA to predict later development into childhood. Follow-up into childhood would allow more subtle motor and cognitive deficits associated with very preterm birth to be identified on standardised assessments.

6.6 Conclusion

Several linear measures of larger brain tissue made from cUS within the first weeks after birth are related to lower risk of atypical neurobehaviour at TEA in infants born at <30 weeks' GA. Further studies are warranted to explore the relationship of early postnatal cUS linear measures with long-term neurodevelopmental outcomes.

Chapter 7

Study 3: Associations of Neurodevelopment at 2 Years with Early Postnatal Cranial Ultrasonography Linear Measures

Chapter Overview

This chapter is presented in manuscript form, as prepared for submission to American Journal of Neuroradiology.

Study 3 turns to the final aim of my thesis, that is, to explore associations between early postnatal cUS linear measures of brain size and brain growth and neurodevelopment at 2 years in infants born at <30 weeks' GA. The prognostic utility of linear measures made from sequential cUS performed in the neonatal period as part of routine clinical care of very preterm infants is tested using their neurodevelopmental outcomes at 2 years. The primary outcomes of interest include performance on the cognitive, language, and motor domains on the Bayley-III. The secondary outcome is risk of adverse neurodevelopment, defined by a composite of either cerebral palsy, any developmental delay, deafness, or blindness.

Title Page

Relationships between Early Postnatal Cranial Ultrasonography Linear Measures and Neurodevelopment at 2 Years in Infants Born <30 Weeks' Gestational Age and Without Major Brain Injury

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Abbreviations: AHW=anterior horn width; BPD=biparietal diameter; BW=birth weight; CCL=corpus callosum length; cUS=cranial ultrasonography; GA=gestational age; IHD=interhemispheric distance; IVH=intraventricular haemorrhage; PNA=postnatal age; PVL=periventricular leukomalacia; TCD=transcerebellar diameter; TEA=term-equivalent age; VH=vermis height; VW=ventricular width.

7.1 Abstract

7.1.1 Background

There are scant data on the relationship between early postnatal brain growth and neurodevelopment at 2 years in very preterm infants.

7.1.2 Objective

To examine the relationships of early postnatal cUS linear measures of brain size and brain growth with neurodevelopment at 2 years in infants born <30 weeks' gestational age (GA) and without major brain injury.

7.1.3 Materials and Methods

Prospective study comprising 144 infants born <30 weeks' GA and free of major brain injury on cUS or anomalies known to affect neurodevelopment. Linear measures of brain tissue and fluid spaces, made from cUS performed as part of routine clinical care, were used to assess brain size at 1-week, 1-month, and 2-months' postnatal age (PNA), and brain growth between 1-week and 1-month, and between 1-month and 2-months' PNA. Primary outcomes were the cognitive, language, and motor scores on the Bayley-III at 2 years. Secondary outcome was risk of adverse neurodevelopment at 2 years, defined by a composite of either cerebral palsy, any developmental delay, deafness, or blindness.

7.1.4 Results

313 scans were evaluated for the 131 children assessed at 2 years. Larger measures of brain tissue at 1-week, 1-month, and 2-months were related to higher cognitive and language scores, and larger measures of brain tissue at 2-months were related to lower risk of adverse neurodevelopment. Faster growth of the transcerebellar diameter between 1-month and 2-months was related to a higher cognitive score and lower risk of adverse neurodevelopment. Unexpectedly, larger

measures of fluid spaces at 1-month and 2-months were related to higher language and motor scores, larger measures of fluid spaces at 1-week and 1-month were related to lower risk of adverse neurodevelopment, and a faster increase of the interhemispheric distance between 1-week and 1-month was related to a higher language score and lower risk of adverse neurodevelopment.

7.1.5 Conclusions

This study supports associations of early postnatal cUS linear measures of brain size and brain growth with neurodevelopment at 2 years in infants born <30 weeks' GA and free of major brain injury. The relationships between larger fluid spaces and better neurodevelopment warrant further exploration.

7.1 Introduction

Cranial ultrasonography (cUS) can be used to reliably detect major brain injury associated with very preterm birth and long-term adverse neurodevelopment, namely high-grade germinal matrix-intraventricular haemorrhage (GMH-IVH), cystic periventricular leukomalacia (PVL), and larger cerebellar haemorrhage.[5, 123] However, the greater proportion of children born very preterm with neurodevelopmental impairments do not have major brain lesions identified on neonatal cUS.[4, 5, 147] Nonetheless, as a widely available, inexpensive, bedside tool, cUS remains the most frequently used brain imaging modality for preterm infants.[94]

There is the potential to explore further the utility of cUS to predict neurodevelopment following very preterm birth using alternative parameters apart from overt, focal brain injury. Neuroimaging studies, using cUS and magnetic resonance imaging (MRI), have shown that very preterm infants have smaller brains and larger ventricular and extra-axial spaces at term-equivalent age (TEA) than term-born controls, and these findings relate to higher risk for adverse neurodevelopment in later childhood.[4, 134, 187] Horsch et al showed that evidence of brain atrophy on cUS at TEA, defined by enlarged extra-axial spaces and less complex gyral folding, with or without ventriculomegaly, was associated with poorer psychomotor and mental development at 3 years in children born very preterm, including those with earlier cUS findings of overt focal pathology.[187] However, the subjective assessment of brain size and implications of atrophy in their study limits its reproducibility, and therefore clinical applicability.

Using linear measures to supplement qualitative assessments of brain size, Skiold et al recently showed cUS to be as sensitive as brain MRI to predict cerebral palsy and severe cognitive delay.[161] In their study, the negative predictive values of a normal or mildly abnormal cUS at TEA for later normal neurologic status or cognitive outcome were 98% and 100%, respectively.[161] Other studies comparing the prognostic utility of contemporaneous cUS and brain MRI at TEA for very preterm infants have largely focused on cUS assessment of brain injury (for example,

cysts), and a limited qualitative assessment of brain size (for example, enlarged extra-axial spaces and ventricuolomegaly), and reported, somewhat marginal, superior sensitivity of brain MRI over cUS.[47, 164] The use of simple linear measures made from routine clinical cUS for the prediction of neurodevelopmental outcomes following very preterm birth warrants further research.

In most neonatal units, cUS is routinely performed for very preterm infants from the first days after birth up to discharge, and in some places also at TEA, affording an opportunity to evaluate early postnatal brain size and brain growth as potential markers of long-term neurodevelopment.[190] We have previously shown that simple and reproducible linear measures of brain tissue and fluid spaces, made from cUS performed as part of clinical care, are related to perinatal determinants of neurodevelopment in very preterm infants free of major brain injury on cUS.[227] In this follow-up study of the same cohort of children, we aimed to explore the relationships of early postnatal cUS linear measures of brain size and brain growth with neurodevelopment at 2 years. We hypothesised that larger measures of brain tissue and smaller measures of fluid spaces made from cUS performed within the first weeks and months after very preterm birth would be associated with higher cognitive, language, and motor scores on the Bayley-III and lower risk of adverse neurodevelopment at 2 years.

7.2 Materials and Methods

7.2.1 Study Participants

Between January 2011 and December 2013, 149 infants born at <30 weeks' GA, and 201 term-born controls, were recruited from The Royal Women's Hospital, Melbourne, into a prospective longitudinal study of neuroimaging, neurobehaviour, and long-term development.[215] Infants with congenital or chromosomal anomalies known to affect neurodevelopment were excluded. For the current study, 5 preterm participants with major brain injury on cUS (grades III or IV IVH [n=5], post-haemorrhagic ventricular dilatation [n=3], and/or cystic PVL [n=1]) were also

excluded, as were a further 5 preterm participants who died during the primary hospitalisation.[93, 98] This study received institutional approval from the hospital's Human Research Ethics Committee, and written informed consent was obtained from the parents of all participants.

7.2.2 Cranial Ultrasonography Linear Measures

Linear measures were made from cUS performed as part of routine clinical care using a Logiq 9 Ultrasound System and an 8 MHz broadband curvilinear transducer (GE Healthcare, Milwaukee, Wisconsin). Eight linear measures – four of brain tissue [biparietal diameter (BPD), corpus callosum length (CCL), transcerebellar diameter (TCD), and vermis height (VH)] and four reflecting fluid spaces [interhemispheric distance (IHD), left and right anterior horn widths (AHWs), and ventricular width (VW)] – were chosen on the basis of their potential clinical importance, ease of recognition of anatomical landmarks on standard imaging planes, and excellent inter-observer agreement (see Chapter 4, Figure 4.2).[190, 227] The methodology and reliability for these cUS linear measures has been described previously.[227]

Linear measures of *brain size* in mm were made from a single scan for each participant at 3 time-points with respect to postnatal age (PNA), including 1-week (days 3-11, inclusive), 1-month (days 21-35, inclusive), and 2-months (days 50-70, inclusive). Linear measures of *brain growth* in mm/week were made from all scans performed for each participant within 2 time-epochs with respect to PNA, namely between the first week and 1-month (days 0-35, inclusive), and between 1-month and 2-months (days 21-70, inclusive).

7.2.3 Neurodevelopment at 2 Years

Participants were assessed at 2 years' corrected age for cerebral palsy, deafness, and blindness by a developmental paediatrician, and for cognitive, language, and motor development by an allied health professional using the Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley-III).[251] Assessors were blinded to the clinical history of all participants, including cUS findings.

Cerebral palsy was defined as loss of motor function accompanied by abnormal tone, posture or tendon reflexes, in the absence of other neurological causes of motor dysfunction.[33] Primary outcomes were the cognitive, language, and motor scores on the Bayley-III. Secondary outcome was risk of adverse neurodevelopment, defined by a composite of either cerebral palsy, any developmental delay (Bayley-III composite score <-1 SD in any domain relative to term-born controls), deafness (required hearing aids or worse), or blindness (visual acuity <6/60 in the better eye).[221]

7.2.4 Data Analysis

Data were analysed using STATA Version 13.1 (StatCorp, College Station, Texas). Relationships of early postnatal cUS linear measures with neurodevelopment at 2 years were explored using linear (for the continuous primary outcomes) and logistic (for the categorical secondary outcome) regression modelling, fitted using generalised estimating equations to account for multiple birth. Firstly, we explored the relationships between linear measures of early postnatal *brain size* and neurodevelopment at 2 years. For each of the 3 time-points, linear measures were included separately in the model, with either the primary or secondary outcomes as the dependent variable, and the linear measure (mm) and PNA at time of scan (days) as covariates. Secondly, we explored the relationships between linear measures of early postnatal *brain growth* and neurodevelopment at 2 years. Brain growth, or rate of change of the linear measure with respect to PNA (mm/week PNA), was evaluated for all linear measures for each participant using linear regression modelling within each of the 2 time-epochs. Then, within time-epochs, the calculated brain growth was included separately in the model, with either the primary or secondary outcomes as the dependent variable, and the rate of change of the linear measure (mm/week) and time between the first and last scans within that time-epoch (days) as covariates. Relationships between linear measures and neurodevelopment at 2 years were then adjusted for GA at birth, BW z score, and sex, known determinants of brain development and later neurodevelopment in very preterm infants.

7.3 Results

One hundred and thirty-one (94%) of the 139 preterm survivors, and 183 (91%) of the 201 term-born controls, included in the current study were assessed at 2 years. Preterm children assessed at 2 years [64 (49%) male] had a mean GA at birth of 27.8 (SD 1.5) weeks and BW 1019 (SD 254) g, compared with term-born children [98 (54%) male) with mean GA at birth 39.8 (SD 1.2) weeks and BW 3524 (SD 444) g. Of the 8 preterm survivors who were not assessed, 4 had withdrawn from the study and 4 were lost to follow-up. There were no significant differences in the perinatal characteristics between the 131 preterm participants who were assessed at 2 years and the 8 not assessed (Supplementary Table 7.1).

7.3.1 Cranial Ultrasonography Linear Measures

Linear measures were made from 313 scans performed in the early postnatal period from the first week up to 2-months' PNA for the 131 preterm participants with complete data.

Overall, linear measures of brain tissue and fluid spaces increased in absolute size across successive time-points from 1-week to 2-months' PNA (Table 7.1). There were many fewer infants who had linear measures available for evaluation at 2-months' PNA (range 34-39) compared with earlier time-points (range 86-112). Early postnatal brain growth within the two time-epochs with respect to PNA is also given in Table 7.1. The BPD, TCD, and VH all increased more in the second time-epoch (1- to 2-months' PNA) compared with the first (birth to 1-month PNA), but the CCL increased more in first time-epoch. The IHD and VW increased more in the second time-epoch compared with the first, but the AHWs increased earlier. Again, fewer infants had linear measures available for evaluation in the later time-epoch (range 32-40) compared with earlier (range 83-94).

Table 7.1 Linear measures of brain size and brain growth

Linear measure	Brain size (mm) Mean (SD)			Brain growth (mm/week) Mean (SD)	
	Time-point (PNA)			Time-epoch (PNA)	
	1-week	1-month	2-months	Birth – 1-month	1-month – 2-months
Biparietal diameter	57.8 (5.1) [n=106]	61.0 (4.8) [n=92]	67.8 (6.8) [n=38]	1.14 (0.86) [n=91]	2.01 (1.04) [n=39]
Corpus callosum length	35.4 (3.0) [n=110]	38.4 (3.3) [n=95]	39.5 (3.4) [n=35]	1.10 (0.48) [n=94]	0.63 (0.46) [n=36]
Transcerebellar diameter	32.3 (3.3) [n=107]	37.4 (4.4) [n=90]	43.8 (5.6) [n=34]	1.69 (0.77) [n=89]	2.17 (0.79) [n=35]
Vermis height	16.7 (1.9) [n=108]	18.1 (1.9) [n=86]	20.2 (2.5) [n=36]	0.46 (0.46) [n=83]	0.79 (0.47) [n=34]
Interhemispheric distance	0.94 (0.50) [n=107]	1.59 (0.74) [n=96]	2.60 (1.22) [n=38]	0.16 (0.25) [n=93]	0.29 (0.33) [n=40]
Anterior horn width, left^	1.44 (1.00) [n=112]	1.65 (0.87) [n=96]	1.95 (1.08) [n=39]	0.15 (0.26) [n=83]	0.09 (0.24) [n=32]
Anterior horn width, right^	1.33 (1.07) [n=112]	1.62 (0.90) [n=96]	1.86 (1.16) [n=39]	0.11 (0.25) [n=85]	0.06 (0.30) [n=32]
Ventricular width	17.6 (2.0) [n=100]	18.5 (1.8) [n=94]	22.0 (2.4) [n=36]	0.46 (0.49) [n=84]	0.82 (0.52) [n=37]

^Excluding participants with an ipsilateral intraventricular haemorrhage (grades I or II). n=number of linear measures available; PNA=postnatal age; SD=standard deviation.

7.3.2 Neurodevelopmental Outcomes

Within each of the three developmental domains of the Bayley-III, the mean scores for preterm participants were well within the test norms but were approximately half a standard deviation lower than those for term-born controls (Table 7.2). Significantly more preterm children had developmental delay (<-1 SD relative to the mean for term-born controls) compared with term-born controls in all three domains. Four (3%) preterm children and no term-born child had cerebral palsy, and no child in either group had deafness or blindness. All 4 preterm children with cerebral palsy were walking (2 diplegia, 1 hemiplegia, and 1 not classified).

Table 7.2 Neurodevelopmental outcomes for preterm participants and term-born controls

Neurodevelopmental outcome	Preterm participants n=131	Term-born controls n=183	MD	OR	95% CI	p value
Primary outcomes						
Cognitive score – mean (SD)	102.8 (12.9) [n=130]	109.1 (13.7) [n=181]	6.2	-	3.2, 9.2	<0.001
<-1 SD^ – n (%)	48 (37)	34 (19)	-	2.53	1.46, 4.39	<0.0001
Language score – mean (SD)	103.4 (17.8) [n=130]	111.3 (16.6) [n=178]	7.9	-	4.1, 11.8	<0.001
<-1 SD^ – n (%)	37 (28)	34 (19)	-	1.69	0.95, 2.98	0.054
Motor score – mean (SD)	104.6 (16.1) [n=128]	111.5 (15.2) [n=177]	7.0	-	3.4, 10.5	<0.001
<-1 SD^ – n (%)	30 (23)	20 (11)	-	2.40	1.24, 4.72	0.005
Any of cognitive, language, or motor scores <-1 SD^ – n (%)	59 (45)	52 (28)	-	2.06	1.25, 3.40	0.002
Secondary outcome						
Risk of adverse neurodevelopment[#]	62 (47)	52 (28)	-	2.26	1.38, 3.72	<0.001

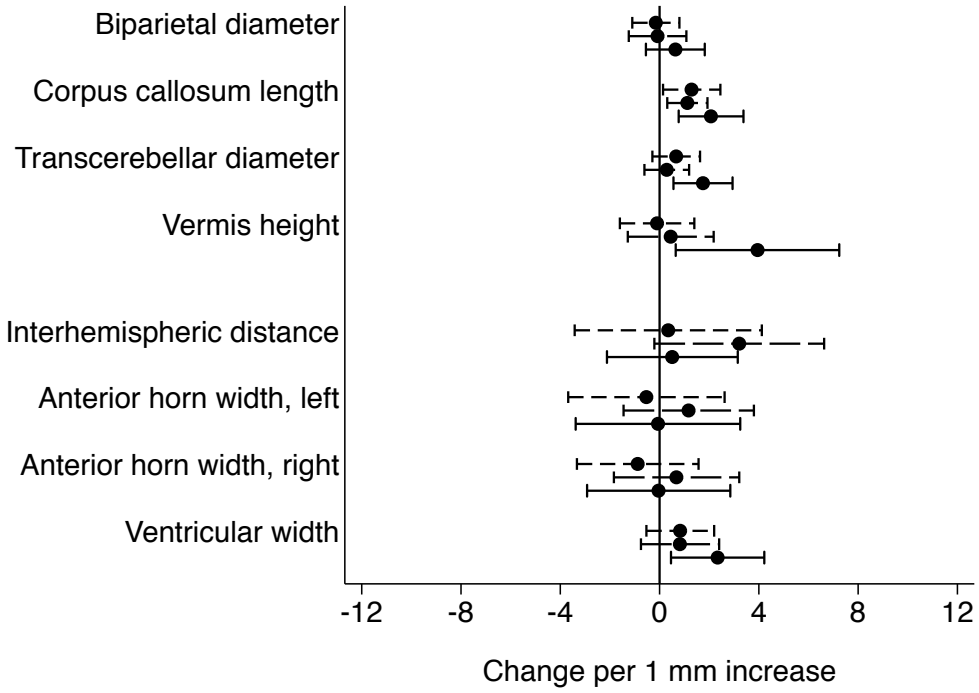
^Relative to the mean for term-born controls. [#]Composite of cerebral palsy, any developmental delay, deafness, or blindness. MD=mean difference; n=number; OR=odds ratio; SD=standard deviation.

7.2.4 Early Postnatal Brain Size and Neurodevelopment at 2 Years

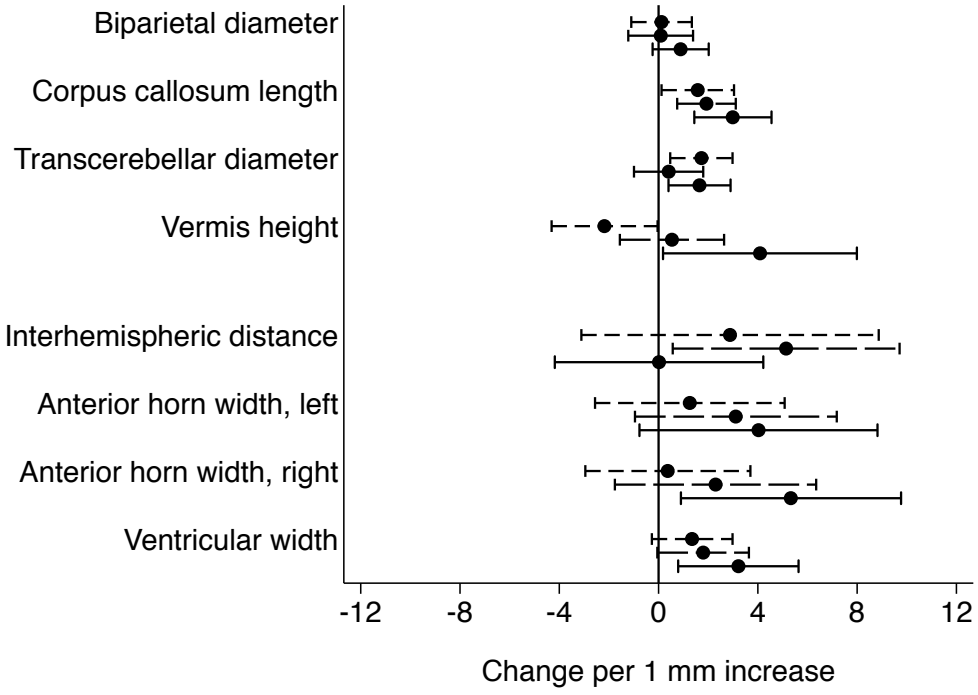
Figures 7.1 and 7.2 (data adjusted for GA at birth, BW z score, and sex) and Supplementary Tables 7.2-7.5 describe the relationships between early postnatal cUS linear measures of brain size and neurodevelopment at 2 years.

Figure 7.1 Change in Bayley-III scores at 2 years per 1 mm increase in early postnatal brain size

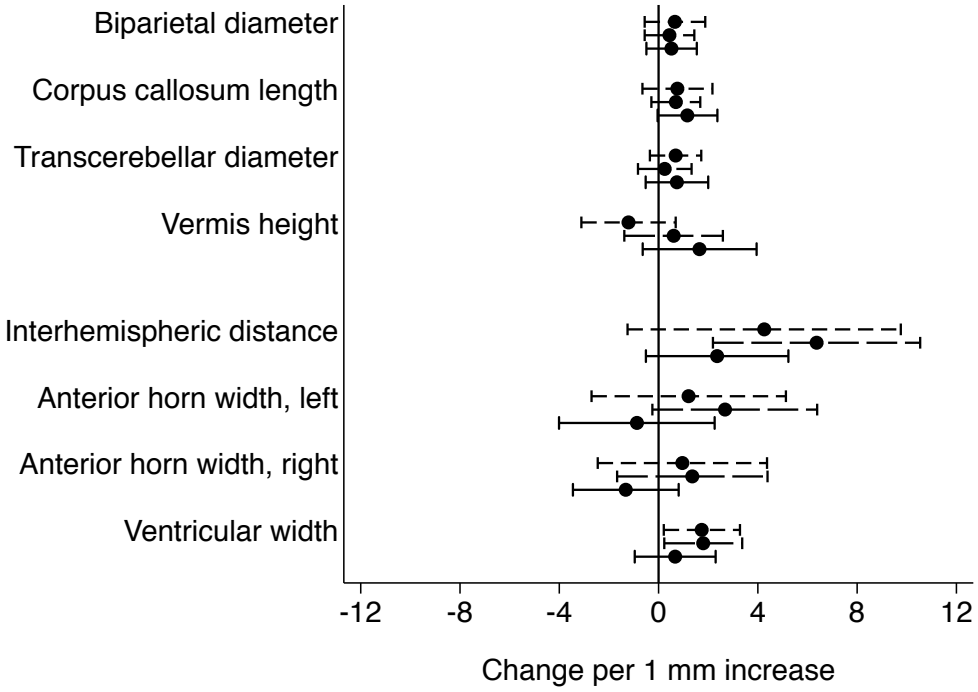
A. Cognitive scores



B. Language scores

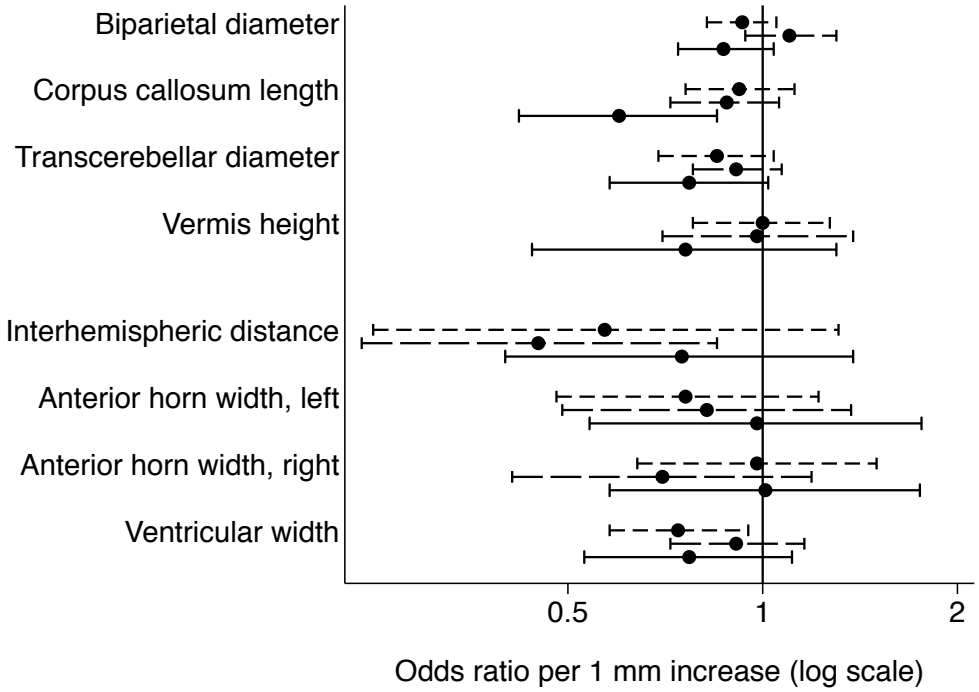


C. Motor scores



Legend: Dot=mean; Line=95th confidence interval; Short dash line=1-week postnatal age; Long dash line=1-month postnatal age; Solid line=2-months' postnatal age. Adjusted for gestational age at birth, birth weight z score, and sex.

Figure 7.2 Odds ratio for risk of adverse neurodevelopment at 2 years per 1 mm increase in early postnatal brain size



Legend: Dot=mean; Line=95th confidence interval; Short dash line=1-week postnatal age; Long dash line=1-month postnatal age; Solid line=2-months' postnatal age. Adjusted for gestational age at birth, birth weight z score, and sex.

Cognitive development. Larger measures of the BPD and VH at 2-months, CCL at 1-week, 1-month, and 2-months, and TCD at 1-week and 2-months were related to higher cognitive scores at 2 years. After adjustment for GA at birth, BW z-score, and sex, only the relationships of the CCL at 1-week, 1-month, and 2-months, and TCD and VH at 2-months with cognitive outcome at 2 years persisted. Larger measures of the VW at 1-week and 2-months were related to higher cognitive scores at 2 years, but only the relationship of the VW at 2-months with cognitive outcome at 2 years persisted after adjustment for GA at birth, BW z score, and sex.

Language development. Larger measures of the BPD at 1-week and 2-months, CCL and TCD at 1-week, 1-month, and 2-months, and VH at 2-months were related

to higher language scores at 2 years. After adjustment for GA at birth, BW z score, and sex, only the relationships of the CCL at 1-week, 1-month, and 2-months, TCD at 1-week and 2-months, and VH at 2-months with language outcome at 2 years persisted, and a relationship between a larger measure of the VH at 1-week and lower language scores at 2 years emerged. Larger measures of the IHD at 1-month, left and right AHW at 2-months, and VW at 1-week and 2-months were related to higher language scores at 2 years. After adjustment for GA at birth, BW z score and sex, only the relationships of the IHD at 1-month, and right AHW and VW at 2-months with language outcome at 2 years persisted.

Motor development. Larger measures of the BPD and VH at 2-months, CCL at 1-week, 1-month, and 2-months, and TCD at 1-week and 2-months were related to higher motor scores at 2 years. After adjustment for GA at birth, BW z score, and sex, there was no evidence for any relationships of brain tissue at any time-point with motor outcome at 2 years. Larger measures of the IHD at 1-month and 2-months, and VW at 1-week, 1-month, and 2-months were related to higher motor scores at 2 years. After adjustment for GA at birth, BW z score, and sex, only the relationships of the IHD at 1-month and VW at 1-week and 1-month with motor outcome at 2 years persisted.

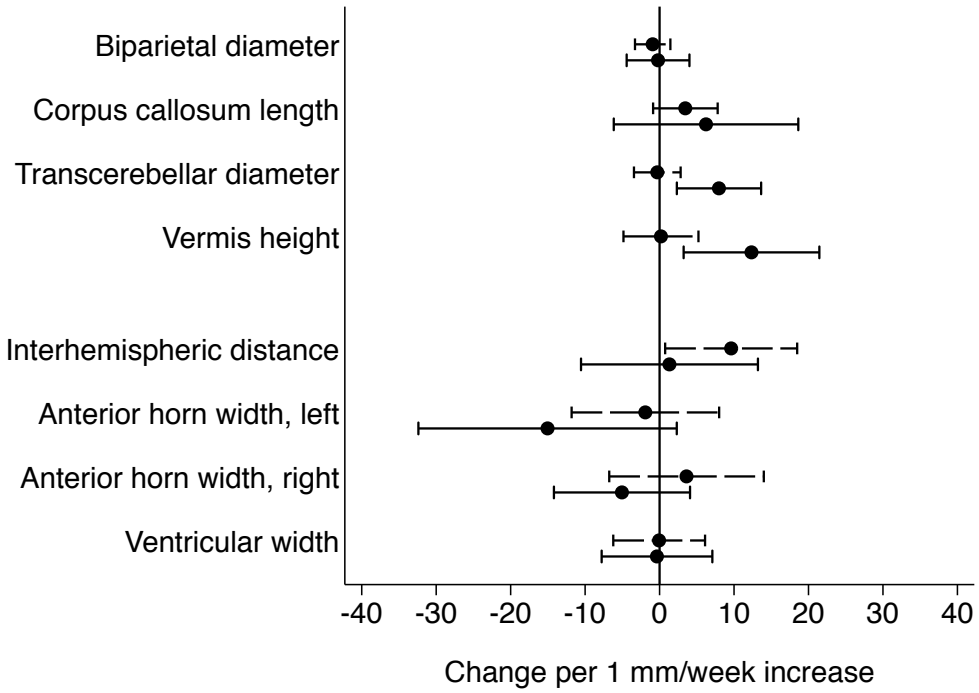
Adverse neurodevelopment. There was no evidence for any relationships of linear measures of brain tissue at any time-point with risk of adverse neurodevelopment at 2 years. However, after adjustment for GA at birth, BW z score, and sex, a larger measure of the CCL at 2-months was related to lower risk of adverse neurodevelopment at 2 years. There was also evidence to support associations between larger measures of the IHD at 1-month and VW at 1-week with lower risk of adverse neurodevelopment at 2 years, both before and after adjustment for GA at birth, BW z score, and sex.

7.2.5 Early Postnatal Brain Growth and Neurodevelopment at 2 Years

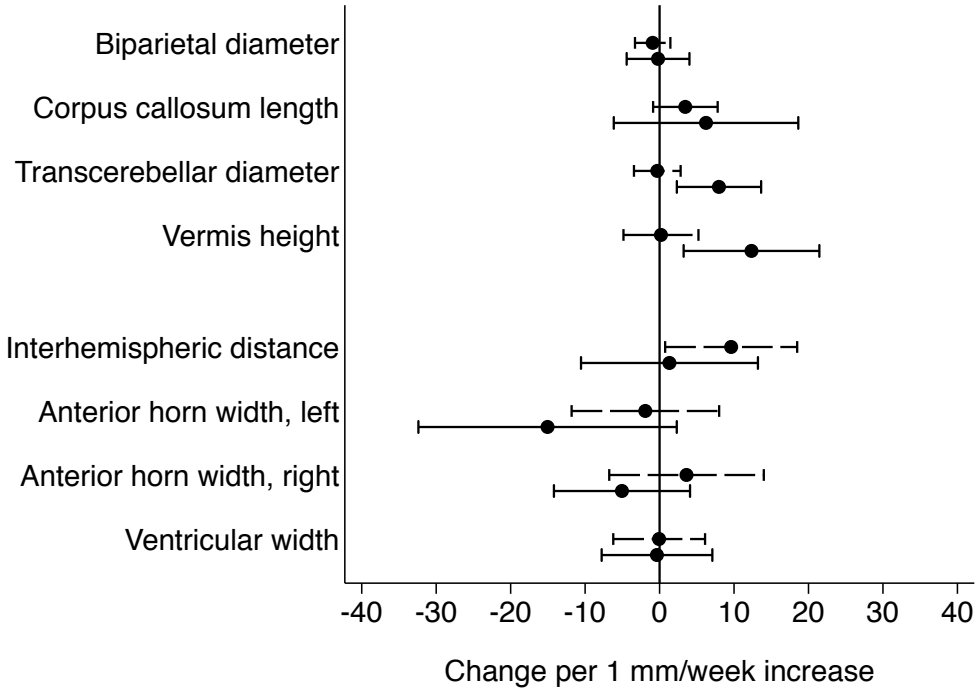
Figures 7.3 and 7.4 (data adjusted for GA at birth, BW z score, and sex) and Supplementary Tables 7.6-7.9 describe the relationships between cUS linear measures of early postnatal brain growth and neurodevelopment at 2 years.

Figure 7.3 Change in Bayley-III scores at 2 years per 1 mm/week increase in early postnatal brain growth

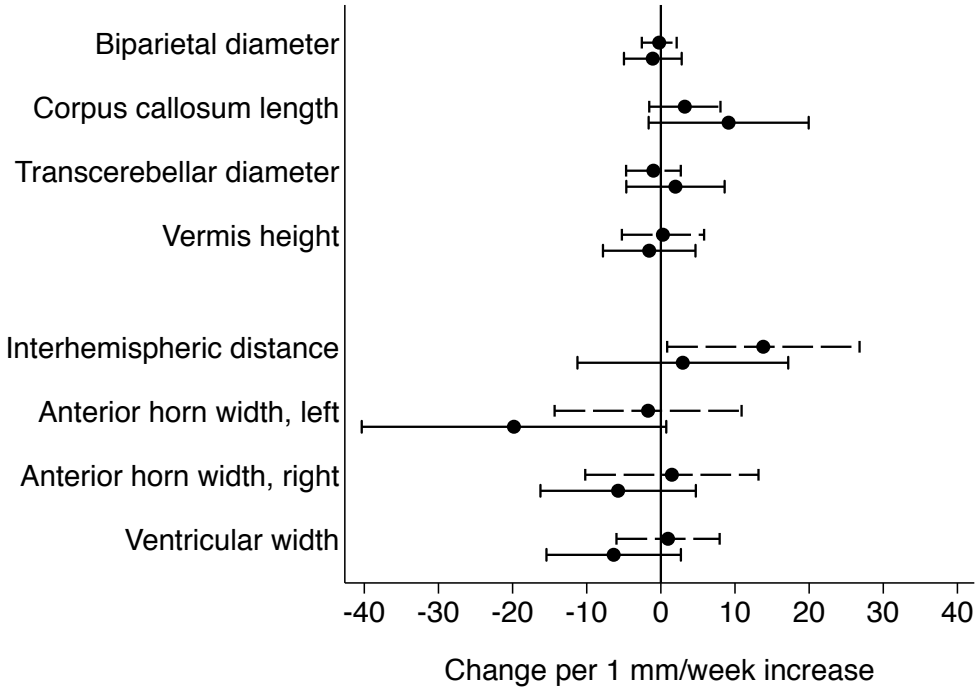
A. Cognitive scores



B. Language scores

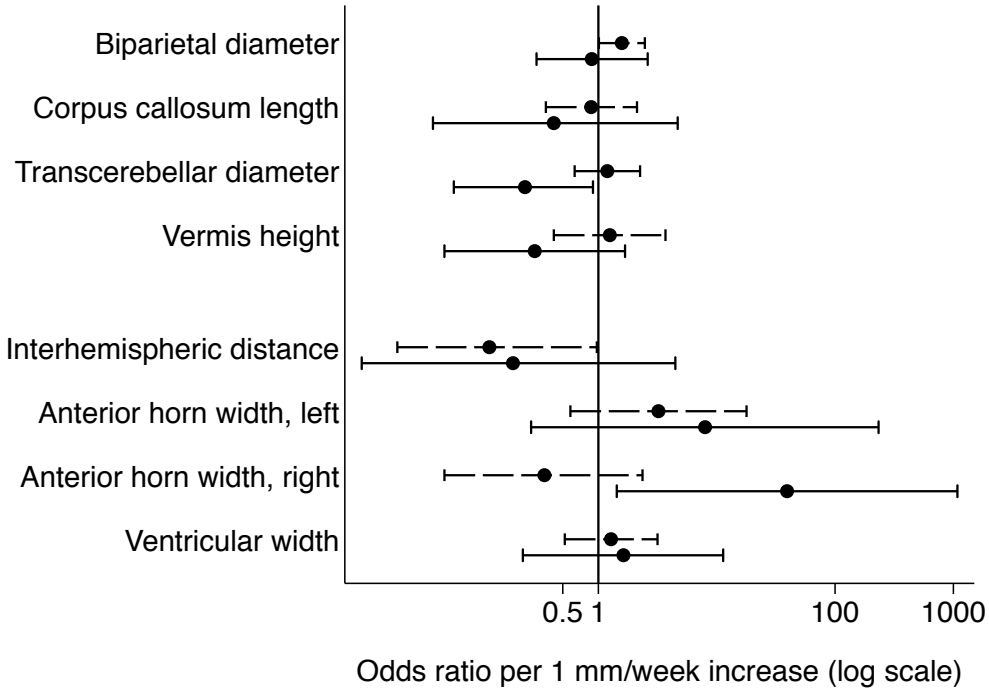


C. Motor scores



Legend: Dot=mean; Line=95th confidence interval; Long dash line=birth to 1-month postnatal age; Solid line=1- to 2-months' postnatal age. Adjusted for gestational age at birth, birth weight z score, and sex.

Figure 7.4 Odds ratio for risk of adverse neurodevelopment at 2 years per 1 mm/week increase in early postnatal brain growth



Legend: Dot=mean; Line=95th confidence interval; Long dash line=birth to 1-month postnatal age; Solid line=1- to 2-months' postnatal age. Adjusted for gestational age at birth, birth weight z score, and sex.

Cognitive development. Faster positive growth of the TCD and VH between 1- and 2-months was related to higher cognitive scores at 2 years, both before and after adjustment for GA at birth, BW z score, and sex. There was no evidence of relationships of rates of change of linear measures of fluid spaces within any time-epoch with cognitive outcome at 2 years, with the exception of a faster rate of positive change of the IHD between birth and 1-month and better cognitive outcome at 2 years after adjustment for GA at birth, BW z score, and sex.

Language development. Faster positive growth of the CCL between birth and 1-month, and the TCD and VH between 1- and 2-months, was related to higher language scores at 2 years, both before and after adjustment for GA at birth, BW z

score, and sex. A faster rate of positive change of the IHD between birth and 1-month, and VW between 1- and 2-months, was related to higher language scores at 2 years, but only the relationship of the IHD with language outcome persisted after adjustment for GA at birth, BW z score, and sex.

Motor development. There was no evidence of relationships between growth of brain tissue within any time-epoch and motor outcome at 2 years. A faster rate of positive change of the IHD between birth and 1-month was related to higher motor scores at 2 years, both before and after adjustment for GA at birth, BW z score and sex.

Adverse neurodevelopment. Faster positive growth of the BPD between birth and 1-month was related to higher risk of adverse neurodevelopment at 2years, before and after adjustment for GA at birth, BW z score, and sex. After adjustment for GA at birth, BW z score, and sex, faster positive growth of the TCD between 1- and 2-months was related to lower risk of adverse neurodevelopment at 2 years. With respect to fluid spaces, faster rates of positive change of the IHD between birth and 1-month, and right AHW between 1- and 2-months, were related to lower and higher risk of adverse neurodevelopment at 2 years, respectively, but only after adjustment for GA at birth, BW z score, and sex.

7.4 Discussion

In this study of children born at <30 weeks' GA and free of major brain injury on neonatal cUS, several cUS linear measures of brain tissue and fluid spaces, measured from as early as the first days after birth and up to 2-months' PNA, were related to neurodevelopment at 2 years. Fewer relationships persisted after adjustment for GA at birth, BW z score, and sex, suggesting that these covariates are more important determinants of neurodevelopment at 2 years than some of the linear measures, or that their relationship with later development is mediated by their influence on early postnatal brain growth.[229]

After adjustment for GA at birth, BW z score, and sex, several linear measures of brain size and brain growth were independently related to neurodevelopment at 2 years. With respect to linear measures of brain size, larger measures of brain tissue at 2-months' PNA were related to lower odds of adverse neurodevelopment at 2 years, and larger measures of brain tissue at 1-week, 1-month, and 2-months' PNA were related to higher cognitive and language scores at 2 years. Unexpectedly, larger measures of fluid spaces at 1-week and 1-month PNA were related to lower odds of adverse neurodevelopment at 2 years, and larger measures of fluid spaces at 1-month and 2-months' PNA were related to higher language and motor composite scores at 2 years. With respect to sequential linear measures of brain growth, faster growth of the TCD between 1-month and 2-months' PNA was related to lower odds of adverse neurodevelopment and a higher cognitive score at 2 years, and a faster increase of the IHD between birth and 1-month PNA was related to lower odds of adverse neurodevelopment and a higher language composite score at 2 years.

The corpus callosum is a major white matter tract, forming the largest commissure of axons connecting the cortex of one cerebral hemisphere to the other. As a higher-order cerebral structure that continues to develop throughout late gestation, the corpus callosum provides a sensitive structural marker of neurological integrity and later neurodevelopment.[175, 235] Thinning of the corpus callosum in very preterm infants at TEA reflects poorer brain growth and maturation, and is associated with adverse neurodevelopment.[4, 134] In a cUS study of very-low-birth weight infants by Anderson et al, a shorter CCL at TEA was associated with lower psychomotor and mental developmental indices at 2 years, and slower growth of the CCL between 2 and 6 weeks' PNA was predictive of motor delay and cerebral palsy.[174, 175] The current study provides further evidence of an association between a larger CCL and better cognitive and language development at 2 years, albeit in relation to measures of the CCL made at earlier time-points of 1- and 2-months' PNA. However, contrary to Anderson et al's study which included infants with major brain injury, there was no evidence in the current study to support a relationship between the CCL and motor development at any time-point or within any time-epoch.[175]

We found that early postnatal cerebellar measures, including the TCD and VH, were related to cognitive and language development at 2 years. The cerebellum, comprised of the cerebellar hemispheres and vermis, undergoes rapid growth and development in late gestation, increasing in volume up to 5-fold between 20 and 40 weeks' gestation.[252] In addition to modulating motor activity, the cerebellum also has a role in higher-order cognitive, language, and behavioural functioning.[253] Cognitive deficits have been reported in up to 50% of very preterm children by school-age, underscoring the importance of identifying infants in the early postnatal period who are at risk of poorer cognitive development.[19] The current study provides further evidence to support the association of cerebellar growth and later cognitive and language development, albeit using simple and reproducible cUS linear measures. cUS measures of the TCD and VH, particularly at 2-months' PNA, should be explored further as potential prognostic markers to identify infants at risk of cognitive and language delay.

The finding of no relationship between early postnatal brain tissue measures and later motor development was not surprising. Major motor deficits in children born very preterm are most often associated with focal brain injury in the motor pathway and such problems at 2 years, in the absence of focal injury, would not be expected. However, less severe motor problems, though common in the older preterm population, are not necessarily detectable at 2 years. Nevertheless, it is interesting that we did not find an association between the cUS linear measures made in the current study and 2-year motor development, but did with cognitive and language development. However, there have been problems with the standardisation of the motor scale on the Bayley-III and at least absolute values are subject to some scrutiny.[254, 255]

The relationships between larger measures of the IHD at 1-month PNA and better language and motor development at 2 years, and larger measures of the VW at 2-months' PNA and better language development, was surprising. We have previously reported excellent intra- and inter-observer reliability for measures of the IHD and VW in this cohort, but the relatively wide confidence intervals for many of the relationships between linear measures of fluid spaces and neurodevelopmental test scores at 2 years may reflect imprecision of these measures relative to their absolute

values; the IHD in particular is very small compared with most of the other measures of brain tissue and fluid spaces, except the AHW. Measurement of the IHD can be arbitrarily influenced by variation in the relationship between the gyri in any given plane. However, unlike the IHD, measures of the VW are relatively larger, and less dependent on the plane of imaging. In addition, biological plausibility for these relationships is lacking. Ventriculomegaly, in the absence of IVH, is generally taken to reflect ex-vacuo dilatation, a marker of cerebral white matter injury and subsequent reductions in brain tissue volume.[134, 144] Contrary to our findings, neuroimaging studies of very preterm infants at TEA have described associations between larger ventricular and extra-axial spaces and adverse neurodevelopment.[4, 169, 187] Thus, we would caution how the relationships between larger fluid spaces measured in the early postnatal period and later neurodevelopment in the current study are interpreted, and suggest that further research is needed.

A strength of this study is its use of simple and reproducible linear measures made from cUS performed as part of routine clinical care. Linear measures were made from standard imaging planes, with easily identifiable anatomical landmarks, to support their applicability to clinical practice. The study also recruited one of the largest cohorts of very preterm infants with excellent follow-up rates and blinded assessors. Most preterm participants were free of major brain injury seen on cUS, but otherwise had similar rates of perinatal morbidities observed in very preterm populations in comparable healthcare settings, affording an opportunity to interrogate the influence of very preterm birth on early postnatal brain growth unencumbered by major brain injury. However, our study has several limitations. Although the majority of the cohort had scans until approximately 33 weeks' post-menstrual age, the more mature infants in the cohort were less likely to have had linear measures beyond the first month after birth as it is standard practice in our tertiary unit to transfer infants to non-tertiary units from as early as 32 weeks' post-menstrual age, if they no longer require intensive care. Thus, infants who had linear measures available at 2-months' PNA were more likely to have been less mature at birth, or to have had greater neonatal morbidity, and consequently required more prolonged hospitalisation beyond the first month after birth.

The current study does not provide absolute values for any linear measure that may help clinicians to identify infants at risk of adverse neurodevelopment. Future studies, specifically designed for research purposes, need to include infants who are scanned frequently from the early days after birth and up to TEA, to devise reference norms for linear measures of postnatal brain growth in very preterm infants. Using these reference norms, there is the potential to evaluate the prognostic utility of linear measures, with regard to absolute size, which may have better clinical applicability. In addition, it is important that infants be followed into later childhood, when more subtle motor and cognitive deficits are more likely to be identified on standardised assessments.

7.5 Conclusions

This study provides evidence to support some associations between early postnatal cUS linear measures and neurodevelopment at 2 years in infants born at <30 weeks' gestation free of major brain injury seen on neonatal cUS. Larger measures of the CCL and cerebellum were related to lower risk of adverse neurodevelopment and better cognitive and language development. The relationships between larger measures of fluid spaces and better language and motor development were unexpected and warrant further exploration.

Supplementary Table 7.1 Participant characteristics

Perinatal variable	Assessed at 2 years		MD	OR	95% CI	p value
	Yes n=131	No n=8				
GA – weeks, mean (SD)	27.8 (1.5)	27.9 (1.3)	0.11	-	-0.96, 1.18	0.84
BW – g, mean (SD)	1019 (254)	1094 (116)	75	-	-112, 261	0.43
BW z score – mean (SD)	-0.49 (1.03)	-0.21 (1.15)	0.28	-	-0.47, 1.03	0.46
SGA – n (%)	14 (11)	1 (13)	-	0.84	0.10, 40.41	0.87
Male – n (%)	64 (49)	3 (38)	-	1.59	0.29, 10.64	0.53
Multiple gestations – n (%)	59 (45)	4 (50)	-	0.82	0.15, 4.60	0.78
Chorioamnionitis^ – n (%)	36/105 (34)	1/2 (50)	-	0.52	0.01, 42.08	0.64
Antenatal corticosteroids – n (%)	122 (93)	7 (93)	-	1.94	0.04, 18.06	0.55
Antenatal magnesium sulphate – n (%)	95 (73)	6 (75)	-	0.88	0.08, 5.22	0.88
Bronchopulmonary dysplasia – n (%)	42 (32)	2 (25)	-	1.42	0.24, 14.88	0.68
Postnatal corticosteroids – n (%)	15 (11)	1 (13)	-	0.91	0.10, 43.47	0.93
IVH, grades I or II – n (%)	21 (16)	2 (25)	-	0.57	0.09, 6.21	0.51
Sepsis and/or NEC – n (%)	27 (21)	1 (13)	-	1.82	0.22, 84.88	0.58

^Histologic chorioamnionitis known for 107 participants. BW=birth weight; CI=confidence interval; GA=gestational age at birth; IVH=intraventricular haemorrhage; MD=mean difference; n=number; NEC=necrotising enterocolitis; OR=odds ratio; SGA=small for gestational age.

Supplementary Table 7.2 Relationships of early postnatal brain size and cognitive scores at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			β	95% CI	p value	β	95% CI	p value
Biparietal diameter	1-week	105	0.37	-0.1, 0.84	0.13	-0.15	-1.1, 0.8	0.76
	1-month	91	0.28	-0.27, 0.83	0.33	-0.08	-1.24, 1.08	0.89
	2-months	42	**0.67**	**0.19, 1.14**	**0.006**	0.64	-0.55, 1.82	0.29
Corpus callosum length	1-week	110	**1.32**	**0.63, 2.01**	**<0.001**	**1.29**	**0.14, 2.45**	**0.028**
	1-month	94	**0.97**	**0.29, 1.65**	**0.005**	**1.12**	**0.31, 1.93**	**0.007**
	2-months	39	**1.86**	**0.65, 3.08**	**0.003**	**2.07**	**0.77, 3.38**	**0.002**
Transcerebellar diameter	1-week	107	**0.88**	**0.22, 1.55**	**0.009**	0.67	-0.29, 1.63	0.17
	1-month	89	0.42	-0.07, 0.9	0.09	0.29	-0.61, 1.19	0.53
	2-months	39	**1.07**	**0.36, 1.78**	**0.003**	**1.75**	**0.56, 2.94**	**0.004**
Vermis height	1-week	107	0.92	-0.37, 2.22	0.16	-0.1	-1.6, 1.4	0.90
	1-month	85	0.5	-0.81, 1.82	0.45	0.45	-1.28, 2.18	0.61
	2-months	39	**2.45**	**0.3, 4.6**	**0.026**	**3.95**	**0.65, 7.24**	**0.019**
Interhemispheric distance	1-week	106	1.15	-2.96, 5.26	0.58	0.35	-3.42, 4.12	0.86
	1-month	95	3.08	-0.49, 6.64	0.09	3.21	-0.21, 6.63	0.07
	2-months	42	1.46	-1.03, 3.95	0.25	0.51	-2.12, 3.15	0.70
Anterior horn width, left^	1-week	97	-0.09	-3.49, 3.32	0.96	-0.53	-3.68, 2.62	0.74
	1-month	83	1.01	-1.78, 3.8	0.48	1.17	-1.45, 3.8	0.38
	2-months	35	0.64	-2.38, 3.67	0.68	-0.06	-3.38, 3.25	0.97
Anterior horn width, right^	1-week	98	0.17	-2.73, 3.06	0.91	-0.88	-3.33, 1.57	0.48
	1-month	85	1.27	-1.23, 3.77	0.32	0.68	-1.84, 3.21	0.60
	2-months	35	0.83	-1.73, 3.4	0.53	-0.04	-2.92, 2.85	0.98
Ventricular width	1-week	99	**1.25**	**0.09, 2.4**	**0.034**	0.83	-0.53, 2.2	0.23
	1-month	93	0.76	-0.84, 2.37	0.35	0.82	-0.75, 2.4	0.31
	2-months	39	**1.87**	**0.3, 3.45**	**0.02**	**2.34**	**0.46, 4.22**	**0.015**

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. β=change in cognitive score per 1 mm increase in linear measure; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.3 Relationships of early postnatal brain size and language scores at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			β	95% CI	p value	β	95% CI	p value
Biparietal diameter	1-week	105	**0.68**	**0.04, 1.31**	**0.036**	0.12	-1.1, 1.34	0.85
	1-month	91	0.46	-0.28, 1.2	0.22	0.09	-1.22, 1.39	0.90
	2-months	42	**0.98**	**0.33, 1.64**	**0.003**	0.89	-0.24, 2.02	0.12
Corpus callosum length	1-week	110	**1.74**	**0.81, 2.68**	**<0.001**	**1.58**	**0.12, 3.04**	**0.033**
	1-month	94	**1.56**	**0.68, 2.43**	**<0.001**	**1.93**	**0.75, 3.11**	**0.001**
	2-months	39	**2.52**	**1.16, 3.87**	**<0001**	**2.99**	**1.44, 4.55**	**<0.001**
Transcerebellar diameter	1-week	107	**1.68**	**0.76, 2.59**	**<0.001**	**1.73**	**0.47, 2.98**	**0.007**
	1-month	89	**0.75**	**0.004, 1.49**	**0.049**	0.41	-0.99, 1.8	0.57
	2-months	39	**1.26**	**0.45, 2.07**	**0.002**	**1.65**	**0.4, 2.9**	**0.01**
Vermis height	1-week	107	0.31	-1.47, 2.09	0.73	**-2.18**	**-4.31, -0.04**	**0.046**
	1-month	85	0.88	-0.72, 2.49	0.28	0.54	-1.56, 2.64	0.61
	2-months	39	**3.45**	**0.99, 5.91**	**0.006**	**4.09**	**0.18, 7.99**	**0.04**
Interhemispheric distance	1-week	106	3.63	-3.26, 10.51	0.30	2.88	-3.11, 8.87	0.35
	1-month	965	**4.69**	**-0.34, 9.73**	**0.07**	**5.14**	**0.57, 9.71**	**0.027**
	2-months	42	2.11	-1.6, 5.82	0.26	0.02	-4.18, 4.22	0.99
Anterior horn width, left^	1-week	97	1.68	-2.43, 5.79	0.42	1.26	-2.56, 5.08	0.52
	1-month	83	2.89	-1.41, 7.18	0.19	3.11	-0.95, 7.18	0.13
	2-months	35	**4.73**	**0.68, 8.78**	**0.022**	4.03	-0.77, 8.82	0.10
Anterior horn width, right^	1-week	98	1.48	-2.22, 5.19	0.43	0.37	-2.95, 3.7	0.83
	1-month	85	2.95	-1, 6.9	0.14	2.3	-1.76, 6.35	0.27
	2-months	35	**5.49**	**1.45, 9.53**	**0.008**	**5.33**	**0.9, 9.77**	**0.018**
Ventricular width	1-week	99	**1.94**	**0.59, 3.29**	**0.005**	1.35	-0.27, 2.98	0.10
	1-month	93	1.69	-0.22, 3.6	0.08	1.8	-0.05, 3.64	0.06
	2-months	39	**3.01**	**1.2, 4.82**	**0.002**	**3.22**	**0.79, 5.64**	**0.011**

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. β=change in language score per 1 mm increase in linear measure; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.4 Relationships of early postnatal brain size and motor scores at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			β	95% CI	p value	β	95% CI	p value
Biparietal diameter	1-week	103	0.65	-0.03, 1.33	0.06	0.66	-0.56, 1.88	0.29
	1-month	90	0.5	-0.22, 1.22	0.18	0.44	-0.56, 1.44	0.39
	2-months	42	**0.93**	**0.35, 1.52**	**0.002**	0.52	-0.49, 1.54	0.31
Corpus callosum length	1-week	108	**0.97**	**0.02, 1.93**	**0.046**	0.76	-0.65, 2.17	0.29
	1-month	93	**0.87**	**0.01, 1.72**	**0.046**	0.7	-0.29, 1.68	0.16
	2-months	39	**1.49**	**0.27, 2.71**	**0.017**	1.16	-0.04, 2.37	0.06
Transcerebellar diameter	1-week	105	**0.95**	**0.05, 1.85**	**0.039**	0.69	-0.35, 1.72	0.19
	1-month	88	0.67	-0.01, 1.36	0.054	0.25	-0.83, 1.33	0.65
	2-months	39	**1.22**	**0.19, 2.26**	**0.02**	0.74	-0.52, 2	0.25
Vermis height	1-week	105	0.48	-1.15, 2.1	0.57	-1.21	-3.11, 0.69	0.21
	1-month	84	0.74	-0.76, 2.24	0.33	0.61	-1.38, 2.59	0.55
	2-months	39	**2.56**	**0.38, 4.74**	**0.021**	1.65	-0.64, 3.95	0.16
Interhemispheric distance	1-week	104	4.56	-1.55, 10.67	0.14	4.26	-1.25, 9.76	0.13
	1-month	94	**5.93**	**1.4, 10.47**	**0.01**	**6.37**	**2.19, 10.54**	**0.003**
	2-months	42	**3.8**	**1, 6.59**	**0.008**	2.36	-0.51, 5.23	0.11
Anterior horn width, left^	1-week	96	1.41	-2.71, 5.53	0.50	1.21	-2.7, 5.13	0.54
	1-month	82	2.56	-1.17, 6.29	0.18	2.68	-0.25, 6.39	0.16
	2-months	35	1.54	-1.62, 4.7	0.34	-0.87	-4.01, 2.26	0.59
Anterior horn width, right^	1-week	97	1.75	-2.01, 5.51	0.36	0.96	-2.45, 4.37	0.58
	1-month	84	1.97	-1.15, 5.09	0.22	1.36	-1.67, 4.39	0.38
	2-months	35	0.26	-1.54, 2.07	0.77	-1.32	-3.45, 0.81	0.23
Ventricular width	1-week	97	**1.9**	**0.48, 3.33**	**0.009**	**1.74**	**0.21, 3.28**	**0.026**
	1-month	92	**1.71**	**0.13, 3.3**	**0.034**	**1.8**	**0.23, 3.37**	**0.024**
	2-months	39	**1.6**	**0.01, 3.19**	**0.049**	0.67	-0.96, 2.3	0.42

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. β=change in motor score per 1 mm increase in linear measure; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.5 Relationships of early postnatal brain size and risk of adverse neurodevelopment at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			OR	95% CI	p value	OR	95% CI	p value
Biparietal diameter	1-week	106	0.96	0.89, 1.04	0.34	0.93	0.82, 1.05	0.24
	1-month	92	1.01	0.92, 1.11	0.78	1.1	0.94, 1.3	0.23
	2-months	43	0.94	0.85, 1.05	0.26	0.87	0.74, 1.04	0.13
Corpus callosum length	1-week	110	0.92	0.8, 1.04	0.18	0.92	0.76, 1.12	0.42
	1-month	95	0.92	0.81, 1.05	0.21	0.88	0.72, 1.06	0.18
	2-months	40	0.79	0.61, 1.01	0.06	**0.6**	**0.42, 0.85**	**0.005**
Transcerebellar diameter	1-week	107	0.88	0.77, 1.01	0.07	0.85	0.69, 1.04	0.12
	1-month	90	0.95	0.86, 1.06	0.35	0.91	0.78, 1.07	0.26
	2-months	40	0.88	0.74, 1.04	0.14	0.77	0.58, 1.02	0.07
Vermis height	1-week	108	0.94	0.77, 1.15	0.55	1	0.78, 1.27	0.97
	1-month	86	1.01	0.79, 1.3	0.92	0.98	0.7, 1.38	0.92
	2-months	40	0.86	0.6, 1.23	0.41	0.76	0.44, 1.3	0.32
Interhemispheric distance	1-week	107	0.6	0.26, 1.36	0.22	0.57	0.25, 1.31	0.18
	1-month	96	**0.48**	**0.26, 0.89**	**0.02**	**0.45**	**0.24, 0.85**	**0.014**
	2-months	43	0.78	0.44, 1.41	0.42	0.75	0.4, 1.38	0.35
Anterior horn width, left^	1-week	97	0.76	0.48, 1.21	0.25	0.76	0.48, 1.22	0.26
	1-month	83	0.83	0.5, 1.37	0.46	0.82	0.49, 1.37	0.45
	2-months	35	0.87	0.43, 1.73	0.69	0.98	0.54, 1.76	0.94
Anterior horn width, right^	1-week	98	0.91	0.6, 1.39	0.67	0.98	0.64, 1.5	0.92
	1-month	85	0.65	0.38, 1.1	0.11	0.7	0.41, 1.19	0.19
	2-months	35	0.75	0.44, 1.27	0.28	1.01	0.58, 1.75	0.98
Ventricular width	1-week	100	**0.77**	**0.61, 0.96**	**0.023**	**0.74**	**0.58, 0.95**	**0.017**
	1-month	94	0.91	0.72, 1.15	0.42	0.91	0.72, 1.16	0.46
	2-months	40	0.85	0.62, 1.16	0.31	0.77	0.53, 1.11	0.16

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. OR=odd ratio for risk of adverse neurodevelopment at 2 years; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.6 Relationships of early postnatal brain growth and cognitive scores at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			β	95% CI	p value	β	95% CI	p value
Biparietal diameter	Birth to 1-month	89	-1.4	-3.63, 0.83	0.22	-0.92	-3.3, 1.45	0.45
	1- to 2-months	38	1.43	-2.64, 5.5	0.49	-0.2	-4.42, 4.01	0.93
Corpus callosum length	Birth to 1-month	92	3.18	-1.34, 7.69	0.17	3.46	-0.87, 7.8	0.12
	1- to 2-months	35	1.23	-8.12, 10.57	0.80	6.24	-6.15, 18.63	0.32
Transcerebellar diameter	Birth to 1-month	87	0.29	-2.7, 3.28	0.85	-0.3	-3.44, 2.83	0.85
	1- to 2-months	34	**8.4**	**3.39, 13.42**	**0.001**	**7.98**	**2.32, 13.64**	**0.006**
Vermis height	Birth to 1-month	81	-0.91	-6.18, 4.37	0.74	0.19	-4.85, 5.22	0.94
	1- to 2-months	33	**12.06**	**3.79, 20.33**	**0.004**	**12.35**	**3.23, 21.46**	**0.008**
Interhemispheric distance	Birth to 1-month	91	8.82	0.01, 17.62	0.05	**9.62**	**0.75, 18.48**	**0.03**
	1- to 2-months	39	7.18	-3.4, 17.76	0.18	1.33	-10.55, 13.21	0.83
Anterior horn width, left^	Birth to 1-month	82	-3.48	-13.82, 6.87	0.51	-1.91	-11.81, 8	0.71
	1- to 2-months	32	-8.45	-24.15, 7.25	0.29	-15.04	-32.39, 2.31	0.09
Anterior horn width, right^	Birth to 1-month	84	3	-7.69, 13.7	0.58	3.62	-6.76, 14	0.50
	1- to 2-months	32	-3.23	-12.52, 6.06	0.50	-5.05	-14.19, 4.09	0.28
Ventricular width	Birth to 1-month	82	-0.87	-7.23, 5.48	0.79	-0.06	-6.22, 6.11	0.99
	1- to 2-months	36	1.62	-5.77, 9.02	0.67	-0.35	-7.78, 7.09	0.93

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. β=change in cognitive score per 1 mm increase in linear measure; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.7 Relationships of early postnatal brain growth and language composite scores at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			β	95% CI	p value	β	95% CI	p value
Biparietal diameter	Birth to 1-month	89	-1.14	-4.47, 2.2	0.50	-0.01	-3.19, 3.17	0.99
	1- to 2-months	38	1.45	-4.78, 7.68	0.65	1.8	-4.33, 7.92	0.57
Corpus callosum length	Birth to 1-month	92	**5.8**	**-0.29, 11.9**	**0.06**	**6.36**	**0.52, 12.2**	**0.033**
	1- to 2-months	35	1.22	-13.09, 15.53	0.87	6.13	-10.47, 22.74	0.47
Transcerebellar diameter	Birth to 1-month	87	0.9	-2.92, 4.73	0.64	-0.85	-5.08, 3.39	0.70
	1- to 2-months	34	**7.43**	**0.74, 14.12**	**0.03**	**7.4**	**0.52, 14.27**	**0.035**
Vermis height	Birth to 1-month	81	0.27	-6.85, 7.4	0.94	0.66	-5.07, 6.38	0.82
	1- to 2-months	33	**15.88**	**3.89, 27.86**	**0.009**	**13.13**	**0.11, 26.15**	**0.048**
Interhemispheric distance	Birth to 1-month	91	**16.41**	**0.64, 32.18**	**0.041**	**17.45**	**2.66, 32.25**	**0.021**
	1- to 2-months	39	7.77	-8.16, 23.71	0.34	-2.18	-18.21, 13.85	0.79
Anterior horn width, left^	Birth to 1-month	82	2.57	-13.93, 19.08	0.76	3.7	-11.72, 19.13	0.64
	1- to 2-months	32	-3.36	-28.04, 21.33	0.79	-8.95	-37.31, 19.41	0.54
Anterior horn width, right^	Birth to 1-month	84	7.82	-6.23, 21.87	0.28	7.31	-6.82, 21.44	0.31
	1- to 2-months	32	7.85	-7.98, 23.68	0.33	3.7	-13.46, 20.87	0.67
Ventricular width	Birth to 1-month	82	0.22	-7.33, 7.78	0.95	1.46	-5.59, 8.51	0.69
	1- to 2-months	36	**10.2**	**0.82, 19.57**	**0.033**	7.12	-2.64, 16.89	0.15

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. β=change in language score per 1 mm increase in linear measure; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.8 Relationships of early postnatal brain growth and motor composite scores at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			β	95% CI	p value	β	95% CI	p value
Biparietal diameter	Birth to 1-month	88	-0.66	-2.66, 1.35	0.52	-0.22	-2.57, 2.13	0.86
	1- to 2-months	38	1.48	-1.42, 4.38	0.32	-1.09	-4.98, 2.81	0.58
Corpus callosum length	Birth to 1-month	91	2.41	-2.48, 7.3	0.33	3.23	-1.57, 8.04	0.19
	1- to 2-months	35	3.9	-5.47, 13.27	0.42	9.14	-1.65, 19.94	0.10
Transcerebellar diameter	Birth to 1-month	86	0.67	-2.94, 4.29	0.72	-1	-4.69, 2.69	0.60
	1- to 2-months	34	2.92	-2.81, 8.65	0.32	1.97	-4.66, 8.6	0.56
Vermis height	Birth to 1-month	80	0.51	-5.92, 6.94	0.88	0.28	-5.26, 5.83	0.92
	1- to 2-months	33	2.43	-5.06, 9.91	0.53	-1.57	-7.81, 4.67	0.62
Interhemispheric distance	Birth to 1-month	90	**13.73**	**0.43, 27.04**	**0.043**	**13.82**	**0.85, 26.8**	**0.037**
	1- to 2-months	39	11.04	-0.85, 22.92	0.07	2.96	-11.25, 17.18	0.68
Anterior horn width, left^	Birth to 1-month	81	-1.21	-14.85, 12.44	0.86	-1.72	-14.33, 10.89	0.79
	1- to 2-months	32	-9.74	-29.8, 10.33	0.34	-19.81	-40.35, 0.73	0.06
Anterior horn width, right^	Birth to 1-month	83	2.25	-9.79, 14.29	0.71	1.49	-10.2, 13.17	0.80
	1- to 2-months	32	0.56	-7.93, 9.05	0.90	-5.76	-16.24, 4.72	0.28
Ventricular width	Birth to 1-month	81	-0.18	-6.63, 6.26	0.96	0.97	-5.99, 7.93	0.78
	1- to 2-months	36	-2.02	-9.76, 5.72	0.61	-6.36	-15.43, 2.7	0.17

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. β=change in motor score per 1 mm increase in linear measure; CI=confidence interval; N=number of participants; PNA=postnatal age.

Supplementary Table 7.9 Relationships of early postnatal brain growth and risk of adverse neurodevelopment at 2 years

Linear Measure	Timing (PNA)	N	Unadjusted			Adjusted[#]		
			OR	95% CI	p value	OR	95% CI	p value
Biparietal diameter	Birth to 1-month	**91**	**1.52**	**1.02, 2.27**	**0.04**	**1.58**	**1.01, 2.47**	**0.043**
	1- to 2-months	39	0.8	0.37, 1.74	0.57	0.88	0.3, 2.61	0.82
Corpus callosum length	Birth to 1-month	94	0.89	0.37, 2.14	0.8	0.87	0.36, 2.12	0.76
	1- to 2-months	36	0.47	0.06, 3.44	0.46	0.42	0.04, 4.68	0.48
Transcerebellar diameter	Birth to 1-month	89	1.06	0.59, 1.93	0.84	1.19	0.63, 2.25	0.60
	1- to 2-months	35	0.35	0.1, 1.25	0.11	**0.24**	**0.06, 0.9**	**0.034**
Vermis height	Birth to 1-month	83	1.19	0.37, 3.85	0.77	1.25	0.42, 3.69	0.69
	1- to 2-months	34	0.29	0.06, 1.45	0.13	0.29	0.05, 1.68	0.17
Interhemispheric distance	Birth to 1-month	**93**	0.13	0.02, 1.01	0.051	**0.12**	**0.02, 0.97**	**0.046**
	1- to 2-months	40	0.2	0.01, 3.01	0.24	0.19	0.01, 4.47	0.31
Anterior horn width, left^	Birth to 1-month	83	2.94	0.49, 17.48	0.24	3.22	0.58, 17.86	0.18
	1- to 2-months	32	3.9	0.2, 76.17	0.37	7.98	0.27, 233.12	0.23
Anterior horn width, right^	Birth to 1-month	85	0.3	0.05, 1.83	0.19	0.35	0.05, 2.36	0.28
	1- to 2-months	32	8.26	0.52, 130.83	0.13	**39.33**	**1.43, 1081.5**	**0.03**
Ventricular width	Birth to 1-month	84	1.34	0.55, 3.25	0.52	1.28	0.52, 3.16	0.59
	1- to 2-months	37	1.1	0.24, 5.02	0.90	1.63	0.23, 11.34	0.62

[#]Adjusted for gestational age at birth, birth weight z score, and sex. ^Restricted to infants without an ipsilateral germinal matrix haemorrhage-intraventricular haemorrhage, grades I or II. OR=odd ratio for risk of adverse neurodevelopment at 2 years; CI=confidence interval; N=number of participants; PNA=postnatal age.

Chapter 8

Discussion, Clinical Implications, Future Directions, and Conclusions

8.1 Summary of Main Findings and their Contribution to the Literature

8.1.1 Reproducibility of Cranial Ultrasonography Linear Measures

Linear measures made from cUS should be simple to make and reproducible if they are to be applied in research and clinical practice. In the current study, 8 linear measures, from a total of 19 linear measures evaluated, were found to be highly reproducible, including 4 of brain tissue (the BPD, CCL, TCD, and VH) and 4 reflecting fluid spaces (the IHD, left and right AHWs, and VW). Each of these linear measures had intra- and inter-observer ICCs >0.80, which are considered to be excellent.

The excellent reproducibility of the 8 linear measures evaluated in the current study is consistent with that reported by others using the same linear measures made from cUS performed in preterm and term-born infants.[173, 174, 190, 217] In a study by Fox et al comprising a cohort of 44 infants born at <30 weeks' GA, the inter-observer ICC of the BPD was found to be 0.97, similar to 0.98 in the current study (see Chapter 5).[190] However, the inter-observer ICCs for linear measures of fluid spaces reported in their study varied somewhat from the current study. For instance, Fox et al reported inter-observer ICCs for the left and right AHWs of 0.78 and 0.72, respectively, and for the VW 0.76, which are considered to be good; in the current study, the inter-observer ICCs of the left and right AHWs, and VW, were excellent at 0.91, 0.95, and 0.89, respectively. On the other hand, Fox et al reported excellent inter-observer ICCs for the left and right anterior horn heights and ventricular mid-bodies, ranging between 0.83 and 0.90, whereas the inter-observer ICCs of these

linear measures in the current study were only moderate to good, ranging between 0.47 and 0.78. Of note, both studies evaluated linear measures using clinical cUS performed in similar cohorts within the same institution, albeit with different pairs of observers. The differences in reproducibility of these linear measures of fluid spaces between studies may be explained by the experience of observers or the methodology for making these measures, but this does not account for why only some measures had better reproducibility in one or the other study. In their study of 106 term-born infants, Hagmann et al reported good to excellent intra- and inter-observer ICCs for the left and right ventricular indices, ranging between 0.66 and 0.86, and excellent intra- and inter-observer ICCs for the thalamo-occipital distance, ranging between 0.83 and 0.92; these results are comparable with the reproducibility reported for these measures in the current study.[217]

The intra- and inter-observer ICCs for the CCL in the current study were 0.99 and 0.98, respectively, with an inter-observer mean agreement of 0.32 mm (95% limits of agreement -1.44, 2.07). Several other studies have demonstrated a similarly high level of reproducibility for the CCL.[173, 174, 256] In their study of 64 very-low-birth weight infants, Anderson et al reported an intra-observer ICC of 0.99, and inter-observer mean agreement of -0.35 mm (SD 0.96). Roelants et al, in a study of 140 infants born at <29 weeks' GA, reported intra- and inter-observer ICCs for the CCL of 0.92 and 0.89, respectively, and inter-observer mean agreement of 0.46 mm (SD 1.85), whilst Hagmann et al reported intra- and inter-observer ICCs for the CCL of 0.93 and 0.86, respectively. Hagmann et al also reported excellent intra- and inter-observer ICCs for the VH of 0.86 and 0.88, respectively, which are comparable with the respective ICCs of 0.81 and 0.91 found in the current study.

The current study puts forward 8 linear measures of brain tissue and fluid spaces that can be reliably made from cUS performed as part of routine clinical care in infants born at <30 weeks' GA. However, there is a modest degree of variability in the reproducibility for some of these linear measures, as reported across several studies, underscoring the importance of assessing the reproducibility of linear measures within any given pool of observers and within one's own institution. It is essential to know, before one embarks on making similar linear measures for the

purposes of research or clinical practice, that the observers making these linear measures can attain a similar and excellent degree of reproducibility.

8.1.2 Evaluation of Early Postnatal Brain Growth using Sequential Cranial Ultrasonography Linear Measures

Simple and reproducible linear measures of brain tissue and fluid spaces made from clinical cUS can be used to quantify early postnatal brain growth from the first days after birth and up to term-equivalent age in infants born at <30 weeks' GA. Overall, the 8 linear measures of brain tissue and fluid spaces chosen to be explored further in the current study – including 4 of brain tissue (the BPD, CCL, TCD, and VH) and 4 reflecting fluid spaces (the IHD, left and right AHWs, and VW) – increased in absolute size across successive time-points with respect to PNA. Rates of change (mm/week PNA) for all linear measures of brain tissue demonstrated variable positive growth velocities across successive time-epochs within the early postnatal period, with the exception of the BPD that showed a negative growth velocity within the first postnatal week and positive growth velocities thereafter; all rates of change of brain tissue were strongly associated with PNA. Rates of change (mm/week PNA) for linear measures of fluid spaces were more variable, with no evidence of any association in their growth velocities with respect to PNA within the first week after birth.

Although cUS affords an opportunity to make sequential measurements from the first days after birth, there are limited published data relating to postnatal brain *growth* for infants born preterm. The current study (see Chapter 5) is the first to describe the growth of the BPD in the early postnatal period in preterm infants using cUS. The decrease in size of the BPD in the first postnatal week in the current study may relate to a reduction in brain water in the immediate postnatal days and deformational changes in head shape.[231] Growth of the BPD after 1-month PNA (2.17 mm/week PNA) observed in the current study approximates to normative reference data for expected fetal growth (2.10 mm/week post-menstrual age).[193] However, relatively slower growth of the BPD was observed between 1-week and 1-month (1.30 mm/week PNA) compared with after 1-month PNA. This may reflect the influence of postnatal morbidities impeding *ex utero* brain growth relative to *in utero* brain growth reported for fetuses of uncomplicated pregnancies in the cross-

sectional study by Kurmanavicius et al.[193] Moreover, preterm participants cared for at this neonatal intensive care unit would most likely have been receiving respiratory support by continuous positive airway pressure delivered by Hudson prongs, an interface which applies an extrinsic force across the bifrontal and biparietal regions of the skull, commonly causing deformational head shaping resembling dolicocephaly.

In the current study, CCL growth was relatively similar from birth to 1-week and from 1-week to 1-month PNA, but slowed by half thereafter. Similarly, Anderson et al showed that postnatal growth of the CCL in very-low-birth weight infants approximated fetal growth (1.4-1.89 mm/week post-menstrual age) within the first 2 weeks after birth, but subsequently slowed to approximately half of that expected.[175, 196, 197] *In utero* growth of the CCL follows a similar pattern, albeit with respect to post-menstrual age, demonstrating slower growth after approximately 30 weeks' post-menstrual age compared with earlier.[196, 197] Whilst a similar pattern of slowing growth of the CCL was observed in the current study, albeit with respect to PNA, rates of *ex utero* growth of the CCL within the first postnatal month (0.99-1.09 mm/week PNA) were slower than expected fetal growth (1.4-1.89 mm/week post-menstrual age) meaning that infants born very preterm would be expected to have smaller CCL at term-equivalent age compared with term-born infants.

The TCD, measured by ultrasonography in the fetus or the newborn within the first postnatal days, is a reliable marker of GA, even in the presence of fetal growth restriction, in small- and large-for-gestational age fetuses, and in multiple gestations (1.25–1.89 mm/week post-menstrual age).[177, 180, 198, 199] Cross-sectional brain MRI studies in preterm infants have reported cerebellar size at varying PNA, but the current study is the first to report postnatal *growth* of the TCD in preterm infants using sequential cUS.[232, 233] Similar to fetal growth patterns, postnatal growth of the TCD observed in the current study progressively increased in velocity across successive time-epochs. Unlike the TCD, growth of the VH increased with PNA at a relatively constant rate of change from birth up to term-equivalent age, approximating normative reference data for fetal growth of the VH (0.55 mm/week post-menstrual age).[213]

Liao et al and Levene et al performed sequential cUS measurements of the ventricular index in preterm infants from birth to 6 weeks and from birth to 6 months' PNA, respectively, reporting rates of change similar to those in the current study for the VW (the sum of left and right ventricular indices).[119, 185] Rates of change for the AHW with respect to PNA were variable, and increased little after the first postnatal week, as reported in other longitudinal studies.[119, 184-186]

8.1.3 Associations of Early Postnatal Cranial Ultrasonography Linear Measures with Perinatal Variables

Several perinatal variables related to long-term development were associated with early postnatal cUS linear measures of brain growth from birth up to term-equivalent age. Bronchopulmonary dysplasia and postnatal corticosteroid administration were associated with slower growth of the CCL and TCD, and postnatal corticosteroid administration was also associated with slower growth of the VH. Sepsis and/or NEC were associated with slower growth of the TCD.

In the current study, higher GA at birth was related to slower growth of the BPD from birth and up to term-equivalent age. This is in keeping with normative reference data for fetal growth of the BPD that demonstrates a deceleration of growth velocity with increasing post-menstrual age.[193, 232] The majority of very preterm infants admitted to this neonatal intensive care unit are transferred to secondary units when they no longer require intensive care, usually from 32 weeks' post-menstrual age. It is therefore plausible that the more mature infants in the study cohort who remained at this neonatal intensive care unit for a longer period, and were therefore more likely to have had more scans, had a greater burden of morbidities impairing their brain growth. It could also be speculated that slower growth of the BPD observed in the more mature infants within the early postnatal period might relate to extrinsic forces leading to deformational changes in head shape, as discussed in Chapter 8, Section 8.1.2. However, infants born at <30 weeks' GA are more likely to have smaller whole and regional brain volumes at term-equivalent age, as shown using brain MRI, than term-born controls, and most are likely to have had slower rates of brain growth in the early postnatal period than normally expected had they developed *in utero*.[159, 232]

In the current study (see Chapter 5), BW z-score <-2 SD was associated with slower growth of the CCL from birth and up to term-equivalent age, a finding consistent with Roelants et al.[173] Bronchopulmonary dysplasia and postnatal corticosteroids were independently associated with slower growth of the CCL and TCD from birth and up to term-equivalent age, and postnatal corticosteroid administration was also related to slower growth of the VH. Tam et al reported a similar association between postnatal corticosteroid use, a marker of severe bronchopulmonary dysplasia, and impaired cerebellar growth in 172 preterm infants (mean GA 28 weeks, SD 2) using sequential brain MRI between 32 weeks' post-menstrual age and term-equivalent age.[234] In another brain MRI study of very preterm infants scanned at term-equivalent age and at 7 years, Thompson et al reported an association between postnatal corticosteroid use and delayed maturation of the posterior corpus callosum, a region that develops later in gestation and contributes to its length.[235] In the current study, sepsis/NEC was related to slower growth of the TCD from birth and up to term-equivalent age. Sepsis/NEC is associated with white matter injury in very preterm infants as determined from brain MRI at term-equivalent age, but its relationship with brain growth and maturation in the early postnatal period remains unclear.[65] The relationship between antenatal corticosteroid exposure and slower growth of the TCD between birth and up to term-equivalent age observed in the current study is novel and needs to be replicated in future studies.

With respect to linear measures of fluid spaces, lower GA was related to a faster rate of change in the VW from birth and up to term-equivalent age, possibly reflecting a greater burden of diffuse cerebral white matter injury in the less mature infants leading to greater *ex vacuo* dilatation. This is supported by cUS and brain MRI studies in infants born at <30 weeks' GA that demonstrate they are more likely to have ventriculomegaly and enlarged extra-axial spaces at term-equivalent age than term-born controls.

8.1.4 Associations of Neurobehaviour at Term-Equivalent Age with Early Postnatal Cranial Ultrasonography Linear Measures

There was evidence of several relationships between early postnatal cUS linear measures of brain tissue and neurobehaviour at term-equivalent age. Larger brain size, assessed by linear measures of the BPD at 1-week and the CCL at 1-month PNA, were associated with a lower risk of a suboptimal HNNE and abnormal GMs, respectively, and faster growth of the CCL and TCD between birth and 1-month were related to lower risk of abnormal GMs at term-equivalent age. There was no evidence to support any relationship between larger fluid spaces at any time-point and greater risk of suboptimal neurobehaviour at term-equivalent age. There was evidence of a relationship between a faster rate of increase of the right AHW between 1- and 2-months' PNA and a lower risk of abnormal GMs, however absolute size of the right AHW at 1- and 2-months' PNA was not associated with atypical neurobehaviour at term-equivalent age.

Atypical neonatal neurobehaviour has been associated with cUS findings of major brain injury in very preterm infants, including GMH-IVH and PVHI, and cystic PVL.[240] However, many more preterm infants demonstrate atypical neonatal neurobehavioural and motor functioning than have cUS evidence of major brain injury. In the current study of preterm infants free of major brain injury seen on cUS, approximately 70% of preterm participants assessed at term-equivalent age had abnormal GMs – the majority of which were categorised as poor repertoire – and 50% had a suboptimal HNNE (see Chapter 6). It stands to reason that the integrity of the developing brain following preterm birth must necessarily be affected by mechanisms other than overt focal pathologies readily detected by cUS. Microstructural brain injury and perturbations in neuromaturational processes following preterm birth have been described using histopathological and advanced brain MRI techniques.[243] The magnitude of disruption to typical brain development is dependent on the timing of the insult with respect to post-menstrual age (in this case, preterm birth and its morbidities), and manifest by variable growth and maturation of regional brain structures.[235, 257]

In a study of the same cohort, albeit including 3 infants with major brain injury seen on cUS, Olsen et al showed that sequential GMs performed from birth and up to term-equivalent age were related to markers of brain injury, growth, and maturation on brain MRI at term-equivalent age.[246] They observed a relationship between a larger TCD at term-equivalent age and a lower risk of abnormal GMs at term-equivalent age, but not with GMs performed at earlier time-points. The current study provides evidence to support a relationship between a faster growth of the TCD between birth and 1-month PNA and a lower risk of abnormal GMs at term-equivalent age, suggesting that insults to the developing cerebellum within the first weeks after preterm birth may be important determinants of cerebellar growth and neurobehaviour at term-equivalent age. In a study of very preterm infants, Spittle et al showed that abnormal GMs at 3 months' post-term-equivalent age – within the "fidgety" period – were related to cerebral white matter injury and a smaller TCD on brain MRI at -age, providing further evidence of the importance of early postnatal cerebellar growth on later neuromotor functioning.[247, 248] Absence of fidgety GMs is highly specific for cerebral palsy, and, apart from sequential GMs, provide the best evidence for the predictive validity for GMs.[249, 250] Although the finding of abnormal GMs at term-equivalent age is not in itself highly predictive of later adverse neurodevelopment, the associations between a larger measurement of the CCL at 1-month, faster growth of the CCL and TCD between 1- and 2-months' PNA, and a lower risk for abnormal GMs at term-equivalent age suggests that these linear measures should be explored further as early markers of long-term development.

There was little evidence in the current study to support associations between early postnatal cUS linear measures and the HNNE at term-equivalent age, with the exception of a larger BPD at 1-week PNA and lower risk of a suboptimal HNNE at term-equivalent age. The HNNE assesses a broad range of neurological functions, including spontaneous movement and antigravity postures, elicited reflexes and responses to environmental stimuli, and behavioural modulation. It is therefore unsurprising that single linear measures of brain tissue or fluid spaces are unrelated to this complex assessment of an infant's biology and behaviour, and how they relate to their environment. Single linear measures of brain size or brain growth lack the sensitivity to discriminate between optimal and suboptimal HNNE.

Olsen et al reported an association between a larger IHD on brain MRI at term-equivalent age and abnormal GMs at all time-points, up to and including term-equivalent age.[246] In the current study, there was no evidence for any relationship between larger fluid spaces up to 2-months' PNA and greater risk of atypical neurobehaviour at term-equivalent age. The reasons for this difference could be explained by the timing of suboptimal brain growth on absolute size of the IHD with increasing PNA, that is, enlargement of extra-axial space may occur later and more reliably assessed at term-equivalent age, or that measurements of the IHD between the two neuroimaging modalities do not compare.

8.1.5 Associations of Neurodevelopment at 2 Years with Early Postnatal Cranial Ultrasonography Linear Measures

There was evidence of several relationships between neurodevelopment at 2 years and cUS linear measures of brain tissues in the early postnatal period. Larger measurements of the CCL at 1-week, 1-month, and 2-months' PNA, and TCD and VH at 2-months, were related to higher cognitive and language scores at 2 years. Larger measures of the TCD and VH at 1-week were related to higher and lower language scores, respectively. A larger measure of the CCL at 2-months was related to lower risk of adverse neurodevelopment at 2 years. There was also evidence of several relationships between cUS linear measures of fluid spaces in the early postnatal period and neurodevelopment at 2 years. A larger measure of the IHD at 1-month PNA was related to higher language and motor scores, and lower risk of adverse neurodevelopment, at 2 years. A larger measure of the VW at 1-week, 1-month, and 2-months was variably related to higher language and motor scores, and lower risk of adverse neurodevelopment. A larger measure of the right AHW at 2-months was related to higher scores. With respect to linear measures of brain growth, faster positive growth of the TCD and VH between 1- and 2-months was related to higher cognitive and language scores. Faster positive growth of the CCL between birth and 1-month was related to a higher language score. Faster positive growth of the BPD between birth and 1-month, and TCD between 1- and 2-months, was related to higher and lower risk of adverse neurodevelopment, respectively. A faster rate of positive change of the IHD between birth and 1-month was related to higher cognitive, language, and motor scores, and lower risk of adverse neurodevelopment. A faster

rate of positive change of the right AHW between 1- and 2-months was related to higher risk of adverse neurodevelopment.

The current study is the first to describe a relationship between the CCL, measured from cUS in the early weeks after preterm birth, and later cognitive and language development. The corpus callosum is a major white matter tract, forming the largest commissure of axons connecting the cortex of one cerebral hemisphere to the other. As a higher-order cerebral structure that continues to develop throughout late gestation, the corpus callosum provides a sensitive structural marker of neurological integrity and later neurodevelopment.[175, 235] Thinning of the corpus callosum in very preterm infants at term-equivalent age reflects poorer brain growth and maturation, and has been associated with adverse neurodevelopment.[4, 134] In a cUS study of very-low-birth weight infants by Anderson et al, a shorter measure of the CCL at term-equivalent age was associated with lower psychomotor and mental developmental indices at 2 years, and slower growth of the CCL between 2 and 6 weeks' PNA was predictive of motor delay and cerebral palsy.[174, 175] The current study did not show a relationship between CCL growth in the early postnatal period and motor development at 2 years. Anderson et al had included 8 infants with IVH in their study of 55 preterm infants, compared with the current study which excluded infants with high-grade IVH and other major brain injury seen on cUS.[175] A limitation of Anderson et al's study is the lack of any discussion about the grading or site of these lesions, and whether the children who developed cerebral palsy and other motor impairments had had IVH detected on their neonatal cUS. Although they had reported no relationship between CCL growth within any time-epoch and IVH. In the current study, cUS was performed as part of routine clinical care, with fewer scans performed after 1-month PNA, and for many less infants, and therefore could not replicate the methodology used by Anderson et al. In the current study, the magnitude of change in higher cognitive and language scores per 1 mm increase in the measurement of the CCL was progressively larger across successive time-points with respect to PNA, suggesting that the trophic effects of preterm birth on growth and maturation of this white matter tract become more apparent with increasing time from birth.

Early postnatal cerebellar measurements of the TCD and VH were related to cognitive and language development at 2 years. The cerebellum, comprised of the cerebellar hemispheres and vermis, undergoes rapid growth and development in late gestation, increasing in volume up to 5-fold between 20 and 40 weeks' gestation.[258] In addition to modulating motor activity, the cerebellum also has a role in higher-order cognitive, language, and behavioural functioning, and its susceptibility to injury following preterm birth is now well recognised.[68] Cognitive deficits have been reported in up to 50% of very preterm children by school-age, underscoring the importance of any prognostic marker that could potentially identify infants at risk of poorer cognitive development in the early postnatal period.[1] The current study not only provides further evidence to support the association between cerebellar growth and later cognitive and language development, but also validates the use of simple and reproducible cUS linear measures of early postnatal brain size and brain growth that could be used to identify infants at risk for cognitive and language delay.

The finding of no relationship between early postnatal brain tissue measures and later motor development was not surprising. Motor deficits in children born very preterm are most often associated with focal brain injury in the motor pathway, often but not always apparent on sequential cUS in the neonatal period.[5] The current study excluded infants with major brain injury seen on cUS.

The relationships between larger measures of the IHD at 1-month PNA and better language and motor development at 2 years, and larger measures of the VW at 2-months' PNA and better language development, was surprising. I have previously reported excellent intra- and inter-observer reliability for measures of the IHD and VW in this cohort, but I speculate that the relatively wide confidence intervals for many of the relationships between linear measures of fluid spaces and neurodevelopmental test scores at 2 years may reflect poor accuracy of these measures rather than imprecision.[227] Observers making measurements of the IHD are biased by the plane in which the cUS image had been acquired. Measurement of the IHD can be arbitrarily influenced by variation in the relationship between the gyri in any given plane. However, unlike the IHD, measures of the VW are relatively larger, and less dependent on plane of imaging. In any case, biological plausibility of

the relationships between larger measures of the IHD and better neurodevelopmental outcomes is lacking. Ventriculomegaly, in the absence of IVH, is generally taken to reflect ex-vacuo dilatation, a marker of cerebral white matter injury and subsequent reductions in brain tissue volume. Contrary to the findings in the current study (see Chapter 7), a multitude of neuroimaging studies of very preterm infants at term-equivalent age have described associations between larger ventricular and extra-axial spaces and adverse neurodevelopment.[4, 134, 144, 169, 187] Thus, there needs to be further exploration on how the relationships between larger fluid spaces in the early postnatal period and later neurodevelopment in the current study are interpreted.

8.2 Strengths of the Study

A major strength of this study includes the use of simple and reproducible linear measures made from cUS performed as part of routine clinical care, using standard imaging planes with easily identifiable anatomical landmarks, which support their generalisability in clinical practice. Only 8 of the 19 linear measures made from cUS were assessed as having excellent intra- and inter-observer reliability, and were carried forward to address the remaining aims of the study. The current study had a pragmatic design, as cUS was not part of the larger study (VIBeS-2) protocol of neuroimaging. The aims of the current study were therefore tested in a "real world" setting, using cUS performed as part of routine clinical care. The study also recruited one of the largest cohorts of very preterm infants, most of who were free of major brain injury seen on cUS but otherwise had similar rates of perinatal morbidities that are observed in comparable healthcare settings, affording an opportunity to interrogate the influence of very preterm birth on early postnatal brain growth unencumbered by major brain injury. Participants recruited to the larger study (VIBeS-2) are also being followed into childhood, permitting further studies to explore the relationships between early postnatal cUS linear measures with later neurodevelopmental outcomes. The study had excellent follow-up rates (94% of survivors at 2 years), in no small measure a reflection of both parental engagement with the broader aims of the study and the persistence of study coordinators.

Moreover, the study used assessors who remained blinded to the clinical course of participants, including cUS.

8.3 Limitations of the Study

There are, however, several limitations in the study. Although the majority of the infants had scans until approximately 33 weeks' post-menstrual age, the more mature infants in the cohort were less likely to have had linear measures beyond the first month after birth as it is standard practice in this tertiary unit to transfer infants to non-tertiary units from as early as 32 weeks' post-menstrual age, if they no longer require intensive care. The smaller number of infants who had scans after the first postnatal month were therefore more likely to have been less mature at birth, or to have had a greater burden of morbidities that led to the need for ongoing tertiary-level care, diminishing the power of the study to explore associations between linear measures made after this time and any of the outcome measures examined in this thesis.

The current study used scans that were performed as part of routine clinical care, and therefore timed to detect major preterm brain injury, not assess brain growth or maturation. There is an inherent bias in this cohort in that infants of lower GA and BW were more likely to have had suspected or established brain injury and therefore have had more scans for the surveillance or monitoring of brain injury.

It is also likely that the timing of cUS evaluation of brain growth in the current study may be too early in the postnatal course of most preterm infants to assess the influence of preterm birth, and its sequelae, on subsequent brain growth and maturation. Preterm infants have been shown to have smaller brain volumes and larger fluid spaces on brain MRI at term-equivalent age than term-controls, and it may be that impaired brain growth, and the consequent reductions in absolute whole and regional brain volumes, is manifest later in the postnatal course, closer to term-equivalent age. This is supported by sequential brain MRI studies in preterm infants

that demonstrate a slowing of brain growth velocity later in gestation and up to term-equivalent age.[232, 245, 259, 260]

8.4 Clinical Implications

The findings of the current study provide evidence for associations between early postnatal cUS linear measures of brain tissue and fluid spaces and risk of cognitive and language impairment at 2 years in infants born at <30 weeks' GA. These novel associations between early postnatal brain growth and neurodevelopment supplement existing evidence that relates cUS findings of major brain injury with cerebral palsy and other motor impairments in very preterm infants. However, the discriminative validity of linear measures made from cUS in the early postnatal period remains to be determined, particularly given that there remains scarce data for typical postnatal brain growth for very preterm infants without major brain injury on cUS, and their relationships with later neurodevelopmental outcomes. It is unlikely that the application of a single, or even several, linear measures made from early postnatal cUS will, in isolation, best identify the infant at risk of adverse development. Neurosensory and motor outcomes for infants born very preterm are related to complex interactions between biological and genetic factors influencing brain injury, growth, and maturation, and their environment. The prediction of neurodevelopmental outcomes following very preterm birth may best be assessed in the neonatal period by multiple modalities, including neuroimaging and neurobehavioural assessments, at several time-points from the early days after birth and up to term-equivalent age.

8.5 Future Directions

Whilst the current study was not designed to provide absolute values for any linear measure that may help clinicians to identify infants at risk of adverse neurodevelopment, it has provided evidence for relationships between several linear measures made from cUS performed in the early weeks after preterm birth and later

development. As the most frequently used neuroimaging modality for preterm infants, and one that can be safely used in the smallest and sickest of infants from the early days after very preterm birth, cUS affords the opportunity to make sequential assessments from birth and up to term-equivalent age. Future cUS studies should be specifically designed to answer these gaps in evidence, whereby a large cohort of very preterm infants are scanned frequently at specified time-points from the early days after birth and up to term-equivalent age, to provide reference data for linear measures of postnatal brain growth in a generalisable cohort of very preterm infants with and without major brain injury. Using these data, there is better potential to evaluate the prognostic utility of linear measures, with regard to absolute size, which may determine the sensitivity and specificity of a given measurement using receiver-operator curves to better discriminate infants at risk of a specified neurodevelopmental outcome.

Linear measures of whole and regional brain growth, reflecting cerebral and cerebellar brain size and ventricular and extra-axial fluid spaces, could be included with markers of brain injury and brain maturation in validating a scoring system using sequential cUS from birth and up to term-equivalent age to predict later development into childhood at school-age. Follow-up into childhood would allow more subtle motor and cognitive deficits associated with very preterm birth to be identified on standardised assessments.

8.6 Conclusions

This study provides evidence that simple and reproducible linear measures of brain tissue and fluid spaces made from cUS can be used to quantify early postnatal brain size and brain growth in infants born at <30 weeks' GA. Several linear measures of early postnatal brain size and brain growth were related to neurobehaviour at term-equivalent age, providing some evidence of the construct validity of these linear measures for the neurological integrity of the developing brain in infants born very preterm. There was also some evidence to support associations of linear measures with neurodevelopmental outcomes of very preterm children at 2

years. Larger measures of brain tissue were associated with better cognitive and language scores, and lower risk of adverse neurodevelopment, at 2 years. The finding of relationships between larger measures of fluid spaces and better neurodevelopment at 2 years was contrary to my hypothesis, and not in keeping with current thinking, and warrants further research.

Bibliography

1. Anderson, P., Doyle, L.W., and Victorian Infant Collaborative Study, G., *Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s.* JAMA, 2003. **289**(24): p. 3264-72.
2. Williams, J., Lee, K.J., and Anderson, P.J., *Prevalence of motor-skill impairment in preterm children who do not develop cerebral palsy: a systematic review.* Dev Med Child Neurol, 2010. **52**(3): p. 232-7.
3. Bosanquet, M., Copeland, L., Ware, R., et al., *A systematic review of tests to predict cerebral palsy in young children.* Dev Med Child Neurol, 2013. **55**(5): p. 418-26.
4. Woodward, L.J., Anderson, P.J., Austin, N.C., et al., *Neonatal MRI to predict neurodevelopmental outcomes in preterm infants.* N Engl J Med, 2006. **355**(7): p. 685-94.
5. De Vries, L.S., Van Haastert, I.L., Rademaker, K.J., et al., *Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants.* J Pediatr, 2004. **144**(6): p. 815-20.
6. Australian Institute of Health and Welfare. National Perinatal Data Collection, 2010-2016. Perinatal Data Visualisations; August 2018.
7. National Institute for Health and Care Excellence. Preterm Labour and Birth; November 2015.
8. Statistics Canada. Preterm live births in Canada, 2000-2013; October 2016.
9. Centres for Disease Control and Prevention. Reproductive Health. Preterm birth; April 2018.
10. National Institute for Health and Care Excellence. Preterm Labour and Birth; November 2015.
11. Craig, E.D., Thompson, J.M., and Mitchell, E.A., *Socioeconomic status and preterm birth: New Zealand trends, 1980 to 1999.* Arch Dis Child Fetal Neonatal Ed, 2002. **86**(3): p. F142-6.
12. Smith, L.K., Draper, E.S., Manktelow, B.N., et al., *Socioeconomic inequalities in very preterm birth rates.* Arch Dis Child Fetal Neonatal Ed, 2007. **92**(1): p. F11-4.

13. Fuchs, F., Monet, B., Ducruet, T., et al., *Effect of maternal age on the risk of preterm birth: A large cohort study.* PLoS One, 2018. **13**(1): p. e0191002.
14. Australian Institute of Health and Welfare. National Perinatal Data Collection, 2010-2016. Perinatal Data Visualisations; August 2018.
15. National Institute for Health and Care Excellence. Preterm Labour and Birth; November 2015.
16. Statistics Canada. Preterm live births in Canada, 2000-2013; October 2016.
17. Centres for Disease Control and Prevention. Reproductive Health. Preterm birth; April 2018.
18. Chow, S.S.W., Creighton, P., Kander, V., Haslam, R., Lui, K. 2018. Report of the Australian and New Zealand Neonatal Network 2016. Sydney: ANZNN.
19. Saigal, S. and Doyle, L.W., *An overview of mortality and sequelae of preterm birth from infancy to adulthood.* Lancet, 2008. **371**(9608): p. 261-9.
20. Mangham, L.J., Petrou, S., Doyle, L.W., et al., *The cost of preterm birth throughout childhood in England and Wales.* Pediatrics, 2009. **123**(2): p. e312-27.
21. Santhakumaran, S., Statnikov, Y., Gray, D., et al., *Survival of very preterm infants admitted to neonatal care in England 2008-2014: time trends and regional variation.* Arch Dis Child Fetal Neonatal Ed, 2018. **103**(3): p. F208-F215.
22. Ancel, P.Y., Goffinet, F., Group, E.-W., et al., *Survival and morbidity of preterm children born at 22 through 34 weeks' gestation in France in 2011: results of the EPIPAGE-2 cohort study.* JAMA Pediatr, 2015. **169**(3): p. 230-8.
23. Helenius, K., Sjors, G., Shah, P.S., et al., *Survival in Very Preterm Infants: An International Comparison of 10 National Neonatal Networks.* Pediatrics, 2017. **140**(6).
24. Stephens, A.S., Lain, S.J., Roberts, C.L., et al., *Survival, Hospitalization, and Acute-Care Costs of Very and Moderate Preterm Infants in the First 6 Years of Life: A Population-Based Study.* J Pediatr, 2016. **169**: p. 61-8 e3.
25. Doyle, L.W., Roberts, G., Anderson, P.J., et al., *Outcomes at age 2 years of infants < 28 weeks' gestational age born in Victoria in 2005.* J Pediatr, 2010. **156**(1): p. 49-53 e1.

26. Data obtained and used with permission from the Victorian Infant Collaborative Studies group, Melbourne, Australia.
27. Larroque, B., Ancel, P.Y., Marret, S., et al., *Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study.* Lancet, 2008. **371**(9615): p. 813-20.
28. van Houdt, C.A., Oosterlaan, J., van Wassenaer-Leemhuis, A.G., et al., *Executive function deficits in children born preterm or at low birthweight: a meta-analysis.* Dev Med Child Neurol, 2019.
29. Rosenbaum, P., Paneth, N., Leviton, A., et al., *A report: the definition and classification of cerebral palsy April 2006.* Dev Med Child Neurol Suppl, 2007. **109**: p. 8-14.
30. Novak, I., Morgan, C., Adde, L., et al., *Early, Accurate Diagnosis and Early Intervention in Cerebral Palsy: Advances in Diagnosis and Treatment.* JAMA Pediatr, 2017. **171**(9): p. 897-907.
31. Australia Cerebral Palsy Register. Australian Cerebral Palsy Register Report 2016. Sydney (Australia): Cerebral Palsy Alliance; 2016.
32. Himpens, E., Oostra, A., Franki, I., et al., *Predictability of cerebral palsy in a high-risk NICU population.* Early Hum Dev, 2010. **86**(7): p. 413-7.
33. Palisano, R., Rosenbaum, P., Walter, S., et al., *Development and reliability of a system to classify gross motor function in children with cerebral palsy.* Dev Med Child Neurol, 1997. **39**(4): p. 214-23.
34. Rosenbaum, P.L., Walter, S.D., Hanna, S.E., et al., *Prognosis for gross motor function in cerebral palsy: creation of motor development curves.* JAMA, 2002. **288**(11): p. 1357-63.
35. Bashir, K., Blizard, B., Bosanquet, A., et al., *The evaluation of a mental health facilitator in general practice: effects on recognition, management, and outcome of mental illness.* Br J Gen Pract, 2000. **50**(457): p. 626-9.
36. Roberts, G., Anderson, P.J., Davis, N., et al., *Developmental coordination disorder in geographic cohorts of 8-year-old children born extremely preterm or extremely low birthweight in the 1990s.* Dev Med Child Neurol, 2011. **53**(1): p. 55-60.

37. American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders: DSM-5. Washington, DC: American Psychiatric Association.
38. Izadi-Najafabadi, S., Ryan, N., Ghafooripoor, G., et al., *Participation of children with developmental coordination disorder.* Res Dev Disabil, 2019. **84**: p. 75-84.
39. Cheong, J.L.Y., Anderson, P.J., Burnett, A.C., et al., *Changing Neurodevelopment at 8 Years in Children Born Extremely Preterm Since the 1990s.* Pediatrics, 2017. **139**(6).
40. Spittle, A., Orton, J., Anderson, P.J., et al., *Early developmental intervention programmes provided post hospital discharge to prevent motor and cognitive impairment in preterm infants.* Cochrane Database Syst Rev, 2015(11): p. CD005495.
41. Biran, V., Phan Duy, A., Decobert, F., et al., *Is melatonin ready to be used in preterm infants as a neuroprotectant?* Dev Med Child Neurol, 2014. **56**(8): p. 717-23.
42. Colella, M., Biran, V., and Baud, O., *Melatonin and the newborn brain.* Early Hum Dev, 2016. **102**: p. 1-3.
43. Fischer, H.S., Reibel, N.J., Buhrer, C., et al., *Prophylactic Early Erythropoietin for Neuroprotection in Preterm Infants: A Meta-analysis.* Pediatrics, 2017. **139**(5).
44. Moore, T., Hennessy, E.M., Myles, J., et al., *Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: the EPICure studies.* BMJ, 2012. **345**: p. e7961.
45. Cheong, J.L.Y., Lee, K.J., Boland, R.A., et al., *Changes in long-term prognosis with increasing postnatal survival and the occurrence of postnatal morbidities in extremely preterm infants offered intensive care: a prospective observational study.* Lancet Child Adolesc Health, 2018. **2**(12): p. 872-879.
46. Pace, C.C., Spittle, A.J., Molesworth, C.M., et al., *Evolution of Depression and Anxiety Symptoms in Parents of Very Preterm Infants During the Newborn Period.* JAMA Pediatr, 2016. **170**(9): p. 863-70.
47. Edwards, A.D., Redshaw, M.E., Kennea, N., et al., *Effect of MRI on preterm infants and their families: a randomised trial with nested diagnostic and*

economic evaluation. Arch Dis Child Fetal Neonatal Ed, 2018. **103**(1): p. F15-F21.

48. Peacock, J.L., Marston, L., Marlow, N., et al., *Neonatal and infant outcome in boys and girls born very prematurely.* Pediatr Res, 2012. **71**(3): p. 305-10.
49. Marret, S., Marchand-Martin, L., Picaud, J.C., et al., *Brain injury in very preterm children and neurosensory and cognitive disabilities during childhood: the EPIPAGE cohort study.* PLoS One, 2013. **8**(5): p. e62683.
50. De Jesus, L.C., Pappas, A., Shankaran, S., et al., *Outcomes of small for gestational age infants born at <27 weeks' gestation.* J Pediatr, 2013. **163**(1): p. 55-60 e1-3.
51. Vollmer, B. and Edmonds, C.J., *School Age Neurological and Cognitive Outcomes of Fetal Growth Retardation or Small for Gestational Age Birth Weight.* Front Endocrinol (Lausanne), 2019. **10**: p. 186.
52. Roberts, D., Brown, J., Medley, N., et al., *Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth.* Cochrane Database Syst Rev, 2017. **3**: p. CD004454.
53. Sotiriadis, A., Tsiami, A., Papatheodorou, S., et al., *Neurodevelopmental Outcome After a Single Course of Antenatal Steroids in Children Born Preterm: A Systematic Review and Meta-analysis.* Obstet Gynecol, 2015. **125**(6): p. 1385-96.
54. Foix-L'Helias, L., Marret, S., Ancel, P.Y., et al., *Impact of the use of antenatal corticosteroids on mortality, cerebral lesions and 5-year neurodevelopmental outcomes of very preterm infants: the EPIPAGE cohort study.* BJOG, 2008. **115**(2): p. 275-82.
55. Crowther, C.A., Anderson, P.J., McKinlay, C.J., et al., *Mid-Childhood Outcomes of Repeat Antenatal Corticosteroids: A Randomized Controlled Trial.* Pediatrics, 2016. **138**(4).
56. Shanks, A.L., Grasch, J.L., Quinney, S.K., et al., *Controversies in antenatal corticosteroids.* Semin Fetal Neonatal Med, 2019.
57. Doyle, L.W., Crowther, C.A., Middleton, P., et al., *Magnesium sulphate for women at risk of preterm birth for neuroprotection of the fetus.* Cochrane Database Syst Rev, 2009(1): p. CD004661.
58. Jensen, E.A. and Schmidt, B., *Epidemiology of bronchopulmonary dysplasia.* Birth Defects Res A Clin Mol Teratol, 2014. **100**(3): p. 145-57.

59. Marshall, D.D., Kotelchuck, M., Young, T.E., et al., *Risk factors for chronic lung disease in the surfactant era: a North Carolina population-based study of very low birth weight infants. North Carolina Neonatologists Association.* Pediatrics, 1999. **104**(6): p. 1345-50.

60. Laughon, M., O'Shea, M.T., Allred, E.N., et al., *Chronic lung disease and developmental delay at 2 years of age in children born before 28 weeks' gestation.* Pediatrics, 2009. **124**(2): p. 637-48.

61. Anderson, P.J. and Doyle, L.W., *Neurodevelopmental outcome of bronchopulmonary dysplasia.* Semin Perinatol, 2006. **30**(4): p. 227-32.

62. Barrington, K.J., *Postnatal steroids and neurodevelopmental outcomes: a problem in the making.* Pediatrics, 2001. **107**(6): p. 1425-6.

63. Doyle, L.W., Cheong, J.L., Ehrenkranz, R.A., et al., *Early (< 8 days) systemic postnatal corticosteroids for prevention of bronchopulmonary dysplasia in preterm infants.* Cochrane Database Syst Rev, 2017. **10**: p. CD001146.

64. Shi, Z., Ma, L., Luo, K., et al., *Chorioamnionitis in the Development of Cerebral Palsy: A Meta-analysis and Systematic Review.* Pediatrics, 2017. **139**(6).

65. Shah, D.K., Doyle, L.W., Anderson, P.J., et al., *Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term.* J Pediatr, 2008. **153**(2): p. 170-5, 175 e1.

66. Robinson, J.R., Kennedy, C., van Arendonk, K.J., et al., *Neurodevelopmental considerations in surgical necrotizing enterocolitis.* Semin Pediatr Surg, 2018. **27**(1): p. 52-56.

67. Rand, K.M., Austin, N.C., Inder, T.E., et al., *Neonatal Infection and Later Neurodevelopmental Risk in the Very Preterm Infant.* J Pediatr, 2016. **170**: p. 97-104.

68. Limperopoulos, C., Bassan, H., Gauvreau, K., et al., *Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors?* Pediatrics, 2007. **120**(3): p. 584-93.

69. Als, H., Tronick, E., Lester, B.M., et al., *The Brazelton Neonatal Behavioral Assessment Scale (BNBAS).* J Abnorm Child Psychol, 1977. **5**(3): p. 215-31.

70. Noble, Y. and Boyd, R., *Neonatal assessments for the preterm infant up to 4 months corrected age: a systematic review.* Dev Med Child Neurol, 2012. **54**(2): p. 129-39.
71. Einspieler, C., Prechtl, H.F., Ferrari, F., et al., *The qualitative assessment of general movements in preterm, term and young infants--review of the methodology.* Early Hum Dev, 1997. **50**(1): p. 47-60.
72. Hamer, E.G., Bos, A.F., and Hadders-Algra, M., *Specific characteristics of abnormal general movements are associated with functional outcome at school age.* Early Hum Dev, 2016. **95**: p. 9-13.
73. Morgan, C., Crowle, C., Goyen, T.A., et al., *Sensitivity and specificity of General Movements Assessment for diagnostic accuracy of detecting cerebral palsy early in an Australian context.* J Paediatr Child Health, 2016. **52**(1): p. 54-9.
74. Olsen, J.E., Brown, N.C., Eeles, A.L., et al., *Trajectories of general movements from birth to term-equivalent age in infants born <30weeks' gestation.* Early Hum Dev, 2015. **91**(12): p. 683-8.
75. Olsen, J.E., Allinson, L.G., Doyle, L.W., et al., *Preterm and term-equivalent age general movements and 1-year neurodevelopmental outcomes for infants born before 30 weeks' gestation.* Dev Med Child Neurol, 2018. **60**(1): p. 47-53.
76. Spittle, A.J., Spencer-Smith, M.M., Cheong, J.L., et al., *General movements in very preterm children and neurodevelopment at 2 and 4 years.* Pediatrics, 2013. **132**(2): p. e452-8.
77. Kodric, J., Sustersic, B., and Paro-Panjan, D., *Assessment of general movements and 2.5 year developmental outcomes: pilot results in a diverse preterm group.* Eur J Paediatr Neurol, 2010. **14**(2): p. 131-7.
78. Hadders-Algra, M., *Early diagnosis and early intervention in cerebral palsy.* Front Neurol, 2014. **5**: p. 185.
79. Bruggink, J.L., Van Braeckel, K.N., and Bos, A.F., *The early motor repertoire of children born preterm is associated with intelligence at school age.* Pediatrics, 2010. **125**(6): p. e1356-63.
80. Ricci, D., Romeo, D.M., Haataja, L., et al., *Neurological examination of preterm infants at term equivalent age.* Early Hum Dev, 2008. **84**(11): p. 751-61.

81. Mercuri, E., Guzzetta, A., Laroche, S., et al., *Neurologic examination of preterm infants at term age: comparison with term infants.* J Pediatr, 2003. **142**(6): p. 647-55.

82. Dubowitz, L., Mercuri, E., and Dubowitz, V., *An optimality score for the neurologic examination of the term newborn.* J Pediatr, 1998. **133**(3): p. 406-16.

83. Eeles, A.L., Walsh, J.M., Olsen, J.E., et al., *Continuum of neurobehaviour and its associations with brain MRI in infants born preterm.* BMJ Paediatr Open, 2017. **1**(1): p. e000136.

84. Spittle, A.J., Walsh, J., Olsen, J.E., et al., *Neurobehaviour and neurological development in the first month after birth for infants born between 32-42 weeks' gestation.* Early Hum Dev, 2016. **96**: p. 7-14.

85. Molteno, C., Grosz, P., Wallace, P., et al., *Neurological examination of the preterm and full-term infant at risk for developmental disabilities using the Dubowitz Neurological Assessment.* Early Hum Dev, 1995. **41**(3): p. 167-76.

86. Bozynski, M.E., DiPietro, M.A., Meisels, S.J., et al., *Cranial sonography and neurological examination at term and motor performance through 19 months of age.* J Dev Behav Pediatr, 1993. **14**(2): p. 112-6.

87. Woodward, L.J., Mogridge, N., Wells, S.W., et al., *Can neurobehavioral examination predict the presence of cerebral injury in the very low birth weight infant?* J Dev Behav Pediatr, 2004. **25**(5): p. 326-34.

88. de Vries, L.S., Dubowitz, L.M., Dubowitz, V., et al., *Predictive value of cranial ultrasound in the newborn baby: a reappraisal.* Lancet, 1985. **2**(8447): p. 137-40.

89. Shuman, W.P., Rogers, J.V., Mack, L.A., et al., *Real-time sonographic sector scanning of the neonatal cranium: technique and normal anatomy.* AJR Am J Roentgenol, 1981. **137**(4): p. 821-8.

90. Johnson, M.L., Mack, L.A., Rumack, C.M., et al., *B-mode echoencephalography in the normal and high risk infant.* AJR Am J Roentgenol, 1979. **133**(3): p. 375-81.

91. Pape, K.E., Blackwell, R.J., Cusick, G., et al., *Ultrasound detection of brain damage in preterm infants.* Lancet, 1979. **1**(8129): p. 1261-4.

92. De Vries, L.S., Regev, R., Pennock, J.M., et al., *Ultrasound evolution and later outcome of infants with periventricular densities.* Early Hum Dev, 1988. **16**(2-3): p. 225-33.
93. de Vries, L.S., Eken, P., and Dubowitz, L.M., *The spectrum of leukomalacia using cranial ultrasound.* Behav Brain Res, 1992. **49**(1): p. 1-6.
94. Ment, L.R., Bada, H.S., Barnes, P., et al., *Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society.* Neurology, 2002. **58**(12): p. 1726-38.
95. Intrapiromkul, J., Northington, F., Huisman, T.A., et al., *Accuracy of head ultrasound for the detection of intracranial hemorrhage in preterm neonates: comparison with brain MRI and susceptibility-weighted imaging.* J Neuroradiol, 2013. **40**(2): p. 81-8.
96. Parodi, A., Rossi, A., Severino, M., et al., *Accuracy of ultrasound in assessing cerebellar haemorrhages in very low birthweight babies.* Arch Dis Child Fetal Neonatal Ed, 2015. **100**(4): p. F289-92.
97. Benders, M.J., Groenendaal, F., and De Vries, L.S., *Preterm arterial ischemic stroke.* Semin Fetal Neonatal Med, 2009. **14**(5): p. 272-7.
98. Papile, L.A., Burstein, J., Burstein, R., et al., *Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm.* J Pediatr, 1978. **92**(4): p. 529-34.
99. Bassan, H., *Intracranial hemorrhage in the preterm infant: understanding it, preventing it.* Clin Perinatol, 2009. **36**(4): p. 737-62, v.
100. Volpe JJ. Intracranial hemorrhage: neurology of the newborn. 5th edition. Philadelphia: W.B. Saunders Co.; 2008. p. 481–588.
101. Philip, A.G., Allan, W.C., Tito, A.M., et al., *Intraventricular hemorrhage in preterm infants: declining incidence in the 1980s.* Pediatrics, 1989. **84**(5): p. 797-801.
102. Horbar, J.D., Badger, G.J., Carpenter, J.H., et al., *Trends in mortality and morbidity for very low birth weight infants, 1991-1999.* Pediatrics, 2002. **110**(1 Pt 1): p. 143-51.
103. Chow, S.S.W., Creighton, P., Kander, V., Haslam, R., Lui, K. 2018. Report of the Australian and New Zealand Neonatal Network 2016. Sydney: ANZNN.

104. Bassan, H., Feldman, H.A., Limperopoulos, C., et al., *Periventricular hemorrhagic infarction: risk factors and neonatal outcome.* Pediatr Neurol, 2006. **35**(2): p. 85-92.

105. Wilson-Costello, D., Friedman, H., Minich, N., et al., *Improved survival rates with increased neurodevelopmental disability for extremely low birth weight infants in the 1990s.* Pediatrics, 2005. **115**(4): p. 997-1003.

106. Chow, S.S.W., Creighton, P., Kander, V., Haslam, R., Lui, K. 2018. Report of the Australian and New Zealand Neonatal Network 2016. Sydney: ANZNN.

107. Ballabh, P., *Intraventricular hemorrhage in premature infants: mechanism of disease.* Pediatr Res, 2010. **67**(1): p. 1-8.

108. Tortora, D., Severino, M., Malova, M., et al., *Differences in subependymal vein anatomy may predispose preterm infants to GMH-IVH.* Arch Dis Child Fetal Neonatal Ed, 2018. **103**(1): p. F59-F65.

109. Tortora, D., Martinetti, C., Severino, M., et al., *The effects of mild germinal matrix-intraventricular haemorrhage on the developmental white matter microstructure of preterm neonates: a DTI study.* Eur Radiol, 2018. **28**(3): p. 1157-1166.

110. Harteman, J.C., Groenendaal, F., van Haastert, I.C., et al., *Atypical timing and presentation of periventricular haemorrhagic infarction in preterm infants: the role of thrombophilia.* Dev Med Child Neurol, 2012. **54**(2): p. 140-7.

111. Heuchan, A.M., Evans, N., Henderson Smart, D.J., et al., *Perinatal risk factors for major intraventricular haemorrhage in the Australian and New Zealand Neonatal Network, 1995-97.* Arch Dis Child Fetal Neonatal Ed, 2002. **86**(2): p. F86-90.

112. Linder, N., Haskin, O., Levit, O., et al., *Risk factors for intraventricular hemorrhage in very low birth weight premature infants: a retrospective case-control study.* Pediatrics, 2003. **111**(5 Pt 1): p. e590-5.

113. Vohr, B. and Ment, L.R., *Intraventricular hemorrhage in the preterm infant.* Early Hum Dev, 1996. **44**(1): p. 1-16.

114. Crowther, C.A., Hiller, J.E., Doyle, L.W., et al., *Effect of magnesium sulfate given for neuroprotection before preterm birth: a randomized controlled trial.* JAMA, 2003. **290**(20): p. 2669-76.

115. Volpe J.J.: Intracranial hemorrhage: germinal matrix-intraventricular hemorrhage of the premature infant: neurology of the newborn.

116. Volpe JJ. Intracranial hemorrhage: neurology of the newborn. 5th edition. Philadelphia: W.B. Saunders Co.; 2008. p. 481–588.
117. Volpe JJ. Intracranial hemorrhage: neurology of the newborn. 5th edition. Philadelphia: W.B. Saunders Co.; 2008. p. 481–588.
118. Leijser, L.M., Miller, S.P., van Wezel-Meijler, G., et al., *Posthemorrhagic ventricular dilatation in preterm infants: When best to intervene?* Neurology, 2018. **90**(8): p. e698-e706.
119. Levene, M.I., *Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound.* Arch Dis Child, 1981. **56**(12): p. 900-4.
120. Davies, M.W., Swaminathan, M., Chuang, S.L., et al., *Reference ranges for the linear dimensions of the intracranial ventricles in preterm neonates.* Arch Dis Child Fetal Neonatal Ed, 2000. **82**(3): p. F218-23.
121. Brouwer, M.J., de Vries, L.S., Pistorius, L., et al., *Ultrasound measurements of the lateral ventricles in neonates: why, how and when? A systematic review.* Acta Paediatr, 2010. **99**(9): p. 1298-306.
122. Limperopoulos, C., Benson, C.B., Bassan, H., et al., *Cerebellar hemorrhage in the preterm infant: ultrasonographic findings and risk factors.* Pediatrics, 2005. **116**(3): p. 717-24.
123. Plaisier, A., Raets, M.M., Ecury-Goossen, G.M., et al., *Serial cranial ultrasonography or early MRI for detecting preterm brain injury?* Arch Dis Child Fetal Neonatal Ed, 2015. **100**(4): p. F293-300.
124. van Wezel-Meijler, G., Steggerda, S.J., and Leijser, L.M., *Cranial ultrasonography in neonates: role and limitations.* Semin Perinatol, 2010. **34**(1): p. 28-38.
125. Sehgal, A., El-Naggar, W., Glanc, P., et al., *Risk factors and ultrasonographic profile of posterior fossa haemorrhages in preterm infants.* J Paediatr Child Health, 2009. **45**(4): p. 215-8.
126. Soudack, M., Jacobson, J., Raviv-Zilka, L., et al., *Cerebellar hemorrhage in very low birth weight premature infants: the advantage of the posterolateral fontanelle view.* J Clin Ultrasound, 2013. **41**(7): p. 395-401.
127. Di Salvo, D.N., *A new view of the neonatal brain: clinical utility of supplemental neurologic US imaging windows.* Radiographics, 2001. **21**(4): p. 943-55.

128. Levene, M.I., Wigglesworth, J.S., and Dubowitz, V., *Hemorrhagic periventricular leukomalacia in the neonate: a real-time ultrasound study.* Pediatrics, 1983. **71**(5): p. 794-7.

129. Graham, M., Levene, M.I., Trounce, J.Q., et al., *Prediction of cerebral palsy in very low birthweight infants: prospective ultrasound study.* Lancet, 1987. **2**(8559): p. 593-6.

130. Pierrat, V., Duquennoy, C., van Haastert, I.C., et al., *Ultrasound diagnosis and neurodevelopmental outcome of localised and extensive cystic periventricular leucomalacia.* Arch Dis Child Fetal Neonatal Ed, 2001. **84**(3): p. F151-6.

131. Allin, M., Walshe, M., Fern, A., et al., *Cognitive maturation in preterm and term born adolescents.* J Neurol Neurosurg Psychiatry, 2008. **79**(4): p. 381-6.

132. Kuban, K.C., Allred, E.N., O'Shea, T.M., et al., *Cranial ultrasound lesions in the NICU predict cerebral palsy at age 2 years in children born at extremely low gestational age.* J Child Neurol, 2009. **24**(1): p. 63-72.

133. O'Shea, T.M., Counsell, S.J., Bartels, D.B., et al., *Magnetic resonance and ultrasound brain imaging in preterm infants.* Early Hum Dev, 2005. **81**(3): p. 263-71.

134. Inder, T.E., Wells, S.J., Mogridge, N.B., et al., *Defining the nature of the cerebral abnormalities in the premature infant: a qualitative magnetic resonance imaging study.* J Pediatr, 2003. **143**(2): p. 171-9.

135. Volpe, J.J., *Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances.* Lancet Neurol, 2009. **8**(1): p. 110-24.

136. Gilles, F., Gressens, P., Dammann, O., et al., *Hypoxia-ischemia is not an antecedent of most preterm brain damage: the illusion of validity.* Dev Med Child Neurol, 2018. **60**(2): p. 120-125.

137. Thompson, D.K., Warfield, S.K., Carlin, J.B., et al., *Perinatal risk factors altering regional brain structure in the preterm infant.* Brain, 2007. **130**(Pt 3): p. 667-77.

138. Peterson, B.S., Anderson, A.W., Ehrenkranz, R., et al., *Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants.* Pediatrics, 2003. **111**(5 Pt 1): p. 939-48.

139. De Vries, L.S., Groenendaal, F., van Haastert, I.C., et al., *Asymmetrical myelination of the posterior limb of the internal capsule in infants with*

periventricular haemorrhagic infarction: an early predictor of hemiplegia. Neuropediatrics, 1999. **30**(6): p. 314-9.

140. Rademaker, K.J., Groenendaal, F., Jansen, G.H., et al., *Unilateral haemorrhagic parenchymal lesions in the preterm infant: shape, site and prognosis.* Acta Paediatr, 1994. **83**(6): p. 602-8.
141. Hoon, A.H., Jr., Stashinko, E.E., Nagae, L.M., et al., *Sensory and motor deficits in children with cerebral palsy born preterm correlate with diffusion tensor imaging abnormalities in thalamocortical pathways.* Dev Med Child Neurol, 2009. **51**(9): p. 697-704.
142. Bassan, H., Limperopoulos, C., Visconti, K., et al., *Neurodevelopmental outcome in survivors of periventricular hemorrhagic infarction.* Pediatrics, 2007. **120**(4): p. 785-92.
143. Adams-Chapman, I., Hansen, N.I., Stoll, B.J., et al., *Neurodevelopmental outcome of extremely low birth weight infants with posthemorrhagic hydrocephalus requiring shunt insertion.* Pediatrics, 2008. **121**(5): p. e1167-77.
144. Miller, S.P., Cozzio, C.C., Goldstein, R.B., et al., *Comparing the diagnosis of white matter injury in premature newborns with serial MR imaging and transfontanel ultrasonography findings.* AJNR Am J Neuroradiol, 2003. **24**(8): p. 1661-9.
145. O'Shea, T.M., Kuban, K.C., Allred, E.N., et al., *Neonatal cranial ultrasound lesions and developmental delays at 2 years of age among extremely low gestational age children.* Pediatrics, 2008. **122**(3): p. e662-9.
146. Wezel-Meijler, G. and de Vries, L.S., *Cranial ultrasound - optimizing utility in the NICU.* Curr Pediatr Rev, 2014. **10**(1): p. 16-27.
147. Laptook, A.R., O'Shea, T.M., Shankaran, S., et al., *Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents.* Pediatrics, 2005. **115**(3): p. 673-80.
148. Steggerda, S.J., Leijser, L.M., Wiggers-de Bruine, F.T., et al., *Cerebellar injury in preterm infants: incidence and findings on US and MR images.* Radiology, 2009. **252**(1): p. 190-9.

149. Tam, E.W., Rosenbluth, G., Rogers, E.E., et al., *Cerebellar hemorrhage on magnetic resonance imaging in preterm newborns associated with abnormal neurologic outcome.* J Pediatr, 2011. **158**(2): p. 245-50.

150. Limperopoulos, C., Soul, J.S., Haidar, H., et al., *Impaired trophic interactions between the cerebellum and the cerebrum among preterm infants.* Pediatrics, 2005. **116**(4): p. 844-50.

151. Allin, M.P., *Novel insights from quantitative imaging of the developing cerebellum.* Semin Fetal Neonatal Med, 2016. **21**(5): p. 333-8.

152. Bolisetty, S., Dhawan, A., Abdel-Latif, M., et al., *Intraventricular hemorrhage and neurodevelopmental outcomes in extreme preterm infants.* Pediatrics, 2014. **133**(1): p. 55-62.

153. Levine, T.A., Grunau, R.E., McAuliffe, F.M., et al., *Early childhood neurodevelopment after intrauterine growth restriction: a systematic review.* Pediatrics, 2015. **135**(1): p. 126-41.

154. Padilla-Gomes, N.F., Enriquez, G., Acosta-Rojas, R., et al., *Prevalence of neonatal ultrasound brain lesions in premature infants with and without intrauterine growth restriction.* Acta Paediatr, 2007. **96**(11): p. 1582-7.

155. Kwon, S.H., Vasung, L., Ment, L.R., et al., *The role of neuroimaging in predicting neurodevelopmental outcomes of preterm neonates.* Clin Perinatol, 2014. **41**(1): p. 257-83.

156. Arichi, T., Counsell, S.J., Allievi, A.G., et al., *The effects of hemorrhagic parenchymal infarction on the establishment of sensori-motor structural and functional connectivity in early infancy.* Neuroradiology, 2014. **56**(11): p. 985-94.

157. Hamrick, S.E., Miller, S.P., Leonard, C., et al., *Trends in severe brain injury and neurodevelopmental outcome in premature newborn infants: the role of cystic periventricular leukomalacia.* J Pediatr, 2004. **145**(5): p. 593-9.

158. Kelly, C.E., Chan, L., Burnett, A.C., et al., *Brain structural and microstructural alterations associated with cerebral palsy and motor impairments in adolescents born extremely preterm and/or extremely low birthweight.* Dev Med Child Neurol, 2015. **57**(12): p. 1168-75.

159. Nguyen The Tich, S., Anderson, P.J., Shimony, J.S., et al., *A novel quantitative simple brain metric using MR imaging for preterm infants.* AJNR Am J Neuroradiol, 2009. **30**(1): p. 125-31.

160. Nosarti, C., Al-Asady, M.H., Frangou, S., et al., *Adolescents who were born very preterm have decreased brain volumes.* Brain, 2002. **125**(Pt 7): p. 1616-23.

161. Skiold, B., Hallberg, B., Vollmer, B., et al., *A Novel Scoring System for Term-Equivalent-Age Cranial Ultrasound in Extremely Preterm Infants.* Ultrasound Med Biol, 2019. **45**(3): p. 786-794.

162. Martinez-Biarge, M., Groenendaal, F., Kersbergen, K.J., et al., *MRI Based Preterm White Matter Injury Classification: The Importance of Sequential Imaging in Determining Severity of Injury.* PLoS One, 2016. **11**(6): p. e0156245.

163. Martinez-Biarge, M., Groenendaal, F., Kersbergen, K.J., et al., *Neurodevelopmental Outcomes in Preterm Infants with White Matter Injury Using a New MRI Classification.* Neonatology, 2019. **116**(3): p. 227-235.

164. Hintz, S.R., Barnes, P.D., Bulas, D., et al., *Neuroimaging and neurodevelopmental outcome in extremely preterm infants.* Pediatrics, 2015. **135**(1): p. e32-42.

165. Benders, M.J., Groenendaal, F., Uiterwaal, C.S., et al., *Maternal and infant characteristics associated with perinatal arterial stroke in the preterm infant.* Stroke, 2007. **38**(6): p. 1759-65.

166. Plaisier, A., Raets, M.M., van der Starre, C., et al., *Safety of routine early MRI in preterm infants.* Pediatr Radiol, 2012. **42**(10): p. 1205-11.

167. Chow ANZNN

168. Tich, S.N., Anderson, P.J., Hunt, R.W., et al., *Neurodevelopmental and perinatal correlates of simple brain metrics in very preterm infants.* Arch Pediatr Adolesc Med, 2011. **165**(3): p. 216-22.

169. Kidokoro, H., Neil, J.J., and Inder, T.E., *New MR imaging assessment tool to define brain abnormalities in very preterm infants at term.* AJNR Am J Neuroradiol, 2013. **34**(11): p. 2208-14.

170. Leijser, L.M., Srinivasan, L., Rutherford, M.A., et al., *Structural linear measurements in the newborn brain: accuracy of cranial ultrasound compared to MRI.* Pediatr Radiol, 2007. **37**(7): p. 640-8.

171. Garel, C., *Fetal cerebral biometry: normal parenchymal findings and ventricular size.* Eur Radiol, 2005. **15**(4): p. 809-13.

172. Horsch, S., Bengtsson, J., Nordell, A., et al., *Lateral ventricular size in extremely premature infants: 3D MRI confirms 2D ultrasound measurements.* Ultrasound Med Biol, 2009. **35**(3): p. 360-6.

173. Roelants, J.A., Koning, I.V., Raets, M.M., et al., *A New Ultrasound Marker for Bedside Monitoring of Preterm Brain Growth.* AJNR Am J Neuroradiol, 2016.

174. Anderson, N.G., Laurent, I., Cook, N., et al., *Growth rate of corpus callosum in very premature infants.* AJNR Am J Neuroradiol, 2005. **26**(10): p. 2685-90.

175. Anderson, N.G., Laurent, I., Woodward, L.J., et al., *Detection of impaired growth of the corpus callosum in premature infants.* Pediatrics, 2006. **118**(3): p. 951-60.

176. da Graca, A.L., Cardoso, K.R., da Costa, J.M., et al., *Assessment of gestational age using cerebellar measurements at cranial ultrasound: what is the best approach?* Early Hum Dev, 2013. **89**(1): p. 1-5.

177. Imamoglu, E.Y., Gursoy, T., Ovali, F., et al., *Nomograms of cerebellar vermis height and transverse cerebellar diameter in appropriate-for-gestational-age neonates.* Early Hum Dev, 2013. **89**(12): p. 919-23.

178. Pogliani, L., Radaelli, G., Manfredini, V., et al., *Height of the cerebellar vermis and gestational age at birth.* Ultrasound Obstet Gynecol, 2008. **31**(4): p. 401-5.

179. Zuccotti, G.V., Pogliani, L., Dilillo, D., et al., *Nomogram of the cerebellar vermis height at birth in small-for-gestational-age neonates.* Acta Paediatr, 2008. **97**(6): p. 745-50.

180. Davies, M.W., Swaminathan, M., and Betheras, F.R., *Measurement of the transverse cerebellar diameter in preterm neonates and its use in assessment of gestational age.* Australas Radiol, 2001. **45**(3): p. 309-12.

181. Cuddihy, S.L., Anderson, N.G., Wells, J.E., et al., *Cerebellar vermis diameter at cranial sonography for assessing gestational age in low-birth-weight infants.* Pediatr Radiol, 1999. **29**(8): p. 589-94.

182. Swaminathan, M., Davies, M., Davis, P., et al., *Transverse cerebellar diameter on cranial ultrasound scan in preterm neonates in an Australian population.* J Paediatr Child Health, 1999. **35**(4): p. 346-9.

183. Armstrong, D.L., Bagnall, C., Harding, J.E., et al., *Measurement of the subarachnoid space by ultrasound in preterm infants.* Arch Dis Child Fetal Neonatal Ed, 2002. **86**(2): p. F124-6.

184. Sondhi, V., Gupta, G., Gupta, P.K., et al., *Establishment of nomograms and reference ranges for intra-cranial ventricular dimensions and ventriculo-hemispheric ratio in newborns by ultrasonography.* Acta Paediatr, 2008. **97**(6): p. 738-44.

185. Liao, M.F., Chaou, W.T., Tsao, L.Y., et al., *Ultrasound measurement of the ventricular size in newborn infants.* Brain Dev, 1986. **8**(3): p. 262-8.

186. Perry, R.N., Bowman, E.D., Murton, L.J., et al., *Ventricular size in newborn infants.* J Ultrasound Med, 1985. **4**(9): p. 475-7.

187. Horsch, S., Muentjes, C., Franz, A., et al., *Ultrasound diagnosis of brain atrophy is related to neurodevelopmental outcome in preterm infants.* Acta Paediatr, 2005. **94**(12): p. 1815-21.

188. Brouwer, M.J., van Kooij, B.J., van Haastert, I.C., et al., *Sequential cranial ultrasound and cerebellar diffusion weighted imaging contribute to the early prognosis of neurodevelopmental outcome in preterm infants.* PLoS One, 2014. **9**(10): p. e109556.

189. Horsch, S., Skiold, B., Hallberg, B., et al., *Cranial ultrasound and MRI at term age in extremely preterm infants.* Arch Dis Child Fetal Neonatal Ed, 2010. **95**(5): p. F310-4.

190. Fox, L.M., Choo, P., Rogerson, S.R., et al., *The relationship between ventricular size at 1 month and outcome at 2 years in infants less than 30 weeks' gestation.* Arch Dis Child Fetal Neonatal Ed, 2014. **99**(3): p. F209-14.

191. Hadlock, F.P., Deter, R.L., Harrist, R.B., et al., *Estimating fetal age: computer-assisted analysis of multiple fetal growth parameters.* Radiology, 1984. **152**(2): p. 497-501.

192. Hadlock, F.P., Harrist, R.B., and Martinez-Poyer, J., *In utero analysis of fetal growth: a sonographic weight standard.* Radiology, 1991. **181**(1): p. 129-33.

193. Kurmanavicius, J., Wright, E.M., Royston, P., et al., *Fetal ultrasound biometry: 1. Head reference values.* Br J Obstet Gynaecol, 1999. **106**(2): p. 126-35.

194. Goldstein, I., Tamir, A., Reece, A.E., et al., *Corpus callosum growth in normal and growth-restricted fetuses.* Prenat Diagn, 2011. **31**(12): p. 1115-9.

195. Zhang, H.C., Yang, J., Chen, Z.P., et al., *Sonographic study of the development of fetal corpus callosum in a Chinese population.* J Clin Ultrasound, 2009. **37**(2): p. 75-7.

196. Achiron, R. and Achiron, A., *Development of the human fetal corpus callosum: a high-resolution, cross-sectional sonographic study.* Ultrasound Obstet Gynecol, 2001. **18**(4): p. 343-7.

197. Malinger, G. and Zakut, H., *The corpus callosum: normal fetal development as shown by transvaginal sonography.* AJR Am J Roentgenol, 1993. **161**(5): p. 1041-3.

198. Chavez, M.R., Ananth, C.V., Smulian, J.C., et al., *Fetal transcerebellar diameter measurement for prediction of gestational age at the extremes of fetal growth.* J Ultrasound Med, 2007. **26**(9): p. 1167-71; quiz 1173-4.

199. Chavez, M.R., Ananth, C.V., Kaminsky, L.M., et al., *Fetal transcerebellar diameter measurement for prediction of gestational age in twins.* Am J Obstet Gynecol, 2006. **195**(6): p. 1596-600.

200. Chavez, M.R., Ananth, C.V., Smulian, J.C., et al., *Fetal transcerebellar diameter measurement with particular emphasis in the third trimester: a reliable predictor of gestational age.* Am J Obstet Gynecol, 2004. **191**(3): p. 979-84.

201. Chavez, M.R., Ananth, C.V., Smulian, J.C., et al., *Fetal transcerebellar diameter nomogram in singleton gestations with special emphasis in the third trimester: a comparison with previously published nomograms.* Am J Obstet Gynecol, 2003. **189**(4): p. 1021-5.

202. Zalel, Y., Pinhas-Hamiel, O., Lipitz, S., et al., *The development of the fetal penis--an in utero sonographic evaluation.* Ultrasound Obstet Gynecol, 2001. **17**(2): p. 129-31.

203. Malinger, G., Ginath, S., Lerman-Sagie, T., et al., *The fetal cerebellar vermis: normal development as shown by transvaginal ultrasound.* Prenat Diagn, 2001. **21**(8): p. 687-92.

204. Vinkesteijn, A.S., Mulder, P.G., and Wladimiroff, J.W., *Fetal transverse cerebellar diameter measurements in normal and reduced fetal growth.* Ultrasound Obstet Gynecol, 2000. **15**(1): p. 47-51.

205. Snijders, R.J., De Courcy-Wheeler, R.H., and Nicolaides, K.H., *Intrauterine growth retardation and fetal transverse cerebellar diameter.* Prenat Diagn, 1994. **14**(12): p. 1101-5.

206. Hill, L.M., Guzick, D., Fries, J., et al., *The transverse cerebellar diameter in estimating gestational age in the large for gestational age fetus.* Obstet Gynecol, 1990. **75**(6): p. 981-5.

207. Hata, K., Hata, T., Senoh, D., et al., *Ultrasonographic measurement of the fetal transverse cerebellum in utero.* Gynecol Obstet Invest, 1989. **28**(2): p. 111-2.

208. Malinger, G., Lerman-Sagie, T., Achiron, R., et al., *The subarachnoid space: normal fetal development as demonstrated by transvaginal ultrasound.* Prenat Diagn, 2000. **20**(11): p. 890-3.

209. Cardoza, J.D., Goldstein, R.B., and Filly, R.A., *Exclusion of fetal ventriculomegaly with a single measurement: the width of the lateral ventricular atrium.* Radiology, 1988. **169**(3): p. 711-4.

210. Denkhaus, H. and Winsberg, F., *Ultrasonic measurement of the fetal ventricular system.* Radiology, 1979. **131**(3): p. 781-7.

211. Tang, P.H., Bartha, A.I., Norton, M.E., et al., *Agenesis of the corpus callosum: an MR imaging analysis of associated abnormalities in the fetus.* AJNR Am J Neuroradiol, 2009. **30**(2): p. 257-63.

212. Paul, L.K., Brown, W.S., Adolphs, R., et al., *Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity.* Nat Rev Neurosci, 2007. **8**(4): p. 287-99.

213. Zalel, Y., Seidman, D.S., Brand, N., et al., *The development of the fetal vermis: an in-utero sonographic evaluation.* Ultrasound Obstet Gynecol, 2002. **19**(2): p. 136-9.

214. Courchesne, E., Press, G.A., Murakami, J., et al., *The cerebellum in sagittal plane--anatomic-MR correlation: 1. The vermis.* AJR Am J Roentgenol, 1989. **153**(4): p. 829-35.

215. Spittle, A.J., Thompson, D.K., Brown, N.C., et al., *Neurobehaviour between birth and 40 weeks' gestation in infants born <30 weeks' gestation and parental psychological wellbeing: predictors of brain development and child outcomes.* BMC Pediatr, 2014. **14**: p. 111.

216. de Vries, L.S., Groenendaal, F., Liem, K.D., et al., *Treatment thresholds for intervention in posthaemorrhagic ventricular dilation: a randomised controlled trial.* Arch Dis Child Fetal Neonatal Ed, 2019. **104**(1): p. F70-F75.

217. Hagmann, C.F., Robertson, N.J., Acolet, D., et al., *Cerebral measurements made using cranial ultrasound in term Ugandan newborns.* Early Hum Dev, 2011. **87**(5): p. 341-7.

218. Cole, T.J., Freeman, J.V., and Preece, M.A., *British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood.* Stat Med, 1998. **17**(4): p. 407-29.

219. Bell, M.J., *Neonatal necrotizing enterocolitis.* N Engl J Med, 1978. **298**(5): p. 281-2.

220. Bayley, N. (2006). Bayley scales of infant and toddler development, third edition. San Antonio, TX: Harcourt Assessment, Inc.

221. Anderson, P.J., De Luca, C.R., Hutchinson, E., et al., *Underestimation of developmental delay by the new Bayley-III Scale.* Arch Pediatr Adolesc Med, 2010. **164**(4): p. 352-6.

222. Spittle, A.J., Spencer-Smith, M.M., Eeles, A.L., et al., *Does the Bayley-III Motor Scale at 2 years predict motor outcome at 4 years in very preterm children?* Dev Med Child Neurol, 2013. **55**(5): p. 448-52.

223. Fairhurst, C., *Cerebral palsy: the whys and hows.* Arch Dis Child Educ Pract Ed, 2012. **97**(4): p. 122-31.

224. Brennan, P. and Silman, A., *Statistical methods for assessing observer variability in clinical measures.* BMJ, 1992. **304**(6840): p. 1491-4.

225. Bland, J.M. and Altman, D.G., *Statistical methods for assessing agreement between two methods of clinical measurement.* Lancet, 1986. **1**(8476): p. 307-10.

226. Bayley, N. (2006b). Bayley Scales of Infant and Toddler Development—Third Edition: Technical manual. San Antonio, TX: Harcourt Assessment.

227. Cuzzilla, R., Spittle, A.J., Lee, K.J., et al., *Postnatal Brain Growth Assessed by Sequential Cranial Ultrasonography in Infants Born <30 Weeks' Gestational Age.* AJNR Am J Neuroradiol, 2018.

228. Leijser, L.M., de Bruine, F.T., van der Grond, J., et al., *Is sequential cranial ultrasound reliable for detection of white matter injury in very preterm infants?* Neuroradiology, 2010. **52**(5): p. 397-406.

229. Doyle, L.W., Cheong, J.L., Burnett, A., et al., *Biological and Social Influences on Outcomes of Extreme-Preterm/Low-Birth Weight Adolescents.* Pediatrics, 2015. **136**(6): p. e1513-20.

230. Bell, M.J., Ternberg, J.L., Feigin, R.D., et al., *Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging.* Ann Surg, 1978. **187**(1): p. 1-7.

231. Williams, J., Hirsch, N.J., Corbet, A.J., et al., *Postnatal head shrinkage in small infants.* Pediatrics, 1977. **59**(4): p. 619-22.

232. Bouyssi-Kobar, M., du Plessis, A.J., McCarter, R., et al., *Third Trimester Brain Growth in Preterm Infants Compared With In Utero Healthy Fetuses.* Pediatrics, 2016. **138**(5).

233. Kyriakopoulou, V., Vatansever, D., Davidson, A., et al., *Normative biometry of the fetal brain using magnetic resonance imaging.* Brain Struct Funct, 2016.

234. Tam, E.W., Chau, V., Ferriero, D.M., et al., *Preterm cerebellar growth impairment after postnatal exposure to glucocorticoids.* Sci Transl Med, 2011. **3**(105): p. 105ra105.

235. Thompson, D.K., Inder, T.E., Faggian, N., et al., *Corpus callosum alterations in very preterm infants: perinatal correlates and 2 year neurodevelopmental outcomes.* Neuroimage, 2012. **59**(4): p. 3571-81.

236. Saigal, S., den Ouden, L., Wolke, D., et al., *School-age outcomes in children who were extremely low birth weight from four international population-based cohorts.* Pediatrics, 2003. **112**(4): p. 943-50.

237. Brown, N. and Spittle, A., *Neurobehavioral evaluation in the preterm and term infant.* Curr Pediatr Rev, 2014. **10**(1): p. 65-72.

238. Hintz, S.R. and O'Shea, M., *Neuroimaging and neurodevelopmental outcomes in preterm infants.* Semin Perinatol, 2008. **32**(1): p. 11-9.

239. Wusthoff, C.J., *How to use: the neonatal neurological examination.* Arch Dis Child Educ Pract Ed, 2013. **98**(4): p. 148-53.

240. Dubowitz, L.M., Dubowitz, V., Palmer, P.G., et al., *Correlation of neurologic assessment in the preterm newborn infant with outcome at 1 year.* J Pediatr, 1984. **105**(3): p. 452-6.

241. Brown, N.C., Doyle, L.W., Bear, M.J., et al., *Alterations in neurobehavior at term reflect differing perinatal exposures in very preterm infants.* Pediatrics, 2006. **118**(6): p. 2461-71.

242. Dewey, D., Thompson, D.K., Kelly, C.E., et al., *Very preterm children at risk for developmental coordination disorder have brain alterations in motor areas.* Acta Paediatr, 2019. **108**(9): p. 1649-1660.

243. Brossard-Racine, M., Poretti, A., Murnick, J., et al., *Cerebellar Microstructural Organization is Altered by Complications of Premature Birth: A Case-Control Study.* J Pediatr, 2017. **182**: p. 28-33 e1.

244. Peterson, B.S., *Brain imaging studies of the anatomical and functional consequences of preterm birth for human brain development.* Ann N Y Acad Sci, 2003. **1008**: p. 219-37.

245. Matthews, L.G., Walsh, B.H., Knutsen, C., et al., *Brain growth in the NICU: critical periods of tissue-specific expansion.* Pediatr Res, 2018. **83**(5): p. 976-981.

246. Olsen, J.E., Brown, N.C., Eeles, A.L., et al., *Early general movements and brain magnetic resonance imaging at term-equivalent age in infants born <30weeks' gestation.* Early Hum Dev, 2016. **101**: p. 63-8.

247. Spittle, A.J., Brown, N.C., Doyle, L.W., et al., *Quality of general movements is related to white matter pathology in very preterm infants.* Pediatrics, 2008. **121**(5): p. e1184-9.

248. Spittle, A.J., Doyle, L.W., Anderson, P.J., et al., *Reduced cerebellar diameter in very preterm infants with abnormal general movements.* Early Hum Dev, 2010. **86**(1): p. 1-5.

249. Prechtl, H.F., Einspieler, C., Cioni, G., et al., *An early marker for neurological deficits after perinatal brain lesions.* Lancet, 1997. **349**(9062): p. 1361-3.

250. Hadders-Algra, M., *Putative neural substrate of normal and abnormal general movements.* Neurosci Biobehav Rev, 2007. **31**(8): p. 1181-90.

251. Bayley, N. (2006b). Bayley Scales of Infant and Toddler Development—Third Edition: Technical manual. San Antonio, TX: Harcourt Assessment.

252. Limperopoulos, C. and du Plessis, A.J., *Disorders of cerebellar growth and development.* Curr Opin Pediatr, 2006. **18**(6): p. 621-7.

253. Parker, J., Mitchell, A., Kalpakidou, A., et al., *Cerebellar growth and behavioural & neuropsychological outcome in preterm adolescents.* Brain, 2008. **131**(Pt 5): p. 1344-51.

254. Burakevych, N., McKinlay, C.J., Alsweiler, J.M., et al., *Bayley-III motor scale and neurological examination at 2 years do not predict motor skills at 4.5 years.* Dev Med Child Neurol, 2017. **59**(2): p. 216-223.

255. Anderson, P.J. and Burnett, A., *Assessing developmental delay in early childhood - concerns with the Bayley-III scales.* Clin Neuropsychol, 2017. **31**(2): p. 371-381.

256. Hagmann, C.F., Halbherr, M., Koller, B., et al., *Interobserver variability in assessment of cranial ultrasound in very preterm infants.* J Neuroradiol, 2011. **38**(5): p. 291-7.

257. Kelly, C.E., Cheong, J.L., Gabra Fam, L., et al., *Moderate and late preterm infants exhibit widespread brain white matter microstructure alterations at term-equivalent age relative to term-born controls.* Brain Imaging Behav, 2015. **10**: p. 41-49.

258. Limperopoulos, C., Soul, J.S., Gauvreau, K., et al., *Late gestation cerebellar growth is rapid and impeded by premature birth.* Pediatrics, 2005. **115**(3): p. 688-95.

259. Wu, P.M., Shih, H.I., Yu, W.H., et al., *Corpus callosum and cerebellar vermis size in very preterm infants: Relationship to long-term neurodevelopmental outcome.* Pediatr Neonatol, 2018. **60**(2): p. 178-185.

260. Gui, L., Loukas, S., Lazeyras, F., et al., *Longitudinal study of neonatal brain tissue volumes in preterm infants and their ability to predict neurodevelopmental outcome.* Neuroimage, 2018. **185**: p. 728-741.

Appendix 1

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ORIGINAL RESEARCH
PEDIATRICS

Postnatal Brain Growth Assessed by Sequential Cranial Ultrasonography in Infants Born <30 Weeks' Gestational Age

R. Cuzzilla, A.J. Spittle, K.J. Lee, S. Rogerson, F.M. Cowan, L.W. Doyle, and J.L.Y. Cheong

ABSTRACT

BACKGROUND AND PURPOSE: Brain growth in the early postnatal period following preterm birth has not been well described. This study of infants born at <30 weeks' gestational age and without major brain injury aimed to accomplish the following: 1) assess the reproducibility of linear measures made from cranial ultrasonography, 2) evaluate brain growth using sequential cranial ultrasonography linear measures from birth to term-equivalent age, and 3) explore perinatal predictors of postnatal brain growth.

MATERIALS AND METHODS: Participants comprised 144 infants born at <30 weeks' gestational age at a single center between January 2011 and December 2013. Infants with major brain injury seen on cranial ultrasonography or congenital or chromosomal abnormalities were excluded. Brain tissue and fluid spaces were measured from cranial ultrasonography performed as part of routine clinical care. Brain growth was assessed in 3 time intervals: <7, 7–27, and >27 days' postnatal age. Data were analyzed using intraclass correlation coefficients and mixed-effects regression.

RESULTS: A total of 429 scans were assessed for 144 infants. Several linear measures showed excellent reproducibility. All measures of brain tissue increased with postnatal age, except for the biparietal diameter, which decreased within the first postnatal week and increased thereafter. Gestational age of ≥28 weeks at birth was associated with slower growth of the biparietal diameter and ventricular width compared with gestational age of <28 weeks. Postnatal corticosteroid administration was associated with slower growth of the corpus callosum length, transcerebellar diameter, and vermis height. Sepsis and necrotizing enterocolitis were associated with slower growth of the transcerebellar diameter.

CONCLUSIONS: Postnatal brain growth in infants born at <30 weeks' gestational age can be evaluated using sequential linear measures made from routine cranial ultrasonography and is associated with perinatal predictors of long-term development.

ABBREVIATIONS: AHW = anterior horn width; BPD = biparietal diameter; BW = birth weight; CCL = corpus callosum length; cUS = cranial ultrasonography; GA = gestational age; NEC = necrotizing enterocolitis; PMA = postmenstrual age; PNA = postnatal age; TEA = term-equivalent age; TCD = transcerebellar diameter

Preterm infants are at risk of long-term neurodevelopmental impairment related to perinatal brain injury and altered brain maturation.[1,2] The optimal technique and timing of neuroimaging for identifying high-risk infants is under debate.[3] While early and sequential cranial ultrasonography (cUS) can be reliably used to detect major brain injury, it is less sensitive than MR imaging for the more prevalent, diffuse white matter injury associated with preterm birth.[1,4,5] Nonetheless, cUS remains the most widely used neuroimaging technique for preterm infants because it is readily available, easily repeatable, and sensitive enough for detecting most major pathology.

In the absence of overt brain injury and where MR imaging is not accessible, there is a need to improve the prognostic utility of cUS, to better understand why some preterm infants without major brain injury seen on cUS later develop motor and cognitive impairments.[1,6] Most infants born preterm have at least 1 early and 1 later neonatal cUS scan, affording an opportunity to quan-

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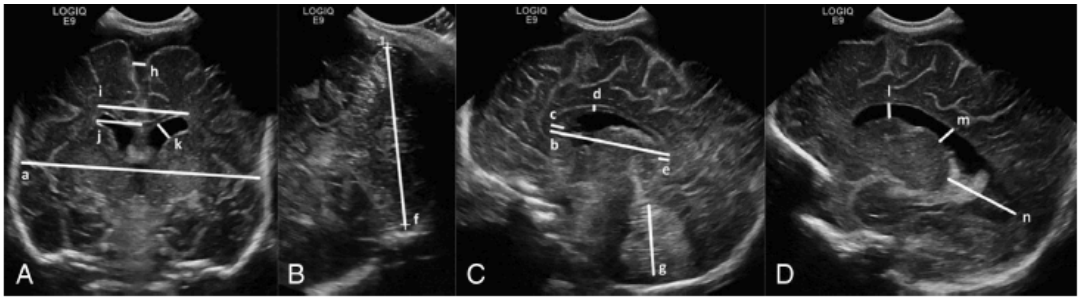

FIG 1. Cranial ultrasonography linear measures: images through the anterior fontanel in the coronal plane at the level of foramina of Monro (*A*) and the sagittal (*C*) and parasagittal (*D*) planes, and an image through the mastoid fontanel in the coronal plane posterior to the fourth ventricle (*B*). Brain tissue: biparietal diameter (a), corpus callosum length (b), corpus callosum genu width (c), corpus callosum body height (d), corpus callosum splenium width (e), transcerebellar diameter (f), and vermis height (g). Fluid spaces: interhemispheric distance (h), ventricular width (i), ventricular index (j), anterior horn width (k), anterior horn height (l), ventricular midbody width (m), and thalamo-occipital distance (n).

tify early brain growth as a potential marker of long-term development that has not been fully exploited.[7]

Linear measures of brain tissue and fluid spaces made from neonatal cUS have been associated with neurodevelopmental outcomes in preterm children.[8,9] However, only 2 studies have evaluated early postnatal brain growth using sequential cUS.[9,10] In a study of 140 infants born at <29 weeks' gestational age (GA), Roelants et al[10] reported normative data for the growth of the corpus callosum length (CCL) and corpus callosum–fastigium length with respect to postmenstrual age (PMA), but they did not relate brain growth with postnatal age (PNA). The study of 61 very-low-birth-weight infants by Anderson et al[9] found that slower growth of the CCL between 2 and 6 weeks' PNA was predictive of motor delay and cerebral palsy at 2 years; however, this study included infants with major brain injury.

To explore further the usefulness of cUS in assessing brain growth in preterm infants without major brain injury, we aimed to achieve the following: 1) assess the reproducibility of linear measures of brain tissue and fluid spaces made from cUS, 2) evaluate brain growth with respect to PNA using sequential cUS linear measures from birth to term-equivalent age (TEA), and 3) explore the associations between perinatal variables and postnatal brain growth measured by cUS.

MATERIALS AND METHODS

Study Participants

One hundred forty-nine infants born at <30 weeks' GA at The Royal Women's Hospital, Melbourne, Australia, between January 2011 and December 2013, were recruited into a prospective longitudinal study of neuroimaging, neurobehavior, and long-term development.[11] Infants with congenital or chromosomal anomalies known to affect neurodevelopment were excluded. For the current study, 5 infants with major preterm brain injury detected on cUS were also excluded, including infants with grades III or IV intraventricular hemorrhage, posthemorrhagic ventricular dilation, and cystic periventricular leukomalacia, leaving 144 infants for analysis.[12,13] The study received institutional approval from the Human Research Ethics Committee of the hospital, and written informed consent was obtained from parents of all participants.

Cranial Ultrasonography

cUS was performed as part of routine clinical care using a Logiq 9 Ultrasound System and an 8-MHz broadband curvilinear transducer (GE Healthcare, Milwaukee, Wisconsin). Standard images were acquired through the anterior fontanel (5 images in different coronal planes, an image in the midsagittal plane, and 2 images in different parasagittal planes both on the left and right) and the mastoid fontanel (1 image in the coronal plane). As per local protocol for the surveillance of preterm brain injury, infants born at <30 weeks' GA were scanned on, or around, days 7, 28, and 60 PNA. Infants born at <28 weeks' GA were also scanned on, or around, day 1 PNA. Additional scans were performed depending on clinical need. The current study included all neonatal scans performed from birth to TEA (<42 weeks' PMA).

Nineteen linear measures of brain tissue and fluid spaces were explored on the basis of potential clinical importance, ease of recognition of anatomic landmarks on standard imaging planes, and evaluation of their reproducibility (Fig 1). A neonatologist (R.C.) blinded to the clinical course of study participants and with 4 years' experience in performing cUS obtained measurements using electronic calipers on stored digital images (Synapse; Fujifilm Medical Company, Minato-Ku, Japan). Thirty scans (7%) were randomly selected to assess the reproducibility of the linear measures. Measurements were made by the same observer (R.C.) twice, at least 1 month apart, and once by another observer (S.R.) with 20 years' experience in performing cUS.

Perinatal Predictors of Long-Term Neurodevelopment

Perinatal variables were chosen a priori on the basis of their known associations with brain injury and long-term neurodevelopmental outcomes following preterm birth.[14] These included GA at birth (determined by first-trimester ultrasonography when available or by menstrual history), birth weight SD score (BW *z* score), sex, multiple gestations, chorioamnionitis (confirmed by placental histology), antenatal corticosteroids (any number of doses of betamethasone) and magnesium sulfate (for maternal or fetal indications), bronchopulmonary dysplasia (defined by oxygen requirement at 36 weeks' PMA), postnatal corticosteroids, confirmed sepsis (blood or CSF culture–positive and the use of antibiotics for ≥5 days) and/or necro-

tizing enterocolitis (NEC; defined by stage II or higher modified Bell criteria), and low-grade intraventricular hemorrhage (grades I or II) seen on cUS (defined by Papile classification).[13,15]

Data Analysis

Data were analyzed using STATA, Version 13.1 (StataCorp, College Station, Texas). Intra- and interobserver agreement was assessed by the intraclass correlation coefficient. Linear measures with both intra- and interobserver intraclass correlation coefficients of >0.80 were considered for inclusion in further analyses. Brain growth (or rate of change) with respect to PNA was assessed using mixed-effects linear regression fitted to all the sequential measurements from all individuals, with PNA as the time variable and a random effect for an individual fitted with an unstructured covariance matrix. The model included a random effect to account for multiple measurements for each infant, with a fixed effect of time. First, overall brain growth was assessed from birth to TEA using a single time variable. Brain growth was then assessed within 3 time intervals to reflect the timing of our routine cUS by fitting separate effects of time in the periods <7, 7–27, and >27 days' PNA. Analyses were repeated with adjustment for GA, BW *z* score, and sex. Associations between perinatal variables and brain growth with respect to PNA (fitting a single effect of time from birth to TEA) were explored separately by including an interaction between the perinatal variable and PNA in the mixed-effects linear regression model. Continuous variables were dichotomized for this analysis: GA group (<28 weeks or 28–29 weeks) and BW *z* score group (<−2 or ≥−2 SDs from the mean). Analyses were repeated with adjustment for GA, BW *z* score, and sex, with the exception that GA and the BW *z* score were not adjusted for when exploring the effect of the GA group and BW *z* score group, respectively. Measurements of the left and right anterior horn widths (AHWs) included the germinal layer hemorrhage when present. Consequently, all analyses involving the AHW were restricted to infants without an ipsilateral intraventricular hemorrhage, to eliminate the influence of the germinal layer hemorrhage on the generalizability of results with respect to rates of change of the AHW with PNA.

Table 1: Infant characteristics

Perinatal Variable	Summary (*N* = 144 Infants)
Gestational age (mean) (SD) (wk)	27.7 (1.5)
Birth weight (mean) (SD) (g)	1017 (259)
Small for gestational age, birth weight <−2 SDs (No.) (%)	16 (11)
Female (No.) (%)	75 (52)
Multiple gestations (No.) (%)	64 (44)
Cesarean delivery (No.) (%)	106 (74)
Antenatal corticosteroids (No.) (%)	134 (93)
Antenatal magnesium sulfate (No.) (%)	103 (72)
Respiratory distress from birth (No.) (%)	141 (98)
Surfactant (No.) (%)	91 (63)
Duration of positive pressure ventilation (median) (25th–75th centile) (days)	19 (0–96)
Duration of supplemental oxygen (median) (25th–75th centile) (days)	19 (4–56)
Bronchopulmonary dysplasia[a] (No.) (%)	46 (32)
Postnatal corticosteroids (No.) (%)	18 (13)
Intraventricular hemorrhage, grades I or II (No.) (%)	25 (17)
Retinopathy of prematurity (No.) (%)	32 (22)
Sepsis confirmed[b] (No.) (%)	20 (14)
NEC (No.) (%)	17 (12)
Sepsis confirmed and/or NEC (No.) (%)	31 (22)
Survived to discharge home (No.) (%)	139 (97)

[a] Defined by oxygen requirement at 36 weeks' postmenstrual age.
[b] Blood or CSF culture–positive and use of antibiotics for ≥5 days.

RESULTS

Characteristics of the 144 infants included in the current study are presented in Table 1. In the 144 infants, 429 scans were performed from birth to TEA. The mean number of scans per infant was 3 (range, 1–8). For the 77 infants born at <28 weeks' GA, 291 scans were assessed (mean, 3.8; range, 1–8 per infant), and 138 scans for the 67 infants born at ≥28 weeks' GA (mean, 2.1; range, 1–6 per infant). Almost two-thirds (n = 272; 63%) of the scans were performed at <28 days' PNA, but most infants had scans up to 33 weeks' PMA.

Table 2: Reproducibility of cranial ultrasonography linear measures

	Intraobserver		Interobserver	
Linear Measure	ICC	95% CI	ICC	95% CI
Brain tissue				
Biparietal diameter	0.98	0.95–0.99	0.97	0.94–0.99
Corpus callosum length	0.99	0.97–0.99	0.98	0.95–0.99
Corpus callosum genu width	0.78	0.53–0.90	0.38	−0.04–0.69
Corpus callosum body height	0.74	0.52–0.87	0.58	0.27–0.78
Corpus callosum splenium width	0.80	0.56–0.92	0.21	−0.22–0.58
Transcerebellar diameter	0.99	0.98–0.99	0.98	0.96–0.99
Vermis height	0.91	0.82–0.96	0.81	0.62–0.91
Fluid spaces				
Interhemispheric distance	0.98	0.96–0.99	0.98	0.95–0.99
Anterior horn width, left	0.94	0.87–0.97	0.91	0.81–0.96
Anterior horn width, right	0.96	0.90–0.98	0.95	0.89–0.98
Ventricular index, left	0.85	0.70–0.93	0.85	0.69–0.93
Ventricular index, right	0.63	0.32–0.82	0.59	0.26–0.80
Ventricular width	0.87	0.72–0.94	0.89	0.76–0.95
Anterior horn height, left	0.86	0.72–0.94	0.76	0.53–0.88
Anterior horn height, right	0.92	0.82–0.96	0.78	0.55–0.90
Ventricular midbody height, left	0.73	0.49–0.87	0.47	0.11–0.72
Ventricular midbody height, right	0.81	0.61–0.91	0.52	0.18–0.75
Thalamo-occipital distance, left	0.93	0.81–0.97	0.80	0.52–0.93
Thalamo-occipital distance, right	0.92	0.80–0.97	0.62	0.22–0.84

Note:—ICC indicates intraclass correlation coefficient.

Intra- and Interobserver Reproducibility

Intra- and interobserver intraclass correlation coefficients are shown in Table 2. Eight linear measures, 4 of brain tissue (biparietal diameter [BPD], CCL, transcerebellar diameter [TCD], and vermis height) and 4 reflecting fluid spaces (interhemispheric distance, left and right AHWs, and ventricular width), had intraclass correlation coefficients of >0.8 both between and within observers and were used to evaluate brain growth.

Brain Growth with Respect to Postnatal Age

There was evidence that all linear measures of brain tissue increased with PNA, before and after adjustment for GA, BW *z* score, and sex, overall and within the 3 time intervals, except for the BPD, which decreased within the first postnatal week (Fig 2 and On-line Table). Faster rates of growth of the BPD, TCD, and vermis height were observed after 27 days' PNA compared with 7–27 days' PNA; however, the rate of growth of the CCL more than halved after 27 days' PNA compared with 7–27 days' PNA (Fig 2 and On-line Table).

Rates of change of linear measures of fluid spaces were variable within the first postnatal week, with little evidence of an association between linear measures and PNA (Fig 2 and On-line Table). The interhemispheric distance and ventricular width increased after the first postnatal week, with faster rates of change after 27 days' PNA (1.5 and 2.7 times, respectively) compared with 7–27 days' PNA. Although the rates of change for the AHW appeared to decrease with successive time intervals, there was little statistical evidence of an association between the AHW and PNA, except for the right AHW between 7 and 27 days' PNA.

Associations between Perinatal Variables and Postnatal Brain Growth

There was strong evidence of associations between many of the perinatal variables and brain growth with respect to PNA, which remained after adjustment for GA, BW *z* score, and sex (Table 3). Infants born at 28–29 weeks' GA showed slower growth of the BPD and ventricular width than infants born at <28 weeks' GA. Postnatal corticosteroid administration was associated with slower growth of the CCL, TCD, and vermis height, and bronchopulmonary dysplasia was associated with slower growth of the CCL and TCD. Infants with a BW *z* score <-2 SDs below the mean showed slower growth of the CCL than infants with BW ≥ -2 SDs below the mean. Sepsis and/or NEC and antenatal corticosteroid administration were associated with slower growth of the TCD.

DISCUSSION

In this study of early postnatal brain growth in infants born at <30 weeks' GA and without major brain injury, several linear measures of brain tissue and fluid spaces were reliably made from routine clinical cUS scans. Most linear measures of brain tissue increased with PNA, but rates of change were more variable for the fluid spaces. Several perinatal variables related to long-term development were associated with postnatal brain growth. Bronchopulmonary dysplasia and postnatal corticosteroid administration were associated with slower growth of the CCL and TCD, and postnatal corticosteroid administration was also associated with slower growth of the vermis height. Sepsis and/or NEC were associated with slower growth of the TCD.

Measurements of brain growth for use in clinical practice should be simple to obtain and reproducible. The cUS linear measures explored in this study had well-defined anatomic landmarks on standard imaging planes obtained during routine clinical care, and several showed excellent intra- and interobserver reproducibility, as also shown by others.[8,10,16,17]

Although cUS affords an opportunity to make sequential measurements from birth, there are limited published data relating to postnatal brain growth in preterm infants. To our knowledge, this study is the first to describe the growth of the BPD in the early postnatal period following preterm birth using sequential cUS. We speculate that the decreasing size of the BPD in the first postnatal week found in the current study may relate to a reduction in brain-water in the immediate postnatal days rather than deformational changes in head shape.[18] Growth of the BPD between 7 and 27 days was slower (1.30 mm/week PNA) than after the first 27 days (2.17 mm/week PNA), from which time postnatal growth of the BPD approximates to expected fetal growth (2.10 mm/week PMA) as reported from cross-sectional data by Kurmanavicius et al.[19] Higher GA at birth related to slower growth of the BPD from birth to TEA, in keeping with normative data for fetal growth of the BPD that demonstrates a slowing of growth with increasing PMA.[19,20] However, infants born at <30 weeks' GA have smaller brains at TEA than term-born controls, and most are likely to have slower rates of brain growth immediately after birth than normally expected in utero.[20,21]

In the current study, CCL growth was relatively constant from birth to 27 days' PNA but slowed by half thereafter. Similarly, Anderson et al[9] showed that postnatal growth of the CCL in very-low-birth-weight infants approximated fetal growth (1.4–1.89 mm/week PMA) within the first 2 postnatal weeks but subsequently slowed to approximately half of that expected.[22,23] Moreover, the current study found that a BW *z* score of <-2 SDs was associated with slower CCL growth from birth to TEA, a finding consistent with that of Roelants et al.[10]

The TCD measured in the fetus, or the neonate in the first postnatal days, is a reliable marker of GA, even with fetal growth restriction, small- and large-for-gestational age fetuses, and multiple gestations (1.25–1.89 mm/week PMA).[24-27] Cross-sectional preterm MR imaging studies have reported cerebellar size, but our study is the first to report postnatal growth of the TCD in preterm infants using sequential cUS.[20,28] Similar to fetal growth patterns, TCD growth in the current study progressively increased between time intervals. Unlike the TCD, the vermis height increased with PNA at a relatively constant rate of change from birth to TEA, approximating fetal growth (0.55 mm/week PMA).[29]

Bronchopulmonary dysplasia and postnatal corticosteroids were associated with slower CCL and TCD growth with PNA, and postnatal corticosteroid administration was also related to slower growth of the vermis height. Tam et al[30] reported a similar association between postnatal corticosteroids, a marker of severe bronchopulmonary dysplasia, and impaired cerebellar growth in very preterm infants using serial MR imaging between 32 weeks' PMA and TEA. In another MR imaging study of very preterm infants scanned at TEA and 7 years, Thompson et al[31] reported an association between postnatal corticosteroids and delayed maturation of the posterior corpus callosum, a region that develops later in gestation and contributes to corpus callosum length. In the current study, sepsis and/or NEC were related to slower growth of the TCD. Sepsis and/or NEC have been associated with preterm white matter injury at TEA, but their relationship with brain growth and maturation in the early postnatal period remains unclear.[32] The relationship between antenatal corticoste-

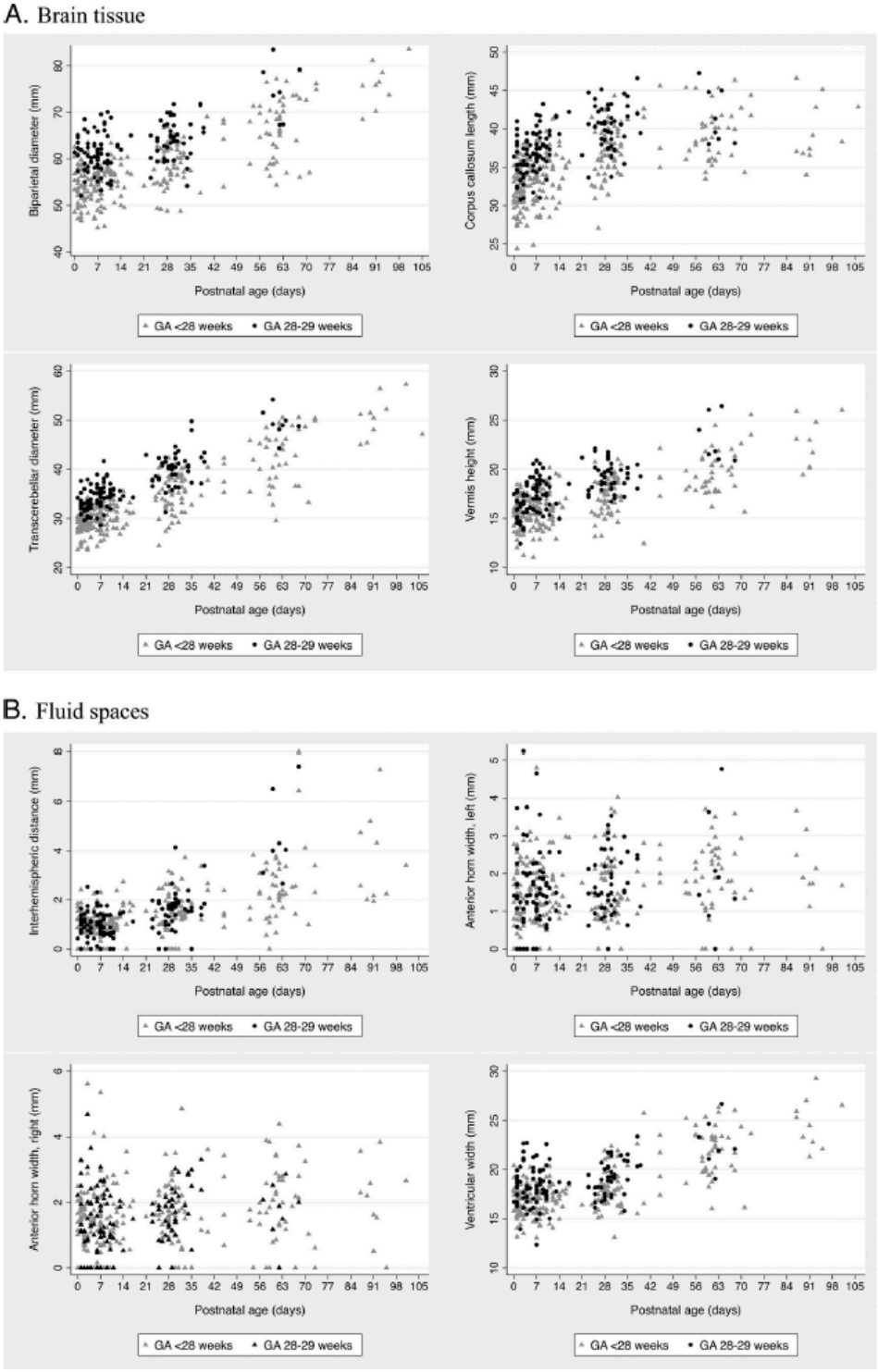

FIG 2. Cranial ultrasonography linear measures with respect to postnatal age. *A*, Brain tissue. *B*, Fluid spaces.

Table 3: Associations between perinatal variables and postnatal brain growth

Perinatal Variable	Adjusted[a] Growth (mm/week PNA) β	95% CI	Interaction Term[b] (*P* Value)
Biparietal diameter			
GA			
<28 weeks	1.88	1.71–2.05	.002
≥28 weeks	1.29	0.96–1.62	
Corpus callosum length			
BW *z* score			
≥−2 SD	0.91	0.84–0.98	.045
<−2 SD	0.73	0.57–0.89	
Postnatal corticosteroids			
No	0.92	0.85–1.00	.046
Yes	0.77	0.63–0.90	
Bronchopulmonary dysplasia			
No	0.98	0.88–1.07	.01
Yes	0.81	0.73–0.89	
Transcerebellar diameter			
Antenatal corticosteroids			
No	2.26	1.81–2.70	.023
Yes	1.73	1.63–1.83	
Postnatal corticosteroids			
No	1.84	1.74–1.95	<.001
Yes	1.41	1.22–1.61	
Bronchopulmonary dysplasia			
No	1.89	1.75–2.02	.008
Yes	1.63	1.49–1.76	
Sepsis/NEC			
No	1.82	1.71–1.93	.016
Yes	1.55	1.36–1.74	
Vermis height			
Postnatal corticosteroids			
No	0.63	0.58–0.69	.011
Yes	0.49	0.39–0.59	
Ventricular width			
GA			
<28 weeks	0.64	0.57–0.71	.004
≥28 weeks	0.41	0.27–0.55	

[a] Adjusted for gestational age, birth weight *z* score, and sex.
[b] *P* value for the interaction between the perinatal variable and postnatal age.

roid exposure and slower TCD growth reported here is novel and needs to be replicated in future studies.

Liao et al[33] and Levene[34] reported serial measurements of the ventricular index in preterm infants from birth to 6 weeks' and 6 months' PNA, respectively, and found rates of change like those in the current study for the ventricular width (the sum of left and right ventricular indices). We found that lower GA was related to a faster rate of change in the ventricular width, possibly resulting from a greater burden of diffuse cerebral white matter injury in the less mature infants leading to greater ex vacuo dilation. Rates of change for the AHW with respect to PNA were variable and increased little after the first postnatal week, as also reported in other studies.[33-36]

A major strength of the current study is its use of simple and reproducible linear measures obtained from standard cUS performed as part of routine clinical care. We use the mastoid fontanel for cerebellar imaging and TCD measurement, a reliable approach that is not technically difficult.[37] Our study, however, has several limitations. We used routine clinical scans timed to detect major preterm brain injury. There is, therefore, an inherent bias in our study cohort because the smaller, less mature infants were more likely to have had a greater burden of brain injury and, subsequently, a greater number of scans for monitoring the progression of any overt findings. It is also likely that these infants had more comorbidities and longer periods of hospitalization. In our neonatal unit, infants who no longer need tertiary-level care are transferred to nontertiary hospitals from as early as 32 weeks' PMA. The more mature infants in our cohort, unless they were very sick, were likely to have had fewer scans, limited to the early days and weeks after birth. However, the mixed-effects model used allows for different infants having different numbers of scans.

The cohort used in the current study will have developmental assessments throughout childhood, which will allow the relationships between sequential cUS linear measures of early postnatal brain growth and neurodevelopment in later childhood to be determined in the future.

CONCLUSIONS

Brain growth in infants born at <30 weeks' GA can be assessed using simple and reproducible linear measures made on sequential cUS. Several perinatal variables already shown to be related to long-term development, including GA, BW *z* score, bronchopulmonary dysplasia, postnatal corticosteroids, and sepsis and/or NEC, were associated with early postnatal brain growth. Further research is needed to evaluate the usefulness of brain growth, as evaluated using sequential cUS linear measures, as a marker of long-term neuro developmental outcome.

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cil, *Comments*: Career Development Fellowship ID 1141354; *UNRELATED*: *Employment*: The Royal Women's Hospital, Melbourne, *Comments*: This is my clinical employer. *Money paid to the institution.

REFERENCES

1. Woodward LJ, Anderson PJ, Austin NC, et al. **Neonatal MRI to predict neurodevelopmental outcomes in preterm infants.** *N Engl J Med*, 2006;355:685–94 CrossRef Medline
2. De Vries LS, Van Haastert IL, Rademaker KJ, et al. **Ultrasound abnormalities preceding cerebral palsy in high-risk preterm infants.** *J Pediatr* 2004;144:815–20 Medline
3. Plaisier A, Raets MM, Ecury-Goossen GM, et al. **Serial cranial ultrasonography or early MRI for detecting preterm brain injury?** *Arch Dis Child Fetal Neonatal Ed* 2015;100:F293–300 CrossRef Medline
4. Leijser LM, de Bruïne FT, van der Grond J, et al. **Is sequential cranial ultrasound reliable for detection of white matter injury in very preterm infants?** *Neuroradiology* 2010;52:397–406 CrossRef Medline
5. Miller SP, Cozzio CC, Goldstein RB, et al. **Comparing the diagnosis of white matter injury in premature newborns with serial MR imaging and transfontanel ultrasonography findings.** *AJNR Am J Neuroradiol* 2003;24:1661–69 Medline
6. Laptook AR, O'Shea TM, Shankaran S, et al. **Adverse neurodevelopmental outcomes among extremely low birth weight infants with a normal head ultrasound: prevalence and antecedents.** *Pediatrics* 2005;115:673–80 CrossRef Medline
7. Ment LR, Bada HS, Barnes P, et al. **Practice parameter: neuroimaging of the neonate: report of the Quality Standards Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society.** *Neurology* 2002;58:1726–38 CrossRef Medline
8. Fox LM, Choo P, Rogerson SR, et al. **The relationship between ventricular size at 1 month and outcome at 2 years in infants less than 30 weeks' gestation.** *Arch Dis Child Fetal Neonatal Ed* 2014;99:F209–14 CrossRef Medline
9. Anderson NG, Laurent I, Woodward LJ, et al. **Detection of impaired growth of the corpus callosum in premature infants.** *Pediatrics* 2006;118:951–60 CrossRef Medline
10. Roelants, JA, Koning, IV, Raets MM, et al. **A new ultrasound marker for bedside monitoring of preterm brain growth.** *AJNR Am J Neuroradiol* 2016;37:1516–22 CrossRef Medline
11. Spittle AJ, Thompson DK, Brown NC, et al. **Neurobehaviour between birth and 40 weeks' gestation in infants born <30 weeks' gestation and parental psychological wellbeing: predictors of brain development and child outcomes.** *BMC Pediatr* 2014;14:111 CrossRef Medline
12. de Vries LS, Eken P, Dubowitz LM. **The spectrum of leukomalacia using cranial ultrasound.** *Behav Brain Res* 1992;49:1–6 CrossRef Medline
13. Papile LA, Burstein J, Burstein R, et al. **Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm.** *J Pediatr* 1978;92:529–34 CrossRef Medline
14. Doyle LW, Cheong JL, Burnett A, et al; Victorian Infant Collaborative Study Group. **Biological and social influences on outcomes of extreme-preterm/low-birth weight adolescents.** *Pediatrics* 2015;136:e1513–20 CrossRef Medline
15. Bell MJ, Ternberg JL, Feigin RD, et al. **Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging.** *Ann Surg* 1978;187:1–7 CrossRef Medline
16. Hagmann CF, Robertson NJ, Acolet D, et al. **Cerebral measurements made using cranial ultrasound in term Ugandan newborns.** *Early Hum Dev* 2011;87:341–47 CrossRef Medline
17. Anderson NG, Laurent I, Cook N, et al. **Growth rate of corpus callosum in very premature infants.** *AJNR Am J Neuroradiol* 2005;26:2685–90 Medline
18. Williams J, Hirsch NJ, Corbet AJ, et al. **Postnatal head shrinkage in small infants.** *Pediatrics* 1977;59:619–22 Medline
19. Kurmanavicius J, Wright EM, Royston P, et al. **Fetal ultrasound biometry, 1: head reference values.** *Br J Obstet Gynaecol* 1999;106:126–35 CrossRef Medline
20. Bouyssi-Kobar M, du Plessis AJ, McCarter R, et al. **Third trimester brain growth in preterm infants compared with in utero healthy fetuses.** *Pediatrics* 2016;138. pii: e20161640 CrossRef Medline
21. Nguyen The Tich S, Anderson PJ, Shimony JS, et al. **A novel quantitative simple brain metric using MR imaging for preterm infants.** *AJNR Am J Neuroradiol* 2009;30:125–31 CrossRef Medline
22. Achiron R, Achiron, A. **Development of the human fetal corpus callosum: a high-resolution, cross-sectional sonographic study.** *Ultrasound Obstet Gynecol* 2001;18:343–47 CrossRef Medline
23. Malinger G, Zakut, H. **The corpus callosum: normal fetal development as shown by transvaginal sonography.** *AJR Am J Roentgenol* 1993;161:1041–43 CrossRef Medline
24. Imamoglu EY, Gursoy T, Ovali F, et al. **Nomograms of cerebellar vermis height and transverse cerebellar diameter in appropriate-for-gestational-age neonates.** *Early Hum Dev* 2013;89:919–23 CrossRef Medline
25. Chavez MR, Ananth CV, Smulian JC, et al. **Fetal transcerebellar diameter measurement for prediction of gestational age at the extremes of fetal growth.** *J Ultrasound Med* 2007;26:1167–71; quiz 1173–74 Medline
26. Chavez MR, Ananth CV, Kaminsky LM, et al. **Fetal transcerebellar diameter measurement for prediction of gestational age in twins.** *Am J Obstet Gynecol* 2006;195:1596–600 CrossRef Medline
27. Davies MW, Swaminathan M, Betheras FR. **Measurement of the transverse cerebellar diameter in preterm neonates and its use in assessment of gestational age.** *Australas Radiol* 2001;45:309–12 CrossRef Medline
28. Kyriakopoulou V, Vatansever D, Davidson A, et al. **Normative biometry of the fetal brain using magnetic resonance imaging.** *Brain Struct Funct* 2017;222:2295–307 CrossRef Medline
29. Zalel Y, Seidman DS, Brand N, et al. **The development of the fetal vermis: an in-utero sonographic evaluation.** *Ultrasound Obstet Gynecol* 2002;9:136–39 Medline
30. Tam EW, Chau V, Ferriero DM, et al. **Preterm cerebellar growth impairment after postnatal exposure to glucocorticoids.** *Sci Transl Med* 2011;3:105ra105 CrossRef Medline
31. Thompson DK, Inder TE, Faggian N, et al. **Corpus callosum alterations in very preterm infants: perinatal correlates and 2 year neurodevelopmental outcomes.** *Neuroimage* 2012;59:3571–81 CrossRef Medline
32. Shah DK, Doyle LW, Anderson PJ, et al. **Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term.** *J Pediatr* 2008;153170–75, 175.e1 CrossRef Medline
33. Liao MF, Chaou WT, Tsao LY, et al. **Ultrasound measurement of the ventricular size in newborn infants.** *Brain Dev* 1986;8:262–68 CrossRef Medline
34. Levene MI. **Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound.** *Arch Dis Child* 1981;56:900–04 CrossRef Medline
35. Sondhi V, Gupta G, Gupta PK, et al. **Establishment of nomograms and reference ranges for intra-cranial ventricular dimensions and ventriculo-hemispheric ratio in newborns by ultrasonography.** *Acta Paediatr* 2008;97:738–44 CrossRef Medline
36. Perry RN, Bowman ED, Murton LJ, et al. **Ventricular size in newborn infants.** *J Ultrasound Med* 1985;4:475–77 CrossRef Medline
37. da Graca AL, Cardoso KR, da Costa JM, et al. **Assessment of gestational age using cerebellar measurements at cranial ultrasound: what is the best approach?** *Early Hum Dev* 2013;89:1–5 CrossRef Medline

Appendix 2

Bland-Altman Plots

The intra- and inter-observer mean differences, and the 95% limits of agreement, are reported for each of the linear measures in Table A2, and their respective Bland-Altman plots are presented in Figure A2. In general, linear measures of brain tissue and fluid spaces of a larger absolute size had proportionally smaller mean differences between measurements made by the same, or different, observers. For all linear measures, variation in the mean difference between measurements appeared constant relative to increasing means of the measurements, with the exception of the IHD between different observers, where observer A consistently made larger measurements of the IHD compared with observer B.

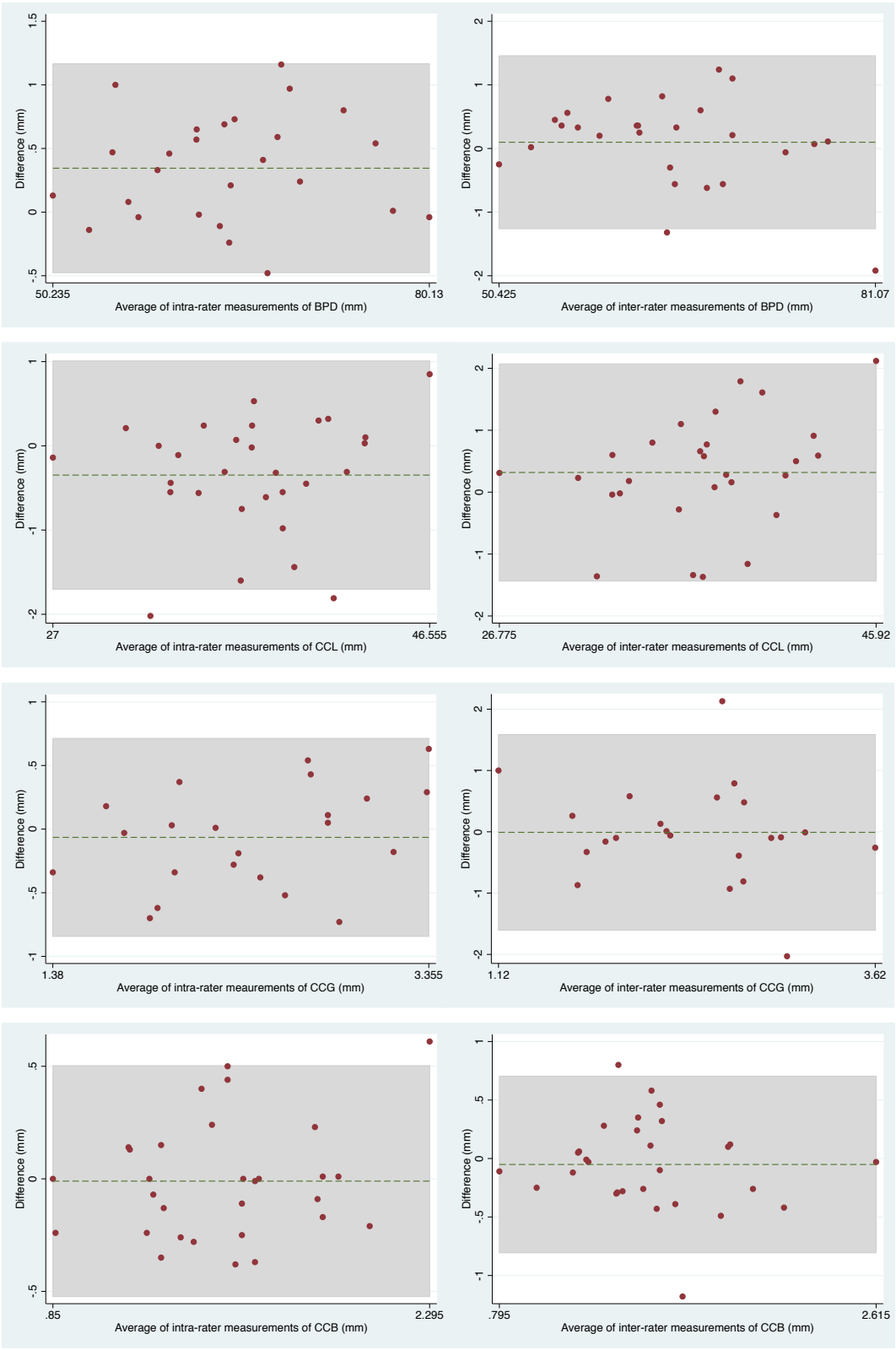
Table A2 Mean differences and the 95% levels of agreement to assess for systematic bias in making linear measures

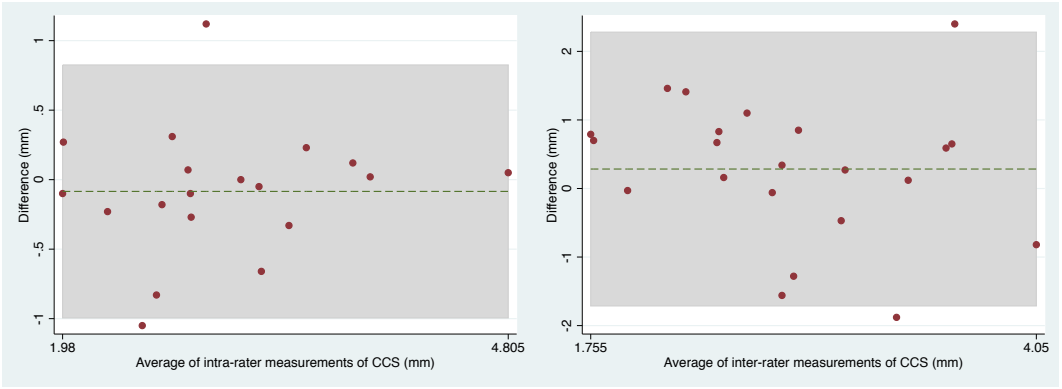
	Intra-observer		Inter-observer	
Linear measure	MD (mm)	95% limits of agreement (mm)	MD (mm)	95% limits of agreement (mm)
Brain tissues				
Biparietal diameter	0.66	-2.48, 3.81	0.42	-3.14, 3.97
Corpus callosum length	-0.35	-1.70, 1.01	0.32	-1.44, 2.07
Corpus callosum genu width	-0.06	-0.84, 0.71	-0.01	-1.60, 1.59
Corpus callosum body height	-0.01	-0.52, 0.50	-0.05	-0.81, 0.70
Corpus callosum splenium width	-0.08	-0.99, 0.83	0.28	-1.71, 2.28
Transcerebellar diameter	0.13	-1.79, 2.04	0.54	-2.06, 3.13
Vermis height	-0.32	-2.19, 1.55	-0.92	-4.01, 2.18
Fluid spaces				
Interhemispheric distance	-0.02	-0.60, 0.55	-0.17	-0.87, 0.52
Anterior horn width, left	0.01	-0.57, 0.58	-0.08	-0.79, 0.63
Anterior horn width, right	-0.003	-0.50, 0.49	-0.001	-0.51, 0.51
Ventricular index, left	0.46	-1.11, 2.04	0.38	-1.19, 1.95
Ventricular index, right	0.54	-1.20, 2.28	0.40	-1.58, 2.37
Ventricular width	0.79	-2.00, 3.57	0.42	-2.27, 3.12
Anterior horn height, left	0.16	-0.54, 0.87	-0.08	-0.92, 1.07
Anterior horn height, right	0.07	-0.55, 0.68	-0.01	-1.02, 1.00
Ventricular mid-body height, left	-0.07	-1.47, 1.33	0.50	-1.89, 2.86
Ventricular mid-body height, right	0.23	-1.30, 1.76	0.89	-2.04, 3.82
Thalamo-occipital distance, left	-0.13	-2.24, 1.98	0.29	-3.11, 3.70
Thalamo-occipital distance, right	-0.83	-3.46, 1.81	0.16	-4.30, 4.61

MD=mean difference.

Figure A2 Bland-Altman plots

A. Brain tissue

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-.5
-1
1.98
4.805
Average of intra-rater measurements of CCS (mm)
Difference (mm)
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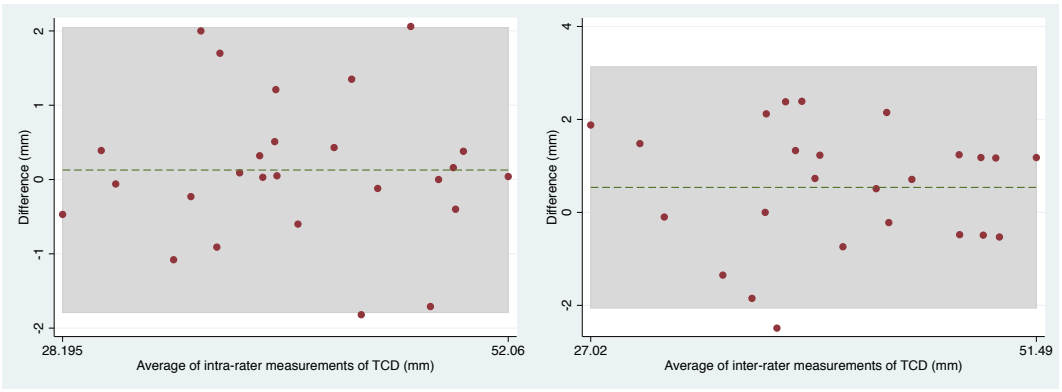
1
0
-1
-2
1.755
4.05
Average of inter-rater measurements of CCS (mm)


Difference (mm)
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-1
-2
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52.06
Average of intra-rater measurements of TCD (mm)
Difference (mm)
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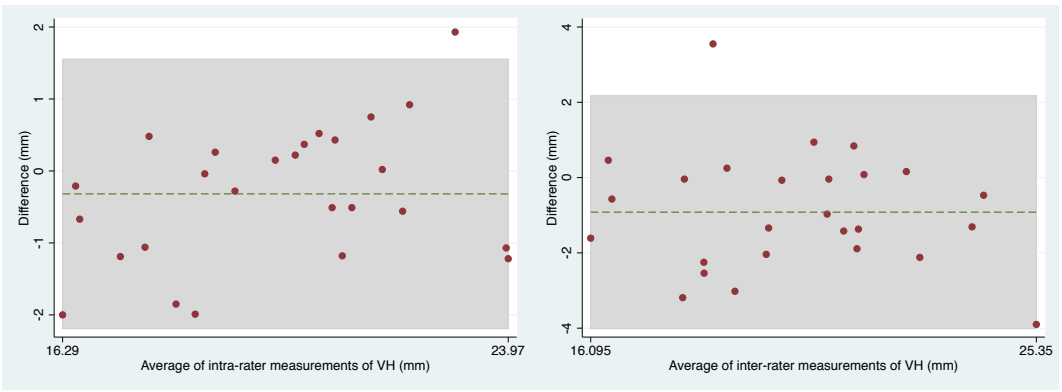
2
0
-2
27.02
51.49
Average of inter-rater measurements of TCD (mm)


Difference (mm)
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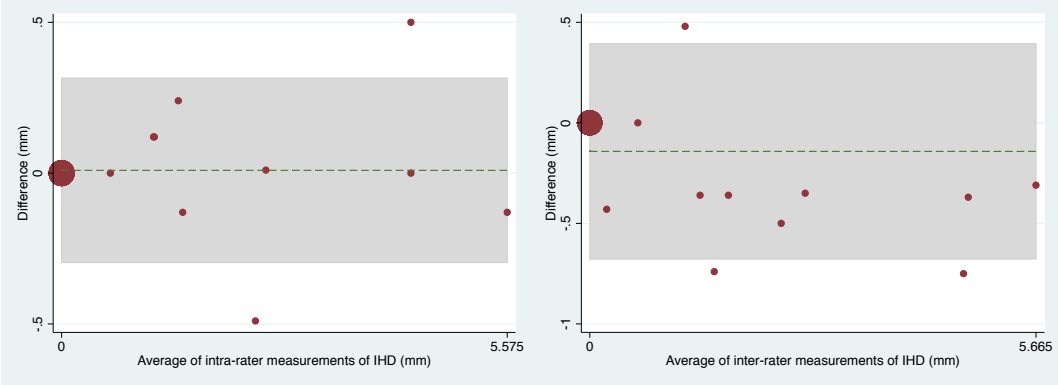
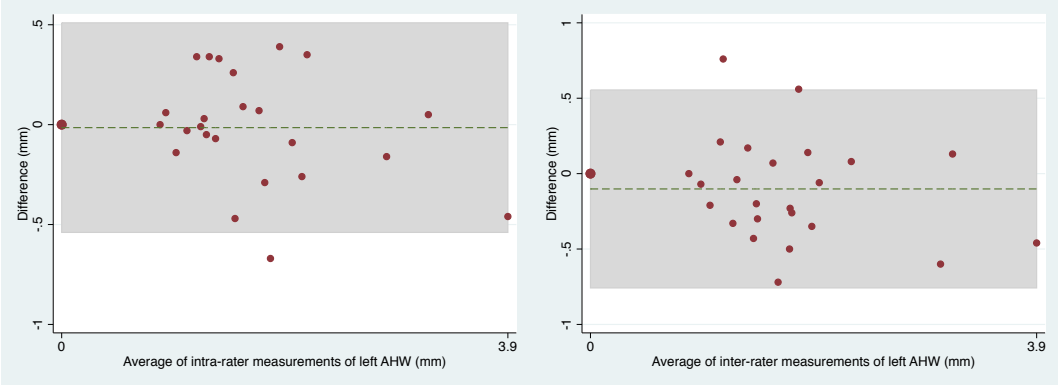
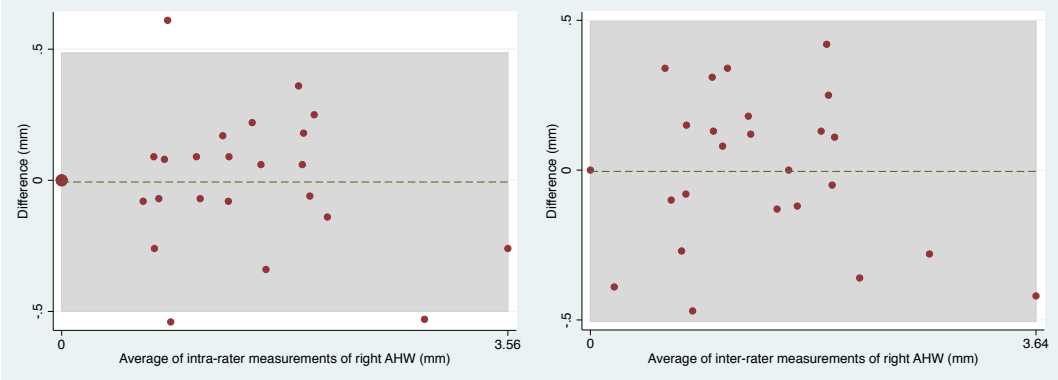
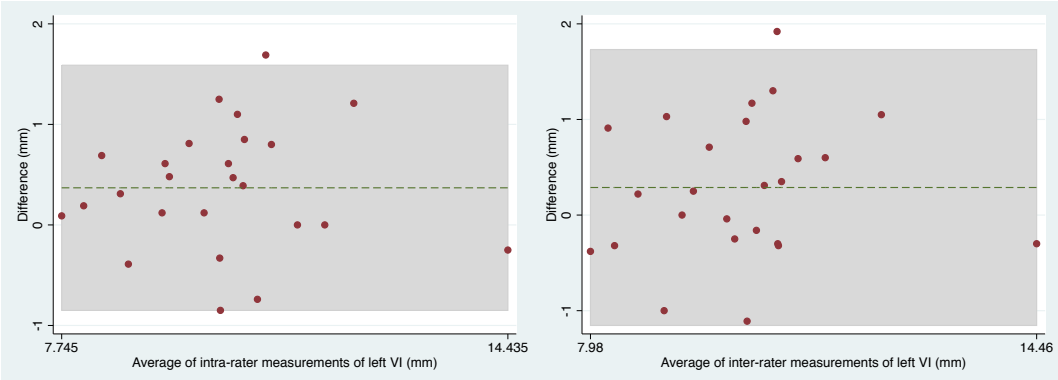
1
0
-1
-2
16.29
23.97
Average of intra-rater measurements of VH (mm)
Difference (mm)
4
2
0
-2
-4
16.095
25.35
Average of inter-rater measurements of VH (mm)

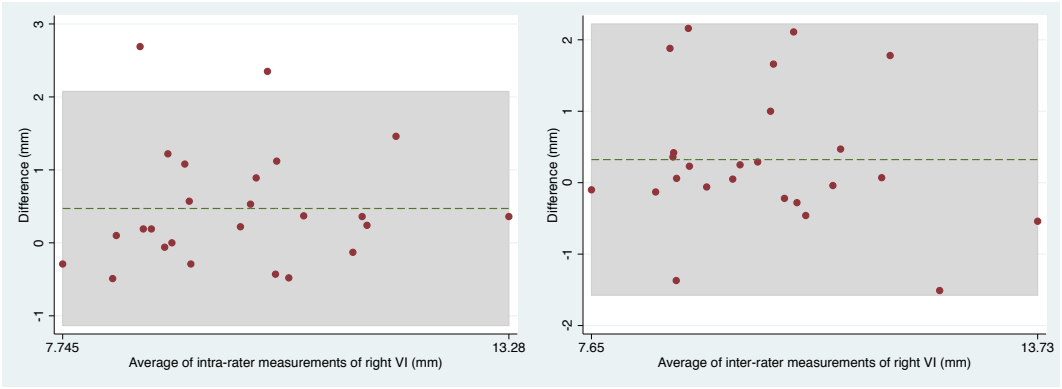
B. Fluid spaces










Difference (mm)
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1
2
3
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13.28
Average of intra-rater measurements of right VI (mm)
Difference (mm)
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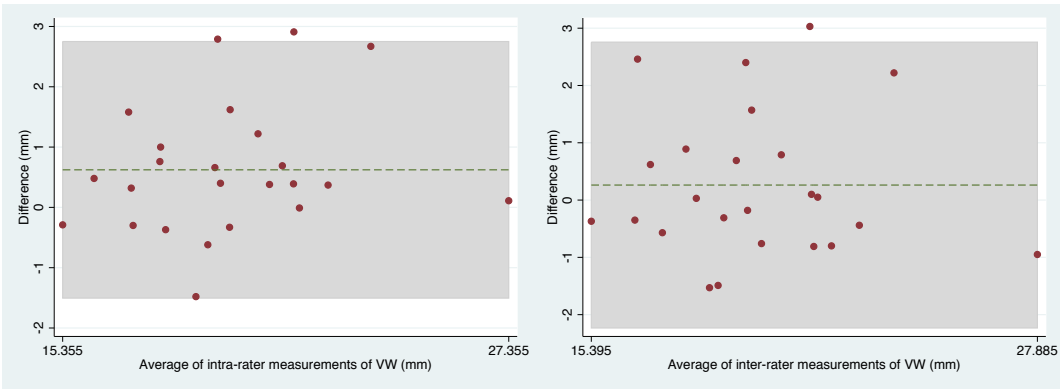
-1
0
1
2
7.65
13.73
Average of inter-rater measurements of right VI (mm)


Difference (mm)
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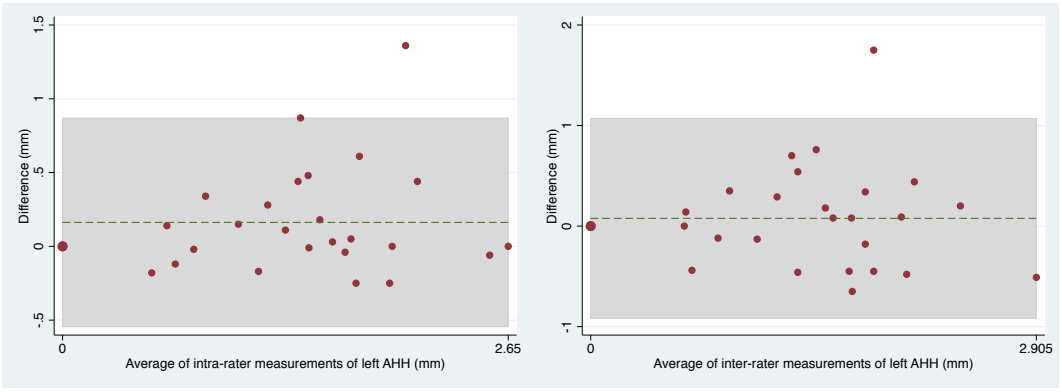
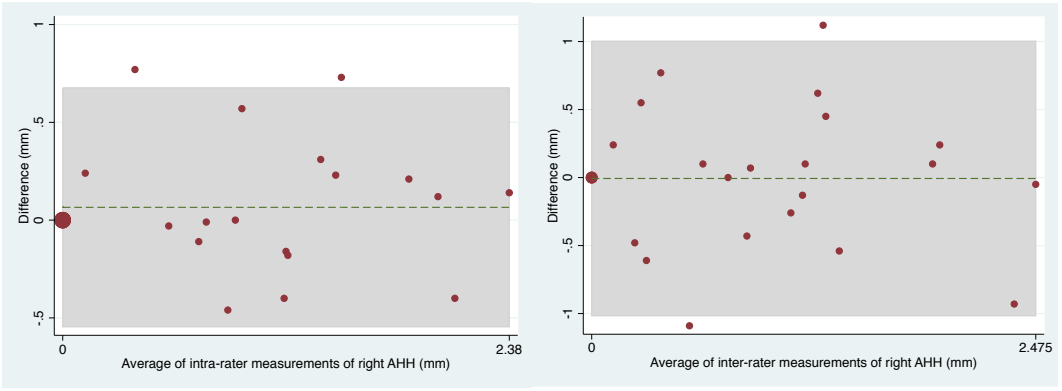
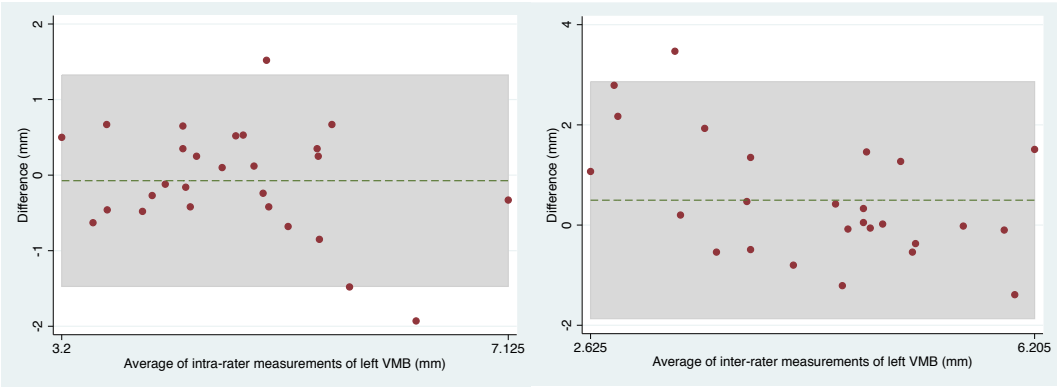
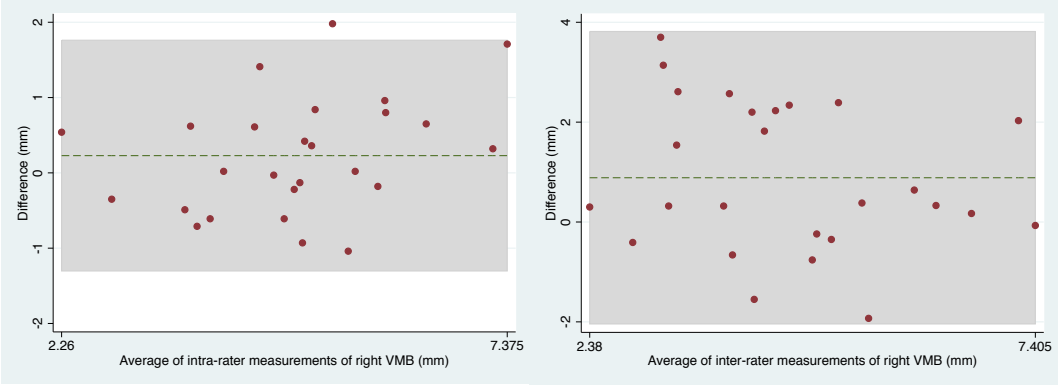
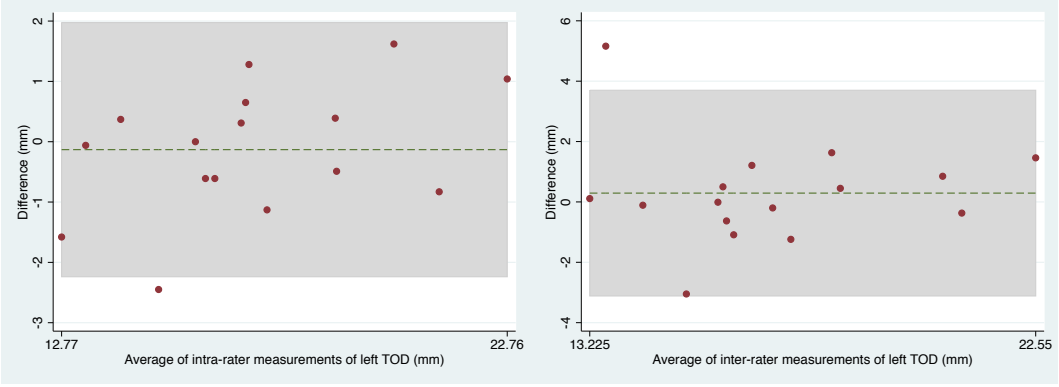
0
1
2
3
15.355
27.355
Average of intra-rater measurements of VW (mm)
Difference (mm)
-2
-1
0
1
2
3
15.395
27.885
Average of inter-rater measurements of VW (mm)


Difference (mm)
-.5
0
.5
1
1.5
0
2.65
Average of intra-rater measurements of left AHH (mm)
Difference (mm)
-1
0
1
2
0
2.905
Average of inter-rater measurements of left AHH (mm)


Difference (mm)
-.5
0
.5
1
0
2.38
Average of intra-rater measurements of right AHH (mm)
Difference (mm)
-1
-.5
0
.5
1
0
2.475
Average of inter-rater measurements of right AHH (mm)


Difference (mm)
2
1
0
-1
-2
3.2
7.125
Average of intra-rater measurements of left VMB (mm)
Difference (mm)
4
2
0
-2
2.625
6.205
Average of inter-rater measurements of left VMB (mm)


Difference (mm)
2
1
0
-1
-2
2.26
7.375
Average of intra-rater measurements of right VMB (mm)
Difference (mm)
4
2
0
-2
2.38
7.405
Average of inter-rater measurements of right VMB (mm)


Difference (mm)
2
1
0
-1
-2
-3
12.77
22.76
Average of intra-rater measurements of left TOD (mm)
Difference (mm)
6
4
2
0
-2
-4
13.225
22.55
Average of inter-rater measurements of left TOD (mm)