RELATIONSHIP BETWEEN BRAIN STRUCTURE AND FUNCTION PRIOR TO OPEN HEART SURGERY IN NEWBORNS WITH COMPLEX CONGENITAL HEART DEFECTS

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ABSTRACT

Infants with complex congenital heart defects (CHD) show evidence of impaired brain growth as well as neurobehavioural abnormalities prior to undergoing open heart surgery. The aim of this study was to evaluate the relationship between neurobehavioural performance and brain volume as well as white matter microstructure in full-term newborns with complex CHD before cardiac surgery using a standardized neurobehavioural assessment and quantitative magnetic resonance imaging (MRI) measures. Thirty-five newborns (>36 weeks gestational age) were evaluated. Advanced three-dimensional, volumetric MRI was used to measure global and tissue-specific brain volumes. In addition, the integrity of the corpus callosum, corticospinal tracts, and optic radiations was quantified using diffusion tensor imaging and post-acquisition fibre-tracking techniques to measure fractional anisotropy (FA) and mean diffusivity (D_{av}). Neurobehavioural status was evaluated with the Einstein Neonatal Neurobehavioural Assessment Scale (ENNAS). Reduced subcortical gray matter volume (SCGM) and increased cerebrospinal fluid volume (CSF) were associated with abnormal behavioural state (SCGM, p=0.04; CSF, p=0.007) and visual orienting (CSF, p=0.003) on the ENNAS. Lower D_{av} in the optic radiations was associated with overall ENNAS score (p=0.02) and active motility (p=0.006). Stratification for cardiac physiology revealed further associations between neurobehavioural performance and brain volumes as well as microstructure. This study demonstrates associations between impaired brain development and neurobehavioural abnormalities in newborns with complex CHD, extending our understanding of early brain structure and function in this population, and emphasizing the importance of early screening and intervention in order to improve developmental outcomes and quality of life.

RÉSUMÉ

Les enfants ayant une malformation cardiaque congénitale (MCC) sont à risque de présenter un trouble de la croissance cérébrale ainsi que des problèmes neurocomporatementaux avant même d'avoir subi une chirurgie cardiaque. L'objectif de cette étude était d'évaluer, dans le contexte préopératoire, les relations existant entre le comportement, le volume cérébral et la microarchitecture de la substance blanche chez les nouveau-nés présentant une MCC complexe en utilisant une évaluation neurocomportementale standardisée et des mesures quantitatives d'imagerie par résonnance magnétique (IRM). Trente-cinq nouveau-nés d'âge gestationnel supérieur à 36 semaines ont été évalués. Une technique de volumétrie cérébrale tridimensionnelle par IRM a été utilisée pour mesurer le volume cérébral régional et total. De plus, l'intégrité du corps calleux, du faisceau corticospinal et de la radiation optique a été quantifiée au moyen de la technique de tractographie par IRM en tenseur de diffusion qui permet de mesurer la fraction d'anisotropie (FA) et la diffusivité moyenne (D_{moy}). Le statut neurocomportemental a été évalué à l'aide du Einstein Neonatal Neurobehavioural Assessment Scales (ENNAS). Une diminution de volume de la substance grise sous-corticale (SGSC) et une augmentation du liquide céphalorachidien (LCR) étaient associées avec des anomalies neurocomportementales hors norme (SGSC, p=0.04; LCR, p=0.007) de même qu'une orientation visuelle déficitaire (LCR, 0=0.003) tels que évalués par le ENNAS. Une faible D_{moy} de la radiation optique était corrélée avec le score global du ENNAS (p=0.02) et avec une plus grande activité motrice (p=0.006). Des analyses de stratification selon le type d'anomalie morphologique cardiaque ont révélé d'autres associations entre la performance neurocomportementale et le volume et la microarchitecture cérébrale. Cette étude confirme de l'existence d'associations significatives entre l'altération du développement du cerveau et la présence d'anomalies neurocomportementales chez les nouveau-nés avec une MCC complexe. Ces résultats apportent une meilleure compréhension du développement des structures cérébrales et de leurs fonctions chez cette population tout en mettant l'emphase sur l'importance du dépistage et de l'intervention précoce afin de permettre un développement harmonieux et d'améliorer la qualité de vie.

CONTRIBUTIONS OF AUTHORS

Mallory Owen, BHSc, MSc (Candidate)

I, the candidate, am the primary author of each manuscript included in this thesis. I recruited all subjects which were eligible postnatally and assisted with antenatal recruitment. I assisted with or personally performed all neurobehavioural assessments as well as coordinating and attending all preoperative brain scans. I conducted all of the manual corrections for the brain volume segmentation masks, as well as all post-acquisition processing of the diffusion tensor imaging data. Finally, I performed the review of the literature, descriptive statistic analysis, data interpretation, and discussion.

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Ms. Bouyssi-Kobar conducted or assisted with all of the preoperative brain scans. She performed the necessary processing of the raw magnetic resonance imaging (MRI) data to allow for subsequent image analysis.

Mary T. Donofrio, MD

Dr. Donofrio was instrumental in facilitating subject recruitment during the antenatal period. She also provided her expertise to establish cardiac diagnoses within the cohort.

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Dr. Limperopoulos conducted or assisted with the administration of the *Einstein Neonatal Neurobehavioral Assessment Scale*. She was also responsible for supervising

MRI acquisitions, clinical data collection, and data analysis. Lastly, she reviewed and appraised all versions of the manuscripts and thesis.

Annette Majnemer, PhD

Dr. Majnemer provided expert guidance for conducting the *Einstein Neonatal Neurobehavioral Assessment Scale*. She also provided content expertise and critical appraisal of the manuscripts.

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Dr. McCarter provided expert guidance and support for the statistical analysis, and critical appraisal of the research manuscript.

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Dr. Shevell provided content expertise and critical appraisal of the manuscripts and thesis.

Gilbert Vezina, MD

Dr. Vezina was responsible for overseeing the review and interpretation of all conventional MRI studies in order to establish the clinical radiological diagnoses of the newborns in the study cohort.

Neil Weisenfeld, PhD

Dr. Weisenfeld developed an automatic segmentation program in order to perform advanced three-dimensional MRI segmentation of the newborn brain. He was responsible for conducting this automatic segmentation for each newborn enrolled in the study.

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ABBREVIATIONS

3D	Three-dimensional
ADC	Apparent Diffusion Coefficient
CGM	Cortical Gray Matter
CHD	Congenital Heart Defect
CSF	Cerebrospinal Fluid
CV	Cerebellar Volume
D_{av}	Mean Diffusivity
DTI	Diffusion Tensor Imaging
EEG	Electroencephalography
ENNAS	Einstein Neonatal Neurobehavioural Assessment Scale
FA	Fractional Anisotropy
HIE	Hypoxic-Ischemic Encephalopathy
HLHS	Hypoplastic Left Heart Syndrome
HNNE	Hammersmith Neonatal Neurologic Examination
ICC	Intraclass Correlation Coefficient
MRI	Magnetic Resonance Imaging
mWM	Myelinated White Matter
PLIC	Posterior Limb of the Internal Capsule
ROI	Region of Interest
SCGM	Subcortical Gray Matter
TBV	Total Brain Volume
TGA	Transposition of the Great Arteries
umWM	Unmyelinated White Matter
WMI	White Matter Injury

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CHAPTER 1

1.1 Rationale

Today, newborn infants with complex congenital heart defects (CHD) are expected to survive beyond infancy and well into adulthood due to continuing advances in fetal and neonatal critical care[1–6]. These survivors, however, are vulnerable to brain injury and neurobehavioural abnormalities as a consequence of various preoperative, intraoperative, and postoperative factors[7–12]. While intraoperative factors have historically been viewed as the primary source of injury, preoperative abnormalities are commonly observed on conventional magnetic resonance imaging (MRI) and during neurological examinations[12–17]. To date, no study has investigated the relationship between brain development and neurobehaviour in newborns with complex CHD prior to cardiac surgery using data from quantitative MRI and a standardized neurobehavioural assessment. Understanding this "structure-function" relationship is an important initial step towards defining a suitable approach to neurologic evaluation and developmental intervention for this high-risk population.

This thesis seeks to investigate the strength of the association between neurobehaviour and brain volume as well as white matter microstructure in newborns with CHD using advanced quantitative MRI techniques as well as a standardized neurobehavioural assessment. Three-dimensional (3D) volumetric MRI and diffusion tensor imaging (DTI) techniques have the capacity to delineate structural and microstructural brain abnormalities that are below the resolution of conventional MRI and not evident even to highly trained neuroradiologists. Correspondingly, neurodevelopment can be evaluated in a standardized and reliable fashion using instruments such as the Einstein Neonatal Neurobehavioral Assessment Scale (ENNAS)[18] which provide quantifiable information about the neurologic and neurobehavioural status of the newborn.

Employing these quantitative and standardized methods for evaluation affords a solid basis for the analysis of the relationship between brain growth and neurobehavioural development in high-risk newborns. Characterizing this relationship may help guide discussions regarding the implementation of appropriate routine screening within the early postnatal period. Early identification of newborns at risk for developmental disabilities is essential for recognizing those who warrant

more extensive neuroradiological investigation and developmental follow-up, or who might benefit from early intervention to optimize their functional independence and enhance their overall health and well-being.

1.2 Objectives

The overall objective of this thesis is to investigate the relationship between brain development and neurobehaviour in newborns with complex CHD before corrective or palliative open heart surgery. A central underpinning of this research is the use of advanced MRI post-processing methodology to quantify structural brain growth and microstructural impairments. Moreover, this work ventures to relate these measurements to performance on a standardized neurobehavioural assessment, further extending the emphasis on objectively acquired information about the developing brain and newborn behaviour.

Two specific aims will be addressed herein: First, this study will examine the relationship between brain volumes computed through 3D volumetric MRI and performance on a standardized assessment of newborn neurobehaviour (the ENNAS). Second, this study will investigate the relationships between the microstructural integrity of select white matter tracts, as measured by DTI and tractography, and neurobehavioural status, as measured by the ENNAS.

The work presented here provides an opportunity to consider the relationship between brain structure and function in newborns with complex CHD. Using a systematic approach in the analysis of the relationships that are explored, the present study endeavors to generate hypotheses to inspire future research.

CHAPTER 2

REVIEW OF THE LITERATURE

Background

As the heart develops, its progression from a simple tube to an intricate, multi-chambered organ is a complex trajectory. Small errors that occur during this process can result in profound consequences, leaving newborn infants ill-equipped to manage the transition from fetal to neonatal circulation. These congenital heart defects are common, affecting nearly 1 in every 100 newborns, with up to 35% requiring corrective or palliative surgery in order to survive[19–22].

At present, newborns with even the most complex defects are expected to survive into adulthood as a result of continued improvements in surgical practices, critical care, and diagnosis. Since the pioneering work of Gross and Hubbard[23], who performed the first ligation of a patent ductus arteriosis in 1938, surgical advancements have permitted increasingly complex procedures. The advent of controlled cross-circulation, introduced by Lillehei and colleagues in 1955[24,25] facilitated the repair of complex defects through direct vision of the heart, which was followed shortly by the reliable application of a cardiopulmonary bypass apparatus developed by Gibbon and later refined by Kirklin[26,27]. With this revolutionary heart-lung machine, surgeons were provided the opportunity to operate in a still and virtually bloodless field.

Advancing the specialty still further, in 1959 Drew and Anderson[28] instituted the use of "profound hypothermia" during cardiopulmonary bypass which extended the permissible interval for cardiac arrest by protecting the vital organs. The use of deep hypothermia, as it came to be called, became especially helpful in performing complicated procedures on newborns and infants; differences in size and metabolism posed particular challenges in this age group, making cardiopulmonary bypass at normal temperatures more difficult[1]. The advantages afforded by extracorporeal circulation and deep hypothermic cardiac arrest promoted the development of surgical techniques to correct previously inoperable defects in an unhurried and deliberate fashion. The following decades witnessed the advent of bold new procedures including the correction of transposition of the great arteries introduced by Jatene in 1976[2], and the staged palliation of hypoplastic left heart syndrome reported by Norwood and colleagues[3] in 1980.

The last 30 years have built on this solid foundation, contributing a wealth of evidence to support the continued use of conventional practices or to justify alternative approaches. Complementary advances in critical care and diagnostic techniques have occurred in parallel, making the provision of acute care safer and more efficient in the perioperative period, and resulting in improved pre- and postoperative status[4,29–31]. Of these advances, the growing rate of prenatal diagnosis may have the highest impact; newborns that are diagnosed prenatally fair better in the early postnatal period and benefit from an improved long-term prognosis[5,6,29–34]. Moreover, surgical innovation has progressed to the point where the antenatal detection of select heart lesions enables prenatal intervention and consequent improvement in heart growth and postnatal outcome[35,36].

Collectively, these innovations have led to a dramatic reduction in mortality among infants with complex CHD[37,38]. Over time, however, as survival rates have gradually improved, there has also been an increase in the recognition of post-operative neurodevelopmental sequelae. Early studies of neurologic outcome following open heart surgery reported delays in gross and fine motor coordination, impaired intellect, learning and behavioural problems, difficulties with language expression and comprehension, as well as seizures, abnormal electroencephalographic (EEG) recordings, cerebral palsy, and varying degrees of choreoathetosis[39–47]. These reports were supported by studies investigating outcomes as they related to potential intraoperative risk factors, in particular the use of circulatory arrest compared to continuous perfusion[7,8,45,48–53]. Many of these studies observed an association between circulatory arrest and an increased incidence of neurologic abnormalities when this approach was compared to continuous perfusion[7,9,45,49,50,54–56]. Furthermore, several studies, including a large randomized clinical trial, demonstrated a relationship between extended circulatory arrest time and increased risk of seizures and abnormalities on MRI, as well as delayed psychomotor development, apraxia, and impaired cognitive ability[7–9,50,54].

Following this wave of research centering on risks incurred intraoperatively, new evidence began to emerge, revealing preoperative neurologic abnormalities and suggesting an antenatal origin to central nervous system impairment[10–16,57–60]. One illustrative finding was the increased frequency of microcephaly, indicating diminished brain growth among newborns with CHD[12,13,58]. Preoperative neuroimaging studies contributed to this notion, noting a high prevalence of

periventricular leukomalacia, a type of white matter injury common in premature infants[11,17,59]. Other markers of brain immaturity were observed as well, including large subarachnoid spaces and incomplete closure of the operculum[11,14,15,17,59]. Studies evaluating neonatal neurologic and neurobehavioural status further corroborated these findings, frequently observing abnormal muscle tone, and poor behavioural state regulation[13,16,57]. Importantly, these early findings have demonstrated associations with later neurodevelopmental outcomes. Studies have shown that abnormal preoperative neurodevelopmental status is significantly associated with neurologic abnormalities, microcephaly, gross and fine motor deficits, and developmental delays at 1 year of age as well as functional independence at 12-18 months[61,62]. Moreover, according to one study, an abnormal preoperative neurological examination is predictive of lower scores for physical well-being at 5 years of age as assessed by parents[63]. A high predictive value has also been reported for a normal newborn neurobehavioural assessment with respect to favourable performance at 8 years on tests of IQ, communication, daily living skills, socialization, motor proficiency, and sensorimotor integration[64].

It is imperative to understand both the timing and extent of neurologic impairment among infants with CHD in order to determine best practices for early screening and intervention. With the increased commitment to research into the neurodevelopmental outcomes of this population, it has become clear that a number of risk factors throughout development and treatment collectively and cumulatively place newborns with CHD at risk for brain injury. An appreciation of the neurologic status of newborns with CHD prior to surgery is especially important, as this information can be used to guide both pre- and intraoperative care, as well as provide a basis for ongoing evaluation post-discharge. The following review article provides an in-depth focus on the neurodevelopmental and neuroimaging findings in newborns with CHD preoperatively.

ABNORMAL BRAIN STRUCTURE AND FUNCTION IN NEWBORNS WITH COMPLEX CONGENITAL HEART DEFECTS BEFORE OPEN HEART SURGERY: A REVIEW OF THE EVIDENCE

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2.1 Abstract

Newborns with complex congenital heart defects are at high risk for developing neurological abnormalities. It is important to understand the timing, progression, and extent of these abnormalities to better elucidate their potential impact on neurodevelopment, and their implications for early screening and intervention. This review synthesizes the recent literature describing neurological and neurobehavioral abnormalities observed in fetuses and newborns before cardiac surgery. A considerable proportion of newborns with complex congenital heart defects exhibit neurobehavioral and electrophysiological abnormalities preoperatively. Likewise, conventional neuroimaging studies reported that a high percentage of this population experienced brain injury. Advanced neuroimaging modalities indicated that fetuses showed delayed third trimester brain growth, and newborns showed impaired white matter maturation, reduced N-acetylaspartate, and increased lactate. These findings suggest a fetal or early postnatal onset of impaired brain growth and development. Consequently, reliable methods for early screening and subsequent developmental intervention must be implemented.

2.2 Introduction

In the United States and Canada, approximately 1 in every 100 newborns is diagnosed with a congenital heart defect[1,2]. Many of these newborns (25%-35%) will require either corrective or palliative open heart surgery[3]. Even with surgical intervention, congenital heart defects remain the most common cause of infant death from birth defects[4]. Until recently, issues related to neurodevelopmental morbidity in the population of infants with complex congenital heart defects were overshadowed by the challenge of achieving survival. With the advent of novel diagnostic techniques and refined surgical interventions, the survival rate of infants with congenital heart defects has dramatically increased[2]. This development has been accompanied by an increasing awareness that newborns diagnosed with congenital heart defects are at risk for neurobehavioural[5–11] and neuroimaging[10–24] abnormalities before open heart surgery. Consequently, there has been a recent shift in focus from cardiac outcomes to neurological and developmental outcomes[25].

The neurologic sequelae of congenital heart defects in infants can arise from a combination of preoperative, intraoperative, and postoperative factors. Until recently, most studies evaluating mechanisms of brain injury in infants with congenital heart defects have focused predominantly on surgical procedures and manipulations (e.g., circulatory arrest, bypass times) as the primary causes of eventual developmental disabilities without careful examination of the potential for brain injury in the preoperative period[26–29]. In recent years, preoperative and antenatal findings have been accorded further attention[9,30–33]. Accumulating evidence demonstrating neuroimaging as well as clinical neurobehavioral abnormalities emphasizes the potential for the antenatal or early postnatal onset of neurologic dysfunction in young infants with congenital heart defects. Despite this increasing awareness, these preoperative abnormalities have not yet been subjected to a structured review process. Hence, the primary objective of this study was to consolidate the existing knowledge pertaining to the structural and functional abnormalities in newborns with congenital heart defects. Studies describing neuroimaging and/or the neurobehavioral abnormalities in newborns with congenital heart defects before open heart surgery published over the past two decades were reviewed. A secondary objective was to inspect the papers included in the review for information regarding risk factors for brain injury in this preoperative population.

2.3 Methods

To better understand the types of structural and functional abnormalities observed preoperatively in newborns with congenital heart defects, a review of the literature was conducted on the neuroimaging and neurobehavioral sequelae in this population. The search of the literature published between October 1990 and September 2010 was conducted using Medline and PubMed search engines. Search terms included the following: congenital heart defect, congenital heart disease; neuroimaging; magnetic resonance imaging; computed tomography; cranial ultrasound; neurologic, neurobehavior, neurodevelopment, development; exam, and assessment. Studies describing acquired structural or functional abnormalities in fetuses or full-term neonates (>36 weeks gestational age) with complex congenital heart defects before open heart surgery were included. Studies were excluded if they described infants older than one month (unless the sample was heterogeneous, and in these cases the studies were included); preterm infants; or subjects with genetic/chromosomal anomalies or brain malformations. One study published in Spanish was excluded given that the resources necessary to properly appraise this article were not available for the purposes of this review. The reference lists of selected articles were also inspected. Using the described search parameters, 1181 records were identified through PubMed and 545 were identified through Medline. A total of 29 papers were included in this review.

2.4 Results

An array of preoperative neurological abnormalities manifest in newborns with congenital heart defects before open heart surgery. Hypotonia, poor state regulation, and microcephaly are the neurodevelopmental sequelae observed most frequently[5–9,11]. The neuroimaging literature demonstrates that more than half of the population of newborns diagnosed with congenital heart defects experience some form of brain injury before cardiac surgery, with ischemic lesions, white matter injury, periventricular leukomalacia, and strokes all evident[10–23]. Given that all of these abnormalities are observed before open heart surgery, it is likely that impaired brain development in this population has an antenatal or early postnatal origin[21].These findings are described below.

2.4.1 Preoperative Clinical Neurologic Dysfunction

Preoperative neurological and neurobehavioral status in newborns with congenital heart defects has been evaluated in several studies, which are summarized in Table 1. One of the first studies to systematically examine preoperative neurological and neurobehavioral status in full-term newborns with congenital heart defects requiring open heart surgery was carried out by Limperopoulos et al[6]. Upon neurological examination, over half of the newborns examined (56%) were classified as ‘‘abnormal.’’ Specific abnormalities included the following: diffuse hypotonia (40%), hypertonia (12%), jitteriness (8%), absent suck (6%), motor asymmetry (4%), decreased muscle power in the upper and lower extremities (10%), cranial nerve abnormalities (4%), lethargy or stupor (14%), and restlessness and agitation (8%). Preoperative seizures were reported in 4 of 56 (7%), and microcephaly (i.e., head circumference at or below the third percentile for age and gender) in over one third (36%). Hypotonia and poor state regulation were the predominant neurological findings in this cohort. Similarly, for the same cohort, using the Einstein Neonatal Neurobehavioral Assessment Scale, there were 19 suspect examinations and 10 abnormal examinations out of 50 newborns examined[6,7,9]. Abnormalities included the following: hypotonia (44%), hypertonia (10%), jitteriness (8%), absent suck (8%), motor asymmetry (4%), lethargy (28%), and irritability (34%). Additionally, poor visual fixation and tracking were observed in 50%, and poor auditory alerting in 66%. The evaluation of general feeding status showed a weak suck in almost one quarter of newborns (23%), no suck in four (7%), and decreased feeding efficiency in 19 (34%). Once again, hypotonia and poor state regulation were predominant findings. Poor orienting responses, along with a weak suck and decreased feeding efficiency, were additional characteristic findings for this cohort.

In addition to the studies by Limperopoulos and colleagues, several other studies reported neurodevelopmental findings of interest in newborns with congenital heart defects before cardiac surgery. Tavani et al evaluated 17 newborns and observed neurological abnormalities in 41%[11]. Two newborns (12%) demonstrated decreased muscle tone in the upper extremities, and five (29%) showed decreased tone in the lower extremities. Upper limb hypertonia was observed in almost one in four newborns (18%) and two (12%) showed increased tone in the lower limbs. In addition, an altered level of consciousness was observed in this group in the form of lethargy (18%). Miller and colleagues reported the findings of the neurological

examination before surgery in newborns with transposition of the great arteries, and summarized the main observations as abnormal tone and reflexes[10]. Specifically, three (30%) of the newborns demonstrated either abnormal tone or abnormal reflexes, while four (40%) demonstrated both abnormal tone and reflexes. There were no focal deficits observed to suggest underlying focal lesions, no deficits or asymmetries of motor power, and no seizures. Chock et al observed acute neurological findings preoperatively in three (3%) newborns with congenital heart disease[34]. These events were defined as seizures, significant hypertonia, hypotonia, or choreoathetosis. In a sample of 10 newborns, El-Naggar et al found that 10% had an abnormal neurological examination, although 60% experienced clinical seizures[35]. Newburger and colleagues determined the definite presence of neurologic abnormalities in 55 (36%) newborns, and possible neurologic abnormalities in 31 (20%) newborns scheduled to undergo surgical correction for transposition of the great arteries[27]. The abnormalities reported in this study were not further defined; these findings were presented as secondary to the main research question.

Finally, several studies demonstrated a high prevalence of microcephaly at birth in infants with congenital heart defects, providing additional objective evidence that impaired brain development in newborns with congenital heart defects can be of fetal origin. Shillingford and colleagues reported that 12% of newborns with hypoplastic left heart syndrome were microcephalic at birth, of which 26% also weighed less than or equal to the third percentile[36]. Overall, the percentiles for head circumferences were disproportionately smaller than the percentiles for both weight and length. A study by Barbu and colleagues showed that fetal head growth was impaired in subjects with congenital heart defects compared with controls[37]. Using measures of head circumference at birth, it was concluded that a higher proportion of subjects with congenital heart defects had microcephaly compared with controls. More specifically, it was noted that there was an increased frequency of microcephaly in subjects with cyanotic congenital heart defects, mixing-type lesions, and right-sided obstructive disorders. When the results were stratified, removing subjects that were small for gestational age, coarctation of the aorta/aortic arch hypoplasia was the only remaining type of congenital heart defect which predicted microcephaly at birth.

A chart review by Manzar et al highlighted a significant difference between the birth head circumference of newborns with hypoplastic left heart syndrome when

compared with newborns with transposition of the great arteries and controls[38]. Likewise, newborns with transposition of the great arteries also had a significantly smaller head circumference than controls. In a chart review conducted to assess fetal growth, Rosenthal illuminated some trends with respect to different congenital heart defect diagnoses[39]. Measurements taken at birth indicated that infants with transposition of the great arteries had smaller head volume (given birth weight), along with smaller head circumference, shorter birth length, and greater birth weight for birth length when compared with controls. Infants with Tetralogy of Fallot also had smaller head circumference than controls, in addition to a lower birth weight and shorter birth length. Infants with hypoplastic left heart syndrome were smaller than controls for all measurements, with a notably small head volume for birth weight. Of interest, infants diagnosed with coarctation of the aorta had a larger head volume for birth weight, lower birth weight, and shorter birth length than controls. One study investigated fetuses and neonates with hypoplastic left heart syndrome and isolated aortic stenosis[40]. Using ultrasound, it was determined that fetuses with hypoplastic left heart syndrome had a normal head size at mid-gestation, and experienced reduced head size and head growth restriction during the latter half of gestation. Postnatally, reduced newborn head circumference was observed in 13% of newborns with hypoplastic left heart syndrome, and 9% of newborns with aortic stenosis.

It should be noted that, while four of the five studies which discussed the incidence of microcephaly performed measurements at birth, one study included measurements collected during the neonatal period and the timing was otherwise not specified. This distinction is important as head circumference measured later in the newborn period can be indicative of postnatally acquired microcephaly. While the basis of the relationship between congenital heart defects and microcephaly remains speculative, the high prevalence of abnormal head growth in this population at birth and prenatally is suggestive of an antenatal origin.

Taken together, the available evidence shows that preoperative neurobehavioral abnormalities are common in this population of newborns, and the abnormalities most frequently observed across studies are hypotonia (28%-44%), poor state regulation (18%-62%), and microcephaly[5–11,27,34–40].

2.4.2 Preoperative Electrophysiologic Abnormalities

The early detection of neurological sequelae in newborns with complex congenital heart defects can be achieved with clinical assessments, as illustrated above, although there are other viable techniques which should also be considered as resources for routine monitoring, namely electrophysiological (Table 2) and neuroimaging modalities (Table 3). Relatively few studies have investigated the use of evoked potentials and electroencephalography (EEG) in newborns with congenital heart defects[8,35,41] and there is a similar dearth of cranial ultrasound studies[21,22,34,35]. In contrast, many studies have outlined the effectiveness of magnetic resonance imaging (MRI) for the diagnosis of neurologic abnormalities related to congenital heart defects[10–20,23,24,35]. The available neuroimaging data will be summarized following a review of the electrophysiological findings.

Measuring somatosensory evoked responses has proven to be an objective, noninvasive approach to assessing neurologic status with demonstrated predictive validity in high-risk infants[42–44]. Limperopoulos and colleagues investigated the incidence of somatosensory and brain stem auditory evoked potential abnormalities in newborns with congenital heart defects perioperatively[41]. The results of this study indicated that 41% of newborns demonstrated somatosensory evoked potential abnormalities, while conduction times for brain stem auditory evoked potentials were normal in all subjects[41]. Both sensitivity and specificity were excellent for the prognostic value of the perioperative somatosensory evoked potentials; all newborns with somatosensory evoked potential abnormalities had neurologic findings 1 year after open heart surgery, and 82% of newborns with normal somatosensory evoked potentials had normal neurologic examinations at 1 year of age[41]. Evoked potentials have not yet been routinely recorded to specifically investigate the population of newborns with complex congenital heart defects before cardiac surgery.

Electroencephalographic findings can also provide important insights into the neurological status of infants with congenital heart defects. One study has specifically examined the prevalence of preoperative EEG abnormalities. Limperopoulos et al demonstrated that 12 of 27 newborns (44%) had an abnormal EEG before surgery, with five (42%) showing epileptiform activity and nine (75%) showing disturbances in background activity[8]. Among newborns with EEG abnormalities, 74% had abnormal neurological examinations, while 45% of newborns with normal EEGs had normal neurological examinations[8]. This indicates that the sensitivity of EEG for

detecting neurologic abnormalities is very good, while its specificity is modest at best. Another study investigated the frequency of abnormal amplitude-integrated EEG using a smaller sample of neonates, determining that 5 of 10 newborns with congenital heart defects had abnormal outcomes[35].

Although the number of studies investigating electrophysiological abnormalities in newborns with congenital heart defects is limited, the cumulative findings suggest that between 41% and 50% of newborns with congenital heart defects have functional deficits.

2.4.3 Preoperative Neuroimaging Abnormalities

A growing number of neuroimaging studies have contributed considerably to the increasing awareness that infants diagnosed with complex congenital heart defects are at risk for acquiring brain injury before open heart surgery. Cranial ultrasound[21,22,34,35] and conventional MRI studies[10–20,23,24,35] have highlighted the magnitude of brain injury in this population, indicating that 28% to 59% of newborns with congenital heart defects experience some degree of brain injury before surgery.

The results of the neuroimaging studies to be discussed here investigated full-term infants diagnosed with congenital heart defects requiring corrective or palliative open heart surgery. Common exclusion criteria included the following: 5-minute Apgar score <5, congenital anomalies (neither neurological nor cardiac in nature), chromosomal abnormalities, congenital infection, birth asphyxia, and preoperative cardiac arrest. Cranial ultrasound studies were performed within the first month of life[21,22,34,35], while MRI studies were carried out on the day of surgery after undergoing anesthesia[11,13–15,20,24], or once infants were clinically stable before surgery (i.e., typically before eight days of life[10,12,16–18,23], although in one study the maximum age at MRI was 36 days of life[19]).

Cranial ultrasound studies have identified several abnormalities including widened ventricles and subarachnoid space, cerebral infarction, cerebral edema, periventricular echodensities, intraventricular hemorrhage, calcifications in the basal ganglia, subarachnoid hemorrhage, cerebral atrophy, linear echodensities in the basal ganglia or thalamus, and intraparenchymal echodensities[21,22]. The most common abnormalities were widened ventricles and subarachnoid space (26%), cerebral atrophy (27%), linear echodensities in the basal ganglia or thalamus (20%), and intraventricular hemorrhage (16%)[21,22]. Abnormal cranial ultrasound findings were

more frequently reported in infants with coarctation of the aorta or hypoplastic left heart syndrome (63%), followed by infants with transposition of the great arteries (14%).

Similarly, MRI studies have also revealed a high incidence of preoperative brain injury including ischemic lesions, intraventricular hemorrhage, intracranial hemorrhage without mass effect, delayed brain maturation, and reduced brain volume[10–20,23,24]. Conventional MRI has frequently shown ischemic lesions in neonates with congenital heart defects before surgery. These lesions are believed to result from deficient blood supply to a region of the brain[45]. Several studies have investigated the prevalence of ischemic lesions in newborns with heterogeneous congenital heart defect diagnoses, while select studies have limited their investigations to newborns with transposition of the great arteries[10,16,20], or transposition of the great arteries and single ventricle physiology[14,18]. Altogether, ischemic lesions appear in 21% to 41% of newborns with congenital heart defects before open heart surgery[10–20,23,24]. Strokes (i.e., infarcts) are prevalent in this population, with up to 31% of newborns affected[10,12,14–21,23,24]. Another common ischemic lesion that has been reported in this vulnerable population is white matter injury. The prevalence of white matter injury, both focal and diffuse, in newborns with congenital heart defects before open heart surgery ranged from 7% to 27%, with mild white matter injury affecting between 57% and 94% of these cases, and moderate to severe white matter injury affecting between 6% and 36%[12,16–19,23,24]. Periventricular leukomalacia, confined to the region bordering the lateral ventricles, is also frequently observed in this high-risk infant population, affecting between 17% and 38% of newborns with congenital heart defects before surgery[13–15,20]. Of these cases, 30% to 57% had mild periventricular leukomalacia, while 43% to 70% had moderate to severe cases.

Intracranial hemorrhage was also observed in newborns before cardiac surgery, affecting up to 54% of this population[10,11,15–17,20]. One study examined intracranial hemorrhage in depth, characterizing hemorrhages according to the region affected[11]. The most common type of hemorrhage was an infratentorial subdural hematoma, found in 46% of newborns. Supratentorial subdural hematomas (23%) and choroid plexus bleeds (27%) were also common. Parenchymal hematomas (4%) and intraventricular bleeds (4%) were observed less frequently. Intraventricular hemorrhage involves bleeding into the ventricles and most often affects premature

infants[46]. Accordingly, the observation of intraventricular hemorrhage in newborns with congenital heart defects suggests delayed brain and cerebrovascular maturation[17]. Several studies observed mild intraventricular hemorrhage in up to 23% of newborns with congenital heart defects before open heart surgery[17–20,23].

In addition to overt brain injury, recent studies have assessed brain maturity in newborns with congenital heart defects before surgery. One such study examined closure of the insular operculum radiologically[14]. An open insular operculum is a structural abnormality that serves as an indicator of delayed brain maturation, and it appears with inconsistent frequency in MRI studies. In two recent studies, an open insular operculum was identified in 16% to 17% of infants[13,15]. Two other studies observed 31% to 33% of infants with incomplete closures of the opercular space[20,47], while one study demonstrated incomplete closure of the opercular space in 86% of infants[14]. A study using a novel statistical framework evaluated the surface complexity of the brain at the insular operculum in neonates with congenital heart defects[48]. It was determined that newborns with hypoplastic left heart syndrome had less complex folding of the insular operculum when compared with newborns with transposition of the great arteries. The latter appeared to have more complex folding of the insular operculum, although the opercular spaces were wider.

The overarching conclusion from the neuroimaging literature appears to be that up to 59% of full-term, newborn infants diagnosed with congenital heart defects experience some form of brain injury before cardiac surgery[10–24]. Ischemic lesions are observed in 21% to 41% of the population[10–20,23,24], with white matter injury occurring in 7% to 27%, periventricular leukomalacia affecting 17% to 38%, and strokes evident in up to 30% of infants[12–15,17–19,23,24]. The loci of these infarcts were varied, although several studies reported a higher prevalence of middle cerebral artery distribution[12,16,17,23]. Intracranial hemorrhage is prevalent in this population, occurring in up to 54% of newborns[11,15,17], with 5% to 23% experiencing intraventricular hemorrhage[16–20]. The most commonly observed types of hemorrhage were supra- and infratentorial subdural hemorrhages[11,16,17]. Given that all of these abnormalities are observed before open heart surgery, it seems plausible that impaired brain development in this population has an antenatal or early postnatal origin[21]. The long-term predictive value of these acute and prevalent neuroimaging abnormalities awaits further study.

2.4.4 Preoperative Quantitative MRI Methods

Quantitative Three-Dimensional Volumetric MRI. Three-dimensional volumetric MRI techniques allow for the objective determination of total and regional brain tissue volumes as well as the volumes of distinct tissue classes[49,50]. The ability to make quantitative measurements of gray and white matter volumes in the high-risk preterm infant has advanced understanding of normal and abnormal cerebral cortical development and myelination[49,51]. A recent fetal study showed evidence of brain growth impairment antenatally, reporting for the first time delayed third trimester brain growth in fetuses with congenital heart defects compared with age-matched controls[30]. These fetuses had shown similar measurements at 25 weeks of gestation for both total brain volume and intracranial volume as controls. It was not until 30 weeks of gestation that variation between fetuses with congenital heart defects and controls emerged. The same study demonstrated that the ratio of cerebrospinal fluid to intracranial cavity volume, a measure that typically decreases over the third trimester, remained significantly higher in fetuses with congenital heart defects compared with controls, suggesting impaired brain development before birth.

Diffusion Tensor Imaging. Two studies have performed diffusion tensor imaging preoperatively on newborns with congenital heart defects. The data generated by diffusion tensor imaging characterize the microstructural development of the brain. In diffusion tensor imaging studies, two primary values are computed: average diffusivity and fractional anisotropy. The first (average diffusivity) measures the degree of diffusion of water molecules within brain tissue. This value normally decreases with maturity as cell membranes develop within the brain[18]. Fractional anisotropy measures the directional dependency of water molecules during diffusion such that a lower fractional anisotropy indicates isotropy (random movement) and a higher fractional anisotropy indicates a move toward anisotropy (complete directional dependence), which is observed with increased myelination (i.e., organization of white matter fiber bundles)[18]. One study measured fractional anisotropy in newborns with congenital heart defects preoperatively and postoperatively to determine the integrity of the pyramidal tracts during maturation in this population[19]. The fractional anisotropy for the pyramidal tracts showed less change over time for newborns with preoperative brain injury on conventional MRI than for those with postoperative injury. Furthermore, change in fractional anisotropy over time was significantly reduced in newborns with brain injury

compared with the change in fractional anisotropy for infants without injury, suggesting impaired white matter maturation[19]. There was no difference in average diffusivity documented. Another study observed significantly higher average diffusivity in newborns with congenital heart defects before open heart surgery versus controls[18]. This study also reported significantly lower fractional anisotropy in newborns with congenital heart defects compared with controls[18]. These data suggest impaired white matter maturation in newborns with congenital heart defects before open heart surgery.

Proton Magnetic Resonance Spectroscopy. Several studies have used proton magnetic resonance spectroscopy to investigate brain metabolism in newborns with congenital heart defects preoperatively. This imaging modality provides clinicians with a safe, non-invasive technique to measure brain metabolites in vivo to follow disease progression, or to track normal developmental changes[52]. N-acetylaspartate (NAA) and lactate are two particular compounds measured by proton magnetic resonance spectroscopy, and they are often reported relative to choline levels, with the assumption that choline levels remain generally constant in the brain[10,53]. The neuronal marker N-acetylaspartate is expected to increase with increasing cerebral maturity; therefore, reduced N-acetylaspartate is indicative of impaired cerebral integrity[54]. Lactate is a product of anaerobic glycolysis, a process which occurs when oxidative metabolism is disrupted; therefore, the presence of lactate could function as a marker for hypoxic–ischemic injury[52]. Choline is an essential component of cell membranes and is fundamental to the make-up of the neurotransmitter acetylcholine[53].

Mahle et al demonstrated that 10 of 19 (53%) newborns with congenital heart defects had elevated lactate before surgery[15]. Elevated lactate in this sample was significantly associated with conventional preoperative MRI abnormalities[15]. Another study observed elevated lactate in 6% of a sample of mixed congenital heart defect diagnoses[24]. A study investigating newborns with transposition of the great arteries before surgery similarly stated that a higher ratio of lactate to choline was evident in newborns with transposition of the great arteries when compared with controls; however, elevation of lactate was diffuse and was apparent in newborns both with and without preoperative stroke[10]. In this study, the N-acetylaspartate/choline ratio did not differ significantly between newborns with transposition of the great arteries and those without. Among newborns with

transposition of the great arteries, however, global N-acetylaspartate/choline levels were significantly associated with preoperative MRI abnormalities. Another study investigated brain metabolism in newborns with heterogeneous congenital heart defect diagnoses and determined that newborns with congenital heart defects had a mean ratio of N-acetylaspartate/choline that was significantly lower than that of control newborns, although the mean ratios of lactate to choline were not significantly different[18]. Importantly, the former indicated that impaired brain metabolism was evident even when conventional MRI did not reveal any abnormalities[18].

In summary, findings from proton magnetic resonance spectroscopy studies indicate that newborns with congenital heart defects often demonstrate reduced N-acetylaspartate and increased lactate (anaerobic metabolism) before open heart surgery lending further credence to the high-risk of brain injury in this vulnerable population even before cardiac surgery.

2.4.5 Risk Factors for Preoperative Neurological Abnormalities

Several clinical factors have been examined by the papers discussed in this review as potential risk factors for preoperative brain injury in newborns with congenital heart defects. Studies investigating these risk factors excluded infants with other known risks for neurodevelopmental sequelae and brain injury (e.g., perinatal asphyxia, known genetic anomaly or syndrome, congenital infection, prematurity, 5-minute Apgar score <5). Patient-related factors including gestational age and the 5-minute Apgar score demonstrate an inverse relationship with the detection of brain injury on preoperative MRI[14,16]. Cardiac-related factors have also been implicated in several studies. Limperopoulos and colleagues observed that newborns with acyanotic lesions were more likely to have an abnormal neurological examination before open heart surgery than newborns with cyanotic lesions[6]. Similarly, a cranial ultrasound study reported an association between acyanotic congenital heart defects and preoperative intraventricular hemorrhage[22]. This study also observed that newborns with coarctation of the aorta or a ventricular septal defect were more likely to have cerebral atrophy or linear echodensities in the basal ganglia or thalamus than newborns with other congenital heart defects.

Additionally, hemodynamic factors have been correlated with preoperative brain injury in newborns with congenital heart defects. According to one study,

decreased carbon dioxide reactivity and low baseline cerebral blood flow were significantly associated with preoperative MRI abnormalities, namely periventricular leukomalacia[13]. In another study, the lowest systemic arterial hemoglobin saturation level documented was predictive of brain injury on preoperative MRI[16]. Similarly, one study found that lower mean arterial oxygenation and a longer wait before surgery, risk factors which are additive, were associated with preoperative MRI detection of periventricular leukomalacia[20].

Conflicting data exist with respect to the role of balloon atrial septostomy as a risk factor for preoperative brain injury. One study demonstrated a strong association between balloon atrial septostomy and preoperative stroke, as detected by MRI, in newborns with transposition of the great arteries. In this study, only newborns that underwent balloon atrial septostomy had preoperative brain injury[16]. Two later studies by this group supported their initial observation[17,23]. However, two further studies contradict this finding. The first noted that the prevalence of brain injury in infants with transposition of the great arteries is similar to that in infants with other complex congenital heart defects; thus, there is no association between balloon atrial septostomy and brain injury in infants with transposition of the great arteries[12]. The second supports this claim, stating periventricular leukomalacia on preoperative MRI is not associated with balloon atrial septostomy. In fact, the authors suggested that balloon atrial septostomy can be protective against periventricular leukomalacia when the procedure results in an increase in cerebral oxygenation[20].

2.5 Discussion

The results of this structured review call attention to the neurobehavioral and neuroimaging abnormalities that infants diagnosed with congenital heart defects are at risk for before undergoing open heart surgery. Conventional neurological examinations along with standardized neurobehavioral assessments reveal a high prevalence of abnormal muscle tone, particularly hypotonia, in newborns with congenital heart defects before surgery[5–11,27,34,35]. Microcephaly is another primary clinical observation in neonates, strongly suggesting impaired brain development of fetal onset[36–40]. Available prenatal studies corroborate this finding, demonstrating that abnormal brain growth is already perceptible in fetuses diagnosed with congenital heart defects[30,40]. Moreover, structural brain abnormalities in this population have

been elucidated by neuroimaging modalities. Strikingly, up to 59% of full-term infants with congenital heart defects have abnormal neuroimaging results including ischemic lesions, white matter injury, periventricular leukomalacia, and strokes[10–24].

As it becomes increasingly evident that brain injury is prevalent in this high-risk population before surgery, it is imperative to identify risk factors separate from those that arise during or after surgery to better understand which infants are most at risk for neurological sequelae and to establish the most appropriate means of early intervention. To date, several potential preoperative predictors of neurobehavioral and structural abnormalities in infants with congenital heart defects have been proposed. These include patient-related factors such as gestational age and the 5-minute Apgar score[14,16]; cardiac factors including the type and category of lesion (i.e., acyanotic versus cyanotic)[6,22]; hemodynamic factors such as decreased carbon dioxide reactivity[13], low baseline cerebral blood flow[13], low arterial oxygenation[16,20]; and procedural factors such as balloon atrial septostomy[16,17,23]. Equally worth exploring is the effect of altered circulatory hemodynamics on the immature brain during the critical transition from intrauterine to extrauterine life. Studies are needed to better elucidate the relative contribution of these putative antenatal and transitional (peripartum) risk factors on neurological sequelae in this population.

Irrespective of the factors that put this population at risk, a considerable volume of data has been amassed with respect to the vulnerability of infants with congenital heart defects to various neurological sequelae. It has also been established that the structural and functional abnormalities present in the preoperative newborn population are predictive of later neurodevelopmental difficulties[32,33,55–58]. Therefore, it is prescient that measures are taken to implement the most accurate and cost-effective screening methods available to this high-risk population.

Until recently, brain injury in high-risk newborns was typically identified with the use of conventional, qualitative MRI techniques, or by neuropathological evaluations. Interestingly, there were no neuropathological reports that matched the search criteria for this review. Recently, innovations in neuroimaging acquisitions and postprocessing techniques have led to the emergence of quantitative MRI methods which allow for the detailed in vivo quantification of brain growth and development. These powerful MRI technologies can now be successfully applied to critically ill newborns, and more recently, fetuses. Specifically, volumetric MRI techniques are designed to objectively determine total brain volume, regional brain

volumes, and the volumes of distinct brain tissue classes. Additionally, diffusion tensor imaging is useful for measuring the impact of brain injury on the microstructural organization of the developing white matter by detecting the preferential diffusion of water molecules in brain tissue. Alternatively, proton magnetic resonance spectroscopy permits the measurement of different brain metabolites. These advanced imaging techniques have provided great insight into the timing and evolution of brain injury preoperatively; however, the long-term predictive value of these acute findings awaits further study. Moreover, the recent successful application of these advanced neuroimaging techniques to the developing fetus with a congenital heart defect will allow us to better understand the nature and progression of impaired brain development before birth.

The accumulating knowledge regarding the relationship between congenital heart defects and brain injury in newborns preoperatively provides invaluable insight into the plight of this new population of survivors. Presently, however, despite the increasing awareness of the vulnerability of this cardiac population to neurological sequelae, these infants are surprisingly not routinely screened for brain injury before open heart surgery. To ascertain the method of routine preoperative screening best suited to newborns with complex congenital heart defects, it is important to characterize the relationship between neuroimaging assessments and neurobehavioral assessments, both of which have independently demonstrated a high prevalence of neurological abnormalities in this population. Should abnormal neurobehavioral assessment scores correlate strongly with abnormal brain structure and microstructure, reliable and inexpensive bedside assessments could be used to routinely screen and monitor all newborns preoperatively. It has been demonstrated that negative scores on neurobehavioral assessments are generally reassuring[43], therefore, this type of assessment could be implemented as a primary tool for analysis, followed by further evaluation with accurate, advanced neuroimaging for infants who had abnormal neurobehavioral examinations. The application of routine preoperative screening will help to identify infants at greater risk for disability and facilitate the implementation of targeted early intervention services.

Alongside the implementation of accurate and cost-effective routine screening methods, it is critical that early intervention exists to prevent or reduce neurodevelopmental morbidity. Tworetzky and colleagues have been refining fetal surgical interventions for the past decade for aortic stenosis with evolving hypoplastic

left heart syndrome[58], and hypoplastic left heart syndrome with intact atrial septum[59]. The results are promising, with most fetuses surviving and being brought to term.

Nonsurgical forms of early intervention must also be made available to families. Although the federally funded Early Intervention Program is provided in the United States, available evidence suggests that early intervention services for children with congenital heart defects are limited[60–62]. It is important that programs are in place to attend to the needs of this high-risk population, just as there are ample services provided to other similar high-risk populations such as premature infants[63]. This type of programming is necessary, given the vulnerability of infants born with congenital heart defects to developing later neurodevelopmental difficulties[8,31–33,43]. With the growing knowledge base concerning newborns diagnosed with congenital heart defects, it is apparent that measures must be taken to ensure that this high-risk population is maximally protected from neurodevelopmental morbidity.

Table 1. Summary of Preoperative Neurological Abnormalities for Newborns with Congenital Heart Defects

Author	**Year**	**Study Design**	**CHD**	**Age at Assessment**	**Sample Size**	**Number Assessed**	**Measures**	**Abnormalities**
El-Naggar et al[35]	2010	Retrospective cohort	Mixed	Neonates (ONS)	10	10	Neurologic exam Clinical seizures	10% 60%
Barbu et al[37]	2009	Retrospective case-control	Mixed	At birth	401	401	HC	14%
Hinton et al[40]	2008	Chart review	HLHS AS	At birth	72 HLHS 34 AS	68 HLHS 32 AS	HC	HLHS 13% AS 9%
Shillingford et al[36]	2007	Chart review	HLHS	At birth	129	129	HC	12%
Chock et al[34]	2006	Chart review	Mixed	Neonates (ONS)	124	95 ANE 80 CUS	ANE CUS	3% 6%
Manzar et al[38]	2005	Chart review	TGA HLHS	At birth	46 TGA 28 HLHS 74 Ctls	46 TGA 28 HLHS 74 Ctls	HC	TGA: ↓HC vs. Ctls HLHS: ↓HC vs. TGA & Ctls
Miller et al[10]	2004	Prospective cohort	TGA	6 days (mean, no injury) 4 days (mean, pre-op injury)	10	10	Neurologic exam	70%
Tavani et al[11]	2003	Prospective cohort	Mixed	9.3 days (mean)	24	17	Neurologic exam	41%
Limperopoulos et al[6]	1999	Prospective cohort	Mixed	13.9 days (mean)	56	50	Neurologic exam ENNAS	56% 58%

(continued)

Author	Year	Study Design	CHD	Age at Assessment	Sample Size	Number Assessed	Measures	Abnormalities
Rosenthal [39]	1996	Chart review	TGA ToF CoA HLHS	At birth	69 TGA 66 ToF 65 CoA 51 HLHS 276 Ctls	69 TGA 66 ToF 65 CoA 51 HLHS 276 Ctls	Anthropometric measures	TGA: ↓HV for BW ToF: ↓HC vs. Ctls HLHS: ↓HV for BW CoA: ↑HV for BW
Newburger et al[27]	1993	RCT	TGA	Neonates (ONS)	180	152	Neurologic exam (Possible abnormalities)	36% (20%)

CHD, congenital heart defect; HLHS, hypoplastic left heart syndrome; AS, aortic stenosis; HC, head circumference; ONS, otherwise not specified; Ctls, controls; ANE, acute neurologic events; CUS, cranial ultrasound; TGA, transposition of the great arteries; ENNAS, Einstein Neonatal Neurobehavioral Assessment Scale; ToF, Tetralogy of Fallot; CoA, coarctation of the aorta; HV, head volume; BW, birth weight

Table 2. Summary of Preoperative Electrophysiology Studies for Newborns with Congenital Heart Defects

Author	Year	Study Design	CHD	Age at Assessment	Sample Size	Number Assessed	Modality	Abnormalities
El-Naggar et al[35]	2010	Retrospective cohort	Mixed	Neonates (ONS)	10	10	aEEG	50%
Limperopoulos et al[8]	2001	Prospective cohort	Mixed	11 days (mean)	36	27	EEG	44%
Limperopoulos et al[41]	1999	Prospective cohort	Mixed	15.8 days (mean, pre-op) 33.9 days (mean, post-op)	27	27 (peri-operative)	SEP	41%

CHD, congenital heart defect; ONS, otherwise not specified; aEEG, amplitude-integrated electroencephalography; SEP, somatosensory evoked potential; BAEP, brainstem auditory evoked potential

Table 3. Summary of Preoperative Neuroimaging Findings for Fetuses and Newborns with Congenital Heart Defects

Author	Year	Study Design	CHD	Age at Assessment	Sample Size	Number Assessed	Abnormalities
Fetal Brain Imaging							
Limperopoulos et al[30]	2010	Prospective cohort	Mixed	31 weeks GA (median)	50 CHD 55 Ctls	50 CHD 55 Ctls	**Total Abnormalities:** 12% ↓TBV and ICV in CHD vs. Ctls ↑CSF:ICV in CHD vs. Ctls
Hinton et al[40]	2008	Chart review	HLHS AS	Fetuses (ONS)	72 HLHS 34 AS	28 HLHS 6 AS	Abnormal Head Size: HLHS (21%) Microcephaly: HLHS (4%), AS (17%)
Neonatal Cranial Ultrasound							
El-Naggar et al[35]	2010	Retrospective cohort	Mixed	Neonates (ONS)	10	8	**Total Abnormalities:** 75% (ONS)
Chock et al[34]	2006	Chart review	Mixed	Neonates (ONS); pre- and post-op	124	80	**Total Abnormalities:** 15% IVH, WMI, hydrocephalus, structural abnormalities
Te Pas et al[21]	2005	Chart review	Mixed	9.6 days (mean)	50	50	**Total Abnormalities:** 21 (42%) Widened ventricles and/or SAS: 13 (26%) Acute ischemic changes (infarction, cerebral edema, PV echodensities, IVH): 4 (8%) LSV: 3 (6%) Basal ganglia calcifications: 1 (2%)
van Houten et al[22]	1996	Chart review	Mixed	4 days (mean)	49 CHD 42 Ctls	49 CHD 42 Ctls	**Total Abnormalities:** 29 (59%) IVH, grade I-IV: 8 (16%) Subarachnoid hemorrhage: 2 (4%) Cerebral atrophy: 13 (27%) Subcortical linear echodensities: 10 (20%) PV echodensities: 4 (8%) IP echodensities: 4 (8%) SAS enlargement: 1 (2%)

(continued)

Author	Year	Study Design	CHD	Age at Assessment	Sample Size	Number Assessed	Abnormalities
Neonatal Computed Tomography							
El-Naggar et al[35]	2010	Retrospective cohort	Mixed	Neonates (ONS)	10	4	**Total Abnormalities:** 50% (ONS)
Neonatal Magnetic Resonance Imaging							
Block et al[23]	2010	Prospective cohort	Mixed	5 days (median)	92	92	**Total Abnormalities:** 43% Stroke: 23 (25%) WMI: 21 (23%) IVH: 7 (8%)
Andropoulos et al[24]	2010	Prospective cohort	SV 2V	7 days (mean) 8 days (mean)	68	68	**Total Abnormalities:** 57% Infarct: 12 (18%) Punctate lesions: 4 (6%) Subdural hemorrhage: 14 (21%) Dural sinovenous thrombosis: 2 (3%) IVH: 2 (3%) Elevated lactate: 4 (6%)
El-Naggar et al[35]	2010	Retrospective cohort	Mixed	Neonates (ONS)	10	2	**Total Abnormalities:** 100% (ONS)
Beca et al[12]	2009	Prospective cohort	Mixed	7 days (mean)	64	64	**Total Abnormalities:** 19 (30%) Stroke: 3 (5%) WMI: 17 (27%)
Licht et al[14]	2009	Cross-sectional	HLHS TGA	4.1 days (mean)	29 HLHS 13 TGA	29 HLHS 13 TGA	**Total Abnormalities:** unspecified PVL: 9 (21%) {HLHS: 4, TGA: 5} Stroke: 4 (9.5%) {HLHS: 4} Open operculum: 36 (86%) {HLHS: 24, TGA: 12}

(continued)

Author	Year	Study Design	CHD	Age at Assessment	Sample Size	Number Assessed	Abnormalities
Petit et al[20]	2009	Chart review	TGA	4.1 days (mean) 5.1 days (mean)	14 BAS 12 Non-BAS	14 BAS 12 Non-BAS	**Total Abnormalities:** unspecified PVL: 10 (38%) IVH (grade I): 6 (23%) Hemorrhage (choroid plexus): 2 (8%) Open operculum: 8 (31%)
McQuillen et al[17]	2007	Prospective cohort	Mixed	Neonates (ONS)	66	62	**Total Abnormalities:** 28 (45%) Ischemic lesions: 23 (37%) (WMI, 11; Stroke, 13) IVH: 5 (8%) Subdural hemorrhage: 11 (18%) ↓ Myelination, immature sulcation: 1 (2%) Globally ↓ parenchymal volume: 1 (2%)
Miller et al[18]	2007	Case-control	TGA SV	5 days (median)	29 TGA 12 SV 16 Ctls	29 TGA 12 SV 16 Ctls	**Total Abnormalities:** 14 (33%) WMI: 4 (10%) {TGA: 3, SV 1} Stroke: 10 (24%) {TGA: 9, SV: 1} IVH: 2 (5%) {TGA: 2} Average diffusivity: CHD 1.28, Ctls 1.35 Mean FA: CHD 0.21, Ctls 0.18
McQuillen et al[16]	2006	Prospective cohort	TGA	Neonates (ONS)	19 BAS 10 Non-BAS	19 BAS 10 Non-BAS	**Total Abnormalities:** 12 (41%) WMI: 2 (7%) Infarct: 5 (17%) IVH: 1 (3%) Infarct and WMI: 3 (10%) Infarct and IVH: 1 (3%) Subdural hemorrhages: 3 (10%)

(continued)

Author	Year	Study Design	CHD	Age at Assessment	Sample Size	Number Assessed	Abnormalities
Partridge et al[19]	2006	Prospective cohort	Mixed	6 days (median, no injury) 4 days (median, pre-op injury) 3.5 days (median, post-op injury)	32	25	**Total Abnormalities:** 7 (28%) WMI: 4 (16%) Infarct: 3 (12%) Subependymal germinal matrix IVH: 2 (8%) FA: lowest in newborns with pre-op injury
Licht et al[13]	2004	Cross-sectional	Mixed	4.7 days (mean)	25	25	**Total Abnormalities:** 13 (53%) PVL: 7 (28%) Open operculum: 4 (16%)
Miller et al[10]	2004	Prospective cohort	TGA	6 days (mean, no injury) 4 days (mean, pre-op injury)	10 CHD 5 Ctls	10 5 Ctls	**Total Abnormalities:** 4 (40%) Stroke: 3 (30%) Hemorrhage: 2 (20%)
Tavani et al[11]	2003	Prospective cohort	Mixed	9.3 days (mean)	24	24	**Total Abnormalities:** 13 (54%) Hemorrhage: 13 (54%) (Subdural, 17; choroid plexus, 7; parenchymal, 1; occipital horns, 1)
Mahle et al[15]	2002	Prospective cohort	Mixed	4 days (mean)	24	24	**Total Abnormalities:** 11 (46%) Ischemic lesions: 6 (25%) (PVL: 4, Infarct: 2) Hemorrhage: 1 (4%) Open operculum: 4 (17%)

CHD, congenital heart defect; GA, gestational age; TBV, total brain volume; ICV, intracranial volume; HLHS, hypoplastic left heart syndrome; AS, aortic stenosis; ONS, otherwise not specified; IVH, intraventricular hemorrhage; WMI, white matter injury; SAS, subarachnoid space; LSV, lenticulostriate vasculopathy; SV, single ventricle; 2V, double ventricle; BAS, balloon atrial septostomy; PVL, periventricular leukomalacia

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CHAPTER 3

Introduction

The review of the literature presents a considerable store of evidence indicating that infants born with heart defects are also vulnerable to acquired brain injury and neurobehavioural abnormalities prior to cardiac surgery. Evidence suggests ischemic lesions such as white matter injury and stroke affect up to one third of these newborns, while more than half experience intracranial bleeding, with intraventricular hemorrhage alone occurring in almost one quarter[11,15–17,59,65–70]. It appears that increased intracranial water content and immature white matter tracts, along with the presence of lactate and reduced N-acetylaspartate levels are also characteristic of this population[15,16,69]. Concurrently, neurological examinations have reported highly prevalent motor abnormalities, as well as microcephaly, abnormal cranial nerve function, and poor behavioural state regulation[13,16,65].

Despite the prevalence of both neuroimaging and clinical findings, the relationship between structural injury and functional abnormalities remains to be determined. It is important to understand the strength of this relationship to ascertain the most effective preoperative screening methods. This knowledge may establish whether neuroimaging can forecast clinical deficits, or conversely, if clinical signs indicate structural impairment. Previous studies have investigated this relationship using conventional MRI techniques, although no significant correlations were observed[16,65].

The following manuscript addresses the two specific aims of this thesis. In newborns with complex CHD that will require corrective or palliative open heart surgery, we aimed first to examine the relationship between brain structure (using 3D volumetric MRI) and neurobehavioural status (using a standardized neurobehavioural assessment) and second, to investigate the corresponding relationship between brain microstructure (using DTI and fibre-tracking techniques) and neurobehaviour using the same standardized assessment. With the aid of advanced MRI techniques which can discern disturbances in brain growth and organization currently beyond detection by conventional MRI capabilities, this study seeks to define the relationship between impaired brain structure and abnormal clinical signs.

NEUROBEHAVIOURAL ABNORMALITIES AND THEIR RELATION TO BRAIN GROWTH AND MICROSTRUCTURAL ORGANIZATION IN NEWBORNS WITH COMPLEX CONGENITAL HEART DEFECTS

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3.1 Abstract

Preoperative brain injury and neurobehavioural impairment are major complications of congenital heart defects (CHD) in infants requiring corrective or palliative open heart surgery. The current study was designed to investigate the relationship between tissue-specific alterations in brain volume as well as white matter microstructure and neurobehavioural status in newborns with complex CHD preoperatively. Full-term newborns (N=35) underwent magnetic resonance imaging (MRI) and standardized neurobehavioural assessment prior to cardiopulmonary bypass surgery. Three-dimensional volumetric MRI was used to calculate tissue-specific brain volumes and diffusion tensor imaging (DTI) data was used to compute tract specific fractional anisotropy (FA) and mean diffusivity (D_{av}) for the corpus callosum, corticospinal tracts, and optic radiations. Neurobehavioural status was assessed using the Einstein Neonatal Neurobehavioral Assessment Scale (ENNAS). Reduced subcortical gray matter (SCGM) volume and increased cerebrospinal fluid (CSF) volume were associated with poor behavioural state regulation (SCGM, p=0.04; CSF, p=0.007) and poor visual orienting (CSF, p=0.003). Mean diffusivity within the optic radiations was negatively associated with overall deviant score on the ENNAS (p=0.02) as well as deviant active motility (p=0.006). In newborns with cyanotic lesions, reduced SCGM was associated with higher deviant ENNAS scores (p=0.001) and poor behavioural state regulation (p=0.04); increased CSF volume with poor behavioural state regulation (p=0.02) and poor visual orienting (p=0.02); and increased FA in the optic radiations and corticospinal tracts with abnormal overall ENNAS scores (p=0.01, p=0.04). Conversely, newborns with acyanotic lesions showed associations between a reduced cerebellar volume (CV) and poor behavioural state regulation (p=0.03) as well as between increasingly abnormal active motility and reduced D_{av} in the optic radiations (p=0.007). Infants with complex CHD have an increased risk of impaired brain development and neurobehaviour which are associated prior to surgery. Moreover, cardiac physiology appears to be a key determinant in the nature of this relationship. This study contributes to a broader understanding of neurologic impairment within this vulnerable population and highlights a need for routine preoperative screening and early intervention to improve neurodevelopmental outcomes.

3.2 Introduction

Brain injury and neurodevelopmental impairments have emerged as salient co-morbidities of congenital heart defects (CHD), especially in newborns with complex cardiac lesions[1–4]. Moreover, there is increasing awareness of neurologic compromise presenting even before corrective or palliative surgery, with up to 60% of newborns demonstrating neuroimaging abnormalities[1–3,5,6], and up to 70% showing signs of neurobehavioural impairment[7–10]. Studies using advanced magnetic resonance imaging (MRI) techniques are adding to this growing body of literature by providing quantitative *in vivo* measurements of brain structure and microstructure[11–14]. Recent studies using three-dimensional (3D) volumetric MRI have shown fetuses with CHD have progressively lower total and tissue-specific brain volumes as well as delayed gyrification in the third trimester when compared to healthy controls[14,15]. Furthermore, diffusion tensor imaging (DTI) studies investigating white matter microstructure in newborns with CHD have reported increased mean diffusivity (D_{av}) (suggesting increased water content) and decreased fractional anisotropy (FA) (suggesting reduced tissue organization) in the optic radiations, as well as the perirolandic, frontal, and posterior white matter when compared to healthy controls[11,13].

The high prevalence of neurologic and neurobehavioural abnormalities, in addition to more recent quantitative indicators of impaired brain growth and development, points to a need for routine monitoring of neurologic status in these high-risk infants. Knowledge of a newborn's preoperative neurologic condition can be used to inform critical care provision, family counselling, clinical decision-making and surgical planning, and to determine whether further diagnostic testing and developmental intervention may be beneficial. More broadly, a solid understanding of the neurologic complications encountered in this population prior to surgery might serve to promote the development of future neuroprotective strategies. Thus, monitoring brain and behavioural development beginning in the preoperative period and continuing post-discharge is vital in the effort to prevent and reduce morbidity in newborns with complex CHD[16].

Neurologic and neurobehavioural assessments are valuable tools which can be used to evaluate neurologic status at the bedside in a comprehensive, non-invasive, and cost-effective fashion[17,18]. Even so, very few studies have investigated the relationship between neurologic status and brain injury in newborns with

complex CHD. Available data are equivocal, with one study reporting reflex and tone abnormalities in newborns with and without injury on MRI[8], and another which focused on intracranial hemorrhage finding no association between such insults and neurologic deficits[9]. No study to date has investigated the relationship between clinical neurobehavioural status and either volumetric brain growth or white matter microstructure in newborns with CHD prior to open heart surgery. A better understanding of the association between early brain structure and function may help to determine the most appropriate methods for evaluating this high-risk population. Therefore, the objectives of this study were two-fold.

The first objective was to examine the association between preoperative neurobehavioural status, as measured by a standardized neurobehavioural assessment, and global and tissue-specific brain volumes as measured using 3D volumetric MRI. The second objective was to assess the relationship between preoperative neurobehavioural status and tract specific measures of white matter integrity (FA and D_{av}).

3.3 Methods

3.3.1 Study Population

The study cohort included full-term newborns (>36 weeks gestational age) diagnosed with complex CHD requiring corrective or palliative cardiopulmonary bypass surgery. Subjects were enrolled prospectively, either antenatally or postnatally. Mothers of fetuses with confirmed complex CHD following a fetal echocardiogram were enrolled as part of a larger, longitudinal study of brain development in fetuses and infants with CHD. Postnatally, newborns were enrolled following admission to the cardiac intensive care unit at our centre with complex CHD confirmed by echocardiogram. Postnatal enrolment took place in the absence of a prenatal diagnosis, when prenatal monitoring was performed at another centre, or when mothers were excluded from the longitudinal study or were not available prenatally.

Newborns were excluded when there was evidence of central nervous system dysfunction which could be plausibly attributed to causes that were unrelated to complications of CHD. This included subjects with central nervous system infections (e.g. TORCH complex of perinatal infections); congenital malformations (e.g.

agenesis of the corpus callosum), known chromosomal anomalies or syndromes (e.g. Trisomy 21); or documented perinatal insults (e.g. perinatal asphyxia).

3.3.2 Procedures

Magnetic Resonance Imaging

Preoperative brain MRI studies were conducted for enrolled newborns according to the standard clinical protocol at our centre for newborns undergoing cardiopulmonary bypass surgery. On occasion, however, studies were performed strictly for research purposes when surgery was scheduled beyond the neonatal period. Studies took place as soon as the clinical care team determined that the newborn was stable enough to be transported to the MRI scanner. In cases where newborns were scanned as outpatients, the MRI study was conducted at the parents' earliest convenience.

No sedation was used during the MRI scan unless it was necessary for clinical reasons. Newborns that were not clinically sedated were quieted by feeding and swaddling, and were immobilized with a vacuum bean bag (Newmatic Medical, Caledonia, MI). All infants were provided with two layers of hearing protection: silicone ear plugs (Beneficial Products Inc., Ashland, OR) and adhesive ear muffs (Natus Medical Inc., Seattle, WA). Oxygen saturation, heart rate, and temperature were monitored and recorded throughout the scanning process by a nurse using a magnetic resonance compatible vital signs monitoring system (Veris, MEDRAD, Inc. Indianola, PA).

MRI pulse sequences were acquired on a 3T MR scanner (Discovery MR750, GE Healthcare, Milwaukee, WI) with a 32 channel receive-only head coil (MR Instruments, Inc., Minneapolis, MN). Two sequences were required to compute brain volumes: a T1-weighted fluid attenuated inversion recovery (FLAIR) sequence with periodically rotated overlapping parallel lines with enhanced reconstruction (PROPELLER), 2mm contiguous axial slices, 38.6 ms echo time (TE), 1525 ms repetition time (TR), 565.42 ms inversion time, 256x256 matrix, one excitation (NEX) and 25.6 cm field of view (FOV); as well as a 2mm axial T2-weighted PROPELLER sequence with TE 83.72 ms, TR 9640 ms, 256x256 matrix, NEX 2, and FOV 25.6 cm. DTI data used in the quantification of white matter tract integrity were obtained using a diffusion-weighted sequence with 27 gradient directions at $b=1000s/mm^2$, three volumes with no diffusion weighting (b=0), 8000 ms repetition

time, 80 ms echo time, 20 cm field of view, 96x96 matrix, and 3mm contiguous slices on the axial plane. A pediatric neuroradiologist blinded to clinical diagnosis and neurobehavioural findings reviewed each MRI study.

Post-Acquisition Processing: Brain Volumes

Image processing was performed on Linux workstations. An automatic segmentation software program developed and validated in newborns by Weisenfeld and Warfield[19,20] was used to segment each brain volume into five major tissue classes (Figure 1): cortical gray matter (CGM), myelinated white matter (mWM), unmyelinated white matter (umWM), subcortical gray matter (SCGM), and cerebrospinal fluid (CSF). Total cerebellar volume (CV) was segmented separately from the cerebral tissue volumes. The resulting images were inspected and corrected manually using MINC software (www.bic.mni.mcgill.ca/software/minc). Manual corrections were performed by a single investigator, and intra-rater reliability was assessed by performing the manual corrections a second time for five cases. Using the intraclass correlation coefficient (ICC), intra-rater reliability was calculated as 0.998 overall (total brain volume (TBV), 0.96; CGM, 0.89; mWM, 0.77; umWM, 0.97; SCGM, 0.91; CV, 0.91). Following automatic segmentation and manual corrections, each tissue volume was determined by counting the number of voxels in the appropriate tissue's segmentation mask (e.g. CGM) and multiplying by the volume of each voxel (www.bic.mni.mcgill.ca/software/minc).

Post-Acquisition Processing: White Matter Microstructure

Microstructural organization was quantified using DtiStudio software[21]. Raw DTI images were registered to the average of the b0 (non-diffusion weighted) volumes using automatic image registration with an affine linear transformation approach and tri-linear interpolation. Motion-corrupted slices were removed using automatic outlier slice rejection (relative error >3%), followed by manual removal if applicable. The remaining slices were used to compute the three diffusion tensor eigenvalues corresponding to the three principal eigenvectors which characterize diffusion along each principal axis (x,y,z), and to calculate the parametric D_{av} and FA maps. Mean diffusivity is the average rate of water diffusion in all directions, a value computed by averaging the three eigenvalues. Fractional anisotropy is a measure of the magnitude of the longitudinal movement or "directionality" of water

molecules during diffusion, with a value of zero representing isotropic diffusion (equal diffusion in all directions), and a value of one representing anisotropic diffusion (diffusion restricted to one axis).

Using the computed FA and D_{av} maps, fibre tracking was performed for five known white matter tracts: the corpus callosum, left and right corticospinal tracts, as well as the left and right optic radiations. These tracts were chosen to represent a cross-section of tract type (commissural, projection, and association, respectively) as well as the potential for varied functional associations. The measurements for the left and right tracts for both the corticospinal tracts and optic radiations were averaged within each newborn. Each tract was defined by fibres passing through two or three regions of interest (ROIs), drawn manually on the FA maps to encapsulate the entire tract circumference (Figure 2). For the corpus callosum, this included one midsagittal and two parasagittal ROIs. The corticospinal tracts were defined by axially drawn ROIs at the level of the cerebral peduncle, posterior limb of the internal capsule (PLIC), and the centrum semiovale anterior to the central sulcus. The two ROIs for the optic radiations were drawn on the coronal plane, encircling the white matter adjacent to the lateral geniculate nucleus and the calcarine fissure. Exclusion masks were also drawn to prevent implausible tracts from forming across anatomical boundaries (e.g. midsagittal plane for the corticospinal tracts and optic radiations). The anatomical validity of the resulting fibres was checked using the D_{av} maps, which delineate anatomical boundaries.

Fibre trajectories were formed along the primary eigenvector (determined by the maximum eigenvalue) between two voxels if the FA value was greater than 0.01. Tracts were terminated if this value dropped below 0.2 for the corpus callosum and the corticospinal tracts, or below 0.1 for the optic radiations which compete with crossing fibres to a greater extent. Tracts were also terminated if the angle formed by the tract was too great. This angular threshold was set at 50 degrees for the corticospinal tracts, and 60 degrees for the corpus callosum and optic radiations to allow for the increased curvature of these tracts. For the corpus callosum and optic radiations, fibres which did not pass through all ROIs were excluded. For the corticospinal tracts, fibres were excluded if they did not pass through either the cerebral peduncle and the PLIC, or the PLIC and the white matter of the motor cortex.

Mean, standard deviation, minimum, and maximum D_{av} and FA values were calculated within each fibre system using the statistical program included in the DtiStudio software. All fibre tracking measurements were performed by a single investigator. Intra-rater reliability was assessed by redrawing the ROIs and repeating the measurements five times for each tract type. Using the ICC, the intra-rater reliability for D_{av} was computed as 0.99 for each of the WM tracts; for FA, the values were 0.80 (corpus callosum), 0.99 (corticospinal tracts), and 0.98 (optic radiations).

Neurobehavioural Assessment

The Einstein Neonatal Neurobehavioural Assessment Scale (ENNAS)[22] was administered by one of two evaluators trained in assessing neonatal neurobehavioural status and blinded to clinical neurologic findings and MRI results. The ENNAS was selected as a comprehensive yet efficient assessment of neonatal neurological and neurobehavioural status which has been previously used to evaluate newborns with CHD[4,7,18,23]. It takes approximately 15 to 20 minutes to perform at the newborn's bedside, and consists of 20 test items which evaluate muscle tone, passive movements (arm recoil, popliteal angle), active motility (head extension, traction, head lag, extremity movement, ventral suspension), primitive reflexes (suck, rooting, grasp, optic blink, Moro, asymmetric tonic neck reflex, withdrawal, rotation), and responses to animate and inanimate visual and auditory stimuli (visual and auditory orienting). In addition, four summary items assess overall impression of "cuddliness," spontaneous movements, tonus, and tremor (both incidence and quality). Of the 24 scored items, 19 contribute to an overall deviant score (number of failed items), with the remaining items informing the summary scores. The infant's behavioural state is also observed throughout the assessment, with predominant state documented independently of the scored items. Assessments with up to two failed items were classified as normal, while three to six failed items constituted a suspect assessment, and greater than six failed items was considered abnormal. Evaluators followed the itemized instructions for administration and scoring, however the order of evaluation remained dependent on the newborn's behavioural state in order to optimize the infant's performance.

The ENNAS has excellent concurrent validity with the standard neonatal neurological examination[23]. The negative predictive value of this instrument is very good (83%-92%) for intellectual function, communication, and socialization, and

good (64-83%) for neuromotor status and daily living skills at school age[24]. The sensitivity of the ENNAS is also good, ranging from 64%-78%, though positive predictive value and specificity remain limited due to a susceptibility for false positive results[24]. We did not determine inter-rater reliability, as the critical condition of the newborns in the present cohort was not compatible with an increase in assessment duration to accommodate both examiners or with conducting two separate assessments between which intervening clinical events might have affected reliability. However, the inter-rater reliability has been previously calculated as 0.97[18].

Clinical Data Collection

Medical records for each newborn were reviewed for demographic and clinical information including gestational age at birth, birth weight, gender, cardiac diagnosis, 5-minute Apgar score, mode of delivery, need for respiratory resuscitation at birth, medications, and any cardiac catheterization procedures prior to evaluation (e.g. balloon atrial septostomy). The Score for Neonatal Acute Physiology-II (SNAP-II)[25] was calculated for each newborn based on information from medical records to assess illness severity within the first 12 hours of life.

Statistical Analyses

Descriptive statistics were developed as means and standard deviations for continuous values, medians and ranges for ordinal values, and proportions for categorical values. Multiple linear regression models, both unstratified and stratified were performed to evaluate the relationships between overall deviant ENNAS scores as well as individual items or clusters (active motility, muscle tone, visual orienting, auditory orienting, and behavioural state) and brain volumes and white matter tract integrity (FA and D_{av}). The stratification factor was cardiac physiology (cyanotic versus acyanotic defects). All analyses controlled for differences in postconceptional age at time of MRI, birth weight, gender, and SNAP-II score to rule out the effects of general illness severity and maturation. In addition, tract length and the number of fibres per voxel were controlled for in the white matter microstructure analyses.

This study was intended primarily to generate hypotheses; as such, all probability values with a significance of 0.05 or less are reported.

3.4 Results

3.4.1 Newborn Characteristics

Of the 35 newborns enrolled, 18 were enrolled antenatally, and 17 postnatally. Table 1 presents characteristics of the cohort. All were full term (mean gestational age 38.8 ± 0.9 weeks). The MRI took place at a median age of 3 days (range 1-21 days) and the neurobehavioural assessment was performed within 36 hours of the MRI in the majority of patients (91%). The span between MRI and neurobehavioural assessment for the remaining newborns was two days (2) and five days (1). Six newborns received an abnormal score (>15) for illness severity on the SNAP-II[25]. One newborn was removed from the microstructure analyses as the DTI data were acquired for this patient using different parameters. This subject had a greater than average birth weight, but did not differ from the rest of the cohort on other baseline perinatal characteristics. Furthermore, newborns enrolled postnatally did not differ from those enrolled prenatally with respect to ENNAS score, conventional MRI findings, brain volumes, or measures of microstructure. Table 2 provides a breakdown of the cardiac diagnoses of enrolled newborns; 26 were classified as cyanotic, while nine were acyanotic.

3.4.2 Preoperative Neurobehavioural Performance

Twenty-five (71%) newborns had a suspect or abnormal neurobehavioural assessment; almost half of the study cohort fell into the suspect category (16) with between three and six failed items, while nine were classified as abnormal with greater than six failed items. Of all newborns studied, muscle tone was abnormal in 18, of which 10 were hypotonic, five were hypertonic, and three had mixed tone. Active motility and primitive reflexes were each abnormal in 21 newborns, while passive movements were abnormal in 23. Visual orienting and auditory orienting were abnormal in 26 and 21 respectively. Twenty-three newborns had difficulty maintaining an alert behavioural state, and were instead drowsy or irritable; in 10 of these cases, newborns fluctuated between drowsiness and irritability. One newborn was microcephalic (head circumference <3rd percentile) and one was macrocephalic (head circumference >97th percentile). Of note, only one newborn was on morphine during the neurobehavioural assessment and this infant received a suspect score on limited examination.

3.4.3 Conventional MRI Findings

Sixteen (46%) newborns had evidence of injury or immaturity on conventional MRI. The most prevalent findings were hemorrhages (9), including four extra axial, two cerebellar, two intraventricular, and one choroid plexus. Other abnormalities included prominent subarachnoid spaces (5), infarction (3), T2 hyperintensity [bilateral anterior limbs of the internal capsule (1), bilateral basal ganglia (1)], white matter injury [deep cerebral white matter (1), periventricular white matter (1)], asymmetric cerebral hemispheres and lateral ventricles (1), delayed opercular development (1), and diffuse edema (1).

3.4.4 Relationship between Neurobehavioural Status and Brain Volume

We did not find evidence of an association between the overall deviant ENNAS scores and total brain or tissue-specific volumes after controlling for postconceptional age at time of MRI, gender, birth weight, and illness severity (SNAP-II score). Independent associations were observed, however, between individual and clustered items on the ENNAS and tissue-specific brain volumes. Newborns that had difficulty maintaining an alert behavioural state had reduced SCGM volume ($p=0.04$) as well as increased CSF volume ($p=0.007$). After stratifying for cyanotic versus acyanotic lesions, these relationships were primarily attributed to newborns with cyanotic defects (SCGM, $p=0.04$; CSF, $p=0.02$). In addition, decreased visual orienting was associated with increased CSF volume ($p=0.003$), especially among the cyanotic newborns ($p=0.02$).

Newborns with cyanotic defects also demonstrated an association between a reduced SCGM and higher deviant ENNAS scores ($p=0.001$). Conversely, an association between decreased cerebellar volume and poor behavioural state regulation ($p=0.03$) was noted among newborns with acyanotic lesions.

3.4.5 Relationship between Neurobehavioural Status and White Matter Microstructure

Newborns with higher (worse) overall deviant scores on the ENNAS had reduced D_{av} in the optic radiations ($p=0.02$). Increasingly abnormal active motility was associated with reduced D_{av} in the optic radiations ($p=0.006$), especially among the acyanotic newborns ($p=0.007$), and possibly among cyanotic newborns ($p=0.06$).

Additionally, for newborns with cyanotic defects, higher deviant scores on the ENNAS were associated with increased FA in the optic radiations. This was true when ENNAS deviant scores were included in the model as continuous values ($p=0.04$), or when they were dichotomized as normal (fewer than three failed items) versus abnormal (suspect and abnormal assessments collapsed, i.e. three or more failed items) ($p=0.01$). Newborns with cyanotic heart defects also demonstrated an association between abnormal ENNAS scores and increased FA in the corticospinal tracts ($p=0.04$).

3.5 Discussion

The results of the present study support mounting evidence demonstrating an increased risk of brain injury and concomitant neurobehavioural impairment in newborns with complex CHD prior to surgery[1–3,5–10]. Almost half of the newborns in our cohort showed signs of brain injury or immaturity on conventional MRI and more than two-thirds had suspect or abnormal neurobehavioural assessments. Moreover, this study describes for the first time an association between abnormal neurobehavioural performance and reduced tissue-specific brain volumes. Also unique to this study, we report associations between neurobehavioural status and tract specific measurements of white matter tract integrity.

As the relationship between neurobehaviour and brain volumes has not been previously examined in this population, a direct comparison of these results is not possible. Watanabe and colleagues[26], however, have examined the connection between regional brain volumes and neurodevelopment in older infants following corrective surgery for ventricular septal defects and critical CHD. Their study described an association between reduced frontal gray matter volume and lower scores on the psychomotor developmental index of the Bayley Scales. In studies of premature infants, another population at risk for neurologic compromise, there is a growing body of evidence of associations between abnormal neurobehaviour and reductions in specific brain tissue volumes[27–29]. At term equivalent age, lower behaviour scores on the Revised Hammersmith Neonatal Neurological Examination have been associated with white matter volume loss[27], and premature infants with abnormal general movements at one and three months of age have smaller cerebellar diameters[29]. Taken together, these data suggest that abnormal neurobehavioural

status is associated with tissue-specific brain growth disturbances in these two high-risk infant populations.

The first aim of the present study was to determine if quantification of brain growth using advanced 3D volumetric MRI is related to newborn function as measured by a standardized neurobehavioural assessment. While there was no relationship between total brain volume and overall score on the ENNAS, there were several observable independent associations between tissue-specific volumes and behavioural items.

In newborns with cyanotic heart defects, those that could not maintain an alert behavioural state throughout the majority of the assessment, and were instead predominantly irritable or drowsy, had increased CSF volumes. A similar relationship was observed for newborns that did not attend to a moving stimulus in the visual field. In typically-developing infants, CSF decreases with increasing gestational age[30]. Correspondingly, increased CSF volume has been correlated with prematurity[31,32]. We postulate that abnormal behavioural state regulation and poor performance on visual orienting items indicate impaired brain development in cyanotic newborns. A potential antenatal origin for this increased CSF volume is supported by a recent study reporting progressively higher volumes of CSF in third trimester fetuses with CHD relative to controls[14].

Our data also demonstrate that newborns with cyanotic heart defects show an association between reduced SCGM volume and higher overall ENNAS scores as well as poor behavioural state organization. Though SCGM was comprised collectively of the thalamus and the basal ganglia in this study, the role of the thalamus as a relay centre between the environment and other brain regions, as well as its strong connections to the "emotion" centres of the limbic system (e.g. periaqueductal gray, hypothalamus) may drive this particular relationship[33]. Since the emotional (and thus behavioural) state is regulated on the basis of external cues, it is conceivable that if the thalamus is under-developed in a newborn, such an infant may become easily overwhelmed and consequently irritable or drowsy. Furthermore, studies of local cerebral metabolic rates for glucose and regional cerebral blood flow have established the thalamus as a region with one of the highest metabolic rates in the newborn[34–36]. We propose that such a metabolically demanding region would be more susceptible to decreased oxygenation resulting from cyanotic CHD, and consequently to impairments in growth and functionality. There is a comparatively

low rate of local glucose metabolism and regional cerebral blood flow in the majority of the neonatal cerebral cortex and the striatum[34–36]. From this, we can infer that the basal ganglia, typically associated with controlling voluntary muscle movements and more recently with learning and memory[37–39], may not be as vulnerable to hypoxia and potential reductions in tissue growth and function. Future studies are needed to address this intriguing question.

Newborns with acyanotic defects demonstrated associations between reduced cerebellar volumes and abnormal behavioural state regulation. This observation is consistent with the evolving view of the cerebellum, which is increasingly recognized as contributing to high-order cognitive and behavioural abilities[40–44]. The cerebellum, and particularly the central vermis, is another region with an active metabolism and one of the highest regional cerebral blood flow requirements during late gestation and the early postnatal period[34–36,45,46]. Therefore, it can be inferred that the increased perfusion requirements within the cerebellum during this transitional period likely render it susceptible to growth disturbances in acyanotic newborns with poor cerebral perfusion.

The second aim of the current study was to investigate the relationship between tract specific measurements of white matter microstructure and neurobehavioural performance. Our results revealed unexpected associations between impaired neurobehaviour and restricted diffusion (D_{av}) as well as increased anisotropy (FA). These findings contrast with studies of other high-risk neonatal populations including premature infants[47–49] and infants with severe motor dysfunction[50,51] which report positive correlations between white matter integrity (i.e. decreased FA, increased D_{av}) and abnormal neurodevelopmental outcome. However, recent studies in newborns with hypoxic-ischemic encephalopathy (HIE) report findings which appear to align with our results[52–55]. These studies have described an association between decreased apparent diffusion coefficient (ADC) values (i.e. restricted diffusion) and poor neurodevelopmental outcome in newborns with HIE when imaged within days of birth following injury[52–55]. The precise mechanism for this early reduction in ADC values is unclear, although it has been proposed that neuronal energy depletion due to ischemic injury may prompt ion channel activity failure in the axons[56]. The resulting neuronal swelling may then diminish the volume of the extracellular compartment and thus the degree of

extracellular diffusion[56]. Energy-dependent axoplasmic transport may also be disrupted, increasing viscosity and restricting water diffusion within the axon[56].

In the present study, we identified a relationship between lower D_{av} values in the optic radiations and higher deviant scores on the overall ENNAS as well as in the active motility cluster in the unstratified cohort. When stratified, acyanotic newborns showed an association between reduced D_{av} in the optic radiations and active motility. We postulate that restricted diffusion in the optic radiations may be indicative of a global brain injury which translates to abnormal neurobehaviour. This relationship between lower D_{av} values and neurobehavioural abnormality may indicate a mechanism of white matter tract injury analogous to that seen in infants with HIE, wherein the acquired injury is secondary to a more prolonged course of hypoxic ischemia. Newborns with cyanotic defects, in contrast, demonstrated relationships between abnormal overall ENNAS scores and increased FA values in both the optic radiations and the corticospinal tracts. A potential mechanism for these relationships remains elusive, however it has been acknowledged that high FA values can be difficult to interpret[57]. Future studies are required to probe these relationships further.

Additional interest is prompted by the different patterns of association which emerged among newborns with cyanotic defects and those with acyanotic defects, as seen in the volume-neurobehaviour analyses. Our findings suggest cardiac physiology may have a role in mediating the relationship between brain development and neurobehaviour; structural and microstructural maturation may indeed differ depending on relative perfusion and oxygenation experienced *in utero* and in the early post-natal period[5,58,59].

In the normal fetal circulation, oxygenated blood is preferentially directed to the brain by means of an interconnected series of shunts, however this streamlined system is disrupted in the presence of a heart defect[60,61]. *In utero*, the network of shunts is capable of compensating for altered hemodynamics to an extent, however it has been established that complex heart defects can impact cerebral blood flow and oxygenation in the fetus[58–60]. This disturbance becomes even more critical in the newborn when the fetal shunts begin to close, leaving the newborn brain vulnerable to diminished oxygen levels or altered blood flow[5,60]. Fetuses and newborns with heart defects resulting in hypoxemia (e.g. hypoplastic left heart syndrome, transposition of the great arteries) exhibit an increase in cerebral blood flow, a

response known as the "brain sparing effect[58,59,62]." Despite this adaptive mechanism, evidence suggests that the severity of the hemodynamic disturbance may be enough to overwhelm the response and compromise brain development[14,63–65]. Both hypoxia and hypoperfusion, as seen in cyanotic and acyanotic defects respectively, have been correlated with brain injury[8,58,63–70].

There is a paucity of research exploring the potential mediating effects of distinct cardiac lesion types on the relationship between brain development and neurobehaviour. Findings from animal studies, however, point to a potential effect of hypoperfusion and hypoxia. Chronic cerebral hypoperfusion in mice has been shown to result in white matter damage in the corpus callosum and internal capsule, which was correlated with deficits in object recognition and spatial memory[66,71]. Furthermore, in studies of mice under hypoxic conditions, reduced regional brain volumes (cerebellum and hippocampus), impaired myelination (corpus callosum and septal nuclei), and decreased FA values (corpus callosum, cingulum, and fimbria of the hippocampus) were observed alongside functional deficits including impaired associative learning, spatial memory, long-term social memory, and consistency of lateralized behaviour[68,72]. Future studies will be necessary to explicitly address the question of potential mediating effects of both hypoxic and hypoperfusion conditions on the relationship between brain injury and neurobehaviour.

This study has several limitations. While newborns enrolled prospectively with a wide range of complex CHD is at first a strength, it may also mask potential effects present within certain diagnostic categories. Although the relatively small sample size precluded analysis of effects within each lesion type, we stratified the cohort according to broad defect classification (cyanotic versus acyanotic) to account for variations in physiology. Studies with larger, equivalent samples of newborns with cyanotic and acyanotic heart defects are needed to further elucidate these initial observations. Another limitation of the study was the necessary restriction of the neurobehavioural assessments in some cases; for example, items assessed in the prone position could not be performed on newborns that were intubated. In these instances, the items were not scored and therefore did not contribute to the infant's overall deviant score, possibly resulting in an underestimation of abnormal findings in these subjects. In the future, an assessment which takes into account the ratio of items performed to items failed may be helpful. With respect to the post-processing of MRI data, due to the small volume of mWM in the neonatal brain and the lower

intra-rater reliability coefficient we obtained for mWM volumes compared to other tissue types, important observations may have been missed between subjects for this tissue class. The inherent challenges of performing fibre-tracking on newborn subjects must also be acknowledged. Relatively large slice thickness with respect to small head size results in larger voxels, introducing a greater potential for intravoxel fibre crossing. Furthermore, fibre-tracking is sensitive to precise head alignment during acquisition which can be difficult to achieve with such young and critically ill subjects. It is also possible that collapsing the right and left corticospinal tracts and optic radiations masked potential lateralized effects. Future studies are necessary to separately relate left and right tracts to neurobehavioural status. Finally, we conducted multiple tests; we consider the results of these exploratory analyses hypothesis generating for future research.

3.6 Conclusions

We have described associations between neurobehavioural status and tissue-specific brain volumes as well as white matter microstructure in newborns with complex CHD. Our findings suggest differences in cardiac lesion type may impact the nature of these relationships. While precise mechanisms remain to be established, the results of this study imply abnormal neonatal neurobehaviour is indicative of an impaired nervous system, providing new insight into early brain structure and function and underscoring the importance of early developmental evaluation in this population. Long-term developmental follow-up of our cohort is currently underway to determine the predictive value of these initial findings.

Table 1: Perinatal Characteristics

Characteristic	**All Newborns (N=35)**
Gestational age at birth, wk, mean ± SD	38.8 ± 0.9
Birth weight, g, mean ± SD	3195.9 ± 528.1
Male gender, n (%)	19 (54)
Cyanotic, n (%)	26 (74)
Normal spontaneous vaginal delivery, n (%)	7 (20)
Induced vaginal delivery, n (%)	15 (43)
Elective c-section, n (%)	10 (29)
Stat c-section, n (%)	3 (9)
Apgar score at 5 min, median (range)	8 (7-9)
SNAP-II score, median (range)	5 (0-28)
Respiratory resuscitation at birth, n (%)	13 (37)
Postconceptional age at MRI, mean ± SD, wk	39.5 ± 1.3
Age at MRI, d, median (range)	3 (1-21)
Postconceptional age at ENNAS, mean ± SD, wk	39.6 ± 1.3
Age at ENNAS, d, median (range)	4 (1-21)
Time between MRI and ENNAS, d, median (range)	1 (0-5)
Pressor support before MRI, n (%)	19 (54)
Prostaglandin E_1 before MRI, n (%)	24 (69)
Balloon atrial septostomy or cardiac catheterization before MRI, n (%)	9 (26)
Time to surgery, d, median (range)	10.5 (4-248)

Table 2: Heart Defect Classifications

Transposition of the Great Arteries
8 TGA
Hypoplastic Left Heart Syndrome
5 HLHS 1 HLHS + DORV + Mitral Atresia
Double Ventricle
4 Tetralogy of Fallot 1 VSD 1 Truncus Arteriosus
Double Ventricle with Coarctation of the Aorta
2 Coarctation + Mitral Stenosis 1 VSD + Coarctation 1 VSD + Interrupted Aortic Arch 1 Ebstein's Anomaly + VSD + Coarctation + Left Ventricle Dysfunction
Single Ventricle
1 Tricuspid Atresia + VSD
Single Ventricle with Coarctation of the Aorta
1 DORV + Atrioventricular Canal Defect + Coarctation 1 DORV + VSD + Coarctation 1 DILV + L-TGA + Aortic Stenosis
Single Ventricle with Pulmonary Stenosis/Atresia
1 DORV + Hypoplastic Right Heart + Pulmonary Stenosis 1 DORV + Ventricular Inversion + Pulmonary Stenosis 1 Pulmonary Atresia + Hypoplastic Right Heart 1 DILV + l-TGA + Pulmonary Stenosis 1 Heterotaxy + Single Ventricle + Pulmonary Atresia 1 Tricuspid Atresia + VSD+ Pulmonary Stenosis

TGA, Transposition of the Great Arteries; HLHS, Hypoplastic Left Heart Syndrome; DORV, Double Outlet Right Ventricle; VSD, Ventricular Septal Defect; DILV, Double Inlet Left Ventricle; L-TGA, Levo-Transposition of the Great Arteries

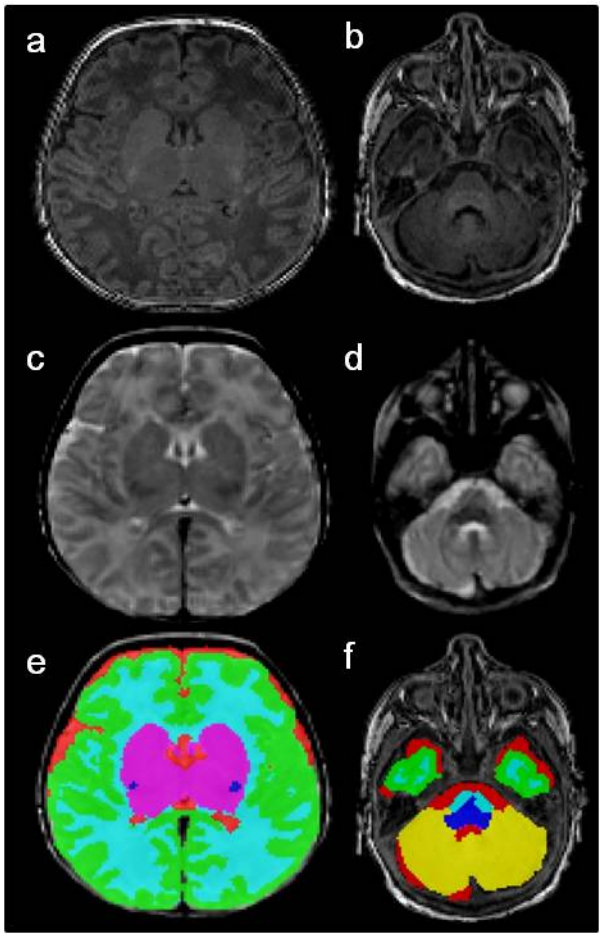

Figure 1. Input and output for the automatic segmentation of brain tissue volumes. a) Axial T1 slice at the level of the thalami and b) cerebellum; c) axial T2 slice at the level of the thalami and d) cerebellum; e) automatic segmentation of brain tissue volumes at the level of the thalami and f) cerebellum. The tissue classes depicted are cortical gray matter (green), unmyelinated white matter (light blue), myelinated white matter (dark blue), subcortical gray matter (magenta), cerebrospinal fluid (red), cerebellum (yellow).

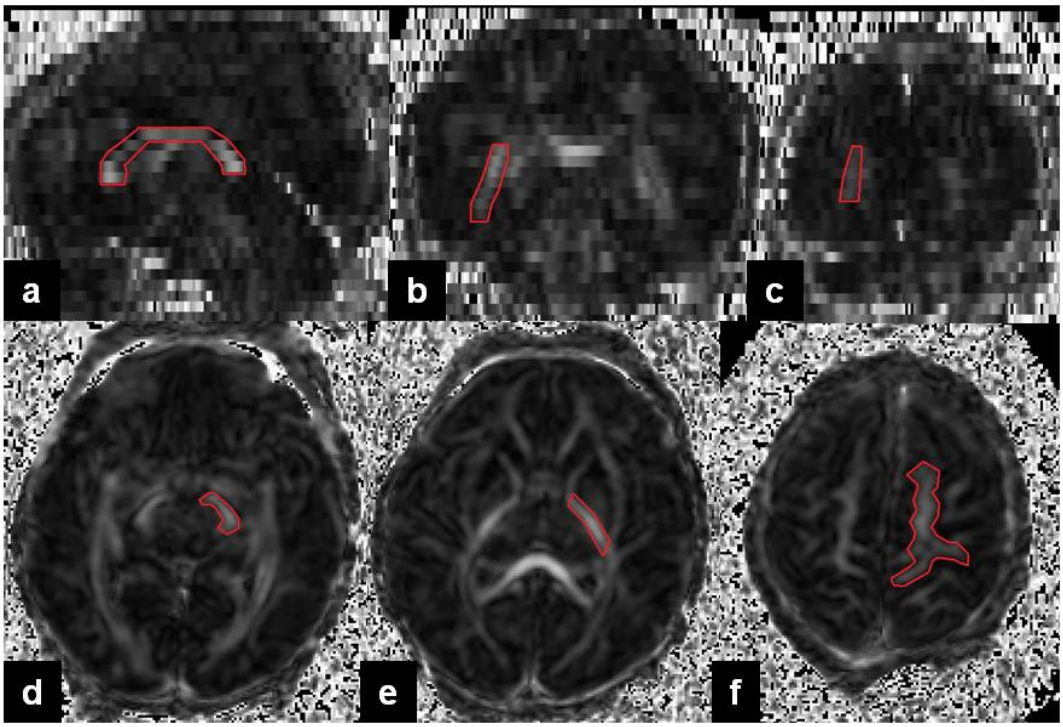

Figure 2. Manually drawn fibre tracking regions of interest on diffusion tensor imaging fractional anisotropy (FA) maps. a) Corpus callosum on the midsagittal plane, b) right optic radiation on the coronal plane adjacent to the lateral geniculate nucleus and c) adjacent to the calcarine fissure, d) left corticospinal tract on the axial plane at the level of the cerebral peduncle, e) posterior limb of the internal capsule, and f) centrum semiovale anterior to the central sulcus.

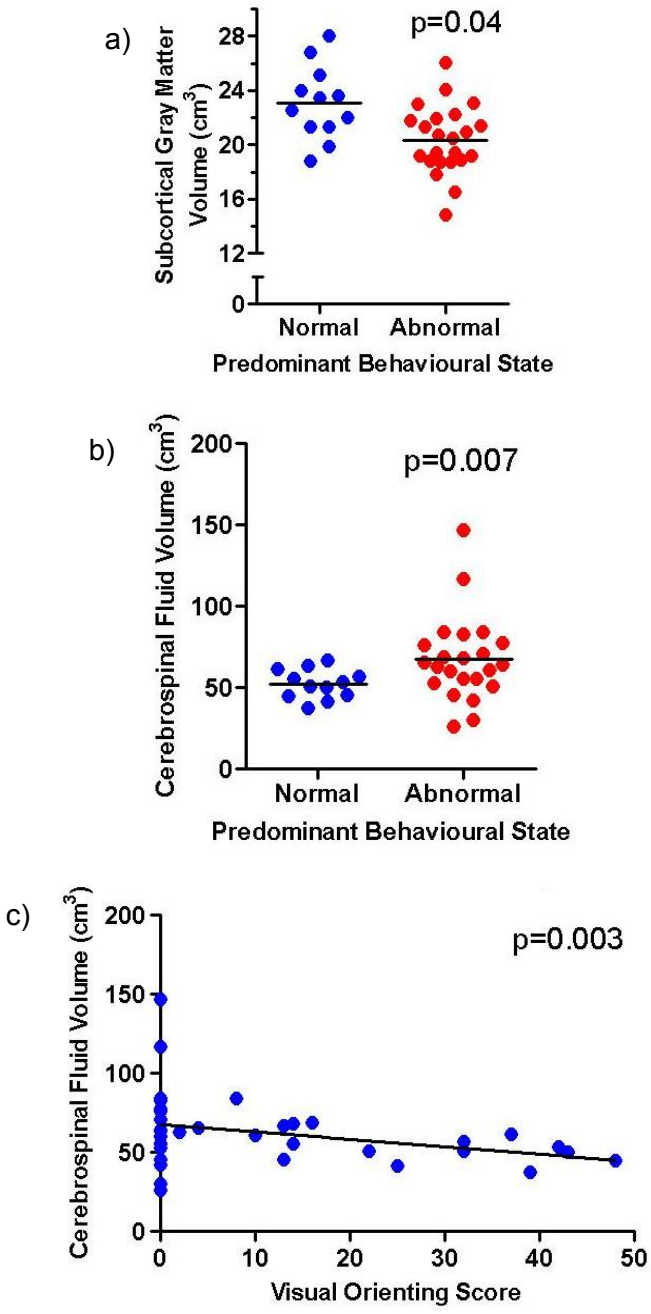

Figure 3. Associations between neurobehavioural performance and tissue-specific brain volumes for all newborns. a) Relationship between subcortical gray matter volume and predominant behavioural state, b) relationship between cerebrospinal fluid volume and predominant behavioural state, c) relationship between cerebrospinal fluid volume and visual orienting score.

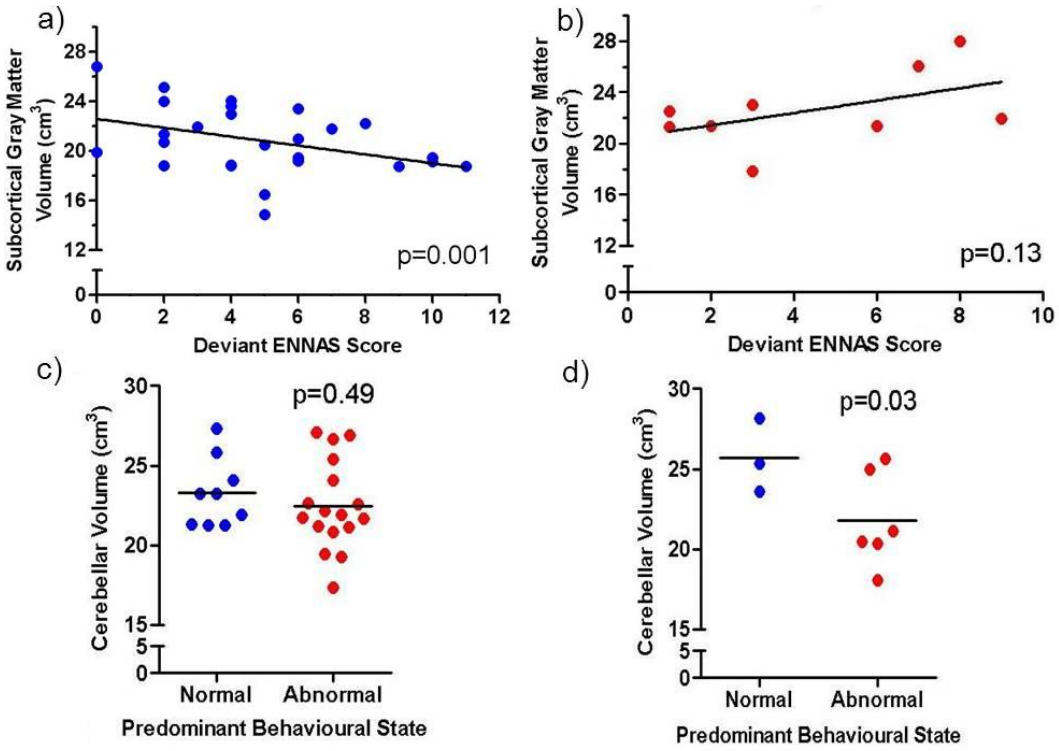

Figure 4. Associations between neurobehavioural performance and tissue-specific brain volumes for cyanotic versus acyanotic newborns. Relationship between subcortical gray matter volume and deviant ENNAS scores in a) cyanotic and b) acyanotic newborns. Relationship between cerebellar volume and predominant behavioural state in c) cyanotic and d) acyanotic newborns.

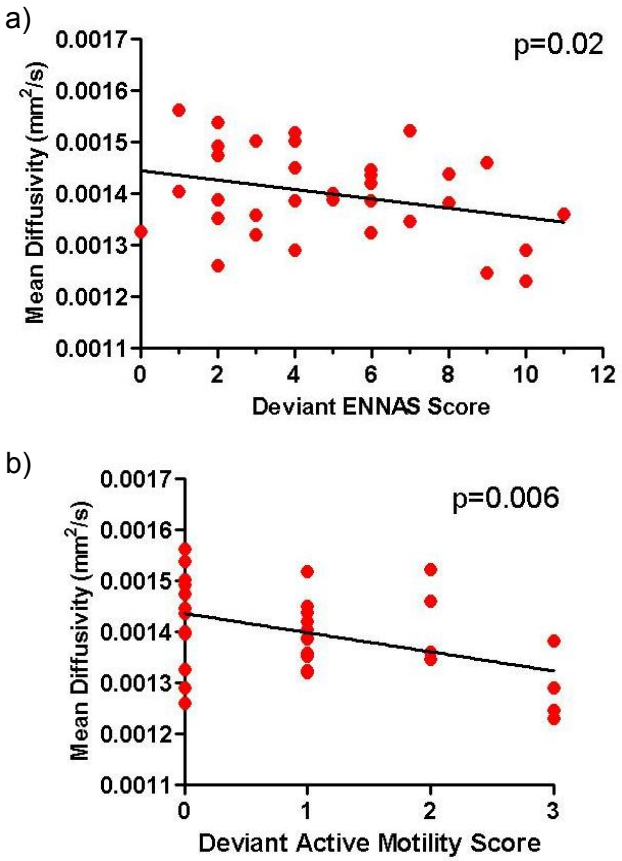

Figure 5. Associations between mean diffusivity in the optic radiations and neurobehavioural performance for all newborns. Relationship between mean diffusivity in the optic radiations and a) deviant ENNAS scores, and b) deviant active motility scores.

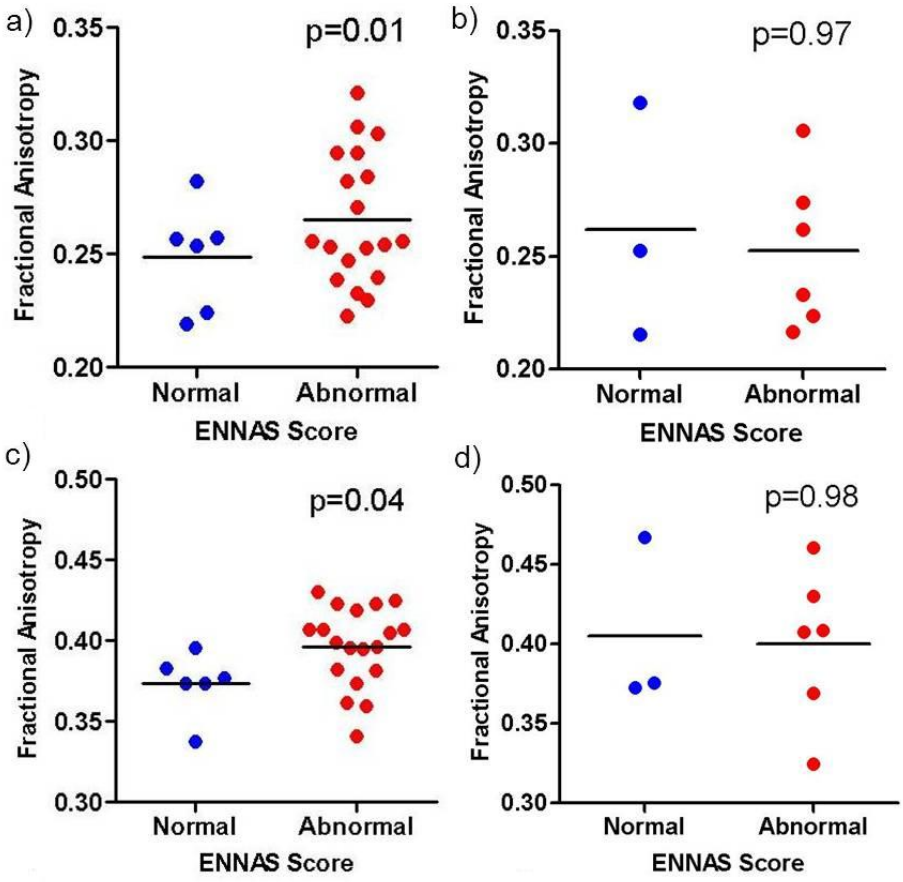

Figure 6. Associations between neurobehavioural performance and fractional anisotropy values for cyanotic versus acyanotic newborns. Relationship between FA in the optic radiations and ENNAS score in a) cyanotic and b) acyanotic newborns. Relationship between FA in the corticospinal tracts and ENNAS score in c) cyanotic and d) acyanotic newborns.

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CHAPTER 4
DISCUSSION

The relationship between neurobehavioural performance and brain development in newborns with complex CHD has been described for the first time within this thesis. With the use of a standardized measure of neurobehaviour and advanced, quantitative MRI techniques, this study compares newborn neurobehavioural status to brain structure and microstructure under a uniquely objective lens. Each specific aim yielded results pointing to clear associations between the neurobehavioural status and underlying brain growth of these critically ill newborns. To begin with, upon analyzing the relationship between brain volumes and neurobehaviour, an association was determined between reduced SCGM volume and inability to regulate behavioural state; likewise there were associations between increased CSF volume and poor behavioural state regulation as well as diminished capacity for visual orienting. Stratification by the general heart defect classifications of cyanotic and acyanotic established each of these relationships to be exhibited primarily by newborns with cyanotic defects. These newborns also displayed a strong relationship between SCGM volume and overall ENNAS scores. Conversely, newborns with acyanotic defects showed an association between reduced cerebellar volume and abnormal behavioural state. The analyses addressing our second specific aim, relating white matter mictrostructure to ENNAS scores, revealed several surprising results. There were associations between reduced D_{av} in the optic radiations and poor overall performance as well as abnormal active motility on the ENNAS. Acyanotic newborns were chiefly responsible for the association between reduced D_{av} in the optic radiations and abnormal active motility, while cyanotic newborns instead showed relationships between increased FA values in the optic radiations and corticospinal tracts and abnormal ENNAS scores. Taken together, these results begin to define the relationship between brain structure and function in a population of newborns that is at risk for neurologic compromise throughout gestation and into the early postnatal period.

4.1 Significance

Over the past two decades, a wealth of evidence has fostered a growing recognition of the high prevalence of preoperative neurologic abnormalities in

newborns with complex CHD, abnormalities which encompass both developmental delays and irregularities on neuroimaging[11,13–16,57,59,60]. In spite of this emergent awareness, little is known about the relationship between brain structure and function in the early postnatal period. Indeed, two previous studies have addressed this question only briefly, as a secondary consideration in works concentrating primarily on neuroimaging findings[16,65]. Neither of these studies observed a relationship between traditional neurological examinations and brain injury on MRI[16,65]. The present work, through focused investigation of this structure-function relationship, has revealed distinct associations between neurobehavioural performance and tissue-specific brain volumes as well as white matter microstructure at this early stage.

The research presented within this thesis offers several contributions to an evolving field. First, the selection of powerful, objective, and non-invasive methods provide an in-depth look at the developing neonatal brain. Advanced, quantitative neuroimaging tools shift the focus of this work away from overt injury, the focus of previous studies, to signs of neurologic impairment which are below the threshold of subjective neuroradiologic detection. To measure global and tissue-specific brain volumes, 3D volumetric MRI was selected as a reliable and accurate approach which was further enhanced by the use of an automatic segmentation program validated in newborns and utilized in other studies of high-risk infants[71,72]. To evaluate the microstructure of the developing white matter, tract specific values were selected over ROI-based values as they provide a more sensitive measurement than the latter, accounting for the length of the entire tract rather than an isolated section[70,73]. Finally, to assess neurobehavioural status, the ENNAS was chosen over other neurologic and neurobehavioural assessments for its comprehensive yet efficient approach, as well as the objectivity afforded by standardized scoring[74]. Each of these modalities allowed for deeper insight into neonatal brain development and behaviour and consequently led to newfound associations between structure and function.

Second, this study shows specific relationships between brain tissue volumes and neurobehavioural performance, in particular, behavioural state regulation and visual orienting. Although no study to date has examined this relationship in newborns with CHD, studies of premature newborns have highlighted similar associations[75–78]. Brown et al[75] assessed premature infants at term equivalent age using the Revised Hammersmith Neonatal Neurological Examination (HNNE) and

a systematic approach to classifying white matter abnormalities which took into account measures of volume loss. The authors reported an association between increasingly severe white matter abnormalities and diminished overall performance on the HNNE, as well as lower scores for spontaneous movements and behaviour. A strong, independent association was also reported between white matter volume loss and lower behaviour scores on the HNNE. In related studies, prematurely born infants with abnormal general movements at one and three months of age demonstrated more severe abnormalities within the white matter (including white matter volume loss and corpus callosum thinning) as well as a smaller cerebellar diameter at term equivalent age[78,79].

Later neurodevelopment also appears to be reflective of reduced tissue volumes at term. In a study of very low birth weight infants, moderate to severe neurodevelopmental disability at 1 year of age was associated with reduced gray matter volume (SCGM and CGM), and increased CSF volume[76]. Studies using the Bayley Scales of Infant Development[80] in preterm-born children at 2 years of age described relationships between poor cognitive[81,82] and motor[82] performance and reduced cerebellar volumes. Abnormal motor performance was also associated with reduced thalamic volume[82]. Similarly, preterm infants with low motor scores on the Bayley Scales at 6 months of age showed lower SCGM volume at term equivalence[83]. In one study, reductions in total brain volume, cerebral volume, cerebellar volume, frontal lobes, basal ganglia and thalami, as well as larger ventricles were correlated with a range of potential neurodevelopmental impairments at 2 years of age in infants that were born preterm[77]. The results of the present study support these findings from a similarly high-risk population.

Third, the current work highlights striking associations between neurobehaviour and white matter microstructure. This is the first report of such relationships in newborns with CHD, and while the direction of the associations observed in this study were somewhat surprising (with restricted diffusion and increased anisotropy relating to poor neurobehavioural status), they were in keeping with similar associations observed in newborns with HIE[84–87]. According to two independent studies, HIE infants with unfavourable neurologic outcomes had significantly reduced ADC values in various brain areas when imaged within either the first four or six days of life compared to those with better outcomes[85,87]. Similarly, in one study which imaged newborns with HIE at a mean age of 5.6 days, those that

went on to develop severe disability had significantly lower ADC values within the PLIC than those with mild or moderate outcomes[84]. The findings from the present study suggest an analogous relationship, begging further study for corroboration and to uncover the underlying mechanisms.

Fourth, by detecting relationships between neonatal neurobehaviour and brain volumes as well as microstructure, this study contributes to a broader understanding of preoperative brain injury in newborns with CHD and provides the beginnings of a foundation on which to base decisions for appropriate early screening regimens. The results of this study suggest an abnormal neurobehavioural assessment (in particular, abnormal behavioural state regulation, visual orienting, and active motility) may be indicative of underlying structural and microstructural abnormalities. In facilities where routine evaluation with MRI is not feasible, performing simple, bedside standardized neurobehavioural assessments may help to determine which patients require further neuroradiological examination and developmental follow-up.

Last, and perhaps most importantly, the results of this thesis provide a platform for further inquiry. In view of the absence of previous research on this subject, all results which demonstrated potential statistical significance ($p<0.05$) were reported. With this approach, we hope to inspire new and interesting hypotheses which will further enhance our understanding of the neurologic status of newborns with complex CHD prior to cardiac surgery.

4.2 Limitations

Although this study has many strengths, the limitations of our approach and consequently of our conclusions deserve mention. To begin, the inclusive, prospective study design evaluates the neurologic compromise of newborns with a range of complex CHD, however the heterogeneous nature of this cohort may have diluted the effects of particular lesion types. Previous studies have shown, for instance, that newborns with transposition of the great arteries are more likely to show injury on MRI than those with single ventricle physiology[17,69]. To account for some of these differences in cardiac physiology, we stratified the cohort by the broad classifications of cyanotic and acyanotic defects, and subsequently found different patterns of association between these groups.

The assessment of neurobehavioural status, while standardized in the form of the ENNAS, was limited by the degree of illness in some newborns which prevented the administration of certain items. Instances which were more common included the preclusion of prone items for newborns that were intubated, and of visual orienting items for newborns that could not open their eyes due either to generalized edema or to a marked inability to regulate behavioural state. Indeed, poor behavioural state regulation was common among this cohort, and this occasionally affected the efficacy of the ENNAS which was designed to be administered on newborns in an optimal state of alertness[18]. Although every effort was made to soothe irritable newborns or further arouse those that were drowsy, the failure to do so in some cases may reflect central nervous system immaturity or an unknown cause and deserves further study. It is possible that the necessary abbreviation of the assessment for some newborns meant certain abnormalities remained unaccounted for, potentially lowering the overall deviant score for those infants. It is also possible that the resulting conservative estimate of fewer abnormal assessments may have been counteracted by the tendency of the ENNAS towards a high prevalence of false positives. We acknowledge that each of these limitations is inherent to neurobehavioural assessments, especially when employed in the evaluation of critically ill newborns.

The advanced methods selected for the computation of brain tissue volume and white matter microstructure were not without their own limitations. The automatic segmentation program[71] used to determine tissue-specific brain volumes performed well, but still required manual correction in order to provide accurate characterization of all tissue classes. The consequence to the introduction of a manual component to this process was the potential for examiner bias, though with the exception of mWM, the intra-rater reliability for this study was high. All ROIs for the fibre-tracking analysis were drawn manually and therefore may also have introduced bias, although the tract specific approach used in this study better accounts for inter-subject variability than single ROI-based values[70]. Furthermore, in the present study, the intra-rater reliability was very high for all FA and D_{av} values. The acquisition of DTI data in newborns has other challenges which are more difficult to overcome, however, including the larger voxel size which results from a small head and relatively large slice thickness; this introduces an increased potential for fibres to cross within voxels, thereby increasing the level of "noise" in the data.

Moreover, precise midline head positioning, crucial for the alignment of fibre tracts, proved to be a challenge in this very young and critically ill cohort. Additionally, in this study, we elected to collapse the right and left corticospinal tracts and optic radiations, which may have masked effects of laterality.

It is also important to acknowledge the multiple analyses conducted within this work in order to generate future hypotheses, as this was the first study of its kind. The results reported here reflect potential significance based on probability values of less than 0.05, although with statistical correction, certain associations may not have been included. The resulting associations should be interpreted with appropriate care.

4.3 Future Directions

This study has the potential to provide a basis for future research into the relationship between brain structure and function in newborns with CHD. Looking ahead, the targeted evaluation of the relationships observed here may lead to a better understanding of the connection between brain and behaviour in this population. Future studies incorporating larger sample sizes permitting subgroup analyses based on heart lesion type, or alternatively, smaller studies which include only one lesion type, would provide a more precise look at the possible mediating effect of cardiac physiology on neurological structure-function relationships. The specific associations observed under the current approach were unanticipated; although we did not harbour *a priori* hypotheses, previous studies of other populations defined patterns presumed to be possible outcomes for the present cohort. We were interested to note, for example, that the volume of CSF maintained a central role in the associations observed in this study, without corresponding associations featuring TBV or CGM as seen in studies of premature infants[76,77]. Future studies might employ ratios of TBV to intracranial volume to determine whether TBV shows a greater interaction with neurobehaviour under these conditions. The increased FA values which related to poorer overall neurobehavioural performance provoked further questions; as higher FA values are typically interpreted as evidence of greater white matter organization, it is difficult to determine the reason for these elevated FA values. Further study is required to understand this particular interaction.

Perhaps the most useful research to follow from this work would be studies which investigate whether the relationships observed here persist in the long-term. This knowledge will lend significance to the associations observed within the

newborn period, and inform future research as well as prospective screening and intervention guidelines. Currently, a longitudinal study is underway which will help contribute to our understanding of the interaction between brain and behavioural development in this cohort with complex CHD.

4.4 Conclusions

Prior to cardiac surgery, newborns with complex CHD demonstrate a number of neurologic sequelae. Abnormalities range from acquired injury (e.g. white matter injury, stroke) and altered cerebral metabolite levels (e.g. presence of lactate), to delayed growth (e.g. reduced brain volumes, open operculae) and microstructural impairment (e.g. increased mean diffusivity, decreased fractional anisotropy), to myriad neurobehavioural deficits (e.g. hypotonia, absent suck, poor behavioural state regulation). The review of the literature which commences this thesis, as well as the results presented within the body of this work underscore the prevalence of neurologic compromise within this group. Despite this accumulation of knowledge, very little is known about the relationship between abnormal brain structure and neurobehavioural status. This thesis presents new information which highlights several associations between tissue-specific brain volumes as well as white matter microstructure and neurobehavioural performance on the ENNAS. From these findings it can be inferred that abnormalities observed during a clinical assessment of neurobehaviour may be suggestive of underlying structural or microstructural impairment and could potentially be used to distinguish infants that might benefit from further evaluation. For this high-risk population, the implementation of early neurologic screening and developmental intervention is essential in the effort to minimize long-term disability, optimize functional outcomes, and improve quality of life. The present study, along with future research embracing similar aims, may be used to guide the implementation of such early screening endeavours and future neuroprotection strategies.

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