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3D Ultrasound in the Management of Post Hemorrhagic Ventricle Dilatation

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Abstract

Enlargement of the cerebral ventricles is relatively common among extremely preterm neonates born before 28 weeks' gestational age. One common cause of ventricle dilatation is post hemorrhagic ventricle dilatation (PHVD) following a bleed in the cerebral ventricles. While many neonates with PHVD will have spontaneous resolution of the condition, severe, persistent PHVD is associated with a greater risk of brain injury and morbidity later in life and left untreated can cause death. The current clinical management strategy consists of daily measurements of head circumference and qualitative interpretation of two-dimensional US images to detect ventricular enlargement and monitoring vital signs for indications of increased intracranial pressure (i.e. apnea, bradycardia). Despite the widespread clinical use of these indicators, they do not have the specificity to reliably indicate when an intervention to remove some CSF is required to prevent brain damage. Early recognition of interventional necessity using quantitative measurements could help with the management of the disease, and could lead to better care in the future. Our objective was to develop and validate a three-dimensional ultrasound system for use within an incubator of neonates with PHVD in order to accurately measure the cerebral ventricle volume. This system was validated against known geometric phantoms as well as a custom ventricle mimicking phantom. Once validated, the system was used in a clinical study of 70 neonates with PHVD to measure the ventricle size. In addition to three-dimensional ultrasound, clinical ultrasound images, and MRIs were acquired. Clinical measurements of the ventricles and three-dimensional ultrasound ventricle volumes were used to determine thresholds between neonates with PHVD who did and did not receive interventions based on current clinical management. We determined image based thresholds for intervention for both neonates who will receive an initial intervention, as well as those who will receive multiple interventions. Three-dimensional ultrasound based ventricle volume measurements had high sensitivity and specificity as patients with persistent PHVD have ventricles that increase in size faster than those who undergo resolution. This allowed for delineation between interventional and non-interventional patients within the first week of life. While this is still a small sample size study, these results can give rise to larger studies that would be able to determine if earlier intervention can result in better neurodevelopmental outcomes later in life.

Keywords

Hydrocephalus, three-dimensional ultrasound, preterm neonate, intraventricular hemorrhage, post hemorrhagic ventricle dilatation, evidence based medicine, classification

Co-Authorship Statement

This thesis is presented in an integrated article format, and the chapters of which are based on the following publications that are either published or in preparation for submission to publish. As first author on the peer-reviewed manuscripts, Jessica Kishimoto made a significant contribution to all aspects of the studies, the manuscript preparation and submission. Specifically, Jessica imaged, or collected the images of all the study participants, and was responsible for the data collection and managing of the database for the studies. She designed the research questions, and was responsible for planning, implementing and analyzing the experiments and data. She drafted and revised all papers prior to submission to publication. All work was performed under the supervision of Dr. Sandrine de Ribaupierre and Dr. Aaron Fenster. Dr. de Ribaupierre was responsible for the initial study conception and design, and defining the research questions and interpretation of results as well as ensuring the study followed Good Clinical Practice. Dr. Fenster supervised and supported the design and validation of the imaging system used to bring Dr. de Ribaupierre's study conception to life. For each manuscript contained in this thesis, all other co-authors contributed to reviewing and editing the manuscript and their specific contributions are described below.

Chapter 2 is an original research article entitled "3D ultrasound system to investigate intraventricular hemorrhage in preterm neonates" and was published in *Physics in Medicine and Biology* in October of 2013. The manuscript was co-authored by Jessica Kishimoto, Sandrine de Ribaupierre, David S.C. Lee, Richa Mehta, Keith St. Lawrence, and Aaron Fenster. David. S.C. Lee and Richa Mehta enrolled patients and collected clinical data. Richa Mehta additionally performed segmentation of some study patient images. Keith St. Lawrence assisted in supervision of the project.

Chapter 3 is an original research article entitled "In Vivo validation of a 3-d ultrasound system for imaging the lateral ventricles of neonates" and was published in *Ultrasound in Medicine and Biology* in April 2016. The manuscript was co-authored by Jessica Kishimoto, Aaron Fenster, David S.C. Lee, and Sandrine de Ribaupierre. David S.C. Lee enrolled patients, managed initial imaging studies by introducing the parents of study participants to the study team, and collected clinical data for study patients.

Chapter 4 is an original research article entitled “Preterm neonatal lateral ventricle volume from three-dimensional ultrasound is not strongly correlated to two-dimensional ultrasound measurements” and was published in the *Journal of Medical Imaging* in October 2016. The manuscript was co-authored by Jessica Kishimoto, Sandrine de Ribaupierre, Fateme Salehi, Walter Romano, David S.C. Lee, and Aaron Fenster. David S.C. Lee enrolled patients, managed initial imaging studies by introducing the parents of study participants to the study team, and collected clinical data for study patients. Walter Romano and Fateme Salehi measured the ventricle index, anterior horn width, thalmo-occipital distance, and third ventricle width on all study participants clinical ultrasounds.

Chapter 5 is an original research article entitled “Quantitative 3D head ultrasound measurements of ventricle volume to determine thresholds for preterm neonates requiring interventional therapies following PHVD” and is in preparation for the journal *Scientific Reports.* The manuscript was co-authored by Jessica Kishimoto, Sandrine de Ribaupierre, David S.C. Lee, and Aaron Fenster. David S.C. Lee enrolled patients, managed initial imaging studies by introducing the parents of study participants to the study team, and collected clinical data for study patients.

Chapter 6 is an original research article entitled “Estimating ventricle volume from lineal ultrasound measurements in neonates with post hemorrhagic ventricle dilatation: benefits and limitations” and is in preparation for the journal *Acta Paediatrica.* The manuscript was co-authored by Jessica Kishimoto, Aaron Fenster. Walter Romano, Fateme Salehi, David S.C. Lee, and Sandrine de Ribaupierre. David S.C. Lee enrolled patients, managed initial imaging studies by introducing the parents of study participants to the study team, and collected clinical data for study patients. Walter Romano and Fateme Salehi measured the ventricle index, anterior horn width, thalmo-occipital distance, and third ventricle width on all study participants clinical ultrasounds.

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List of Abbreviations

2D – two dimensional

3D – three dimensional

AHW – anterior horn width

AMOSO - Academic Medical Organization of Southwestern Ontario

AUC – area under curve

CIHR - Canadian Institutes of Health Research

COV – coefficient of variation

CPAP – continuous positive airway pressure

CPC - choroid plexus cauterization

CSF – cerebral spinal fluid

CT - computed tomography

DRIFT - drainage, irrigation, and fibrinolytic therapy trial

ETV - endoscopic third ventriculostomy

EVD – external ventricle drain

GA – gestational age

HC – head circumference

ICC – intra-class correlation

ICP - intracranial pressure

IV - intravenous

IVH – intraventricular hemorrhage

LP – lumbar puncture

MDD – minimum detectable distance

MRI - Magnetic Resonance Imaging

MSE – mean squared error

MSR – mean squared rater

MSS – mean squared subject

NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells

NICU – neonatal intensive care unit

NIRS – near infrared spectroscopy

PDA - Patent Ductus Arteriosus

PHH – post hemorrhagic hydrocephalus

PHVD – post hemorrhagic ventricle dilatation

ROC – receiver operator curve

SEM – standard error of measurement

TFNα - tumor necrosis factor alpha

TOD – thalamo- occipital distance

TPN - choroid plexus cauterization

TVV – total ventricle volume

US – ultrasound

VADs – ventricular access devices

VI – ventricle index

VLBW – very low birth weight

VP - ventriculo-peritoneal

VSGS - ventriculosubgaleal shunt

VT – ventricle tap

VV – ventricle volume

WD – water displacement

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Chapter 1

1 Introduction

Post hemorrhagic ventricle dilatation in preterm born neonates has highly variable outcomes and management strategies. This chapter will give a brief outline of the disease, its outcomes, the clinical management practices, and image based monitoring.

1.1 Overview

Very low birth weight (VLBW) neonates (less than 1500g) represent 1.2% of all live births. In recent decades, due to changes in perinatal management such as increased use of antenatal steroids to speed up lung development, use of surfactant to improve lung function after birth, and delayed (umbilical) cord clamping, the rates of neonatal mortality have been substantially decreasing in this population.[1] However, even with these improvements, almost half of all VLBW neonates in Canada will either die or survive with major neonatal morbidity including, but not limited to, severe intraventricular hemorrhage (IVH).[2]

IVH, a bleed located inside the lateral cerebral ventricles, is a common disease found in VLBW neonates with occurrence around 20-30% in this population.[3] The incidence and severity of the hemorrhage increases with the degree of prematurity of the neonate, with those born earliest having the highest risk.[4] The clots and blood breakdown products from a severe hemorrhage can block the flow of the cerebral spinal fluid (CSF), leading to post hemorrhagic ventricle dilatation (PHVD), which is the abnormal, progressive enlargement of the ventricular system. This enlargement can compress the surrounding brain and can lead to major disability and even death. In its most severe state, PHVD is known as post hemorrhagic hydrocephalus (PHH). Occurrence of PHH is 9-25% of all patients with IVH.[5,6] While the mean direct costs of initial hospitalization for VLBW preterm neonates with IVH is $300,000 USD, in patients with PHH the cost increases to $495,000 USD.[6] Neonates with PHH would tend to have a more complicated care course than neonates without PHH, and these numbers only reflect the initial stay within the

neonatal intensive care unit (NICU) and not the prolonged care required for preterm patients after discharge, which adds considerably to the cost of care.

Neonates with PHH tend to have poor developmental outcome and higher mortality rates, thought to be caused by injury to the white matter.[7] While cranial ultrasound (US) has been used to diagnose and monitor IVH and PHVD, there is currently no consensus on how and when to treat neonates with this disease in a manner that optimizes neurological outcomes and quality of life.

1.2 Anatomy

1.2.1 The neonatal brain

The preterm neonatal brain has many significant differences when compared to an adult, or even a term age neonate. The most apparent is the less prominent sulci and gyri (cortical folds) in comparison to the term age neonate, or adult (Fig. 1-1).[8] In addition, the germinal matrix located inferior to the lateral ventricles, and superior to the caudate nucleus is present in the early neonatal period. This structure is a highly vascularized region from which neurons migrate to the cortex during brain development. Generally, the most active part of this cell migration occurs while the fetus is *in utero* for neonates born at term equivalent age.[9] However, very preterm neonates are born during this very active cell proliferation and migration, and angiogenesis (creation of new blood vessels), and this is the primary site of hemorrhage in many IVH cases.

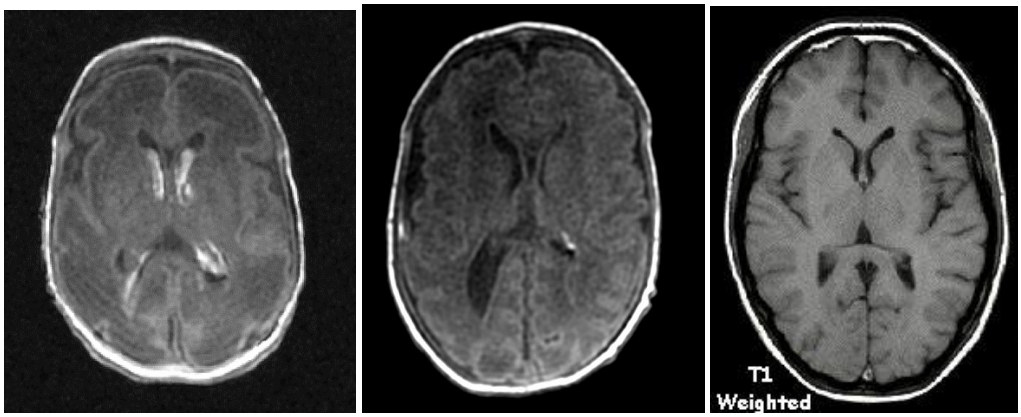

Figure 1-1 MR images of brains. a) preterm brain at 28 weeks GA with a grade III IVH, b) preterm born neonate at 38 weeks GA (term equivalent age) and c) and adult brain. Adapted with permission by Kieran Maher using Graphic Converter from Applied Imaging Technology

As the germinal matrix is adjacent to the lateral ventricles, hemorrhages stemming from this region tend to pour into the ventricles causing IVH. (See Fig. 1-2)

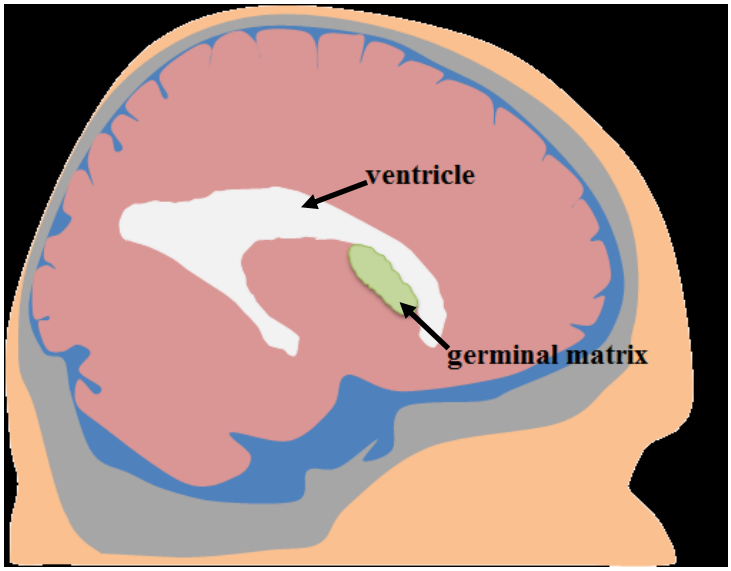

Figure 1-2 Approximate anatomy of ventricle and germinal matrix. Germinal matrix is indicated next to the lateral ventricles in the sagittal plane.

1.2.2 The ventricle system and flow of cerebral spinal fluid

The ventricular system allows for the generation of CSF which is involved in the mechanical protection of the brain and functions to regulate the cerebral environment.[10] The cerebral ventricles themselves are four communicating chambers that house the CSF generating choroid plexus. Under normal conditions, CSF is generated by specialized cells that filter blood plasma into the ventricles and this CSF is then circulated through the ventricles into the subarachnoid space surrounding the brain and also within the spinal cord, both of which house areas that the CSF can be reabsorbed into the blood stream. Visual representation of the flow of CSF can be found in Fig. 1-3.

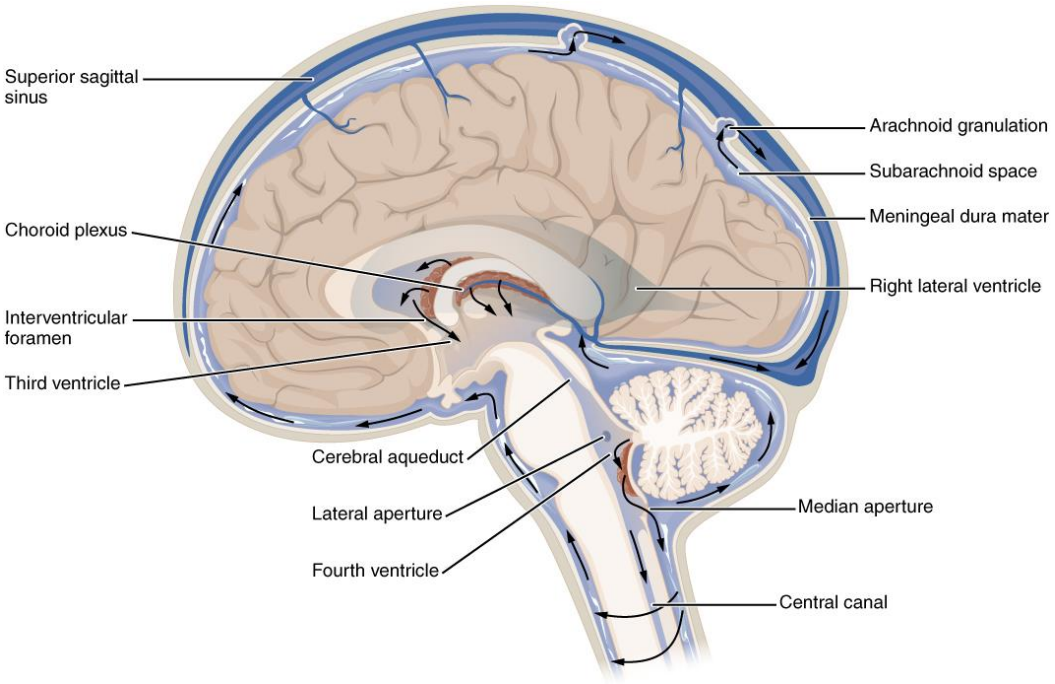

Figure 1-3 Flow of CSF. Used with permission from Textbook OpenStax Anatomy and Physiology as Published May 18, 2016 at: https://commons.wikimedia.org/wiki/File:1317_CFS_Circulation.jpg

The lateral ventricles are the two largest chambers on either side (left and right) of the brain wrapping around the thalamus in a C-shape. They can be subdivided into the anterior, temporal (inferior), and posterior (occipital) horns and house the largest portions of choroid plexus. In the anterior horn region of the lateral ventricles, the Foramen of

Monroe communicate with the third ventricle. The third ventricle is located at the mid-line of the brain between the two thalami. From the third ventricle CSF can drain into the fourth ventricle through the Aqueduct of Sylvius, which is the narrowest channel in the ventricular system and is highly susceptible to blockage. From the fourth ventricle, which is also located at mid-brain, the CSF then drains into the subarachnoid spaces through the lateral foramina of Luschka. Image of the ventricle anatomy can be seen in Fig. 1-4.

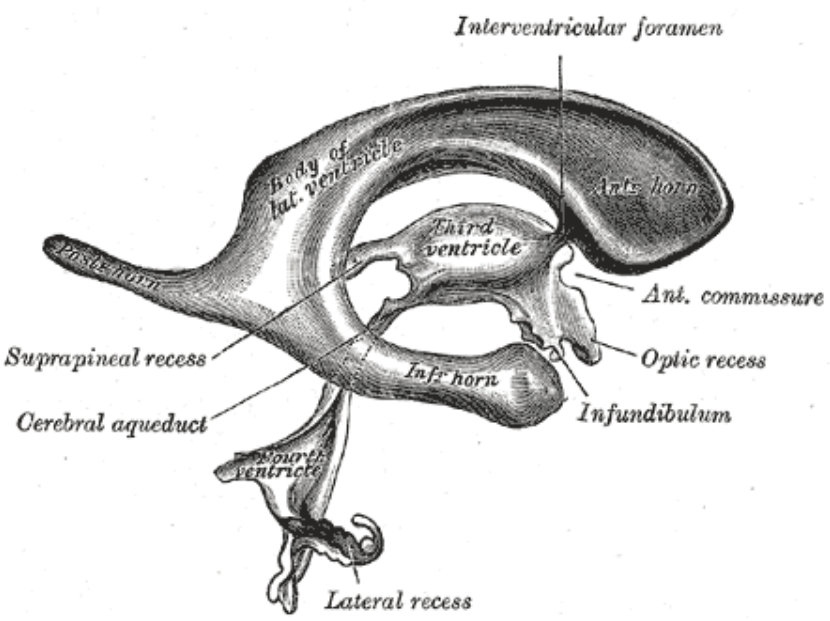

Figure 1-4 Drawing of a cast of the ventricular cavities, viewed from the side. From Anatomy of the Human Body by Henry Gray. Image is a scan of a piece of work that is part of public domain.

The choroid plexus generates CSF and is located within the lateral ventricles, the roof of the third and forth ventricles. The choroid plexus can generate upwards of 500 mL of CSF per day in healthy adults, and therefore any blockage in the flow of the CSF from the choroid plexus containing ventricles to the subarachnoid space where it is reabsorbed can result in CSF gathering in the ventricles leading to ventricle dilatation.

1.3 Risk factors

The major risk factor for severe IVH is very low gestational age[11] and very low weight at birth with neonates born closest to viability at highest risk.[12,13] Additional antenatal risk factors include no antenatal steroid treatment and/or no magnesium sulfate therapy to the mother and having been conceived via fertility treatment (especially *in vitro* fertilization).[14,15] Perinatal risk factors include early sepsis, high fraction of ventilated oxygen in the first day of life, pneumothorax (air or gas between the lungs and the chest wall), born outside of level three NICUs, treatment for hypotension in first days of life, and acidosis.[14-16] Neonates born after maternal substance abuse, specifically cocaine use, are at an increased risk of IVH, and increased severity of the disease even after accounting for gestational age.[17-19] These risk factors can generally be considered to cause fluctuations in blood flow, high venous pressure, and/or abnormal blood pressure, and as the vasculature in the germinal matrix is particularly fragile and not able to adapt to rapidly changing hemodynamic environments, this can cause the vessels to burst and a hemorrhage to occur. [20] Although the mechanism is not totally understood, male sex is associated with having more severe IVH even after confounding factors are controlled.[21]

1.4 Pathophysiolgy of IVH to PHVD and hydrocephalus

For most very preterm patients, IVH occurs within the first 72 hours of life with the subsequent ventricle dilatation occurring soon after the hemorrhage. Generally, the hemorrhage is thought to stem from the germinal matrix, which is a site of rapid cell division and migration, as neuronal progenitor cells are generated and moved to their eventual site (the cerebral cortex). Rapid angiogenesis occurs within the germinal matrix during this early neonatal period to feed the cell division. These rapidly generated vessels are fragile, immature, and lack structural support (such as smooth muscles or collagen) of more developed vasculature.[20] Additionally, the germinal matrix is located between relatively large arterials and veins. This makes it a prime site for rupture should any fluctuation in blood pressure or flow occur. When born preterm, the neonate has many major stressors as mentioned previously that cause various amounts of extra stimuli to their system. The poor capacity of the preterm neonate to adapt and regulate the body's

cardiovascular systems to outside stimuli (autoregulation) causes surges in blood flow and/or pressure in the vessels of the brain instead of the body automatically tempering the system to a lower flow/pressure which could be accommodated by the fragile vessels.

Following an IVH, red blood cells are destroyed (hemolysis), and hemoglobin is released into the ventricular space. The commonly held belief was that IVH induces hydrocephalus due to blockage of the flow of CSF from the ventricles into the subarachnoid space. Through this line of thought, blood clots can acutely block smaller aqueducts between the lateral ventricles and third and fourth ventricle. This would cause 'obstructive' hydrocephalus, an enlargement of the ventricles as the CSF is continually generated by the choroid plexus, but cannot flow to where it can be reabsorbed. Over time, as the blood clots have been broken down, hemosiderin, an end product of hemoglobin breakdown, can become lodged in the subarachnoid space and reduce the amount of CSF that can be absorbed. If the CSF flow had been restored between the ventricles and the subarachnoid space, the enlargement of the ventricles from this lack of reabsorption could cause 'communicating' hydrocephalus.

Between the acute blood clot phase, and the hemosiderin deposit phase, many pro-inflammatory pathways are induced as the hemoglobin breaks down into different blood breakdown products. Over time, the visual appearance of the CSF change to reflect the breakdown of the hemorrhage and clearance of the breakdown products (Fig. 1-5). While research into the particular processes of the hemorrhage breakdown following IVH causing induction of inflammation in the preterm neonatal brain is ongoing, a few pathways and factors have been elucidated.

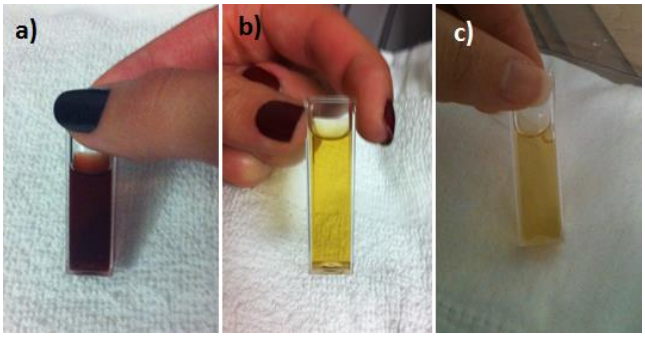

Figure 1-5 CSF from patients with PHVD. CSF colour change as a hemoglobin is broken down and the blood break down products are slowly removed from the CSF.

Specifically, methemoglobin, a hemoglobin breakdown product, has been shown to form pro-inflammatory cytokines (tumor necrosis factor alpha, TNFα) in a rabbit model, and the same cytokine, TNFα, was observed to increase in concentration as the amount of methemoglobin increased in CSF samples of preterm neonates following IVH.[22] Additionally, the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) protein complex was found to be significantly more activated in barrier cells of the choroid plexus in a rat model of IVH with marked ventricle dilatation.[23] The author's posited that the activation of this complex could lead to barrier cell, and tight junction dysfunction leading to abnormally high transfer of proteins into CSF, and perhaps the increased generation of CSF.[23]

Hemolysis was determined to be a factor in the increase in size of ventricles in a rat model, while intact red blood cells injected into the ventricles did not show a similar increase in ventricle dilatation.[24] Iron, also released as a part of hemolysis, has also been implicated in the development of hydrocephalus.[24,25] While specific pathways that seem to cause the inflammation from hemoglobin breakdown products have not been fully investigated, inflammation, and subsequent tissue scarring, is certainly a likely cause of stenosis and blockage of the smaller channels (i.e., cerebral aqueduct) that CSF must go through in order to become reabsorbed.

In general, irrespective of the mechanism of action, there are three clinical courses of PHVD, 40% of patients spontaneously resolve, 10% rapidly progress, and 50% persistently but slowly progress.[5] Infants who have rapidly progressing PHH and some

with persistent, slowly progressing PHH will require interventions to prevent further brain damage.[26]

1.5 Treatment of PHVD

Definitive, curative medical treatment for PHVD as of yet does not exist for routine clinical use. However, there have been a few prior studies investigating potential therapies, as well as surgical therapies that are the standard of care for neonates with PHVD that does not resolve.

1.5.1 Prior medical treatments, the DRIFT trial, current stem cell therapies

For many years, to minimize the production of CSF, and hopefully reduce the number of complications and surgical procedures, neonates with progressive hydrocephalus were treated with diuretics.[27] Diuretics such as acetazolamide and furosemide cause a reduction in CSF production, and one uncontrolled study suggested that there was a reduction in surgical shunt placement.[28] However, this drug-based therapy came with side effects that could increase risk of morbidity and mortality. In one multicenter randomized, control trial of the efficacy of the treatment, it was found that neonates with PHVD treated with acetazolamide and furosemide had no difference in shunt placement, but had an increase in neurological morbidity.[29,30] This type of therapy is no longer standard care, or recommended.[31]

As it was thought that the cause of PHVD was primarily caused by a blockage of the flow of CSF from the ventricles to areas of reabsorption, medication to break up the blood clots were administered.[32] The intraventricular administration of the thrombolytic agent streptokinase was seemingly promising in an early, non-randomized study;[32] however, the results of a case controlled follow up study did not find any benefit to the therapy, and indeed more of the treated patients required shunt surgery than those who had standard care.[33] Following this, and with the hypothesis that both the blood clots as well as pro-inflammatory cytokines are causing the PHVD, the drainage, irrigation, and fibrinolytic therapy trial (DRIFT trial) was proposed.[34] The surgical insertions of two catheters into the lateral ventricles (one right frontal, one left posterior) was followed by fibrinolytic

therapy to break up blood clots, then after a period of time a constant, slow infusion of artificial CSF was administered until the draining fluid cleared to the appearance of "white wine".[34] While the randomized, controlled study was suspended due to an increase in secondary intraventricular bleeding within the treatment arm, among those patients who survived and completed the trial, a lowered rate of severe cognitive disability and higher mental developmental index was seen in the treatment group in comparison to control when the patients were followed up at 2 years of age.[35] In spite of this, due to the significant risk of meningitis from the indwelling catheters and increased secondary hemorrhage, DRIFT therapy is not recommended.[31,36]

Some current research into treatment of IVH include the use of umbilical cord blood derived mesenchymal stem cells to attenuate inflammation after a severe IVH.[37] Pre - clinical results in a rat model of PHH have shown an attenuation in development of PHH, brain injury, and impaired behavioral test.[38] A single center clinical trial is ongoing.[39]

1.5.2 Surgical Intervention

A number of procedures exist to alleviate increased intracranial pressure (ICP) caused by the excess CSF from PHVD. The most commonly used temporary methods include lumbar puncture, external ventricle drainage, ventricular access devices (reservoirs), and ventriculosubgaleal shunts. In addition, our center uses ventricle taps for temporary ICP relief. Additional, slightly more invasive procedures include the valveless ventriculo-peritoneal (VP) shunt and endoscopic third ventriclostomy. There is no consensus of evidence into which of these methods are the best for temporary CSF diversion[40,41] with all procedures linked to poorer neurodevelopmental outcomes.[42] Often the end point 'permanent' surgical intervention is a VP shunt that is valve regulated and this can remain in the patient for their entire lifespan. Patients with VP-shunts tend to have poorer outcomes than those without shunts.[43] Generally, the progression of treatment ranges from least invasive (lumbar puncture), to most invasive (VP-shunt); however, during the course of each type of treatment, a small subset of neonates will have spontaneous resolution of the symptoms from increased ICP and require no further therapy while in the NICU. Of those patients who are non-symptomatic after some treatment, a small

number will once again require treatment later in life, often once the skull sutures close. All mentioned techniques will be outlined briefly.

1.5.2.1 Serial CSF tapping – Lumbar Puncture and Ventricle Tap

Serial tapping allows for the temporary relief of increased ICP as well as analysis of CSF to determine the amount of protein. A needle is inserted either in the lumbar spine (lumbar puncture), or transfontelle into the ventricle space through the frontal lobe (ventricle tap, VT). Lumbar puncture had previously been shown to improve hydrocephalus in patients who appeared to have communicating hydrocephalus (not a full blockage in the flow of CSF) at least temporarily;[44] however, after a systematic review of literature, this is no longer advised for most patients with PHH as generally, they showed no improvement in rates of VP shunt placement, death, or disability in comparison to conservative treatment.[45] The ventricle tap is the first line of treatment in our center as the nursing care and management of the neonate post-ventricle tap is somewhat less complicated than in neonates with drains or access devices (there are not catheters or skin irritation from a constantly growing subcutaneous reservoir). This is especially important for those patients with extremely low birth weights who might have sensitive skin and require minimal handling. Ventricle taps do however result in needle cysts that can be seen on imaging in between serial VTs (Fig. 1-6). It should be additionally noted, that the neonate is experiencing periods of high ICP followed by low ICP immediately following the intervention and this change in pressure can be experienced multiple times a week when serial tapping is occurring. Certainly, this is not optimal management, though that has not been definitively determined through scientific study.

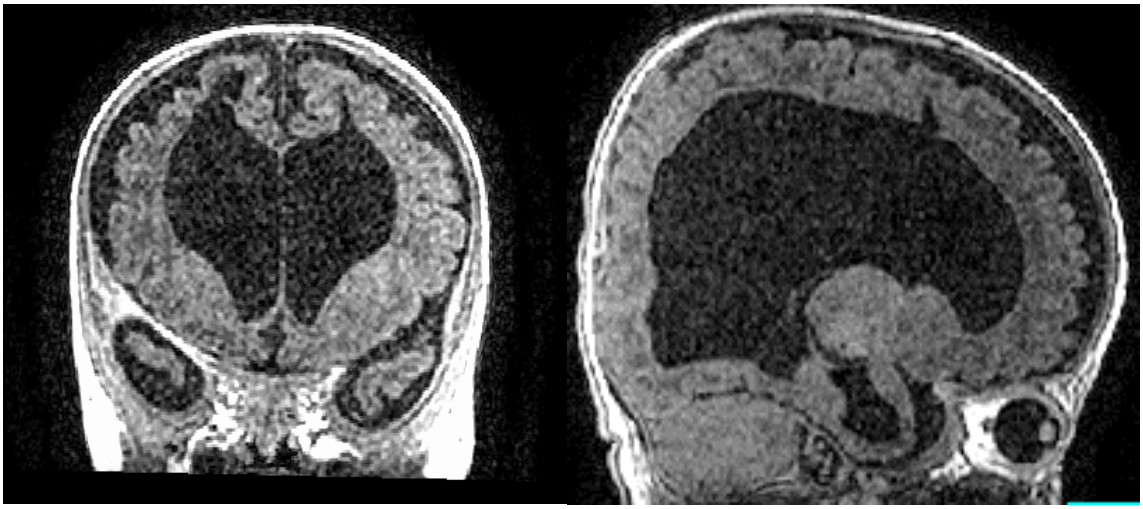

Figure 1-6 MR images of needle track cysts from serial VTs. MRI was taken prior to VP-shunt insertion from a neonate at term equivalent age who had PHVD following grade III IVH.

1.5.2.2 External Ventricle Drainage

An external ventricle drainage (EVD) system allows for the constant removal of CSF in order to keep a consistently lowered ICP. A catheter is inserted through the fontanelle into the anterior horn of one of the lateral ventricles and this is generally done within the NICU. The end of the catheter is placed within a bedside, external, closed drainage system which can be raised or lowered to adjust the amount of CSF drained. A neonate cannot go home with an EVD as even within the hospital setting the risk of infection has been reported to be quite high (26%).[46] However, it has been reported that the use of EVD reduces VP shunt placement in a third of neonates with PHH.[47-49] Clinical judgement is generally indicated to determine if an EVD is used.[31]

1.5.2.3 Ventricle Access Devices and Reservoirs

Ventricular access devices (VADs) have a catheter placed into the anterior horn of the lateral ventricle much like EVDs; however, instead of an external container constantly collecting CSF at the end, there is a subcutaneous reservoir that collects CSF. This reservoir can be aspirated through a needle puncture to remove CSF daily, and has the advantage of allowing a slower, more controlled removal of CSF in compared to VT or lumbar punctures. In theory, this would mediate the extreme high and low ICP

experienced in the latter procedures. The major risks and complications from VADs are peri-operative infection, skin defects, and CSF leaking.[50]

1.5.2.4 Ventriculosubgaleal Shunts

A ventriculosubgaleal shunt (VSGS), has a catheter, which shunts CSF from the lateral ventricles into a large 'pocket' made in the subgaleal potential space by the surgeon.[51] This allows for temporary continuous drainage until the pocket becomes full of fluid and stops draining, or the PHVD resolves. In the former case, the VSGS can be converted into a VP shunt. There is some evidence from a single center study that a VSGS results in a lesser VP shunt rate than using VAD as well as requiring less often CSF aspirations.[31,50] It should be noted that the complications that VADs have held true for VSGS; however, due to the less often CSF aspirations, perhaps there is a lowered infection risk. Despite the benefits, the unsightly cosmetic appearance of the pocket protruding from the patient's head is possibly a factor in the reduced use of this type of shunt.

1.5.2.5 Endoscopic Third Ventriculostomy

The endoscopic third ventriculostomy (ETV) is a technique where a hole is made on the floor of the third ventricle through an endoscopic approach so that CSF can drain into the basal cistern where it can be reabsorbed by the arachnoid granulations.[52] This technique was typically used in patients with non-communicating hydrocephalus (CSF is blocked from draining into the arachnoid granulations, often through aqueduct stenosis). PHH is thought to be both communicating (where there is an increased production of CSF) as well as non-communicating (having a blockage of CSF flow). Since, PHH is not easily categorized as either for any given patient, and indeed it can be both in the same patient, choroid plexus cauterization (CPC) which decreases the amount of CSF produced is often combined with ETV. There are ongoing trials into the use of ETV/CPC in patients with PHH;[53,54] however, there is no data from randomized controlled trials at this time.

1.5.2.6 Ventriculo-peritoneal Shunt

The ventriculo-peritoneal (VP) shunt is often the definitive surgery for patients with PHH. In this, a catheter is placed between the ventricles and the abdominal cavity and can be

seen in Fig. 1-7. A valve is set to either maintain a certain pressure or flow of CSF so that the ventricles are not over drained when a patient sits or stands. The VP shunt is however prone to failure[55], whether through infection, obstruction, or over drainage. Generally, infection and obstruction requires shunt revision surgery and patients who undergo VP shunts from PHH are at a high risk of complications requiring revision.[55] VP shunt insertion is correlated with poorer outcomes, and with every additional revision increases the risk for morbidity. Although some criteria such as patient weight over 1500g and/or CSF protein count <100mg/dL has been used to determine when shunts are inserted, there is no evidence from randomized controlled trials to back up these criteria.[31] It has previously been noted through laboratory experiments that blood cells impact shunt valves more than protein levels, though there may be a correlation between the two, this has not be definitively shown.[56]

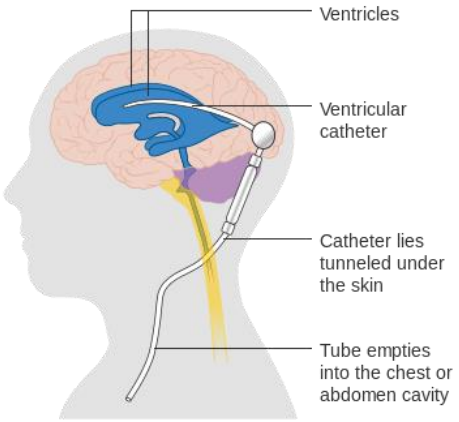

Figure 1-7 Diagram of a VP-shunt. Fair use under the Creative Commons attribution international license (attribution to: Cancer Research UK / Wikimedia Commons)

1.5.2.7 Valveless Shunts

A valveless shunt is much like a VP-shunt, except it lacks a valve to maintain pressure or flow of CSF. While this type of shunt has been shown to reduce neurosurgical hospitalizations in adult patients with PHH, the use in preterm neonates with PHVD requires either the removal or conversion to a valve regulated VP-shunt once the infant

become ambulatory enough to sit upright.[57] As this type of shunt might allow for infants to go home earlier than waiting for a VP-shunt, clinical judgement is required to determine if a patient is well enough to leave the NICU, and if parents are able to return to the hospital for a revision surgery once the infant is sitting upright. Complications are similar to that of VP-shunts with valves; however, they hold a larger risk of over drainage.

1.6 Current Monitoring and Management

Neonates at risk of PHVD are screened using transfontanelle head ultrasound. The exact time frame for when this screening occurs varies widely from hospital to hospital. At our center, neonates born at less than 1500g are screened during the first week of life as well as the fourth week of life if no pathology is present on the first image. As most IVHs are evident by the third day of life[58] the first week scan often detects the presence of severe IVH before they become obvious from the clinical status of the patient. More frequent ultrasound scanning occurs at the discretion of the clinical team. Once PHVD is diagnosed, neurological exams are routinely performed to assess for worsening signs and symptoms of increased ICP. In general, a full and tense fontanelle, splaying skull sutures, and increased in head circumference are used to determine raised ICP. Additionally, clinical signs and symptoms such as increased spells of apnea and bradycardia, lethargy, and decreased activity could be signs of increased ICP, but are non-specific as other common co-morbidities in this patient population could also cause these symptoms. Additionally, there are some preterm neonates who have no clinical signs and symptoms and who only are diagnosed upon screening imaging.

1.7 Imaging Neonatal Brains

Imaging preterm neonates is challenging for many reasons. Those born the most preterm (about 24-28 weeks gestational age (GA)), are those who should be screened most often; however, they are also the population who will more likely have spells of apnea, unstable heart rate (bradycardia and/or tachycardia), and who could require resuscitation and increased oxygen just from handling (touching, moving the neonate). Additionally, these patients are incubator bound, requiring outside temperature regulation, in addition to ventilation for their immature lungs. A large fraction of these patients will also have IVs,

for various different reasons, such as requiring IV nutrition as they are not able to suckle (total peripheral nutrition (TPN)), for antibiotics, for treatment for other co-morbidities (such as patent ductus arteriosus), or for blood infusions. Therefore, methods that require the least amount of handling, and can be done with the patient inside the incubator are highly preferred for this patient population. Additional factors, such as the cost of care and time investment, need to be weighed against the potential value of an imaging session.

1.7.1 X-ray based methods

X-ray based imaging techniques have the ability to generate 3D images of the head through computed tomography (CT) and are relatively cost and time efficient. The first images of the preterm neonatal brain were performed using CT in the late 1970s, and the IVH grading system still used to this day was developed using this modality.[59] However, CT images quickly went out of favour, and are not generally done in the neonatal population anymore due to concerns over potential DNA damage from ionizing radiation. Neonates are growing at very fast rates, with many organ systems undergoing rapid cell division and this makes them at risk candidates for dose-related induction of cancer later in life. Additionally, CT would require moving the neonate into an imaging facility, which is often not feasible.

1.7.2 Magnetic Resonance Imaging (MRI) Methods

MRI methods for imaging preterm neonates is an ongoing area of research and clinical interest. MRI allows for better structural visualization of the brain and has high tissue contrast allowing for the differentiation of white and grey matter along with hemorrhages. Fig. 1-8 illustrates MR images from preterm neonates at term equivalent age. Additionally, 3D images can be acquired to determine the size of brain structures including the ventricles following PHVD. However, there are many challenges associated with MRI that are specific to the neonatal patient population.

To image neonates who are incubator bound, they must be moved from the incubator in the NICU into a MRI compatible incubator. These incubators are relatively expensive and are generally only available in research centers. Preterm neonates are therefore only

imaged sparingly in these specialized centers after careful consideration to patient risk if imaged. The transfer to the MRI incubator and then to the MRI suite requires a dedicated staff member which increases costs, and increasing the amount of patient handling considerably.

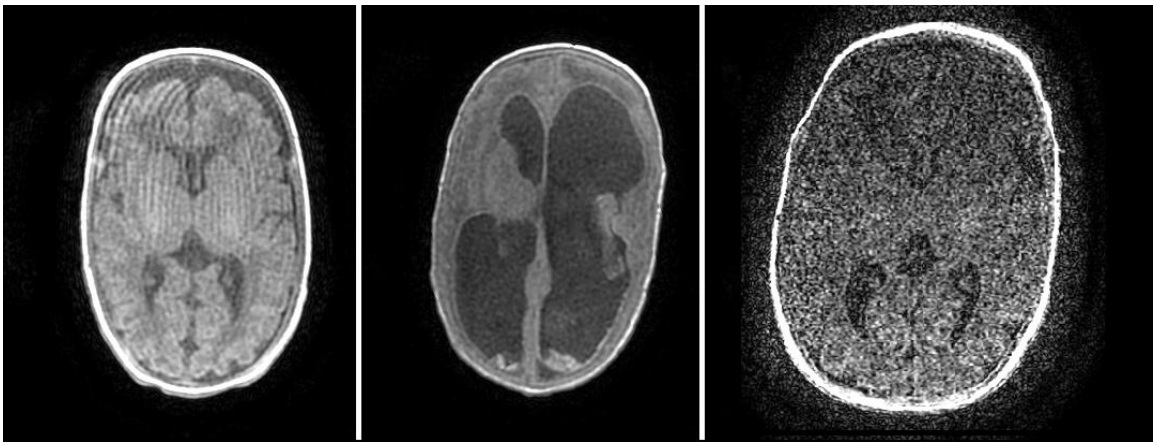

Figure 1-8 MR images of neonatal brains. From left to right: Motion artifact, hemorrhage and ventricle dilatation, poor image quality due to low signal to noise ratio

Motion is another particularly difficult issue to overcome since breath holding and other immobilization techniques are not feasible. Immobilization blankets and swaddling have been shown to work well for patients less than three months' age; however, this does not work for all neonates. Motion artifacts can be seen in Fig. 1-8a. Thermoregulation, if not done through an MRI-compatible incubator, is an additional challenge as MRI rooms are relatively cool. Neonates must be monitored closely for signs of distress from cold.

In general, the largest problem with imaging preterm neonates is the use of medical equipment such as IVs, ventilation devices, infusion pumps, and resuscitation devices that either are not safe to use in the MRI suite, or function inappropriately during scanning. Changing all medical devices to MRI compatible versions is expensive and challenging, and often this means delaying scans until the neonate is no longer reliant on ventilation or certain IV pumps.

Specialized head coils for neonates are not widely commercially available, which limits the highest quality imaging to centers that can develop these coils themselves, or to use sub-optimal set ups that worsen image quality. As such, imaging sequences for neonates are often limited by the hardware, and better imaging is only possible if the technology was developed in house.

1.7.3 Ultrasound Imaging Methods

Ultrasound imaging is particularly well posed for this preterm neonatal population in that it allows for rapid acquisition of images while at the bedside of the patient without using any ionizing radiation. Additionally, the imaging is relatively cost-efficient, which allows for serial exams to be performed to detect changes that can occur during both the initial stay in the NICU and during follow up. For these reasons, historically, US has been the imaging of choice for first line diagnosis of cerebral pathologies in incubator bound neonates.

2D US imaging is performed using mid-frequency, small footprint, curvalinar transducers with wide views. The small footprint allows for the use of the anterior fontanelle as an acoustic window as the skull would prove difficult to image through due to its high speed of sound (4000 m/s) and therefore high attenuation of ultrasound in comparison to soft tissues and bodily fluids (1480-1570 m/s). The frequency of the probes are between 5-8 MHz which allows for adequate depth of penetration of the tissues (between 7-14 cm). The images are generally acquired at a temporal frame update rate of 10-15Hz depending on the depth setting. Due to CSF having low scatter in US, the ventricles tend to appear hypoechoic (black) in images, while the solid blood clot from IVH appears hyperechoic (white) and the surrounding brain is a grey color. PHVD then appears as an increasing large CSF and blood presence surrounded by the brain. 2D US images of patients with mild, moderate, and severe PHVD can be seen in Figure 1-9.

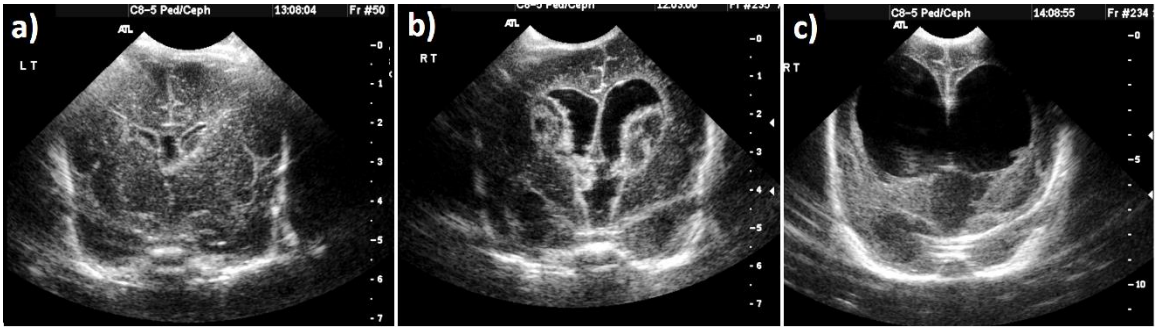

Figure 1-9 2D ultrasound images of neonatal brains. Para-coronal 2D US images of a) mild, b) moderate and, c) severe PHVD. Patients in b) and c) required interventions, and eventually had VP-shunts inserted.

Initially, ultrasound was performed through the temporal bone which allowed for a measurement across the anterior horns.[60] A normative curve was generated for patients born 25 weeks GA and older which allowed for the detection of ventricle dilatation and hydrocephalus in patients who were significantly above the normal range (the 97th centile).[60] Later, the anterior fontanelle was used as an acoustic window for imaging. The previously used anterior horn measurement was later appropriated to this new acoustic window though now measured on a para-coronal plane and is now known as the ventricle index (VI) (Fig. 1-10a). In some patients, the ventricles do not expand laterally and the VI might not change much during ventricle dilation, but instead balloon out into a more rounded shape.[61] For these patients, the anterior horn width (AHW),[62] which is measured diagonally across the largest part of the ventricle on the same para-coronal plane as VI (Fig. 1-10a) might be a better indicator of progressing ventricle dilatation. Since the ventricles are a highly irregular 3D structure, the anterior horns are not always the portion of the ventricles, which undergo dilatation. In 2000, Davies et al. generated reference curves for both the VI and AHW as well as for the 3rd ventricle width (Fig. 1-10a) and the thalamo-occipital distance (TOD) (Fig. 1-10b) to better address patients who could have ventricles dilating in different areas. The TOD is the only measurement made on the oblique sagittal plane instead of the oblique coronal and could be a better measurement to determine the course of PHVD in a sub-set of patient with ventricle dilatation primarily in the posterior horn. The 3rd ventricle width could potentially determine the site of the blockage of the flow of CSF, as it likely only undergoes dilatation if the blockage is in

the cerebral aquaduct, while would be less likely to dilate if the blockage is in the foremen of Monroe.

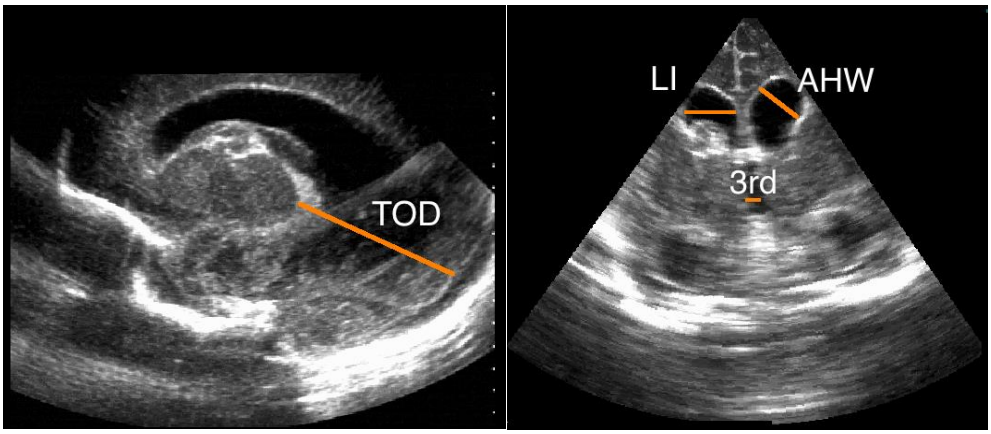

Figure 1-10 Ultrasound images of different linear ventricle measurements a) VI, AHW and 3rd b) TOD

While these quantitative measurements are better than qualitative estimations of ventricle dilatation, they still are limited by the intra- and inter- variability of each measurement. Specifically, while there is error in performing the measurement itself, there is additional error in the plane selection that an ultrasound technician records. This is confounded by the inherent limitation of using linear measurements to quantify the 3D irregularly shaped ventricles.

To address this, Brann et al. developed a cylindrical coordinate estimate of VV from 2D US images.[63] VV was estimated assuming that the technician was able to record 2D planes at equal angular spacing and found good agreement to phantoms[63] as well as to *in vivo* measurements of removed fluid during interventions.[64] They were able to create guidelines from a small pool of patients (N=48) with severe IVH and then prospectively used these guidelines predict which infants would go on to require interventions in a follow up study.[65] Although this work was performed in the late 1980 and early 1990s and was remarkably good at characterizing which patients would require treatment, it was never brought into clinical practice. Likely, this was due to the amount of time required to segment the ventricles from 2D US, and the training required to reproduce ultrasound planes that were spaced out at regular angular intervals.

1.7.4 Emerging 3DUS Imaging and Ventricle Volume Estimates

In the late 1990s, 3D US imaging began to look more feasible in a clinical research setting as 3D US systems became more readily available. Imaging was accomplished initially using a mechanical device to rotate a conventional US transducer to generate the third dimension[66] and this system was based on one used for cardiac imaging. Another group tracked a 2D US transducer during free hand scanning through electromagnetic tracking to generate 3D US images.[67] Other groups have had success acquiring 3D US images of neonatal ventricles using commercial tilting transducers,[68-70] which are not currently used as standard of care.

Mechanically rotated transducers use a motorized device to translate, tilt, or rotate a transducer during image acquisition in order to capture the volume of interest. These captured images are recorded by a computer, and because the scanning protocol (i.e., the amount rotated and the speed of rotation) is already known and precisely controlled, the captured 2D images have a known relative position to one another.[71] The computer that recorded the images and controlled the motor can then reconstruct the 3D volume. An electromagnetic tracking system in comparison is used to track the relative position of each image plane by tracking the location of a hand held transducer in space and relating that to where the recorded images would be located in relation to one another.[71] Again, the anatomy is imaged during acquisition and manipulation of the handheld transducer through the region of interest, but as the tracked images are not predefined to one degree of freedom, reconstruction of a tracked transducer takes longer than a mechanical rotation.

More recently, commercially available matrix transducers[72] have been used to image the neonatal ventricles.

Mclean et al. have found that 2D US measurements (the VI and AHW) can be made accurately on 3D US images. Salerno et al in a single center study on 30 patients, and Romero et al in a separate single center study on 59 patients found diagnostic agreement for ‘normal’ as well as diagnosis of hydrocephalus, IVH, periventricular leukomalacia, and subdural collection between 2D and 3D ultrasound images made on the same patient.

Images for both studies were taken immediate after one another. In general, the time per scan is reduced when a 3D US image is acquired in comparison to 2D US images (2 minutes for 3D US vs 5-10 minutes for 2D US).[70,72,73]

Some previous work has been performed acquiring ventricle volumes (VV) from 3D US images, including a study on 'normal' preterm ventricles[68] and those with mild ventricle dilatation[69] although this work has not been done in patients with PHVD.

While the commercially available 3D US systems are high performing, their high cost may limit their use, especially in the developing world. The previous studies using conventional 2D transducers (Abdul-Khaliq et al., 2000; Nagdyman et al., 1999) reconstructed the 3D US images offline, well after the patient had been imaged due to the time involved in reconstructing the image. Offline reconstructions resulted in examinations not encompassing the full ventricle volume in some patients, or the images were otherwise not of sufficient quality to be used in the study. While all previous studies have found 3D US to be feasible in a clinical setting, a system that could be used in conjunction with a conventional clinical 2D transducer to generate full 3D images at the bedside (incubator) would allow centers without a 3D US capable machine to confidently acquire 3D ventricle volumes.

1.8 Research Hypothesis and Objectives

The overall objective of the thesis was to develop a 3D US system that can accurately measure and monitor the ventricle volume of neonates with PHVD. Once developed, the system could then be used in a pilot study of patients with PHVD to develop thresholds for treatment and help guide clinical management of at risk neonates. The specific objectives and hypotheses tested in each chapter are briefly outlined below.

In Chapter 2, our objective was to develop a 3D US system and validate the system in the lab through various phantom experiments. We wanted to determine if the system was generating geometrically correct images that could provide volume measurements of irregular 3D structures, such as the lateral ventricles. We expected to find the system was able to measure linear strings in three orthogonal directions ±5% of the actual distance

between strings. Additionally, we made a phantom approximating the neonatal head with ventricles of known volume and hypothesized our 3D US system would be able to measure the phantom ventricle volume ±5% of the actual volume. As a proof of concept, three neonates were imaged and their ventricle volumes were segmented by three different observers to determine intra- and inter- observer variance of the measurement.

In Chapter 3, our objective was to further validate the 3D US system through *in vivo* experiments within neonates with PHVD in the NICU. We hypothesized that ventricle volumes from gold standard 3D MRI and 3D US from our system if acquired within 24 hours of each other will be highly correlated (Pearson correlation, $R^2 > 0.9$). Additionally, we hypothesize the difference between the volume of CSF removed from the ventricles during a ventricle tap will highly correlate ($R^2 > 0.9$) with the difference in VV of 3D US images taken immediately prior to and within an hour after the ventricle tap.

In Chapter 4, our objective was to compare 3D US VV against previously reported 2D US based measurements that were made on images taken on the same day. Additionally, we compared between changes in VV estimates and changes in 2D US measurements made between serial US imaging sessions. We hypothesized that although VV is strongly correlated with 2D US measurements, as had been previously shown using 3D MRI VV, the change in 2D US measurement between imaging sessions might not be strongly correlated with change in VV. We hypothesized this is due to higher variability in 2D US measurements than 3D US VV.

In Chapter 5, our objective was to determine thresholds for treatment based on our pilot study 3D US VV data. We hypothesized that neonates who require interventions will have both larger VV and higher change in VV when measuring the VV over time than neonates who have PHVD but do not require interventions. While image-based guidelines exist to determine when an initial intervention for PHVD exist, follow up interventions are often not able to be done based on quantitative imaging. Given the previous 2D US based work, we believe that 3D US based estimates of VV should be able to determine thresholds for treatment in neonates with PHVD assuming those who

require treatment have ventricle dilatation that occurs significantly more rapidly than patients who undergo spontaneous resolution of PHVD.

In Chapter 6, our aim was to expand upon previously published work[74] that had generated an equation to estimate ventricle volume from 2D US measurements. Our aim was to investigate this technique using a larger patient population and test whether the measurement could predict surgical interventions, which was not performed in the original study. We were able to corroborate their initial results showing strong correlations between 3D US VV and this 2D US-based estimate of VV (R^2=0.74). We hypothesized that although strong correlations have been found between this technique and 3D US VV, the relatively high variance in 2D US measurements will mean 2D US-based VV is less sensitive to predict whether a neonate received an intervention.

In Chapter 7, an overview and summary of the important findings of Chapters 2-6 will be presented. The limitations of the current findings, study specific limitations, and potential solutions will be highlighted. Directions for future research to begin to address these limitations, as well as potential follow up studies that can build on the foundation of this knowledge will be addressed at the end.

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Chapter 2

This chapter will discuss the in vitro validation experiments and initial patient experiences with the three-dimensional ultrasound system.

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2 3D Ultrasound System to Investigate Intraventricular Hemorrhage in Preterm Neonates

2.1 Introduction

Very low birth weight infants (less than 1500g) account for 1.4% of all births in the United States[1] and, of those, 20-30% will develop intraventricular hemorrhage (IVH).[2] Once detected, the grade of the hemorrhage is ranked clinically from I-IV (least to greatest severity)[3] and patients with grade II or higher tend to have a period of ventriculomegaly (ventricle dilation).[4] Patients with mild IVH (grade I) usually have good outcomes, whereas those with severe IVH (grades III and IV) can go on to develop hydrocephalus and cerebral palsy.[5] Additionally, nearly one in four infants with hydrocephalus will also progress to develop epilepsy in later life.[6]

Patients may progress to a hydrocephalic state by different mechanisms. This progression can be due to hemosiderin (a hemoglobin breakdown product) blocking the subarachnoid granulation causing a decrease in the re-absorption of cerebral spinal fluid (CSF) leading to communicating hydrocephalus, or from membranes within the ventricular system causing stenosis of the aqueduct of Sylvius leading to obstructive hydrocephalus[4] The timing of treatment of hydrocephalus in IVH infants is not well standardized and as the progression of the disease is not constant, monitoring of patients with post hemorrhagic hydrocephalus (PHH) who might require a timely intervention is critical to patient outcome.[7]

2D cranial ultrasound (US) imaging is performed as standard care on preterm neonates to diagnose and monitor IVH and ventriculomegaly. The selection of 'slices' of brain imaged in 2D vary from technician to technician, which can lead to variability and ambiguous grading of IVH, as the location of the hemorrhage may not be apparent after the initial US exam. In addition, clinical US lacks sensitivity to changes in ventricular volume, as 2D images alone cannot provide accurate quantitative measures of irregular structures such as the ventricular system.

Previous work has been performed on neonates to evaluate the utility of 3D US for cranial imaging exams. Imaging was accomplished using a mechanical device to move a conventional 2D transducer to generate the third dimension[8] or free hand electromagnetic tracked 3D US scanning.[9] Other groups have had success acquiring 3D US images of neonatal ventricles using commercially available matrix transducers[10] or commercial tilting transducers[11-13], which are not currently used as standard of care. While these commercially available systems are high performing, the high cost may limit their use, especially in the developing world. The previous studies using conventional 2D transducers[8,9] reconstructed the 3D US images offline, well after the patient had been imaged due to the time involved in reconstructing the image. Offline reconstructions resulted in examinations not encompassing the full ventricle volume in some patients, or the images were otherwise not of sufficient quality to be used in the study. All of these studies have found 3D US to be feasible in a clinical setting; however, a system that could be used in conjunction with a conventional clinical 2D transducer to generate full 3D images at the bedside (incubator) would allow centers without a 3D US capable machine to confidently acquire 3D ventricle volumes.

We have developed a hand-held mechanical 3D US scanner system that can be coupled to any clinical 2D US machine with an appropriate conventional transducer used for imaging neonatal' brains. This system makes use of the mechanical "tilting" scanner system for 3D US imaging as described in Fenster et al. (2001),[14] but constructed to suit a neonatal cerebral scan in the confines of an incubator. Since this system acquires images using the same transducer used in clinical scans, we are able to make direct comparisons to clinical

images and the estimates from those images as to whether or not ventricular dilation is occurring.

In addition to the 3D US system, we have developed a volumetric phantom using three-dimensional rapid prototyping technology to mimic a neonatal head with both mild and severe ventriculomegaly. Previously reported volume phantoms of ventricles[15] did not have transfontanelle properties, and previous US phantoms of neonatal skulls have not been for volumetric measurements, and therefore did not include ventricles.[16] Using geometries segmented from MR images of infants with IVH, we built US phantoms with ventricles of known volumes that allowed us to validate the volume measurements of our system.

In this paper, we describe our new 3D US system and report on the validity of its volumetric measurements of phantom ventricles of known volumes. We verify the ability to image *in vivo* the ventricles of preterm neonates using this system and analyze volumetric measurements made from these images.

2.2 METHODS

2.2.1 3D US System

Although our system can be used with any US machine and appropriate transducer, we tested it with a HDI 5000 (Philips, Bothel WA) and C8-5 (Philips, Bothel WA) curved array 5-8 MHz broadband transducer, which is used in the NICU in our hospital. The 3D US scanner consists of a motorized transducer housing powered by a Faulhaber IE2400 1409 dc motor with a 2100:1 gear ratio (MicroMo Electronics, Clearwater, FL) and a MCDC controller for the motor. Conventional 2D US images were acquired using an Epiphan VGA2USB LR frame grabber (RB Computing, Nepean, ON). A diagram of the transducer attachment, as well as a photograph of the transducer with attachment placed on approximately the anterior fontanelle inside an incubator is shown in Figure 2-1.

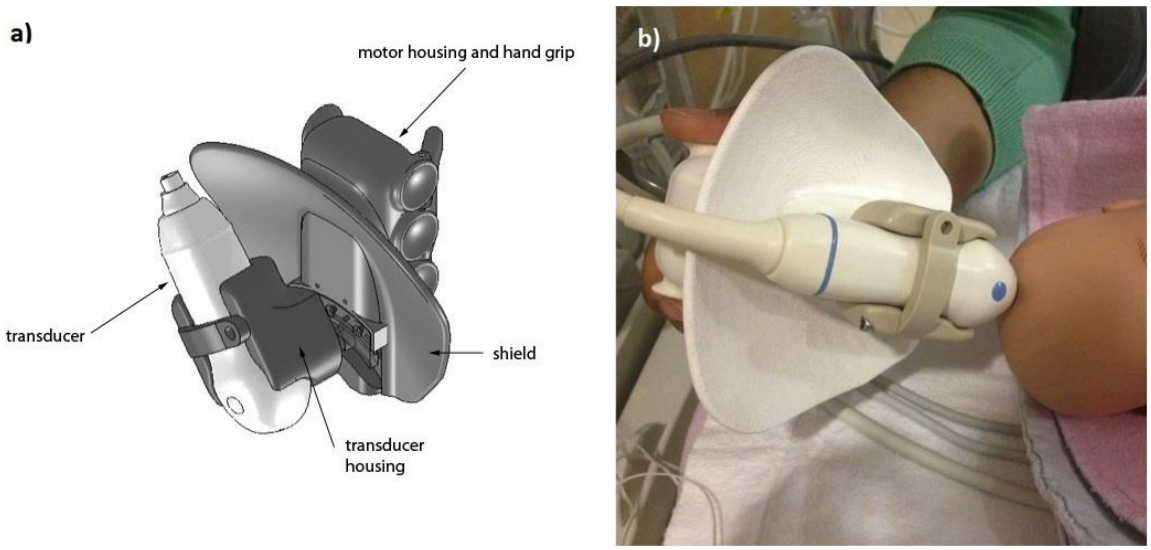

Figure 2-1 The handheld motorized 3D ultrasound system. a) The handheld motorized transducer housing with transducer (C8-5, Philips) attached. The "shield" region protects user from making contact with the moving transducer and indicates where the probe will be at maximum scan angle allowing optimal positioning prior to scanning a patient who may be lying prone, or on their side. b) The motorized transducer at the anterior fontanelle of a mannequin illustrating a scan inside an incubator.

To perform a 3D US scan, the motor controller, which is connected to the computer via USB, tilts the transducer, while the frame grabber acquires 2D images from the video output of the US machine throughout the scan. Software developed in our laboratory controls the motor's movements, accepts the 2D US images as they are acquired by the frame grabber, and reconstructs the 2D images into a 3D image as the 2D US images are acquired.[14] Thus, the acquired 2D US images are spaced at a regular angular interval arranged in a fan-like form. Bilinear interpolation is used to fill in gaps between successive 2D images to generate a full 3D volume that can be manipulated through any plane.

During image acquisition, the technician or physician locates the middle of the target of interest, and then firmly holds onto the motor encasement (see Fig. 1b) while the device tilts the transducer on an axis at the probe tip, which is held against the patient's head. The angular spacing between images can be chosen to be between 0.2 and 0.3 degrees, and

images are acquired at 25 frames/second over a scan angle of 30-72 degrees making total image acquisition between 4-14 seconds. Typically, for neonates in our study, we have used an angular spacing of 0.3 degrees and a total scan angle of 65 degrees, for a scan time of 8.7 s, and a 3D US image size of about 400 x 350 x 350 voxels. The voxel size for the most commonly used depth setting of 7.9 cm was 0.22 x 0.22 x 0.22 mm.

Patient images used in this study were collected following protocols approved by the Research Ethics Board of Western University (London, ON, Canada) and informed parental consent.

2.2.2 Geometric Validation of Image Reconstruction

To ensure our reconstructed 3D US geometries are correctly calibrated, linear measurements in all three scanning directions (axial, lateral, and elevation) must be validated. To test the accuracy of these measurements, a phantom made of strings spaced 1 cm apart in each direction was constructed and scanned in a solution of distilled water and 7% glycerol by weight (speed of sound 1540m/s).[17] The 3D US scanner was stabilized and the transducer face was completely submerged in the glycerol solution during image acquisition. The string phantom was then scanned at every calibrated depth setting (4.9 cm to 16.0 cm). The distance between strings was measured manually using our visualization software. Specifically, we measured from one intersection of a string to the next closest intersection in the axial, lateral, or elevation direction four times per direction per depth. Ten depth settings were calibrated, for a total of 40 measurements per orthogonal direction. As for all data analysis, we used SPSS Statistics v. 20 (IBM) to perform t-tests between the measured data and the expected measurement of 10 mm. Additionally, a two-way ANOVA was performed between direction and depth to ensure distance measurements at all depth settings were the same.

2.2.3 Ventricle Volume Validation

2.2.3.1 Development of Ventricle Mimicking phantom

Images from a 1.5T Signa HDxt MRI scanner (General Electric Health Care, Milwaukee,WI) of infants with severe IVH were collected after their parents gave consent.

Axial T1 weighted images (Ax FSPGR 3D) were used to design the ventricle phantoms. The interior surface of the skull and the lateral ventricles were manually segmented in parallel slices with 1 mm between slices. We generated a model of the skull after adding an additional 3.5–4 mm margin around the skull surface, which is approximately the thickness of a neonatal skull.[18,19] To model a neonate of 30 weeks gestational age, an anterior fontanelle measuring 32 mm anterior to posterior and 30 mm left to right was added to the model using values from a recent study.[20] STL files were generated from the segmented ventricle surfaces and the skull and those models were manufactured using rapid prototyping (A FORTUS 400mc 3D Production System, Stratasys, Eden Prairie, MN).

A positive model of the lateral ventricles and the skull (Fig. 2-2) were generated in ABS-M30i (acrylonitrile butadiene styrene). Using these, negative moulds of the ventricles were produced by casting the positive ventricle model in silicone SILBIONE® RTV 4420 A/B (Bluestar Silicone, East Brunswick, NJ) to create a two-part mould for the large ventricles (288 cm^3) and a four-part mould for the more intricate small ventricles (51 cm^3).

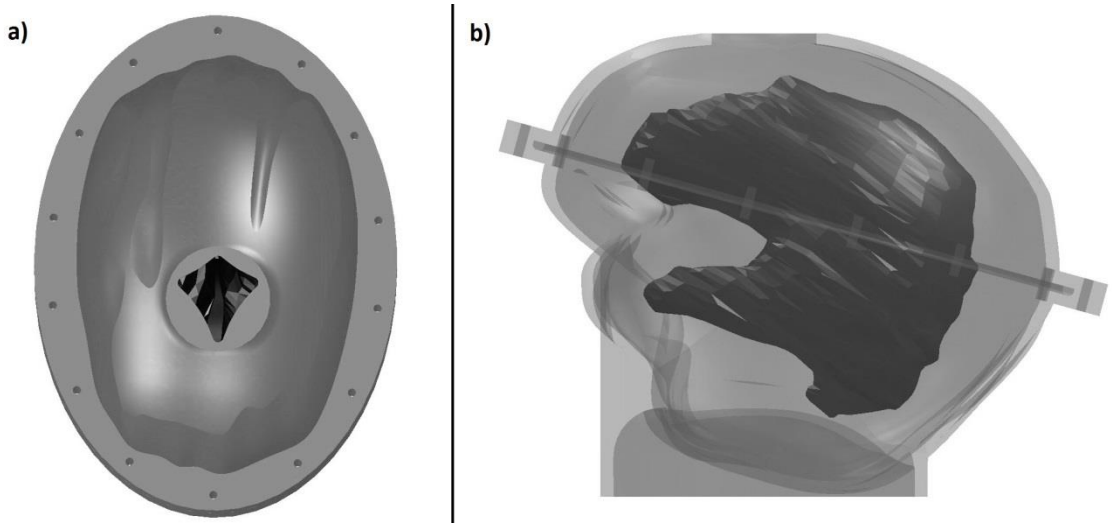

Figure 2-2 Model of the ventricle phantom. a) a top-down view of the anterior fontanelle and b) profile of the skull and attached base (semi-transparent) and the position of the large lateral ventricle phantom (dark region) inside the model skull.

An agar mixture of 1L distilled water, 35 g of agar powder (high strength A-6924, Sigma Chemicals, St Louis, MO) and 80 mL glycerol (EMD Chemicals Inc. Gibbstown, NJ) was

heated to 85˚C. The ventricle was composed of 500 mL of this agar mixture combined with 10 g of 100 mesh tungsten powder (Alfa Aesar, Ward Hill, MA) to generate contrast in MR images for volume validation. The brain-like medium was prepared using the other 500 mL of agar mixture with 15 g of SigmaCell (Sigma Chemicals, St Louis, MO) to generate soft-tissue mimicking scatter in US images.[21] The volumes of the ventricles were determined by water displacement measurements prior to embedding into the brain-like agar. Photographs of the agar ventricles are shown in Figure 2-3.

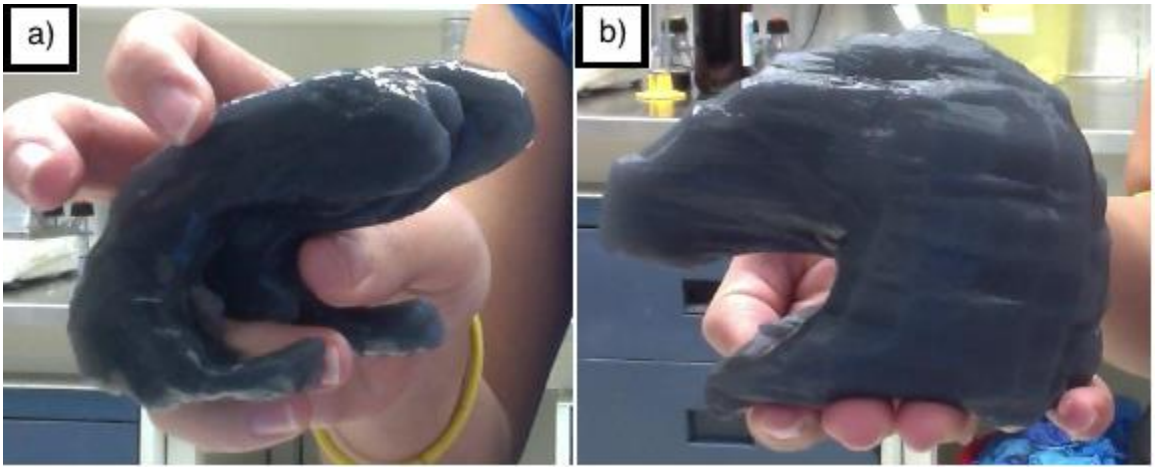

Figure 2-3 Photographs of the phantom models of the ventricles once molded in an agar/tungsten compound. a) small ventricle (51 mL) and b) large ventricles (288 mL)

2.2.4 Imaging of the Volume Phantom

The ventricle phantoms were scanned using a 3.0T Discovery 750MR scanner (General Electric Health Care, Milwaukee, WI). Sagittal T2 weighted 3D fast spin echo sequence was used (TR 2500ms, TE 65.6 ms, echo train 100, scanning matrix 160x160, slice thickness 1mm) with geometric correction to give accurate volume measurements. The phantom was also scanned using our 3D US system through the fontanelle. A 60 degree tilting sweep was sufficient for the smaller ventricles, while a 72 degree sweep was required for the larger ventricles. Images were acquired with 0.3 degrees between frames, and at 25 frames per second.

Ventricles were manually segmented five times by a single observer (JK) in parallel slices with 1 mm between slices through the sagittal plane in both MR and 3D US images. Two-way, repeated measures ANOVA was performed to determine if there were statistically significant differences between volumetric measurements in MRI, 3D US and the gold standard of water displacement.

2.2.5 In vivo Imaging and Volume Measurements

After an initial conventional 2D US clinical exam showed an IVH of at least grade II, parents of the neonate were asked for their consent to image their child. Following a routine cranial 2D US exam, the US transducer was placed into the 3D US scanner housing (Fig. 1b) and the 3D US image was acquired. The clinical US technician or a physician located the midline of the lateral ventricles through the anterior fontanelle. A 3D US image was then acquired while the 3D US scanner was held firmly and the transducer tip was monitored to ensure constant contact with the patient's head while maintaining a low level of pressure applied to the fontanelle. The 3D US system acquired 2D US sagittal slices at a depth of 5-15 cm (usually 7.9cm) and were reconstructed into a 3D image as the 2D images are acquired. Scans were performed with a 60-72 degree scan angle, a step size of 0.3 degrees at a frame rate of 25 frames/s. Scans were completed once a 3D US image of sufficient quality was acquired (one with limited movement, and the full ventricular volume). Generally, this was obtained in 1-4 acquisitions of 3D US images with the entire investigation, including routine US acquisition, lasting 10-25 minutes.

To determine the variability of the measured ventricle volumes, an evaluation of the ventricles of three patients with similar grades of IVH were segmented by one physician examiner (SdR) and two trained observers (JK and RM). The 3D US images were randomly ordered and manual segmentation was done on each 3D US image 5 times (a total of 45 image segmentations). Each manual segmentation required 20-30 minutes to complete and was performed at least 24 hours apart from previous segmentations to avoid memory bias. Two-way, repeated measures ANOVA was performed to determine if there were significant differences between repeated measurements of the three patient images by three different observers. Intra-class correlation (ICC) was performed to assess the agreement between observers.

2.2.6 Intra- and Inter-observer Variability Measurements and Measurement Error Analysis

The inter- and intra-observer variability was measured as the standard error of measurement (SEM) from the means squared calculated from the repeated measures ANOVA as cited in Eliaszim et al. (1994).[22]

Equation 2-1

$$SEM_{\text{intra}} = \sqrt{MSE}$$

Equation 2-2

$$SEM_{\text{inter}} = \sqrt{[(MSR + (n-1)MSSR + n(m-1)MSE]/[mn]}$$

Where MSE is the mean squared error, MSR is the mean squared rater (observer), MSS is the mean squared for subject (patient), and MSSR is the mean squared interaction between the subject and the rater for n subjects segmented m times.

The minimal detectable difference (MDD) between measurements for the same observer was also calculated from equation 2-3.[22] The critical value is ($Z_{\alpha/2}$) is 1.96 corresponding to α = 0.05. The measurement of MDD is the smallest measured volume difference between segmentations that can be detected with 95% confidence. Thus, if the measured change between two time points is greater than this value, then one can be confident (at 95%) there has been a volume change.

Equation 2-3

$$MDD_{\text{intra,inter}} = Z_{\alpha/2}\sqrt{2}SEM_{\text{intra,inter}}$$

As volume changes less than 1 cm^3 would not be clinically relevant in this patient population, a MDD of less than 1 cm^3 will be considered acceptable.

2.3 RESULTS

2.3.1 Geometric Validation using String Phantom Measurements

The string phantom as well as a 3D US image of the phantom is shown in Figure 2-4. The measured values and t-test results are shown in Table 2-1. T-tests failed to show statistically significant differences between the manufactured distance of 10 mm and the mean measured distances from 3D US images of the string phantom. This is indicative of a correctly calibrated geometry in our 3D reconstruction. As the head grows due to either normal brain development or ventriculomegaly, US exams will require different settings to best visualize the ventricles, so it is important to ensure that there are no discrepancies between the geometric calibrations between different depth settings (4.9-16.0 cm). An additional two-way ANOVA to determine if there were discrepancies in measurements at different calibrated depth settings failed to find statistical significant differences between distance measurements made at different depth settings for any of the three orthogonal directions (p=0.603, 0.622 and 0.568 for axial, lateral and elevation respectively).

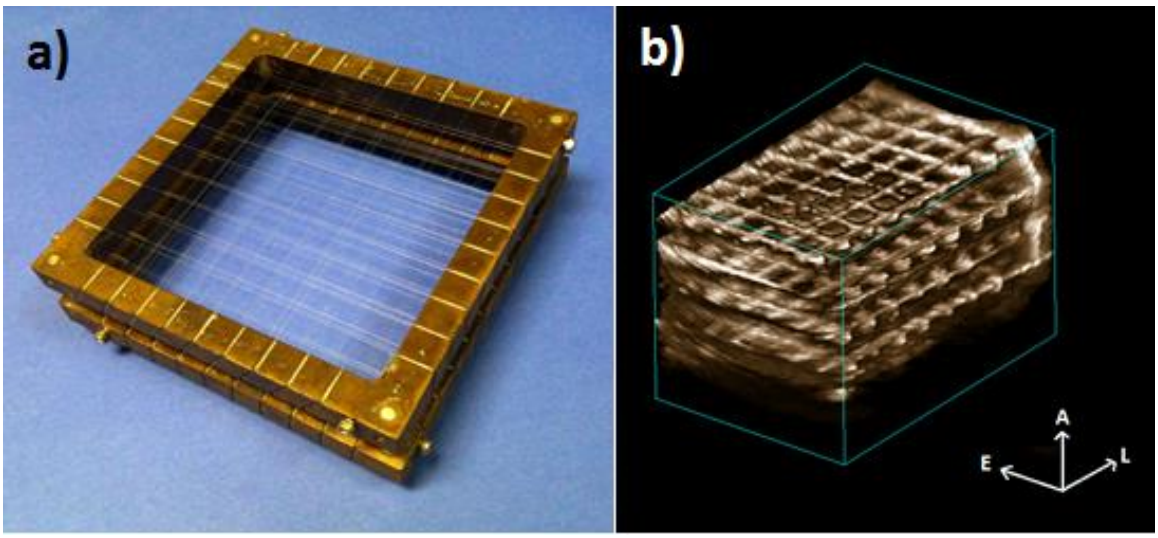

Figure 2-4 The string phantom. a) Photograph of string phantom prior to imaging with 3D US. b) String phantom imaged in 3D US with A – axial, L – lateral and E – elevation directions indicated

Table 2-1 Results from the string phantom. Results of 40 manual measurements of the distances between strings in the 3 orthogonal directions along with confidence intervals. T-test results listed are for comparisons between measured and manufactured distance between the strings, which was 10 mm, and no significant differences were found between them (p>0.05).

Direction	Mean (mm)	St. dev. (mm)	95% CI (mm)	p-value (α=0.05)
Axial	10.02	0.14	10.00-10.09	0.280
Lateral	10.01	0.04	10.00-10.02	0.269
Elevation	10.01	0.16	9.97-10.08	0.590

2.3.2 Volume Validation using Ventricle-like Phantom

Sagittal image showing the mid-lateral left ventricle and a coronal image showing anterior and inferior horns of the lateral ventricles at approximately the same plane in 3D US and MR images are shown in Figure 2-5. Ventricle volume measurements from all three methods (water displacement, MRI, 3D US) are shown in Table 2-2 shows the imaging methods of volume measurement compared to the gold standard. ANOVA performed between the methods of measurement and the volumes calculated for each phantom failed to find a statistical significant differences between them with p>>0.05 (Table 2-2). The coefficient of variation (COV) was 4.1% and 0.8% for the small phantom and 1.7% and 2.1% for large phantom for segmentations in 3D US and MRI respectively.

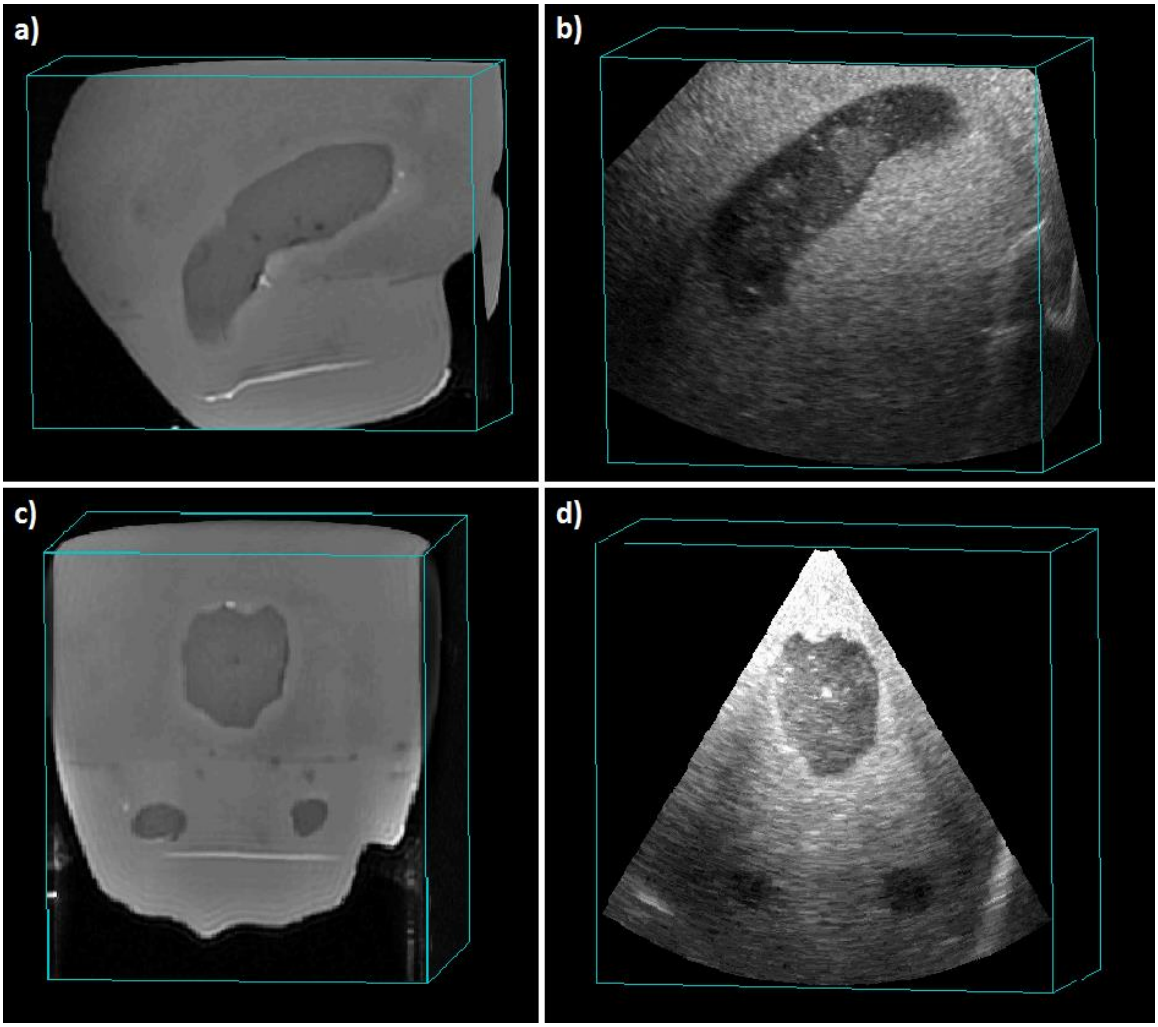

Figure 2-5 3D images in 3D US and MRI of a ventricle volume phantom. The sagittal and coronal planes of the 'small' ventricle phantom in approximately the same slice in both MRI (a and c) and 3D US (b and d).

Table 2-2 Volume measurements of two ventricle-like phantoms. Water displacement (WD) and manual segmentation in MRI and 3D US images. ANOVA between the manual segmentations and water displacement found no significant differences (p>0.05). Measurement ± standard deviation

Phantom	WD (cm^3)	MRI (cm^3)	3D US (cm^3)	p-value (α=0.05)
Large	287.9 ± 2.0	289.8 ± 6.2	286.5 ± 4.9	0.71
Small	51.2 ± 1.0	49.9 ± 0.4	49.4 ± 2.0	0.31

2.3.3 *In vivo* Volume Measurements

Figure 2-6 shows 3D US images of a neonate with bilateral grade II IVH along with the segmented ventricle model from that image with a mean volume of 35.2 cm^3. Figure 2-6d shows the 'cube view', which is immediately available to view at the bedside manipulate through any 2D plane (coronal, sagittal, axial or oblique) and examine for image quality.

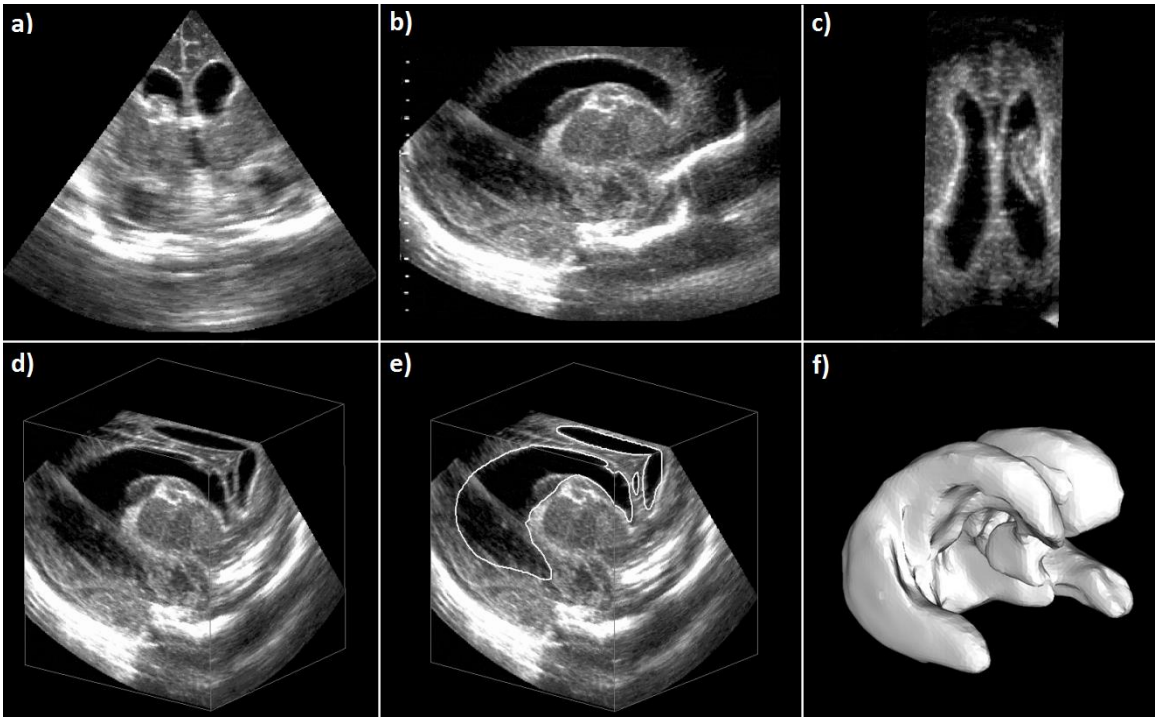

Figure 2-6 In vivo imaging of a neonatal patient. Axial (a), sagittal (b), and coronal (c) views of the cerebral ventricles of patient 1. Cube view (d) with segmented outline of the ventricular system (e) and the ventricle surface made from the segmentation (f). Calculated ventricle volume was 35.2 cm^3.

Table 2-3 summarizes the 3D US ventricle volume measurements along with the coefficient of variation (COV) of the three different IVH patients as segmented by 3 different observers multiple times. The COV was < 5% for all patients and all observers indicating relatively low measurement variance. The ICC between the measurements made by the three different observers was found to be a high 0.97. A two-way repeated measures ANOVA failed to find a statistical significant differences (p-values >>0.05) between the volumes segmented between the three observers (Table 2-3).

Table 2-3 Inter-intra observer measurements from neonatal images. Manual segmentations of the total ventricle volume (± standard deviation) of three IVH patients done by three different observers. Segmentations were performed five separate times per observer. Performed repeated measures ANOVA found no significant differences between the volume measurements made for the different observers (p>0.05).

Patient	Observer	Volume (cm^3)	COV (%)	p-value (α=0.05)
1	1	35.7 ± 2.2	6.2	
	2	35.1 ± 0.83	2.4	
	3	34.9 ± 0.62	1.8	
	total	35.2 ± 1.41	4.0	0.60
2	1	11.1 ± 0.70	6.3	
	2	10.7 ± 0.43	4.0	
	3	10.4 ± 0.41	3.9	
	total	10.7 ± 0.53	4.9	0.14
3	1	9.60 ± 0.39	4.1	
	2	9.45 ± 0.42	4.5	
	3	9.17 ± 0.18	1.9	
	total	9.41 ± 0.35	3.7	0.19

2.3.4 Inter- and Intra- Observer Variation and Minimal Detectable Difference

Mean squared values as used to calculate intra- and inter-observer variation are shown in Table 2-4. The inter observer SEM was 0.24 and intra observer SEM was 0.243 as calculated from equations 2-1 and 2-2. The MDD_{intra} was found to be 0.63 cm^3 and the MDD_{inter} was 0.68 cm^3 both calculated from equation 2-3.

Table 2-4 Calculated minimum detectable difference. Calculated values for error terms from repeated measures ANOVA, and minimal detectable difference (MDD). Where MSE is the mean squared error, MSR is the mean squared rater (observer), MSS is the mean squared for subject (patient), and MSSR is the mean squared interaction in cm6. The standard error of measure (SEM) for intra- and inter-observer variability is calculated from equations 1 and 2 respectively is in cm3. MDD are calculated from equation 3 assuming a 95% confidence interval and are also in cm^3.

Calculated Term	Value
MSS	$3.17x10^3$
MSR	0.0996
MSSR	1.73
MSE	0.451
SEM_{intra}	0.23
SEM_{inter}	0.24
MDD_{intra}	0.63
MDD_{inter}	0.68

2.4 DISCUSSION

We have shown that our 3D US system can measure ventricle volumes through a fontanelle in neonates accurately. We were able to show that ventricular volumes could be measured precisely between different observers with mean COV ranging between 1.8 and 6.3% and ICC of 0.97. IVH patient's ventricles tend to be larger than normal, for example, patients in our study had mean ventricle volumes between 9.0-36.0 cm^3 while patients with average sized ventricles at the same gestational age would have ventricle volumes between 0.5-2.0 cm^3.[11] As was reported in Brann et. al. 1991, patients with ventriculomegaly have daily increases in ventricle volume in excess of 1 cm^3/day. This is larger than the MDD calculated for our system (MDD_{intra} = 0.63), indicating that our 3D US system can be used in a study focusing on monitoring IVH patients' ventricular volumes change both in response to treatment and as a result of spontaneous recovery.

We have extended the phantom development reported by Gatto et al. who developed an US cranial phantom with a fontanelle. While we also used 3D rapid prototyping technology

to manufacture the skull, the inclusion of ventricles that could be imaged in both US and MRI is important in the validation of the 3D US approach for monitoring patients with IVH. This type tungsten-agar phantom was developed previously for a CT and US compatible prostate phantom[23] since embedded tungsten powder also increases the Hounsfield units of the agar. The quantity of the tungsten component of their phantom was much lower than in our phantom to avoid significant artifact in CT. We have shown, however, that this type of tungsten-agar phantom could also be used to validate systems for imaging pathologies commonly scanned in both US and MRI. As agar does not require time-consuming freeze/thaw cycles as would a PVA based phantom, the development of phantoms simulating changes in IVH can be done much faster and more economically. Tungsten powder does not diffuse through agar like gadolinium based contrast agents, which makes it more reliable as a contrast agent when volumes need to be validated with MRI and 3D US. It does seem plausible to create a triple modality phantom in the future using agar and tungsten, however, tungsten concentrations must be sufficiently low so as not to cause significant artifacts in the CT and MR images, but high enough to create contrast.

Much like the findings of Abdul-Khaliq et al., we found mechanically moving a 2D transducer to generate a 3D US image is feasible in a clinical setting; however, their patients were already sedated due to other procedures, and therefore patient motion was not present in their images. Additionally, the scanner described in[8] rotated a 2D probe 180 degrees around a central axis, which may have caused the 2D probe to image the skull at some portions on the scan, causing shadowing. Our scanner tilts the US transducer through a maximum of 72 degrees with the transducer face held along the long axis of the fontanelle. This approach allows good contact with the fontanelle during the entire scan, and the ability to acquire full ventricle volumes in a shorter time than a 180 degree rotation approach. The free-hand system with position sensors described by Kampmann et al. is smaller than our system, and had volume measurements in phantoms with a COV ranging from 3.6-10%. This was comparable to values we found in our phantoms (COV of 4.1% for the phantom with a volume corresponding closest to the ones measured in Kampmann et al.) However, due to the more complicated reconstruction of a 3D US image from position sensor encoded 2D slices, this was done offline after scans were complete in their

study. In this study, only well after the procedure was completed could users verify whether or not the full ventricle volume was acquired, and so not all patients scanned were included.[15] We were able to overcome this limitation, as the reconstruction of our 3D US images is performed at the bedside, much like the more expensive commercially available systems. The user can view the reconstruction of the 3D US image as it is being acquired and can examine the complete 3D US image immediately after the scan, and can conclude whether or not another image is required. Thus, the user can limit scan time and ensure that high quality images of the full ventricular system are obtained prior to leaving the patient's bedside.

The main limitation of our 3D US system is the current requirement for manual segmentation of the ventricles for patients who have abnormal US scans. Bleeding in IVH creates a hyperechoic region around the relatively hypoechoic region of the CSF filled ventricles making simple threshold or boundary based segmentation algorithms not useful in this patient population (see Figure 2-7). Though we have shown manual segmentation to be accurate and precise, it is time consuming and would not be feasible for routine clinical use. Automated segmentation of 3D US images has been previously performed successfully in other organ systems (bladder, and fetus) and has been made into commercially available product BladderScan® (Verathon, Bothell, WA). In addition, automated segmentation of the CSF and blood in the ventricles could be successfully segmented using raw US (RF) acoustic signal, but most clinical 2D US systems do not allow raw data capture in real time, or at all. We therefore acquired our images using a video frame grabber, which could potentially be another limitation to this system. The development of a stable automated or semi-automated segmentation algorithm for the cerebral ventricles, could make the 3D US scanning approach much more feasible clinically once segmentation times are reduced to a more acceptable sub-minute per patient.

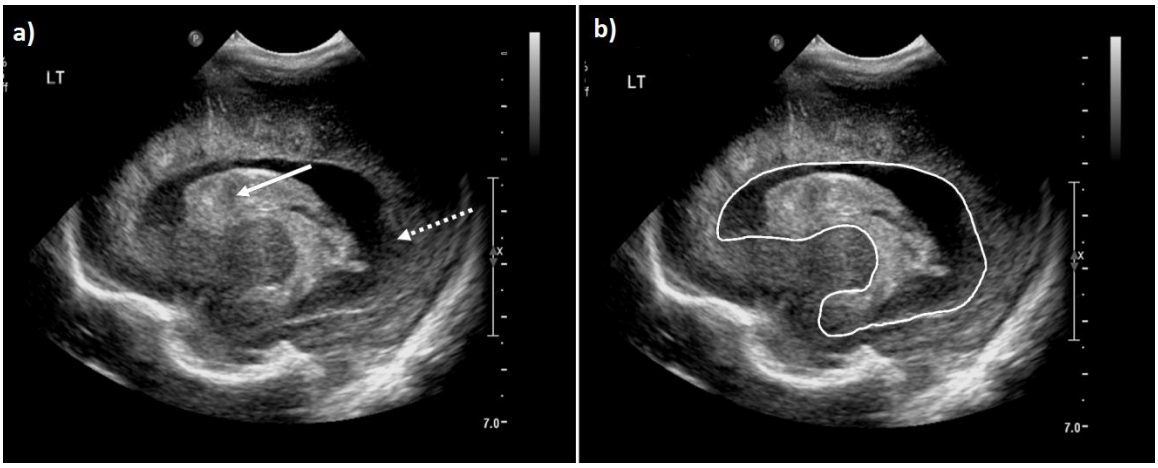

Figure 2-7 Ultrasound image of a preterm neonate with IVH including the boundaries of the ventricle and hemorrhage. a) Parasagittal image of the left lateral ventricle in an infant with grade IV IVH at 7 days of life. b) The same image with approximate boundary of the ventricle. Notice the ambiguous boundary of the posterior horn (dashed arrow) as well as the hyper-echoic region from IVH that is similar in intensity to some surrounding brain tissue (white arrow).

As other authors[8,11] have discussed, fontanelle size is also a limiting factor in the quality of images. As the patients get older and the fontanelle shrinks, images will reduce in quality as the skull starts to cause shadowing on the edges of the image frame, by effectively reflecting most of the US signal before it can image the brain. This would cause the boundary of the posterior horns to become almost imperceptible. Neonates with inherently small fontanelle will pose a challenge for acquiring high quality 3D US images. Nevertheless, 3D US examinations require less scanning time than conventional US examinations, and can provide information for the full volume of the ventricular system.

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Chapter 3

This chapter will discuss the in vivo validation experiments used to validate the three-dimensional ultrasound system in a clinical setting.

The contents of this chapter were previously published in the journal Physics in Medicine and Biology: J. Kishimoto, A. Fenster, D.S.C. Lee, S. de Ribaupierre. In Vivo Validation of a 3D Ultrasound System for Imaging the Lateral Ventricles of Neonates. Ultrasound in Medicine and Biology 42(4) (2016) 971-79. doi: 10.1016/j.ultrasmedbio.2015.11.010. Permission to reproduce this article was granted by the Elsevier and is provided in Appendix A.2.

3 *In Vivo* Validation of a 3D Ultrasound System for Imaging the Lateral Ventricles of Neonates

3.1 INTRODUCTION

As the survival of very low birth weight (VLBW) infants (birth weight less than 1500g) has increased over time, so has the necessity for better neonatal monitoring in order for clinicians to manage physiological stability and to screen for pathological conditions. The VLBW infant is most at risk for intraventricular hemorrhage (IVH), as a result of bleeding from the immature vascular structure of the germinal matrix located near the medial aspect of the cerebral ventricles. Across Canada, IVH occurs in 20-30% of VLBW infants[1] and 25% of these may develop the secondary complication of post-hemorrhagic ventricle dilatation (PHVD) as a result of interruption/blockage of the flow of cerebral spinal fluid (CSF).[2] The development of PHVD has been linked to higher mortality and poor neurodevelopmental outcome later in life, thought to be caused by injury to the periventricular white matter.[3] In general, there are three clinical courses of PHVD: 40% of patients spontaneously resolve, 10% progress rapidly, and 50% progress persistently but slowly.[2] Infants who have rapidly progressing PHVD and some with persistent, slowly progressing PHVD will require interventions, including serial lumbar punctures or ventricle taps (VT), ventricular drainage, or shunting of the CSF to relieve the elevated intracranial pressure (ICP) in order to alleviate symptoms, and prevent

further brain damage.[4] At our center, ventricle taps are used as an initial form of intervention.

Since a large percentage of PHVD spontaneously resolve without intervention, current clinical management is to follow VLBW neonates with serial head ultrasound (US) and head circumference (HC) measurements to assess for ventricular dilatation, and to monitor for signs of raised ICP (tense, bulging fontanels, apnea and bradycardia). However, the timing of intervention remains imprecise as the clinical signs of increased ICP are nonspecific in the preterm infant and could be due to other co-morbidities. Further, 2D US cannot provide accurate quantitative measurements of irregular volumes, such as the cerebral ventricles. Brann *et al*. (1990) have found that the amount of CSF removed during serial lumbar punctures or ventricular drainage is significantly correlated (r^2=0.84) with changes in ventricular volume (VV) as calculated from 2D US taken before and after intervention.[5] Though this was very promising work, their approach never translated into clinical practice. To the best of our knowledge, no work has been done to date comparing 3D US ventricle volumes with volume of CSF removed in serial ventricle taps (VTs).

Previous work with 3D US has been attempted in neonates.[6-10] While this imaging technique was shown to be feasible, its clinical utility has not been established. A systematic bias has been found towards lower volumes in US-based measurements compared to gold standard MRI measurements when evaluating different organ systems.[11,12] Nevertheless, Gilmore and Gerig (2001) have found good agreement between 3D US measurements of VV and MRI measurements when evaluating neonates with mild ventricular dilatation at term equivalent age.[7] However, the study had limited variation in VV (10-18 cm^3) and had a very small size of 7 patients. It is important to determine if the previous findings are applicable to PHVD patients who can have VV upwards of 100 cm^3.

A hand-held mechanical 3D US scanner system has been previously developed and validated on phantoms and a small set of patient images.[13] This system can be coupled to any clinical 2D US machine with an appropriate transducer used for imaging the neonatal

brain. This system is akin to the mechanical "tilting" scanner system for 3D US imaging as described in Fenster *et al.*[14] but constructed to suit a neonatal cerebral scan in the confines of an incubator.

The 3D system used in this *in vivo* study is described briefly, and its validity in measuring ventricle volumes in neonates with non-resolving PHVD is compared to the gold standard MRI VV to further validate the image acquisition and volume determination using manual segmentations of the ventricles. Further validation is performed by comparing volume measurements from 3D US images obtained before and after VT with the reported volume of CSF that was removed from the tap.

3.2 MATERIALS & METHODS

As part of a larger, ongoing, observational study on the potential for new technologies (near infrared spectroscopy, 3D US, and functional MRI) to allow for better monitoring of patients with PHVD, patients were enrolled after informed parental consent once a positive diagnosis of IVH was made on routine clinical head US. Protocols used in this study were approved by the Research Ethics Board of Western University. Generally, enrollment was within two weeks after birth for patients between 25-34 weeks gestational age (GA), with pre-term birth considered to be any neonate born earlier than 37 weeks GA.

3.2.1 3D US image acquisition and segmentation

Images were acquired using a Philips HDI 5000 US machine with a C8-5 transducer set to Pediatric Cerebral scan. 3D US imaging was performed after placing the US transducer in a motorized housing that tilted the transducer about the axis at the probe tip (Figure 3-1).[15] In-house software reconstructed the 2D ultrasound frames into a 3D US image as images were received into the computer.[14] Each 2D US frame was acquired every 0.3 degrees at 25 frames/s over a scan angle of 60-72 degrees, making total image acquisition between 8-9.6 seconds. Due to patient motion images of both lateral ventricles were obtained in 1-8 attempts, for a total bedside scan time of 2-15 minutes. Images were manually segmented in parallel sagittal slices of the 3D US image, 1 mm apart between adjacent slices, with manual adjustments to the lateral margins of the contours done in the

coronal plane to ensure that the full ventricle was segmented. A trained observer (JK) segmented all the images, and boundaries were verified by a clinical collaborator (SdR). Each image required between 20-45 minutes to segment.

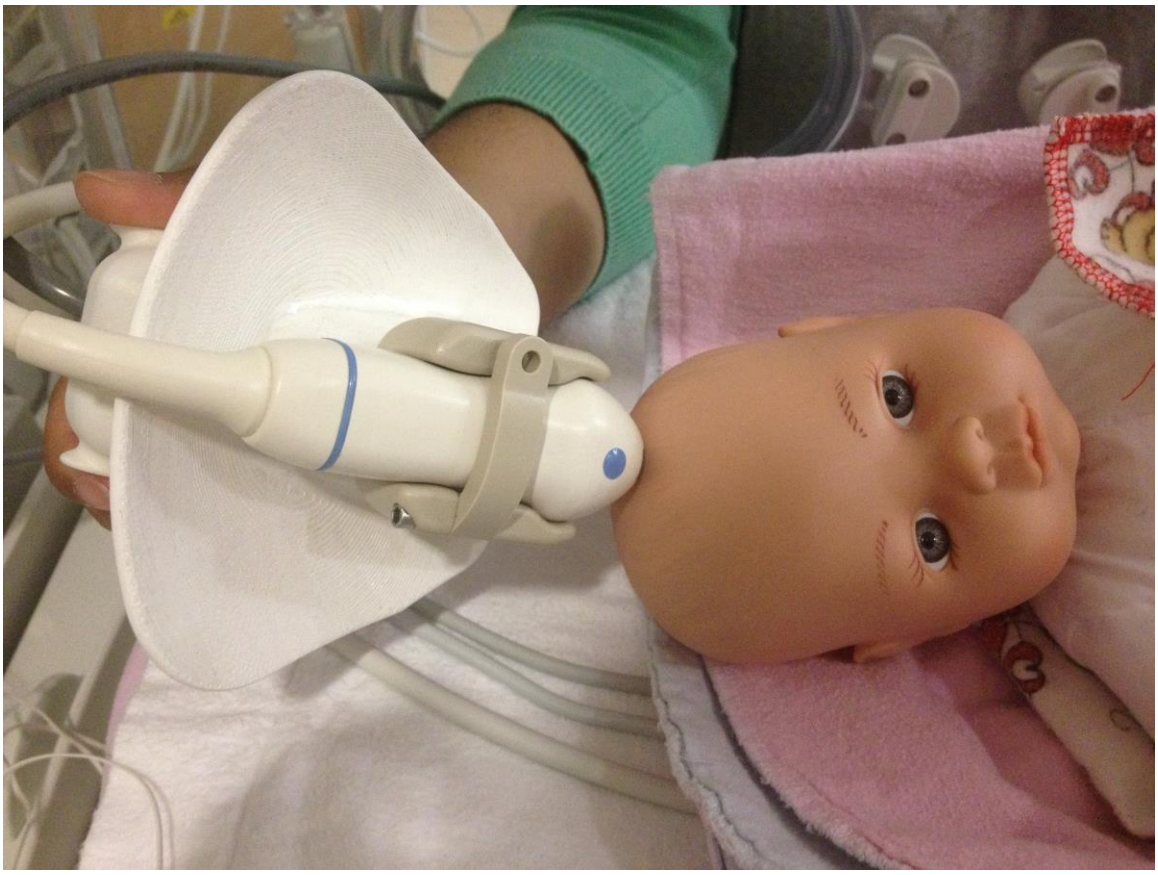

Figure 3-1– Mechanical 3D US transducer housing with transducer in place. This entire housing/transducer set up done through incubator port holes in order to mimic scan with minimal disruption to patient.

3.2.2 MR Imaging of Patients

Patients were imaged without sedation with a 1.5T Signa HDxt MRI scanner (General Electric Health Care, Milwaukee, WI) upon reaching term equivalent age (GA >37 weeks), and limited oxygen dependency inside a MedVac blanket used to reduce patient movement (CFI Medical Solutions Inc, Fenton, MI). MRI and 3D US images were acquired within 3 days of each other if the ventricle size had been clinically stable, but for those with prior interventions and/or if the patient was suspected of having progressive PHVD, images were acquired on the same day. 3D T1-weighted MR images

of the brain were acquired in the axial plane (TR = 9.29 ms, TE = 4.20 ms, scan time ~ 9 minutes) and these were manually segmented by the same observer who performed the 3D US segmentation in parallel sagittal slices using the same in-house software and settings as was used for 3D US images. The observer was not blinded to the patient IDs, but were blinded to TVV for any of the segmentations until all segmentations were completed.

3.2.3 Interventional Ventricle Tap Validations

Ventricle taps (VT) are procedures in which a small needle is inserted through the fontanel of a patient into one of the lateral ventricles in order to drain excess CSF. The clinical team was blinded to the 3D US images and VV until after patient discharge, and as such, all taps were performed based on clinical 2D US scans, and signs and symptoms of increased ICP such as apnea, bradycardia and a full, tense fontanel. Once the decision to perform a VT, enrolled subjects were imaged using the 3D US system 15-60 minutes prior to the intervention, and 15-60 minutes afterward. Reported CSF removed during the tap was measured in specialized vials as the CSF was being removed by the neurosurgeon consult and recorded and stored until after images had been segmented to avoid bias. The difference between pre-tap and post-tap ventricle volume as measured using the 3D US images was calculated and compared to CSF removed during the procedure. Patients with grade IV IVH who had developed porencephalic cysts (Figure 3-2) were not included in the analysis when CSF was removed from the ipsilateral side of the cyst, as the CSF would have primarily been removed from the cyst and not the ventricles; however, the reported pre-post ventricle volumes were reported. Ventricles were segmented such that the hemorrhage and choroid plexus was included within the volume, but the grade IV hemorrhage and resultant porencephalic cysts were not included.

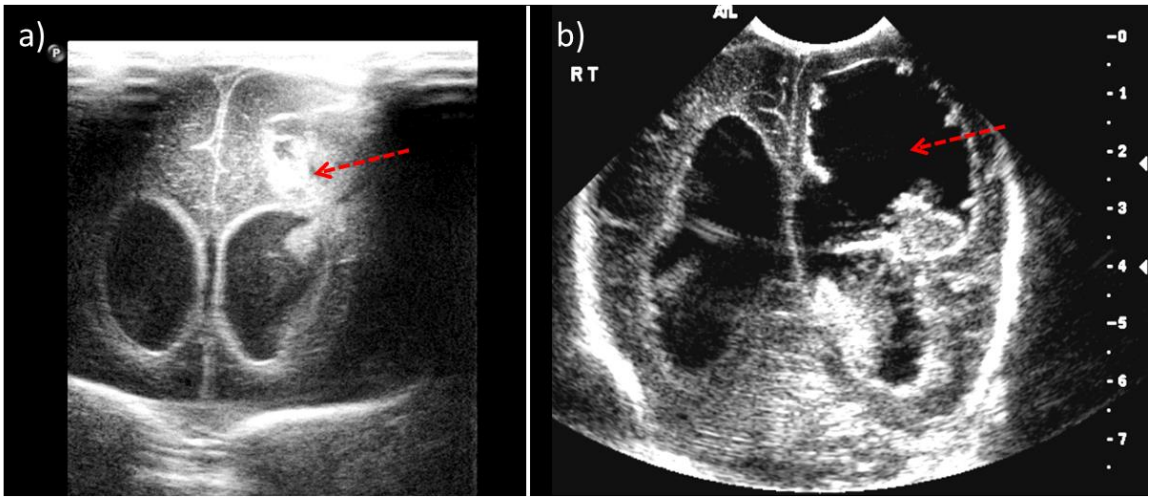

Figure 3-2 2D ultrasound images of a neonatal patient with a grade IV IVH. a) shows a grade IV hemorrhage (arrow in a) on the 7th day of life using a linear transducer, and b) shows an image of the same patient from a curvilinear transducer with the developed porencephalic cyst (arrow in b) on the 27th day of life.

3.2.4 Statistical Methods

Pearson correlation, as well as Bland-Altman plot to determine the bias between MRI and 3D US, were performed using GraphPad Prism (GraphPad Software, San Diego, Calif). Additionally, Pearson correlation and Bland-Altman plot were performed between pre-post VT difference in VV and the recorded volume of CSF removed. The slope of the regression was tested against the unity line (y=x) via t-test with $\alpha = 0.05$.

3.3 RESULTS

3.3.1 3D US volume validation against MRI

Twenty patients were used to analyze the relationship between 3D US and MRI VV. Two patients were not used as one had insufficient quality MRI (significant motion artifact) and one had insufficient quality 3D US (too much motion throughout scans). Images were usually acquired within one day (N=15) and all patients with prior interventions and/or suspected progressive PHVD were scanned on the same day. Three additional patients who were not scanned within 1 day had a history of mild/moderate IVH, had no prior interventions for PHVD, and were considered to clinically have had stabilized

ventricle size. The descriptions of the included 18 patients who were studied with 3D US and MRI are described in Table 3-1 with example MRI and 3D US images of a patient with grade IV(left), II (right) IVH are shown in Figure 3-3.

Table 3-1 Characteristics of patients who had magnetic resonance imaging and 3D ultrasound (US) images at term equivalent age (37-40 weeks gestational age). The intraventricular hemorrhage (IVH) grade was based on the severity scale by Papile [23]. ** Interventions included were ventricle tap, ventricle drain, shunt, or any combination.

	Mean ± Stdev	range
Gestational Age (weeks)	26 ± 1.3	23 6/7-35 4/7
Birth Weight (g)	1200 ± 572	640-2430
Weight at MRI (g)	3036 ± 863	1250-5275
	N (total=18)	%
IVH		
Grade I	2	11%
Grade II	5	28%
Grade III	5	28%
Grade IV	6	33%
Required Interventions** (%)	8	44%

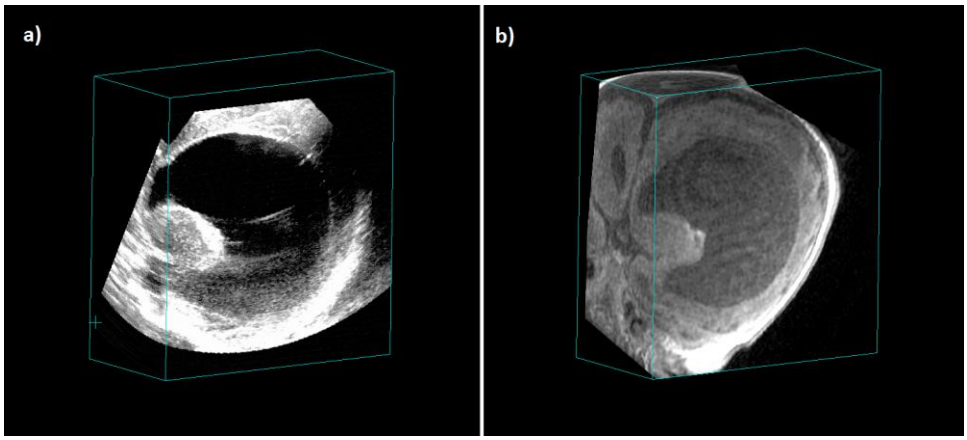

Figure 3-3 Para-sagittal slices of the right lateral ventricle with coronal anterior horn cut though a) 3D US and b) T1-weighted 3D MRI image of a neonate. This patient had grade IV IVH in the left ventricle and grade II IVH in the right ventricle. The VV measure from MRI was 157.7 $cm^3$and from 3D US was 147.7 cm^3.

Figure 3-4a shows the plot of the correlation between 3D US and MRI based VV and a very high correlation between the two modalities is present ($R^2 = 0.99$). The slope of the best-fit line was 0.87, which was significantly different than 1 (95% CI of 0.83-0.90). MRI VV was higher than 3D US for almost all patients. Bland-Altman plot indicates a bias towards lower 3D US VV at larger VV (Figure 3-4b).

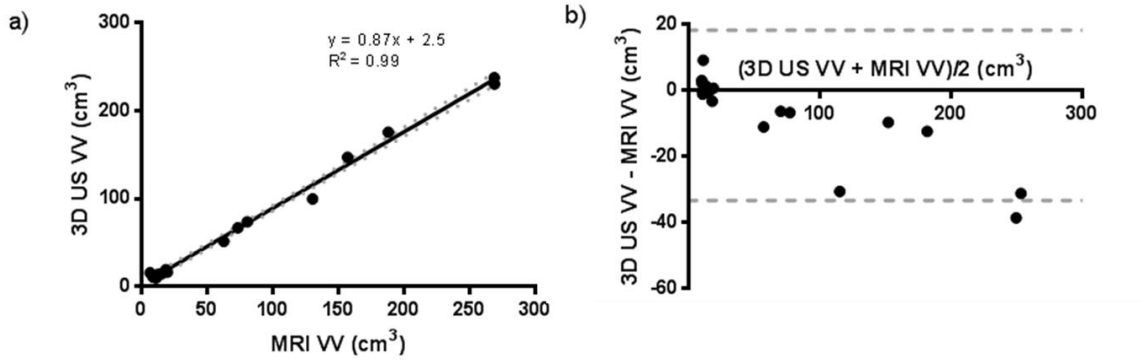

Figure 3-4 Ventricle Volumes as measured through manual segmentation of 3D US and MR images. a) Linear regression line is shown in bold with 95% confidence intervals of the regression are shown as the dotted lines. b) Bland-Altman plot of the difference in 3D US compared against the mean in 3D US and MR ventricle volumes.

3.3.2 Ventricle Tap Volume Validation

Eight different IVH patients are described in Table 3-2 with their pre- and post-ventricle tap ventricle volume measurements shown in Figure 3-5. Of those 8 patients, 6 had multiple VTs (Table 3-2) during the course of the stay in the NICU, resulting in a total of 25 VT studied. It was noted that during 5 of the studied procedures, CSF was taken from the ipsilateral side of in a patient who had developed unilateral porencephalic cysts, which left 20 taps to be included the regression analysis.

Table 3-2 Patient characteristics for those requiring ventricle taps. GA is gestational age indicating how premature the patient was (with 37-40 weeks being accepted as a term birth), and intraventricular hemorrhage (IVH) grade is based on the severity scale by Papile et al.[23]

Patient	Taps monitored	age at first tap (days)	GA at birth (weeks)	Birth weight (g)	IVH grade
P007	1	27	27	920	IV(R)/III(L)
P012	5	15	25	800	III(R)/III(L)
P019	2	19	27 2/7	1080	III(R)/III(L)
P041	5	16	25 3/7	910	III(R)/III(L)
P043	4	16	27 3/7	1280	IV(R)/III(L)
P052	1	16	30 2/7	1280	III(R)/III(L)
P055	4	7	29	1210	IV(R)/III(L)
P057	3	35	24 6/7	750	III(R)/IV(L)

For all patients and all taps, the pre-tap ventricle volume was larger than the post-tap ventricle volume (Figure 3-5). Figure 3-6a shows a plot of the least squared fit of the difference between pre- and post-tap ventricle volumes as calculated from 3D US images against the reported volume of CSF removed. When CSF was taken from the contralateral ventricle of the grade IV IVH, or when the patient had hemorrhage contained within the ventricles, the calculated pre-post difference in VV is highly correlated with the reported amount of CSF removed ($R^2 = 0.92$). A t-test comparing the pre-post tap volume difference with the CSF removed failed to show a statistically significant difference between the slope and 1 (slope 95% confidence interval 0.94 - 1.26) and failed to show a statistically significant difference between the y-intercept and 0 (y intercept 95% confidence interval -4.54 to 0.82). Bland-Altman plot found limited bias (0.32 ± 1.98) with only one point falling outside the 95% confidence interval and about the same number of points above and below the x-axis (Figure 3-6b).

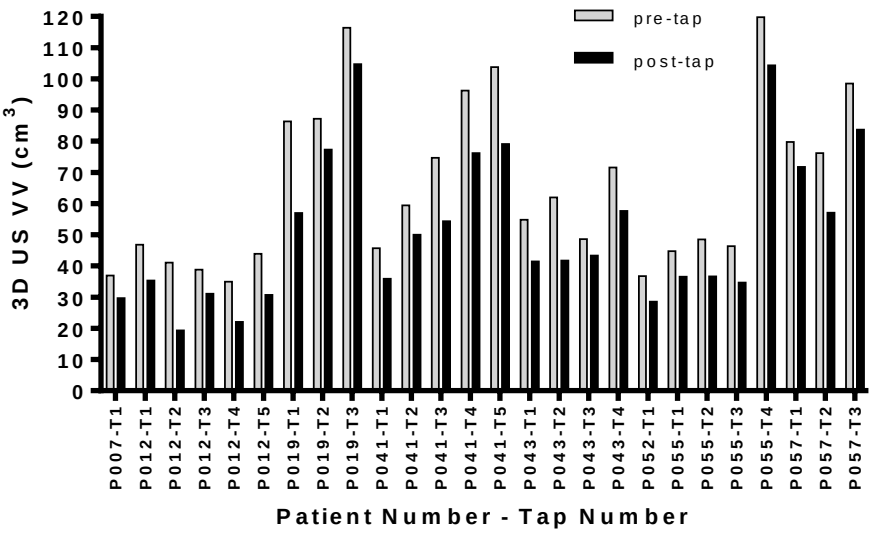

Figure 3-5 Ventricle volumes from 3D US for neonates who required ventricle taps (VTs) for clinical reasons. Pre- (grey) and post- (black) VT and the ventricle volumes (cm^3) segmented from each 3D ultrasound (US) image are graphed. As most patients in this study had multiple VTs, patient number followed by the serial tap number is indicated on the x-axis.

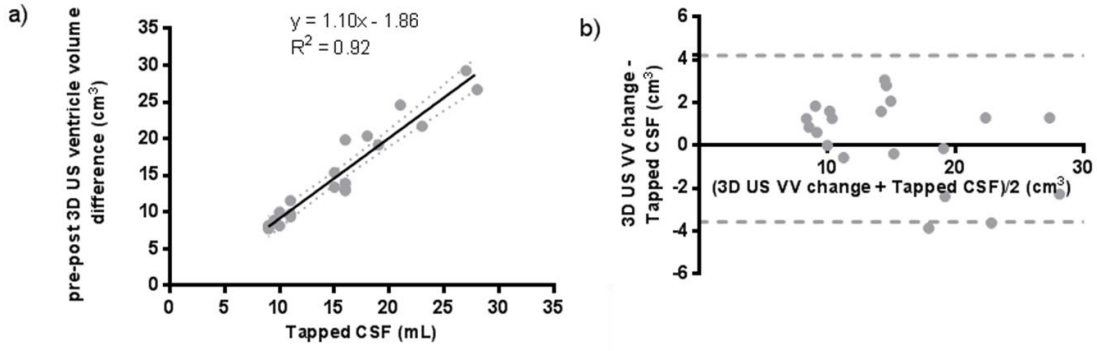

Figure 3-6 Change in ventricle volume before and after ventricle tap compared to measured amount of CSF removed during the VT. a) Least squares fit (bold line) with 95% confidence intervals plotted as dotted lines. Difference between pre- and post-tap measurements is along the y-axis and the reported volume of CSF removed during intervention is along the x-axis. b) Bland-Altman plot of the difference in calculated change in ventricle volume compared to measured volume of CSF removed.

3.4 DISCUSSION

We have shown that there is a systematic bias towards lowered VV using 3D US-based measurements as compared to MRI when the ventricle size becomes increasingly enlarged from PHVD (See Fig. 3-4). However, when comparing between 3D US images, such as before and after VT, the volume difference correlates significantly and matches well to the amount of CSF reported removed by the surgical team (See Fig. 3-6). The change in VV in neonatal patients is more clinically important than knowing the actual amount of fluid present in the system as it could inform clinicians how to optimize interventions, since there is no consensus on how much CSF to remove. While a reported study has shown that a minimum of 10 mL of fluid should be removed for every 1 kg of body weight,[16] optimal amounts and upper limits have not been studied. Excess CSF removal can cause apnea and bradycardia after the intervention [17], but removal of too small amounts may not alleviate clinical signs and symptoms and may lead to excessive repeated interventions if there is no improvement in clinical status. Thus, a more informed approach in how much CSF is being collected within the ventricles could lead to more optimized, personalized patient care. If necessary to compare to a previous MR image VV to 3D US VV, a simple calculation from the strong linear regression (Fig. 3-3) could be performed, although this requires further validation before routine use can be advised.

In vivo neonatal VV validation during ventricle tap has only been previously used by Brann *et al.* [5] using their 2D US cylindrical coordinate method to estimate VV. While we found a very strong Pearson correlation coefficient of $R^2 = 0.92$ comparing VV measurements before and after VT, Brann *et al*. found a correlation of $R^2 = 0.84$.[5] Brann *et al*'s cylindrical coordinate method relied on free-hand 2D US image slices acquisition that were exactly the same angular spacing apart from each other. While this is a reasonable assumption for a highly trained US technician, it is a potential source of error and variability and cannot be relied upon to provide accurate and reproducible volume measurements. [14] Discrepancy between their results and the improved results in our study can be partially accounted for by the fact that our study used a motorized system to acquire the images, which is less user dependent and more precise. [13] The technique

proposed by Brann *et al.* required a highly trained individual to draw manual contours on each 2D slice, which is both time and resource expensive and likely the reason that even though this technique was strongly predictive of interventional treatment necessity, it was never adopted as standard care.

The validation of the 3D US system against MRI images of IVH patients with resolved ventricle dilatation as well as PHVD is an extension of previous research done on neonates. The previous paper outlined a small-scale study of 7 patients with slightly enlarged ventricles (Gilmore *et al*. 2001) and compared segmented 3D US ventricle volumes to those measured in MRI and found high agreement. It should be noted that patients in that study had a maximum ventricle volume of 18 mL, with most ventricles much less than 10 mL, while patients in our study had a much wider range from 8 mL to 230 mL (in 3D US).

While we have shown that there is a bias towards lower VV using 3D US-based measurement compared with MRI, this is systematic, and can be taken into account through calibration. We hypothesize that this bias is due to the acquisition of the images through the anterior fontanelle combined with characteristics of the neonatal brain at mid-line, which is known to cause issues in visualization of some midline structures that are near the posterior fossa. [18,19] In the case of patients with severe PHVD, the posterior horns of the lateral ventricles themselves would be in this difficult-to-visualize area.

We investigated the potential for boundary visualization discrepancies using a fiducial based rigid registration of 3D US to MRI in a few cases of PHVD. In 3D US, as most patients with PHVD had two separate images for left and right ventricle, four landmarks: a) the most anterior point of the anterior horn, b) midpoint of thalamus and choroid plexus intersection boundary, c) foramen of Monro, and d) middle of corpus callosum were manually chosen (See Fig. 3-7) from each ventricle resulting in eight landmarks for the two ventricles in each image. Eight corresponding initial landmarks were also manually chosen from the time-matched MRI. A rigid transformation was then calculated from those corresponding point pairs, which were used to roughly rigidly register the 3D US to the MR images. This rough registration was then manually adjusted following

alpha blending of the overlapped, registered image volumes. Once the images were manually adjusted to obtain a closer registration, the rigid registration was again performed using four points placed in a single slice outside of the ventricles in MRI with corresponding points found on the alpha-blended, registered MRI/US volume. This was iterated multiple times until a visual, qualitative 'good' registration was achieved.

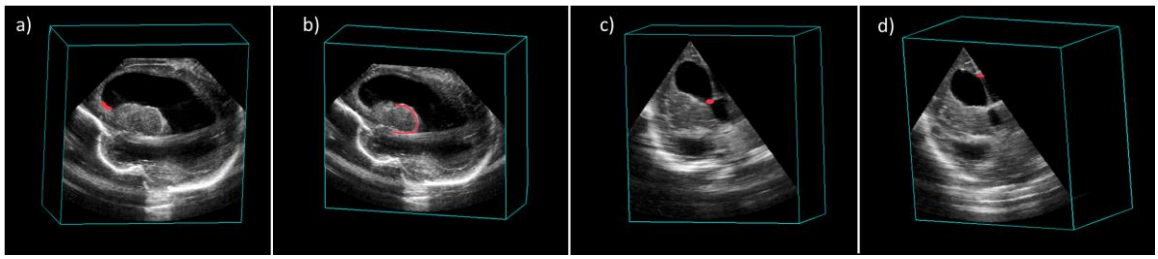

Figure 3-7 Examples of the described fiducial points used in the registration of 3D US to MRI. Fiducials presented on 3D US images from a patient who had PHVD following bilateral grade II IVH. a) the most anterior point of the anterior horn, b) midpoint of thalamus and choroid plexus intersection boundary, c) foramen of Monro, and d) middle of corpus callosum were manually chosen from each ventricle in a resulting in eight landmarks for the two ventricles. a) and b) are mid-ventricle in the sagittal plane while c) and d) are in the coronal plane at the level of the foramen of Monro.

Registered images from three patients in the study can be found in Figure 3-8. Using these registered images, we showed that at the medial most part of the lateral ventricles (mid-line) boundaries are obscured when patients have highly enlarged ventricles (Figure 3-8 f,i). Thus, a portion of the most medial part of each lateral ventricle was systematically missed in the manual 3D US segmentation, but was segmented in the MR image. In patients with mild PHVD, the lateral ventricles would have smaller portions missed or would have no lateral ventricle portions near mid-line (Figure 3-8c), likely closer to that of segmentation error, and as such, we saw no bias when the ventricles were smaller than 50 cm^3 (Figure 3-3b). Additionally, the lateral edge of the ventricles were much harder to visualize in 3D US in comparison to MRI (Figure 3-8a,d,g); however, the

mid-ventricle images had fairly easy to visualize borders in both modalities (Figure 3-8b,e,h). This hypothesized visualization-based bias will be investigated further in future studies, though is out of scope of this paper.

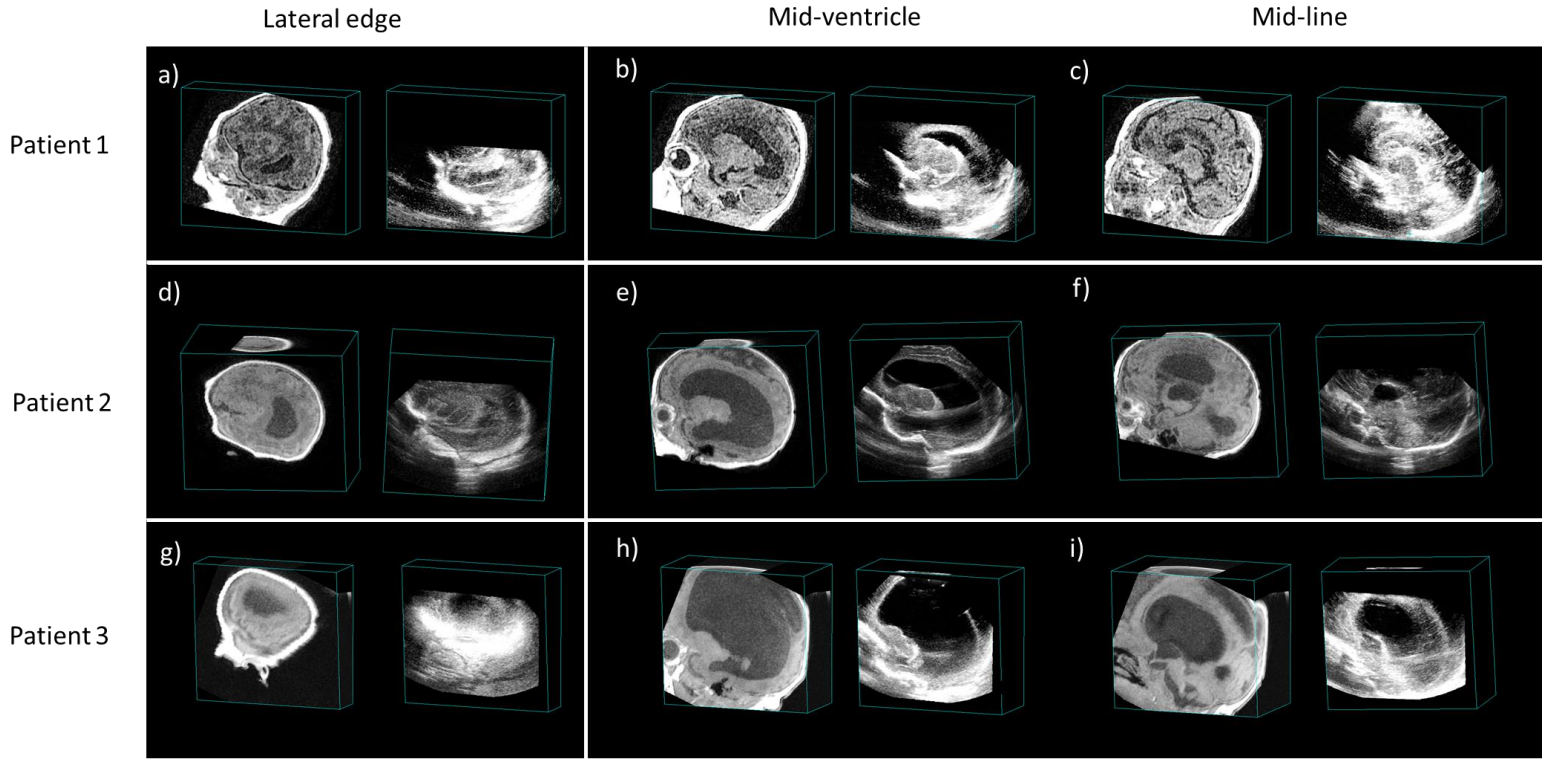

Figure 3-8 Registered 3D US and MR images sets of three IVH patients at the lateral edge of the lateral ventricle (left), mid-ventricle (center) and mid-line of the brain (right) all in the sagittal plane. a) shows a patient with grade II IVH who did not require interventional therapy, b) shows a patient with bilateral grade II IVH who required a single ventricle tap and shunt while c) shows a patient with grade IV (right) and grade III (left) IVH who required multiple ventricle taps and a shunt.

A limitation in the current study was the small number of neonates who required interventions (N=8). As the enrolment period of the study performed by Brann *et al.* was performed in the late 1980s when severe IVH, and PHVD was more common, they were able to recruit many more neonates who required interventions. While the MRI to US validation study had more patients than the study published by Gilmore and Gerig (N=7), the current study still had a relatively small sample size (N=18). Additionally, our MRI study could only be performed on preterm born neonates at term equivalent age. Future research investigating MRI and 3D US in low birth weight preterm neonatal patients which are incubator bound, or on extensive respiratory assistance would allow for a more complete validation of the system. Unfortunately, most centers, including our own, lack the equipment necessary to safely image incubator bound preterm neonates in an MRI facility, as that would require an MR compatible incubator and preterm head coil. While stable preterm neonates can be imaged with MR in other facilities, to perform MRI before and after a ventricle tap would almost certain be unattainable for most neonates who require a tap as they will have become unstable with spells of apnea and bradycardia making transfer to an imaging suite even more dangerous and time consuming. In addition, it is unrealistic to expect that all centers worldwide will ever be able to monitor IVH infants with repeated MRI, from a resources point of view (time and cost). Therefore, an US-based method will remain the method of choice for monitoring PHVD. That said, this validation study using the amount of tapped fluid is the also most feasible for extremely preterm neonates unable to be moved into an imaging suite, as the imaging study can be performed while the infant is in the incubator.

The main limitation of implementing the 3D US system into clinical practice is the requirement for manual segmentation of the ventricles. As this can take up to 45 minutes per patient and seems to be not feasible in a clinical setting, a semi-automated segmentation approach to segmentation has been developed in our laboratory to address this issue. [20] The segmentation algorithm specifically for neonatal cerebral 3D US images has been shown to reduce segmentations times to 2-5 minutes, which is much more

reasonable to use as a tool to follow PHVD patients. We are currently integrating the segmentation software into our 3D US system so that it can be used in a prospective study of patients with PHVD.

There are a few potential clinical applications for our 3D US system other than to monitor IVH patients' progression. Infantile hydrocephalus can also occur following meningitis, other infections, or from congenital malformations. These patients would be good candidates to monitor before and after surgery with this non-invasive imaging tool, which could be done during routine follow up. Additionally, patients who have been diagnosed with ventricle dilatation *in utero* could be followed on antenatal exams using this technology. As many of these patients with *in utero* ventricle dilatation end up having some mental health and cognitive problems in later life,[21,22] ventricle volume measurements might be able to indicate the severity of the defects to expect, which could inform parents as to what they should be expecting from their child and inform follow-up care.

Our 3D US system was validated with patients ranging from <1000g, confined to incubators to >5000g and stable enough to be on room air and a crib. As there was such a variation in patient size and general stability, we believe that we have captured the majority of what we could be reasonably expected to see in future studies of preterm neonates.

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Chapter 4

This chapter will discuss the comparison of ultrasound measurements of the cerebral ventricles in neonates using clinical two-dimensional ultrasound as well as the validated three-dimensional ultrasound system.

The contents of this chapter were previously published in the journal Physics in Medicine and Biology: J. Kishimoto, S. de Ribaupierre, F. Salehi, W. Romano, D.S.C. Lee, A. Fenster. Preterm neonatal lateral ventricle volume from three-dimensional ultrasound is not strongly correlated to 2D ultrasound measurements. J. Med. Imag. 3(4) (2016) 046003. doi: 10.1117/1.JMI.3.4.046003. Permission to reproduce this article was granted by the Society of Photo-Optical Instrumentation Engineers (SPIE) and is provided in Appendix A.3.

4 Preterm neonatal lateral ventricle volume from 3D ultrasound is not strongly correlated to 2D ultrasound measurements

4.1 Introduction

Post hemorrhagic ventricle dilatation (PHVD) is characterized by an enlargement of the cerebral ventricles and commonly occurs in preterm neonates with moderate to severe intraventricular hemorrhages (IVH), grades II-IV, as grade by the clinical standard system.[1] PHVD can lead to lifelong neurological complications such as cerebral palsy as well as visuospatial disorders and lower developmental quotient.[2,3] Trans-fontanel cranial ultrasound (US) is sensitive and specific for detection of PHVD, and is used clinically to diagnose and monitor this condition.[4] However, 2D images cannot provide accurate volume measurements, and so ventricle volume (VV) is often estimated and monitored qualitatively. To address this, several quantitative measurements (ventricle index, anterior horn width, thalamo-occipital distance, 3rd ventricle width) have been derived for 2D US images.[5] These measurements rely on linear widths of ventricles to estimate the changes in VV as the disease progresses. Such measurements have not been standardized across neonatal intensive care units (NICUs), and therefore there is a wide variation in the interpretation of 2D US-based measurements' utility. This is further confounded by the user dependent nature of 2D ultrasound as the technician only records some image planes, and, furthermore, there has been no consensus on what should be measured by a radiologist to best illustrate VV.[6]

The ‘gold standard’ to quantify VV makes use of 3D MR images but can only be acquired once the patient is stable enough to be transferred to the imaging suite - often weeks after initial diagnosis and intervention for PHVD. Indeed, only a few studies directly comparing 2D US and MRI measurements of the ventricles have been done.[7,8] Specifically, Horsch et al found that the anterior horn width was strongly correlated with MRI VV ($R^2 = 0.88$) when preterm born infants with varying ventricle size were imaged at term equivalent age.[7]

In contrast to the ‘gold standard’ MRI-based measurements, 3D US images can be attained multiple times per week – early after birth - with limited adverse effects to the patient, as the imaging procedure is done within the incubator, and takes only a few minutes to complete.[9] Additionally, serial 3D US is far less expensive than longitudinal monitoring with MRI.

3D US imaging studies have been able to attain VV measurements from preterm neonates with both normal ventricles and those with mild ventricle dilatation throughout the course of stay in the NICU.[10-14] Recently, a study found that 2D measurements can be made using a reconstructed 3D US image with reasonable inter-observer variability and with no significant differences from measurements made on 2D US images from the same day[9] and another study has found excellent agreement for the diagnosis of different neonatal pathologies using 3D US images compared to 2D US obtained at the same time.[15] Additionally, when directly comparing VV made from 3D US images and MRI, high interclass correlations (ICC) were found (ICC = 0.92) in term born patients with mild ventricle dilation.[10] We have previously found very high correlations between MRI and 3D US VV in preterm born infants with PHVD when imaged at term equivalent age ($R^2 = 0.99$).[14]

However, in most centers, even in those with 3D US imaging capabilities, to guide treatment and therapy of patients with enlarged ventricles the only quantitative measurements of ventricle size routinely performed are 2D US measurements. While there are commercially available 3D US probes that perform well, the high cost may limit their use. We have previously developed and validated a low cost 3D US system.[14,16] Since this system acquires images using the same 2D US transducer used in clinical

scans, we are able to make direct comparisons to clinical images and the estimates of ventricle size from those images.

In this paper, we compare 3D US VV to previously reported 2D US based measurements made on the same day. Additionally, we compare the change in 2D US measurements to changes in VV estimates from 3D US since serial changes in 2D US could determine whether treatment is performed on a neonate with symptoms of increased intracranial pressure stemming from PHVD.

4.2 Methods

4.2.1 Selection of subjects

The research protocol was approved by the local human Research Ethics Board as part of a larger study investigating the development of patients with IVH. Patients with a positive diagnosis of IVH on an initial clinical head US exam were prospectively recruited following informed consent from their parents. Once enrolled, patients underwent serial US exams 1-2 times per week until discharge from the neonatal intensive care unit (NICU), or transfer to a secondary care center. Infants with congenital brain abnormalities were excluded from the study. Decisions on when to intervene in patients with severe PHVD were based on clinical assessment of the patient (neurological exam, palpation of the fontanelle, increase in head circumference measurements, monitoring for increased spells of apnea and bradycardia) and qualitative viewings of 2D US images. The care team was blinded to 3D US images and measurements.

4.2.2 Ultrasound Imaging

Patients were imaged by a trained clinical ultrasound technician who performed a 2D head US exam prior to a 3D US scan.

4.2.2.1 2D US

Imaging was performed at the bedside in the NICU using a HDI 5000 US system (Philips, Bothel WA) and a C8-5 curved array 5-8 MHz broadband transducer (Philips, Bothel WA). 2D US exams were performed using a standard technique, acquiring images

in coronal and sagittal planes through the anterior fontanelle, with a few screen captures chosen by the technician over the relevant landmarks as per the standardized exam.[17] The 2D US exams required approximately 10-15 minutes. As the neonates' heads grow during the study (both due to normal development and PHVD), depth settings were adjusted accordingly to capture the ventricle system. As such, images were acquired between 7.1 cm and 9.9 cm in depth and 2D US images were 640 x 476 pixels from 0.0200 x 0.0200 mm per pixel for 7.1 cm depth to 0.0266 x 0.0266 mm per pixel for 9.9 cm depth.

4.2.2.2 3D US images

3D US images were acquired using a 3D US motorized attachment developed in our laboratory, which housed the US transducer (Fig 4-1a). Technical details of the 3D US system were previously described.[16,18,19] The 3D US images were acquired by locating the midline in the sagittal plane, then firmly holding the motor encasement to allow the device to mechanically tilt the transducer about the axis at the probe tip along parasagittal slices, while the technician's hand remained as still as possible.

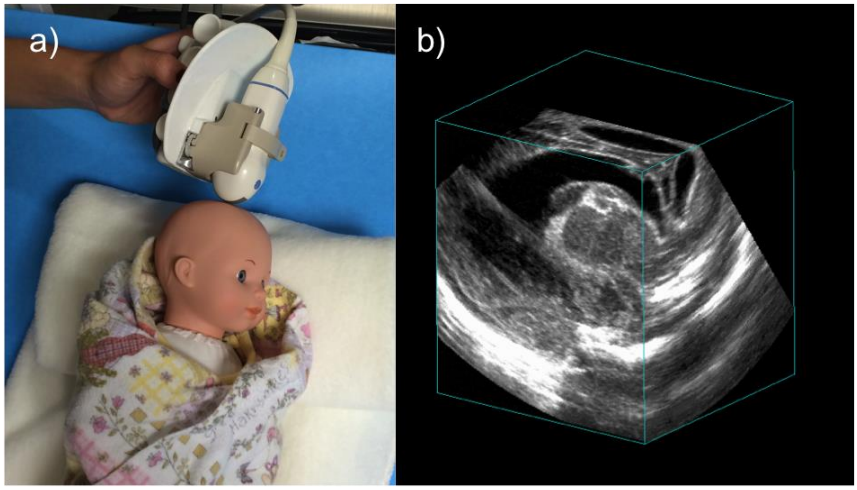

Figure 4-1 Motorized 3D ultrasound system and image. a) The mechanical 'tilting' 3D US transducer device being held in position next to a preterm sized mannequin. This is approximately the position used for neonates scanned while intubated or while on CPAP during a 3D US acquisition. b) A representative 'cube' view cut through three orthogonal planes of a 3D US image of a patient with PHVD

This image can be viewed through any reconstructed plane (i.e., sagittal, axial, coronal, or oblique) using a multi-planar reformatted 3D visualizer (See Fig. 4-1b) at the bedside to determine that the full ventricle volume was acquired with limited motion from either the patient or the technician during the scan.[20] An example of motion artifact that would require additional scans can be seen in Figure 4-2a, whereas Figure 4-2b shows the same patient's 'acceptable' 3D US image from the same scanning session. Imaging specifications are as such that 2D US images were acquired with an angular spacing of 0.3 degrees at 25 frames/s over a scan angle of 60-72 degrees, making total 3D image acquisition between 8-9.6 seconds. 3D US images ranged from 300x300x300 to 400x400x400 pixels with voxel sizes ranging from 0.2x0.2x0.2 mm for 7.1 cm depth to 0.24x0.24x0.24 mm for 9.9 cm depth. The same depth settings were used in 2D and 3D images on the same day for the same patient. Under ideal circumstances a single sweep of the mechanical tilting device is able to capture the entire ventricular system in 3D US. Generally, an acceptable image (Fig. 4-2b) was acquired in 2-3 sweeps of the device, taking between 2-10 minutes for the entire 3D US examination. Some patients with extremely dilated ventricles required acquisition of two separate 3D US images to capture the entire ventricular system, one of the right lateral ventricle, and one of the left.

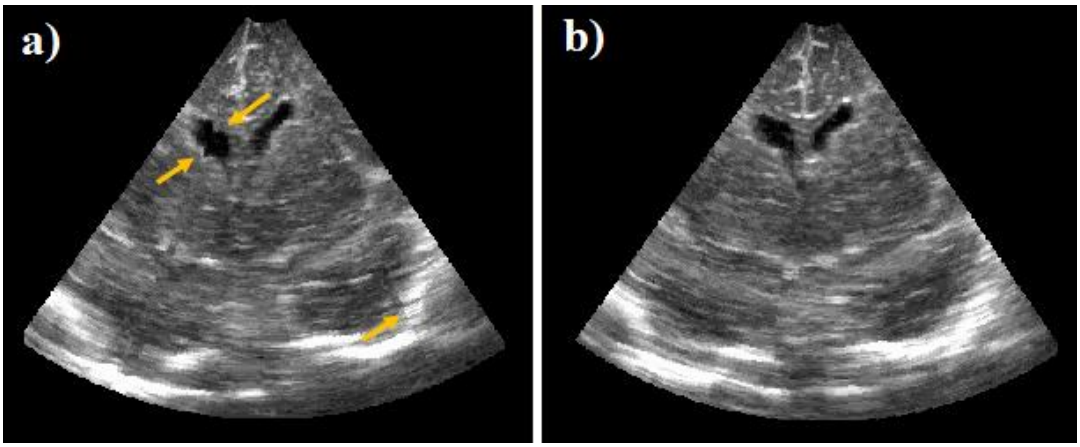

Figure 4-2 Reconstructed coronal slice of 3D US images of a preterm patient with ventriculomegaly following a bilateral grade II IVH. a) arrows showing motion 'shifts' that disrupt the smooth ventricle boundary (left side of image) as well as the skull reflection (right side of image) in comparison to b) which has much less motion and would be considered an acceptable image.

4.2.3 Image analysis and ventricle segmentation

4.2.3.1 2D US

Two observers (FS and WR) analyzed the 2D US images and reported the ventricle index (VI) and anterior horn width (AHW) for the left and right lateral ventricles as well as the third ventricle width (3rd) and the largest thalamo-occipital distance (TOD). Reporting a single TOD was the standard at our institution, and was used as there was often large differences between the left and right side, with one much larger than the other.[21] Therefore, to attain the best correlation possible, we used the largest TOD, as we believed this would be a better representative of both ventricle volume and change in the ventricle volume. Measurements are described in detail in Davies *et al.*[5] and examples are shown in Figure 4-3. The differences (Δ) obtained between consecutive time points (i.e., between scans 2 and 3 and scans 3 and 4 but not between 2 and 4) were calculated for all 2D US based measurements.

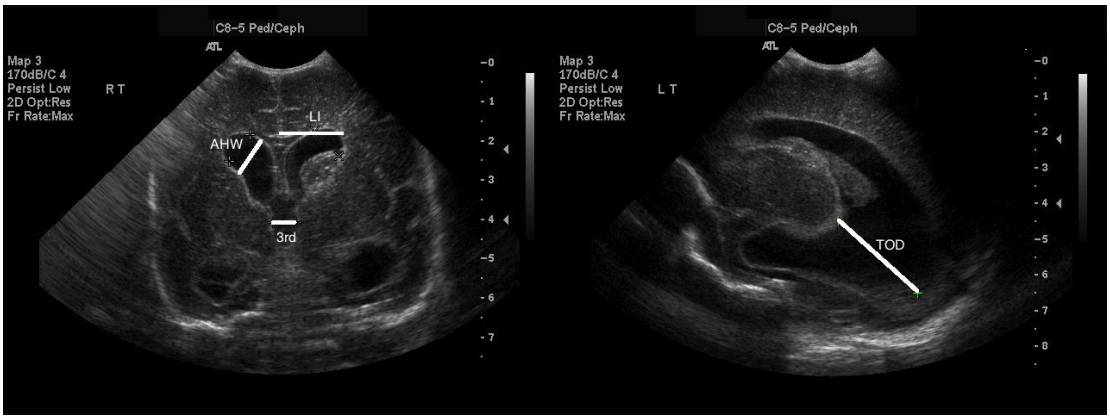

Figure 4-3 2D US images of a preterm patient with ventriculomegaly following a grade II bilateral IVH. a) coronal plane at the level of the foramina of Monro indicating LI, AHW and 3rd ventricle width, and b) parasagittal plane indicating TOD.

4.2.3.2 3D US

Ventricle volume (VV) was obtained by manually segmenting the ventricles from the 3D US images by a single trained observer (JK) and verified by an experienced clinician (SdR). Due to the high volume of images to be segmented (N=255), each image was only

segmented once in order to mitigate user-fatigue which can cause an increase in variance of segmentation.[22] The segmentation was performed on parallel sagittal slices on the reconstructed 3D image. The manually segmented boundaries included the intraventricular blood clot as well as the choroid plexus, but not porencephalic cysts if they were present. Slice spacing was set at 0.5 or 1 mm depending on the amount of ventricular dilatation. Patients with smaller ventricles (that could be captured in a single image) required smaller slice spacing (0.5mm) due to the highly irregular shape, as well as the thinness of the anterior and temporal horns. Patients with severe PHVD who required two 3D US images to fully capture the ventricles (one image for right and one for the left lateral ventricle) had slice spacing set at 1 mm as the ventricles were much less irregular in shape and the segmentation of these patients required the most time and most contours to segment for determination of VV. For these patients, each of the left and right lateral ventricle volumes were segmented from separate images and added together. Each image set required an average of 20 minutes to segment, with 30-70 contours segmented per 3D US image set. Figure 4-4 shows 3D US segmented image and volume rendered ventricle surface for a patient with mild ventricle dilatation as well as a patient with PHVD. The difference in 3D US ventricle volumes (ΔVV) between successive time points were calculated and recorded.

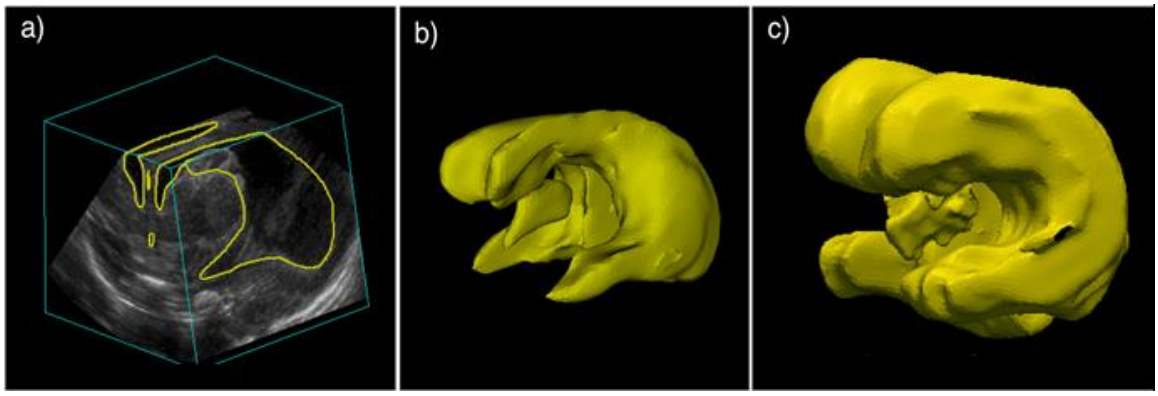

Figure 4-4 3D US images and segmented ventricle surfaces. a) 3D US image through the anterior fontanelle of a patient with bilateral grade II IVH. a) depicts the segmented region within the 3D US image, b) is the ventricle model generated from the segmented boundaries in a, and c) depicts a 3D US ventricle model generated from 2 3D US images of a patient with PHH following a bilateral grade III IVH. Volume was measured to be b) 20.2 cm^3 and c) 45.7 cm^3.

4.2.4 Observer Agreement in reported 2D US measurements

As there were two observers (FS and WR) for the 2D US images, a small subset of the images was measured by both observers to determine measurement agreement. Seventeen images were analyzed, and they were all from different neonates with varying severities of IVH and ventricle dilatation. VI and AHW for the left and right lateral ventricles as well as the 3rd and the largest TOD were reported by the two observers.

4.2.5 2D US measurements made on 3D US images

In order to further validate our 3D US system, VI, AHW, and TOD were measured on approximately the same plane on the 3D US images as would be used for 2D US. 3rd was excluded as images were taken too close to mid-line to visualize this structure in most 'acceptable' 3D US images. A single observer (F.S.) performed measurements on both the 3D US images and time matched 2D US images. Twenty-eight image sets from different neonates with varying severities of IVH and ventricle dilatation were analyzed for this study.

4.2.6 Statistical Analysis

Linear regression (R^2) were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA). A regression of $R^2>0.7$ was considered strong, 0.7-0.5 moderate, and <0.5 weak. Observer agreement between the two observers on the same 2D US images was performed through absolute agreement, two-way random intraclass correlation (ICC) using SPSS v.20 (IBM Corp, Armonk, NY). Absolute agreement, two-way random ICC was additionally performed to determine agreement between 2D US images made on both 2D and 3D US for the same observer.

4.3 Results

4.3.1 Patient Characteristics

Forty-two neonates were enrolled into the study following an initial diagnosis of IVH and subsequent ventricle dilatation between April 2012 and February 2015. The median age of enrollment was 9 days of life (range 4-30 days). Patient characteristics are summarized in Table 4-1. A total of 265 image sets (2D and 3D US images acquired on the same day) were collected for this study. 255 2D/3D US exams provided all the required US measurements, with 8 image sets missing 3rd measurement and 2 missing TOD. Scans were performed between 25 and 42 2/7 weeks corrected GA with the median GA at imaging being 30 3/7 weeks (Figure 4-5).

Table 4-1 Clinical characteristics of the study population. Interventions include ventricle taps, external ventricle drains and VP-shunts. Direct Comparison indicates

all patients used to study VV to 2D ultrasound measurements of the ventricles, while Changes is the patients who had more than one imaging session and therefore changes in VV as well as 2D ultrasound measurements of the ventricles between imaging sessions could be calculated and correlated.

	Direct comparisons (N = 42)	Changes (N = 35)
Gestational Age	27.6 ± 3.0	27.3 ± 3.1
Birth Weight (g ± SD)	1105 ± 450	1062 ± 480
Sex (M:F)	30:12	25:9
Patent Ductus Arteriosus (%)	30 (68%)	24 (71%)
Antenatal Steroids (%)	24 (37%)	20 (57%)
IVH		
Grade I	7 (11%)	4 (11%)
Grade II	17 (58%)	16 (46%)
Grade III	10 (21%)	9 (26%)
Grade IV	8 (11%)	6 (17%)
Required Intervention		
Yes	10 (16%)	9 (26%)
No	32 (84%)	26 (74%)

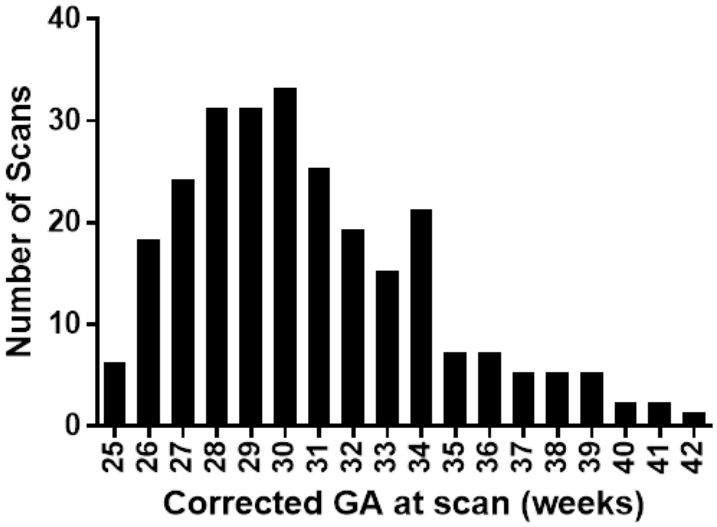

Figure 4-5 - The number of 2D/3D US image sets. Patients were separated by given corrected gestational age (GA) as reported on the chart on the day of the scan.

To compare changes between images, patients required at least two 3D/2D US image sets, and 7 patients were discharged to a secondary care center or home after only a single imaging session leaving 35 to be used in the change study, with their 248 images for a total of 213 comparison points with a median of 6.5 days in between scans (range 3-46 days). These change scans were over the same GA range as the direct comparisons (25-42 2/7 weeks). Of the 35 patients who had >1 imaging session the median number of images per patient was 7 (range 2-14).

Ventricle volumes ranged from 2.7 cm^3 (GA at scan 25 weeks) to 96.2 cm^3 (GA at scan was 26 5/7 weeks) where the overall median was 10.3 cm^3 for patients ranging in GA from 25 - 42 2/7 weeks. In comparison, normal preterm neonates without IVH between 25 - 40 weeks GA would have ventricle volumes in the range of 0.4 - 2 cm^3.[11]

4.3.2 Observer Agreement

There was a high ICC (> 0.95) between the two observers for all 2D ultrasound measurements and can be seen in Table 4-2.

Table 4-2 Inter-observer agreement. Intraclass correlation (ICC) of the ventricle index (VI) and anterior horn width (AHW) for the left and right lateral ventricles as well as the third ventricle width (3rd) and the largest thalamo-occipital distance (TOD). 95% confidence intervals (95% CI) are within [].

	VIR	VIL	AHWR	AHWL	3rd	TOD
ICC	0.98	0.99	0.97	0.99	0.95	0.96
[95% CI]	[0.99-0.91]	[0.99-0.98]	[0.99-0.92]	[0.99-0.98]	[0.98-0.86]	[0.98-0.89]

4.3.3 2D US measurements on 3D US images

There was a high ICC (>0.70) between measurements made by a single observer on both 2D and 3D US images for VI, AHW and TOD which is presented in Table 4-3.

Table 4-3 - Intra-observer agreement though intra-class correlation (ICC) between 2D and 3D US images. ICC of the ventricle index (VI) and anterior horn width (AHW) for the left and right lateral ventricles as well as the third ventricle width (3rd) and the largest thalamo-occipital distance (TOD). 95% confidence intervals (95% CI) are within [].

	VIR	VIL	AHWR	AHWL	TOD
ICC	0.70	0.70	0.85	0.87	0.77
[95% CI]	[0.91-0.50]	[0.90-0.54]	[0.93-0.70]	[0.93-0.73]	[0.89-0.56]

4.3.4 Ventricle Volumes compared to 2D US parameters

Linear regressions were performed between 3D US VV and the corresponding 2D US based measurements recorded at the same time. Figure 4-6 shows the linear regressions (bolded line) and 95% confidence intervals (thin lines) of VV as measured using 3D US images against the corresponding 2D US based measurements (VI, AHW, 3rd and TOD). While there were trends towards increasing 2D US measurements with increased VV, the Pearson correlation coefficients were between 0.69 and 0.36. For the left lateral ventricle measurements, there was a single patient (open circles) who tended to have high 2D US measurements in the coronal plane (LI, AHW), but relatively low VV (Figure 4-6 b,d). This appeared to be due to a very large porencephalic cyst on the left side that was communicating with the ventricular system and was erroneously included in the coronal measurements. This patient was removed from the analysis, the correlation for LIL and AHWL became 0.65 and 0.69 respectively, and the linear regression line on Figure 4-6 reflects this patient being removed from analysis.

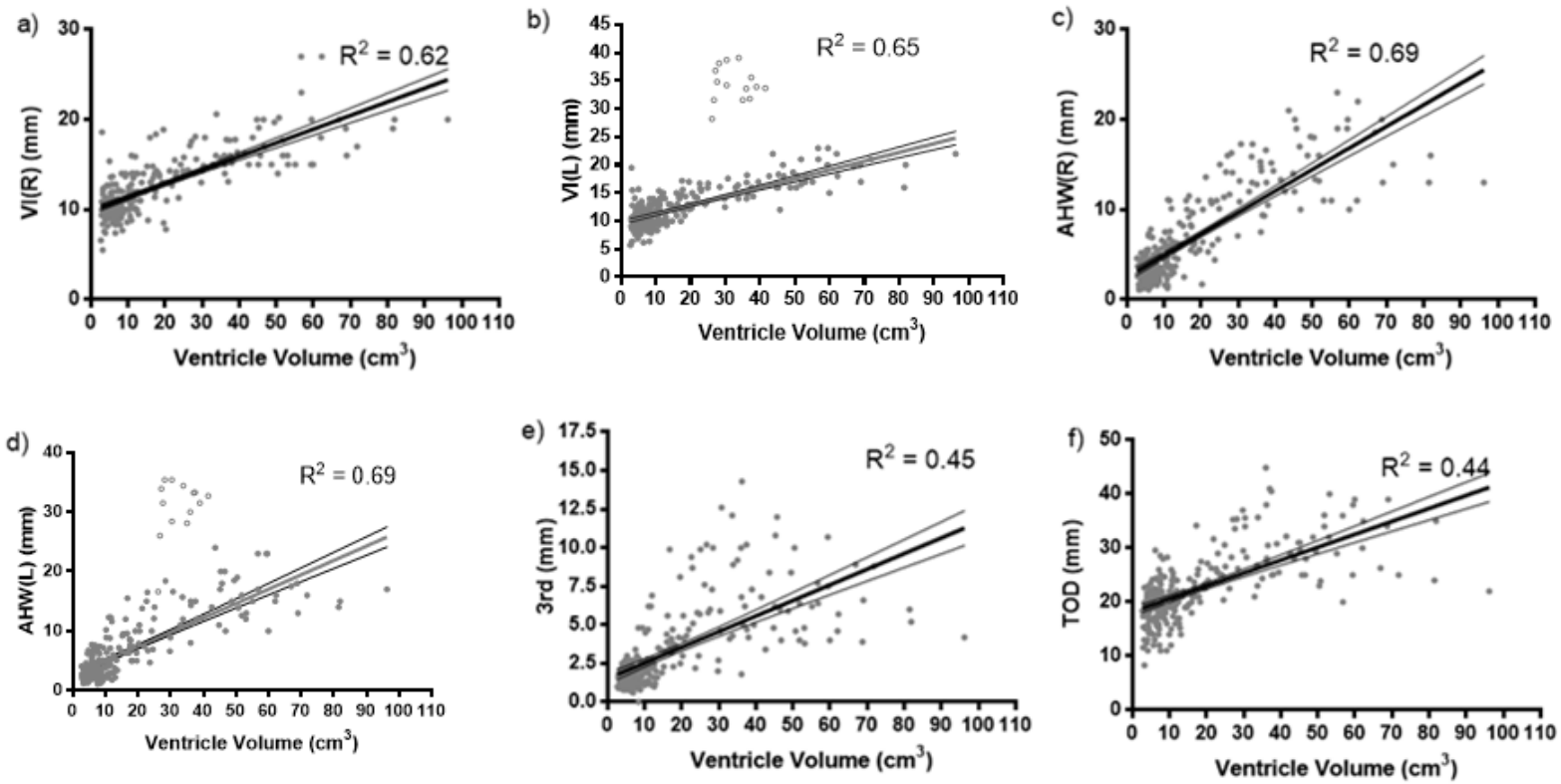

Figure 4-6 Correlation graphs for ultrasound measurements. Ventricle index (VI) a) right (R) and b) left (L), Anterior Horn Width (AHW) c) right (R) and d) left (L), e) third ventricle width (3rd) and f) thalamo-occipital distance (TOD) measurements against VV from 3D US. Linear regression is indicated by the bolded line along with 95% confidence interval (thin lines). A single patient was excluded from regression analysis for VIL and AHWL (b,d) as the measurements included a left side porencephalic cyst and are represented as the cluster of points well above the linear regression

4.3.5 Changes in Ventricle Volumes compared to changes 2D US parameters

We investigated the differences in 2D and 3D measurements between successive imaging sessions as an indication of how ventricular size changes post-IVH. Since there can be substantial differences in rates of change from one patient to the next, and due to the various number of imaging points per patient, linear regression between adjacent time points was used instead of multilevel modeling, or ANOVA. We believed that linear regression on the difference of measurements at two time points would be fairly representative of what would happen on a day to day use of measurements when comparisons are made to the previous scans. Figure 4-7 shows the linear regressions (bolded line) and 95% confidence intervals (thin lines) of ΔVV as measured using 3D US images against the corresponding change in 2D US based measurements. The Pearson correlation coefficients found between ΔVV estimated using 3D US and the measured change in 2D US parameters were between 0.13 and 0.02.

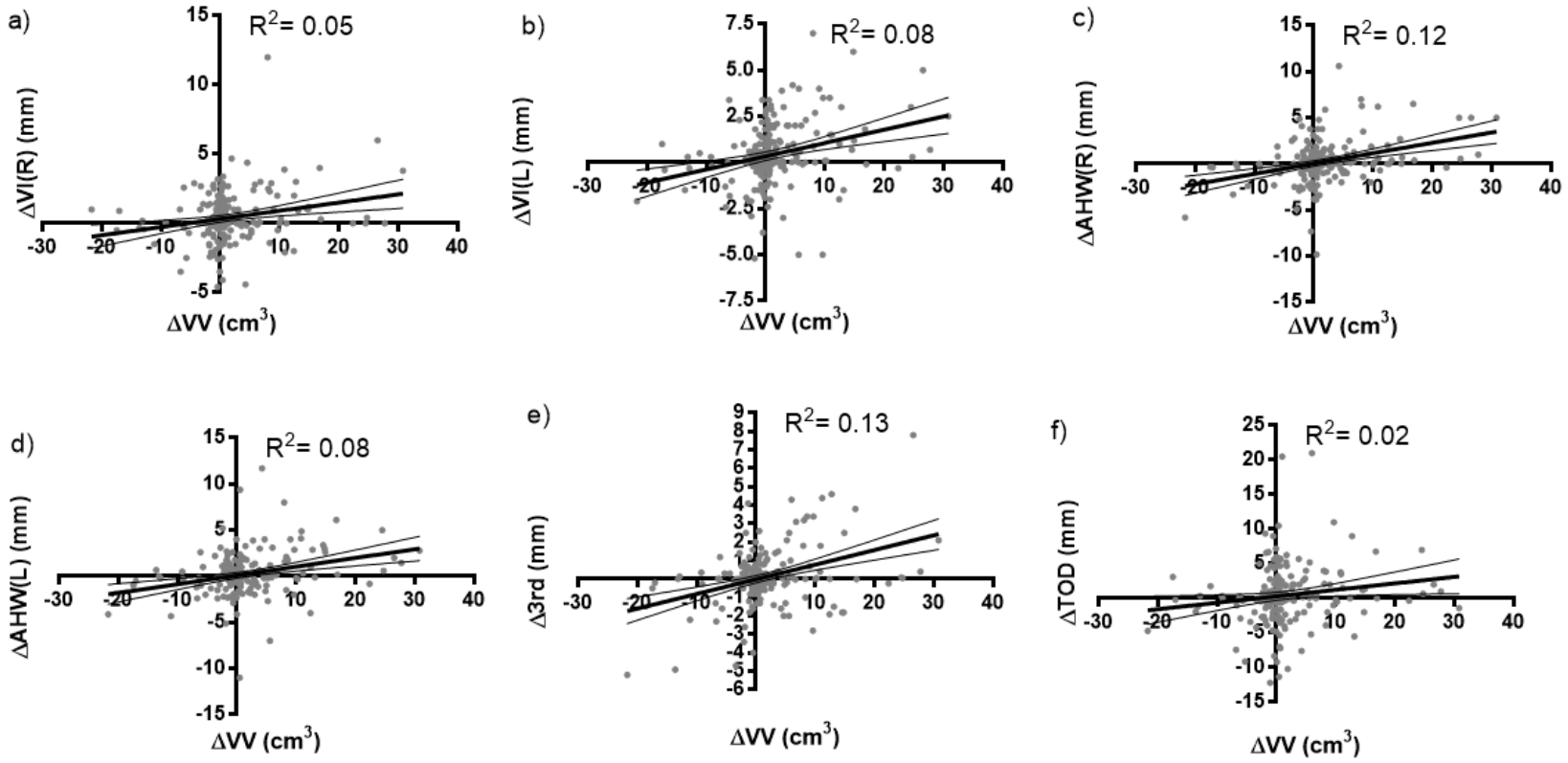

Figure 4-7 Correlation graphs for change in ultrasound. Change in ventricle index a) right and b) left, Anterior Horn Width c) right and d) left, e) third ventricle width and f) thalamo-occipital distance measurements (ΔLI, ΔAHW, Δ3rd, and ΔTOD respectively) against the change in ventricle volume (ΔVV) from 3D US. Linear regression is indicated by the bolded line along with 95% confidence interval (thin lines).

4.4 Discussion

As expected, there was a trend towards increasing 2D US-based measurements with increased VV (Fig. 4-6), though there was no 2D US measurement that 'best' correlated with 3D US VV. Additionally, due to the longitudinal nature of this study, we were able to show that changes in serial 2D US measurements are weakly correlated to changes in VV and indeed, there was not an obvious trend. This is possible due to the variability in 2D US-based measurements. Previously reported standard deviations of measurements ranged from 2 mm for the AHW[9] to 2.5 mm for the VI and 4 mm for the TOD,[8] whereas the mean absolute change between imaging sessions was 1.4 mm for AHW, 1.2 mm for VI, and 2.7 mm for TOD – less than measurement variability. In comparison, we have previously found intra-user variance (standard error of measure, SEM) to be 0.23cm^3 for intra-user and 0.24cm^3 for inter-user through a study of 3D US ventricle segmentation using three different observers.[16] An additional study found variability in 3D US as measured through standard deviation to be an average of 0.95 cm^3 (range 0.31-2.03 cm^3) for preterm patients born less than 32 weeks GA with ventricles that ranged in size from 5-34cm^3.[2] Given that a single observer, who was one previously used in the prior published work,[16,23] was used in the present study for 3D US VV, the mean absolute change in VV of 4.1 cm^3 was much higher than the intra-user variability reported in either previous study.

Attempting to estimate change in an irregular 3D volume, such as the lateral ventricles, with a linear measurement obtained from 2D US has obvious limitations other than high measurement error as well. This could explain why, to our knowledge, no previous papers have found rates of change in 2D US parameters that predict which patients will go on to develop PHVD, and why there is a reliance on qualitative viewings of 2D US scans to determine if an intervention is required.

A previous study[9] has shown that the 2D US measurements of the VI and AHW can be made on 3D US images with reasonable reproducibility and accuracy. While they looked specifically at the AHW and VI, we additionally found reasonable agreement with the TOD between 2D and 3D US. We found the agreement (measured through ICC) is lower when comparing between 2D

US and 3D US than between users for the same 2D US images and we believe this could be because the measurements were not able to be made on exactly the same planes. While we are able to reproduce oblique planes using our 3D US system, the plane selection is still a potential source of error in this study. Further research into how to best select and present para-sagittal and para-coronal planes from 3D US images is necessary to make robust 2D US measurements as well as indicate other planes of interest such as those with cysts or other abnormalities. This is likely necessary to support translation of 3D US imaging into the clinical setting.

In contrast to what had previously been shown using MRI VV and 2D US images by Horsch et al.,[7] we did not find strong correlations between 3D US VV and linear measurements. This could partially be due to a higher variance in volume measurement made on 3D US in comparison to MR images as the boundaries are less clear in US images.[14] In Horsch et al.'s MRI based VV study they were only able to make one comparison at a single time point per patient at term equivalent age, and only 4 of 28 patients analyzed had ventricle dilatation. As most of the patients in that study would have clustered near the lower 'normal' end of VV, the 4 with ventricle dilatation would have driven the linear regression and strengthened the correlation in that study, especially with such a relatively low sample size. In comparison, in the present study, we had patients with ventricle sizes along the continuum of both normal (2.7 cm^3) to very large (96.2 cm^3) and included a total of 255 image sets from 42 patients.

An additional strength of the present study is that we were able to make 3D US-based VV measurements prior to term equivalent age in every preterm neonate studied with varying severities of ventricle dilatation throughout the course of their stay in the NICU as opposed to only when stable enough to be transferred to an MRI suite. This is in keeping with when neonates would be monitored most closely to detect IVH as well as to monitor for PHVD and to determine potential initial interventional therapy. A previous study examining normal preterm patients' VV from 3D US found that small increases in VV occurred in the preterm period (from medians of 0.25 cm^3 at 25 weeks GA to a median of 0.56 cm^3 at 37 weeks GA).[11] However, the main limitation in comparing those values to ones reported in our study is that the previous paper excluded the choroid plexus in their segmentation, and would make for smaller than actual VV. To our knowledge, that is the only study on normal VV from 3D US in neonates. In comparison, a review paper noted that over the course of GA 24-42 weeks, the VI was the only measurement

that increased with GA, whereas AHW and TOD remained fairly constant, with the exception of a single paper, which the review paper's authors' noted as having a remarkably small standard deviation and did not agree with their experience or other published works.[8] In a follow up paper,[23] this group developed new reference values for VI, AHW and TOD for patients without US abnormalities born at varying GA (25-42 weeks) and found that increased GA was related to increased VI and TOD, but not AHW. However, studying neonates born <30 weeks GA and comparing VI, AHW and TOD near birth to that at term equivalent age (GA 37-40 weeks), the authors found increases over time for all ultrasound measurements.[23]

Recent studies[6,24] have advised using the >97th percentile of 2D US measurements as the 'action line' for when interventional therapies should begin. While this may provide guidance as to when the first intervention should occur, often patients with severe PHVD will remain over the 97th percentile even after intervention. Thus, subsequent interventions can no longer be guided by 2D US measurements. The change in 3D US-based VV as opposed to 2D US-based measurements might be a better indicator as to when subsequent interventions are required and would provide another metric to those already proposed. Although outside the scope of this study, a future study should be performed using 3D US VV to determine thresholds for interventional therapy while the patient is in the NICU and during follow up to determine whether or not a neonate requires shunting, or whether or not the shunt has failed, as there are also no consensus imaging guidelines for that intervention.

The main limitation in our study from a translational standpoint is that the methodology involves manual segmentation of the images off-line and does not provide timely data for routine patient care. For 3D US to become a viable method for determining the VV at the bedside, an automated or semi-automated approach must be developed. This was beyond the scope of this pilot study. Neonates with PHVD pose a particularly challenging segmentation problem as the ventricles change fairly significantly in shape as well as echogenicity over time. For example, the ventricles become rounder over time, and the bleed, initially hyperechoic, slowly breaks down over time and becomes indistinguishable from the hypoechoic CSF (Fig. 4-8) The change in echogenicity over time makes simple thresholding or local optimization based methods like active contour or level set methods not useful for this application, while the dramatic change in shape makes atlas based approaches challenging. Given the inherent variability and prohibitively long segmentation

times in manual segmentation, our lab has developed a semi-automated approach based on convex optimization with some encouraging preliminary results[25] and we have integrated this new software into our acquisition software, and are currently piloting this new protocol in a small sample of patients. The semi-automated segmentation algorithm has been validated through a multi-observer study and compared to multi-user manual segmentation.[26] The segmentation time was reduced to approximately 2 minutes in comparison to an average of 20 minutes for manual segmentation. In Chen et al., a new framework for future validation of both manual and algorithm based ventricle segmentation was presented and gave us knowledge on areas of local segmentation variability that were not easily seen using global metrics such as VV agreement, or Dice Coefficient. In particular, the temporal and posterior horns have the largest segmentation variability in both manual and semi-automated algorithm segmentation,[26] and this information can be used to generate better segmentations in the future. Additionally, an offline, atlas initialized automated segmentation method[27] was recently developed, though has yet to be integrated into our protocols.

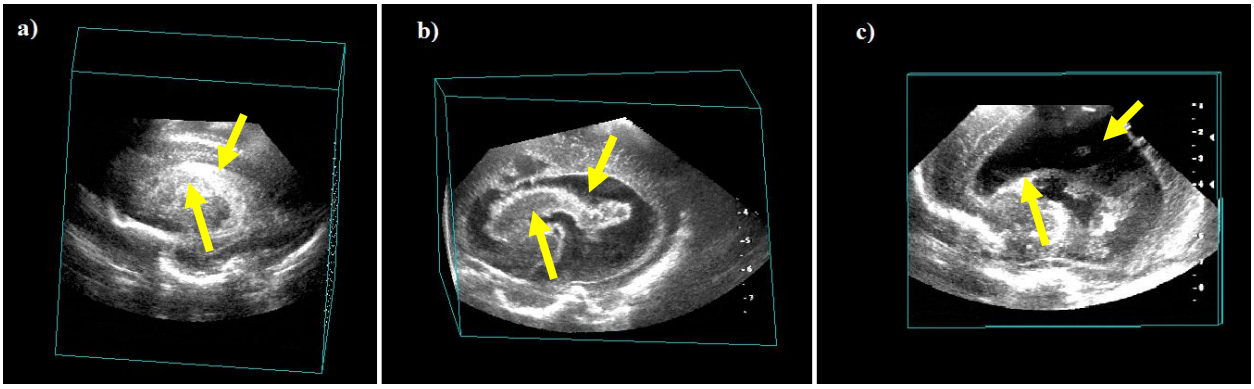

Figure 4-8 - The progression of PHVD in a patient with grade III/IV IVH in 3D US images. All images show a parasagittal view of the right ventricle. a) At 8 days of life, the ventricle is nearly completely filled with blood and is hyperechoic in comparison to surrounding brain. b) at 25 days of life the bleed is beginning to break down and appears nearly the same echogenicity as surrounding brain. c) at 64 days of life the bleed is still breaking down, however, most of it cannot be distinguished from hypoechoic CSF. Ventricle volumes were measured to be 11.4, 62.0, and 83.7 cm^3 respectively.

Another limitation to direct translation to other centers, is the use of a non-commercial system. As our 3D US has been validated both using phantom[14] and patient images,[16] we would anticipate that there should not be a discrepancy between the VV attained from our system in comparison to a commercial system; however, we were not able to definitively prove this as our center does not have an appropriate commercial 3D US transducer for this type of imaging. The HDI 5000 US system used in this study is the most easily assessable unit within the NICU at our center and there is no 3D US transducer within the frequency range for neonatal head ultrasound. However, the semi-automated segmentation software we have developed would be able to accommodate 3D US images from commercial systems, so there is potential for a future study to use this software along with a commercial 3D US transducer. Any patient/technician motion (Fig. 2a) identified through our 3D US system, could be addressed by a commercial system 2D array transducer as the whole 3D US volume is performed in real time (in 33 ms) instead of over 8s. While beyond the scope of this study, detecting and analyzing motion artifacts during acquisition that both can and cannot be detected upon visual inspection would be interesting future work to both understand how this can be adjusted for and how it impacts volume measurements from 3D US.

If 3D US based measurements of VV are a more accurate and precise measurement of ventricle size than 2D based measurements, and they have moderate-weak correlation to 2D US measurements, this suggests that 2D US measurements alone might not be enough to determine the progression of PHVD. This is further shown given the weak correlations between changes in serial 2D US to changes in VV for neonatal patients. This could help explain why there is no consensus image based standard on when PHVD is occurring at a rate that merits intervention. Previous studies[9,15] have shown the time per exam is reduced when acquiring a 3D scan instead of 2D imaging sets, and the diagnostic accuracy is maintained. Therefore, a 3D US approach would preserve the ability to obtain the clinical standard set of measurements, while potentially enhancing the ability to determine whether or not a new clinical standard could be developed using 3D US-based VV estimates. We have presented further evidence that 3D cranial US has potential as valuable tool; however, a future study with a larger cohort would be necessary before guidelines for intervention could be suggested for the clinical management of PHVD.

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Chapter 5

This chapter will discuss the ability of three-dimensional ultrasound based ventricle volume measurements of the cerebral ventricles of preterm neonates to predict whether a neonate will receive a ventricle tap.

The contents of this chapter have been submitted to the journal Scientific Reports in January 2017: J. Kishimoto, A. Fenster, D.S.C. Lee, S. de Ribaupierre. Quantitative 3D head ultrasound measurements of ventricle volume to determine thresholds for preterm neonates requiring interventional therapies following PHVD.

5 Quantitative 3D head ultrasound measurements of ventricle volume to determine thresholds for preterm neonates requiring interventional therapies following post hemorrhagic ventricle dilatation: A single center study

5.1 Introduction

Despite advances in neonatal care and improved evidence-based perinatal management guidelines, preterm neonates are still at a high risk of morbidity. Intraventricular hemorrhage (IVH), bleeding inside the cerebral ventricles, has been decreasing in prevalence yet remains as a common morbidity among preterm born neonates.[1] The risk of long-term disabilities such as cerebral palsy and cognitive impairment increases significantly with the occurrence of post-hemorrhagic hydrocephalus (PHH), the abnormal enlargement of the ventricles, which typically occurs in 25-28% of IVH patients with severe bleeds (Grade III and IV by the Papile scale[2]).[3] Generally, PHH is thought to be caused by the blood clots and inflammatory reactions from blood breakdown products causing obstruction of the flow of CSF which normally flows from the ventricles where CSF is generated to the subarachnoid space where it is reabsorbed into the blood stream.

While the definitive treatment for PHH is the placement of a ventriculoperitoneal shunt (tubing between the ventricles and the abdomen), placement of the shunt is often delayed

by several weeks in order to allow for the blood clot to resolve, the CSF proteins to decrease (below 1000mg/dL) and the weight of the infant to increase (at minimum above 800g-1kg). However, during this delay in treatment, the increase in ventricle size, also known as post-hemorrhagic ventricle dilatation (PHVD), can cause dangerously increased intracranial pressure (ICP), which can limit blood flow to the brain. Any ischemia or decreased blood flow during this critical period can be detrimental to the developing brain and can lead to impairment later in life. Due to this risk, neonates who have rapidly progressing PHVD and some with persistent, slowly progressing PHVD will require temporary interventions to relieve the elevated ICP. These interventions include lumbar/ventricle puncture, external ventricle drainage, ventricular access devices (reservoirs), and ventriculosubgaleal shunts. There is no consensus of evidence as to which of these methods are the best for temporary CSF diversion.[4,5]

Currently, clinical experience, as well as daily head circumference and transfontanelle brain ultrasound (US), are used to determine when neonates receive temporary interventions to removed built up CSF.[6] However, it has been shown that head circumference does not accurately correlate with increases in clinical US measurements (Evan's ratio)[7] or with ventricular volume.[8] Additionally, clinical signs of increased ICP (apnea, bradycardia) are nonspecific in the preterm infant and could be due to other co-morbidities. Early detection of interventional necessity could potentially lead to better management of the condition, while early detection of resolution could provide comfort to the clinician and parents that intervention is unlikely to be necessary. Additionally, since many cases of PHVD resolve after a temporizing CSF diversion procedure and do not then require a shunt, we aimed to characterize an ultrasound-based metric to determine the resolution of PHVD versus continual, progressive ventricle dilatation leading to PHH, which may receive additional interventions.

Previous work with transfontanelle brain 3D US has been attempted in neonates.[9-13] While this imaging technique was shown to be feasible, gives similar measurements[14] and diagnosis[12,15] as 2D US, its clinical utility above and beyond 2D has not been established. Recent studies have had success acquiring 3D US images of neonatal ventricles using commercial systems;[9,10,12,14,15] however, these transducers are not part of

standard-of-care and must be bought separately. The expense of these systems may limit their use, especially in the developing world. A system that could be used in conjunction with a conventional clinical 2D transducer to generate full 3D images would allow centers without a 3D US capable machine to confidently acquire 3D ventricle volumes. As hydrocephalus is far from only an issue in the developed world with 6000 cases per year diagnosed in East Africa,[16] we developed a relatively low-cost 3D US system that can be modified to generate 3D US images from virtually any conventional 2D US system.[17] From these 3D images, we measure the volume of the infants' ventricles. This system has been validated both using test phantom ventricles of known volume[17] and *in vivo* against the volume of CSF withdrawn during a tap as well as against MRI.[18]

In this paper, we present for the first time a preliminary study used to examine whether a 3D US-based ventricle volume and ventricle volume change measurements allow us to prognosticate which infants with PHVD will receive a temporary intervention and who will have a spontaneous resolution of PHVD.

5.2 Methods

As part of a larger study, between April 2012 and May 2016, after an initial diagnosis of IVH[1] was made during standard screening head ultrasound exam, preterm neonatal patients were enrolled with informed parental consent. 3D US images and all experimental protocol were acquired in accordance with a protocol approved by the Research Ethics Board at the University of Western Ontario. Imaging was performed twice per week with 3-4 days between imaging sessions.

Interventions were performed based on clinical judgement, with the clinician using the head circumference growth over time, the increase in ventricular dilatation on the 2D US, as well as sometimes, but not necessarily, clinical signs and symptoms of increased intracranial pressure such as apnea, bradycardia, and a full, tense fontanelle. Interventions were not based on 3D US images collected during the study, as the clinical team was blinded to all 3D US images during the course of the stay in the neonatal intensive care unit (NICU). The initial intervention at our center is a ventricular tap (VT) since it can be done at the bedside and doesn't require to go to the operating room (whereas the

Ommaya catheter insertion would); but if taps are thought to be needed frequently, then an external ventricle drain is usually inserted. For the purpose of this study, only patients born <32 weeks gestational age (GA) were included in the analysis.

5.2.1 3D US image acquisition

Images were acquired using a Philips HDI 5000 US machine with a C8-5 transducer. 3D US imaging was performed after placing the ultrasound transducer in a hand-held motorized housing that tilted the transducer about the axis at the probe tip for 8-9 sec.[2]

During image acquisition, 2D US images were collected on a laptop computer as the motor tilted the transducer over 9.6 sec and in-house software reconstructed the 3D US image in real time as images were received into the computer.[3] Technical details can be found in Kishimoto *et al.* (2013).[17] This system has been validated both for geometric fidelity and volume measurements.[17]

5.2.2 3D US image segmentation

The lateral ventricles were manually segmented in parallel sagittal slices with 1 mm gaps between adjacent slices. A trained observer (JK) segmented all the images and a paediatric neurosurgeon (SdR) verified the boundaries after the patient had left the NICU. Each image required between 20-45 minutes to segment. The 3D US imaging system and ventricle segmentation have been previously validated using phantom and patient images.[2] An example of segmented ventricles from a patient with PHVD following IVH, is shown in Figure 5-1.

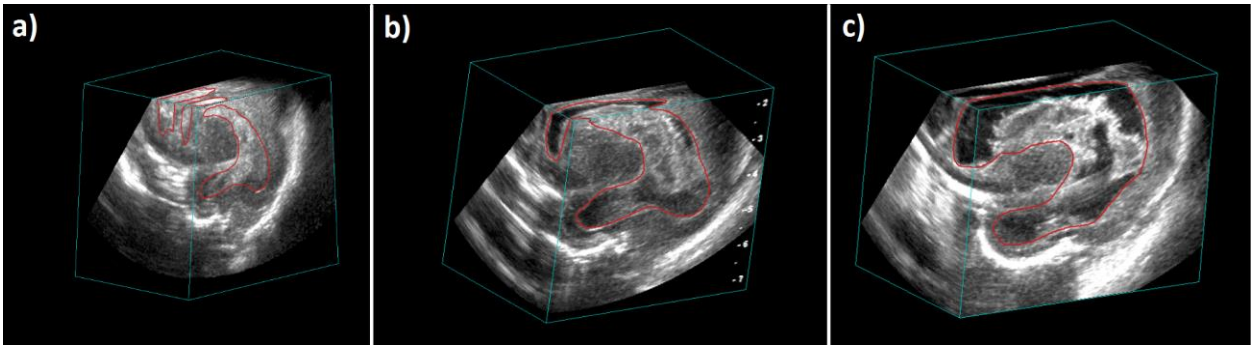

Figure 5-1 3D US images of a pre-term neonate with PHVD over the first three weeks of life. This patient required multiple VT to treat the rapid increase in ventricle size. a) week one, b) week two, and c) week three. Ventricles have been segmented as indicated by the red outline.

5.2.3 Determining which neonates with PHVD receive an initial ventricle tap

We performed the analysis between patients who did and did not receive interventional therapies for PHVD. The maximum ventricle volume (VV) for each scan was recorded during the first month of life for each patient as well as whether or not they eventually received a VT.

5.2.3.1 Volume measurement as a predictive marker

The ventricle volume for age ranges <7, 8-11, 12-15, 16-19, 20-22, 23-27 days of life was calculated and recorded for each patient. Volume measurements were not always attainable for every patient at each time point, as some enrolled late, or were too unstable to be scanned that day. Given the study population leaned towards extremely preterm neonates with at minimum an IVH and often with other morbidities related to prematurity, instability following minimal handling (such as during a head US) was more common in this population than it would be for most NICU patients. For some patients, two measurements were made within the time periods (i.e., days 8 and 11) and both were used as independent points.

5.2.3.2 Maximum rate of change of ventricle volume

We calculated the maximum rate of change of ventricle volume between 2 scans, and analyzed it for the first week (day <8), second week (day 8-14) and third week (day 15-21) of life. The neonatal 'week of life' was categorized depending on the week of the second scan (ie: if scans were obtained on days 3 and 7 of life, this would be considered 'first week of life' but if scans were obtained on days 6 and 10 of life, this would be 'second week of life').

5.2.4 Determining which neonates with PHVD will receive follow-up interventions

Since most patients with PHVD who have an initial VT will go on to have multiple VTs during the course of the stay in the NICU, we investigated whether or not imaging based measurements could detect the requirement for further treatments. Thus, we analyzed ventricle volume as well as ventricle volume change using the images obtained closest to the initial VT but not on the day of, and within the week after the VT.

5.2.5 Data Analysis

Receiver operator curves (ROC) were generated between patients who did and did not receive interventions using 3D US based ventricle volumes. In the second study in patients who had previous interventions, ROC analysis was performed using 3D US-based ventricle volumes, between patients who did not receive further interventions, and those that received at least one additional intervention. For each parameter (volume and volume change), optimal threshold for intervention was estimated through the highest product of sensitivity and specificity. Additionally, the area under the ROC curve (AUC), sensitivity and specificity were calculated. Assuming a Bonferroni correction, two sided *t*-tests were performed between all previously described compared groups of patients with an $\alpha=0.05$. All analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

5.3 Results

5.3.1 Patient Characteristics

Thirty-eight neonates were enrolled prior to the second week of life (before most patients with PHVD would receive an intervention) and were used to determine thresholds in this study. Of those, 14 patients received at least one VT. Mean age of enrolment was 9 days of life, and 12 patients were enrolled in the first week of life (7 did not receive interventions, 5 had at least a single VT). Descriptions of the all study patients are shown in Table 5-1.

Table 5-1 - Patient characteristics for those who did and did not receive interventional therapies

	Intervention (N = 14)	No Intervention (N =24)
Gestational Age (weeks ± SD)	27.2 ± 2.3	27.3 ± 2.7
Birth Weight (g ± SD)	1060 ± 300	1040 ± 490
Sex (M/F)	10/4	16/8
IVH Grade[3] (I/II/III/IV)	0/1/7/6	3/13/6/2

5.3.2 Determining which neonates with PHVD require an initial ventricle tap.

5.3.2.1 Volume measurements as predictive marker

Table 5-2 shows the threshold in ventricle volume used to determine which patients did or did not receive a VT intervention for PHVD for a given patient's age in days. Sensitivity and specificity for the threshold used as well as area under the ROC curve (AUC) is also given in Table 5-2. Figure 5-2 shows the VV in neonates with PHVD during the different time periods previously specified as well as the calculated threshold from ROC analysis. In general, through the use of VV we found the thresholds that characterized patients that received interventional therapy with high sensitivity (100-91.7%) and high specificity (100-92.9%) during the first three weeks of life (Table 5-2). The most ambiguity came during the second week of life (Table 5-2, 8-11 and 12-16

days) when a portion of the patients who did not receive interventions had transient increases in ventricle size, reflected in a small increase in mean volume from 7 to 8 cm^3. After this time, those patients who did not receive interventions and who transiently saw small increases in VV had VV which decreased or stabilized with the average volume remaining at about 7 cm^3. The patients who had interventions saw a continued increase in VV at every time interval and this can be seen in Fig. 5-2. The group differences between patients who did and did not receive interventions were reflected in the increase in the threshold between the two groups, as well as the increase in sensitivity and specificity after day 17 (Table 5-2).

Table 5-2 - Optimal sensitivity and specificity using the maximum single measurement of 3D US-based VV, area under the ROC curve (AUC), and volume threshold obtained from ROC curve specificity/sensitivity maximum. The number of patients for each time interval is indicated as recruitment often happened after the first week of life, and not all patients were stable enough to image at every time interval.

Age of Patient	Interventional patients (N)	No Interventions (N)	Sensitivity (%)	Specificity (%)	AUC	Threshold used (cm^3)
<7 days	7	8	100	100	1	> 9.4
8-11 days	14	12	91.7	100	0.98	> 10.6
12-16 days	14	22	100	92.9	0.99	> 20.3
17-20 days	8	13	100	100	1	> 20.4
21-27 days	5	17	100	100	1	> 20.9

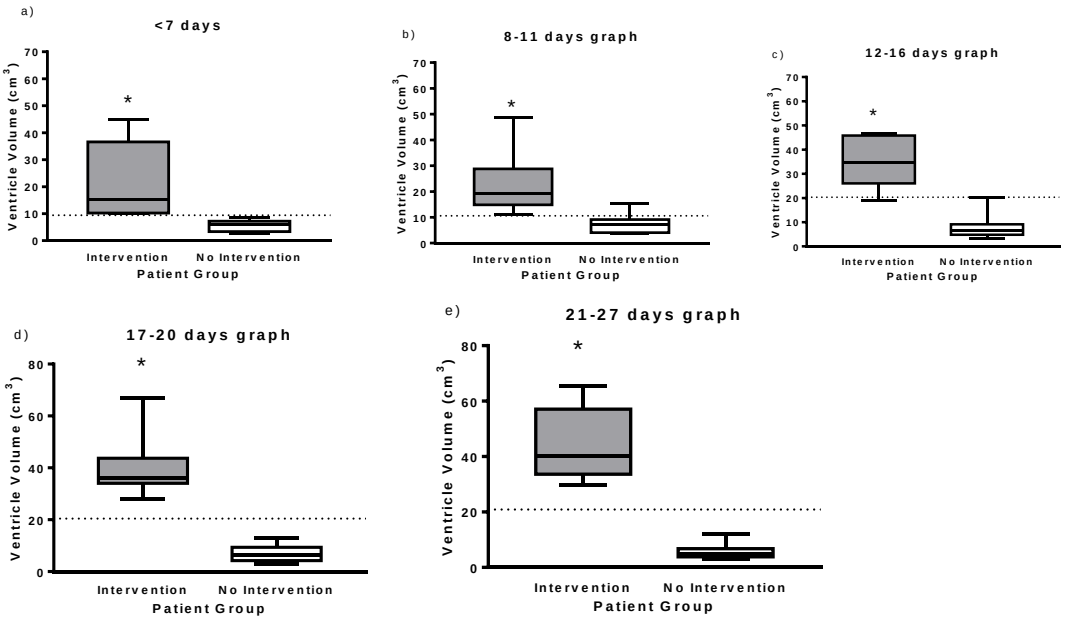

Figure 5-2 Box and whisker plots of the ventricle volume of neonatal patients who received an interventional ventricle tap for clinical reasons (Intervention), compared to those who did not require intervention (No Intervention).

Images obtained at a) 7 b) 8-11 c) 12-16 d) 17-20 and e) 21-27 days of life for patients born <32 weeks gestational age. Threshold from ROC analysis is marked as a dotted line. Sig. differences from *t*-test are indicated as * over the graph.

5.3.2.2 Maximum rate of change of ventricle volume as a predictive marker

Table 5-3 shows the threshold in the rate of change ventricle volume to determine, which patients did or did not receive a temporary intervention for PHH for different age ranges in days. Sensitivity and specificity for the threshold used as well as the area under the ROC curve (AUC) are also given in Table 5-3. Figure 5-3 shows the rate of change in ventricle volume in neonates with PHVD between those who did and did not receive interventions during the different age ranges previously specified. Additionally, the threshold calculated in ROC analysis is indicated in Fig. 5-3 for ease of comparison between time periods.

The rate of change in ventricle volume was highly sensitive for detecting which patients received interventions (100% sensitivity) during all age intervals monitored (Table 5-3). The ROC in VV additionally allowed for more specific thresholds (100% specificity) to be generated for patients who received interventional therapy in comparison to a single measurement of VV for all age ranges except for those of <11 days, which had a sensitivity of 75% (Table 5-3). From day 7 to 11, some patients who did not receive intervention had a transient period of ventricle dilatation that appeared to resolve by the second week of life when the rate of change stopped increasing or increased by a small amount (Figure 5-3). The threshold for 7 days of life had a very low N (5 patients total), which should be taken into consideration as this might not be representative of the total patient group studied (Table 5-3).

Table 5-3 - Optimal sensitivity and specificity using the maximum rate of change in the first three weeks of life in 3D US VV is reported along with area under the curve (AUC) from ROC curve, and threshold is reported from ROC curve specificity/sensitivity maximum. The number of patients for each time point is indicated as recruitment often happened after the first week of life, or patients only had a single VV recorded during their time in the study.

Age of Patient	Interventional patients (N)	No Interventions (N)	Sensitivity (%)	Specificity (%)	AUC	Threshold used (cm^3/day)
<7 days	2	3	100	100	1	> 0.25
<11 days	8	9	100	75	0.82	> 1.35
2 weeks	10	18	100	100	1	> 2.20
3 weeks	10	19	100	100	1	> 2.20

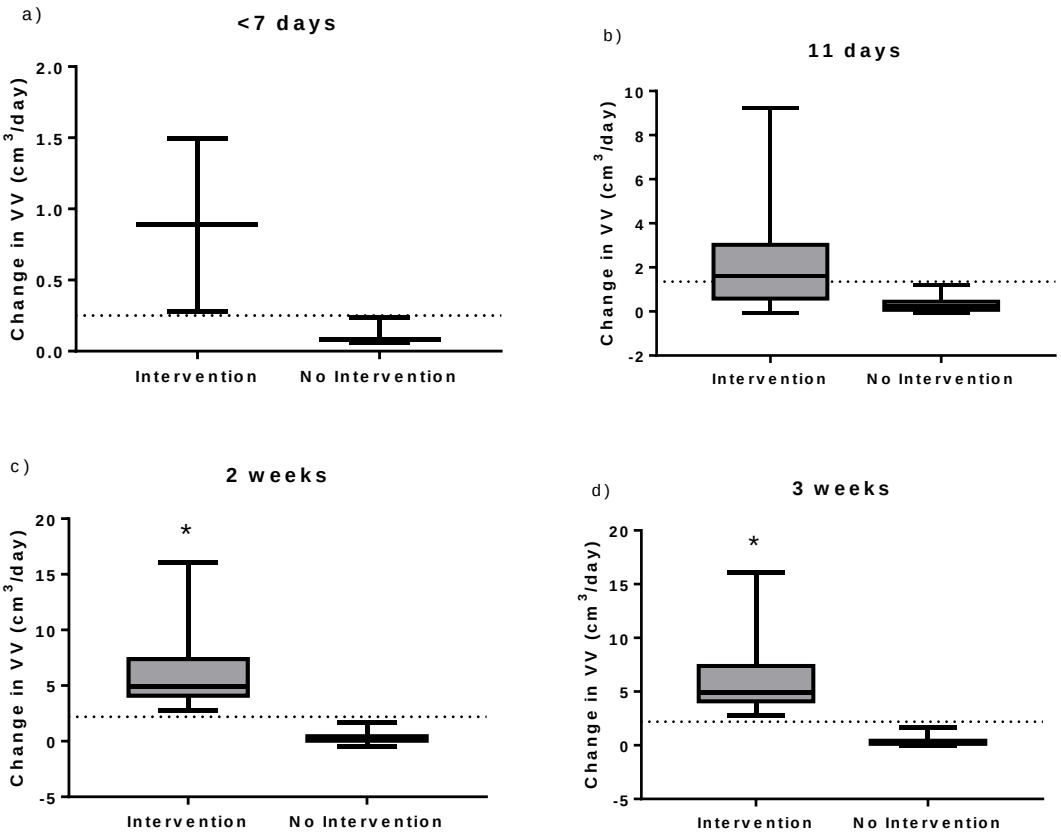

Figure 5-3 - Box and whisker plot of the change in ventricle volume between consecutive imaging sessions for preterm neonates born <32 weeks gestational age who required an interventional ventricle tap for clinical reasons (Intervention), compared to those who did not require intervention (No Intervention). Scans taken at a) 1 week of life b) 11 days of life c) second week of life and d) third week of life. Threshold from ROC analysis is marked as a dotted line. Sig. differences from *t*-test are indicated as * over the graph.

5.3.3 Determining which neonates with PHVD will receive follow-up interventions

For this study, we examined only the patients who already had a previous VT. Of the 11 patients with a previous VT, 4 resolved clinically without further interventions during their NICU stay. Table 5-4 shows 3D US-based thresholds between patients who received at least one additional VT, and patients who had PHVD, which resolved after a single VT. Figure 5-4 shows the 3D US-based VV (left) as well as the change in VV

(right) between neonates who previously had VT who received repeated VT and those who had resolving PHVD. Ventricle volume appeared to remain high in 3D US images acquired after a first intervention, and there was no clear threshold between those patients who received further interventional management. (Figure 5-4, left). Indeed, there was no sig. difference between the average VV for patients who received additional VT (48.8 ±12.5 cm^3 stdev) and patients who were undergoing resolution of PHVD (45.1 ± 16.1 cm^3 stdev). However, when the patient was monitored for an additional imaging session and the change of volume could be calculated, it was clear that patients who did not receive further VT had a decrease in ventricle size (Figure 5-4, right). The threshold for change in ventricle volume (<0.31 cm^3/day) was able to characterize patients who were resolving vs those receiving further monitoring with a sensitivity and specificity of 100% (Table 5-4).

Table 5-4 Optimal sensitivity and specificity using the maximum ventricle volume (VV) and maximum rate of change in ventricle volume (ΔVV) in the week following initial VT from 3D US is reported along with area under the curve (AUC) from ROC curve, and threshold is reported from ROC curve specificity/sensitivity maximum.

Measurement	Sensitivity (%)	Specificity (%)	AUC	Threshold used
VV	75	71.4	0.61	< 45.45 cm^3
ΔVV	100	100	1	<0.31 cm^3/day

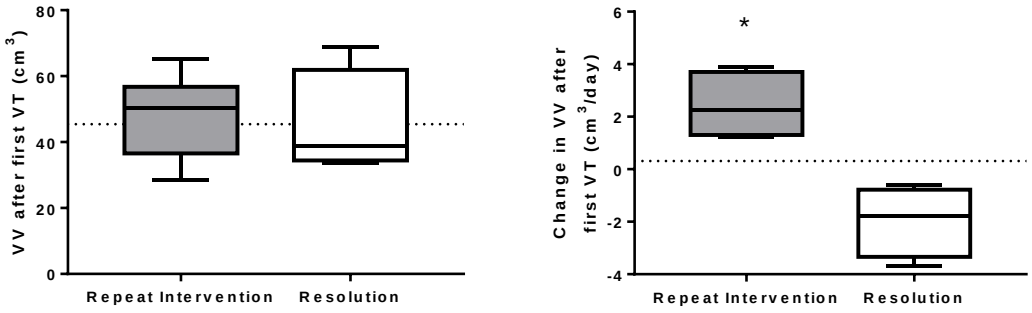

Figure 5-4 - Box and Whisker plot of the ventricle volume in the imaging session immediately after VT (left) as well as the change in ventricle volume in consecutive imaging session after VT (right) for patients who required either multiple interventions, or had resolving ventricle dilatation after a single intervention. Threshold from ROC analysis is marked as a dotted line. Sig. differences from *t*-test are indicated as * over the graph.

5.4 Discussion

In our preliminary study, we presented the potential for 3D US-based measurements of ventricle volumes from neonatal brain images to characterize whether or not patients with PHVD will receive interventions. Specifically, neonates who go on to receive interventions have larger ventricle volumes, (mean 21 ± 14 cm^3 for patients who received interventions, vs. 5.6 ± 2.1 cm^3 for patients who did not receive interventions during the first week of life (Figure 5-1a). The patients who received interventions tend to have larger increases in VV change over time (Figure 5-2).

3D US-based VV acutely remains very high in the week following an intervention regardless of whether or not the patient is undergoing resolution of PHVD (interventional patients had VV of 48.8 ±12.5 cm^3 (mean±stdev) whereas patients undergoing resolution were 45.1 ± 16.1 cm^3 (mean±stdev)). However, neonates who have PHVD that is resolving do show a *change* in VV (-1.9±1.3 cm^3/day, mean±stdev) from one 3D imaging session to the next that is indicative of a reduction in VV or no change from previous image even in the week immediately after an intervention. This is in comparison to the

increasing change in VV shown in patients that receive additional VT (2.4±1.1 cm^3/day, mean±stdev). Though calculating the change in VV requires multiple imaging sessions, this may prove to be a better indicator for when to schedule therapies to drain CSF than using VV alone. IVH patients receiving interventions, and certainly those receiving repeated VTs tend to have serial US images performed as part of clinical care. Furthermore, given the presented data, there is a high likelihood that even immediately after an initial VT even if the ventricles are stable or resolving, the VV could very well be above a threshold VV. This could be due to how much CSF can be drained at any given VT. Removing larger amounts of CSF occasionally results in infants transiently appearing ashen, and in some cases the drop in blood pressure, heart rate, and oxygen saturation is large enough that the patients require resuscitation after the procedure. Knowing how much the VV has increased following a tap might help better inform how much CSF to drain during subsequent interventions, in order to mediate adverse effects. Currently, it is not known how much CSF is optimal to drain and practices vary greatly between neurosurgeons; however, it is known that too little drained (<10 ml per kg of body weight) has no effect on ventricle size or ICP.[19]

Previous mathematical estimates of VV based on 2D US images found that there was a significant difference between patients who received interventions and those who did not.[20] This model received 2D US images spaced at regular angular intervals and a calculation involving solving an integral using cylindrical coordinates.[21] While fairly simplistic to solve for those with a background in calculus and geometry, this is a non-trivial level of knowledge to expect any given user to have. They found very good classification for patients who received treatment at 21 days of life using 30mL VV or greater as a threshold, whereas we found a lower threshold of 20.9 cm^3 at 21-27 days (Table 5-2). Brann et al additionally found the change in VV was more predictive than VV alone with 100% sensitivity and specificity in both retrospective and prospective analysis,[20] which concurs with the results of the present study. Admittedly, change in VV is a more challenging metric as it requires multiple 3D images. Additionally, it should be noted that although this previous work was performed in the late 1980s, these criteria never made it into clinical practice. Perhaps the relative difficulty in generating this measurement was the reasons this method did not translate into general clinical practice.

Perhaps there is less user error in the acquisition of a 3D US image than attempting to acquire 2D US images at equal angular spacing, and therefore, a 3D US approach is more realistic to translate into practice.

Neonatal patients in the NICU are also at risk of having prenatal ventriculomegaly and this finding could be indicative of abnormalities such as neural tube defects or trisomy, which require follow up after birth. For these neonates with larger than normal ventricles, the rate of change in VV might be a better indicator of which patients have progressive VD and not just large ventricles which are stable in volume and are likely not to contribute to signs and symptoms of increases in ICP. Therefore, while our study did not include patients with prenatal ventriculomegaly, 3D US VV should be examined in a cohort of patients with this indication, as it could impact how these patients' care is managed.

The relative difficulty of generating VV through manual contouring a 3D US image might prove too difficult for translation into practice and this is a limitation of the current work. In light of this, work in our lab has been performed to speed up the segmentation of ventricles from the 3D US images. Both a semi-automated segmentation algorithm that can be used online at the bedside and an automated segmentation algorithm that can be run offline with no user input have been developed. [22,23] Further research is required to determine best practices in the implementation of these new methods; however, the semi-automated segmentation has been integrated into our acquisition software and is ongoing a pilot study to test and improve upon the software.

Additional limitations of this work include the use of VT as the initial intervention and not using 2D ultrasound linear measurements to determine interventional necessity. There is a wide variation between centers as far as what intervention is performed with lumbar puncture, ventricular taps, ventricular reservoirs, third ventriculostomy, subgaleal shunts and/or external ventricle drains all being used. Further confounding the use of image based metrics, 3D US might be easier or harder to obtain depending on the ease of access to the fontanelle after different types of treatments have been performed. Furthermore, the criteria for intervention in this study is based highly on clinical judgment with

multiple clinicians deciding on whether or not to tap; and not on quantitative measurements, which could hinder the reproducibility depending on the degree of clinical variation in practice. These limitations and questions of the validity of 3D US based VV in this patient population can only be elucidated in a larger study with more variation in clinical practice accounted for.

As such, we have presented a potential clinical use for 3D US within the NICU. This type of imaging appears to be promising to be able to help generate management strategies for neonates at risk of PHVD and the subsequent potential brain damage from elevated ICP. A larger, multicenter study is required to generate guidelines as this will increase the patient pool and better account for differences in clinical practice that occur in different centers.

5.5 Acknowledgements

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Chapter 6

In this chapter, we discuss the potential for two-dimensional ultrasound based estimates of cerebral ventricle volumes to three-dimensional ultrasound estimates. In addition, the chapter discusses the differences between two-dimensional and three-dimensional ultrasound based ventricle volume estimates to predict interventions in neonates with post hemorrhagic ventricle dilatation.

The contents of this chapter are in preparation to be submitted to the journal Acte Paediatrica in June 2017: J. Kishimoto, A. Fenster, D.S.C. Lee, S. de Ribaupierre. Estimating ventricle volume from lineal ultrasound measurements in neonates with post hemorrhagic ventricle dilatation: benefits and limitations.

6 Estimating ventricle volume from lineal ultrasound measurements in neonates with post hemorrhagic ventricle dilatation: benefits and limitations

6.1 Introduction

While intraventricular hemorrhage (IVH), has been decreasing in prevalence in infants, this type of hemorrhage remains common among preterm born neonates.[1] Posthemorrhagic ventricle dilatation (PHVD) due to IVH often requires treatment and has a complicated management course with highly variable practices.

The definitive treatment for severe, unresolved PHVD is the placement of a ventriculoperitoneal (VP) shunt, but its placement is often delayed several weeks to allow for the neonate to grow. VP shunt placement in these neonates, especially placement that requires revision surgery, is a well-known marker of poor outcomes.[2] Currently, a clinical exam to test for signs and symptoms of increased intracranial pressure, as well as daily head circumference and transfontanelle brain ultrasound (US) are used in order to diagnose and manage PHVD.[3] Several ultrasound-based measurements have been proposed to quantify ventricle dilatation, with the most commonly used being ventricle index, anterior horn width, and thalamo-occipital distance.[3,4]

Previous work with transfontanelle brain 3D US has been attempted in neonates.[5-9] While this imaging technique gives similar measurements[10] and diagnosis[8,11] as 2D US, its clinical utility above and beyond 2D has not been established. Recent studies have had success acquiring 3D US images of neonatal ventricles using commercial systems;[5,6,8,10-12] however, these transducers are not part of standard-of-care and must be purchased separately. Previously, we have developed a relatively low-cost 3D US system that can be modified to generate 3D US images from virtually any conventional 2D US system.[13] This system has been validated using test phantom ventricles of known volume[13] and *in vivo* against the volume of CSF withdrawn during a tap as well as against MRI.[14]

Previously, studies have been performed with small numbers of neonates to evaluate the relationship between ventricle volume (VV) and ventricle linear measurements. Comparison between MRI and ultrasound has found a strong correlation between linear measurements and ventricle volume (R^2= 0.37-0.88); however, even stronger correlations were found in the same study when combining linear measurements (R^2 = 0.94).[15] 3D US to 2D linear measurements have been investigated in two independent studies and were found to have correlations of 0.70-0.69 for anterior horn width, 0.65-0.62 for ventricle index and 0.72-0.44 for the thalamo-occipital distance.[12,16] In one of the previously mentioned studies, an equation was proposed to accurately estimate ventricle volume using a significant multilevel mixed-effects linear regression model; however, this was developed using a small patient set (N=7).[12]

In this paper, we test the equation proposed in Benavente-Fernandez et al.[12] against an independent data set of 2D/3D US pairs, and in addition, we investigate the ability of other combinations of 2D US measurements to predict 3D US ventricle volume measurements. We also test the ability of the proposed equation calculated VV to detect whether a neonate received an intervention in a retrospective sample of 42 patient images.

6.2 Methods

After an initial diagnosis of IVH[1] was made during a standard screening head ultrasound exam, preterm neonatal patients were enrolled with informed parental consent. As part of

a larger study investigating novel techniques (3D US, fMRI, and near infrared spectroscopy) 2D and 3D US images were acquired and all experimental protocols were performed in accordance with a protocol approved by the Research Ethics Board at the University of Western Ontario. The clinical team was blinded to all 3D US images during the course of the patients' stay in the neonatal intensive care unit (NICU). The patients in this study were enrolled between April 2012 and February 2015. Imaging was performed 1-2 times per week for the duration of the stay in the NICU until transfer to a secondary care center or discharge from the unit.

6.2.1 Two-dimensional ultrasound

Patients were imaged by a trained ultrasound technician using a standard 2D US technique, acquiring images in coronal and sagittal planes through the anterior fontanelle, with a few screen captures chosen by the technician over the relevant landmarks as per the standardized exam.[17] Imaging was performed at the bedside in the NICU using an HDI 5000 US system (Philips, Bothel WA) and a C8-5 curved array 5-8 MHz broadband transducer (Philips, Bothel WA). The 2D US exams required approximately 10-15 minutes.

6.2.2 Two-dimensional ultrasound analysis

The ventricle index (VI) and anterior horn width (AHW) for the left and right lateral ventricles and the thalamo-occipital distance (TOD) were recorded for all 2D US exams in the study. Measurements have been previously described in Davies *et al.*[4] While two different radiologists made measurements in this study, these observers have previously shown agreement with intraclass correlation on neonatal head US measurements (ICC = 0.96-0.99 for VI, AHW and TOD).[18]

Given the improved correlations previously found by combining multiple linear measurements in both Horsch[15] and Benavente-Fernandez[12], we combined linear measurements as described in Table 1. The equation provided by Benavente-Fernandez to generate VV estimates from these linear 2D US measurements is:

Equation 6-1

$$VV = -11.02 + 0.668 \times VI + 0.817 \times AHW + 0.256 \times TOD$$

where linear measurements are in mm, VV is in cm^3 and this is for a single lateral ventricle.[12]

Table 6-1 - Abbreviations for combined linear 2D US measurements

Abbreviation	Combination of measurements
VI*AHW	the product of the ventricle index and the ipsilateral anterior horn width
VI*TOD	the product of the ventricle index and the ipsilateral thalamo-occipital distance
AHW*TOD	the product of the anterior horn width and the ipsilateral thalamo-occipital distance
VI*AHW*TOD	the product of the ventricle index, anterior horn width, and the thalamo-occipital distance

6.2.3 Three-dimensional ultrasound image acquisition

3D US imaging was performed after placing the ultrasound transducer in a hand-held motorized housing that tilted the transducer about the axis at the probe tip for 8-10 sec.[2] Motorized transducer housing and the clinical transducer is shown in Figure 6-1.

Figure 6-1 - Clinical 2D US transducer within the hand-held motorized transducer housing simulating a scan of a preterm sized mannequin.

During image acquisition, 2D US images were acquired on a laptop computer as the motor tilted the transducer over 9.6 sec, and in-house software reconstructed the 3D US image in real-time as images were received into the computer.[3,19] The total image acquisition lasted between 3-10 minutes and was highly dependent on patient motion. Technical details can be found in Kishimoto *et al.* (2013).[13] This system has been validated both for geometric fidelity and volume measurements accuracy.[13]

6.2.4 Three-dimensional ultrasound image segmentation

The lateral ventricles were manually segmented offline by a trained observer in parallel sagittal slices with 1 mm gaps between adjacent slices. Each 3D ultrasound image required between 20-45 minutes to segment both lateral ventricles (10-25 minutes per ventricle) and the time depended on image quality and ventricle size. The 3D ultrasound imaging system and ventricle segmentation have been previously validated using phantom and patient images.[2] An example of segmented ventricles from a patient with PHVD following IVH is shown in Figure 6-2.

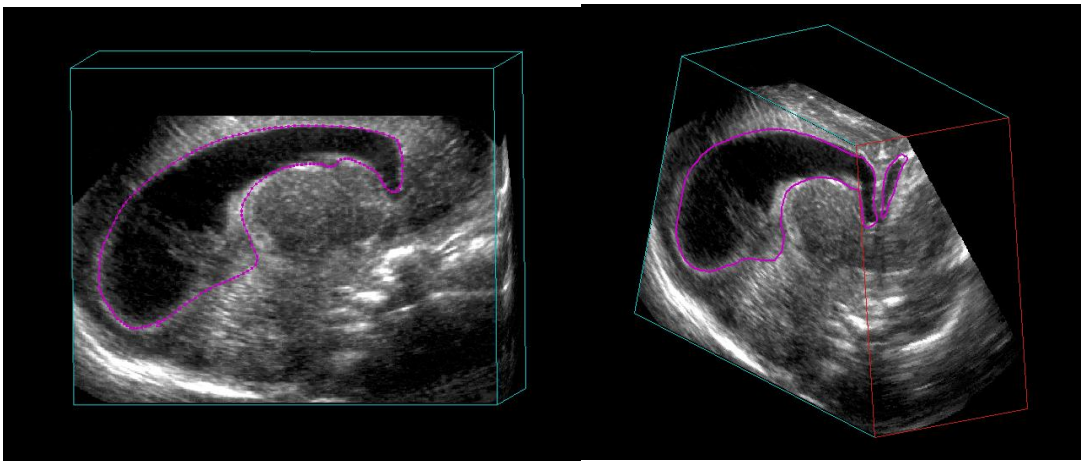

Figure 6-2 - 3D US images of a pre-term neonate with PHVD. Manually segmented contour (left) and segmented surface on 3D ultrasound image cut through oblique planes (right).

6.2.5 Data Analysis

For the purpose of this paper, ventricle volume (VV) will describe a single lateral ventricle, and total ventricle volume (TVV) will describe the sum of the left and right lateral ventricles. The rate of change in TVV (ΔTVV) was recorded between each imaging time point for 3D US and equation based TVV and will be measured in cm^3/day. The subscript $_{eq}$ will denote VV made from the equation, while the subscript $_{3DUS}$ will denote VV from 3D US images.

All analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA). Statistical analysis was performed using SPSS v.20 (IBM, Armonk, NY). Correlations for regressions were performed between each combination of 2D US parameter and VV_{eq}. Linear regression was used to test the proposed hypothesis that 2D US will be strongly correlated with 3D US VV. A regression of $R^2>0.7$ was considered strong. The significance of the regression was determined for an $\alpha < 0.05$. In addition to a Pearson correlation, a Bland-Altman plot was generated for VV_{eq} and VV_{3DUS}.

Receiver operator curves (ROC) were generated between patients who did and did not require interventions using the TVV_{eq}. In this study, for patients who had previous interventions, ROC analysis was performed using the TVV_{eq} and ΔTVV_{eq} between

patients who did not require further interventions, and those who required at least one additional intervention. For each parameter (TVV_{eq} and ΔTVV_{eq}) optimal threshold for intervention was estimated using the highest product of sensitivity and specificity. Additionally, the area under the ROC curve (AUC), sensitivity and specificity were calculated. Using a Bonferroni correction, two-sided *t*-tests were performed between all previously described compared groups of patients with an uncorrected $\alpha=0.05$.

6.3 Results

6.3.1 Patient Characteristics

Forty-two neonates with previously diagnosed IVH were enrolled in the study following informed parental consent. Descriptions of all study patients are shown in Table 6-2. A total of 265 image sets (2D and 3D US images acquired on the same day) were collected for this study. All required US measurements were available providing 263 2D/3D US exams, with 2 missing TOD due to poor image quality. Enrolled patients had serial US exams performed between 25 and 42 2/7 weeks corrected gestational age with the median gestational age at imaging of 30 3/7 weeks.

Table 6-2- Patient characteristics for neonates diagnosed with IVH and enrolled into the study

Patient Characteristic	N = 42
Gestational age at birth (weeks ± SD)	27.6 ± 3.0
Birth weight (g ± SD)	1105 ± 450
Sex (M/F)	30/12
Patent ductus arteriosus (%)	30 (68%)
antenatal steroids (%)	24 (37%)
IVH Grade[3] (I/II/III/IV)	7/17/10/8

6.3.2 Ventricle Size Measurements from 2D and 3D ultrasound

For ease of comparison to previous papers[12], VV will be discussed in this section. VV is compared to 2D US measurements made on the same ventricle (ie. right VI and right lateral ventricle VV are compared and left VI and left lateral ventricle VV are compared).

The median VI was 11.7 mm (range 5.5 - 39.1), the median AHW was 4.7 mm (range 1.1 - 35.4), and the median TOD was 21.4 mm (range 8.3 - 44.9). Ventricle volume (VV_{3DUS}) ranged between 1.1 - 55.3 cm^3 with a median of 4.6 cm^3. In general, the left lateral ventricles were larger than the right lateral ventricles with the average left VV_{3DUS} of 8.9 ± 9.9 cm^3 and the average right VV_{3DUS} of 7.7 ± 7.9 cm^3. Moderate correlations were found between lateral VV_{3DUS} and 2D US linear measurements as can be seen in Fig. 6-3 (R^2 = 0.43-0.67). Similar regressions were found between left and right sided measurements, so the reported regressions are the amalgamation of right and left ventricle measurements.

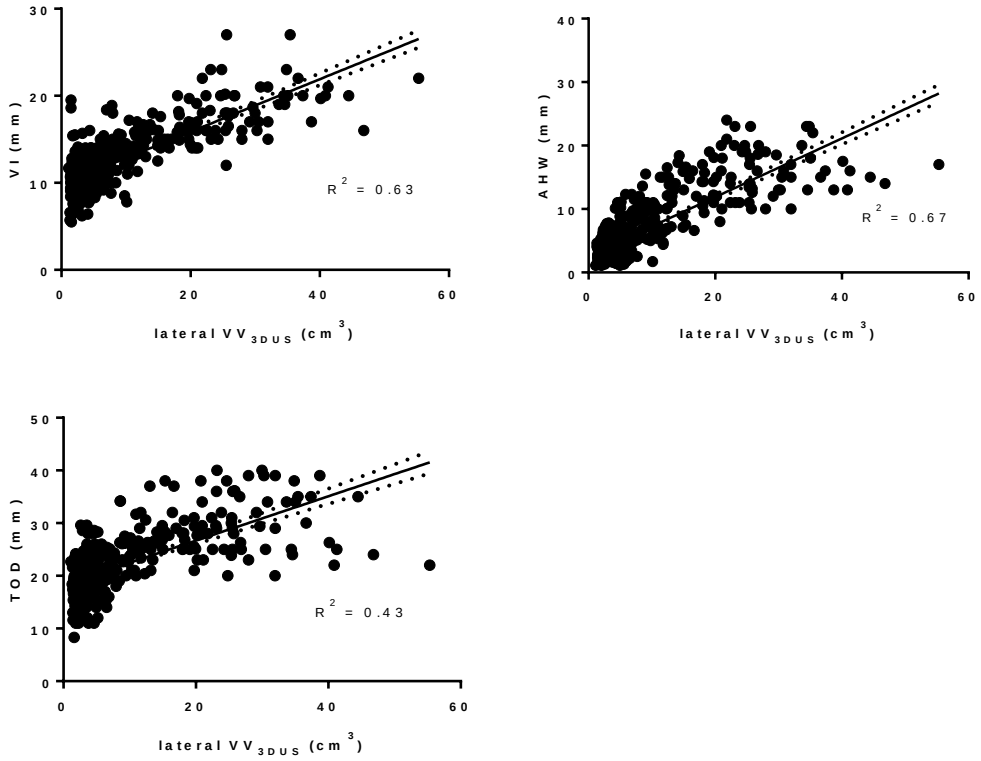

Figure 6-3 - Linear regression for 3D US generated VV and 2D US measurements when comparing ipsilateral 2D US measurements to left or right lateral ventricle volumes taken from 3D US.

6.3.3 Combining 2D US measurements to predict 3D US ventricle volume

Combined 2D US linear measurements described in Table 6-1 had strong-moderate correlations with VV_{3DUS} (R2=0.68-0.72) and are shown in Fig. 6-4. These correlations were all higher than using a single linear measurement, but were comparable to the highest performing single measurement, AHW (Fig. 6-3).

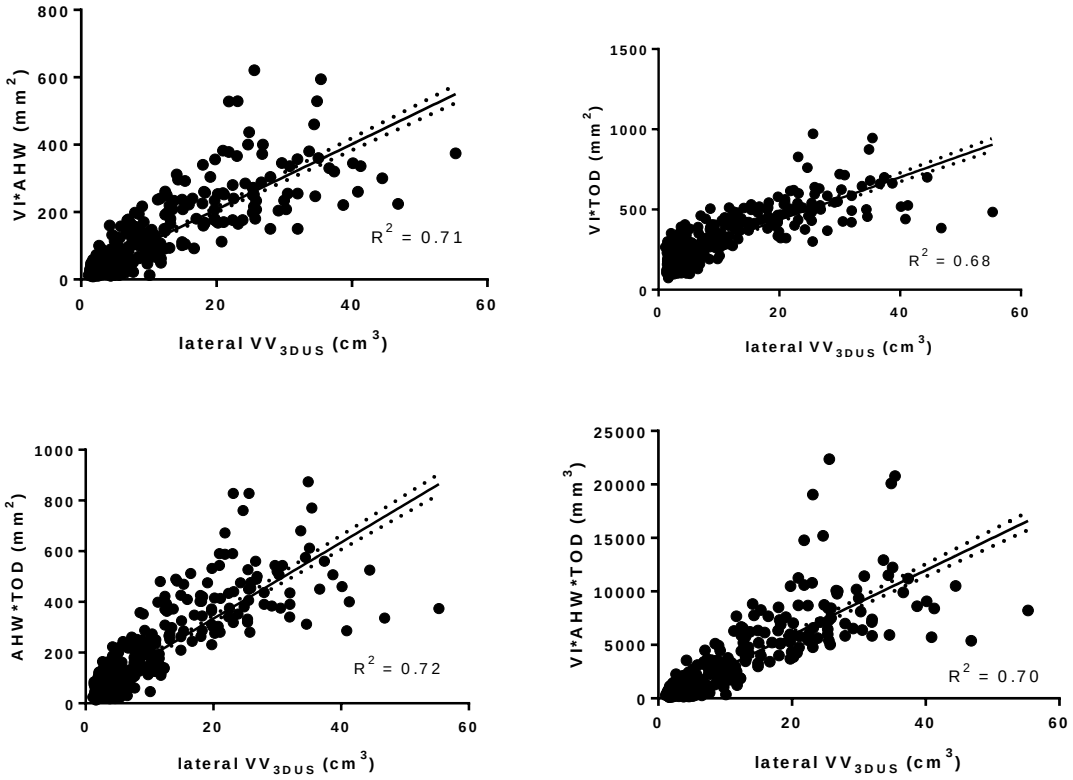

Figure 6-4 - Linear regression for 3D US generated VV and combined 2D US measurements when comparing ipsilateral 2D US measurements to left or right lateral ventricle volumes taken from 3D US.

6.3.4 Equation based VV estimates compared to 3D US VV

Strong correlations were found between the VV_{eq} and VV_{3DUS} (Fig. 6-5a) with R^2 = 0.71-0.74. There was some bias detected for larger values of VV, where the VV_{eq} was more likely to be outside the 95% CI (Fig. 6-5b).

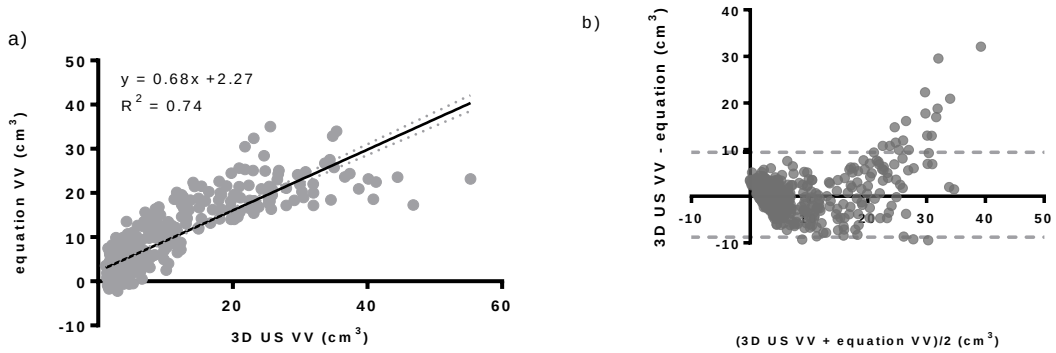

Figure 6-5 - Equation-based VV compared to 3D US based VV. a) Linear regressions comparing 2D US equation based lateral VV to 3D US lateral VV. b) Bland-Altman plot comparing equation based VV and 3D US based VV (dashed lines are the 95% confidence intervals).

6.3.5 Determining whether PHVD received interventions – retrospective study using equation-based ventricle volumes

Previous papers have examined this patient population and used thresholds for intervention based on total ventricle volume (TVV) (sum of left and right lateral ventricles), so for ease of comparison, the results in this section will be given in TVV.[20,21] Previously, we had shown that TVV from 3D US was predictive of both whether a neonate received an initial intervention and whether or not follow-up intervention was received with very high sensitivity and specificity.[21] Of the 42 neonates in the study, 7 had documented interventions.

6.3.5.1 Initial intervention requirement

For initial intervention, we examined the TVV of neonates with PHVD at the maximum TVV_{eq} during the first three weeks of life. Patients who received interventions had higher TVV_{eq} than those who did not receive interventions with no overlap in TVV_{eq} between the groups (Fig. 6-6a). There was a high sensitivity and specificity of 100% (Fig. 6-6a,b) using a threshold of 27.6 cm^3 to determine whether the patient was one who had received an intervention or not. The maximum ΔTVV_{eq} over this time period was slightly less specific (91.7%), but as sensitive (100%) when using a threshold of 2.14 cm^3/day (Fig. 6-6c,d).

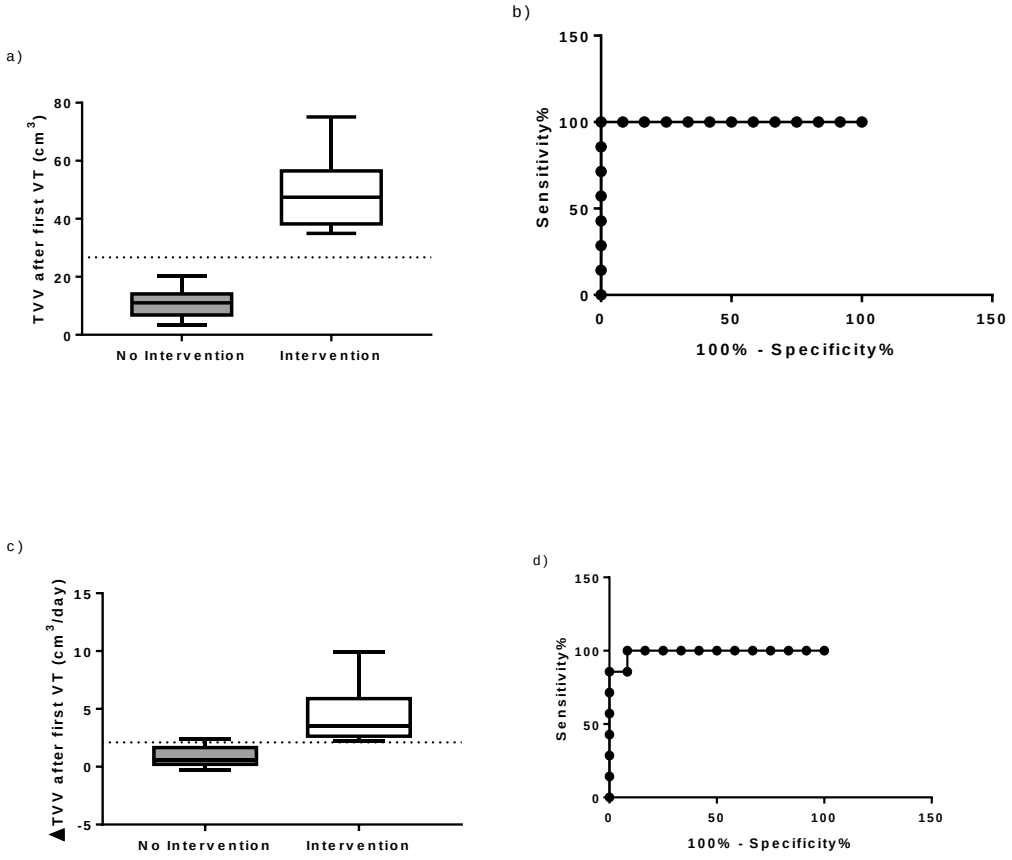

Figure 6-6 a) Box and whisker plot, and b) ROC curve describing equation based TVV to predict whether an initial intervention was received by a neonate with PHVD. c) Box and whisker plot, and d) ROC curve describing the equation-based rate of change in TVV to predict whether an initial intervention was received by a neonate with PHVD.

6.3.5.2 Serial Interventions received

Since we had previously observed that TVV_{3DUS} measurements were able to predict whether a neonate received serial interventions,[21] we investigated the utility of the TVV_{eq} to do the same.

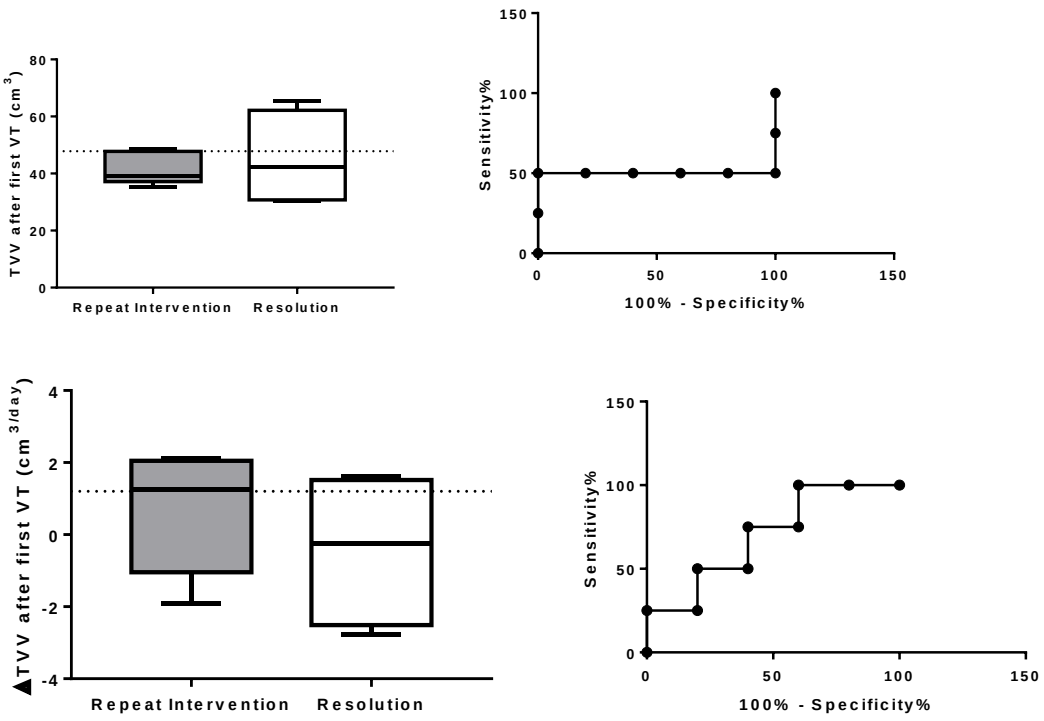

Figure 6-7 – a) Box and whisker plot, and b) ROC curve describing equation based TVV to predict whether a repeat intervention was received by a neonate with PHVD. c) Box and whisker plot, and d) ROC curve describing the equation-based rate of change in TVV to predict whether a repeat intervention was received by a neonate with PHVD.

We used the TVV_{eq} to predict interventional necessity in patients who previously had at least one VT and found that this measurement was not very sensitive, but had high specificity (50% sensitivity and 100% specificity) if a high enough threshold was used (Threshold 50.5 cm^3, Figure 6-7a). We also used the ΔTVV_{eq} to predict whether repeat interventions were received and also found that it was not very sensitive or specific (75% sensitivity and 60% specificity) (Threshold 1.2 cm^3/day, Figure 6-7c).

6.4 Discussion

We analyzed 263 2D/3D US image sets from 42 neonates with PHVD and found moderate to strong correlations between 2D US linear measurements and VV_{3DUS}. These correlations increased slightly when multiple 2D US measurements were included, which has been previously shown in 3D US[12] and MRI[15].

The novelty of this study was the use of TVV_{eq}[12] to predict whether or not a neonate with PHVD would receive an interventional VT. Using TVV_{eq}, we were able to retrospectively predict with very high sensitivity and specificity (100%), which patient would receive an initial intervention, which was similar to previously reported values we found using estimates of TVV_{3DUS} and a method of acquiring TVV estimates from regularly spaced 2D US (cylindrical coordinate method).[16,20] Furthermore, we used the TVV_{eq} to predict whether neonates with PHVD who previously had at least one intervention would receive additional interventions. Deciding when to perform additional interventions when ventricle size is already large is challenging, as even immediately post-intervention, the size of the ventricles can be above the threshold to intervene (such as when the VI 97+4mm mark is used).[3] We found that the TVV_{eq} was not very sensitive, but could be fairly specific if a high enough threshold was used (50% sensitivity and 100% specificity for a threshold 50.5 cm^3, Fig 6-7). In comparison, TVV_{3DUS} had 75% sensitivity and 71.4% specificity using 20.9 cm^3 as a threshold.[21] Comparisons between these three studies' thresholds can be found in Table 6-3.

Table 6-3 - Equation based thresholds, sensitivity, and specificity compared to previous literature for initial intervention and repeated interventions based on estimated ventricle volumes. Equation 6-1 (eq)[12], CC – cylindrical coordinates[20], 3D US based. CC TVV did not report specificity.

Intervention	Method	Threshold	Sensitivity (%)	Specificity (%)
Initial	TVV_{eq}	27.6 cm^3	100	100
	CC TVV	30 mL	90	
	TVV_{3DUS}	20.9 cm^3	100	100
Serial	TVV_{eq}	50.5 cm^3	50	100
	TVV_{3DUS}	45.5 cm^3	75	71

We also used the ΔTVV_{eq} to predict initial and serial interventional necessity. While in general, in all studies ΔTVV was comparable or slightly worse than using TVV to differentiate between neonates who received at least one intervention and those who never had an intervention, previously we had found ΔTVV_{3DUS} to be more predictive of serial interventions than TVV_{3DUS}.[21] We found that ΔTVV_{eq} was not very sensitive or specific to identify patients who received serial interventions and those who had resolution after a single intervention (75% sensitivity and 60% specificity, threshold 1.2cm^3/day, Fig. 6-7). Comparisons between the three studies measures of ΔTVV for initial and serial interventions can be found in Table 6-4.

Table 6-4 - equation based thresholds, sensitivity and specificity compared to previous literature for initial interventions and repeated intervention based on rate of change in ventricle volume. Equation 6-1 (eq)[12], CC – cylindrical coordinates[20], 3D US based.

Intervention	Method	Threshold	Sensitivity (%)	Specificity (%)
Initial	TVV_{eq}	2.14 cm^3/day	100	91.7
	CC TVV	0.55 mL/day	98	
	TVV_{3DUS}	2.2 cm^3/day	100	100
Serial	TVV_{eq}	1.2 $cm^{3/}$day	75	50
	TVV_{3DUS}	0.3 cm^3/day	100	100

Given the variability in linear measurements performed on 2D US, TVV_{eq}, as a product of 2D US measurements would have even higher variability than any one 2D US measurement. Perhaps these results are not surprising in that context. For example, in some patients who are undergoing ventricle dilatation, the TVV_{eq} will show a decline in TVV_{eq} from one day to the next where this is not seen in TVV_{3DUS}, or vice versa (See Fig. 6-8).

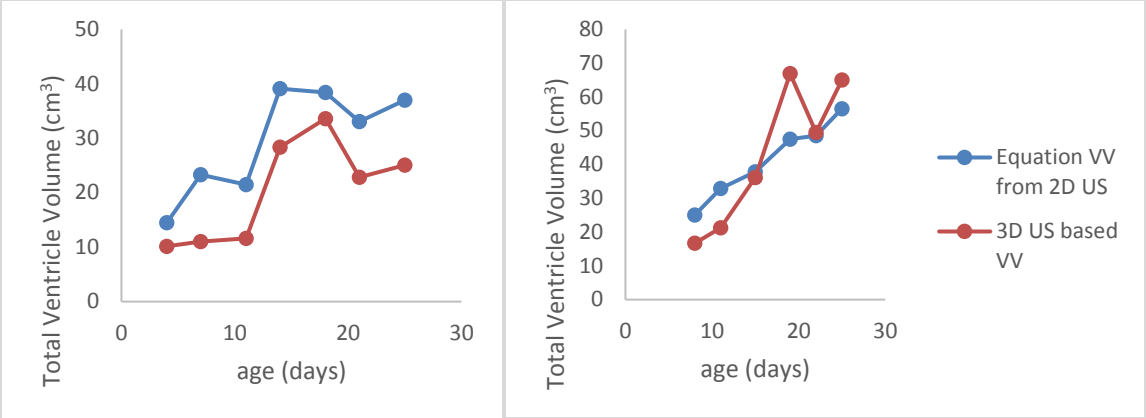

Figure 6-8 - Equation based (blue) and 3D US based TVV estimates in two patients who eventually required surgical intervention for PHVD. Both patients had bilateral grade III IVH and received multiple VT during their stay in the NICU.

While not performed in this study, determining how the inter- and intra-reader variability of 2D US measurements effects the TVV_{eq} variability is necessary to determine how much confidence a clinician can have in this type of measurement. The variance in VV_{eq} can be calculated analytically if the variance (var) and co-variance (covar) of the three 2D US measurements is known:

Equation 6-2:

$$Var_{VVeq} = var_{VI} + var_{AHW} + var_{TOD} + 2(covar_{VI,AHW} + covar_{VI,TOD} + covar_{AHW,TOD})$$

However, previously published studies of variance[4,22,23] in 2D US linear measurements have not published the covariance between the measurements, and this is required to determine the variance in VV_{eq} as it is known that the 2D US variables are not independent of one another (they are linearly correlated, as was described in Benavente-Fernandez et al.[12]). Our single center study only had two observers making one measurement per patient (vs repeated measurements), and as such is very limited in what kind of variance we can report. A study with more representation from different observers would be required to report the inter-observer variance in $VV_{eq.}$ In order to calculate intra-observer variance of VV_{eq}, a single observer would need to make multiple measurements on the same patient imaging sets (e.g. measure 20 patients 5 times each

with days in between each measurement), which was not performed in this study. Ideally, multiple observers would be involved to be able to delineate between inter- and intra-observer variance. We do not have enough expert observers (radiologists) involved in this project to accomplish this, which is a limitation of the current work.

Additional limitations of the present study include being a single center study with a small sample of intervention patients, although this study does reinforce results found in another completely independent single center study.[12] However, the work on using TVV to assist with management of interventional therapies for patients with PHVD will need to be repeated in a multi-center study before recommendations can be made to use TVV in clinical practice.

Another limitation of the study is the use of manual segmentations to generate VV_{3DUS} as these are very time consuming and can only be performed by a relatively skilled technician or clinician. While we have developed and validated semi-automated[24] and automated[25] techniques for segmenting neonatal ventricle volumes, this is just beginning to be implemented into the image acquisition system at our center and is not yet commercially available.

We have presented a potential clinical use for both 2D US and 3D US-based VV within the NICU while independently validating results from a previous study. In addition to the validation study, TVV_{eq} seems to be able to predict initial intervention in neonates with PHVD, and TVV_{3DUS} seems to be able to predict both initial and repeated interventions. This type of imaging appears to be promising to be able to help generate management strategies for neonates at risk of PHVD and the subsequent potential brain damage from elevated ICP. A larger, multicenter study is required to generate guidelines as this will increase the patient pool and better account for differences in clinical practice that occur in different centers.

6.5 Acknowledgements

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Chapter 7

The final chapter of this thesis reexamines the research objectives and provides a summary of findings for Chapters 2-6. The chapter addresses the limitations of the study, and potential solutions. Additionally, this chapter will illustrate some of the potential future directions for this work.

7 Conclusions and Future Directions

7.1 Overview of Rationale and Research Questions

Clinical 2D ultrasound remains a very important part of the diagnosis and management of IVH and resulting PHVD. While ultrasound is very good at detecting IVH and PHVD, there are ongoing questions regarding the type of treatment that is most effective and when to perform interventions. As such, clinical 2D US cannot differentiate between neonates with PHVD who will receive interventions versus those that will undergo spontaneous resolution of PHVD.

Accordingly, the overarching objective of this thesis was to develop a 3D US system to detect when a neonate will require interventional procedures and surgery with greater confidence than currently used clinical US techniques. Five objectives were focused on: 1) the development and *in vitro* validation of the 3D US system to measure the volume of the ventricles in neonates along with intra- and inter- observer variability measurements using patient images (**CHAPTER 2)**; 2) the *in vivo* validation of ventricle volume measurements from this 3D US system **(CHAPTER 3)**; 3) the comparison of 3D US based ventricle volumes to 2D US measurements of the lateral ventricles **(CHAPTER 4)**; 4) using retrospective 3D US based ventricle measurements of ventricle volume to predict interventional requirement and resolution **(CHAPTER 5)**; comparing 2D US based estimates of ventricle volume to 3D US VV and using 2D US based estimates of ventricle volumes predict interventional requirement and resolution **(CHAPTER 6)**.

7.2 Summary and Conclusions

In Chapter 2, we described the development and *in vitro* validation of a 3D US system using neonatal head phantom experiments. The system was found to be geometrically accurate in all three orthogonal directions based on comparison to known manufactured distances of 10 mm with no statistically significant difference found ($p>>0.5$, $\alpha=0.05$). Additionally, the 3D US-based volume measurement of a ventricle-like phantom (50 cm^3 and 290 cm^3) was compared to MRI and water displacement, and results failed to show a statistically significant difference when compared to the other two modalities using ANOVA ($p>> 0.3$, $\alpha=0.05$).

The minimum detectable difference (MDD) is a measure of how much ventricle volume must change in order to statistically determine whether a change as occurred above the level of measurement variance. An intra- and inter- observer study in three neonates found MDD to be 0.63 cm^3, much less than the previously determined 2 cm^3/day increase in VV seen in patients with PHVD who received interventions.[1] This validation study indicated that 3D US-based VV could be usable in a clinical setting to monitor patients with PHVD.

In Chapter 3, we validated the 3D US system further in *in vivo* experiments with 18 patients. The comparison between CSF drained by a ventricle tap and the difference in measured ventricle volume before and after the tap was strongly correlated ($R^2 = 0.92$). The comparison between 3D US and MRI showed very high correlation ($R^2 = 0.99$); however, 3D US ventricle volumes appeared to be lower than MRI. This latter finding was followed up by investigating whether certain regions of the ventricles were systematically not being segmented in US using a manual, point-=based 3D US to MRI registration. Our results showed that the mid-line ventricle structures in patients with severe ventricle dilatation are not visualized in 3D US, though further investigation is required. These findings suggest that direct MRI to 3D US ventricle volume comparison should not be made without accounting for image based bias, however, US to US ventricle volumes comparisons appear to be valid.

In Chapter 4, we compared 3D US ventricle volumes to linear measurements made using 2D US as previously described in other clinical papers[2-4] in 42 patients with IVH. Our results showed that there was a trend towards larger 2D US measurements reflecting larger 3D US VV (R^2 = 0.69-0.44). However, when examining changes in measurements from successive imaging sessions from the same patient, there was no strong correlation between changes in linear 2D measurements and changes in VV (R^2 = 0.13-0.02). 2D US measurements might not be accurately reflecting a change in ventricle size due to measurement error, plane selection error, and even that the ventricles are not expanding in the area being measured. These results are important, as PHVD is a progressive disease and serial US sessions are often acquired, with changes in qualitative and quantitative estimates of ventricle size impacting patient management. If the currently used imaging metrics are not sufficiently accurate to detect changes in ventricle size from day to day, then this would explain the lack of adoption of image-based thresholds for intervention.

In Chapter 5 we describe the results of a retrospective pilot study attempting to determine 3D US volume-based threshold for interventional ventricle tap in 38 neonates with IVH. 3D US VV was able to characterize patients who required at least one intervention with very high sensitivity and specificity (>90%). The change in VV was able to allow determination between whether a follow-up to intervention was required, or if the patient had at least intermittent resolution of PHVD with very high sensitivity and specificity, in fact, no patient in the study was misclassified using a threshold of 0.31 cm^3/day. These results are sufficient to suggest that a larger study is indicated to develop image-based guidelines for these patients.

In Chapter 6 we were able to independently verify a previously reported method to estimate ventricle volume using 2D US measurements.[5] We found strong correlations (R^2=0.74) between 2D US estimates of VV compared to 3D US VV. In addition to this, we used 2D US estimates of VV to predict whether a neonate with PHVD will receive an intervention, as this had not been performed in the previous study by Benavente-Fernandez *et al.*[5] Equation based VV was able to predict whether a neonate received an initial intervention using a threshold of 27.6 cm^3 with very high sensitivity and specificity (100%). The equation generated TVV estimates were not able to predict that additional

interventions would be received by neonates who had previously had one intervention as the highest sensitivity was 50% and required a very high threshold (50.5cm^3).

In summary, we have developed and validated a 3D US system for imaging the brains of preterm neonates, compared the system to currently used clinical measures of ventricle size, and performed a small clinical pilot study with promising results.

7.3 Limitations

In this section, some of the limitations of the studies presented in Chapters 2-6 are discussed. While the study specific limitations are presented in the discussion section of the respective Chapters, we will detail more general limitations common to Chapters 2-6 in this section.

7.3.1 General Limitations

The major limitation with this 3D US system is that it is still a fairly large peripheral addition to a 2D US unit. While large strides have been made to make the system have a smaller footprint, there is definitely room for improvement, for instance, by mounting it onto the 2D US system so that it is easier to maneuver in a tightly packed NICU environment.

Another limitation is the amount of brain that can be interrogated in a single scan. Since our maximum scan angle is 70 degrees, in patients with severe PHVD, the entire ventricle system cannot be captured in a single scan (Fig. 7-1). This limits segmentation algorithms, as atlas based initialization takes much longer when the ventricles could be in multiple images. Additionally, in a small number of extremely severe cases of PHVD, a 70-degree scan was insufficient to scan even a single lateral ventricle.

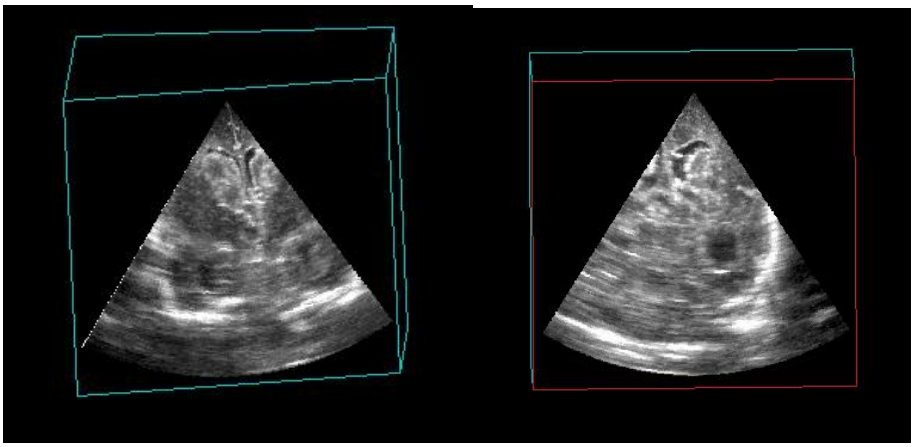

Figure 7-1 – 3D US images of a neonate with PHVD. This patient had ventricles enlarged enough that a single scan could not fully encompass the entire ventricle system, but each lateral ventricle could be imaged separately. a) right lateral ventricle b) left lateral ventricle with some overlapping regions within the middle portion of the brain/ventricle system.

The majority of the 3D US segmentations were made by a single trained user at a single center institute. The validity of this technique can only be fully realized if these results are confirmed by additional users at multiple centers.

In addition to this, some patients were not imaged due to handling concerns by the parents or nurses. While it would have been feasible to perform the imaging if necessary for a clinical reason, as the 3D US was only being performed for research purposes, there were a few patients with complicated medical courses in the NICU who were not represented in this data set. While the 'skipped' patients' PHVD was not particularly severe, and none of the 'skipped' patients had interventional VT or VP-shunts, this could potentially cause some bias in this sample.

7.4 Future Directions

7.4.1 Prospective Prediction of VT using 3D US thresholds

The next step that will be occurring is a prospective study performed using thresholds proposed in this thesis. At our center, enrolled neonates with PHVD will be monitored

using the developed 3D US system and VV will be measured using semi-automated segmented contours of the ventricles. Clinicians will be unblinded to the 3D US data and will make decisions based on current clinical practice with the addition of the 3D US data and thresholds.

7.4.2 Multi-center study using 3D US for interventions

3D US VV must be used in a multi-center study in order to gather a large enough sample size and obtain a variation of clinical practice in managing PHVD. As 3D US is becoming a far more accessible modality, we expect that this study could be performed in the near future.

7.4.3 Image Stitching

For the patients with severe PHVD, a single imaging volume is insufficient to capture the ventricles. As such, multiple 3D US images are acquired to fully capture the ventricles and surrounding brain regions of interest. Stitching these images into a large 'panoramic' 3D image will allow better visualization of the ventricles over time as well as allow for the creation of stitched images taken from different fontanelles. For example, images acquired from the posterior fontanelle allow for better visualization of the posterior horns and the cerebellum, which is currently not imaged with this system due to the poor visualization of the majority of the ventricle system. If the anterior fontanelle view could be combined with the posterior horn view, the images could allow for easier diagnosis of a wider variety of brain abnormalities.

We have developed a landmark-based registration similar to that used in the discussion section of Chapter 3 and are working towards generating an automated stitching program based on image-based registration of the midline overlap of images acquired through the anterior fontanelle.

7.4.4 Diagnosing VP-Shunt failure and requirement

Infantile hydrocephalus is a highly variable disease with numerous causes outside of IVH. These causes can be congenital, such as from spina bifida, or from genetic abnormalities, but also can be acquired after meningitis.[6] Infantile hydrocephalus from all

causes occurs in about 1.1 in 1000 infants.[7] For many of these patients, neurosurgical insertion of a VP shunt that constantly drains CSF into the abdomen is used to prevent further brain damage and alleviate symptoms of increased intracranial pressure (apnea, bradycardia). Despite the widespread clinical use of VP shunts, 31% of shunts will fail within the first year and require revision surgery.[8] Often shunt failure only becomes apparent when clinical symptoms occur, such as when an infant is overly irritable, lethargic, nauseous, ataxic, and has failure to thrive. Especially in infants who are not able to communicate with their caregivers that they have a headache, these symptoms are not specific to VP-shunt failure.

3D US can be performed in a neurosurgical clinic both before VP shunt insertion and at follow-up for much less than the cost of other 3D medical images, and in fractions of the time in order to measure changes in the ventricle volume. Given that the fontanelle does not close until 18 months of age, our 3D US system would provide non-invasive images of the ventricles at the time when acute shunt failure is likely. Given that patients with working shunts should undergo decreased CSF accumulation, we hypothesize that ventricle volumes measured by 3D head US will detect acute shunt failure as continued or sudden increases in ventricle volume at follow-up. Additionally, the 3D US images might be able to detect subdural hemorrhage/hematoma, which is a condition that can occur due to over-drainage of CSF. Other than the hemorrhage, over-drainage can lead to slit-ventricle syndrome, which can be a chronic cause of migraines in these patients. We have performed 3D US in the neurosurgical follow-up clinic up in 5 patients with hydrocephalus to assess shunt function and found that this intervention seems entirely feasible to study, though to date we have not seen acute shunt failure.

Some infants with hydrocephalus will not have shunts implanted until clinical symptoms appear after some skull sutures have closed. These infants present with enlarged ventricles during the peri-neonatal period. We hypothesize that infants with progressive increases in ventricle volume during the peri-neonatal period will go on to require shunts once all skull sutures have closed, while those with decreases or stabilization in ventricle size will not require further interventions. Assessing these at-risk patients during normal

follow-up appointments should provide good indicators towards which of these patients will require VP-shunts later in life.

7.4.5 Using imaging to as a priori information for near infrared spectroscopy

Although not in the body of this thesis, near infrared spectroscopy (NIRS) measurements were taken on some of the same days as 3D US imaging was performed. Generally, NIRS measurements are associated with oxygenation measurements due to the difference in NIRS signal from oxy- and deoxy-hemoglobin (Fig. 7-2).

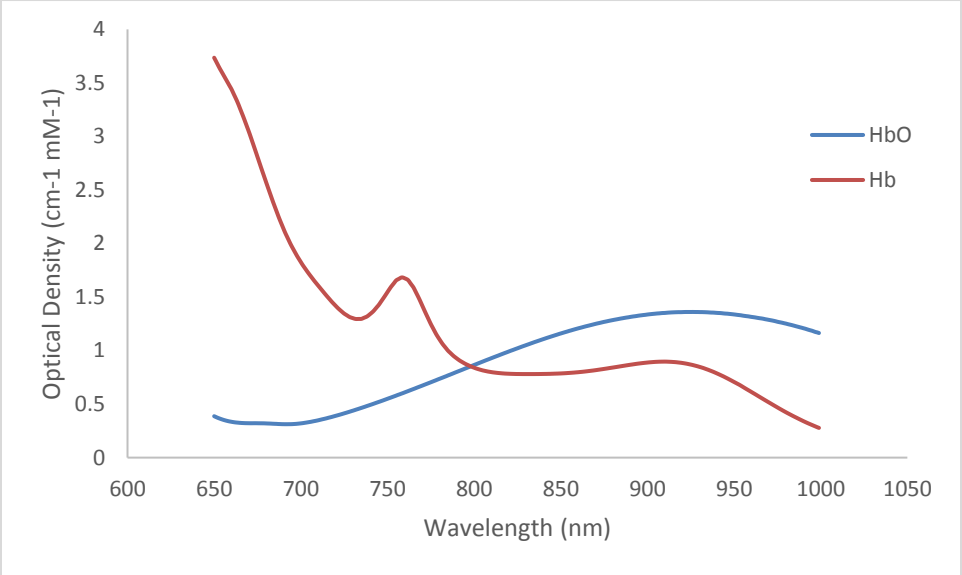

Figure 7-2 Differing near infrared absorption spectra of oxygenated hemoglobin (HbO) and deoxygenated hemoglobin (Hb)

However, due to the multi-modality nature of the study, we were able to detect 'ventricle contamination' of the NIRS signal in patients with IVH.[9] Specifically, we showed qualitatively that there was noticeable signal contamination stemming from what appeared to be decrease in brain mantle thickness due to compression from PHVD (Fig. 7-3). It was proposed that this contamination is from the ventricles where blood breakdown products appear to be similar to deoxy-hemoglobin, as well as from decrease photon scatter in CSF in comparison to tissues.

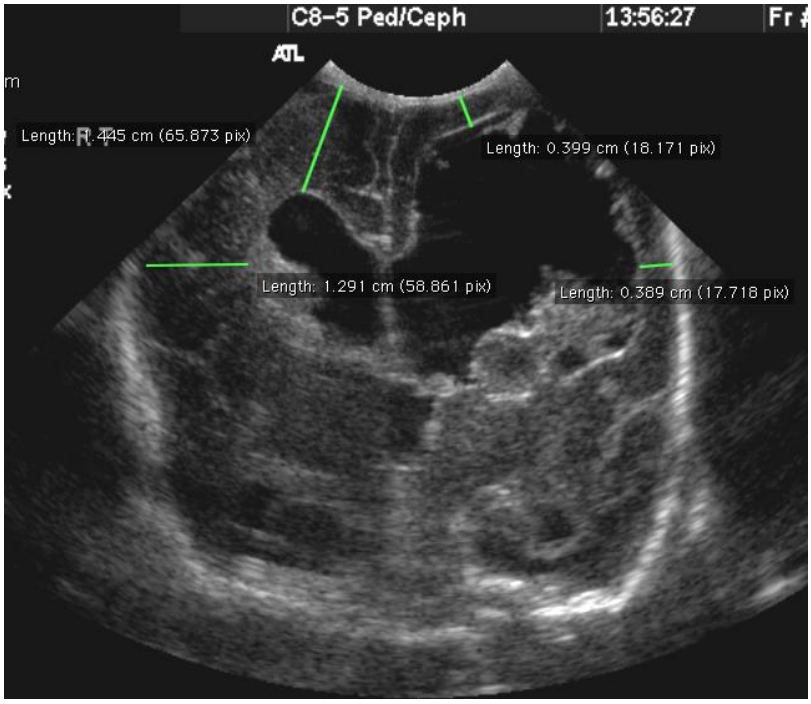

Figure 7-3 Smaller cortical mantel thickness after PHVD as seen on a coronal ultrasound image.

Neonates and infants with PHVD would likely be the patients that benefit the most from neuromonitoring during the neonatal period in order to better manage their condition through therapies and surgeries aimed at alleviating elevated ICP. Since most NIRS systems and algorithms assume homogenous tissue as the region being interrogated by the light, and these patients appear to violate those assumptions, a better model is required to produce accurate and reproducible NIRS measurements in patients with PHVD. A two-layered model, including a top layer for 'tissue' and a bottom layer for 'CSF' could solve this issue. 3D ultrasound would allow for a model of the brain to be generated and to determine how thick the top layer should be. As such, this imaging data could provide the necessary *a priori* information to develop a two-layer model of the brain/ventricles and could assist in analyzing NIRS signal from neonates with PHVD in order to more accurately monitor brain oxygenation despite variations in brain thickness over time.

Currently, a study is being performed using Monte Carlo simulations on a two-layered model with various tissue thicknesses to determine if this previously acquired NIRS/3D US data can be analyzed more appropriately.[10]

7.5 Significance and Impact

Management of PHVD is a complex process with outcomes often not known for years until the patient reaches school age. Current monitoring is based on clinical exams and 2D US images, and while able to diagnose the disease, these methods are not specific enough to generate threshold-based guidelines for treatment past an initial intervention.

This thesis illustrates the potential for 3D US-based ventricle volumes to be used to generate a more specific criterion for interventions and allow for more precise monitoring. The potential for improved outcomes for these children based on better evidence and more clinician confidence could result in less delayed treatments. While still unknown, this thesis will allow for a better definition of 'early' treatment (i.e., when the ventricle's rate of dilatation is over a certain threshold), and could directly lead to a rigorous study on whether or not earlier treatment can lead to better outcomes.

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APPENDIX

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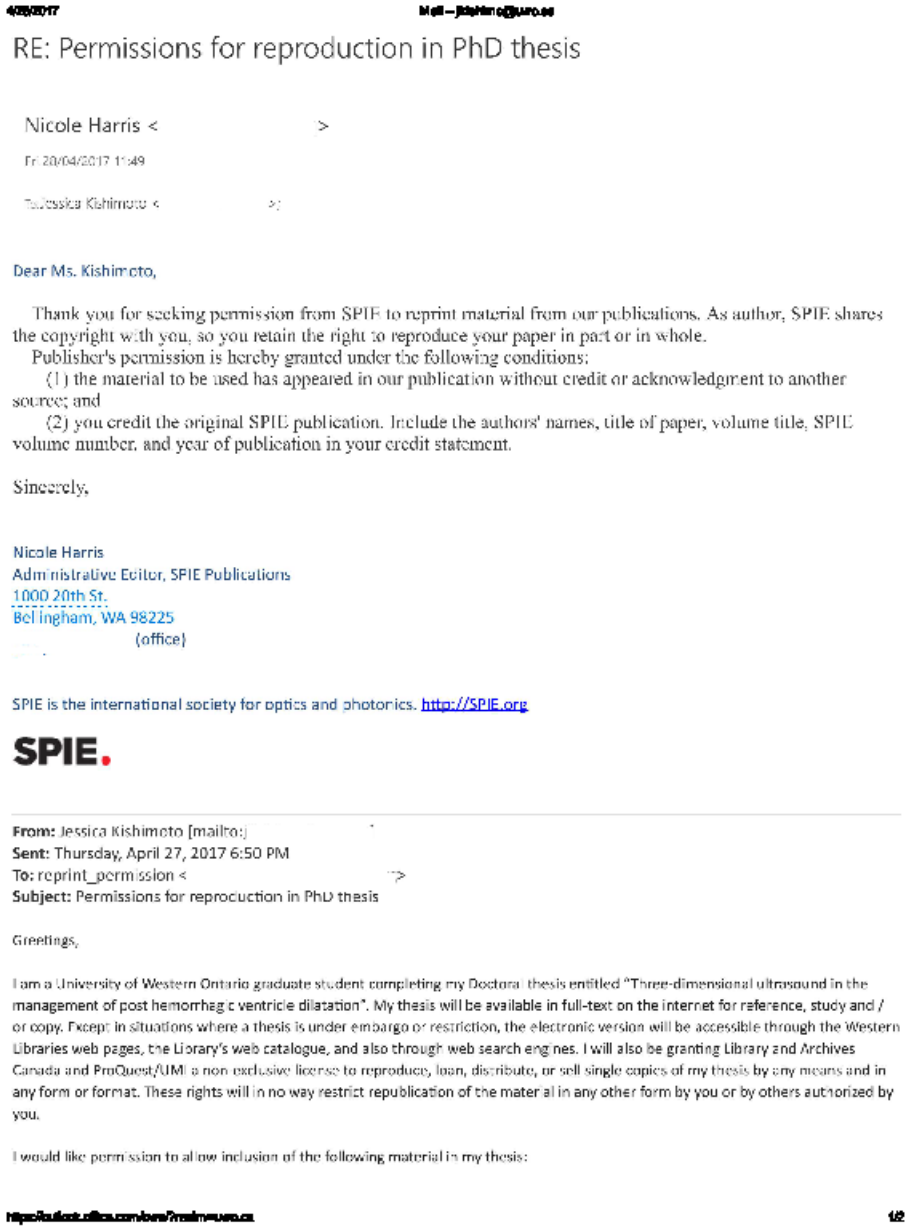

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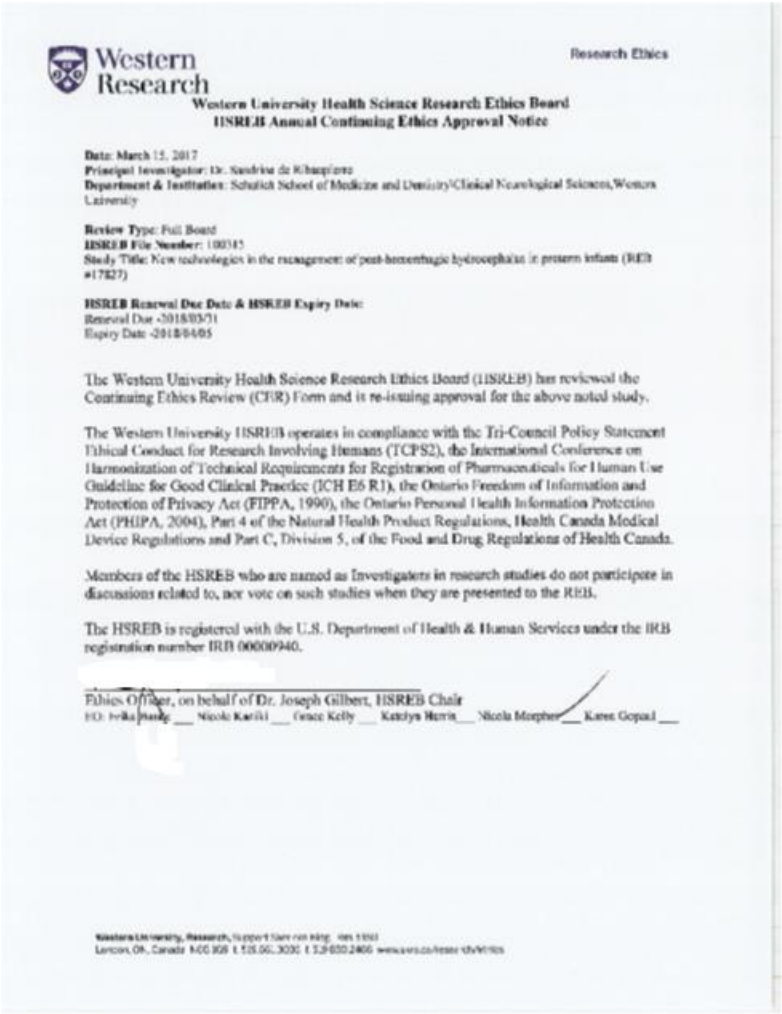
Appendix B - Research Ethics Board Approval Notice



Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Annual Continuing Ethics Approval Notice

Date: March 15, 2017
Principal Investigator: Dr. Sandrine de Ribaupierre
Department & Institution: Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,Western University

Review Type: Full Board
HSREB File Number: 108345
Study Title: New technologies in the management of post-hemorrhagic hydrocephalus in preterm infants (REB #17827)

HSREB Renewal Due Date & HSREB Expiry Date:
Renewal Due: 2018/03/31
Expiry Date: 2018/04/05

The Western University Health Science Research Ethics Board (HSREB) has reviewed the Continuing Ethics Review (CER) Form and is re-issuing approval for the above noted study.

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The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair
EO: Erika Basile ___ Nicole Kotski ___ Grace Kelly ___ Katelyn Harris ___ Nicola Morphet ___ Karen Gopaul ___

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London, ON, Canada N6G 0G8 t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/ethics

Curriculum Vitae

EDUCATION

Sept 2011- summer 2017 **Doctor of Philosophy**
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada
Thesis: Three-dimensional ultrasound for the management of post-hemorrhagic ventricle dilatation
Supervisors: Dr. Sandrine de Ribaupierre and Dr. Aaron Fenster

Sept 2007- April 2011 **Bachelor of Medical Science**
Honours Specialization Medical Biophysics (Medical Science Concentration)
The University of Western Ontario, London, Ontario, Canada
Thesis: The development of a bi-axial dynamic strain platform for live cell microscopy
Supervisors: Dr. David Holdsworth and Dr. S. Jeffery Dixon

RESEARCH POSITIONS

Sept 2011- Summer 2017 **Graduate Research Assistant, Doctoral**
Department of Medical Biophysics
The University of Western Ontario, London, Ontario, Canada
Supervisors: Dr. Sandrine de Ribaupierre and Dr. Aaron Fenster
Project: New technologies in the management of post hemorrhagic hydrocephalus

May 2011- Aug 2011 **Summer Research Student**
Robarts Research Institute, London, Canada
Supervisors: Dr. David Holdsworth and Dr. S. Jeffery Dixon
Project: Vibration and high speed microscopy to study mechanotransduction in osteoblasts

Sept 2010- April 2011 **Forth Year Undergraduate Honours Thesis Student**
Robarts Research Institute, London, Canada
Supervisors: Dr. David Holdsworth and Dr. S. Jeffery Dixon
Project: Development of a bi-axial strain platform for live cell microscopy

RESEARCH SPECIFIC SCHOLARSHIPS AND AWARDS

A. Scholarships

2015-2016 Ontario Graduate Scholarships

- A merit based scholarship (based on academic standing) that encourages excellence in graduate studies and is available to students in all disciplines **$15,000**

2014 Internal Research Fund Studentship
- Awarded to a student supervised by a member of London Health Sciences center or St. Joseph's hospital in a medical science related field. Awarded based on research achievement and proposal design to support stipend $15,000

2016-2011 Western Graduate Research Scholarship
- Awarded for entering and maintaining an overall average of 80% during graduate studies to support tuition **$1,500-$2,900 per term**

2011 Summer Research Studentship - CIHR JuMP (Joint Motion Program)
- Awarded to a summer research student involved in musculoskeletal research for stipend support **$5000**

2008 Evelyn Moxley Bursary
- For achieving over 80% cumulative average in undergraduate studies in addition to financial need **$500**

2007-2010 Queen Elizabeth II Aiming for the Top Scholarship
- For achieving over 80% cumulative average in undergraduate studies **$2,885 per year**

2007 Elgin St. Thomas Medical Scholarship
- Awarded to a single outstanding high school student within Elgin county entering into a medical related undergrad program **$1,000**

B. Awards

2016 **Cum Laude Abstract** – Imaging Network Ontario.

2014 **CIHR Travel Award** to Canadian Student Health Research Forum. Winnipeg, MB. **$833**

2014 **First place, Oral Presentations – Clinical.** Paediatric Research Day, London, ON. **$200**

2014 **Alfred Jay Award for Translational Research.** A.C. Burton Day – Medical Biophysics. **$1500**

2014 **First place, Poster Competition** - Imaging Network Ontario. **$300**

2014 **Second place, Platform competition. Oral Presentation.** London Health Research Day **$600**

2013 **CHRI Translational Research Award. Oral Presentation.** Paediatric Research Day. **$500**

2013 **Cum Laude Poster Presentation.** SPIE Medical Imaging 2013. Ultrasonic Imaging, Tomography, and Therapy Session. Orlando, FL, USA.

SCIENTIFIC REVIEWER

Journal of Medical Imaging 2015, 2016

PUBLICATIONS AND PRESENTATIONS

A. Referred Journal Manuscripts (9 Published, 2 In Review)

In review

1. **J. Kishimoto**, A. Fenster, D.S.C. Lee, S. de Ribaupierre. Quantitative 3D head ultrasound measurements of ventricle volume to determine thresholds for preterm neonates requiring interventional therapies following PHVD. (Scientific Reports)
2. P. McLachlan, **J. Kishimoto**, M. Diop, D. Milej, S. de Ribaupierre, D.S.C. Lee, K. St. Lawrence. Investigating the Effects of Cerebrospinal Fluid Removal on Cerebral Blood Flow and Oxidative Metabolism in Infants with Post-Hemorrhagic Ventricular Dilatation (Pediatric Research)

Published

1. W. Qiu, Y. Chen, **J. Kishimoto**, S. de Ribaupierre, B. Chiu, A. Fenster, J. Yuan. Longitudinal Analysis of Pre-term Neonatal Cerebral Ventricles from 3D Ultrasound Images Using Spatial-Temporal Deformable Registration. IEEE Trans. Med. Imaging. 36(4):1016-1026
2. **J. Kishimoto**, S. de Ribaupierre, F. Salehi, W. Romano, D.S.C. Lee, A. Fenster. Preterm neonatal lateral ventricle volume from 3D ultrasound is not strongly correlated to 2D ultrasound measurements. J. Med. Imag. 3(4) (2016) 046003
3. W. Qiu, Y. Chen, **J. Kishimoto**, S. de Ribaupierre, B. Chiu, A. Fenster, J. Yuan. Automatic Segmentation Approach to Extracting the Neonatal Cerebral Ventricles from 3D Ultrasound Images. Medical Image Analysis 35 (2016) 181-191
4. **J. Kishimoto**, A. Fenster, D.S.C. Lee, S. de Ribaupierre. *In Vivo* Validation of a 3D Ultrasound System for Imaging the Lateral Ventricles of Neonates. Ultrasound in Medicine and Biology 42(4) (2016) 971-79.
5. Y. Chen, W. Qiu, **J. Kishimoto**, Y. Gao, R. Chan, S. Ribaupierre, A. Fenster, B. Chiu. A framework for quantification and visualization of segmentation accuracy and variability in 3D lateral ventricle ultrasound images of preterm neonates. Medical Physics 42(11) (2015) 6387-405.

6. M. Diop, **J. Kishimoto**, V. Toronov, D. S. C. Lee, K. St. Lawrence. Development of a combined broadband near-infrared and diffusion correlation system for monitoring cerebral blood flow and oxidative metabolism in preterm infants. Biomedical Optics Express. 6(10) (2015) 3907-3918
7. W. Qiu, J. Yuan, M. Rajchl, **J Kishimoto**, Y. Chen, S. de Ribaupierre, B. Chui, A. Fenster 3D MR Ventricle Segmentation in Pre-Term Infants with Post-Hemorrhagic Ventricle Dilatation (PHVD) Using Multi-Phase Geodesic Level-Sets. NeuroImage 118 (2015) 13–25.
8. W. Qiu, J. Yuan, **J Kishimoto**, J. McLeod, Y. Chen, S. de Ribaupierre, A. Fenster. User-Guided Segmentation of Preterm Neonate Ventricular System from 3D Ultrasound Images Using Convex Optimization Ultrasound in Medicine and Biology. 41(2) (2015) 542–556.
9. **J. Kishimoto,** S. de Ribaupierre, R. Mehta, K. St. Lawrence, D.S.C. Lee, A. Fenster. 3D ultrasound system to investigate intraventricular hemorrhage in preterm neonates. Phys. Med. Biol. 58 (2013) 7513-7526.

B. Published Refereed Conference Papers (15)

1. **J. Kishimoto**, A. Fenster, F. Salehi, W. Romano, D.S.C Lee, S. de Ribaupierre. Quantitative head ultrasound measurements to determine thresholds for preterm neonates requiring interventional therapies following intraventricular hemorrhage. SPIE Medical Imaging 2016. February 27 – March 3 2016, San Diego, CA (Oral Presentation)
2. W. Qiu, J. Yuan, **J. Kishimoto,** Y. Chen, M. Rajchl, E. Ukwatta, S. de Ribaupierre, A. Fenster. Automatic 3D US Ventricle Segmentation in Pre-term Neonates Using Multi-Phase Geodesic Level-Sets with Shape Prior. MICCAI 2015. October 5-9 2015, Munich, Germany (Poster Presentation)
3. W. Qiu, J. Yuan, **J. Kishimoto,** M. Rajchl, E. Ukwatta, S. de Ribaupierre, A. Fenster. Longitudinal Analysis of Pre-Term Neonatal Ventricle in Ultrasound Images Based on Convex Optimization. MICCAI 2015. October 5-9 2015, Munich, Germany (Oral Presentation)
4. **J. Kishimoto**, A. Fenster D.S.C. Lee, S de Ribaupierre. Characterization of neonatal patients with intraventricular hemorrhage using 3D ultrasound cerebral ventricle volumes. SPIE – Medical Imaging 2015. February 21-26 2015. Orlando, FL. (Oral Presentation)
5. W. Qiu, J. Yuan, **J. Kishimoto**, Y. Chen, S. de Ribaupierre, A. Fenster. 3D MR Ventricle Segmentation in Pre-term Infants with Post-Hemorrhagic Hydrocephalus. SPIE – Medical Imaging 2015. February 21-26 2015. Orlando, FL. (Oral Presentation)
6. Y. Chen, **J. Kishimoto**, B. Chiu, W. Qiu, S. de Ribaupierre, A. Fenster. Quantification of Cerebral Ventricle Volume Change of Preterm Neonates Using 3D Ultrasound Images. SPIE – Medical Imaging 2015. February 21-26 2015. Orlando, FL. (Oral Presentation)
7. **J. Kishimoto**, M. Diop, P. McLachlan, S. de Ribaupierre, D.S.C. Lee, K. St Lawrence. Evidence of ventricular contamination of the optical signal in preterm neonates with post hemorrhagic ventricle dilation. SPIE – Photonics West 2015. February 7-12 2015. San Francisco, CA. (Oral Presentation)
8. W. Qiu, J. Yuan, M. Rajchl, **J. Kishimoto,** E. Ukwatta, S. de Ribaupierre, A. Fenster. Cerebral Ventricle Segmentation from 3D Pre-term IVH Neonate MR Images Using Atlas-Based Convex Optimization. Computer-Assisted and Robotic Endoscopy Workshop, MICCAI, Sept 14-19 2014 Boston, MA. (Workshop)
9. W. Qiu, J. Yuan, **J Kishimoto**, S de Ribaupierre, E. Ukwatta, A. Fenster. Semi-automatic Segmentation of Pre-term Neonate Ventricle System From 3D Ultrasound Images. IEEE

International Symposium on Biomedical Imaging (ISBI), Apr 28-May 2, 2014. Beijing, China (Poster Presentation)

10. **J. Kishimoto**, J. T. Elliot, P. McLachlan, M. Diop, S. de Ribaupierre, D.S.C. Lee, K. St. Lawrence. Monitoring Cerebral Blood Flow in Preterm Infants with Post-Hemorrhagic Hydrocephalus. The Optics Society – Biomedical Optics. April 26-30 2014. Miami, FL. US (Poster Presentation)
11. **J Kishimoto,** Fenster, A, Chen, N, D.S.C. Lee, S de Ribaupierre. *In Vivo* Validation of a 3D Ultrasound System for Imaging the Lateral Ventricles of Neonates. Proceedings of SPIE Volume 9036 Feb 15-21 2014. San Diego, California. (Oral Presentation.)
12. W. Qiu, J. Yuan, **J Kishimoto**, E. Ukwatta, A. Fenster. Lateral Ventricle Segmentation of 3D Pre-term Neonates US Using Convex Optimization. MICCAI 2013. Sept 21-26 2013. Nagoya, Japan (Poster Presentation)
13. M. Diop, **J. Kishimoto**, D. S. C. Lee, T-Y. Lee, K. St. Lawrence. Cerebral Blood Flow Monitoring in Preterm Infants by Diffuse Correlation Spectroscopy The Optics Society – Biomedical Optics. April 26-30 2014. Miami, FL. US (Poster Presentation)
14. **J Kishimoto**, D.S.C. Lee, K. St. Lawrence, A. Fenster, S de Ribaupierre. Development of a 3D Ultrasound System to Investigate Post- Hemorrhagic Hydrocephalus in Pre-term Neonates. SPIE Medical Imaging. Proceedings of SPIE Volume 8675. Feb 9-14 2013, Orlando Florida, US. (Poster Presentation)
15. D.W. Holdsworth, H. Nikolov, J. Au, K Beaucage, **J Kishimoto,** S.J.Dixon. Simultaneous vibration and high-speed microscopy to study mechanotransduction in living cells. SPIE Medical Imaging. Proceedings of SPIE Volume 8317. Feb 4-9 2012, San Diego, California. (Oral Presentation)

C. Peer Reviewed Oral Presentations (10)

1. **J Kishimoto,** A Fenster, F. Salehi, W. Romano, D.S.C Lee, S. de Ribaupierre. Cranial ultrasound in 2D and 3D to guide treatment in preterm neonates with post-hemorrhagic ventricular dilatation. Paediatric Surgery Day. February 6 2017, London, ON
2. P. McLachlan, **J. Kishimoto**, S. de Ribaupierre, D.S.C. Lee, M. Diop, K. St. Lawrence. Development of a NIRS method to quantify cerebral perfusion and oxidative metabolism in preterm neonates with post hemorrhagic ventricle dilation. SPIE Photonics West 2017. January 28 – February 2 2017, San Francisco, CA
3. **J. Kishimoto,** W. Romano, D.S.C. Lee, A. Fenster, S. de Ribaupierre. To tap or not to tap: A comparison of cerebral 3D ultrasound to 2D ultrasound in extremely preterm neonates with post-hemorrhagic ventricle dilation to predict the necessity of interventional ventricular tap. World Congress on Medical Imaging. June 7-12 2015, Toronto, ON.
4. **J Kishimoto,** A. Fenster, D.S.C. Lee, S de Ribaupierre. Development of an image based classification method for the interventional treatment of preterm neonates with intraventricular hemorrhage using 3D ultrasound. Imaging Network Ontario. March 30 - 31, 2015. London, ON.
5. **J. Kishimoto,** D.S.C. Lee, W. Romano, A. Fenster, S. de Ribaupierre. Improved Characterization of Post Hemorrhagic Ventriculomegaly using 3D head Ultrasound. Paediatric Research Day. May 21 2014. London, ON.
6. **J Kishimoto,** A. Fenster, N. Chen, D.S.C. Lee, S de Ribaupierre. *In Vivo* Validation of a 3D Ultrasound System for Imaging the Lateral Ventricles of Neonates. Imaging Network Ontario. March 24 - 25, 2014. Toronto, ON.

7. **J Kishimoto,** A. Fenster, N. Chen, D.S.C. Lee, S de Ribaupierre. Development of a 3D ultrasound system for the measurement of ventricle dilation in preterm patients: In Vivo Validation Studies. London Health Research Day. March 18th 2014. London, ON
8. **J. Kishimoto,** W. Romano, A. Fenster, D.S.C. Lee, S. de Ribaupierre. Comparison of clinical US measurements of the ventricles to 3D US ventricle volumes in IVH patients. RSNA. Chicago, IL. Dec 1-6 2013
9. **J. Kishimoto**, S. de Ribaupierre, W. Romano, K. St. Lawrence, M. Diop, S. Levin, V. Han, A. Fenster, D. S. Lee. 3-D volumetric assessment of cerebral ventricles and quantitative cerebral blood flow in the management of posthemorrhagic hydrocephalus in preterm infants. Canadian Pediatric Society. Edmonton, AB. June 19-22, 2013.
10. **J Kishimoto,** D.S.C. Lee, M. Diop., A. Fenster, K. St. Lawrence, Romano, W., S. Levin, V. Han, S de Ribaupierre. Bedside monitoring of post-hemorrhagic hydrocephalus: 3D US to measure ventricular volumes and NIRS to track changes in cerebral blood flow. Pediatrics Research Day. London, ON., May 29 2013.

D. Peer Reviewed Poster Presentations (24)

1. A. Harris, **J. Kishimoto,** L. Gardi, S de Ribaupierre, D.S.C. Lee, A. Fenster. Automatic Image Registration and Stitching in 3D Ultrasound for Monitoring of Neonatal Post-Hemorrhagic Ventricle Dilation. Imaging Network Ontario. March 15 - 16, 2017. London, ON.
2. **J. Kishimoto,** A. Fenster, D.S.C. Lee, S de Ribaupierre. Three dimensional head ultrasound for the detection of shunt failure in infants with hydrocephalus: pilot study. Imaging Network Ontario. March 15 - 16, 2017. London, ON.
3. **J. Kishimoto**, A. Fenster, F. Salehi, W. Romano, D.S.C Lee, S. de Ribaupierre. Quantitative 3D and 2D head ultrasound to determine thresholds for intervention in preterm neonates with post hemorrhagic ventricle dilation. Canadian Pediatric Society. June 22-25 2016, Charlottetown, PEI
4. **J. Kishimoto**, A. Fenster, F. Salehi, W. Romano, D.S.C Lee, S. de Ribaupierre. Quantitative 3D and 2D Ultrasound to Guide Treatment in Neonates With Post-Hemorrhagic Ventricle Dilatation: Pilot Study Results. Pediatric Academic Societies. April 30-May 3 2016, Baltimore, MD
5. **J. Kishimoto**, A. Fenster, F. Salehi, W. Romano, D.S.C Lee, M Bhaduri, S. de Ribaupierre. 3D ultrasound to guide IVH treatment in neonates. ARRS 2016. April 17-22 2016. Los Angeles, CA
6. **J. Kishimoto**, A. Fenster, F. Salehi, W. Romano, D.S.C Lee, S. de Ribaupierre. Cranial ultrasound in 2D and 3D to guide treatment in preterm neonates with posthemorrhagic ventricular dilatation. Imaging Network Ontario. March 30 & 31 2016, Toronto, ON
7. **J. Kishimoto,** S. de Ribaupierre, J.T. Elliot, D.S.C. Lee, M. Diop, K. St. Lawrence. Preterm neonates with post hemorrhagic ventricle dilation show improvements in cerebral perfusion after ventricle tap: a near infrared spectroscopy study. London Health Research Day. March 29 2016. London, ON
8. M. Diop, **J. Kishimoto**, V. Toronov, D.S.C. Lee, K. St. Lawrence. Using a hybrid optical system to detect the uncoupling of cerebral blood flow and cerebral oxidative metabolism in preterm infants undergoing treatment for patent ductus arteriosus International Symposium on Cerebral Blood Flow, Metabolism and Function. June 27-30 2015, Vancouver, BC

9. **J. Kishimoto**, K. St. Lawrence, M. Diop, V.K.M. Han , D.S.C. Lee, S. de Ribaupierre. Limitations of Cerebral Monitoring with Near Infrared Spectroscopy in Neonates with Post Hemorrhagic Ventricular Dilatation. Pediatric Academic Society. April 25-28 2015. San Diego, CA
10. P. McLachlan, **J. Kishimoto**, K. St. Lawrence, M. Diop, V.K.M. Han , D.S.C. Lee, S. de Ribaupierre. Pitfalls of Cerebral Oxygenation Monitoring in the Presence of Ventricular Dilatation. Pediatric Academic Society. April 25-28 2015. San Diego, CA
11. **J. Kishimoto**, M. Diop, K. St. Lawrence, V.K.M. Han , D.S.C. Lee. Validation of a Non-Invasive Optical Method, Diffuse Correlation Spectroscopy (DCS), for Measuring Cerebral Blood Flow in Preterm Neonates with PDA undergoing Indomethacin Treatment. Pediatric Academic Society. April 25-28 2015. San Diego, CA
12. **J. Kishimoto,** W. Romano, D.S.C. Lee, A. Fenster, S. de Ribaupierre. To tap or not to tap: A comparison of 3D ultrasound to 2D ultrasound in extremely preterm neonates with post-hemorrhagic ventricle dilation to predict the necessity of interventional ventricular tap. London Health Research Day. April 1 2015. London, ON
13. **J. Kishimoto,** M. Diop, P. McLachlan, S. de Ribaupierre, D.S.C. Lee, K. St Lawrence. Clinical Evidence of Ventricular Contamination in a NIRS Study of Post-Hemorrhagic Hydrocephalus in Preterm Infants. fNIRS 2014. October 10-12. Montreal, QU.
14. **J. Kishimoto,** D.S.C. Lee, W. Romano, A. Fenster, S. de Ribaupierre. Improved Characterization of Post Hemorrhagic Ventriculomegaly using 3D head Ultrasound. Pediatric Academic Society. May 3-6 2014. Vancouver, BC, Canada.
15. P. McLachlan, **J. Kishimoto**, J. T. Elliot, M. Diop, S. de Ribaupierre, D.C. Lee, K. St. Lawrence. Path length correction using Monte Carlo modelling for near-infrared spectroscopy measurements of cerebral blood flow in preterm infants with intraventricular hemorrhage. London Health Research Day. March 18th 2014. London, ON
16. **J. Kishimoto**, S. de Ribaupierre, K. St. Lawrence, M. Diop, A. Fenster, D. S. C. Lee. The role of 3D US and Near Infrared Spectroscopy in Monitoring Post-Hemorrhagic Ventriculomegaly in Preterm Neonates. Canadian Perinatal Research Meeting in Banff, Feb. 12-15 2014
17. **J. Kishimoto**, S. de Ribaupierre, K. St. Lawrence, M. Diop, A. Fenster, D. S. C. Lee. Improving the Management of Hydrocephalus by Monitoring Ventricle Volume with 3-D Ultrasound and Cerebral Blood Flow by Near-Infrared Spectroscopy. International Conference on Brain Monitoring and Neuroprotection in the Newborn. Florida, US. January 16-18 2014.
18. **J Kishimoto,** N. Chen, D.S.C. Lee, A. Fenster, S de Ribaupierre. Quantification of neonatal cerebral ventricular volume using 3D US before and after ventricular tap in patients with post hemorrhagic hydrocephalus. London Imaging Discovery Conference. London Health Sciences Centre, London, ON. June 13 2013.
19. **J Kishimoto**, G. Jawa, D.S.C. Lee, K. St. Lawrence, S de Ribaupierre., A. Fenster Development of a 3D Ultrasound System to Investigate Post-Hemorrhagic Hydrocephalus in Pre-term Neonates. London Health Research Day. London, ON March 19 2013.
20. **J Kishimoto**, D.S.C. Lee, K. St. Lawrence, A. Fenster, S de Ribaupierre., The role of 3D US and Near Infrared Spectroscopy to Investigate Post-Hemorrhagic Ventriculomegaly in Pre-term Neonates. Imaging Network Ontario Symposium. Feb 4-5 2013, Toronto, ON
21. S de Ribaupierre, **J Kishimoto**, G. Jawa, K. St. Lawrence, A. Fenster D.S.C. Lee. Alternative ways for hydrocephalus monitoring in IVH infants. International Society for Pediatric Neurosurgery: 40th Annual Meeting. Sydney, Australia. September 9-13 2012.

22. **J Kishimoto,** G. Jawa, D.S.C. Lee, W. Romano, K. St. Lawrence, A. Fenster, S de Ribaupierre Development of a 3D Ultrasound System to Investigate Post-Hemorrhagic Hydrocephalus in Pre-term Neonates. London Imaging Discovery Conference. London Health Sciences Centre, London, ON. June 27 2012.
23. **J. Kishimoto,** K. Beaucage, H. Nikolov, S.J. Dixon, D.W. Holdsworth.., Development of a bi-axial strain platform for live-cell microscopy CIHR Joint Motion Training Program in Musculoskeletal Health Research and Leadership: Summer Research Symposium. University of Western Ontario, London, ON. Aug 4 2011.
24. **J. Kishimoto,** K. Beaucage, H. Nikolov, S.J. Dixon, D.W. Holdsworth. Development of a bi-axial radial strain platform for high speed live-cell microscopy CIHR Joint Motion Training Program in Musculoskeletal Health Research and Leadership: Annual Retreat. University of Western Ontario. London, ON. May 13 & 14, 2011.

E. Invited Talks and Presentations (4)

1. **In Vivo Validation of a 3D Ultrasound System for Measuring the Cerebral Ventricle Volume in Preterm Neonates.** Canadian Student Health Research Forum, Winnepeg, MB, June 10-12 2014 (Poster)
2. **Assessment of ventricle dilation in preterm infants following intraventricular hemorrhage: Comparison of 2D and 3D cranial ultrasound.** Mini-Symposium on Imaging of Mothers, Fetuses, Infants and Children, Western University London, ON May 20th 2014
3. **The role of 3D Ultrasound in the Monitoring of Post Hemorrhagic Hydrocephalus**: Mind and Brain Institute, Western University, London ON. Nov 9 2012.
4. **The role of 3D Ultrasound in the Monitoring of Ventriculomegaly following IVH**: Research Rounds. Victoria Hospital, London, ON. Oct 22 2012.

POST GRADUATE EDUCATION DEVELOPMENT

Sept 2014-April 2017 Graduate Student Supervisor

Andrew Harris, BSc, PhD Student, Medical Biophysics
PhD Student (Sept 2017-present)
Project: Automated image registrtion and stitching of 3D ultrasound images

Duncan Riviere, Medical Physics Undergraduate Student
Volunteer Researcher (Sept 2016-April 2017)
Project: Further development of a semi-automated segmentation algorthm for 3D ultrasoud images of neonatal cerebral ventricles

Peter McLachlan, BMSc, Master's Candidate, Medical Biophysics
Master's Student (Sept 2014-May 2017)
4th year thesis student (Sept 2013-April 2014)
Project: Investigating potential improvements in cerebral blood flow and oxidative metabolism in infants with post hemmorhagic hydrocephalus

Yang (Tracy) Wang, Eletrical Engineering Undergraduate
Summer Student (May 2015-Sept 2015)
Project: Manual registration of time matched 3D ultrasound and MRI of preterm born infants with post hemmorhagic ventricle dilatation

Yufeng (Nancy) Chen, Medical Student (Sept 2012-May 2016)
Volunteer Researcher
Project: Manual segmentation of 3D ultrasound images of preterm neonates with post hemmorhagic ventricle dilatation who received ventricle taps

Sept 2013-
April 2016 Graduate Teaching Assistant
4930 Selected Topics in Medical Sciences
Undergraduate Medical Sciences
The University of Western Ontario

RESEARCH FUNDING APPLICATIONS

Lawson Internal Research Fund Studentship Application – Spring 2014 (*Successful)*
New Technologies to monitor preterm infants with intraventricular hemorrhage
May 2014

PROFESSIONAL MEMBERSHIPS

2012- 2017 The Society of Photo-Optical Instrumentation Engineers
Student Member