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Monitoring intraventricular hemorrhage in preterm infants

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

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Abstract

Germinal Matrix-Intraventricular hemorrhage (GMH-IVH) remains a significant cause of adverse neurodevelopmental outcomes in preterm infants. Current management options for GMH-IVH rely on serial 2-dimensional cranial ultrasound (2D cUS) ventricular measurements and clinical signs. A need exists for reliable biomarkers to aid in the early detection of posthemorrhagic ventricular dilatation (PHVD) and cerebral palsy (CP). We incorporated 3-dimensional cranial US (3D cUS) and functional infrared spectroscopy (fNIRS) to monitor ventricle volumes (VV) and spontaneous functional connectivity (sFC) in preterm infants with GMH-IVH. Infants with severe GMH-IVH who underwent cerebrospinal fluid diversion showed larger VV, which correlated with decreased sFC. Our findings of increased ventricular volume in preterm neonates and reduced fNIRS-based functional connectivity suggest that regional disruptions of ventricular size may impact the development of the underlying grey matter. Findings suggest that 3D cUS and fNIRS are promising bedside tools for monitoring the progression of GMH-IVH in preterm infants.

Summary for Lay Audience

Despite improved care, infants born early still face complications related to being born prematurely. One of these complications is germinal matrix-intraventricular hemorrhage (GMH-IVH) or bleeding into the infant's lateral ventricles of the brain (a space in the brain prone to bleeding). The blood can block the reabsorption of fluid within the ventricles, called cerebrospinal fluid (CSF), which can lead to enlargement of the ventricles. Among infants born early, some key adverse long-term outcomes of GMH-IVH include difficulties with movement (cerebral palsy) and learning. This means that children with GMH-IVH may be more likely to experience early diversity at school-going age than children without. Once born, during their admission to the neonatal intensive care unit (NICU), these preterm infants undergo screening for GMH-IVH. Clinicians rely on 2-dimensional cranial ultrasound (2D cUS) to measure the size and shape of the ventricles and clinical signs. However, these tools are inadequate. A gap exists for more reliable tools to aid in the early detection of ventricular dilatation after GMH-IVH to allow for early intervention. Our study used a combination of bedside tools: three-dimensional cranial US (3D cUS) - to monitor the ventricle volumes (VV); and functional infrared spectroscopy (fNIRS) - to monitor oxygen delivery to the brain. We found that increased ventricle volumes and impaired oxygen delivery to the infant's brain among infants with GMH-IVH suggest impairment to the growing brain. Our findings suggest that 3D cUS and fNIRS are promising bedside tools for monitoring the progression of GMH-IVH in preterm infants. These bedside tools provide additional information regarding the structure and function of the preterm infant's brain.

Keywords

Geminal Matrix-Intraventricular Hemorrhage

Preterm Infants

Three-Dimensional cranial Ultrasound

Functional Infrared Spectroscopy

Co-authorship statement

Chapter 2 contains a manuscript titled “Three-dimensional cranial ultrasound and functional near-infrared spectroscopy for bedside monitoring of intraventricular hemorrhage in preterm infants”. A manuscript version is currently being considered for submission for peer review. The co-authors are Kevin Stubbs, Marcus Lo, Sarah Abu Al-Saoud, Bradley Karat, Keith St Lawrence, Sandrine de Ribaupierre, and Emma G. Duerden. The study conception was by Drs. de Ribaupierre and Duerden. The data were collected by Marcus Lo, Sarah Abu Al-Saoud and Bradley Karat. Kevin Stubbs was involved in pre-processing of the fNIRS data. I, Lilian Kebaya, was involved in the study design and conception, data collection and analysis, and manuscript writing. All my co-authors provided valuable edits and feedback on the manuscript.

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List of Abbreviations

AAP: American Academy of Pediatrics

CBF: Cerebral blood flow

CPS: Canadian Paediatric Society

CP: Cerebral Palsy

CSF: Cerebrospinal fluid

CT: Computed Tomography

LBW: Low Birth Weight

ELBW: Extremely Low Birth Weight

ELGA: Extremely Low for Gestational Age

fNIRS: Functional Near Infrared spectroscopy

GA: Gestational age

GM: Germinal matrix

ICP: Intracranial Pressure

IVH: Intraventricular hemorrhage

LHSC: London Health Sciences Centre

MRI: Magnetic Resonance Imaging

NDI: Neurodevelopmental impairment

PVHD: Posthemorrhagic ventricular dilatation

PVHI: Periventricular hemorrhagic infarction

RDS: Respiratory distress syndrome

TEA: Term equivalent age

US: Ultrasound

VLBW: Very Low Birth Weight

WHO: World Health Organization

Chapter 1

1.1 Introduction

Germinal matrix-intraventricular hemorrhage (GMH-IVH) remains a significant cause of death and disability in very preterm neonates. (1, 2) In the past two decades, perinatal and neonatal improvements have led to increased survival of very preterm neonates and an overall reduction in severe forms of GMH-IVH. That said, however, with more extremely low birth weight (ELBW) neonates (< 1000g) surviving, there has been an upward trend in the incidence of severe forms of GMH-IVH in this population, with up to one-half developing severe GMH-IVH. (3) Posthemorrhagic ventricular dilatation (PHVD) is a common complication of severe forms of GMH-IVH. (4) Untreated PHVD, characterized by cerebrospinal fluid (CSF) accumulation and progressive dilatation of the ventricles, can result in death and neurodevelopmental impairment (NDI), specifically, cerebral palsy (CP), cognitive disability and epilepsy. (5, 6)

1.1.1 Germinal matrix-intraventricular hemorrhage: how does brain injury occur?

The germinal matrix (GM) - the origin of GMH-IVH - comprises a highly cellular and vascular network. In early fetal life, the GM plays a vital role in neuronal maturation. It is also where cells migrate out from (to form the layers of the cerebral cortex and deeper nuclear structures), with migration complete by 26 weeks gestation. (7-9) After that, the GM involutes around 32 weeks gestation to term equivalent age (TEA). (10) This is why GMH-IVH remains rare in term infants. (11) The structural fragility of the germinal matrix is due to a fragile basal lamina, a structure that confers compliance properties, enabling autoregulation; decreased expression of pericytes and glial fibrillary acidic protein (GFAP). (12, 13) In the presence of insults, such as hypoxia or systemic hypotension, the aforementioned structural vulnerabilities provide fertile ground for rupture and progression to GMH-IVH in preterm infants. (14)

Animal and human studies confirmed the role of blood and its products in the formation of GMH-IVH. (15-17) The breakdown of red blood cells with the subsequent release of hemoglobin and iron forms the basis of injury. (18) Hemoglobin and iron are taken up by neurons and microglial cells, triggering injury through oxidative stress, glutamate toxicity and inflammation. Plasma

components of blood, such as thrombin, activate inflammation and gliosis. Further, studies conducted in the premature neonatal brain with GMH-IVH show the following changes: disruption of the ventricular zone; impaired proliferation, maturation and renewal of oligodendroglial and neuronal precursor cells; axonal injury and microglial activation. (10, 19, 20) Over time, blood clots hinder CSF flow in the ventricular system, with subsequent development of hydrocephalus. (18)

1.1.2 Germinal matrix-intraventricular hemorrhage: risk factors

The World Health Organization (WHO) defines "preterm birth" as a live birth occurring < 37 weeks gestation. (21) Prematurity remains a global problem, as fifteen million babies are born early annually, accounting for 11% of total births. (22) In Canada, 8% of all pregnancies result in preterm birth. (23) Worldwide, prematurity significantly contributes to death in children < 5 years of age. (24) The incidence of GMH-IVH is disproportionate to the gestational age (GA), with one in every fifth very preterm neonate will develop GMH-IVH and half of the neonates < 26 weeks GA developing severe forms of GMH-IVH. (3)

Obstetrical and neonatal care advances have undoubtedly led to improved survival of very preterm infants. (25) Antenatal strategies (antenatal corticosteroid therapy, timely treatment of maternal infections) and postnatal strategies (delayed cord clamping, neonatal care bundles) have led to decreased complications related to prematurity, particularly GMH-IVH. (26-29)

Prematurity presents a delicate transition period for neonates, predisposing their immature organs and systems to various postnatal complications and comorbidities. For instance, premature neonates are more likely to undergo aggressive resuscitation at birth than their term counterparts. (10) During their neonatal intensive care unit (NICU) stay, preterm infants have poorer weight gain secondary to feed intolerance and gut immaturity, which can hinder optimal brain growth. (30) Preterm infants are also likely to develop infections due to their prolonged hospital stay and immature immune defense - another contributor to NDI. (31)

Dysfunction of cerebrovascular autoregulation (a physiologic system that maintains stable cerebral blood flow) is also implicated in the development of GMH-IVH in the preterm brain. (32) To begin

with, the immature vessels in the germinal matrix have limited autoregulation capacity. Hence, conditions contributing to fluctuations in cerebral blood flow (CBF), e.g., vaginal birth, respiratory distress syndrome (RDS), hypo/hypertension, pneumothorax, hypoxia, hypo/hypercapnia, seizures, patent ductus arteriosus (PDA), infections, are critical risk factors for the development of GMH-IVH. (12, 33)

Lastly, maternal factors, such as pre-pregnancy obesity, infections, extremes of maternal age, illnesses and placental factors, are associated with the development of GMH-IVH. (34-37)

1.1.3 Grading of GMH-IVH and outcomes

Cranial ultrasound (cUS) is the most used tool for screening GMH-IVH. (2, 38-40) Table 1 captures the two grading systems, starting with the Papile grading system (grade I-IV, with grade I - mild and grade IV - most severe). (41) Volpe reclassified the Papile grade IV IVH to periventricular hemorrhagic venous infarction (PVHI), chiefly to distinguish that grade IV was not a progression of grade III IVH but a different entity altogether. (42) Grade I hemorrhage is restricted to the GM. Papile classified hemorrhage extending into the lateral ventricles as grade II (without ventricular dilatation) and grade III (in the presence of ventricular dilatation).

In contrast, Volpe classified grade II IVH as IVH with 10-50% of the lateral ventricle filled with blood (without ventricular dilatation) and graded III IVH - as IVH with >50% of lateral ventricle filled with blood (with ventricular dilation). (1) The most severe form - grade IV, now PVHI - results from a venous stroke affecting the periventricular white matter. (43) Severe grades (grade III-IV) are often associated with poor outcomes, including death and NDI. (43) (Figure 1)

Table 1 GMH-IVH grading

Severity	Papile	Volpe
Grade 1	Germinal matrix hemorrhage	Germinal matrix hemorrhage with or without IVH (< 10% of ventricle filled with blood)
Grade 2	IVH without ventricular dilation	IVH (10%–50% of ventricle filled with blood) typically without ventricular dilation
Grade 3	IVH with ventricular dilation	IVH (>50% of ventricle filled with blood) typically with ventricular dilation
Grade 4	IVH with ventricular dilation and parenchymal hemorrhage	Periventricular hemorrhagic infarction

Mild IVH is generally defined as grade 1 or 2, and severe IVH is generally defined as grade 3 or 4.

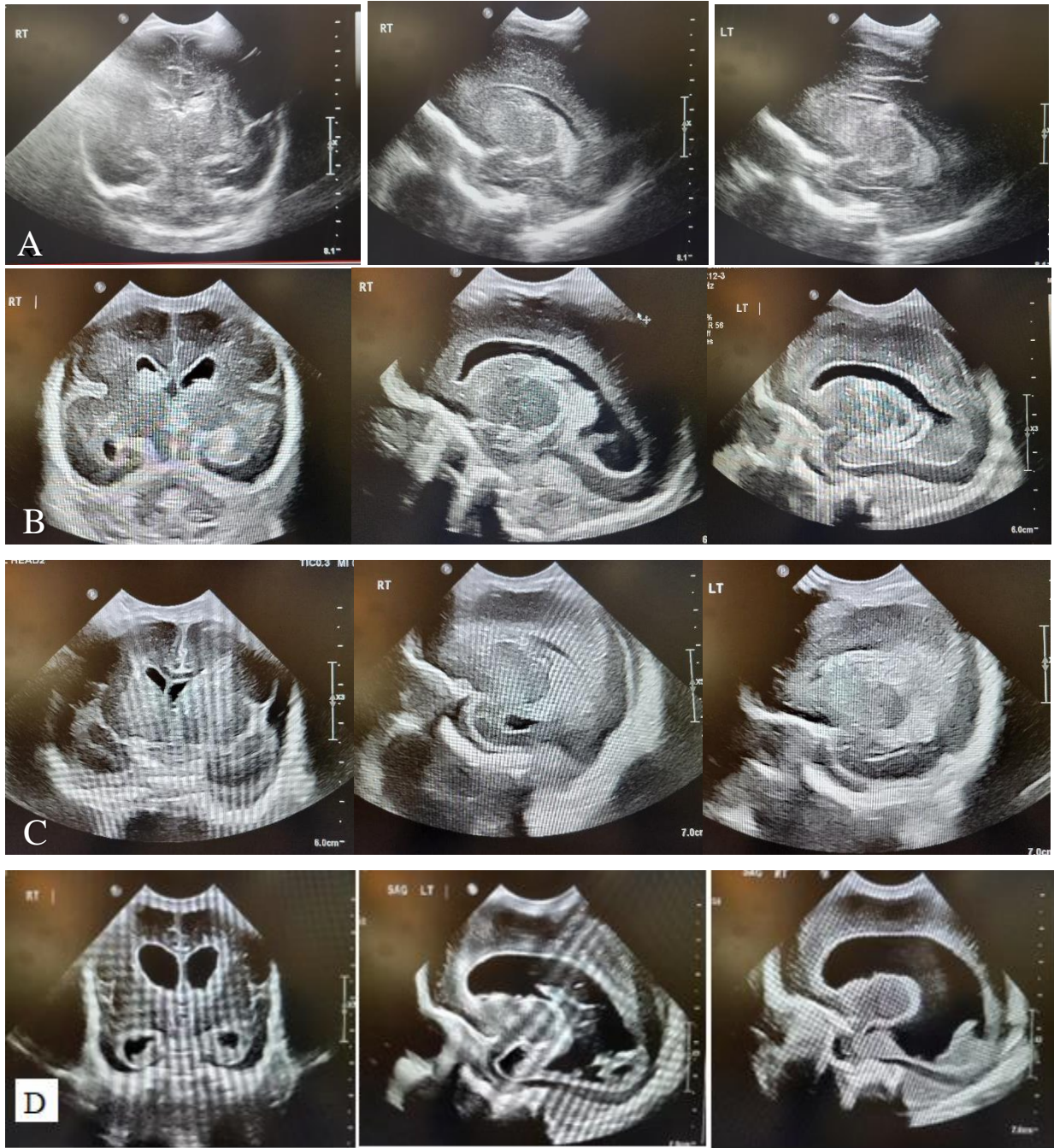


Figure 1 GMH-IVH grading

A: Coronal and sagittal cUS scans of a preterm neonate, born at 26 weeks and 6 days, and scanned on day 7 of life, with left grade I GMH-IVH; **B:** coronal and sagittal cUS scans of a preterm neonate, born at 26 weeks and 6 days, and scanned on day 7 of life, with bilateral grade II GMH-IVH; **C and D:** Coronal and sagittal cUS scans of a preterm neonate, born at 25 weeks and 3 days, with grade bilateral III GMH-IVH, and PHVI on the left. Repeat scan at 1 week of life shows acute distension of the lateral ventricles (PHVD).

GMH-IVH occurs during the first week of life, with half of all GMH-IVH cases occurring on the first day. (1, 44) Up to 90% of GMH-IVH cases occur by the third day. (45, 46) The likelihood of onset of hemorrhage on the first day of life was inversely related to birth weight, with more than half of all bleeds seen on the first day in neonates weighing < 700 g. (1) Progression of GMH-IVH occurs in 20% to 40% of affected neonates, with the maximal extent of GMH-IVH seen within 3 to 5 days of the initial diagnosis. (1) Recent evidence shows that GMH-IVH can occur antenatally, although most had coexisting conditions. (47, 48)

Overall, outcomes in very preterm infants with mild GMH-IVH (grade I and II), including death and morbidity, result from prematurity and related conditions rather than the GMH-IVH. Hence, these outcomes are similar to those of the general population born at similar GA. (49) On the contrary, severe GMH-IVH (grade III and PHVI) is associated with a higher death rate of up to 40%. (50) For the survivors, Hollebrandse et al. showed higher rates of NDI in motor function and intellectual ability at school-age in ELBW infants with severe GMH-IVH. They also showed a higher rate of CP but not intellectual ability in infants with mild GMH-IVH. (51)

1.1.4 Neuroimaging modalities for detecting and monitoring GMH-IVH

Cranial ultrasound (cUS), computed tomography (CT) and brain magnetic resonance imaging (MRI) are the primary neuroimaging tools for demonstrating brain structure. Of the three modalities, cUS is the most widely used at the bedside in NICUs. Despite its high resolution, brain MRI is not the first choice due to high costs and impractical use at the bedside. On the other hand, a CT scan generates radiation; hence it is generally only recommended in neonates for specific and emergent purposes.

Two-dimensional cranial ultrasound imaging (2D cUS)

With an overall GMH-IVH incidence of 25% amongst very preterm neonates, the Canadian Paediatric Society (CPS) and the American Academy of Pediatrics (AAP) recommend routine cUS for all neonates born $\leq 31+6$ weeks' GA. The first cUS is performed in the first 4-7 days to detect GMH-IVH, and a repeat cUS at 4 to 6 weeks of age to detect white matter injury (WMI). (52, 53) Imaging protocols for most NICUs also perform cUS at term equivalent age (TEA) for the

extremely low for gestational age (ELGA) infants (< 26 weeks' GA). Recent evidence recommends more frequent scanning in neonates for timely recognition of GMH-IVH and complications. (54-56)

cUS is highly sensitive in detecting GMH-IVH. (39) cUS imaging uses the fontanelles (anterior and mastoid) to provide windows into the neonatal ventricular system, white matter and cerebellum. (52). cUS provides a safe, quick, easy and portable bedside assessment of GMH-IVH with little stress for very preterm infants. (4, 55)

Limitations of cUS include dependency of image quality on the operator. (4, 57, 58) Compared to MRI, cUS is less sensitive in detecting WMI, cerebellar and low-grade bleeds. (55) Another cUS limitation is its inaccuracy in predicting long-term neurodevelopmental outcomes: with mild GMH-IVH linked to NDI and severe GMH-IVH – good outcomes; inconsistencies which were attributed to the inability of cUS to take into account WMI, and in the latter – site and extent of the lesion. (59) Moreover, cUS cannot predict sensorineural deficits and learning disabilities, much more common problems among high-risk neonates with GMH-IVH than the development of CP. (51) Another limitation of the cUS is the inability to provide accurate lateral ventricular volume measurements – an area of ongoing research. (54)

Brain magnetic resonance imaging

Compared to cUS, MRI has the advantage of high resolution and contrast of inner structures. Brain MRI provides more details to quantify and grade hemorrhage and other associated abnormalities, including WMI and cerebellar hemorrhages. (55) MRI is also superior to cUS for predicting neurodevelopmental outcomes in preterm infants. (60) Despite this, controversy remains regarding early MRI before TEA in preterm infants for various reasons. First, it can be challenging to maintain stability during an MRI examination. There are potential risks during the transport of unstable and critically ill infants (from NICU to the radiology department for imaging), infant movement and sometimes, the need for sedation. (53) Besides, MRI is also time-consuming, expensive and reliant on expertise for interpretation. Moreover, MRI cannot be easily repeated for monitoring and follow-up. Recently, some NICUs are embracing in-NICU MRI scanners to scan

preterm infants at the most significant risk for brain injury. (61) Early MRI scans are helpful during counseling with parents regarding outcomes.

Computed tomography

CT is limited in identifying GMH-IVH in preterm infants, primarily due to concerns about ionizing radiation's effect on the neonatal brain. (62) Second, the high-water content of neonatal brains makes image interpretation difficult. Similar to MRI, it would require transporting the neonate from the NICU to the CT scanner - a cumbersome exercise for the sick, very preterm neonates. Nevertheless, a head CT scan is the ideal neuroimaging tool in neonates for the following conditions: acute trauma, for detecting acute injuries such as skull fractures, posterior fossa and intracranial bleeds; in cases of VP shunt dysfunction; intracranial calcifications resulting from infective, genetic or vascular causes; or, meningitis for detecting complications.

1.1.5 Posthemorrhagic ventricular dilatation: how it occurs, monitoring, management, and outcomes

Approximately 25% of infants with GMH-IVH develop PHVD. The risk of PHVD is higher following severe GMH-IVH (grade III GMH-IVH or PHVI). (63) During prematurity (a time of rapid brain development), the pre-oligodendrocyte (pre-OL) susceptibility to injury, coupled with the activation of pro-inflammatory immature microglia and astrocytes, forms the basis of injury in PHVD. Hence, injury happens due to mechanical and inflammatory insults, disrupting normal CSF production, flow and absorption. (64, 65) Ventricular dilatation begins with the hemorrhage, leading to compression of the adjacent white matter and ischemia. PHVD develops following obstruction in CSF flow, resulting in increased ICP, decreased blood flow to the cortex, axonal disruption, and WMI. (66) Untreated ventricular dilatation is detrimental to the developing brain, with death or severe disability.

The degree of PHVD is based on 2D cUS ventricular measurements: Ventricular Index (VI), Anterior Horn Width (AHW) and Thalamo-Occipital Distance (TOD). (67) Levene produced reference ranges for the VI (distance measured in the coronal plane at the level of the foramen of Monro, from the falx to the lateral wall of the body of the lateral ventricle) according to GA. (68)

Hence, VI > 97th centile for the given GA is considered high. Other reference values exist for lateral ventricular size. (67, 69, 70) Despite this standardization, inconsistencies exist regarding the reference values, particularly in ELBW neonates. First, the VI is often increased in more severe ventricular dilatation and thus may fail to identify neonates with mild dilatation. Second, the occipital horns are often more dilated than the frontal and temporal horns. For these reasons and with the increased survival of ELBW neonates, Goeral et al. established new reference charts based on ELBW. (71)

Besides cUS measurements, clinical measurements indicative of increasing ICP are helpful the monitoring and managing PHVD: splayed sutures, increasing head circumference, tense anterior fontanelle, vomiting, and cardio-respiratory changes. Though reliable, physical exam findings often present late. Moreover, clinical measurements may vary depending on the user, in addition to poor correlation between head size and ventricle size on cUS. All these factors could delay CSF diversion if needed. (72-74)

PHVD develops 1-3 weeks following GMH-IVH diagnosis and can progress either slowly or rapidly. Amongst slow progressors, PHVD eventually resolves (40% spontaneously and 15% after non-surgical treatment). (5) Conversely, 35% of infants with rapid progression in ventricular dilatation will require surgical treatment, and another 10% will die. (6) Rapid progression is related to the severity of GMH-IVH, with the onset of signs of increased ICP within days to a week. (55) Rapid progression is usually secondary to impaired CSF absorption caused by a blood clot. Long-term, PHVD is associated with poor outcomes, specifically CP, cognitive dysfunction and epilepsy.

Removal of CSF may improve neurodevelopmental outcomes by decreasing ICP and brain oedema and restoring normal CSF drainage. The definitive treatment of PHVD is the placement of a ventriculoperitoneal (VP) shunt. However, due to the associated risk of complications (blockage, infections, amongst others) in preterm infants, temporizing CSF diversion procedures during the neonatal period are preferred until TEA or when safe to place a VP shunt. Sometimes, temporizing CSF-diversion procedures have prevented the need for a VP shunt. Temporizing CSF-diversion procedures for progressive PHVD include lumbar puncture (LP), ventricular access device (VAD), ventricular tap (VT), and an external ventricular drain (EVD). A practice variation exists amongst

paediatric neurosurgeons regarding preferred temporizing procedures. (75) None of these temporizing CSF procedures has proved superior. (76) Also, controversy among specialists remains regarding the timing of intervention for PHVD, with most centres leaving the decision to the discretion of the treating paediatric neurosurgeon. That said, recent studies favour an early approach to a later-timed approach. (77, 78) In 2020, El-Dib et al. proposed new guidelines for managing PHVD that have since been adopted in most NICUs. (63)

1.1.5 Existing controversies in the management of GMH-IVH and PHVD

In summary, the survival of very preterm infants has improved in the past two decades. Similarly, there has been an overall decline in the GMH-IVH cases. All these gains result from neonatal and perinatal advancements, including the establishment of neonatal care bundles and standardization of screening for early identification of GMH-IVH in preterm infants. (79) Despite these gains, some of the challenges faced in managing GMH-IVH and PHVD include inconsistencies regarding the optimal timing of CSF diversion interventions. Second, limitations in the current diagnostic tools could delay identifying neonates requiring CSF diversion, potentially impacting their long-term neurodevelopmental outcomes. Therefore, despite extensive research, the management of GMH-IVH and PHVD remains challenging. In light of the increasing survival rates of very preterm neonates, there is an urgent need to develop better tools for monitoring GMH-IVH and PHVD.

1.1.6 Applications of 3D cranial ultrasound (3D cUS) in preterm neonates with GMH-IVH

Precise measurement of the degree of ventricular dilatation to guide timely intervention is critical. A single-centre study showed that a smaller ventricular size before VP shunt placement was associated with larger hippocampal volumes on TEA MRI and improved motor, language and cognitive outcomes at two years of age. (80) 3D cUS has the advantage of providing ventricular volumes (VV), which can prove helpful in determining the timing of intervention for CSF diversion in PHVD management. (81-83) In their study comparing longitudinal 2D cUS ventricular measurements and 3D cUS VVs in preterm neonates with PHVD, Kishimoto et al. found that monitoring preterm infants with PHVD using 2D cUS measurements alone could not accurately represent ventricular dilatation. (82) Hence, they proposed a 3D cUS volumetric measure to be used instead. In addition, Kishimoto et al. found high correlations between TEA

MRI and 3D cUS VV in preterm infants with PHVD. (84) Further, based on 3D cUS measurements of VVs, Kishimoto et al. could determine whether or not preterm neonates with PHVD would receive interventions. (85)

From this group, a follow-up study (at two years of age) of preterm neonates with PHVD who underwent 3D cUS (divided into low-volume < 20cc and high-volume > 40cc) showed that infants in the low-volume group had better neurodevelopmental outcomes compared to those in the high-volume group. (86)

1.1.7 Applications of functional Near-Infrared Spectroscopy in preterm neonates with GMH-IVH

Functional near-infrared spectroscopy (fNIRS) is a non-invasive technique that has increasingly gained popularity as a neuroimaging tool in paediatric research. (62, 87) fNIRS determines local cerebral oxygenation by measuring the living tissue's absorption properties in the near-infrared range (650-950 nm) to measure alterations in the local concentrations of oxygenated (HbO) and deoxygenated hemoglobin (HbR). (88) fNIRS is performed by emitting near-infrared light into the scalp and detecting the transmitted light at certain positions. fNIRS measures brain activity relying on neurovascular coupling (the relationship between local neuronal and vascular activity). An elevation in brain activity leads to an increase in local glucose consumption, inducing an increase in oxygen use, local CBF and oxygen delivery. (94) Hence, a typical hemodynamic response to neuronal activation shows increased CBF, leading to an increase in HbO, a decrease in HbR and an increase in HbT.

Compared with functional MRI (fMRI), fNIRS has several advantages: easy portability, affordability, and safety (radiation-free) - properties making it suitable for neonates. (62) NIRS studies have been trialed in both term and preterm neonates. (89) Postnatally, NIRS has been used to assess CBF and autoregulation and their correlation with hemorrhage. (90) Specifically, in GMH-IVH, several studies have shown that ventricular dilatation was associated with decreased cerebral oxygenation. (91, 92) In addition, CSF drainage was associated with improved cerebral oxygenation for those preterm infants with PHVD requiring a CSF diversion procedure. (93) Hence, fNIRS provides additional clinical value in preterm neonates with GMH-IVH and PHVD.

In addition to hemodynamics, functional connectivity (FC) analysis provides new insights for studying neonatal brain injury. FC indicates the intrinsic functional correlation between spatially separated cortical regions, forming a massive network for the whole brain cortex. Typically, FC is derived from fMRI data as the correlation between blood oxygen level dependent (BOLD) sequences. Recently, the framework was expanded to fNIRS sequences of concentration change of HbO, HbR, and HbT due to their similar hemodynamic origin to BOLD (95). Recent advances have shown that neonatal fNIRS resting-state FC can also be informative on the development of the functional organization and indicative of alterations. Homae et al. (95) identified age-related regional connectivity change indicating the effect of neuron proliferation. Fuchino et al. (96) described decreased connectivity within the parietal and temporal lobule among preterm neonates.

1.2 Aims

1. *Examine the association of ventricular morphology with spontaneous functional connectivity (sFC) in preterm neonates assessed using 3D cUS and multichannel fNIRS.*

No previous study has incorporated 3D cUS and fNIRS to monitor very preterm neonates with GMH-IVH. Previous studies have used 2D cUS and fNIRS or either of these tools in isolation. The first aim of the current thesis was to examine the potential relationship between the ventricular morphology (volume) and spontaneous functional connectivity changes in very preterm neonates with GMH-IVH using 3D cUS and multichannel fNIRS.

2. *Examine the longitudinal assessments of 3D cUS ventricular morphology in neonates with GMH-IVH and PHVD and how they will predict spontaneous functional connectivity.*

Recent evidence recommends more frequent neuromonitoring in neonates with GMH-IVH for timely recognition of complications, namely PHVI, PHVD or periventricular leukomalacia. With this in mind, we aimed to perform regular measurements from enrolment until discharge to see how the ventricular morphology would change (with or without CSF diversion procedures) relative to the sFC changes over time.

1.3 Hypotheses

Our overall hypothesis is that 3D cUS would monitor ventricular changes, and fNIRS reliably detect regional changes in spontaneous functional connectivity predictive of intervention thresholds. Based on previous literature, our predictions for the results of this thesis were:

Hypothesis 1: Ventricular size and volume will predict regional changes in spontaneous functional connectivity.

Serial 3D cUS measurements of the lateral ventricles play an essential role in deciding the optimal timing for intervention and monitoring ventricular size. Hence, we predicted that ventricular size and volume and sFC could provide early objective markers of PHVD.

Hypothesis 2: Neonates whose ventricular volumes decrease over time spontaneously or due to CSF diversion interventions will demonstrate increased sFC.

For neonates with PHVD, we predicted that before the CSF diversion procedure, they would demonstrate larger ventricle volumes and impaired sFC. Conversely, following the CSF diversion procedure, we predicted they would show smaller ventricle volumes and increased sFC.

1.4. The current thesis

GMH-IVH is a significant contributor of death and NDI in preterm neonates. Improvements in perinatal and neonatal care have increased the survival of very preterm infants, a population disproportionately affected by GMH-IVH and PHVD. Prompt management of GMH-IVH and PHVD is crucial to optimizing the long-term developmental outcomes of preterm infants. While the current tools – clinical and radiological – have helped monitor these neonates, in this thesis, we use a blend of bedside tools to monitor preterm neonates with GMH-IVH and PHVD. By conducting this study, we hope to understand how best to care for very preterm neonates with GMH-IVH. Incorporating additional non-invasive neuromonitoring modalities available at the bedside in the management of GMH-IVH, in particular 3D cUS and fNIRS, can guide management and outcome.

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Chapter 2

2.1 Three-dimensional cranial ultrasound and functional near-infrared spectroscopy for bedside monitoring of intraventricular hemorrhage in preterm infants

Germinal matrix-intraventricular hemorrhage (GMH-IVH) is the most common neurological complication faced by preterm born infants (≤ 32 weeks gestation), affecting 25% of very low birth weight (VLBW) neonates (weighing $< 1500\text{g}$). (1-3) Preterm birth, defined as a live birth occurring ≤ 37 completed weeks of gestation (GA), remains the most critical risk factor for the development of GMH-IVH. (4, 5) Yearly, 15 million babies are born early, accounting for 11% of births worldwide and 8% in Canada. (6-8) Advancements in obstetrical and neonatal care have led to improved survival of preterm neonates; (9-16), especially the extremely low birth weight (ELBW) neonates, who are most affected by GMH-IVH (incidence of 45%). (17, 18) Hence, despite improved survival of preterm infants, there has been an increase in severe forms of GMH-IVH in ELBW infants, which is associated with a high risk of death and neurodevelopmental impairment (NDI). (19, 20)

Preterm infants with GMH-IVH face complications: posthemorrhagic ventricular dilatation (PHVD) in the short term, cerebral palsy (CP), hearing and vision deficits, and cognitive and learning disabilities in the long term. (21-24) Preterm infants are susceptible to GMH-IVH due to immature germinal matrix (GM), maternal conditions, impaired autoregulation, alongside changes in blood flow. (25-29) Moreover, the first 72 h to 1 week after birth presents a delicate period of transition that ultimately predisposes these infants to complications, coinciding with the timing of GMH-IVH. (20, 30-32) As a result, routine cranial two-dimensional ultrasound (2D cUS) examination is recommended for all neonates ≤ 32 weeks GA within one week of life to identify and grade GMH-IVH; and subsequently at one month of age and term equivalent age (TEA) to identify white matter injury (WMI). (33, 34) cUS imaging is preferred due to its bedside availability, affordability and lack of ionizing radiation. cUS image quality, however, is dependent on the operator. Another cUS limitation is its inaccuracy in predicting long-term outcomes: mild GMH-IVH linked to NDI and severe IVH – good outcomes. Inconsistencies were attributed to the inability of cUS to consider subtle WMI and, in the latter -the site and extent of the lesion. (35, 36)

Lastly, cUS is limited in predicting sensorineural deficits and learning disabilities – equally important problems in the preterm infant. Compared to cUS, MRI has the advantage of providing more detail to quantify and grade IVH and other complications. However, MRI use is limited by its high cost and the need for expertise in interpretation. Similarly, the need for sedation and transport of sick infants makes MRI less ideal. (37, 38) Bedside tools are therefore needed to monitor hemorrhagic dilation of the lateral ventricles and changes in cerebral hemodynamics in the preterm neonate.

A promising bedside tool to monitor cerebral hemodynamics in preterm neonates is functional near-infrared spectroscopy (fNIRS). (38) This brain imaging technique is comparable to fMRI because it is sensitive to changes in blood oxygenation levels, an indirect marker of neuronal activity. Like fMRI, fNIRS can calculate functional connectivity, a key measure of regional brain health. However, recent advances in fNIRS have overcome several limitations of neonatal MRI, in that fNIRS is portable, relatively insensitive to motion and has an excellent temporal resolution.

Within 1 to 3 weeks of GMH-IVH, 25% of GMH-IVH cases progress to PHVD - seen on serial cUS as increasing dilatation of ventricles. (17) Worsening PHVD leads to decreased blood flow to the cortex and WMI. (39) 35% of infants with progressive PHVD require surgical treatment, while 10% die. (40) Long-term, PHVD is associated with NDI. (41) Management of progressive PHVD includes temporizing measures, such as lumbar taps, ventricular taps (VT), Ommaya reservoirs or external ventricular drains (EVD), and subsequently, a permanent shunt placement, if needed. However, there is little consensus regarding the timing of intervention for PHVD (42), with proponents for an earlier approach while others encourage a later-timed surgical approach. (43) Despite extensive research, managing PHVD remains challenging in the preterm neonate.

Precise measurement of the degree of PHVD to guide timely intervention is vital. Ongoing research has shown the utility of three-dimensional cranial US (3D cUS) volumetric thresholds to guide intervention. (44) A follow-up study of infants with PHVD showed that ventricular volume (VV) affected outcomes. (45) Findings suggest that VV volumes may be an important biomarker for preterms with severe GMH-IVH. (44)

With more preterm infants surviving, the ultimate goal of this longitudinal study was to identify 3D cUS and fNIRS markers that predict intervention thresholds for infants with PHVD. Hence, we used 3D cUS and fNIRS to monitor preterm infants (≤ 32 weeks GA) with GMH-IVH. The overall hypothesis was that 3D cUS VV changes would be associated with regional changes in spontaneous functional connectivity (sFC). Improved understanding of the association between VV and sFC using bedside monitoring methods may improve the prediction of intervention thresholds in neonates with GMH-IVH. We had two main aims for this study. The first aim was to assess the association of ventricular morphology with sFC in preterm neonates assessed using 3D cUS and multichannel fNIRS (infants who did not require CSF diversion). The second aim was to examine longitudinal assessments of 3D cUS ventricular morphology in neonates with GMH-IVH and PHVD and how they predict sFC (infants requiring CSF diversion).

2.2 Methods

2.2.1 Study Setting and patients

This prospective cohort study was conducted at the Level 3 Neonatal Intensive Care Unit (NICU) in Southwestern Ontario, Canada, from January 1, 2021, to June 30, 2022. Families provided informed consent. Infants (≤ 32 weeks' GA) receive a routine cUS within one week of age as part of standard care. (33) Study participants were infants ≤ 32 weeks' GA, born at or referred to London Health Sciences Centre (LHSC) NICU, and admitted with a diagnosis of GMH-IVH (46) made by the most responsible physician on the infant's first routine 2D cUS. The Health Sciences Western University's Research and Ethics Board approved the study.

2.2.2 Data collection

2.2.2.1 Demographic and clinical data

Maternal and neonatal data were abstracted from electronic medical records and charts.

Antenatal and perinatal data: maternal age, medical conditions, place of birth, corticosteroids administration, mode of delivery, GA at birth, birth weight, sex, delayed cord clamping, invasive ventilation at birth, cord pH, Apgar score at 1 and 5 minutes.

Prematurity-related clinical morbidities: patent ductus arteriosus (PDA) requiring treatment, sepsis, hypotension requiring inotropes, bronchopulmonary dysplasia (the need for supplemental oxygen at 36 weeks' postmenstrual age), periventricular leukomalacia (PVL).

Ventricular measurements and fNIRS data: 2D cUS GMH-IVH staging (right, left), 3D cUS ventricle volumes (left, right and total), postnatal course during each measurement and resting state fNIRS data. For infants with PHVD, before and after each CSF diversion procedure: head circumference, weight, hemoglobin, respiratory support, tap volume, the need for VP shunt and brain MRI for those infants who went on to have one.

2.2.2.2 3D cUS system and ventricle volume acquisition

We used a 3D cUS system to image the lateral ventricles attached to a clinical 2D cUS machine (HDI 5000, Philips, Bothel WA) with an appropriate conventional probe (C8-5, Philips, Bothel WA). (47, 48) The 3D cUS system comprises a handheld motorized device housing a 2D cUS probe (Figure 1). The probe was placed on the infant's anterior fontanelle (the soft part of the brain), with the infant lying supine. After initiating the scan, the device tilted the probe on an axis at the probe tip. 2D cUS images were acquired into a computer via a digital frame grabber (Epiphan DVI2USB 3.0) and reconstructed into a 3D image. (44) Total bedside scan lasted 5-10 minutes, accounting for infant movement and large ventricles. We manually segmented cUS images using in-house developed software. Segmentation of the lateral ventricles was performed in two sagittal and coronal planes by trained researchers (LMK., M. L, B.K. and S.A.) and verified by a Paediatric Neurosurgeon (SdR). The software created a 3D image of the lateral ventricles, from which a VV measurement was provided. (49) This system has been validated for geometric validity and volumetric measurements. (48) Depending on VV, the infants underwent sequential 3D cUS, weekly or biweekly, until discharge or TEA, whichever came first.

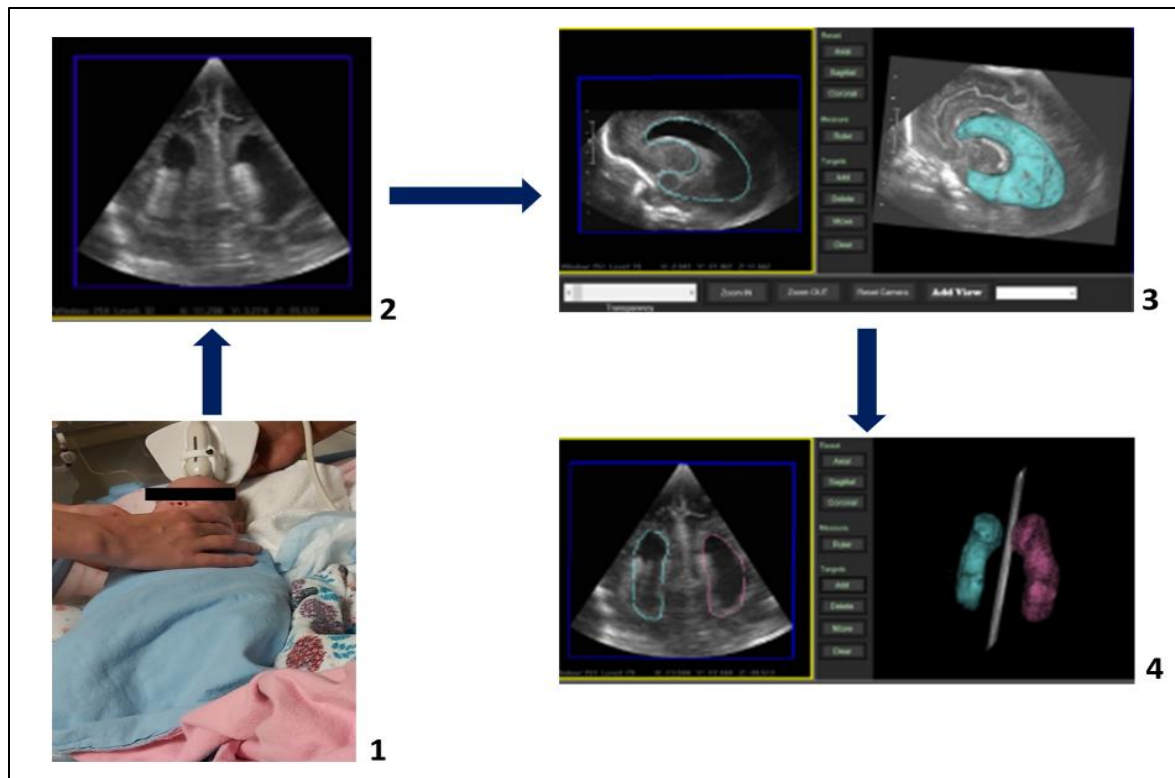


Figure 1: Three-dimensional cUS acquisition process: 1) neonate laying supine in the cot, with US probe placed on anterior fontanelle. 2) cUS image 3) lateral ventricle segmentation 4) segmented ventricles (left – pink; right – blue), from which a VV is provided.

2.2.2.3 fNIRS data acquisition

While infants lay in the incubator, we acquired fNIRS signals using a multichannel NIRSport2 system (NIRStar Software v14.0, NIRx Medical Technologies LLC, Berlin, Germany) at a sampling rate of 10Hz, (Figure 2). We inserted eight sources and eight detectors into predefined cap areas, with ten channels (with 22mm separation) defined for each hemisphere (20 total). Early recordings used a slightly different montage, so all datasets have been merged to contain 20/24 possible channels (supplementary figure 1). Each light source contains two LEDs that emit at 760 nm and 850 nm. According to international standards, the fNIRS cap was positioned on the infant's head. (50) Resting state fNIRS were acquired for a minimum of 2.5 minutes and a mean of 6.8 minutes. (51)



Figure 2: Image of fnirs cap on infants' heads; photo in the middle shows cap attached to the system (photos taken with permission from parents)

2.2.2.4 Measurements' sequence and timing

We performed the fNIRS recording first, followed by 3D cUS at the infant's bedside. Infants with severe GMH-IVH who demonstrated worsening PHVD, requiring a VT or EVD, underwent 3D cUS and fNIRS before and after the intervention (on the same day). Hence, all subjects had multiple 3D cUS VV and fNIRS measurements.

2.2.2.5 fNIRS patient exclusions and subgroups

Three patients with fewer than two viable sessions were excluded. The remaining patients were divided into two groups: those who did not undergo CSF diversion procedures and those who did require CSF diversion procedures. We analyzed these groups separately because it was unknown what effect the CSF diversion procedures would have on sFC.

2.2.2.6 fNIRS subsample selection

Each recording was reduced to the contiguous 2.5-minute subsample (51) with the highest data quality using in-house scripts and metrics adapted from QT-NIRS (also known as NIRSPLOT). (68)

First, abrupt motion spikes were interpolated using an established spline-based method adapted from Homer2. (52, 53) Scalp coupling index and peak spectral power were calculated in 5-second windows with 50% overlap for a cardiac range of 90 to 210 beats per minute. Selections were then made such that scalp coupling index and peak spectral power were maximized while segments with signal dropout (e.g., from optodes lifting off the scalp) were avoided.

2.2.2.7 fNIRS channel exclusions

We excluded channels within each dataset where cardiac pulsation was absent or other issues were manually identified. Cardiac pulsation was detected using a novel method that combines the scalp coupling index with patterns in the frequency domain. Manual corrections were applied based on visual inspection. 14.8% of channels were excluded due to absent cardiac pulsation, excessive motion, and/or signal dropout. An additional 7.3% of channels were excluded due to a hardware malfunction in source 8 that impacted 3 channels in the right hemisphere. These exclusions resulted in variable channel validity across sessions and between patients. Channels that did not meet a minimum sample size threshold (10/23 or 5/7) were indicated as “low confidence” and were excluded from analyses.

2.2.2.8 fNIRS data preprocessing

The data were converted to optical density and then to HbO and HbR using the Brain AnalyzIR Toolbox. (69) Age-appropriate partial pathlength factors (0.1063 for 760nm and 0.0845 for 850nm) were used in the modified Beer-Lambert Law, which yields estimates of relative concentrations of oxygenated (HbO) and deoxygenated hemoglobin (HbR). (54) Additionally, channel lengths were scaled to the cap size used during each session. The data were then converted to total hemoglobin (HbT = HbO+HbR), which has been shown to have improved reproducibility across sessions and subjects. (53) Nuisance regression was performed, which applied temporal filtering (roughly equivalent to a bandpass around 0.004 Hz to 0.09 Hz) and was also the primary means of motion correction. (55) The final data were resampled to 1 Hz before calculating correlations (see supplementary figures 2 and 3).

2.2.2.9 Spontaneous functional connectivity

The Brain AnalyzIR Toolbox calculated sFC using a robust correlation method. (69) Prewhitening was not applied. (56) To validate the sFC, group summary figures were generated as follows: correlations were first averaged across sessions to yield one mean value per patient channel pair; a single-sample t-test was performed on each channel pair to evaluate $sFC \neq 0$; the resulting t-map was drawn over the montage.

Relating spontaneous functional connectivity to ventricle volumes

We used absolute sFC correlations ($|sFC|$) because we expected impairments to involve reductions in the positive correlations and increases in the anti-correlations (i.e., all correlations trending towards zero, a decrease in magnitude). The relation of $|sFC|$ to VV was assumed to be linear and evaluated by estimating the slope of $|sFC| / VV$. We did not use correlation because several patients had as few as two sessions. The $|sFC| / VV$ slope was estimated using three incremental methods. All three methods were calculated independently for each channel pair using VV from the corresponding hemisphere/ventricle. The first method used simple linear regression to estimate each patient's slope. The second method used linear mixed-effects modelling (LME), which was less sensitive to outlier patients. The third method used LME and accounted for changes in sFC and VV across GA.

A t-map was generated for each method for $|sFC| / VV \neq 0$. Positive values indicate where $|sFC|$ increases/decreases with VV, and negative values indicate where the relation is inverted (e.g., decreasing $|sFC|$ as VV increases). For a broad comparison, we performed a subsequent t-test ($|sFC| / VV \neq 0$) on the group-level slopes from each channel-pair (separate for each hemisphere, for each method). This test indicates where significant trends occurred at the hemisphere level.

Longitudinal case studies in patients requiring CSF diversion procedures

For each case study, we present $|sFC|$ and VV sessions across GA and indicated the times of each diversion procedure. As a middle ground between presenting every channel pair and collapsing to hemispheres, $|sFC|$ are presented from four fully symmetrical functional clusters identified in the

sFC validation t-maps. Each cluster contains four channel pairs derived from five channels. (see supplementary figure).

2.2.3 Statistical analysis

SPSS v.28 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses of demographic data. Continuous variables were summarized using means and standard deviations (medians and interquartile ranges for non-normal distributions), and categorical variables were summarized using frequencies and percentages.

For fNIRS, we used a combination of Brain AnalyzIR Toolbox (69), Homer2 (52), and in-house MATLAB scripts.

To address the first aim, we examined the association between VV and sFC by estimating the linear relation (slope) of |sFC| across VV. To address our second aim, we additionally investigated |sFC| and VV across time as individual case studies for infants who underwent CSF diversion procedures. Models were adjusted for relevant covariates and confounding variables. As we had a single hypothesis and did not make direct comparisons across the groups or methods, an alpha level α of 0.05 was considered statistically significant.

2.3 Results

2.3.1 Study population

During the study period, 43 preterm infants (GA \leq 32 weeks, with GMH-IVH) were eligible for 3D cUS and fNIRS monitoring. We excluded two neonates (extremely low for gestation (ELGA), $<$ 26 weeks) following their death during the first week of life. The neonates died after a decision for palliative care in the NICU and before data acquisition. The decision to redirect care was based on the degree of prematurity, the severity of GMH-IVH and other prematurity-related complications. We excluded seven neonates because they lacked fNIRS measurements and another one - 3D cUS measurements. In addition, three patients with fewer than two viable sessions were excluded. Thus, our study population consisted of 30 preterm infants (mean GA 26.6 weeks, mean birth weight 1.002.37g) with 3D cUS and fNIRS measurements. Figure 3 is the study flow diagram. The remaining 30 patients were divided into two groups: those who did not undergo CSF diversion procedures (n=23, "No CSF Diversion" group) and those who required CSF diversion procedures (n=7, "CSF Diversion" group).

Most infants were born at LHSC (24, 80.0%), and 16 (53.3%) were female. Only twelve mothers (40.0%) received a complete course of antenatal corticosteroids. Most mothers presented in spontaneous preterm labour and did not benefit from this intervention. A higher proportion of infants (20, 66.7%) remained intubated during the first week of life. Out of the 30 neonates enrolled, 19 (63.3%) had mild GMH-IVH, while 11 (36.7%) had severe GMH-IVH. Severe GMH-IVH was defined as GMH-IVH grade III or PHVI. In addition, the majority of the study participants had prematurity-related complications.

Seven infants had temporizing CSF procedures (ranging from 2 to $>$ 15). Of these, 3 infants (42.9%) had a VP shunt inserted before discharge. The "CSF Diversion" group had a mean GA of 26.6 weeks and a mean birth weight of 1088.7g. Only 1 infant in the CSF diversion group received a complete course of antenatal corticosteroids. Of the seven neonates, 2 (28.6%) had grade II IVH, 2 (28.6%) had grade III IVH, and 3 (42.9%) had PHVI. Table 1 shows demographic characteristics and clinical data.

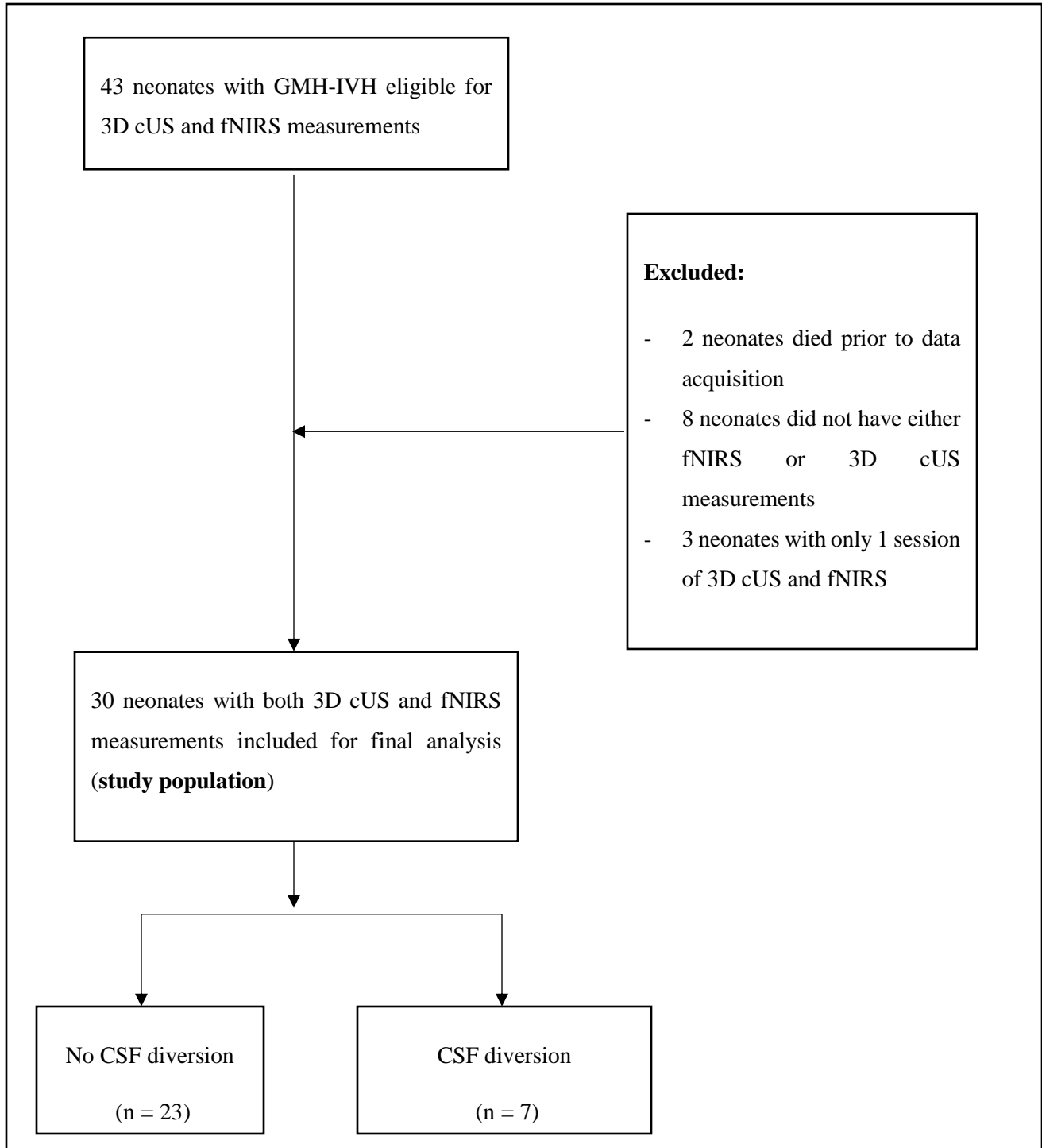


Figure 3: Flow diagram

Table 1: Demographic and clinical data of the study cohort (n = 30)

Characteristics	No CSF Diversion (n = 23)	CSF diversion (n = 7)
Neonatal characteristics		
Gestational age at birth (weeks, SD)	26.6 ± 1.06, SD = 2.59	26.6 ± 2.30, SD = 3.10
Birth weight (g)	976.1 ± 125.2, SD = 306.4	1,088.7 ± 392.2, SD = 529.43
Head circumference at birth (cm)	24.8 ± 1.09, SD = 2.7	26.9 ± 2.2, SD = 3.1
Male sex (%)	10 (43.5)	4 (57.1)
Apgar 5 min	6.09 ± 0.99, SD = 2.4	7 ± 1.54, SD = 2.1
Inborn	21 (91.3)	3 (42.9)
Cesarean section delivery (%)	9 (39.1)	2 (28.6)
Maternal characteristics		
Antenatal corticosteroids (complete course)	11 (47.8)	1 (14.3)
Maternal age (years)	31.48 ± 2.65, SD = 6.5	29.57 ± 4.9, SD = 6.6
Neonatal complications		
Invasive ventilation > 1 week (%)	15 (65.2)	5 (71.4)
hsPDA (%)	12 (52.2)	5 (71.4)
GMH- IVH grade⁽⁴²⁾	Grade I: 7 (30.4) Grade II: 10 (43.5) Grade III: 4 (17.4) Grade IV: 2 (8.7)	Grade I: 0 (0.0) Grade II: 2 (28.6) Grade III: 2 (28.6) Grade IV: 3 (42.9)
Culture-positive or clinical sepsis (%)	19 (82.6)	6 (85.7)
Hypotension, inotrope use (%)	6 (26.1)	4 (57.1)
BPD (%)	8 (34.8)	4 (57.1)
PVL (%)	7 (30.4)	5 (71.4)

Characteristics	No CSF Diversion (n = 23)	CSF diversion (n = 7)
VP shunt (%)	0 (0.0)	3 (42.9)
Death before discharge (%)	0 (0.0)	1 (4.3)
Number of sessions (Mean, median)	3.7, 3	9.6, 11

RDS, respiratory distress syndrome; **hsPDA** - hemodynamically significant patent ductus arteriosus, **PVL** - periventricular leukomalacia; **VP** shunt - ventriculoperitoneal shunt

2.3.2 Validation of spontaneous functional connectivity

It was important first to establish the presence of sFC and identify any existing trends. Figure 4 represents t-maps for both group-level sFC (the top row represents the group that did not require CSF diversion, and the bottom row represents – the CSF diversion group). Both t-maps include clusters of adjacent channels that are positively correlated, anti-correlations between those clusters, and many significant anti-correlations between hemispheres (see supplementary figure 4 for anti-correlations between hemispheres).

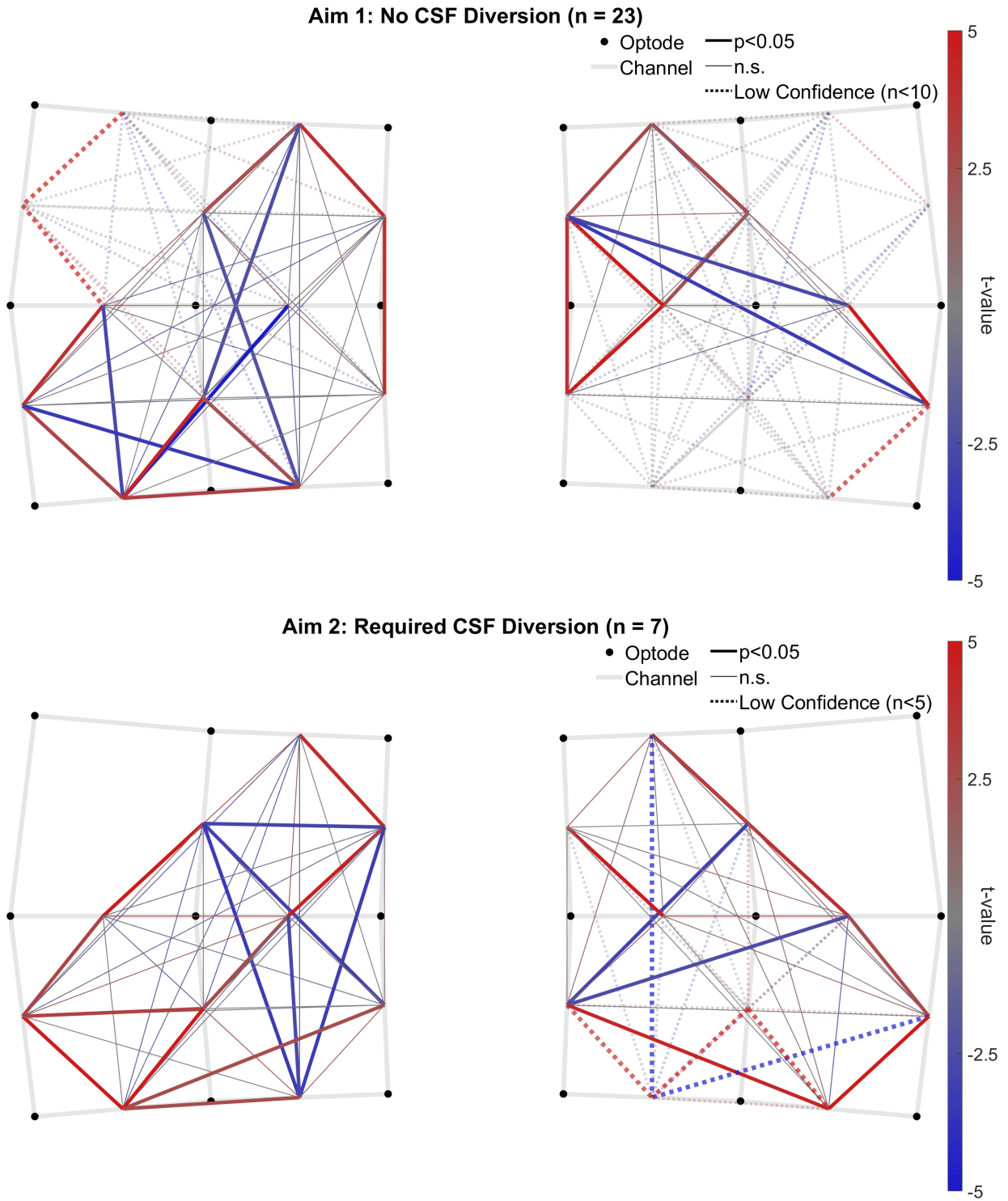


Figure 4: Group level sFC t-maps (top row, “No CSF Diversion group”, n = 23; bottom row, “CSF Diversion” group, n= 7).

2.3.3 Ventricular morphology and spontaneous Functional Connectivity

To assess our first aim, the association of ventricular morphology with sFC in preterm neonates assessed using 3D cUS and multichannel fNIRS was examined. In the “No CSF Diversion” group, the linear regression method produced a widespread negative $|sFC| / VV$ (see Figure 5) that was significantly less than zero in the left hemisphere (-0.0729 , $p < 0.001$) and trending similarly in the right (-0.1030 , n.s.). All results are outlined in Figure 6.

In the “Required CSF Diversion” group, $|sFC| / VV$ trended negatively in the left hemisphere across linear regression (-0.0032 , n.s.), LME (-0.0012 , $p < 0.001$), and LME additionally accounting for GA (-0.0159 , $p < 0.001$). Several mostly-adjacent channel pairs were independently significant in both LME results. However, the right hemisphere was inconsistent across all three methods.

To provide context to the results of $|sFC| / VV$, we performed a post-hoc investigation of possible trends in VV and $|sFC|$ across GA in each group. The only significant finding was a decrease in VV across GA in the left hemisphere of the “No CSF Diversion” group (-0.3869 VV / Week, $p < 0.05$), but this was not maintained when accounting for patient variability. Overall, there were no reliable main effects of GA on VV or $|sFC|$ in this clinical population.

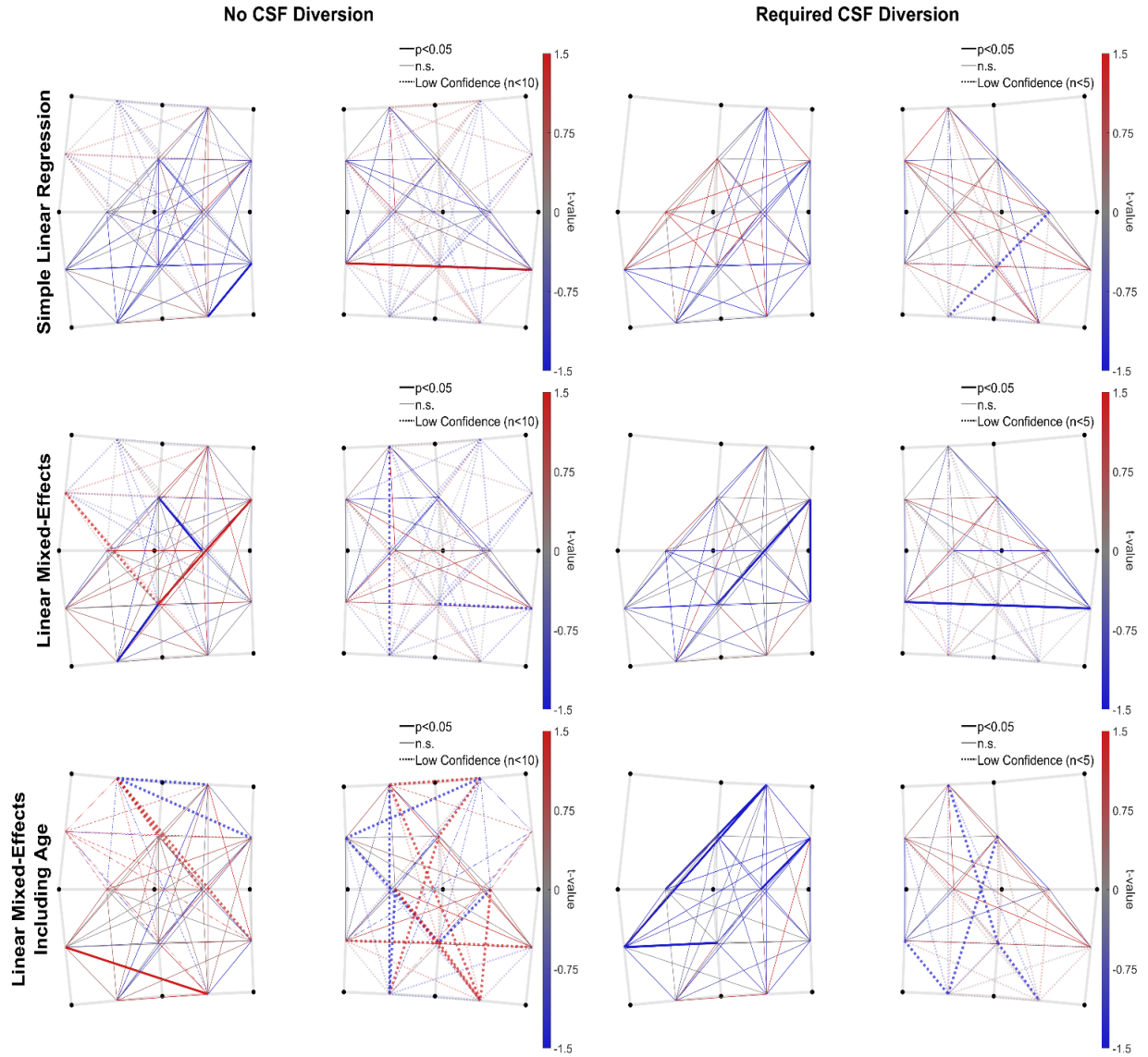


Figure 5: Group level |sFC|/VV t-maps from each slope method (left = “No CSF diversion” group, n = 23; right = “Requiring CSF diversion” group, n = 7); Rows: top = simple linear regression, middle = LME, bottom = LME + GA

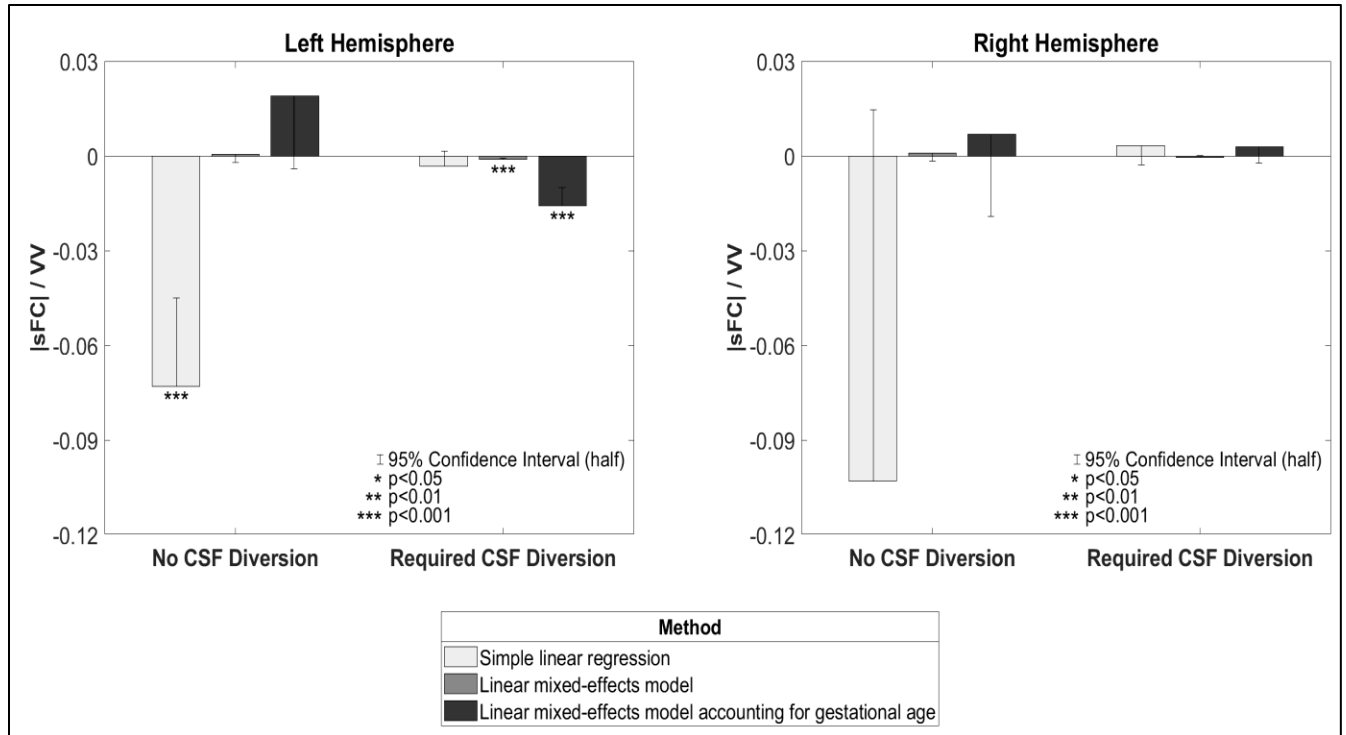


Figure 6: Hemispheric trends in group level $|sFC|/VV$ from each slope method

2.3.4 Longitudinal assessments of 3D cUS ventricular morphology and sFC prediction

To address our second aim, concerning longitudinal assessments of 3D cUS ventricular morphology in neonates with GMH-IVH and PHVD, we examined how they covary with sFC. Two of seven case studies from participants requiring CSF diversion procedures are presented in Figure 7 (see supplemental material for additional figures). An inverse relation between $|sFC|$ and VV is evident even when CSF is diverted. Furthermore, increases in $|sFC|$ following CSF diversions were often observed shortly after each procedure.

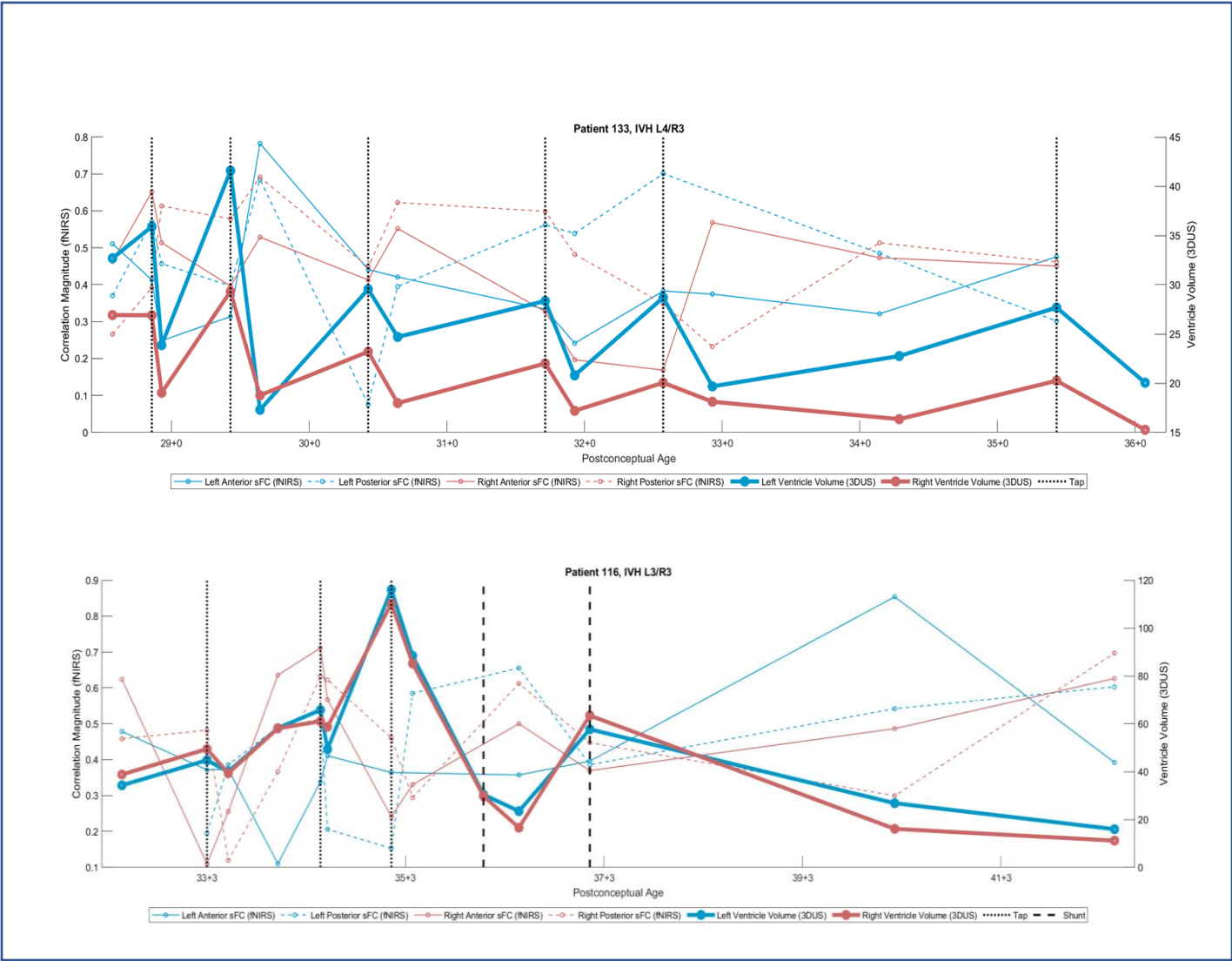


Figure 7: Two case studies from patients requiring CSF diversion

2.4 Discussion

Despite obstetric and neonatal health improvements, germinal matrix-intraventricular hemorrhage remains unacceptably high in preterm neonates. This study used a combination of bedside tools - 3D cUS and fNIRS to monitor preterm infants with GMH-IVH from diagnosis until discharge. To our knowledge, no other study has incorporated these tools simultaneously. Our study aimed to determine if ventricular size in preterm infants with GMH-IVH was associated with changes in sFC. We observed an inverse relation between |sFC| and large VV: increases in VV were associated with decreases in |sFC|. Our findings of increased ventricular volume in preterm neonates and reduced fNIRS-based functional connectivity suggest that regional disruptions of ventricular size may impact the development of the underlying grey matter. (71) These results bear clinical significance given that the preterm neonate's brain is rapidly growing and at risk for injury. (72) Furthermore, severe GMH-IVH and PHVD have been linked to adverse ND outcomes. (75, 76)

In our first aim, in participants who did not have CSF diversion, the linear regression method produced a significant negative |sFC| / VV slope in the left hemisphere with a similar trend in the right, which indicates impaired sFC in preterm neonates with GMH-IVH. We also observed similar but more compelling results in the "Required CSF Diversion" group: consistently negative across all the three methods used. The slopes in the "No CSF Diversion" group switched to positive under the LME-based methods, which suggests that most infants in this group had a positive relation (likely because most of these infants had mild GMH-IVH) and that the few negative outliers likely drove the initial linear regression result. Our findings suggest that increasing ventricular dilatation is associated with reduced |sFC| in preterm infants with GMH-IVH. To put this into context, the larger the ventricle, the more likely sFC is impaired. Our study findings are not surprising, given that the pathological and functional consequences of GMH-IVH depend on its severity. (71) Previous resting-state FC fMRI studies have reported similar findings, showing resting-state network development was affected depending on WMI severity and location. (57, 58) In addition, in a recent study, Tortora et al. showed regional impairment of cortical and regional grey matter perfusion in preterm infants with mild GMH-IVH. (73)

As expected in our second aim, in the "Required CSF Diversion" group, we observed elevated VV and reduced |sFC| right before the CSF diversion procedures. Conversely, after the diversion, VV decreased, and |sFC| often increased. Observed increases in |sFC| were observed shortly after each CSF diversion procedure, possibly correlating to a decrease in ICP. Our findings are consistent with others. In their study of 9 preterm infants, Norooz et al. reported an increase in the mean regional cerebral oxygen saturation (rcSO₂) value after decompression ($42.6 \pm 12.9\%$ before vs $55 \pm 12.2\%$ after decompression). (59) Our findings are also comparable to Kochan et al. Their study, which included 20 VLBW premature infants with GMH-IVH, demonstrated that ventricular dilatation was associated with lower cSO₂, suggesting a decrease in cerebral perfusion. (60) Other studies in preterm infants with PHVD requiring CSF diversion procedures have consistently reported similar findings. (61-63) These studies differ from ours since they used a single probe sensor placed on the infant's forehead, while ours uses a multichannel system. Nonetheless, both methods assess cerebral oxygenation.

Our findings show that fNIRS may provide additional clinical information, particularly in preterm infants with PHVD, to help determine the optimal time for CSF diversion. While the definitive treatment of PHVD is VP shunt placement to divert CSF, this procedure is often delayed. During this delay, temporizing procedures are done to decrease ICP. However, a prolonged increase in ICP can lead to brain damage – both periventricular WM and cerebral grey matter (71). Furthermore, the timings of the temporizing CSF diversion procedures mentioned above are unpredictable and rely on subjective clinical signs, symptoms and 2D cUS measurements. fNIRS measurements, therefore, have the potential to aid in timely decision-making.

Our study has some strengths that are worth highlighting. A key strength of this study was the serial measurements. To date, 2D cUS remains the neuroimaging modality for bedside GMH-IVH diagnosis. Current guidelines recommend the first 2D cUS by the first week of life to screen for GMH-IVH. After that, repeat cUS is completed at 4-6 weeks of life to screen for complications, mainly periventricular leukomalacia. Recent evidence recommends more frequent screening, particularly amongst very preterm infants, to help identify these complications and for timely intervention for those needing it. (64) Moreover, timely diagnosis of these complications provides a window of opportunity to have not only meaningful conversations with parents regarding

prognosis but also an early opportunity for intervention for the affected infants. (65) Another strength of our study was the demonstration of functional connectivity in preterm neonates using multichannel fNIRS, enabling us to cover multiple brain areas simultaneously. Lastly, our NICU and Paediatric Neurosurgery team have incorporated 3D cUS for monitoring preterm infants with GMH-IVH (44, 45, 47, 48) for the past decade. Our current 3D cUS is semi-automated, requiring time to segment the lateral ventricles. For 3D cUS to play a role in diagnosing and monitoring PHVD in other clinical settings, an efficient and fully automated segmentation algorithm is crucial. Several other groups have adapted this technique, given its reliability in VV estimation. (66, 67) Nevertheless, we still achieved reliable VV estimates using our current system.

2.4.1 Future directions and limitations

Some limitations of this study need to be acknowledged. First, the variability in the number of study measurements each participant had could have led to variability in the results. Our analysis showed a more robust and reliable sFC pattern when the study participants had three or more sessions. More sessions for each patient and fewer missing/excluded data would have enabled us to use more robust methods such as graph theory. Despite this, we still observed meaningful sFC patterns. Second, given the limited period, including follow-up data from this cohort was not possible. Morbidity is substantial in preterm infants with severe GMH-IVH and PHVD. Grade III and PHVI have been linked to CP, neurosensory problems and cognitive impairments; hence, it is only fitting that these outcomes are reported. Participants from this study are currently undergoing follow-up in the developmental follow-up and Paediatric Neurosurgery clinics. Their ND outcomes will provide additional information regarding the clinical application of these tools and their implications. Third, the right hemisphere was inconsistent across linear regression in the "No CSF Diversion" group. This inconsistent finding could be attributed to a poor signal in one of the sources during the initial data acquisition period that may have influenced the results in the right hemisphere. Another plausible reason could be heterogeneity in our study participants (63.6% had mild GMH-IVH). Future prospective studies should examine larger populations of infants with all grades of GMH-IVH.

We included a model with gestational age because it is reasonable to expect an increase in both VV and |sFC| across GA. We wanted to investigate their separate relationship from this trend. However, we did not observe any reliable main effects of GA, even in the milder “No CSF Diversion” group. It is likely that these effects were present but could not be detected due to a combination of low sample size and the noise introduced by IVH-related fluctuations in VV and |sFC|. In addition, these findings are not surprising given that with mild GMH-IVH, we expect to see the resolution of the bleeds with time and hence restoration of sFC. Previous studies have shown the maturation of sFC with advancing GA. Further studies are required to determine if GA should be included in the model.

Other points are poor spatial resolution and limited penetration depth (especially in preterm infants with dependent scalp oedema). Such issues, however, are common in fNIRS studies, particularly in the very preterm population where the fit of the caps may be insufficient to make proper contact with the scalp. Therefore, future studies with larger cohorts are needed to confirm our results.

2.4.2 Conclusions

In conclusion, our study shows that 3D cUS and fNIRS are promising bedside tools for monitoring GMH-IVH and PHVD in preterm infants, providing complementary information about the infant's structure and function. Future research should explore the potential role of lateral ventricle volumes and spontaneous functional connectivity in the diagnostic and therapeutic approach to PHVD.

2.4.3 Acknowledgements

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Chapter 3: Study Outcomes

In this work, we examined the relationship between the ventricular volumes and spontaneous functional connectivity changes in preterm neonates with GMH-IVH using 3D cUS and multichannel fNIRS. Our study showed an association between increasing lateral ventricle volumes and impairments in spontaneous functional connectivity in preterm infants with GMH-IVH. This trend was pronounced in infants with larger ventricle volumes undergoing CSF diversion procedures.

3.1 Implications

Our first hypothesis that three-dimensional cUS ventricle volume changes would be associated with regional changes in spontaneous functional connectivity was supported based on the results of our analysis. Our findings align with recent literature showing that preterm infants with GMH-IVH have reduced cerebral oxygenation. (1-6) Furthermore, as expected in our second aim, in preterm neonates with PHVD undergoing CSF diversion procedures, we observed elevated VV and reduced |sFC| right before the CSF diversion procedures, and the reverse - after the diversion, VV decreased, and |sFC| often increased. Second, increases in |sFC| were observed shortly after each CSF diversion procedure, possibly correlating to a decrease in ICP. This latter finding is important because we still do not know the implications of prolonged increased ICP on the developing neonatal brain. Previous studies have shown that although an improvement in the metabolic rate of oxygen was seen after VP shunting, the cerebral blood flow did not return to normal, suggesting a deterioration in the developing brain with PHVD. (7) Similarly, a study showed improved cerebral oxygenation and neurological function following CSF removal in adults with chronic hydrocephalus. (8) In this study, however, the improvement in neurological function decreased with a prolonged period of hydrocephalus, suggesting an association with irreversible brain injury. Suppose these data can be extrapolated to the neonatal population. Then there could be a window of opportunity to prevent further brain injury in preterm infants with PHVD with appropriately timed interventions.

The above-mentioned findings suggest the need for sensitive neuromonitoring tools – to detect changes in the ventricle volumes and assess changes in sFC in a timely manner, amenable to early interventions.

3.2 Future directions

As previously outlined in this thesis, GMH-IVH remains prevalent in the preterm population, affecting 25% of very preterm infants. Consequently, severe forms of GMH-IVH are associated with high rates of NDI, although recent literature suggests that even mild GMH-IVH is associated with adverse outcomes. (9). Prevention and reduction of GMH-IVH are critical. For those with PHVD, more sensitive neuromonitoring tools should be adopted, especially for determining the optimal timing of CSF diversion in these infants. While 2D cUS is the most widely used neuroimaging modality to diagnose GMH-IVH due to its high sensitivity for detecting hemorrhagic brain injury, we explored its limitations previously in this thesis.

One limitation of the current study was that neurodevelopmental outcome data from this cohort was impossible, given the time. That said, participants from this study are currently undergoing follow-up in the developmental follow-up and Paediatric Neurosurgery clinics. Their neurodevelopment outcomes will provide additional information regarding the clinical application of 3D cUS and fNIRS tools and their implications.

Future prospective studies should examine larger populations of infants with GMH-IVH so that all grades can be studied separately. We found that infants with severe GMH-IVH and PHVD requiring CSF diversion procedures had impaired sFC. That said, it is worth separating grades III and PHVI. Although they originate from the germinal matrix, the exact pathophysiology of the injury is different: grade III is associated with compression, but no parenchymal involvement, while PVHI – is attributed to compression of the terminal vein that drains the GM and the adjacent WM. (10, 11) In addition, recent evidence also shows impaired cerebral oxygenation in mild GMH-IVH compared to their healthy counterparts. (12) The evidence in our study provides insight into structural and functional changes in the brain and the need for timely interventions for those needing them.

Lastly, although previous studies have shown the maturation of sFC with advancing gestation age, we did not observe the same in our study. It is likely that these effects were present but could not be detected due to the low sample size. Further studies are required to determine if GA should be included in the model.

3.3 Conclusions

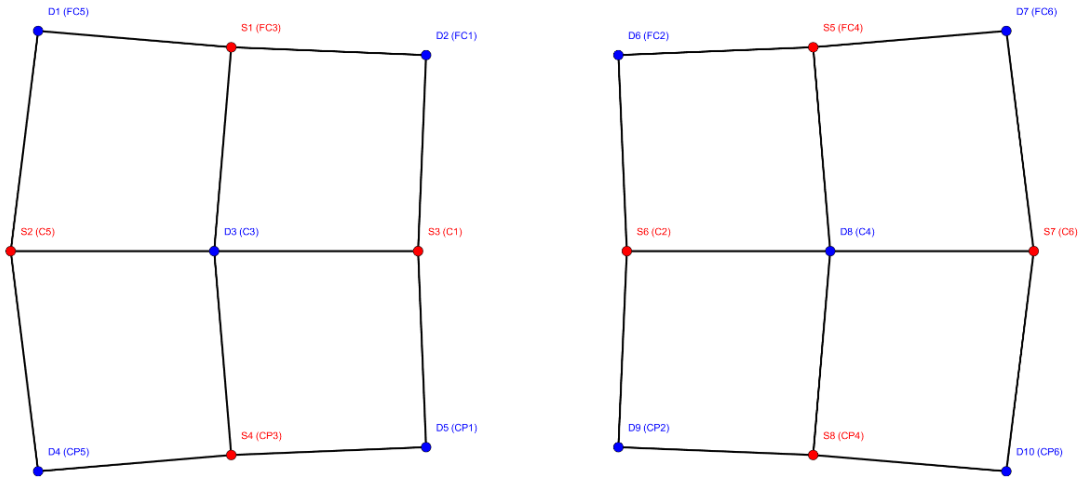
With significant advances in neonatology, preterm neonates with GMH-IVH continue to receive better care to improve their neurodevelopment outcomes. However, more effort must be employed to improve screening, monitoring and timely detection of complications since we know an early diagnosis of neurodevelopment deficits and referral to an early intervention program is crucial to enhance the long-term outcome. Hence, this thesis shows that three-dimensional cUS and fNIRS are promising bedside tools for monitoring GMH-IVH in preterm infants.

3.4 References

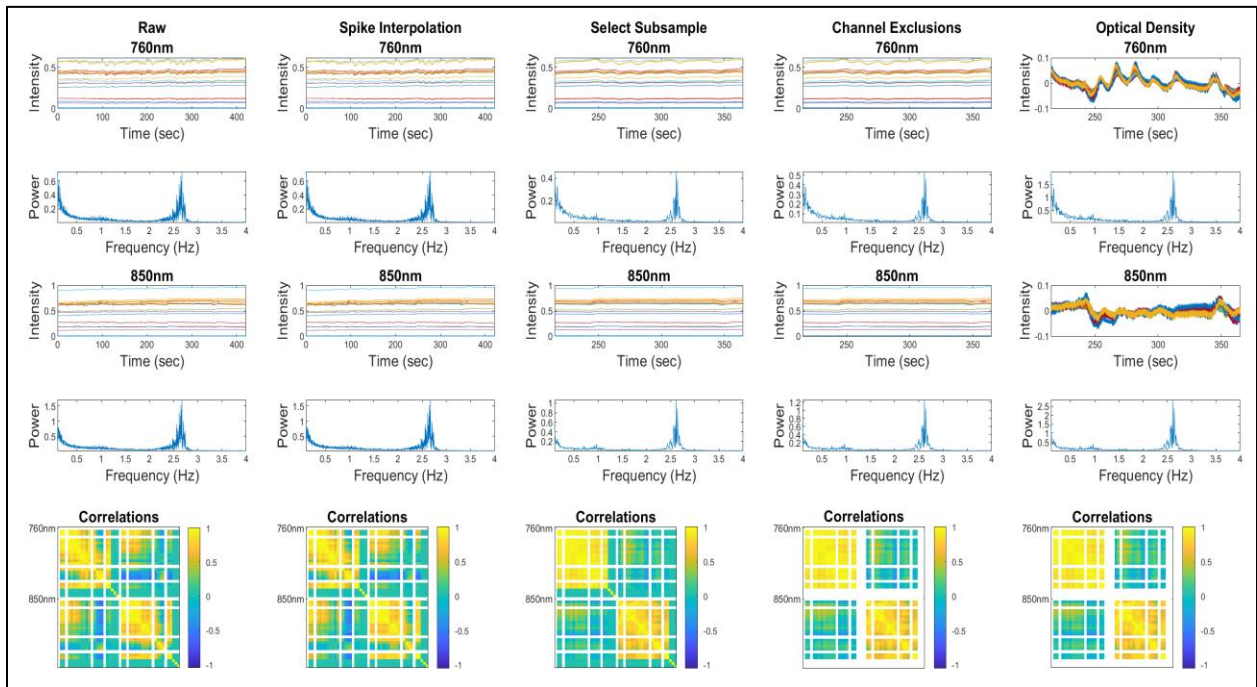
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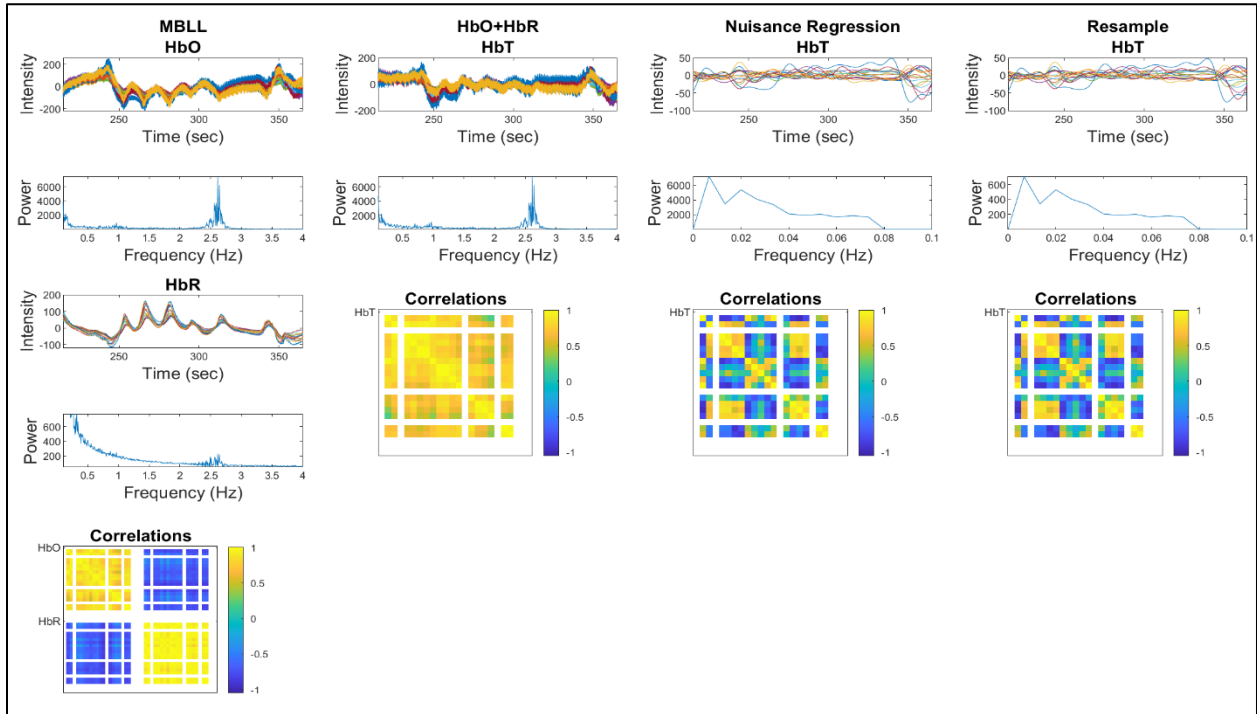
Appendix I: Supplementary figures



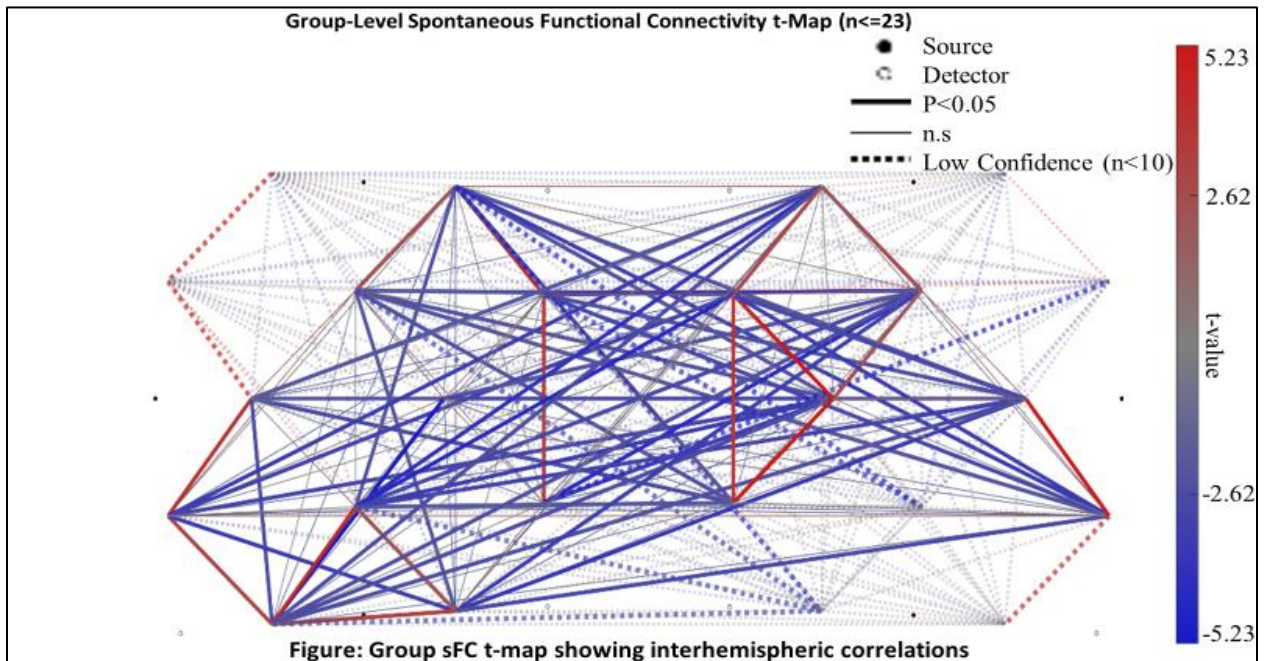
Supplementary Figure 1: Montage



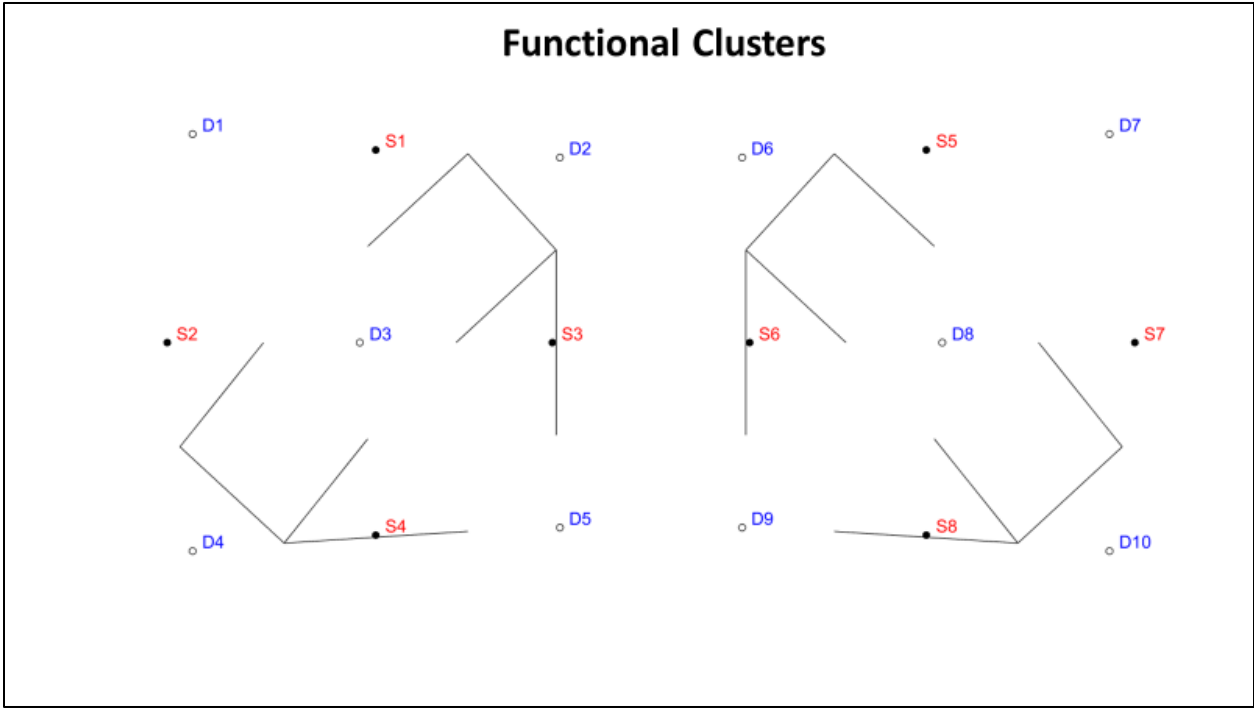
Supplementary Figure 2: Preprocessing pipeline, from the acquisition of raw data to optical density



Supplementary Figure 3: Preprocessing pipeline (continued)



Supplementary Figure 4: Group t-map showing interhemispheric correlations



Supplementary Figure 5: Four functional clusters

Appendix II: REB Approval form



Western Research

Date: 23 August 2022

To: Dr. Sandrine De Ribaupierre

Project ID: 100315

Review Reference: 2022-100315-70039

Study Title: New technologies in the management of post-hemorrhagic hydrocephalus in preterm infants (REB #17827)

Reference Number/ID: NA

Application Type: HSREB Amendment Form

Review Type: Delegated

Full Board Reporting Date: 13/Sept/2022

Date Approval Issued: 23/Aug/2022 10:55

REB Approval Expiry Date: 05/Apr/2023

Dear Dr. Sandrine De Ribaupierre ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

Documents Approved:

Document Name	Document Type	Document Date
IVH_infants_HSREB_28-07-2022	Protocol	28/Jul/2022

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Electronically signed by:

Karen Gopaul , Ethics Officer on behalf of Dr. Philip Jones, HSREB Chair, 23/Aug/2022 10:55

Reason: I am approving this document.

Note: *This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).*

Appendix III: Curriculum Vitae

Lilian M N Kebaya
Western University

Education

Masters in Neuroscience Western University, Schulich School of Medicine, London, ON, Canada	2020 - 2022
Clinical Fellow: Neonatal-Perinatal Medicine Western University, Schulich School of Medicine, London, ON, Canada	2018 – 2022
Masters of Medicine: Paediatrics and Child Health The University of Nairobi, School of Medicine, Nairobi, Kenya	2011 – 2014
Doctor of Medicine Rostov State Medical University, Russia	2001 – 2007

Research Experience

Three-dimensional cranial ultrasound and functional near-infrared spectroscopy for bedside monitoring of intraventricular hemorrhage in preterm infants. <i>Western University, London, ON</i> Supervisors: Dr. Sandrine de Ribaupierre and Dr. Emma G. Duerden	2021 - 2022
Functional near-infrared spectroscopy in neonates at risk for neurological injury. <i>Western University, London, ON</i> Study PI: Dr Emma G. Duerden, Developing Brain Lab	2019 - 2022
Relationship between early laboratory measures and neurological injury in neonates undergoing therapeutic hypothermia. <i>Western University, London, ON</i> Supervisor: Dr Soume Bhattacharya	2019 - 2021

Publications

Kebaya L., Kariuki C., Coughlin K., St-Laurent A. Desaturations in a newborn with bowel obstruction, an autonomic connection. *UTMJ* (June 2022 issue)

Rajaram A., **Kebaya L.**, St Lawrence K., et al. Assessing cerebral blood flow, oxygenation and cytochrome c oxidase stability in preterm infants during the first 3 days after birth. *Sci Rep.* 2022 Jan 7; 12 (1):181. doi: 10.1038/s41598-021-03830-7

Kebaya L., Wamalwa D., Nduati R. et al. Efficacy of mobile phone use on adherence to Neviparine prophylaxis and retention in care among HIV-exposed infants: a randomized controlled trial, *BMC Pediatrics* 21, Article number: 186 (2021).

Kebaya, L., Kiruja J., Munn Z., et al. Implementation of basic newborn resuscitation guidelines among health care providers in Maragua District Hospital: a best practice implementation project, *JBIC Database System Rev Implementation*, 2018, Rep 16 (7): 1564-1581

Barasa AK. Kitonyi GW. Rajab JA. Kariuki C., **Kebaya L.** (2014). Orbital myeloid sarcoma preceding acute myeloid leukemia: a case report, *East African Journal of Pathology*, 2014, (1) 41-44

Submitted for publication

Kebaya L., de Ribaupierre S, Duerden E., et al. Three-dimensional cranial ultrasound and functional near-infrared spectroscopy for bedside monitoring of intraventricular haemorrhage in preterm infants. *Submitted to Scientific reports. November 2022.*

Grants and Awards

Whaley and Harding Fellowship, Master's in Neuroscience (\$40000/year) Competitive grant awarded following a successful application for a project entitled "imaging motor activity and brain growth in neonates" (IMAGINE)	2021-2023
Division of Neonatal-Perinatal Medicine Research Grant, London Health Sciences Centre – support for a fellowship project (\$2000)	2019
AAP Section on Hospital Medicine's International Travel Grant – support to attend and give an oral presentation	2016
American Pediatric Association Global Health Research Award – support to attend and give an oral Presentation	2015
CROI Young Investigator award – support to attend and present a poster Presentation	2015
Best oral thesis presentation award, Master of Medicine in Paediatrics	2014
Distinction. Doctor of Medicine, Rostov State Medical University, Russian federation	2007

Conference Presentations

Pediatric Academic Societies. Three-dimensional cranial ultrasound and functional near infrared spectroscopy for bedside monitoring of intraventricular hemorrhage in preterm infants. Poster presentation. **Kebaya L.**, de Ribaupierre S., Duerden E., et al. 2022

- Pediatric Academic Societies. Altered resting state functional connectivity in newborns with hypoxic-ischemic encephalopathy assessed using high-density functional near-infrared spectroscopy. Poster presentation. Tang L., **Kebaya L.**, Duerden E., et al. 2022
- Biophotonics Congress. Hybrid hsNIRS/DCS system for assessing cerebral blood flow and cytochrome c oxidase stability in preterm infants. Oral presentation. Milej D., **Kebaya L.**, St Lawrence K., et al. 2022
- Society for Functional near infrared Spectroscopy. Imaging brain growth in preterm infants with Intraventricular Hemorrhage. Poster presentation. **Kebaya L.**, de Ribaupierre S., Duerden E., et al. 2021
- Canadian Paediatric Societies. Relationship between Early Laboratory Measures and Neurological Injury in Neonates undergoing Therapeutic Hypothermia. Oral presentation. **Kebaya L.**, Levin S., Bhattacharya., et al. 2021
- Pediatric Academic Societies. Relationship between Early laboratory Measures and Neurological Injury in Neonates undergoing Therapeutic Hypothermia. Poster presentation. **Kebaya L.**, Levin S., Bhattacharya., et al. 2021
- Canadian Neonatal-Perinatal Research meeting. Relationship between Early laboratory Measures and Neurological Injury in Neonates undergoing Therapeutic Hypothermia. Poster presentation. **Kebaya L.**, Ee Mong, Bhattacharya., et al. 2021
- SPIE BiOS Photonics. NNeMo (Neonatal NeuroMonitor): A non-invasive brain monitor for continuous acquisition of cerebral blood flow and cytochrome c oxidase in the premature brain. Oral presentation. Rajaram A., **Kebaya L.**, St. Lawrence K., et al. 2020
- Child Health Research Day, Western University, London, ON. *Cerebral Blood Flow and metabolic neuromonitoring immediately following premature birth.* Oral presentation. Rajaram A., **Kebaya L.**, St. Lawrence K., et al. 2020
- Global Evidence Summit. Implementation of basic newborn resuscitation guidelines among health care providers in Maragua District Hospital: a best practice implementation project. Poster presentation. **Kebaya L.**, Kiruja J., Munn Z., et al. 2017
- Conference on Retroviruses and Opportunistic Infections (CROI). Efficacy of Mobile Phone Use on Adherence to Nevirapine Prophylaxis and Retention in Care among HIV-Exposed Infants, A Randomized Controlled Trial. Poster presentation. **Kebaya L.**, Wamalwa D., Nduati R. et al. 2015
- American Academy of Pediatrics (AAP) Conference. Efficacy of Mobile Phone Use on Adherence to Nevirapine Prophylaxis and Retention in Care among HIV-Exposed Infants”, A Randomized Controlled Trial. Oral presentation. **Kebaya L.**, Nduati R. et al 2015
- AAP National Conference and Exhibition. The recognition, Assessment and Management of Diarrheal Illness in Children Under 5 years at the Community and Health facility level in Kisii, Kenya. Poster presentation. Grant A, **Kebaya L.** 2014

Selected Workshops and Training

Newborn Resuscitation Program (NRP) Instructor Course. <i>Western University</i>	2022
Advances and Controversies in Neonatal Neurocritical care. SickKids Neonatal Neurology Symposium	2022
Writing in the Sciences Course. <i>Stanford University</i>	2021
Neonatal Neurology: HIE-focused Project Learning. <i>PAS Postgraduate Course</i>	2021
Tri-Council Policy Statement: Ethical Conduct for research Involving Humans (TCPS2). <i>Western University</i>	2021
Teacher Boot Camp using the Train-the-Trainer Model. <i>Schulich School of Medicine and Dentistry, Canada</i>	2020
Sonographic Clinical Assessment of the Newborn (SCAN) Workshop. <i>University of Calgary SCAN Program</i>	2020
Canadian National Perinatal Research Meeting of Care Workshop. <i>POCUSNeo International Group</i>	2020
Crucial Conversations in Neonatal-Perinatal Medicine. <i>London Health Sciences Centre/Western University</i>	2019 - 2022
Core Curriculum Workshop - Navigating the Ethical landscape of Pediatric Clinical Research, Recognizing Common Biostatistical Errors, and Grant writing. <i>PAS Fellows Series, Baltimore, MD, USA</i>	2019

Employment History

Neonatal-Perinatal Medicine Fellow. <i>Western University, London, ON</i>	2018 - 2022
Consultant Pediatrician. <i>Ministry of Health, Kenya</i>	2015 - 2018
Paediatric Resident. <i>Department of Pediatrics & Child Health. University of Nairobi, Kenya</i>	2011 - 2014
Deputy Provincial Antiretroviral (ART) Officer. <i>Ministry of Health, Kenya</i>	2010 - 2011
Medical Officer. <i>Accident & Emergency Department. Jaramogi Oginga Odinga Teaching and Referral Hospital, Kisumu, Kenya</i>	2009 - 2010
Medical Officer Intern. <i>Jaramogi Oginga Odinga Teaching & Referral Hospital, Kisumu, Kenya</i>	2008 - 2009

Qualifications, Certification, and Licenses

Subspecialty Affiliate (SEAP) , Royal College of Physicians and Surgeons of Canada. #2543312	2021 - Present
Clinical Fellow , College of Physicians and Surgeons of Canada. #116533	2018 - 2022
Licensure , Kenya Medical Practitioners and Dentists Council. #A6551	2009 - Present