SERIOUS BACTERIAL INFECTIONS AMONG UGANDAN NEONATES: AETIOLOGY, CLINICAL FINDINGS AND ONE YEAR OUTCOMES.

A THESIS SUBMITTED IN ACCORDANCE WITH THE REQUIREMENTS OF THE UNIVERSITY OF LIVERPOOL FOR THE DEGREE OF DOCTOR OF PHILOSOPHY BY

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DECLARATION OF WORK DONE

The CONSHA study was funded by the National Institute for Health, United States and Professor Steve Schiff was the chief investigator. The main study protocol was designed by Professor Schiff and the CONSHA team. The additional brain imaging hypotheses and the cranial ultrasound protocol were led by me.

CONSHA was a multi-site study and I was the site principal investigator for CONSHA in Mbale. I was responsible for the study set-up, recruitment and follow-up. I was also responsible for the supervision and overall training of the research staff.

All members of the study team and I undertook patient recruitment in the neonatal unit. Consent was undertaken by a team member who was fluent in the language of the participant. The clinical evaluation and management of the infants was done by the neonatal clinical team at Mbale Regional Referral Hospital under my supervision and leadership.

I was responsible for the creation of the CONSHA sampling standard operating procedures (SOPs) and for the training of the CONSHA study staff across all study sites. Study samples were only removed by a trained member of the study team or me. All laboratory testing was done by MBN laboratory in Uganda.

All cranial ultrasounds were undertaken by a trained study doctor or me. Dr Cornelia Hagmann trained all staff through a combination of theoretical and bedside teaching. The images were reviewed independently by Dr Cornelia Hagmann and Dr Frances Cowan.

Training in developmental assessments was provided by a local recognised trainer in Bayley Scales of Infant Development-3rd edition (BSID-III). The developmental assessments were carried out by one of the trained study staff, either in the follow-up clinic or in the community.

The data was double entered by the CONSHA data entry team. I undertook the data cleaning and performed all of the statistical analysis using SPSS® with statistical advice from the CONSHA biostatistician, Mr Joe Paulson.

DEDICATION

I would like to dedicate this thesis to my mother and father, my husband and my three children Eseld, Lowenna and Bryluen. I thank you all for your endless love and encouragement in this and everything I do.

ABBREVIATIONS

CONSHA	Control of the Neonatal Septisome and Hydrocephalus in sub-Saharan Africa
CNS	Central nervous system
CMV	Cytomegalovirus
cPVL	Cystic periventricular leukomalacia
CRF	Case report form
CSF	Cerebral spinal fluid
cUS	Cranial ultrasound
DNA	Deoxyribonucleic acid
EV	Enterovirus
GBS	Group B streptococcus
GMH	Germinal matrix haemorrhage
HIC	High income country
HIV	Human immunodeficiency virus
IVH	Intraventricular haemorrhage
LIC	Low income country
LP	Lumbar puncture
MIC	Middle income country
MR	Magnetic Resonance
MRRH	Mbale Regional Referral Hospital
NE	Neonatal encephalopathy
NEC	Necrotising enterocolitis

NNU	Neonatal Unit
PCR	Polymerase chain reaction
PDD	Pervasive development disorder
pSBI	Possible serious bacterial infection
RSV	Respiratory syncytial virus
RNA	Ribonucleic acid
SDG	Sustainable Development Goal
SSA	Sub-Saharan Africa
WHO	World Health Organization
WM	White matter

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Abstract

Title: Serious bacterial infections among Ugandan neonates: Aetiology, clinical findings and one year outcomes.

Author: K L Burgoine

Background:
Globally, serious bacterial infections, such as sepsis, pneumonia and meningitis, are a leading cause of neonatal mortality. In sub-Saharan Africa (SSA) there are believed to be up to 2.6 million cases of neonatal serious bacterial infections every year, leading to an estimated 250,000 deaths. Diagnosis is challenging since signs and symptoms are often non-specific and laboratory facilities are frequently limited. In low-resource settings, the diagnosis of a possible serious bacterial infection (pSBI) relies primarily on clinical algorithms. Cranial ultrasound (cUS) is a relatively cheap, safe and portable method of assessing the neonatal brain that could be used to detect findings indicative of central nervous system involvement. There are also limited data on the outcomes of pSBI survivors. This is one of the first studies in SSA to assess the role of cUS in the evaluation of infants admitted with pSBI and the early developmental outcome of these infants.

Aims:
In term neonates presenting with pSBI to a neonatal unit in eastern Uganda:

- Describe the clinical presentation, aetiology and neonatal outcomes
- Describe findings on cUS scans at presentation and correlate the imaging findings with presentation, CSF analysis and neonatal mortality
- Compare cUS findings to a cohort of similar aged well term neonates
- Describe findings on serial cUS scans up to 28 days after presentation
- Evaluate mortality, growth and developmental impairment up to 12 months of age and compare it to the contemporaneous control cohort
- Investigate the risk factors that contribute to poor early childhood outcome in term-born infants that experienced a neonatal pSBI

Methods:
Over a 1-year period, any term neonates presenting to the neonatal unit at Mbale Regional Referral Hospital who met the definition of pSBI were screened for inclusion. We described the microbiological aetiology using blood and CSF culture, the presenting clinical features and the neonatal outcomes. Each neonate had a standard cUS examination performed. The images were interpreted systematically by one of two experts blinded to the clinical details. A contemporaneous cohort of well term neonates were recruited. They underwent the same clinical and cUS examination.

Surviving infants were seen at 2, 6 and 12 months of age to evaluate survival, growth and development. The Bayley Scales of Infant Development-3rd edition (BSID-III) was used. Developmental impairment was defined as a scaled-score <-1SD below the mean. Poor outcome was defined as death, hydrocephalus, post-neonatal seizures or developmental impairment at 12 months of age.

Results:
214 neonates with pSBI were recruited (Figure i). Definite or possible pathogens were identified in 5.6% (12/214) of blood cultures. The most common pathogens isolated were *Staphylococcus Aureus, Klebsiella* and *Escherichia coli.* Potential pathogens were isolated in 0% (0/189) of CSF cultures. The overall neonatal mortality was 9.3% (20/214). The neonatal mortality from neonatal meningitis was 22.2% (6/27).

Early cUS scans were available for 196/214 (91.6%) neonates with pSBI. There was no observed association between cUS findings at presentation and neonatal mortality. Moderate and severe cortical and/or white matter (WM) echogenicities were significantly associated with abnormal CSF analysis. The presence of signs suggestive of encephalopathy or meningism were associated with abnormal cortical, WM, basal ganglia and thalamic echogenicity, ventricular dilatation and bright ventricular lining.

At 12 months 164/188 survivors of pSBI were available for developmental assessment; 4/188 infants had died during the post-neonatal period. Developmental impairment was evident across all domains of the BSID-III and the rates of impairment ranged from 7.9% to 14.6%. 24/44 control infants were available for assessment and none of these infants were impaired in any of the 5 domains. The raw scores and the scaled scores for all five neurocognitive domains were significantly lower for survivors of pSBI compared to control infants. Survivors of neonatal meningitis, had the highest rates of developmental impairment, being 24%, 35% and 24% in cognitive, language and motor domains respectively. Survivors of neonatal meningitis had a 12 to 18-fold increased risk of developmental impairment across all domains.

The 12-month outcome of 177/196 infants with an early cUS were known and 29.9% (53/177) of these infants had a poor outcome (death or developmental impairment). After adjustment for sex, age and weight, the following factors increased the risk of poor outcome: age <48 hours at presentation, respiratory distress (aOR 2.7, 95%CI 1.2-6.2), neonatal seizures (aOR 13.0 (5.2-32.4)), opisthotonus (aOR 9.5 (3.5-27.0)), hypotonia (aOR 3.0 (1.1-8.3)) and raised CSF protein (aOR 9.5 (2.3-38.6)). The cUS findings at presentation that were significantly associated with poor outcome were abnormal cortex (aOR 6.9 (2.0-23.5)), abnormal white matter (aOR 2.0 (1.0-3.9)), abnormal basal ganglia (aOR 13.6 (2.7-68.2)) and abnormal thalami (aOR 5.28 (1.8-15.2)).

Conclusion:
It is clear that a pSBI during the neonatal period, even without meningitis, may have a substantial public health and economic burden in SSA. Presentation before 48 hours of age, lower weight, several readily recognisable clinical signs as well as raised CSF protein and cortical, white matter, and central grey matter abnormalities seen on cUS, were all significant predictors of poor outcome. These risk factors will enable us to better consider which infants need intensive follow-up, early intervention and support. Improving our understanding of the aetiologies associated with mortality, developmental impairment and post-infectious hydrocephalus, is necessary to inform prevention strategies and treatment approaches.

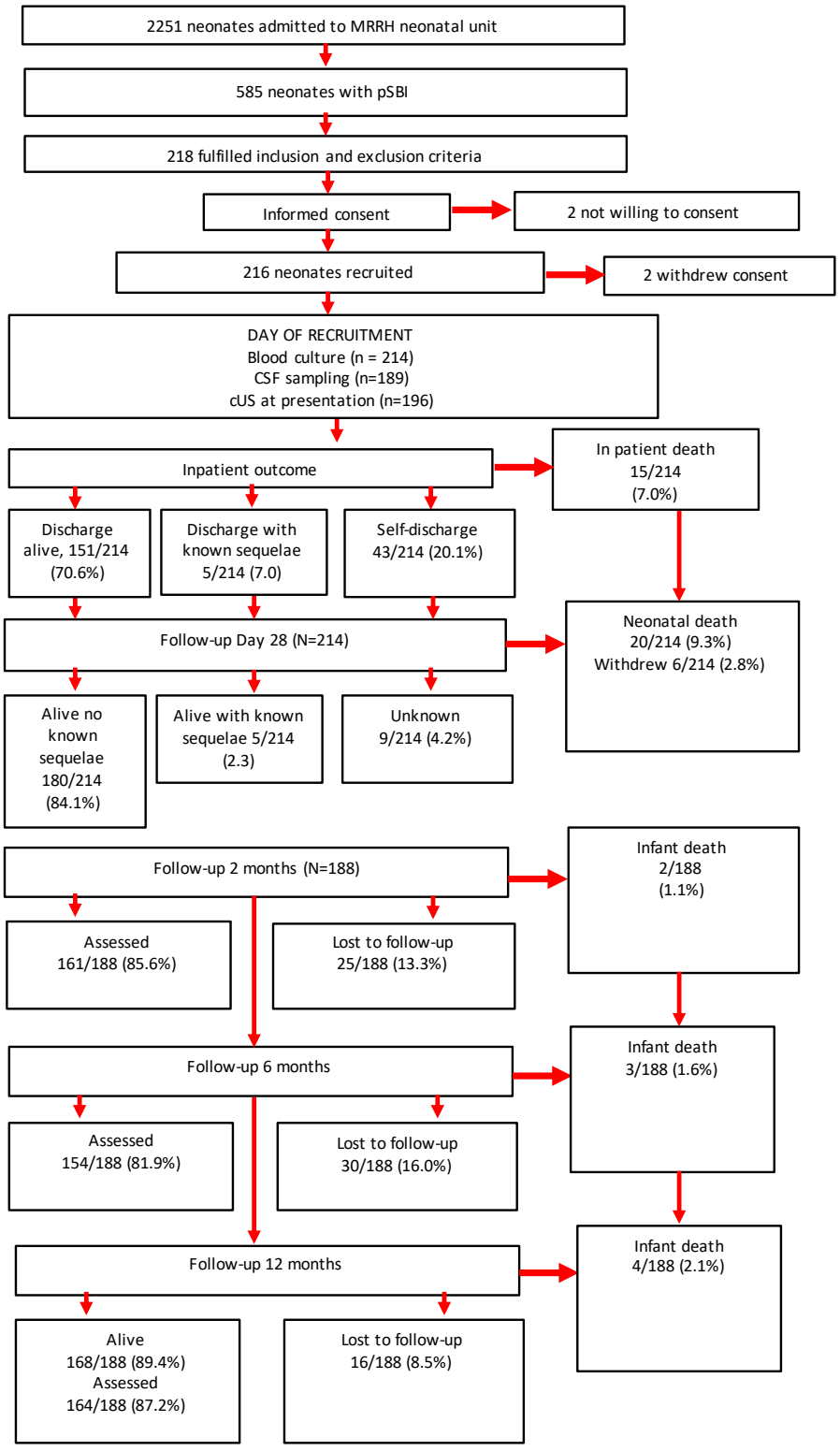


Figure i: Flow chart of patients in the study from recruitment to 12 months

CHAPTER 1 - INTRODUCTION

BURDEN OF NEONATAL MORTALITY

Worldwide, over 5 million children under the age of 5 die every year, and nearly all of these deaths occur in low-income countries (LIC) (Liu et al., 2015, UNICEF, 2020). Neonatal deaths are the predominant cause of these deaths (Liu et al., 2016, UNICEF, 2020). Sub-Saharan Africa (SSA) bears a disproportionate burden of global neonatal mortality and, in Uganda, the neonatal mortality rate (deaths in the first 28 days after birth) has not changed over 2 decades, remaining high at 28/1000 live births (UBoSUa, 2017). The main causes of neonatal deaths in LICs are prematurity, intrapartum complications and serious bacterial infections (Liu et al., 2015, Liu et al., 2016).

DEFINING SERIOUS BACTERIAL INFECTIONS

Neonatal sepsis is classically defined as the presence of symptoms or signs of systemic infection within 28 days after birth accompanied by the bacteriological isolation of a pathogen from the blood or CSF (Qazi and Stoll, 2009). The ‘gold-standard’ for the diagnosis of neonatal sepsis is the isolation of bacteria from the blood culture of a neonate with a clinical diagnosis of sepsis. Although, blood cultures have a high specificity, their sensitivity in neonates is particularly poor and putative pathogens are rarely recovered (Vergnano et al., 2011). In low-resource settings, laboratory facilities are frequently limited, therefore blood cultures are rarely available, and the diagnosis of neonatal serious bacterial infection typically relies on clinical algorithms. Possible serious bacterial infection (pSBI) is a clinical syndrome

that is used in the Integrated Management of Neonatal and Childhood Illness (IMNCI) to identify neonates who need urgent referral to hospital and to direct their treatment and management (Young Infants Clinical Signs Study, 2008). This pragmatic approach uses clinical symptoms and signs to ensure high sensitivity rather than specificity in the detection of what can be a devastating disease. It is therefore the approach that continues to be used in the majority of LICs, including Uganda.

BURDEN OF NEONATAL SERIOUS BACTERIAL INFECTIONS

It is estimated that serious bacterial infections account for 21% of neonatal deaths worldwide and they are considered to be one of the leading causes of neonatal deaths in Uganda (Liu et al., 2016, Health, 2008b). It is estimated that in SSA there are up to 2.6 million cases of pSBI every year, leading to an estimated 250,000 deaths (Seale et al., 2014). The challenges in the diagnosis of serious bacterial infections and the under-reporting of neonatal deaths mean that these estimates could be a gross underestimate of the true burden.

BURDEN OF NEONATAL MENINGITIS

Neonatal sepsis can lead to infection of the central nervous system (CNS) causing inflammation of the meninges (meningitis) and ventricles (ventriculitis) (Barichello et al., 2013). Neonatal meningitis is a devastating illness and carries a higher risk of mortality and subsequent neurodevelopmental impairment than neonatal sepsis (Furyk et al., 2011). It is not always possible to diagnose neonatal meningitis clinically. The ‘gold-standard’ for the

diagnosis of neonatal meningitis is “the isolation of bacteria from the cerebrospinal fluid (CSF) of a neonate with a clinical diagnosis of neonatal sepsis”. As with blood cultures, even when suitable laboratory facilities exist, only a minority of pathogens are identified by CSF culture. In the absence of a positive CSF culture, elevated CSF white blood cell (WBC) count, elevated protein concentration and low glucose concentration can support a diagnosis of neonatal meningitis (Laving et al., 2003, Thomson et al., 2018). In Uganda and similar settings, there are also many cultural fears and concerns that surround lumbar punctures, which without education and careful informed consent, can further hinder the procedure (Thakur et al., 2015). Many cases of neonatal sepsis in low-resource settings do not undergo a lumbar puncture for CSF sampling, therefore the diagnosis of neonatal meningitis is often overlooked and the exact incidence of neonatal meningitis is not certain. Two studies in East Africa that undertook lumbar punctures in neonates presenting with clinical sepsis report a prevalence of meningitis of 3-18% in neonates with sepsis (Laving et al., 2003, Talbert et al., 2010).

THE ROLE OF CRANIAL ULTRASOUND

Affordable and feasible point-of-care tests are urgently needed to optimize the detection of neonatal meningitis and therefore improve outcomes in low-resource settings. Cranial ultrasound (cUS) is a relatively cheap, safe and portable method of imaging the neonatal CNS. In HICs, cUS already plays an important role in monitoring for complications such as post-infectious hydrocephalus and abscesses in confirmed cases of neonatal meningitis. It is conceivable that cUS could be used to detect findings indicative of CNS

involvement in cases of neonatal sepsis and improve the detection and management without having to rely on CSF sampling, expensive laboratory facilities and experienced personnel. Such an approach has the potential to be feasible, affordable and sustainable in a low-resource setting.

This novel study seeks to characterize the abnormalities on serial cUS examinations in term infants presenting with pSBI and describe the correlation between abnormalities on cUS images, clinical presentation, CSF culture and analysis, mortality, developmental impairment and post-infectious hydrocephalus.

CHAPTER 2 - LITERATURE REVIEW

GLOBAL NEONATAL MORTALITY

Globally, an estimated 5.2 million children under 5 years of age die every year (UNICEF, 2020). Almost half of these deaths occur during the first month of life (Liu et al., 2016, UNICEF, 2020)(Figure 1). The top three causes of neonatal death in LICs are; complications of prematurity, intrapartum complications and serious bacterial infections (Liu et al., 2015, Liu et al., 2016).

Neonatal mortality is the major barrier to a further reduction in under-five mortality. The third United Nations Sustainable Development Goal (UNSDG) seeks to end preventable deaths of neonates and reduce global neonatal mortality to 12 deaths per 1000 live births by 2030 (Ghabouli Shahroodi et al., 2016). The disparity in outcome between countries is vast. The NMR ranges from 2 per thousand live births in high-income countries (HICs) up to 42 per thousand live births in some LICs. Nearly all neonatal deaths occur in LICs, making neonatal care a prominent example of the global health inequality that exists.

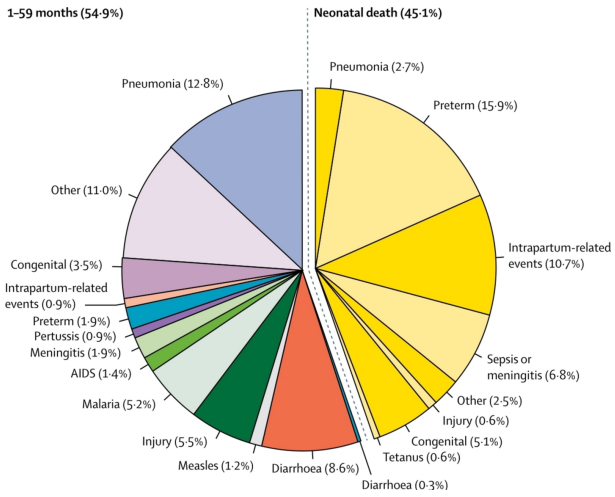


Figure 1: Primary causes of all under-five mortality, 2000-2015 (Liu et al., 2016)

DEFINING SERIOUS BACTERIAL INFECTIONS

Neonatal sepsis is defined as the presence of symptoms or signs of systemic infection within 28 days of birth accompanied by the isolation of a pathogen from the blood or CSF (Qazi and Stoll, 2009). Challenges exist with this definition as firstly; the signs and symptoms of neonatal sepsis are often non-specific and secondly bacterial isolation is rare. In low-resource settings, this is further compounded by the limited availability of laboratory facilities. Clinicians in low-resource settings typically rely on clinical algorithms to diagnose neonatal serious bacterial infections. Making a clinical diagnosis of neonatal sepsis can be challenging since the presentation can be subtle and the clinical features are non-specific and overlap with non-infectious aetiologies, such as prematurity, neonatal encephalopathy and cardiac failure (Camacho-Gonzalez et al., 2013). The Young Infants Clinical Signs Study identified seven clinical signs; difficulty in feeding, lethargy, convulsions,

respiratory rate >60 breaths per minutes, chest indrawing, axillary temperature >37.5°C or <35.5°C - that were sensitive clinical indicators for pSBI (Young Infants Clinical Signs Study, 2008). Together with the presence of jaundice, which is associated with neonatal sepsis, these eight signs are known as "neonatal danger signs". A pSBI is a clinical syndrome, based on these neonatal danger signs, that has been widely used in the Integrated Management of Neonatal and Childhood Illness (IMNCI) to identify neonates who require urgent referral to hospital and to help direct their management (Young Infants Clinical Signs Study, 2008). This pragmatic approach ensures a high sensitivity rather than a high specificity to improve the detection of what can be a devastating disease.

DIAGNOSIS OF NEONATAL SERIOUS BACTERIAL INFECTIONS

The 'gold-standard' for diagnosis of neonatal sepsis is the isolation of bacteria from the blood culture of a neonate with a clinical diagnosis of sepsis. Although, blood cultures are relatively easy to obtain and have a high specificity, their sensitivity in neonates is particularly poor and putative pathogens are rarely recovered (Vergnano et al., 2011). A large study of young infants with symptoms and/or signs of systemic infection (sepsis, meningitis or pneumonia) in multiple LMICs, isolated bacteria from less than 10% of cases using blood culture, many of which may actually be contaminants, leaving the aetiology of the majority of cases unknown (Hamer et al., 2015). Often insufficient volumes of blood are removed from neonates and additionally, neonates become symptomatic at relatively low levels of bacteraemia. The majority of neonates (68%) get septic with a colony count of less than 10

colony-forming units (CFU) per ml and almost half (42%) have counts ≤1CFU/ml (Kellogg et al., 1997). The automated colorimetric blood culture system, the BacTec/Alert system, will detect growth rapidly if one or two viable CFUs are in the blood inoculated into culture media (Schelonka et al., 1996, Brown et al., 1995). Ideally for neonates one millilitre of blood should be inoculated into the culture media for adequate sensitivity, however in reality most samples that are taken are less than 0.5ml (Neal et al., 1986). This means that the number of CFUs inoculated into the culture media may not be high enough for an automated blood culture system to detect bacterial growth (Schelonka et al., 1996, Brown et al., 1995, Kellogg et al., 1997).

The sensitivity of blood cultures is further reduced by the use of intrapartum antibiotics or prior over-the-counter antibiotic use in the neonate in the community, which is common in low-resource settings like Uganda (Kiwanuka et al., 2013). Cases of pSBI with negative blood cultures, may also be due to viral or parasitic pathogens or bacteria that are not easily grown using traditional microbiological techniques. A further challenge of blood cultures is their minimum reporting time of 48 hours, meaning that a neonate may have already succumbed to their infection before the results of a positive blood culture are available. Lastly, contamination of blood cultures from skin flora regularly arises during sampling or specimen processing and can lead to false positive results (Hall and Lyman, 2006). This is particularly important in settings like Uganda, where the skin of neonates is often heavily contaminated. Rigorous antisepsis procedures can help minimise contamination during sampling.

The lack of specificity of a positive blood culture for diagnosing a serious bacterial infection was demonstrated in a multi-site study of neonatal infections in hospitalised infants <2 months of age in six LMICs (Hamer et al., 2015). A negative blood culture did not exclude life-threatening neonatal infection. The mortality rates in those with probable pathogens detected on blood culture (7.0%) were similar to those with negative cultures (7.5%).

THE USE OF BIOMARKERS IN THE DIAGNOSIS OF NEONATAL INFECTIONS

In light of the challenges of traditional microbiological techniques outlined above, alternative methods of diagnosing neonatal sepsis have been investigated. In the absence of a positive blood culture, the presence of raised inflammatory markers such as c-reactive protein (CRP), procalcitonin and white blood cells can support a probable diagnosis of neonatal sepsis. These additional tests are infrequently available in low-resource settings and even when they are, they still have limited sensitivity and are not considered sufficiently accurate to detect all cases of neonatal sepsis (Brown et al., 2019, Vouloumanou et al., 2011).

A single value of CRP has low sensitivity, especially early in an infection, with CRP concentrations taking at least 12 hours to rise (Black et al., 2004, Benitz et al., 1998, Pourcyrous et al., 1993). CRP values may also be inaccurately interpreted due to a physiological rise in CRP the first days after birth or by maternal causes such as; premature rupture of membranes, maternal fever, meconium aspiration and fetal distress (Pourcyrous et al., 1993, Hofer et al.,

2011). A variety of cut-off levels have been used for CRP, but the most widely accepted level is 10mg/l (Meem et al., 2011). A recent Cochrane review of CRP in the initial evaluation of late-onset neonatal sepsis (LONS), found it to have a low sensitivity (0.62, 95%CI 0.50 to 0.73) and low specificity (0.74) (Brown et al., 2019). Undertaking repeat CRP measurements, at presentation and 24-48 hours after the onset of symptoms, has been shown to improve sensitivity and specificity to 74-89% and 74-95% respectively (Benitz et al., 1998, Pourcyrous et al., 1993).

Procalcitonin is another acute phase marker that has been utilized in the diagnosis of neonatal sepsis, however it is not used routinely due to cost. Although levels of procalcitonin rise faster than CRP, it is still affected by postnatal age of the neonate and gestational age (Chiesa et al., 2011). Overall sensitivity and specificity of procalcitonin are 81% and 79% respectively (Vouloumanou et al., 2011). Sensitivity is much lower (70-77%) in early-onset neonatal sepsis (EONS), however procalcitonin is more sensitive (82-92%) than CRP in diagnosis of LONS (Vouloumanou et al., 2011).

The interpretation of white blood cell counts in the neonatal period is challenging because it varies significantly with postnatal age and gestational age (Camacho-Gonzalez et al., 2013). The presence of lymphopaenia, neutropaenia and high immature/total ratio are all associated with EONS. In contrast to older patients, high levels of white blood cells and neutrophils are not helpful in reaching a diagnosis of EONS (Hornik et al., 2012). In the case of LONS, high or low white cell counts, high neutrophil counts, high

immature/total ratio and low platelet counts are associated. For both EONS and LONS the sensitivities of these findings are low and should not be relied upon (Hornik et al., 2012).

It is possible that inflammatory markers, like CRP and procalcitonin, could eventually prove to be useful adjuncts to differentiate between bacterial and viral infections, however, without an effective "gold standard" for the diagnosis of serious bacterial infections, it remains hard to establish the exact role of any of these tests.

THE USE OF MOLECULAR DIAGNOSTICS IN THE DIAGNOSIS OF NEONATAL INFECTIONS

Significant advances in molecular diagnostics have been made through the use of a number of techniques which include; quantitative PCR (qPCR), 16S rDNA amplicon sequencing, and sequencing of bulk DNA or RNA. Unlike culture, these techniques allow detection of both viable and non-viable bacteria, as well as difficult-to-culture pathogens. qPCR targets predefined bacterial species using specific primers and fluorescent probes and allows quantification of the target bacteria. This means that it will not identify previously uncharacterized or unusual pathogens. Alternatively, broad-range 16S rDNA PCR targets highly conserved regions of the 16S ribosomal subunit and can identify any bacterial DNA in a clinical sample. In 16S rDNA PCR, variable regions within these subunits can then be sequenced and compared with known nucleotide sequences to allow novel bacteria to be identified. 16S rDNA PCR is vulnerable to contamination as all bacterial DNA in the sample are amplified, including those from reagents.

In the absence of a rapid, sensitive and specific test for severe bacterial infections in neonates, clinicians continue to rely on a combination of clinical and laboratory findings (Zea-Vera and Ochoa, 2015). In low-resource settings, where little or no laboratory support exists, the diagnosis continues to rely almost entirely on clinical findings (Young Infants Clinical Signs Study, 2008).

THE BURDEN OF NEONATAL SERIOUS BACTERIAL INFECTIONS

It is estimated that serious bacterial infections account for 21% of neonatal deaths worldwide (Liu et al., 2016). These estimates are based on a systematic review of published data which then used verbal autopsy data to estimate the proportions of neonatal deaths. A robust meta-analysis estimated 6.9 million cases (5.5 – 8.3 million) of pSBI in neonates >1500g at birth with 680,000 (0.45-0.91 million) associated neonatal deaths per year (Seale et al., 2014). The authors estimated rates of up to 2.6 million cases of pSBI in SSA alone with an estimated 250,000 neonatal deaths (Seale et al., 2014). Due to challenges in diagnosis and under-reporting of neonatal deaths, these numbers are likely to be gross underestimates of the true burden.

NEONATAL MORTALITY AND THE ROLE OF NEONATAL SEPSIS IN UGANDA

Infant and child mortality rates are basic indicators of a country's socioeconomic status. Uganda has seen a dramatic reduction in mortality of children less than five years, however there has been no concurrent drop in neonatal mortality (ICF, 2017). Neonatal mortality rate (NMR) is defined as the

number of deaths before 28 days of age per 1000 live births. SSA bears a disproportionate burden of neonatal mortality and the NMR in Uganda has not changed for 2 decades (UBoSUa, 2017).

The leading causes of neonatal mortality in Uganda are similar to those seen globally; prematurity, perinatal asphyxia and neonatal sepsis (Health, 2008a). All three of these causes are intimately related to the quality of obstetric care available. Antenatal care (ANC) from a skilled provider is important to monitor a pregnancy and reduce the morbidity and mortality of both the mother and the baby during the pregnancy, delivery and postnatal period. Almost all women in Uganda (97%) attend at least one ANC clinic, however only 60% of women attend the recommended four ANC clinics during their pregnancies (ICF, 2017). Women residing in urban areas, those with higher household wealth and those with higher levels of educations are more likely to attend four or more ANC clinics.

Improving access to healthcare workers and clean deliveries can help reduce the risk of maternal and neonatal complications (Table 1) (Lawn et al., 2009). In 2016, only 73% of women in Uganda delivered in a health facility (ICF, 2017). Even for those who deliver in a facility, the level of infection prevention is still frequently not adequate.

Neonatal Mortality Rate (NMR)	Level of skilled birth attendant
Very low (≤ 5)	100%
Low (6-15)	99%
Moderate (16-30)	88%
High (31-45)	52%
Very high (≥ 45)	46%

Table 1: Association between level of Skilled Attendance at Birth and Neonatal Mortality Rate (Lawn et al., 2009)

THE GLOBAL INCIDENCE OF NEONATAL INFECTIONS

In HICs, estimates of the incidence of neonatal sepsis are relatively accurate due to good quality neonatal care and data systems and access to suitable laboratory tests. Incidence in such settings is less than one per 1000 live births (Stoll et al., 2011, Vergnano et al., 2011). In LICs, limited access to reliable laboratory testing means that the diagnosis of neonatal sepsis is based on clinical algorithms alone (Health, 2008a). The majority of estimates of incidence of neonatal sepsis are based on individual community-based studies where heterogeneous study designs make comparison challenging. In LICs, pSBI has however been estimated to occur in 49-170 per 1000 live births, culture-proven neonatal sepsis in 16 per 1000 live births and neonatal meningitis in 0.8-6.1 per 1000 live births (Liu et al., 2016, Seale et al., 2014, Seale et al., 2013, Thaver and Zaidi, 2009).

RISK FACTORS FOR NEONATAL SEPSIS

Neonates are more susceptible to infections than children of any other age (Camacho-Gonzalez et al., 2013, Levy, 2007, Malek et al., 1996). Neonates are born without endogenous microbial flora and they are quickly colonised with microbes from the maternal genital tract and their postnatal environment (Zaidi et al., 2005). Neonates are particularly susceptible to developing invasive infections if exposed to pathogenic bacteria due to their immature immune systems and incompletely developed skin barriers (Levy, 2007). In preterm neonates this risk is even higher due to lower levels of circulating maternal antibodies, a more immature innate immunity and more fragile skin (Malek et al., 1996). Known risk factors for neonatal sepsis include prolonged rupture of membranes, maternal chorioamnionitis, intrapartum fever and other maternal infections, low birthweight (<2.5kg) and maternal group B streptococcal carriage.

In low-resource settings, the risk of acquiring an infection in neonates is higher. Lack of skilled attendants encourages harmful practices such as cutting and tying the cord with non-sterile equipment. Mothers delivering in non-facility settings are also more likely to adopt harmful practices such as applying ash or cow dung to the umbilical stump, increasing risk of neonatal sepsis and neonatal tetanus (Burgoine et al., 2019, UBoSUa., 2017). For those hospital-born neonates or hospitalized neonates, there is a huge risk of nosocomial infection from various sources including colonised hands of healthcare workers, contaminated shared intravenous fluids and medications. Overcrowding, limited staffing, unreliable water supply, lack of alcohol-based

hand wash and lack of bleach and soap for basic cleaning of equipment further exacerbate poor sterilisation and disinfection practices.

Interventions can be introduced in antenatal and perinatal care to help reduce the transmission of infection. For mothers with a prolonged rupture of membranes, fever or signs of chorioamnionitis intrapartum, intravenous antibiotics can be given to reduce the risk of sepsis in the neonate. Community-based maternal education about neonatal danger signs has been shown to improve maternal knowledge and reduce neonatal mortality (August et al., 2016, Bhutta et al., 2011). Improved training for healthcare workers in the recognition of a sick neonate can improve the diagnosis and appropriate treatment of neonatal infections (Bulto et al., 2019, Kibaru and Otara, 2016).

Neonatal tetanus is a specific neonatal infection that is a major cause of neonatal deaths in LICs. In 2015, it was estimated that neonatal tetanus accounted for 34,000 neonatal deaths worldwide, although this is likely a gross underestimate, as many cases of neonatal tetanus occur in the community and their deaths go unreported (Khan et al., 2015). Neonatal tetanus can be prevented effectively by public health measures, including maternal immunisation, clean facility-based delivery and safe cord care (Khan et al., 2015). Maternal immunisation with the tetanus vaccine confers passive immunity to neonates and is estimated to reduce neonatal tetanus mortality by 94% (Blencowe et al., 2010). In Uganda, up to 29% of mothers do not receive adequate tetanus vaccinations (ICF, 2017).

CLASSIFICATION OF NEONATAL INFECTIONS – EARLY AND LATE ONSET NEONATAL SEPSIS

Conventionally, neonatal sepsis is divided into early-onset (EONS) and late-onset neonatal sepsis (LONS). Previous studies of neonatal sepsis have used a wide range of ages to stratify the onset of neonatal infections varying from 24 hours to 7 days (Newton and English, 2007). EONS is believed to be of maternal origin before or around the time of delivery. Chorioamnionitis, maternal intrapartum fever, prematurity, prolonged rupture of membranes and inadequate intrapartum antibiotic prophylaxis all increase the risk of EONS (Puopolo et al., 2011). Conversely, LONS presents after 48 hours and is more likely to be acquired from a hospital or community source after delivery. Traditionally the different origins of EONS and LONS lead to differences in the causative pathogens. In LICs, the distinction between the pathogens are less apparent, as described below. For these reasons, many researchers in LICs now prefer to classify neonatal sepsis as community-acquired versus hospital-acquired rather than the traditional nomenclature of EONS and LONS.

DEFINING NEONATAL MENINGITIS

Neonatal sepsis can lead to infection of the CNS causing inflammation of the ventricles (ventriculitis) and meninges (meningitis) (Barichello et al., 2013). Although neonatal meningitis can be isolated, it is believed to usually be secondary to an initial bacteraemia, with seeding of the CNS via the choroid plexus (Yikilmaz and Taylor, 2008). The infection then spreads into the

cerebral spinal fluid (CSF) causing ventriculitis and meningitis (Berman and Banker, 1966). Bacterial meningeal inflammation is also known to extend to the brain parenchyma (cerebritis) and cause cerebral oedema through disruption of the blood-brain barrier. There is also potential for the development of cerebral vasculopathy depending on the location of the inflammation (Berman and Banker, 1966, DiNubile et al., 1990). In combination with the prothrombotic states of sepsis, these vasculopathies have the potential to result in arterial and/or venous infarction. Purulent exudate can also obstruct the normal circulation of CSF through the aqueduct or foramina of the 4th ventricle resulting in post-infectious hydrocephalus (Perlman et al., 1992). Post-infectious hydrocephalus can also result from impaired resorption by inflamed arachnoid channels.

Neonatal meningitis can be a devastating illness and the mortality from neonatal meningitis remains higher than that of neonatal sepsis. A cohort study from the UK reported an overall mortality for neonatal meningitis of 6.6%, with the highest mortality associated with GBS (12%) and *E. coli* (15%) meningitis (Holt et al., 2001). In HICs, the mortality from neonatal meningitis has been associated with the presence of coma, delay in antibiotic administration, increasing prematurity and low birthweight (<2000g) (Holt et al., 2001). In should be noted too that the use of steroids has also been observed to increase mortality, this is possibly confounded by the fact that it is often the sickest neonates who receive steroids (Holt et al., 2001, de Louvois et al., 1991). The mortality from neonatal meningitis in LICs is estimated to be up to

58%, almost 9 times higher than that reported in HICs (Polin and Harris, 2001, Furyk et al., 2011).

The early diagnosis of neonatal meningitis is key to the prevention of death and neurological sequelae (Furyk et al., 2011). Unfortunately, the clinical presentation of neonatal meningitis can be subtle. Typical features of meningitis, such as bulging fontanelle, opisthotonus and seizures, that one might observe in an older child, are not always present (Mwaniki et al., 2011). The 'gold-standard' for diagnosis of neonatal meningitis is therefore the isolation of bacteria from the CSF of a neonate with a clinical diagnosis of neonatal sepsis. Selective criteria for performing an LP can delay or miss a diagnosis of meningitis especially in very low birthweight infants (Stoll et al., 2004a, Wiswell et al., 1995). Lumbar punctures have not been shown to be associated with increased mortality, however meningitis increases mortality (Stoll et al., 2004a). A lumbar puncture should therefore be performed to obtain a sample of CSF in all neonates where there is a suspicion of neonatal sepsis. Ideally a LP should be done prior to the administration of antibiotics if there are no contraindications and its performance does not substantially delay the treatment of the neonate. As with blood cultures, even when suitable laboratory facilities exist, only a minority of pathogens are identified by CSF culture. In the absence of a positive CSF culture, an elevated white blood cell (WBC) count, elevated protein concentration and reduced glucose concentration can help support a diagnosis of neonatal meningitis (Laving et al., 2003).

The incidence of neonatal meningitis in HICs is reported to be 0.21 - 0.39 per 1000 live births (Holt et al., 2001). Data from cohort studies in England and Wales show an incidence of neonatal meningitis in HICs is 0.27-0.44 per 1000 live births (de Louvois et al., 2005, Holt et al., 2001). The incidence increases up to 14 per 1000 for infants admitted to a neonatal unit and for preterm neonates (Stoll et al., 2004a, Sheth, 1998). Although bacterial CNS infection in neonates is not a common problem in HICs, in LICs the exact incidence of neonatal meningitis is hard to evaluate as many cases of neonatal sepsis in low-resource settings do not undergo a lumbar puncture for CSF sampling. Two Kenyan studies undertook lumbar punctures in neonates presenting with clinical sepsis and report a prevalence of neonatal meningitis of between 3-18% (Talbert et al., 2010) (Laving et al., 2003). The first study diagnosed meningitis in 3% of young infants based on one or more of positive CSF culture, bacteria on Gram stain, positive CSF antigen test for *Haemophilus influenzae* type b or *Streptococcus pneumoniae*, or white cell count ≥50 cells/µl (Talbert et al., 2010). The second study defined meningitis as one or more of positive CSF culture, bacteria on Gram stain or antigen test for *Haemophilus influenzae* type b, *Streptococcus pneumoniae,* GBS and *E. coli* (Laving et al., 2003). In both of these studies, CSF cultures were only positive in 4% of lumbar punctures, the remaining diagnoses were made based on CSF analysis, gram stain or antigen testing. It is clear that using CSF culture alone is not sufficient to diagnose neonatal meningitis and additional parameters such as white cell count and antigen testing should be considered to improve the detection. It should also be noted that there are data to suggest meningitis

can occur in the presence of normal CSF WBC, glucose and protein levels (Garges et al., 2006).

There are limited data available on accurate normal ranges for CSF WBC count, protein concentration and glucose concentration primarily because it is neither acceptable nor ethical to perform lumbar punctures in a healthy neonate. Various reference manuals and small studies provide CSF reference values for neonates (Ahmed et al., 1996, Martin-Ancel et al., 2006, Nascimento-Carvalho and Moreno-Carvalho, 1998, Byington et al., 2011, Kestenbaum et al., 2010, Shah et al., 2011). A recent large multicentre study of presumptively uninfected neonates has established age-specific reference values for CSF WBC counts, protein concentrations, and glucose concentrations in neonates (Thomson et al., 2018). The upper bound values for protein concentration and WBC count were; 127mg/dl and 15cells/mm^3 respectively. The lower bound value for glucose concentration was 25 mg/dL (1.4mmol/L). These are the values that will be assumed for interpretation in this study.

A diagnosis of neonatal meningitis has implications for dosage and duration of empirical antimicrobial therapy. Neonatal meningitis has a both higher mortality than neonatal sepsis and a higher risk of subsequent neurological impairment. In low-resource settings, without access to a lumbar puncture and CSF investigations, many cases of neonatal meningitis go undiagnosed, leading to inappropriate or inadequate treatment and subsequently a higher mortality and morbidity (Berkley et al., 2004). The early recognition and

appropriate treatment of meningitis are vital to improve these outcomes. Affordable and feasible point-of-care tests are therefore urgently needed to optimize detection and therefore the outcome of neonatal meningitis, especially in a low-resource setting.

THE AETIOLOGY OF SERIOUS BACTERIAL INFECTIONS

Knowledge of the pathogens that cause neonatal infections is essential for appropriate prevention and treatment strategies to be developed, and yet our knowledge of these pathogens remains limited. Although the sensitivity of blood culture is low, studies that have isolated bacteria have demonstrated a broad range of Gram-positive and Gram-negative pathogens. As previously mentioned, EONS is conventionally considered to be maternally-acquired and LONS is considered to be environmentally acquired. In HICs the most common cause of EONS is Group B *streptococcus* (GBS), which accounts for the majority of cases (43-58%), followed by *Escherichia coli (18-29%) (Stoll et al., 2011).* The other commonly identified pathogens include *Staphylococcus aureus,* Coagulase-negative *staphylococcus* (CoNS), *Listeria monocytogenes* and other gram-negative bacteria. In HICs, LONS predominantly affects hospitalised preterm infants (Stoll et al., 2002, Vergnano et al., 2011). The main pathogen responsible for these episodes of LONS is CoNS (39-54%), which is associated with prolonged hospitalisations, the use of central lines, parenteral feeding, prematurity and mechanical ventilation. One would therefore not expect to find such a high incidence of CoNS in a LIC where these risks are minimal. A high incidence of *Candida* infections are also reported in neonatal units in HICs (Fridkin et al., 2006, Brian Smith et al., 2005,

Fernandez et al., 2000). Similar to CoNS, the invasive therapies used to improve survival of neonates in HICs contribute to this. Other pathogens commonly detected in LONS in HICs are *E. coli* and *Klebsiella.* Whilst less commonly detected pathogens are *S. aureus, Enterococcus* sp and *Pseudomonas aeruginosa.*

In LICs, the distinction between the pathogens in EONS and LONS is less apparent. It is very clear from the studies described below that, for both EONS and LONS, the vast number of isolates are either *S. aureus* or gram-negative organisms such as *E. Coli., Klebsiella* spp., *Acinetobacter* spp. (Hamer et al., 2015, Group, 1999c, Waters et al., 2011, Zaidi et al., 2009). This is likely due to unclean delivery practices and poor infection control in health-facility deliveries leading to early nosocomial infections (Zaidi et al., 2005). In LICs, a high rate of home deliveries exposes neonates to community-acquired pathogens much earlier in the neonatal period (Zaidi et al., 2005). It is also possible that due to the unhygienic delivery, poor cord care and postnatal practices both at home and in health facilities, that many more of the EONS infections in LICs are contracted from the environment rather than vertically from the mother (Zaidi et al., 2009).

To date, many studies in LICs have focused on hospital deliveries due to the challenges of implementing sufficient laboratory support in lower level health facilities. In addition, in many hard to reach rural areas numerous neonates delay or even fail to seek medical care and therefore succumb to neonatal sepsis before reaching a health facility. Given the potential difference in the

aetiology of neonatal sepsis depending on the place of birth, it is vital that in LICs where home births contribute to a large proportion of births, studies of neonatal sepsis consider neonates born both at home and in health facilities.

COMMUNITY-ACQUIRED NEONATAL SEPSIS

In LICs, both in community-based and hospital-based studies, the most common pathogens reported are *E. coli, Klebsiella* and *S. aureus* (Zaidi et al., 2005). Waters et al. undertook a review of the aetiology of community-acquired neonatal sepsis in developing countries and included 2066 cases where organisms were isolated from blood and/or CSF (Waters et al., 2011). Globally they reported the most prevalent pathogens to be *S. aureus (15%), E. coli (12%) and Klebsiella (12%).* When they analysed their 583 cases from African studies, *S. Aureus* was the leading pathogen (15%), followed by *E. coli* (11%). GBS (7%) and *Streptococcus pyogenes (7%)*. In their comparison of pathogens detected before and after 7 days of age, the leading pathogens were almost identical (*S. aureus, E. coli, Klebsiella, Pseudomonas* and GBS). This supports the hypothesis that the timing of sepsis in such settings is not so important and that the source of the infection, community versus hospital, is likely to be more significant.

In another meta-analysis of community acquired neonatal infection in LMICs, 2594 bacterial isolates from cases of presumed community-acquired neonatal sepsis, pneumonia and meningitis were identified (Zaidi et al., 2009). Again, the common pathogens isolated were *Escherichia Coli* (17%), *Klebsiella* species (13%), *Staphylococcus aureus* (13%) and GBS (8%). The subset of

results from Africa were similar to the overall global meta-analysis, however *Streptococcus pneumoniae* and *Salmonella* species also contributed substantially in this region. There were 1058 bacterial isolates from the African region and the most common pathogens isolated were *Escherichia Coli* (9%), *Klebsiella* species (8%), and *Staphylococcus aureus* (11%), GBS (15%), *S. Pneumoniae* (12%), *Salmonella* spp. (11%).

Hamer et al., undertook a large multi-site study of community acquired infections in young infants (0-59 days) in Bangladesh, Bolivia, Ghana, India, Pakistan and South Africa (Hamer et al., 2015). They actually only undertook blood cultures in 54% (784) of infants admitted and probable pathogens were only identified in 10.6% of blood cultures (Hamer et al., 2015). Again, *S. aureus* was the most commonly isolated pathogen (43%). Similar to the previous review, gram negative bacteria, *Acinetobacter* spp., *E. Coli.* and *Klebsiella* spp., made up almost half (47%) of the other probable pathogens detected. Again, there was no apparent difference in the aetiology between the younger (0-6 days) and older (7-59 d) infants in this study.

One factor that should be considered in all of the above studies is the potential for contamination during the collection of the blood cultures. It is feasible that a large number of the *S. Aureus* cultures reported by these studies were actually contaminants due to the challenges of sample collection described above and the relative ease of culturing *S. Aureus* compared to other more fastidious bacteria such as GBS. Although gram-negative bacteria are less likely to be contaminants, it is also possible. Any future studies should carefully

consider the blood culture results in light of the treatment administered, the sensitivities of the bacteria identified and the clinical response (Saha et al., 2018). Such an approach would help exclude those bacteria that are likely to be contaminants and is the approach that was adopted in this thesis.

HOSPITAL-ACQUIRED NEONATAL SEPSIS

Studies using microbiological techniques that have focused on hospital-acquired neonatal infections in Africa, have also primarily cultured *Escherichia coli* (5-16%), *Klebsiella* (16-28%), *Staphylococcus aureus* (8-22%) (Zaidi et al., 2005, Zaidi et al., 2009, Downie et al., 2013, Stoll et al., 2011). Other frequently isolated pathogens in hospital-acquired neonatal infections include GBS (8.5%), CoNS (7-28%), S pneumoniae (2%), *Pseudomonas* spp. (3-10%), *Citrobacter* spp. (3%) and *Enterobacter* spp. (4-12%) (Zaidi et al., 2005, Zaidi et al., 2009, Downie et al., 2013).

Due to the challenges in infection prevention in many low-resource settings, many early-onset neonatal infections in hospital-born babies may actually be hospital-acquired, rather than vertically acquired. *S. Aureus* is mainly spread through poor handwashing of healthcare workers, a situation that is often exacerbated by lack of water and/or alcohol-based hand rub. *Klebsiella* forms part of the normal maternal gastrointestinal and vaginal flora, however in many instances the *Klebsiella* that are isolated are highly drug resistant, suggesting perhaps that these are environmentally acquired in the hospital. Infections due to CoNS are normally associated with intensive and invasive neonatal care, particularly in preterm infants. Although the use of invasive therapies in

neonatal units in Africa is limited, CoNS infections may represent the increasing availability of neonatal care in these regions, together with poor aseptic techniques around the time of insertion of intravenous cannulae or medication and fluid administration. It may also be due to contamination at the time of sampling. The role of CoNS in low-resource settings therefore needs further exploration to be fully understood.

Group B Streptococcus

Maternal colonisation with GBS is the main risk factor for neonatal GBS infection. Studies using microbiological techniques have shown that the rates of maternal GBS colonisation in SSA (19-34%) are similar to rates in HICs (Stoll and Schuchat, 1998, Suara et al., 1994, Dawodu et al., 1983, Monyama et al., 2016, Medugu et al., 2017). Although a more recent Kenyan study that used whole-genome sequencing to detect maternal GBS colonisation reported a relatively lower prevalence (12%) of maternal colonisation (Seale et al., 2016).

GBS is a leading cause of neonatal sepsis in HICs, but until recently, GBS was seldom reported as a bacterial cause of neonatal infection in LICs (1999). There are various explanations for the apparent low incidence of neonatal GBS infections. It is possible that GBS infections may be underrepresented in many studies because up to 90% of cases present within 12 hours of birth and therefore neonates infected with GBS might die before reaching a health-care facility (Seale et al., 2016, Airede, 1992, Quiambao et al., 2007) The fastidious nature of the bacteria means that it may also go undetected, particularly if

laboratory facilities are not adequate. In addition, previous studies of maternal GBS colonisation in SSA have found less virulent serotypes (type V) of GBS, which may be a reason for low levels of GBS infections in neonates (Suara et al., 1994). Other studies in SSA have reported higher rates of GBS infections in neonates, similar to rates seen in HICs (Berkley et al., 2005, English et al., 2003, Gray et al., 2007, Laving et al., 2003, Madhi et al., 2003, Nathoo et al., 1991). The incidence of early-onset GBS infection was found to be 2/1000 live births in South Africa and 0.9/1000 live births in Malawi (Gray et al., 2007, Madhi et al., 2003). Unfortunately, the case-fatality rates of GBS infection were 20% and 38% respectively, which are much higher than in HICs. The Kenyan study that used whole-genome sequencing also reported a similarly high incidence of early GBS infection, 0.8/1000 live births, with an associated mortality of 47% (Seale et al., 2016). Based on these findings, it is now estimated that globally GBS may cause up to 319,000 cases of neonatal infection each year, with almost half of cases (169,000) occurring in SSA (Seale et al., 2017). These GBS infections may account for up to 90,000 neonatal deaths globally each year, with the majority, 54,000 occurring in SSA (Seale et al., 2017).

CONGENITAL MALARIA

It is estimated that malaria in pregnancy accounts for 100,000 neonatal deaths every year (Eisele et al., 2012). Placental malaria significantly increases the risk of preterm birth, low birth weight, intrauterine growth restriction and intrauterine fetal death *(Osungbade and Oladunjoye, 2012, Olupot-Olupot et al., 2018)*.

Transplacental transmission of malaria parasites from the mother to the baby in utero or during delivery can result in congenital malaria. The diagnosis is made upon detection of asexual forms of malaria parasites on a blood smear of the peripheral blood of the neonate, or later if there is no possibility of postpartum infection (Menendez and Mayor, 2007). Congenital malaria still remains an important diagnosis to consider in any neonate with signs of neonatal infection who is born to a mother in a malaria-endemic area (Olupot-Olupot et al., 2018). In neonates, the most common symptom of malaria is fever, other symptoms overlap with sepsis and can include anaemia, jaundice, diarrhoea, vomiting, lethargy, convulsions, irritability, tachypnoea, respiratory distress and hepato-splenomegaly *(Covell, 1950, D'Alessandro et al., 2012, Olupot-Olupot et al., 2018)*. To maximise the chances of early detection of congenital malaria, neonates born to mothers with malaria in the last 7 days before delivery, irrespective of the clinical picture, should be investigated with a blood film for malaria parasites at birth and weekly for the first month (Olupot-Olupot et al., 2018). In practice it is often not routine to test neonates for malaria, and therefore, many cases may be overlooked.

IMPROVING PATHOGEN DETECTION IN NEONATAL INFECTIONS

Overall, the results of these aforementioned studies demonstrate clearly that the vast majority of cases of pSBI have no detectable pathogen using these standard microbiological techniques. This supports the urgent need for improved diagnostic tests in pSBI in order to identify other potential pathogens. A recent large study in Bangladesh, the ANISA study, undertook both blood

culture and species-specific PCR for 15 bacterial and 13 viral pathogens on blood and respiratory samples in cases of neonatal serious infections (Saha et al., 2018). Using this combined approach, they detected pathogens in up to 28% of 6022 episodes of pSBI, 16% were bacterial and 12% were viral. The leading pathogen detected in these cases of pSBI was respiratory syncytial virus (RSV), followed by *ureaplasma* spp. (Saha et al., 2018). Despite this combined approach of culture and PCR, the majority of cases of pSBI still had no identifiable pathogen. This suggests two key possibilities, firstly that a substantial proportion of pSBI may not actually be due to bacterial or viral infection and secondly, that we have not yet identified the causative pathogens. Although species-specific qPCR is highly sensitive and specific for the identification of anticipated pathogens, novel and unexpected pathogens will still be missed. Alternatively, 16s rDNA PCR can be used to identify any bacterial DNA present in the blood or CSF and the variable regions can then be sequenced to identify previously uncharacterized or unusual pathogens. Although this method holds promise for detection of pathogens in future studies of pSBI, it is not without complications as it is highly susceptible to contamination.

In the absence of satisfactory diagnostic tests for pSBI and given that pSBI is a high-risk disease that is associated with poor outcomes, many clinicians continue to over treat for fear of missing a treatable infection with possible fatal or serious consequences. This approach has its own risk of unnecessary and often prolonged exposure to antibiotics, which in turn increases antibiotic resistance. It can also disrupt the normal development of the gut microbiome.

AETIOLOGY OF MENINGITIS

Data on the aetiology of neonatal meningitis in LICs are even more limited, firstly because many cases of neonatal sepsis in low-resource settings do not undergo a lumbar puncture for CSF sampling and secondly due to the poor sensitivity of CSF culture. Older studies from Kenya that used CSF culture, reported *Klebsiella* to be the most common pathogen (Kasirye-Bainda and Musoke, 1992, Musoke and Malenga, 1984). The addition of latex particle agglutination assays to CSF culture in a later Kenyan study found *E. coli.,* GBS and *Klebsiella pneumoniae* to be the most common isolates in cases of neonatal meningitis (Laving et al., 2003). A 10-year review of neonatal meningitis in Ethiopia, identified 55 cases and reported that over two-thirds of the cases were accounted for by *K. pneumoniae (30%), E. coli(23%) and Enterobacter* species (13%) (Gebremariam, 1998). In Malawi, a study of 60 culture positive isolates from CSF in neonates <7 days of age found GBS (45%), *Strep. Pneumoniae* (22%) and nontyphoidal *Salmonella enterica* (12%) to be the most common pathogens (Swann et al., 2014). Their findings were similar for the 191 older cases aged 7 days to <2 months: GBS (20%), *Strep. Pneumoniae* (42%) and nontyphoidal *Salmonella enterica* (18%). A study of neonatal meningitis from Nigeria reported *E. coli* (11/50) and *Staphylococcus aureus* (13/50) to be the predominant pathogens detected whilst *N. meningitidis* and *H. influenzae* were also detected (Airede et al., 2008).

The majority of data on the aetiology of neonatal meningitis originates from HICs and studies report GBS and *E. coli* to be the most commonly identified

causes of neonatal meningitis (Kimberlin, 2002, Sheth, 1998). A study from the United States reported *E. coli, Klebsiella* and GBS to account for 19%, 8% and 53% respectively of the positive CSF isolates (Kimberlin, 2002). In the UK, a study of neonatal meningitis found GBS (28%), *E. coli* (18%) and *Listeria monocytogenes* (5%) to account for the majority of organisms cultured from CSF (de Louvois et al., 1991). Similarly, a more recent report of neonatal meningitis in the UK also found GBS (42%) and *E. coli* (16%) to be the most commonly isolated organisms on CSF culture. *Haemophilus influenza type B, Neisseria meningitides and Streptococcus pneumonia* often cause meningitis in older infants and children but are rarely found in neonatal cases. In addition, coagulase negative staphylococcus (CoNS) is occasionally cultured (0.4-1.5%) from CSF in HICs. As discussed above, in LICs many of the neonates admitted to a neonatal unit will not experience the risk factors associated with CoNS infections in HICs.

One complication of meningitis is the development of cerebral abscesses. These are well-circumscribed collections of purulent fluid within the brain parenchyma. Brain abscesses are typically associated with gram-negative bacteria including *Acinetobacter, C. Enterobacter, Salmonella, Serratia, Proteus and Pseudomonas.* Cerebral abscesses have been reportedly associated with the gram-negative bacilli, *Citrobacter* (Algubaisi et al., 2015, Agrawal and Mahapatra, 2005).

Certain pathogens are also known to cause haemorrhagic meningoencephalitis. *Serratia marcescens,* or *Citrobacter* are two such organisms (Ariel et al., 1986, Kline, 1988). Infections with *Citrobacter* are often acquired from the environment, however they can be vertically acquired in the perinatal period. In two reviews of cases of *Citrobacter* meningitis, almost all of the cases (86-91%) were caused by *Citrobacter koseri* and three-quarters had evidence of brain abscesses *(Graham and Band, 1981, Doran, 1999). C. koseri* resides primarily in neutrophils and rapidly penetrates the CSF allowing the bacteria to survive and replicate despite treatment. Infection with *C. koseri* meningitis in neonates often causes poor feeding, vomiting, lethargy, apnoea, seizures, irritability and bulging fontanelle (Algubaisi et al., 2015). Neonatal *Citrobacter* infections have a high morbidity and mortality. *Citrobacter* neonatal meningitis is associated with a 30% mortality and over 80% of survivors have abnormal neurology or neurodevelopmental delay (Doran, 1999, Agrawal and Mahapatra, 2005).

The gram-positive bacteria, *Bacillus cereus* and *Listeria monocytogenes* can also be responsible for meningoencephalitis. *Bacillus cereus* is a rare cause of neonatal meningoencephalitis but causes such extensive damage and necrosis of the brain that in most cases it is fatal (Chu et al., 2001, Hendrickx et al., 1981, Weisse et al., 1991). The organism swarms out from the veins into the tissues and the enterotoxin, phospholipases, proteases and haemolysins induce widespread liquefactive necrosis. A case series of three preterm neonates with *Bacillus cereus* meningoencephalitis reported deaths in all three cases despite appropriate treatment (Lequin et al., 2005).

Symptomatic infection with *L. monocytogenes* is 18 times more common in pregnant women than nonpregnant women and can lead to spontaneous abortion, preterm delivery, stillbirth or neonatal infection (Lamont et al., 2011). Neonatal listeriosis can present with one or more of sepsis, meningitis and pneumonia. Early-onset neonatal listeriosis has a higher mortality of up to 40% and neurological sequelae are reported in 13% of survivors (Lamont et al., 2011, Okike et al., 2013, Ahlfors et al., 1977). Late-onset neonatal listeriosis, has a mortality of less than 10% and neurological sequelae are relatively uncommon.

FUNGAL CENTRAL NERVOUS SYSTEM INFECTIONS IN THE NEONATE

Candida has also been shown to cause meningitis, ventriculitis and parenchymal infections of the neonatal brain (Faix and Chapman, 2003). CSF findings in *candida* infection are variable and normal CSF parameters do not exclude CNS involvement (Fernandez et al., 2000). In addition although less common, brain abscesses have been shown to be a complication of neonatal candida infection (Pahud et al., 2009). Neonatal *candida* infection of the CNS has an associated high mortality and high rate of developmental delay and neurological impairment in survivors (Moylett, 2003, Fernandez et al., 2000, Pahud et al., 2009).

VIRAL CENTRAL NERVOUS SYSTEM INFECTIONS IN THE NEONATE

Although bacterial neonatal meningitis is traditionally perceived as the most dangerous form of neonatal meningitis, it is likely that viruses contribute to a large proportion of neonatal meningitis cases, either as the sole pathogen or as a co-infection. Even in HICs, it is likely that viral meningitis is under reported as it is not often tested for. A study from England and Wales reported 56% of culture negative CSF samples to be positive for viruses (de Louvois et al., 1991, Holt et al., 2001).

Enterovirus (EV) infection in the neonate can range from asymptomatic to life-threatening disease (Abzug, 2004). Severe disease from EV infections is more frequent in the first 2 weeks of life and can lead to meningitis as well as myocarditis, pneumonia and hepatitis (Abzug, 2004). EV meningitis is a common, although perhaps an underdiagnosed cause of neonatal meningitis. Neonatal EV infections are associated with significant mortality rates (12%) and neurological sequelae in survivors (Ghabouli Shahroodi et al., 2016, Abzug, 2004). A study in Iran that used real-time PCR detected EV in the CSF of one-third (13/37) of neonates with neonatal meningitis (Ghabouli Shahroodi et al., 2016). Interestingly, only one quarter of these neonates had a concurrently elevated WBC count in the CSF. One case also had bacterial meningitis and two cases had concomitant bacterial sepsis.

Globally, CMV is the leading cause of congenital infections. The prevalence of CMV infections in adults is higher in LICs than in HICs, however the incidence of congenital CMV infection remains poorly described (Manicklal et al., 2013).

In LICs, the prevalence of congenital CMV has been reported to be as high as 6-14% (Madrid et al., 2018, Cannon and Davis, 2005). CMV can be spread transplacentally and may result in symptomatic or asymptomatic infection in the neonate. In-utero, the fetus can be infected by either a newly acquired (primary) maternal infection or a recurrent (reactivated) maternal infection. The risk of contracting congenital CMV is highest for infants of mothers who acquire a de-novo CMV infection in the first trimester, for such neonates one-quarter will develop sensorineural hearing loss and one-third will develop CNS sequelae. Recurrent infection is the main contributor to the total number of congenital CMV infections worldwide although the risk of transmission is much lower than in primary infection. A recent study of hydrocephalus in Ugandan infants, detected CMV in the CSF of 12% of infants with post-infectious hydrocephalus but none of the infants with congenital hydrocephalus (Paulson et al., 2020). Thus, suggesting that CMV may play a role in the development of neonatal meningitis and subsequent post-infectious hydrocephalus.

NEUROIMAGING CHARACTERISTICS OF NEONATAL INFECTIONS

As previously discussed in '*DEFINING NEONATAL INFECTIONS', m*any infants with sepsis present with non-specific symptoms and signs such as poor feeding, lethargy, fever and hypothermia. Similarly signs of meningitis such as bulging fontanelle, seizures and irritability are not always present in neonates. Despite their limitations, ideally neonates with signs of infection should be thoroughly investigated including blood culture, CSF culture and CSF analysis. For neonates with confirmed meningitis neuroimaging for initial screening of complications such as abscess formation is indicated. Although magnetic

resonance (MR) imaging is the diagnostic method of choice, it is expensive and has logistical limitations, therefore cranial ultrasound (cUS) is normally the method used (Algubaisi et al., 2015). The major advantages of cUS are the relative affordability, the size and portability of the equipment allowing point-of-care examinations, the rapidity with which the images can be obtained, the lack of ionizing radiation and lack of need for sedation. In HICs, cUS routinely plays an important role in the evaluation of sick neonates particularly in cases of prematurity, encephalopathy, seizures and meningitis. Neonatal meningitis can severely damage the developing brain and bedside cUS of these neonates allows the early detection of CNS pathology such as ventricular dilatation, calcification, haemorrhage, cysts, white matter (WM), central grey matter and cortical changes, and cerebellar abnormalities. In HICs, cUS is usually the first imaging modality used in the evaluation of neonatal meningitis. The primary ultrasound establishes the presence of normal anatomy, evidence of any longstanding injuries such as cysts or calcifications and any acute injuries such as haemorrhages or infarctions (Algubaisi et al., 2015). As described below, serial scans enable screening for the development of complications such as cystic change, abscesses and post-infectious hydrocephalus (Algubaisi et al., 2015).

Introduction to Cranial ultrasound

During a cUS the examiner scans the entire brain via the anterior fontanelle in both the coronal and sagittal planes to allow visualization of the cerebral structures and any abnormalities. A minimum of 11 images are then normally recorded, including 6 coronal images, one midline sagittal, two left and two

right parasagittal views. Images of any potential pathologies are also recorded in both planes. The cUS takes a “slice” through the structures resulting in a 2-dimensional image of a 3-dimensional structure. Most cUS examinations are performed through the anterior fontanelle, this is the standard acoustic window for imaging the neonatal brain (Figure 2). If better visualization of the posterior fossa structures is needed, the posterior and mastoid fontanelles can be used (Figure 2). Images of the neonatal brain are obtained in both the sagittal and coronal planes to ensure a complete examination (Figure 3). If images are not obtained in both planes, then pathology can be missed, or false diagnoses can be made. Higher-resolution images can be obtained using high-frequency curvi-linear transducers (5-7.5KHz) though this may not be suitable for delineating deep structures. If near-field scanning is needed in order to evaluate superior sagittal sinus thrombosis, extra-axial collections or cortical infarctions, then it is better to use a linear array, higher frequency transducer (7-12MHz). High-quality images are best achieved if the baby is calm or sleeping. The quality of the images can be limited by excessive movement of the neonate, a small fontanelle and thick hair.

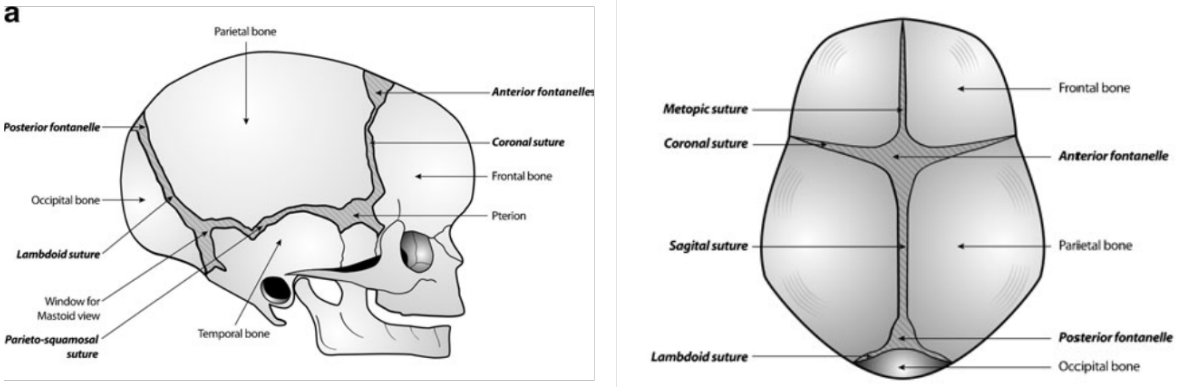


Figure 2: Acoustic windows for neonatal cranial ultrasound (Janet M. Rennie, 2009)

NORMAL CRANIAL ULTRASOUND

For a comprehensive examination of the neonatal brain, the whole brain should be scanned via the anterior fontanelle in both the sagittal and coronal planes. This allows the examiner to visualize the cerebral structures and any abnormalities. A minimum of six images should then be obtained in the coronal plane and a minimum of five images in the sagittal plane (Figure 3). Images in both planes should also be obtained for any potential pathologies.

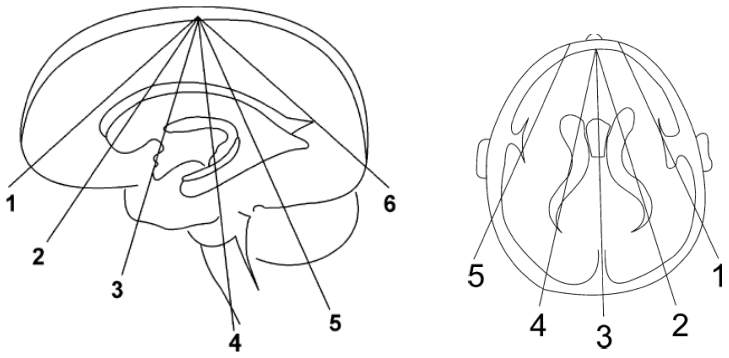
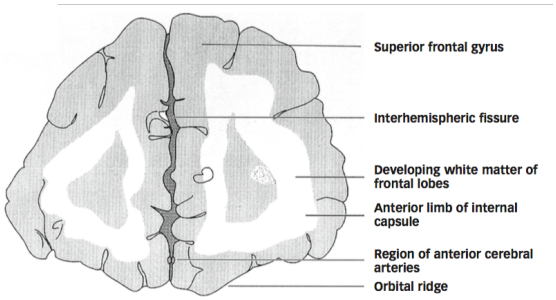


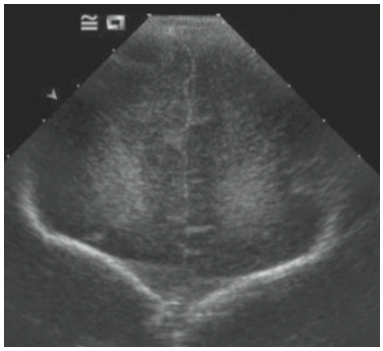
Figure 3: Standard images in coronal (left) and sagittal (right) planes via the anterior fontanelle (Meijler, 2012)

For the coronal images, the examination begins anteriorly and sweeps posteriorly. Although there is a minimum of six images, the operator should ensure additional images are recorded of any abnormalities noted in between the six images. Traditionally, the first and most anterior image obtains an image of the frontal lobes of the cerebral cortex and is taken just anterior to the frontal horns of the lateral ventricles (Figure 4). The second image is just posterior and demonstrates the corpus callosum, frontal horns of the lateral

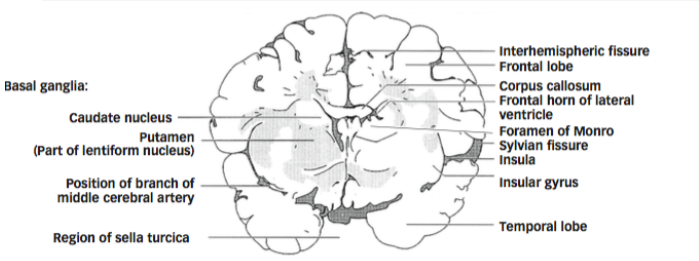
ventricles and temporal lobes. The CSF in the ventricles appears dark. The cavum septum pellucidum lies between the lateral ventricles and can be large in preterm infants. The third image demonstrates the basal ganglia, lateral ventricles, corpus callosum, septum pellucidum and the third ventricle below the lateral ventricles. It also demonstrates the Sylvian fissures as echogenic linear structures separating the frontal lobes from the temporal lobes. The fourth image demonstrates the parietal lobes, basal ganglia and thalami, the lateral ventricles and corpus callosum. More posteriorly in the fifth image, the corpus callosum is less clearly seen, the trigone of the lateral ventricles and the choroid plexus are seen. The choroid plexus fills the lateral ventricles in this view and is particularly prominent in preterm infants. The white matter in this view can sometime appear bright and is called periventricular flare. In the sixth and most posterior image the parieto-occipital lobe is visualised.

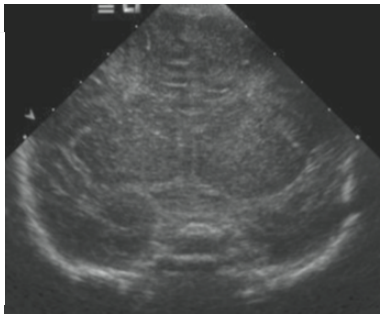
1. *First coronal plane at the level of the frontal lobes*



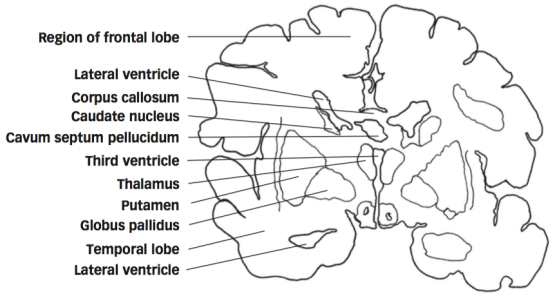


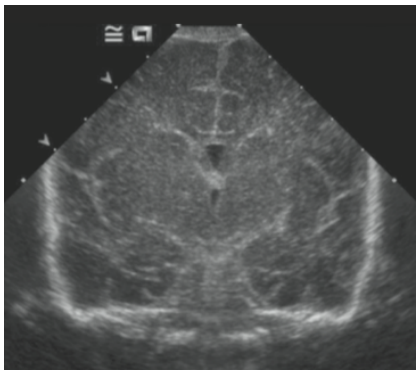
2. *Second coronal plane at the level of the frontal horns of lateral ventricles*



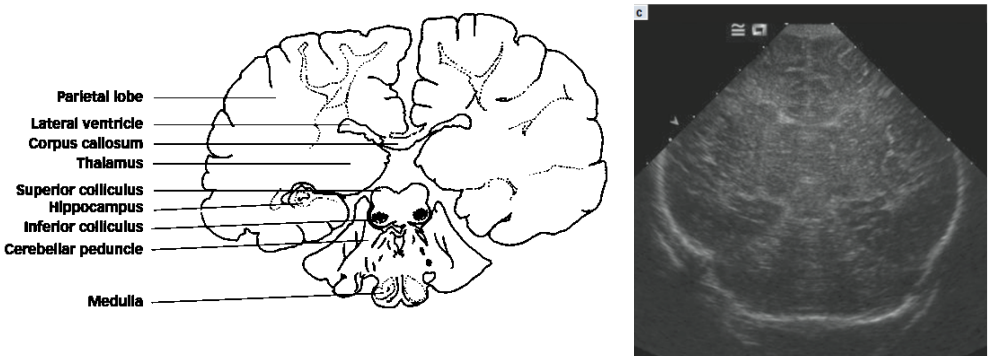


3. *Third coronal plane at the level of the third ventricle*

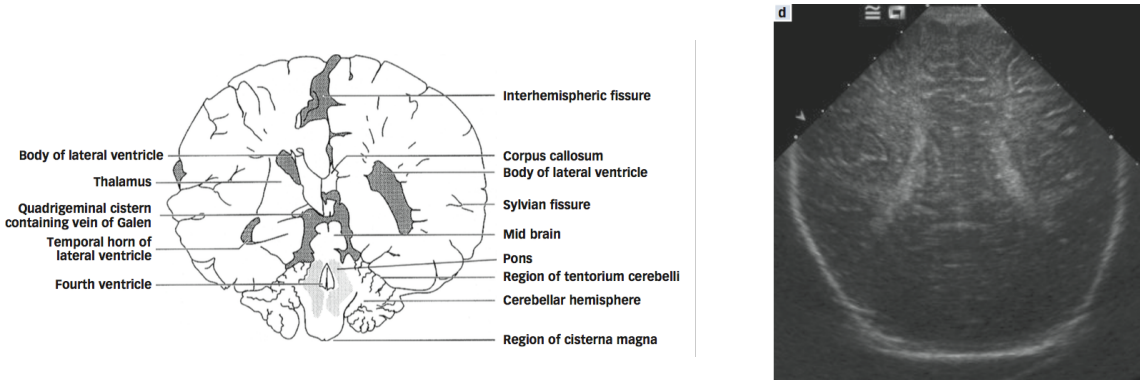




4. *Fourth coronal plane at the level of the cerebellum*



5. *Fifth coronal plane at the level of the trigone of the lateral ventricles*



6. *Sixth coronal plane through the parieto-occipital lobes*

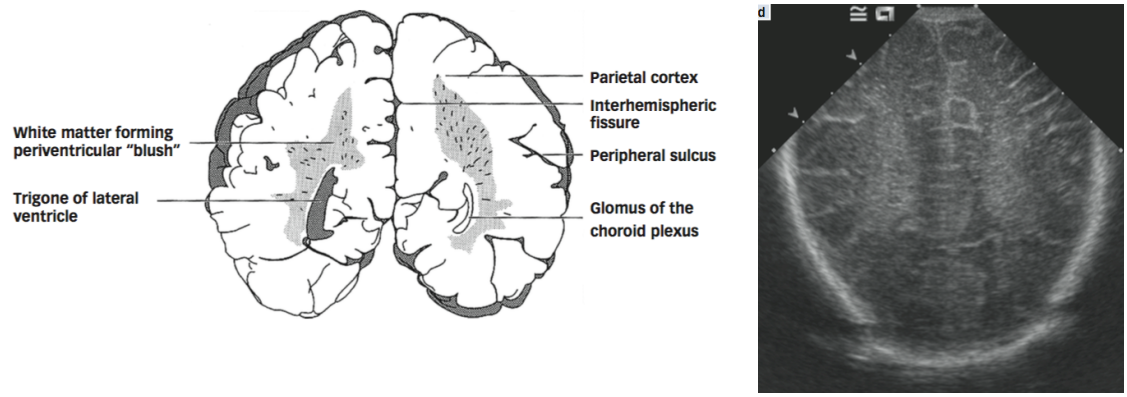
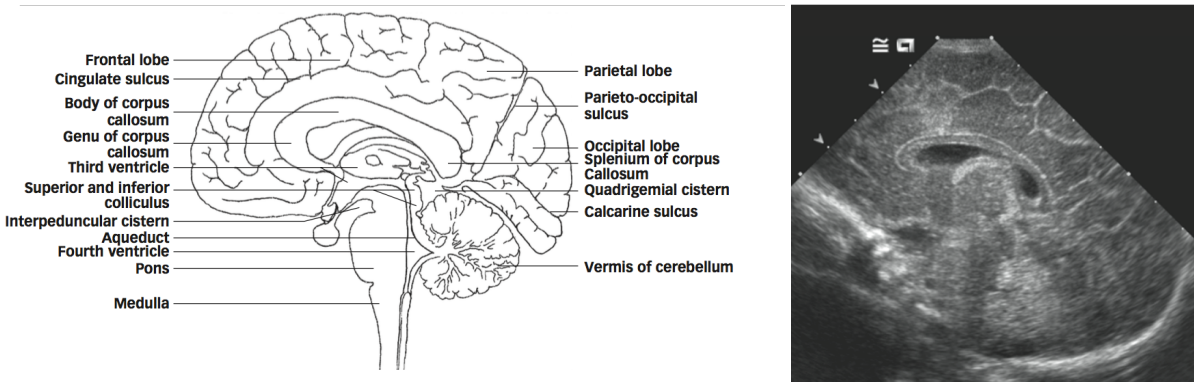


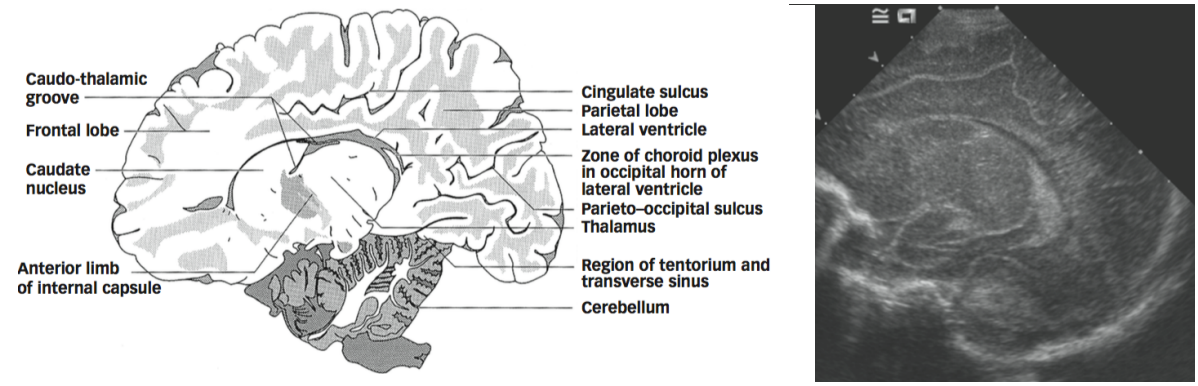
Figure 4: Normal anatomy of images in the coronal planes (Janet M. Rennie, 2009)

For the sagittal images, the examination begins in the midline and sweeps laterally right and left (Figure 5). Again, although there is a minimum of five sagittal images, the operator should ensure there are additional images of abnormalities noted between the five images. Traditionally, the first image is in the midline noting corpus callosum extending from anterior to posterior with the cingulate gyrus superior and parallel to the corpus callosum. It also allows visualization of the cerebellar vermis as an echogenic image in the posterior fossa. The 4th ventricle lies in front of this. The second image is undertaken bilaterally through the right and left lateral ventricles and is the parasagittal. It is identified by the shape of the lateral ventricle. The head of the caudate nucleus lies below the floor of the frontal horn and the thalamus lies behind it with a “notch” in between called the caudo-thalamic notch, a common site for germinal matrix haemorrhage in preterm infants. The choroid plexus starts in the caudothalamic notch and extends posteriorly. In preterm infants the choroid plexus is particularly prominent, and it can sometimes be hard to differentiate bulky choroid from haemorrhage. In the parasagittal image, a clear view on the thalami and the basal ganglia as well as the periventricular white matter is possible. The third image is the angled tangential parasagittal image, which visualizes the white matter adjacent to the lateral ventricles out to the Sylvian fissure. In order to view the Sylvian fissure and cortex well a view that is further lateral is needed.

1. *Midline sagittal image*



2. *Parasagittal image through the lateral ventricles*



3. *Angled tangential parasagittal image*

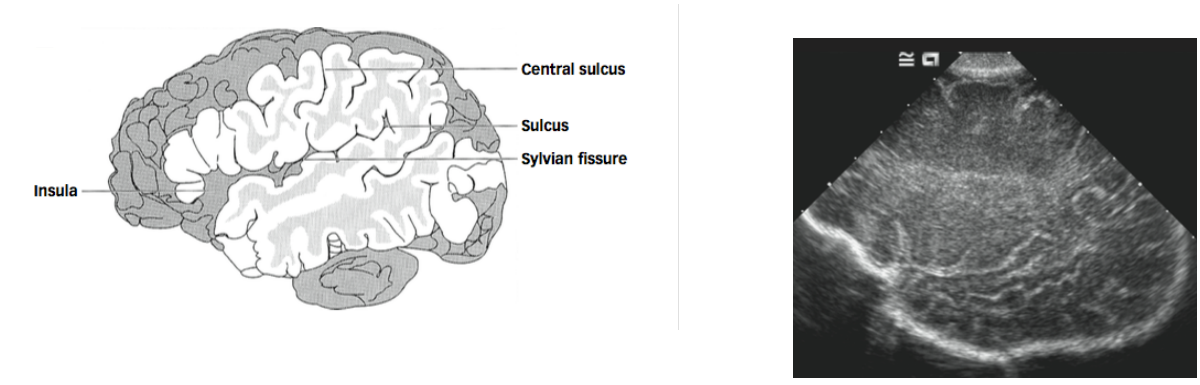


Figure 5: Normal anatomy in the sagittal images (Janet M. Rennie, 2009)

The addition of a high-frequency linear-array probe allows more detailed imaging of the cortex improving visualisation of cortical pathology, most especially cortical infarcts and extra axial collections (Figure 6).

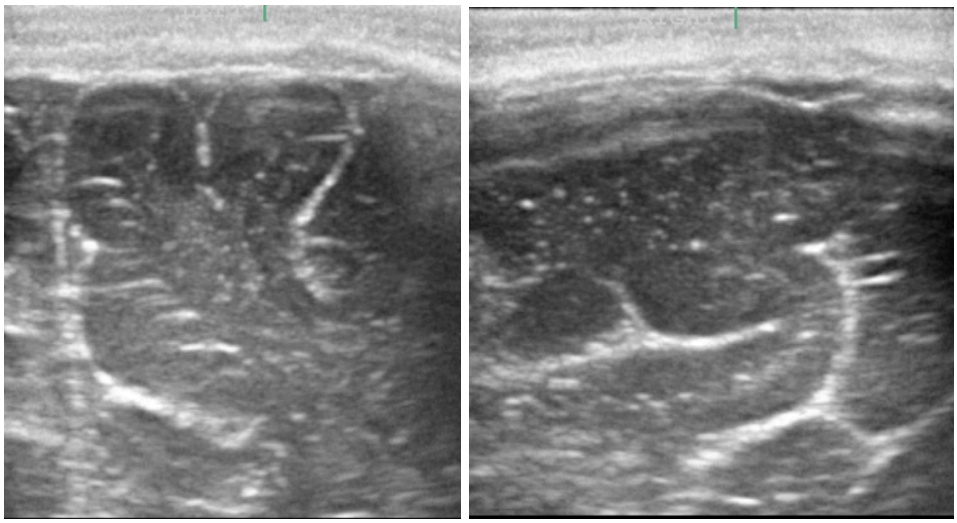


Figure 6: Images of the cortex using a high-frequency linear array probe demonstrating normal cortex in the coronal plane (left) and in midline sagittal (right) (*Credit K Burgoine*)

THE ROLE OF TRANS-FONTANELLE DOPPLER STUDIES

Doppler blood flow velocity studies can be performed to evaluate the flow velocities in major cerebral vessels (Figure 7). The anterior fontanelle is the most commonly used acoustic window for these studies. A midline sagittal scan allows visualisation of the anterior cerebral arteries and evaluation of the resistive index (RI). The RI is defined as (peak systolic velocity – end-diastolic velocity) / peak systolic velocity. The normal RI ranges are between 0.55 and 0.9. A low RI can be seen in perinatal birth asphyxia, whilst a patent ductus arteriosus is associated with an elevated RI. Using the higher frequency linear

array probe through the anterior fontanelle allows evaluation of the superior sagittal sinus aiding in the diagnosis of venous thrombosis.

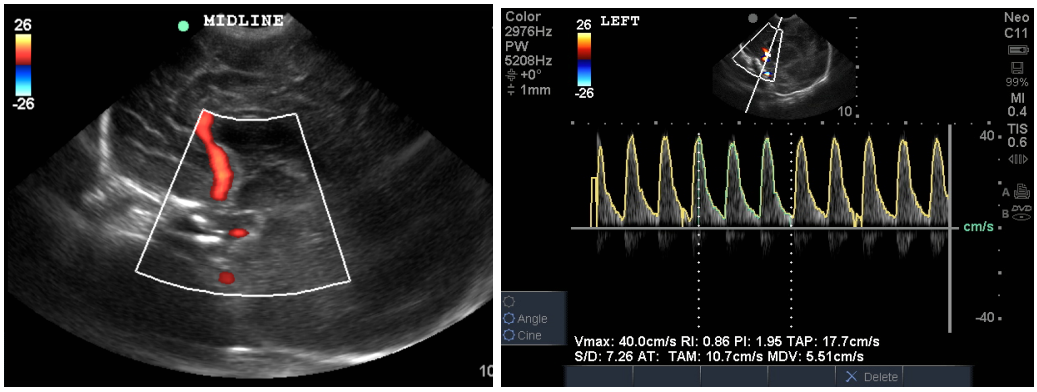


Figure 7: Colour image of blood flow through anterior cerebral artery from a midline sagittal image (left) and associated Doppler flow-velocity recording showing a normal Resistive Index of 0.86 (right) (*Credit K Burgoine)*

CRANIAL ULTRASOUND PATHOLOGY

As previously mentioned, cUS is already well established as the initial brain imaging examination of choice for preterm neonates, those neonates suffering from perinatal asphyxia, those with seizures or abnormal neurology or those with congenital infections. Not only does cUS allow detection and an assessment of the extent of abnormalities at presentation, it has an important role in monitoring evolution and complications.

In preterm neonates, haemorrhages are common and cUS is highly accurate for detecting germinal matrix haemorrhage (GMH), intraventricular haemorrhage (IVH), cerebellar haemorrhages and cystic periventricular leukomalacia (cPVL) (Maalouf et al., 2001). A grade I haemorrhage is confined

to the germinal matrix and is often located in the caudo-thalamic notch. A grade II is defined as bleeding into the lateral ventricle, not causing dilatation. Grade III is defined as intraventricular bleeding causing acute ventricular dilatation and grade IV is a haemorrhagic infarction in the parenchyma usually adjacent to a large GMH-IVH (Papile et al., 1978). Examples of different grades of IVH are shown in Figure 8.

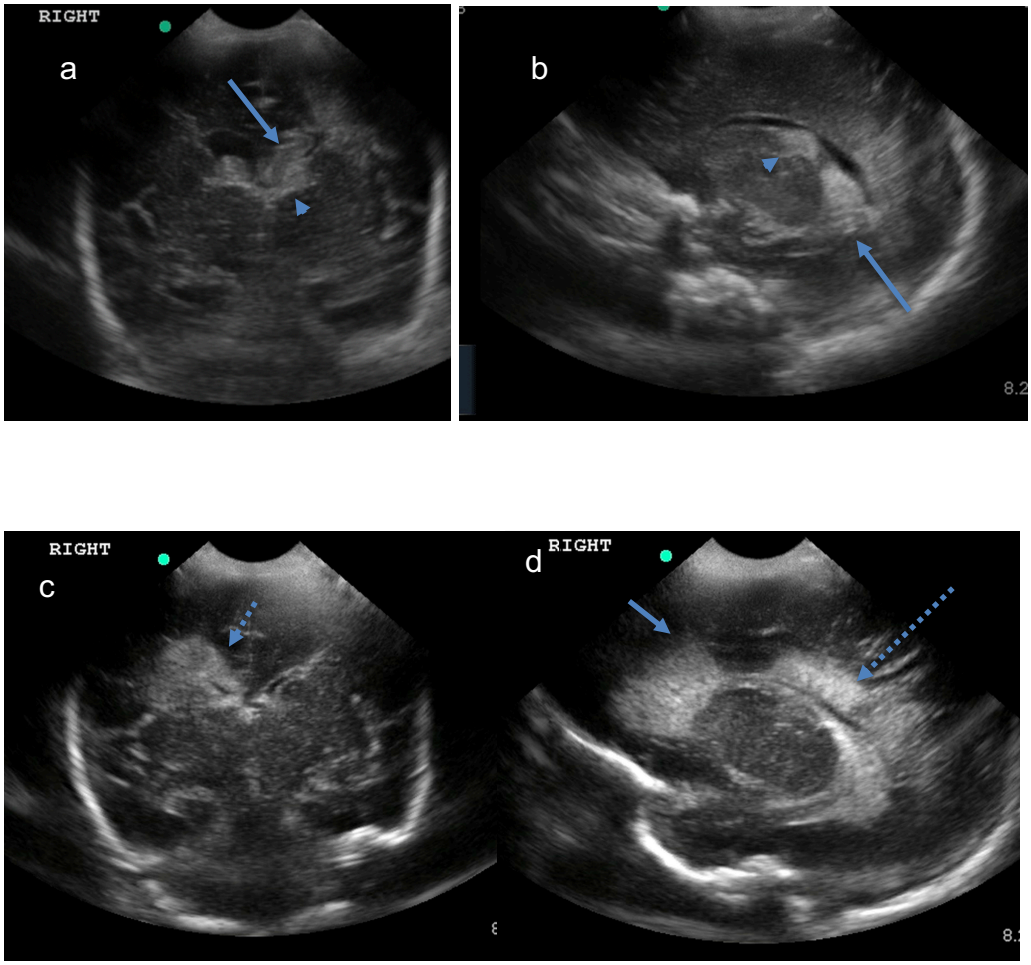


Figure 8: Cranial ultrasound scans showing a grade II haemorrhage with germinal matrix haemorrhage (arrowhead) and limited intraventricular haemorrhage (arrow) on a coronal image (a) and sagittal image (b). A Grade IV haemorrhage shown in a coronal (c) and sagittal (d) image with haemorrhagic infarction in the frontal (solid arrow) and parietal lobes (dotted arrow)

Hypoxic-ischaemic injury in term neonates can lead to white matter, basal ganglia-thalamic or cortical injury depending on the nature of the insult (Tann et al., 2016, Bano et al., 2017). Different patterns of injury can result from different types of hypoxic-ischaemic insult. Chronic hypoxic-ischaemic injury often produces watershed zone infarcts involving the cortex and subcortical white matter. Acute hypoxic-ischaemic injury often affects the basal ganglia, thalami, brainstem and parasagittal cortex and can be seen as increased echogenicity within these areas together with a relatively hypoechogenic internal capsule (Figure 9). Severe global hypoxia can lead to widespread oedema, which will be seen as increased echogenicity with loss of grey-white matter differentiation and obliteration of CSF containing spaces such as slit like ventricles (Figure 10).

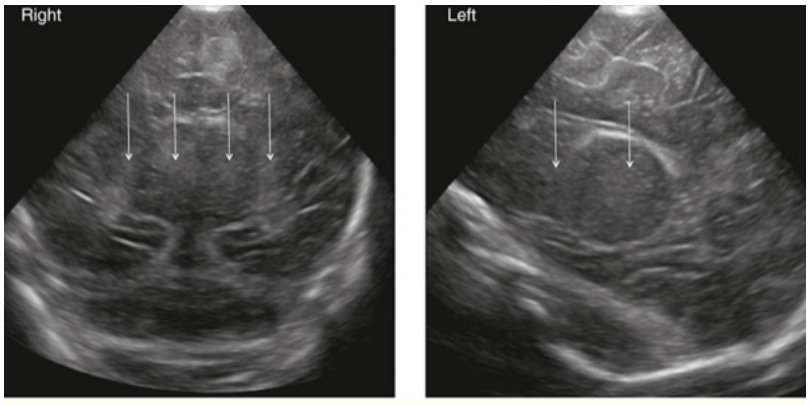


Figure 9: Bilateral basal ganglia and thalamic echogenicity on a coronal and parasagittal images in a patient with hypoxic-ischaemic encephalopathy (Hagmann et al., 2010, Tann et al., 2016)

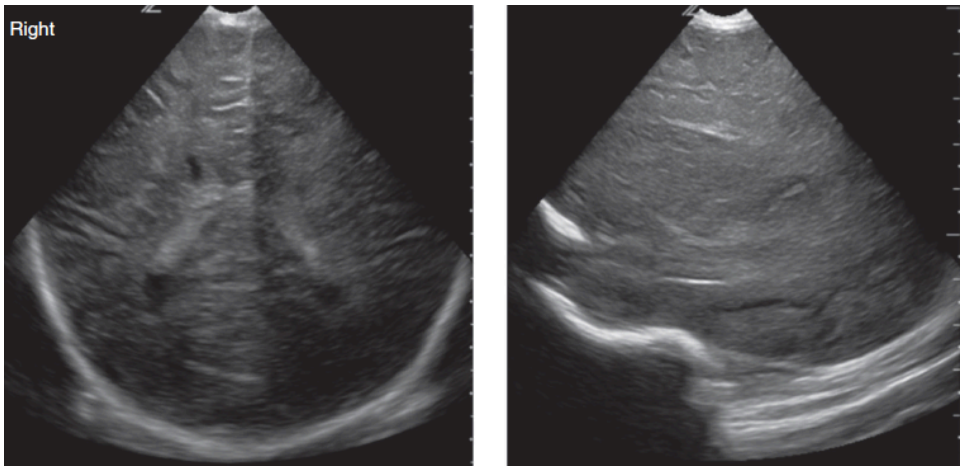


Figure 10: Coronal and parasagittal images of a neonate with hypoxic-ischaemic encephalopathy demonstrating diffuse white matter echogenicity with loss of differentiation (Tann et al., 2016)

Congenital malformations of the brain can also be diagnosed using cUS, these include congenital ventricular dilatation, agenesis of the corpus callosum, cerebellar hypoplasia and other malformations, and posterior fossa cysts. Isolated cysts in the choroid plexus, subependymal, caudo-thalamic cysts can also be detected (Tann et al., 2016, Hagmann et al., 2010). Lenticulostriate vasculopathy is seen on cUS as a unilateral or bilateral branching, punctate or linear increased echogenicity within the basal ganglia and related to the walls of medium sized lenticulostriate vessels, which are early branches of the middle cerebral artery. Their hypoechoic appearance is likely secondary to intramural and perivascular deposits of amorphous basophilic material in the lenticulostriate vessels. Although lenticulostriate vasculopathy is generally considered a benign finding, it is more common in a variety of disorders including CMV infection, toxoplasmosis, trisomy 13, trisomy 21, prenatal drug exposure and metabolic disorders (Cantey and Sisman, 2015, Maayan-

Metzger et al., 2016, Robinson et al., 2017, Sisman and Rosenfeld, 2015). In many cases no underlying cause of lenticulostriate vasculopathy is identified.

CRANIAL ULTRASOUND FINDINGS IN NEONATES WITH CNS INFECTIONS

CNS infections can arise in the antenatal period, usually from the TORCH spectrum of pathogens, which includes toxoplasmosis, other (syphilis, varicella-zoster, parvovirus B19), rubella, cytomegalovirus (CMV), and herpes infections. In-utero infections can cause severe damage to the developing brain. Early on in pregnancy, infection with these pathogens can lead to abnormal brain development and cause congenital malformations such as lissencephaly (smooth brain surface with an absence of sulcation), holoprosencephaly, polymicrogyria (multiple small abnormal gyri), and cerebellar hypoplasia (Barkovich and Lindan, 1994). Infections contracted later in pregnancy cause destructive lesions such as cerebral atrophy and cysts.

Globally, CMV is the leading cause of congenital infections and imaging findings include intracranial calcification, ventricular dilatation, ventricular strands, white matter disease and neuronal migrational disorders (Fink et al., 2010). Intracranial calcifications are the most common finding and can be found in the periventricular area, the basal ganglia and the parenchyma. Lenticulostriate vasculopathy is also associated with congenital CMV infection (Fink et al., 2010).

For neonatal infections, the majority of cUS data that are available focus on neonates with proven CNS infections, specific pathogens and particular pathologies (de Vries et al., 2004, Lequin et al., 2005, Renier et al., 1988, Shah et al., 2005, Verboon-Maciolek et al., 2006). In neonates presenting with seizures and abnormal neurology there can be imaging abnormalities in up to 100% of the cases (Mahajan et al., 1995). Even in the absence of these specific clinical signs, imaging abnormalities can be present in two-thirds of neonates with meningitis (Soni et al., 1994) (de Vries et al., 2006). Typical findings in CNS infections include ventriculitis, cerebritis, abscess and ventriculomegaly as described below.

In normal neonates, the pia-arachnoid membrane is seen as a thin echogenic line over the surface of the brain. The normal thickness of the membrane should be less than 1.3 mm from the surface of a frontal gyrus or less than 2mm within a sulcus. In meningitis, accumulation of inflammatory exudate within the sulci leads to widening and increased echogenicity. In fact, echogenic widening of the brain sulci, or meningeal thickening is the most common and earliest sign of meningitis on cUS and is seen in up to 83% of cases in neonates (Figure 11) (Arrumugham et al., 1994, Han et al., 1985, Kapoor et al., 1989, Littwin et al., 2018, Yikilmaz and Taylor, 2008).

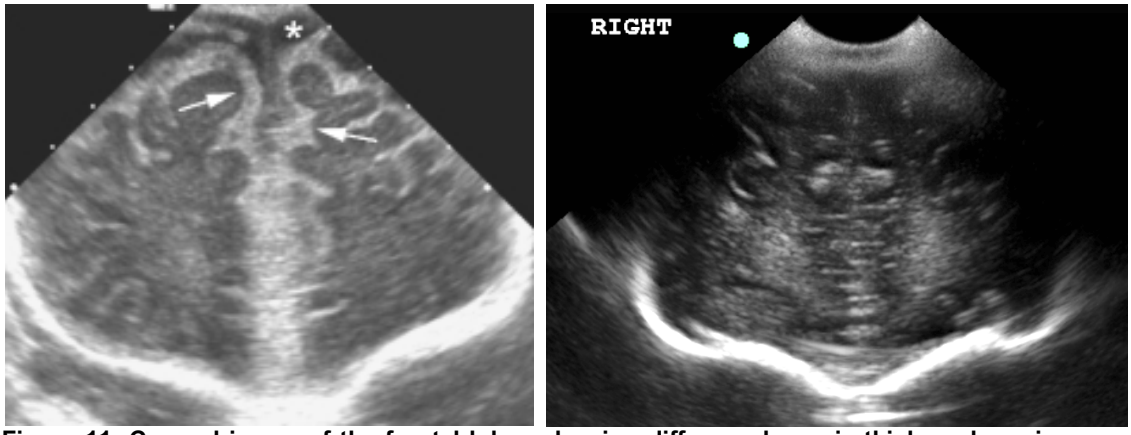


Figure 11: Coronal image of the frontal lobes showing diffuse echogenic thickened meninges *(arrows)* and increased extra-axial fluid spaces (*) on left (Yikilmaz and Taylor, 2008), and normal image (right)

Intraventricular debris, intraventricular strands and irregular and echogenic ependymal are all highly suggestive of ventriculitis (Lequin et al., 2005, Littwin et al., 2018, Soni et al., 1994, Yikilmaz and Taylor, 2008). Purulent exudate can obstruct the CSF circulation through the aqueduct or foramina of the 4th ventricle or the subarachnoid space (Kahle et al., 2016). Ventriculomegaly will follow and be readily detectable on cUS and if it progresses, will result in post-infectious hydrocephalus (Warf, 2005).

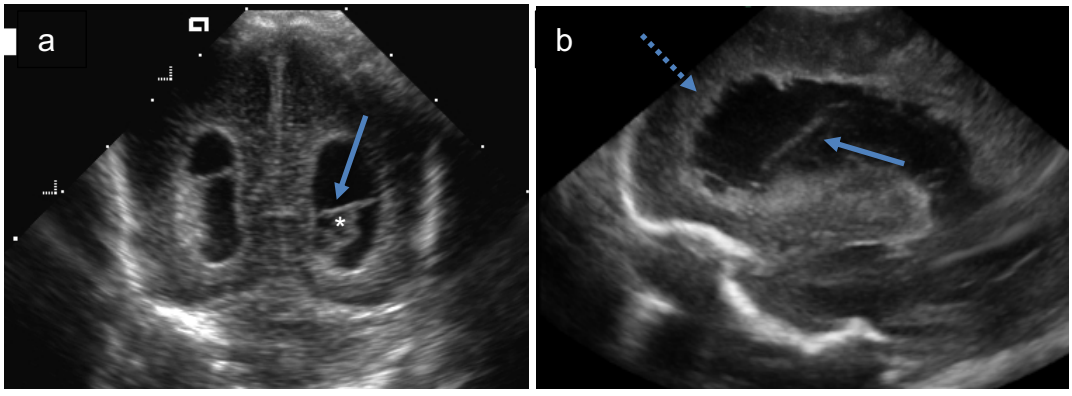


Figure 12: A coronal image showing ventriculomegaly of the lateral ventricles with strands (arrow) and debris (*) in both ventricles (a). A sagittal image showing ventricular debris, strands (arrow), dilatation, bright and irregular ependymal margin (dotted arrow) and abnormal shape (b). (*Credit C Hagmann and K Burgoine)*

Meningeal inflammation can also extend to the brain parenchyma causing a focal parenchymal infection, termed cerebritis or a more widespread parenchymal infection, termed meningoencephalitis. This can cause cerebral oedema through the disruption of the blood-brain barrier (Han et al., 1985, Mahajan et al., 1995). Cerebritis indicates a highly destructive bacterial infection of the brain parenchyma and often precedes the development of an abscess. In cerebritis, hyper echogenicity of the white matter is seen. A study of MR findings of six neonates with *E. coli* meningitis from Australia reported significant white matter injury in all cases (Shah et al., 2005). Some pathogens are known to cause meningoencephalitis in the neonatal period, the most common of which are GBS and *E.* coli. Meningoencephalitis has also been reportedly caused by *Bacillus cereus*, *Serratia marcescens* and *Citrobacter. Bacillus cereus* is a rare but often fatal cause of meningoencephalitis (Lequin et al., 2005, Chu et al., 2001). It has typical patterns of haemorrhage, early and rapid cavitation and selective white matter destruction seen as areas of hypo echogenicity inside the hyperechogenic white matter (Figure 13). Haemorrhagic meningoencephalitis leads to extensive parenchymal damage and necrosis due to the toxins released by *B cereus* (Lequin et al., 2005). It should be noted that hypoxic-ischaemic encephalopathy can also typically cause abnormal hyper echogenicity in the areas of the major arteries and then slowly develop necrosis. This can be contrasted to the very rapid destruction of the brain that is seen in bacterial meningoencephalitis.

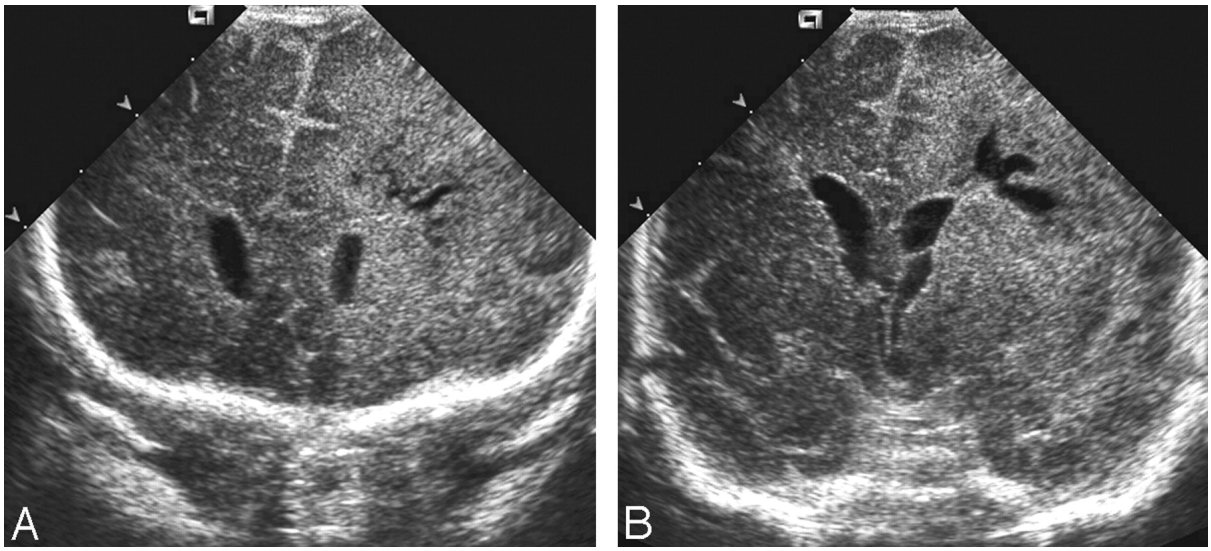


Figure 13: Example of haemorrhagic meningoencephalitis demonstrating hyper echogenicity of the white matter with a midline shift and necrosis of the left parietal lobe. A ventricular strand can be seen in the left ventricle (Lequin et al., 2005)

Cerebral abscesses are a serious but thankfully uncommon complication associated with neonatal meningitis and often follow cerebritis. In isolation, they have a predilection for the frontal lobes, especially the left. Cerebral abscesses are reported to have a high associated mortality and high-rate of complications. They likely originate following the development of ventriculitis, with subsequent disruption of the ventricular ependymal lining and then direct extension of the infection into the surrounding parenchyma. They are usually well-circumscribed collections of purulent fluid within the brain parenchyma seen as irregular or round lesions with hypoechoic centre and echogenic periphery and absent Doppler flow within the lesion (Figure 14).

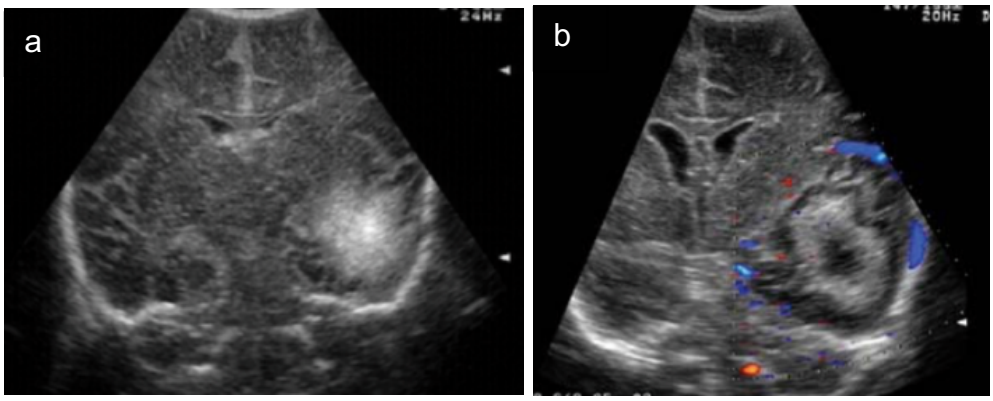


Figure 14: Coronal cranial ultrasound images showing an early abscess with hyperechoic area in left temporal lobe (a) and subsequent development of temporal abscess with hypoechoic centre and echogenic rim, absent Doppler flow within (b). (Ioana Alina Anca, 2009)

Neonates with brain abscess may initially exhibit no neurological symptoms except irritability (Algubaisi et al., 2015). In fact, neonatal brain abscesses may grow very large before clinical signs become apparent, therefore imaging in cases of neonatal meningitis is vital for the diagnosis of an abscess. Specific pathogens known to cause abscesses are the gram-negative bacilli, *Klebsiella* and *Citrobacter* (Algubaisi et al., 2015, Agrawal and Mahapatra, 2005, Doran, 1999, Vaz Marecos et al., 2012, Etuwewe et al., 2009, Ioana Alina Anca, 2009). Although it is a rare cause of neonatal meningitis, it is reported that the majority (68-75%) of cases of *Citrobacter* meningitis are complicated by brain abscesses (Vaz Marecos et al., 2012). It is therefore not surprising that *Citrobacter abscesses* have a high mortality and morbidity rate: 30% mortality and up to 50% neurological sequelae in survivors (Doran, 1999). *Proteus* is also a recognised cause of neonatal brain abscesses (Juyal et al., 2013, Renier et al., 1988). A case series of 30 neonates with brain abscesses identified *Proteus* in 27 neonates. Abscesses were usually found in the frontal region (22/30) and were often enormous and in over half of cases were

multiple (Renier et al., 1988). In *candida* infections, the CNS is the one of the most commonly involved organs, reportedly in 10-67% of cases. *Candida* CNS involvement usually causes meningitis, ventriculitis, or cerebritis, however multiple micro abscesses have been reported in cases of neonatal candidal meningitis (Pahud et al., 2009, Benjamin et al., 2003).

The CNS inflammation can also lead to vasculopathy and prothrombotic states resulting in infarction (Figure 15). A study of 166 cases of meningitis in infants and children found evidence of infarction in 10% of cases, mainly located in the frontal, temporal and occipital lobes and the basal ganglia (Chang et al., 2003). Other small case series have also observed infarctions in cases of neonatal meningitis (Fitzgerald and Golomb, 2007, Chang et al., 2003, Rodrigues et al., 2014). In a series of four cases of *C. Koseri neonatal meningitis*, one case had evidence of frontal echogenicity on cUS suggestive of infarction (Rodrigues et al., 2014). GBS meningitis is also known to cause cerebral infarction as demonstrated by a study using MR imaging that compared findings between 57 cases of GBS meningitis and 50 cases of *E. coli* meningitis (Kralik et al., 2019). This study found significantly more infarcts in the GBS group, 40% vs. 14% (*P= 0.038).* Two distinct patterns have been reported in neonatal GBS meningitis: firstly, deep perforator focal infarction of the basal ganglia, thalamus and periventricular white matter and secondly, focal cortical infarctions (Hernandez et al., 2011). In a study of eight cases of GBS meningitis with associated infarction, all patients presented in shock, six had seizures and the majority (75%) developed severe disability or death (Hernandez et al., 2011). Although more commonly recognised as a cause of

anaemia, hydrops and stillbirth, CNS infection with parvovirus B19 has been shown to cause neonatal and/or fetal stroke (De Haan et al., 2006).

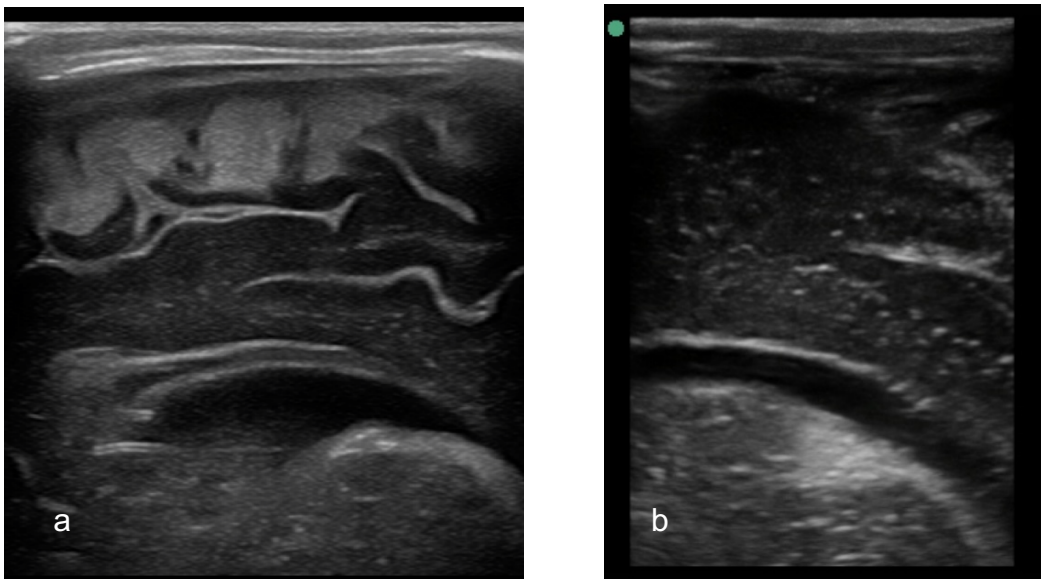


Figure 15: Sagittal view of the cortex visualized with a linear probe showing cortical infarct (a), normal cortex (b) (*Credit C. Hagmann).*

LATER COMPLICATIONS OF NEONATAL MENINGITIS

Later complications of meningitis include post-infectious hydrocephalus, cerebral cortical and white matter atrophy, extra-axial collections, poor brain growth, and multi-cystic encephalomalacia (Kalsbeck et al., 1980). Post-infectious hydrocephalus occurs when the normal circulation of CSF is obstructed by the purulent exudate anywhere from its point of secretion by the choroid plexus, during its circulation and through to its absorption (Kahle et al., 2016). Although the exact aetiology is yet to be described, a particularly high incidence of neonatal post-infectious hydrocephalus has been reported in East Africa where over 60% of infantile hydrocephalus is due to neonatal sepsis (Warf, 2005) (Warf and East African Neurosurgical Research, 2010). A study

of infants with post-infectious hydrocephalus at the children's neurosurgical hospital in Uganda detected bacterial DNA in the CSF of 94% of the infants and these cases often had a prior history of neonatal sepsis (Warf, 2005, Li et al., 2011). Ventriculoscopy in these cases of post-infectious hydrocephalus also allowed direct observation of choroid plexus scarring, post-inflammatory aqueductal obstruction and intraventricular deposition of pus (Warf, 2005). Post-infectious hydrocephalus seems to be predominantly caused by gram-negative bacteria (Agrawal and Mahapatra, 2005, de Vries et al., 2006, Renier et al., 1988, Warf and East African Neurosurgical Research, 2010). In a case series of 30 neonates with brain abscesses, the majority caused by *Proteus* (27/30), half (14/30) of these infants went on to develop post-infectious hydrocephalus (Renier et al., 1988). In a review of 96 cases of neonatal meningitis in The Netherlands, 5 cases developed ventriculomegaly and the responsible pathogens were identified as *E. coli, C. koseri* and *E. cloacae (de Vries et al., 2006).* An Australian study of six infants with *E. coli* meningitis found three to develop gross ventriculomegaly (Shah et al., 2005). The retrospective study that compared brain MR imaging findings between 57 cases of GBS meningitis and 50 cases of *E. coli* meningitis, found significantly more cases of early hydrocephalus in the *E. coli* group, 22% vs. 0% (*P= 0.0014) (Kralik et al., 2019).* A recent study using genomic sequencing in Uganda found that compared to non-post infectious hydrocephalus, infection with *Paenibacillus*, together with frequent cytomegalovirus (CMV) coinfection, was associated with post-infectious hydrocephalus (Paulson et al., 2020).

Although cystic encephalomalacia is perhaps more commonly reported in intrauterine infections such as herpes simplex virus (HSV), rubella and cytomegalovirus (CMV), multi-cystic encephalomalacia is also reported as a complication of neonatal meningitis. In a case series of infants with *C. Koseri* neonatal meningitis, one developed multi-cystic encephalomalacia (Rodrigues et al., 2014). Another case report of *C. koseri* showed multiple brain abscesses in the temporal lobe, which again developed into multi-cystic encephalomalacia (Vaz Marecos et al., 2012).

Cerebral atrophy is often a result of damage to cortical neurons and periventricular white matter injury (Shah et al., 2005). It can be visualized on cUS as a progressive dilatation of the ventricular system and increased extracerebral space, such as ventricular dilatation, without associated increase in the infant's head circumference.

CRANIAL ULTRASOUND FINDINGS IN NEONATAL SEPSIS

To date there are no studies that have routinely undertaken cUS examinations in cases of neonatal sepsis in term neonates. There are however data available from HICs comparing brain imaging, including cUS and MR imaging, between preterm infants with and without neonatal infections. The brains of preterm infants are already susceptible to brain injury but the data show that neonatal infections are associated with an increased incidence of abnormalities on cUS, most especially white matter (WM) injury (Mitha et al., 2013). This is important as neonatal cerebral WM injury represents a major

precursor for cerebral palsy and neurodevelopmental impairment as discussed below.

A prospective study of 192 preterm infants found that those who experienced sepsis or necrotizing enterocolitis (NEC) had significantly more WM injury compared to those with no episodes of sepsis or NEC (Shah et al., 2008). A retrospective case-study of 150 preterm infants with WM injury, characterized by cystic periventricular leukomalacia or ventricular dilatation from WM atrophy, compared to 150 preterm infants without, found that those with WM injury, had a significantly higher rate of positive blood (OR 2.0), CSF (OR 3.8) or tracheal cultures (OR 3.1) (Graham et al., 2004). Another study which undertook an early MR imaging and term-equivalent MR imaging of 133 preterm infants, found an association between infection and WM injury, when comparing those infants with >1 infection and ≤ 1 infection (Glass et al., 2008). In a study of 117 preterm infants, WM injury was reported on the MR imaging scans in one-third (29%) and this risk of WM injury was three-fold higher in those with a culture-positive infection, all four infants with meningitis had WM injury (Chau et al., 2012). The risk of cerebellar haemorrhage was also 9-times higher. Another study sought to differentiate between the imaging findings in infants with clinical signs of sepsis compared to confirmed sepsis. They used cUS to examine the brains of 117 preterm infants <32 weeks, 86 with proven LONS and 31 with clinical LONS (Claessens et al., 2017). They found no significant difference ($p=0.624$) in the prevalence of abnormalities between the two groups. This is likely due to the poor sensitivity of the diagnostic techniques described above meaning it is likely that the majority of these

infants had infection despite being unable to identify the cause. This suggests that any preterm infant with clinical signs suggestive of infection, whether confirmed or not, should undergo routine cUS examinations.

More recently a study of 72 extremely preterm (<28 weeks) infants with culture-proven sepsis, where MR imaging was used to look for WM injury, found an inversely proportional relationship between postmenstrual age at sepsis diagnosis and the severity of the WM injury (Heo et al., 2018). If increasing maturity of the neonatal brain is protective against damage from neonatal infections, it is possible that neonatal infections may not create the same level of damage to the mature brain of a term infant. There are limited data available on the brain imaging findings in term infants who experienced neonatal infections. This novel study will seek to describe the abnormalities on cUS in term infants with clinical sepsis.

Cerebral WM injury, identified by cUS or MRI, is a strong predictor of poor neurodevelopmental outcome. Studies of preterm infants have shown a correlation between neonatal sepsis, WM injury and poor neurodevelopmental outcome (Mitha et al., 2013). A study of 162 preterm infants found that sepsis or NEC significantly increased the risk of WM injury as seen on MR imaging. At 2-years of age, those infants who had experienced neonatal sepsis or NEC had delayed cognitive and motor development, a difference which disappeared on adjustment for WM injury, suggesting this developmental delay was mediated by WM injury (Shah et al., 2008). In addition, a large study

of newborn infants with CNS infection showed a correlation between mortality and increased echogenicity and ventricular dilatation (de Vries et al., 2006).

NEONATAL INFECTION AND NEURODEVELOPMENTAL OUTCOME

Mortality is not the only burden of neonatal infections. Even neonates who receive timely and effective treatment and survive, have an increased risk of neurodevelopmental impairment and neurological disability, especially those who are born preterm (Stevens et al., 2003). There are more data available on the long-term sequelae of neonatal meningitis than for neonatal sepsis. Despite reductions in mortality in HICs, substantial long-term morbidity associated with neonatal meningitis persists. In cases of neonatal meningitis in HICs, complications such as ventriculitis, convulsions, post-infectious hydrocephalus and neurodevelopmental impairment are reported in up to 26% of cases and these complications all increase the risk of mortality (Holt et al., 2001). A recent meta-analysis identified 8 studies that included a total of 451 survivors of neonatal meningitis and estimated that 23% (95%CI: 19-26%) of neonates who survive meningitis have moderate to severe neurodevelopmental impairment (Seale et al., 2013). In addition, they identified 5 studies, including 311 neonatal meningitis survivors, that estimated 12% (95%CI: 5-19%) had mild impairment. A large case-control study of 1584 children who suffered from meningitis in infancy in England and Wales from 1985-1987 evaluated the neurodevelopmental outcome of the survivors at 5 years of age compared to 1391 controls (Bedford et al., 2001). The risk of moderate or severe disability in the survivors was 10-fold higher (RR 10.3, $P<0.001$) than that of the children in the control group. They reported moderate

or severe disability in 15.6% of survivors compared to 1.5% of controls. There was a wide variation in the incidence of disability depending on the aetiological agent identified. Moderate or severe disability were reported in 24.3% of cases due to *E. coli* and 30% of cases due to GBS, however the highest rates were in those caused by other gram-negative bacteria (56.3%).

GBS meningitis is a known and important cause of neurodevelopmental impairment. An older study of 38 survivors of GBS meningitis in infancy, found that 29% had severe neurological sequelae when evaluated between 3 to 9 years of age (Edwards et al., 1985). Another recent study from the United States on the long-term outcomes of GBS meningitis, reported that 44% of 43 affected term infants had neurodevelopmental impairment when evaluated between 3 to 12 years of age including 25% with mild-to moderate impairment and 19% with severe impairment (Libster et al., 2012). A recent meta-analysis of survivors of GBS meningitis in middle- and high-income countries found 32% had neurodevelopmental impairment at 18 months, including 18% which were moderately to severely impaired (Kohli-Lynch et al., 2017).

Data from SSA on the long-term outcomes of neonatal meningitis survivors are scarce. Available data report neurodevelopmental impairment in 21-83% of survivors of neonatal meningitis. This wide variation likely represents the differences in the study designs and the level of clinical care available. A retrospective study of 55 cases of neonatal meningitis in Ethiopia reported a 40% mortality and neurological complications including post-infectious hydrocephalus, cerebral palsy and seizures in 21% of survivors

(Gebremariam, 1998). A study from Nigeria reported the incidence of neurodevelopmental impairment in survivors of neonatal meningitis (Airede et al., 2008). From 69 cases, they found 40 survivors and 33 (83%) of them had neurodevelopmental impairment at 24 months. Three of these cases had sensorineural hearing loss, 2 had developed post-infectious hydrocephalus and 1 had a hemiparesis. An observational study of neonatal meningitis in Malawi reported an inpatient mortality of 20% and neurodevelopmental impairment in up to 47% of the 20 survivors at 6 months of age and up to 60% at 12 months of age (Dube, 2014).

The relationship between infection and adverse neurological and neurodevelopmental outcomes in preterm infants has been explored and well documented. The developing preterm brain is already vulnerable to cytotoxic, hypoxic and ischaemic injuries and therefore preterm infants are already at increased risk of cerebral palsy and neurodevelopmental delay. A large cohort study of 6093 extremely low birth weight infants (<1000g), found increased risk (OR 1.4-1.7) of neurodevelopmental impairment and cerebral palsy at 18 to 22 months in those who suffered from a neonatal infection including sepsis, meningitis and necrotizing enterocolitis (Stoll et al., 2004b). A Swiss cohort of 541 extremely preterm infants (<28 weeks) also reported a three-fold increased risk of cerebral palsy at 2 years of age for those infants who suffered proven sepsis (Schlapbach et al., 2011). In a French cohort of 2665 very preterm infants, 1769 were examined for cerebral palsy and 1495 underwent cognitive assessment at 5 years of age (Mitha et al., 2013). Overall 9% had cerebral palsy and 12% had cognitive impairment. Although there was no

reported increase in cognitive impairment, the risk of cerebral palsy was almost double in those that experienced EOS or LOS compared to children uninfected during the neonatal period. A small Dutch cohort study of 117 preterm infants below 32 weeks gestational age, 85 with culture-proven LOS and 32 with clinical LOS, examined their neurodevelopment at 24 months corrected age (Zonnenberg et al., 2019). They observed no differences in the neurodevelopment, again this is likely due to the poor sensitivity of blood culture.

The long-term survival for term infants after neonatal sepsis can also be complicated by childhood mortality, post-infectious hydrocephalus, cerebral palsy and neurodevelopmental impairment (Figure 16). There are however limited data available on the incidence and level of these sequelae. A recent retrospective study from America looked at the 5-year outcome of patients who suffered from confirmed sepsis (190) or suspected sepsis (3449), including 2677 term infants, compared to control infants (Savioli et al., 2018). Overall developmental delay was diagnosed in 22.2% of controls, 28.2% of infants with suspected sepsis and 50.5% of infants with confirmed sepsis. In a sub-group analysis of the term born infants, the overall risk of developmental delay was 1.7 times higher in cases of confirmed sepsis compared to control term infants and affected almost all developmental domains, especially communication, learning, motor and pervasive development disorders (PDD). Suspected sepsis still carried a significantly higher risk of learning delay and PDD than found in term controls.

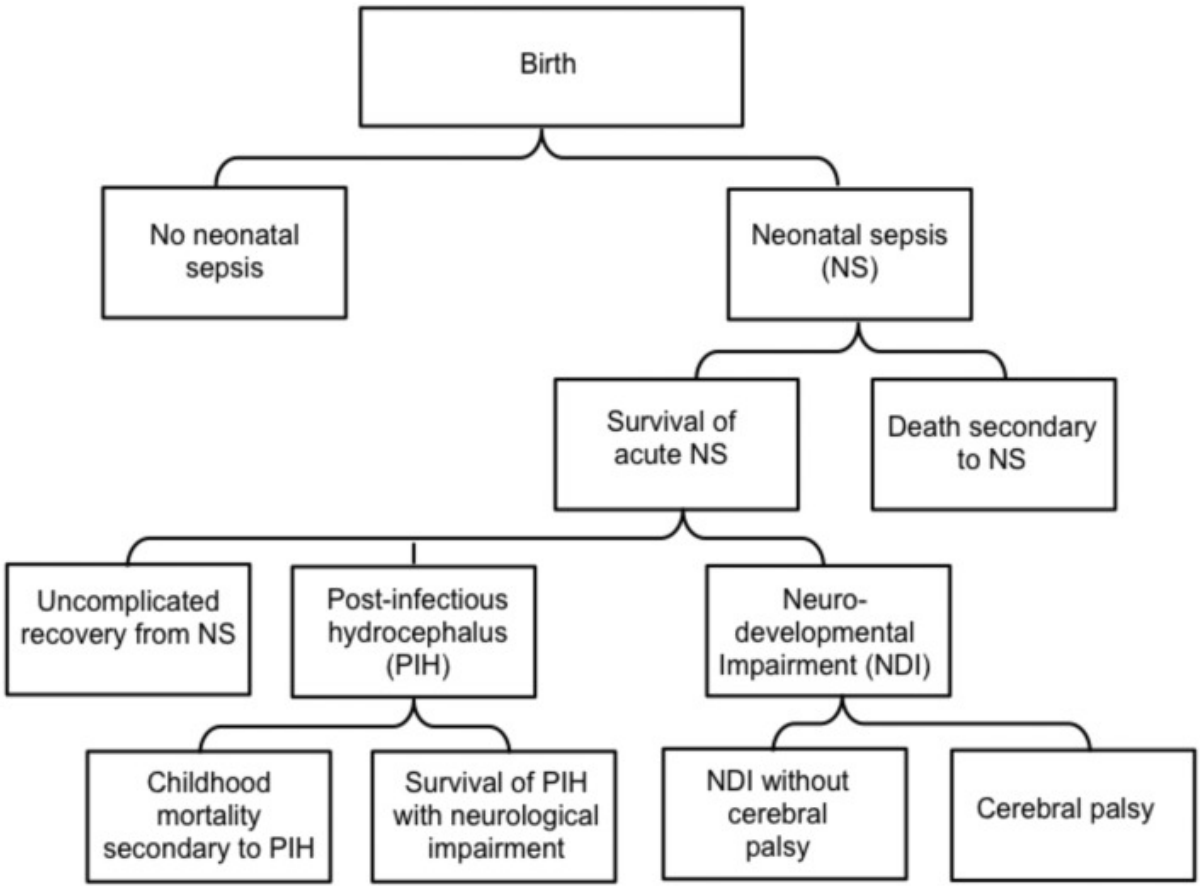


Figure 16: Possible outcomes for survivors of neonatal sepsis (Ranjeva et al., 2018)

A small retrospective study from Kenya of 24 term infants with a clinical diagnosis of neonatal sepsis found gross and fine motor impairment was significantly higher when compared to community controls at 18-32 months (Gordon et al., 2005). Overall 16% of the survivors were unable to stand/walk and 21% were unable to take a lid off a jar using two hands, compared to 0% for both activities in the control group. A study from Malawi that assessed 62 cases of neonatal sepsis, 19 cases of meningitis and 164 controls, found significantly higher levels of neurodevelopmental impairment in gross motor,

fine motor and cognitive domains for those with neonatal meningitis at 12-months of age (Dube, 2014). Compared to controls, neonatal meningitis increased the risk of delay by up to 17-fold at 12 months of age and neonatal sepsis increased the risk of fine motor and cognitive delay by up to 6-fold at 12 months of age. A more recent clinical trial of young infants <60days with pSBI in Malawi, reported a 12% inpatient mortality, reaching 15% within 6 months of discharge (Molyneux et al., 2017). They reported sequelae in 12.6% at 6 months of age including hearing loss, blindness, global delay and hydrocephalus. It is possible that in settings of high infectious morbidity, such as Uganda, neonatal sepsis may be contributing to a large burden of post-infectious hydrocephalus, cerebral palsy and poor neurodevelopmental outcome (Dube, April 2014, Warf, 2005).

SUMMARY

Neonatal infections are increasingly recognized as a major and under-represented global health challenge. SSA carries a disproportionate burden of neonatal mortality and in Uganda the NMR has not changed over two decades, remaining high at 28/1000 live births. The main causes of neonatal deaths in LICs like Uganda are considered to be prematurity, intrapartum complications and severe bacterial infections, including sepsis, meningitis and pneumonia.

Neonatal sepsis can lead to infection of the CNS including meningitis and ventriculitis. Neonatal meningitis is a devastating illness with known complications such as neurodevelopmental impairment including cerebral

palsy and post-infectious hydrocephalus. In LICs the incidence of neonatal sepsis and meningitis remains high and both are still associated with a high mortality.

The diagnosis of both neonatal sepsis and neonatal meningitis in LICs is problematic and there is a great and urgent need for improved diagnostics. This is particularly true in countries with a high burden of neonatal severe bacterial infections and associated mortality such as Uganda. The ideal diagnostic test would be rapid, sensitive, specific and not affected by prior maternal or neonatal antibiotic administration. Such a test would aid appropriate management and antibiotic choice for cases of neonatal sepsis or neonatal meningitis. But many hospitals in LICs lack the equipment needed to perform blood and CSF cultures and in addition have inadequate laboratory support to process these investigations. In contrast many hospitals in such settings do have access to radiographers and have ultrasound scanners which require minimal maintenance and few ongoing supplies. Given the imaging characteristics described in neonatal meningitis and other CNS infections above, the use of cUS is an attractive possibility for improving the detection of neonatal meningitis. It would also provide the potential to monitor complications, thus hopefully reducing both the morbidity and mortality.

Survivors of neonatal meningitis are at high risk of neurodevelopmental impairment, although data from LICs are more limited. It is also apparent that the longer-term complications of neonatal sepsis are not well described in any setting, and it is possible that they are likely to be a significant cause of

impairment and disability, especially in low-resource settings. Improved data on the long-term sequelae following neonatal infections are therefore needed in order to estimate the true burden of neonatal sepsis. This novel study will use serial cUS examinations to improve our understanding of the development of cerebral complications such as atrophy, cysts and post-infectious hydrocephalus and also to evaluate the relationship between findings on neuroimaging and longer-term outcomes.

CHAPTER 3 - OBJECTIVES

OVERALL OBJECTIVE

To study the clinical features, aetiology, imaging findings and early childhood outcomes of possible serious bacterial infections (pSBI) among neonates presenting to a regional referral hospital in eastern Uganda over a 12-month period.

HYPOTHESES

In neonates, in a low-resource setting in Uganda, who suffer an episode of pSBI:

- All those with a CSF analysis and/or culture results suggestive of CNS infection will have pathological findings on cUS at presentation
- A cUS at presentation will detect CNS involvement more reliably than CSF analysis and CSF culture alone
- There will be an increased risk of post-neonatal mortality, developmental impairment, poor growth and post-infectious hydrocephalus at 12 months of age compared to infants who did not suffer from pSBI during the neonatal period.
- The presence of moderate to severe abnormalities on cUS will predict those infants at an an increased risk of post-neonatal mortality, developmental impairment, poor growth and post-infectious hydrocephalus at 12 months of age.

SPECIFIC OBJECTIVES

In neonates presenting with possible serious bacterial infections to a regional referral hospital in eastern Uganda over a 12-month period:

1. Describe the clinical features at presentation
2. Describe the aetiology using traditional blood and CSF cultures
3. Describe the findings on cranial ultrasound scan at presentation
4. Describe the progressive changes on cranial ultrasound 3, 7 and 28 days after presentation
5. Determine the inpatient, neonatal and infant mortality
6. Evaluate the incidence of neurodevelopmental impairment at 2, 6 and 12 months of age
7. Describe the clinical, laboratory and imaging predictors of poor outcome by 12 months of age

THESIS PLAN

Chapter 4 will provide an overview of the methods used in this thesis. It will describe in detail the study site, study design, recruitment and follow-up. The results will then be set out in chapters 5 to 9 and will cover the following:

- Chapter 5 - Serious bacterial infections among Ugandan neonates: clinical features, aetiology and neonatal outcomes.
- Chapter 6 - Cranial ultrasound findings on admission among term neonates presenting with possible serious bacterial infection (pSBI) in Uganda

- Chapter 7 - Progressive changes on cranial ultrasound among neonates admitted with possible severe bacterial infection (pSBI) in Uganda
- Chapter 8 - Post-neonatal mortality, morbidity and developmental outcome after possible severe bacterial infection (pSBI) in Ugandan neonates: A prospective hospital-based cohort study with external controls.
- Chapter 9 - Clinical, laboratory and imaging predictors for poor early childhood outcome among Ugandan neonates with possible severe bacterial infection (pSBI).

In chapter 10, the overall thesis will be summarised and discussed in the context of the current literature.

CHAPTER 4 – METHODS

STUDY AREA

Uganda is a land-locked country in East Africa (Figure 17). It is bordered by Kenya in the east, by South Sudan in the north, by Rwanda and the Democratic Republic of the Congo in the west and by Tanzania in the south. Uganda lies within the Nile basin and the southern part of Uganda includes a substantial portion of Lake Victoria. The country is located on the equator and lies between latitudes 4°N and 2°S and longitudes 29°E and 35°E. It averages about 1,100m above sea level. The climate is tropical and is generally rainy and there are two dry seasons from December to February and June to August.

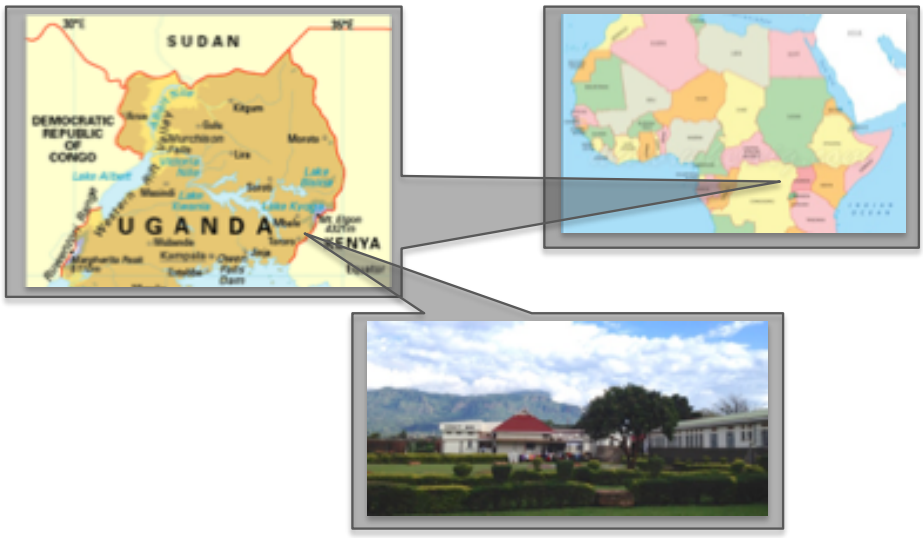


Figure 17: Maps showing geographical location of Mbale, Uganda

Uganda is one of the least developed countries in the world and in 2019 was ranked number 159 out of 188 countries on the Human Development Index by

the United Nations Development Programme Report. Uganda is home to 39 million people. Poverty remains entrenched in rural Uganda, which is where the vast majority of the population resides. Improvement in healthcare in Uganda has been limited by inaccessibility, limited transport and cost. In 2014, Uganda was estimated to have the fifth highest total fertility rate in the world, with each woman delivering 5.97 children.

Mbale is a city in the eastern region of Uganda. The city stands at an altitude of 1131 metres above sea level at the base of Mount Elgon, one of the highest peaks in East Africa. It lies at 1°N and 34°E and longitudes 29°E and 35°E. The temperature averages 23°C, with an average maximum of 30°C and minimum of 17°C. The national population census estimated the population of Mbale to be 96,189 (UBoSUa., 2017).

STUDY SITE

The study was conducted in a government hospital in eastern Uganda. Mbale Regional Referral Hospital (MRRH) is a busy regional hospital that serves a population of about 4.5 million people in the east of Uganda, serving 14 districts and hundreds of lower level health facilities. The labour ward at MRRH has nearly 10,000 deliveries a year. Being the only hospital with a neonatal unit and neonatal specialists in the region, the hospital also receives neonates referred from district hospitals and health centres well beyond its catchment area. In addition, a high rate of home deliveries still occur in rural Uganda and some of these neonates are brought directly from home (Inc, 2007, Akinyo, 2009-10). MRRH is a public hospital, which offers free healthcare service. It

also serves as a teaching hospital for Busitema University.

Since May 2015, MRRH has had a dedicated Neonatal Unit (NNU) that provides Level II Neonatal Care including intravenous fluids, oxygen, nasogastric tube feeding and phototherapy (Figure 18). The availability of six high-dependency cots supported by bubble continuous positive airways pressure (bCPAP) began in July 2016 (Okello et al., 2019). The NNU is staffed by a full-time paediatrician, a neonatal clinical officer, 6 neonatal nurses and a rotating intern. The NNU admits around 2000 neonates annually, of which almost half are admitted with pSBI. The current NMR in the NNU is 15%.

Figure 18: The neonatal unit in Mbale Regional Referral Hospital

STUDY POPULATION

The study reported in this thesis recruited infants less than 28 days of age, weighing >2000g, who presented to the MRRH-NNU with pSBI as defined below.

STUDY DESIGN

This work reported in this thesis had two components: a cross-sectional study and a prospective cohort study. The cross-sectional study allowed the aetiological, laboratory and neuroimaging findings to be described at presentation. The prospective cohort study allowed the in-patient, neonatal and infant mortality to be evaluated alongside the progressive neuroimaging changes during the 28 days after presentation and the developmental outcomes at 2, 6 and 12 months (Figure 19).

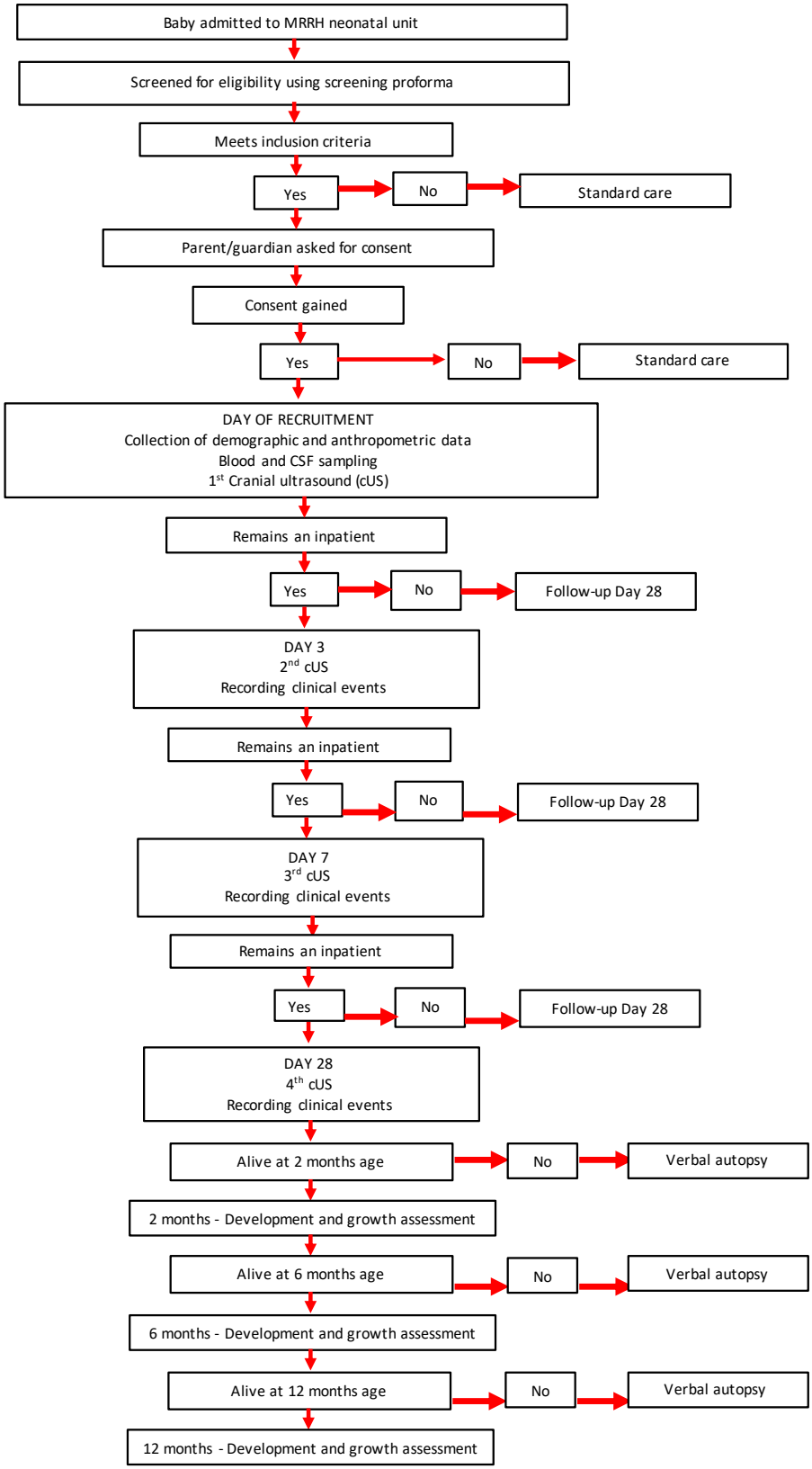


Figure 19: Flow chart of the overall study design showing the proposed timings of infant evaluations for cranial ultrasound, growth and developmental assessments

STUDY DURATION

Infants were recruited over a 1-year period from December 2016 until December 2017. Each infant was followed-up until 12-months of age.

STUDY RECRUITMENT AND CONSENT

Infants were only recruited to the study Monday to Friday due to the limitations of laboratory support on weekends. Each weekday, if available, up to three consecutive cases of pSBI that fulfilled both the inclusion and exclusion criteria were invited to enrol in the study.

STUDY PROCEDURES

SCREENING

All neonates (less than 28 days) who presented to the NNU at MRRH were assessed for symptoms and signs of pSBI as described in more detail below. Neonates who fulfilled the inclusion and exclusion criteria described below were invited to join the study.

CLINICAL DIAGNOSIS OF NEONATAL SEPSIS

Unfortunately, the clinical features of pSBI can overlap with a number of different neonatal pathologies, the most common of which are complications of prematurity and neonatal encephalopathy due to perinatal asphyxia. Uganda has a high incidence of preterm births and preterm infants often suffer from respiratory distress syndrome, environmental hypothermia, apnoea of

prematurity, poor feeding and jaundice, all of which can be present in infection (McGuire et al., 2004). In this study we chose to exclude those with a birthweight <2000g in order to be more confident that these clinical signs were secondary to infection and not prematurity. There is also a high incidence of perinatal asphyxia and neonatal encephalopathy (NE) in Uganda and similar settings (Tann et al., 2014). Although infection is an important risk factor for developing NE, we wanted to exclude those with NE and focus on those with neonatal infection alone (Tann et al., 2014). We only included those who had a normal Apgar score at birth and/or did not require significant resuscitation at birth. In addition, in order to define a group that unequivocally required hospital-based treatment and had severe disease that was most likely due to infection, we defined pSBI using a combination of signs and symptoms from the WHO Young Infant study (Group, 1999b). The definition of pSBI was the presence of one of the following three combinations of symptoms and signs in any infant less than 28 days at presentation:

a) Fever (>37.5°C), lethargy and poor feeding
b) Hypothermia (<35.5°C) , lethargy and poor feeding
c) Full fontanelle and/or seizures, fever, poor feeding

A standardised case report form allowed the study team to assess for and record the presence of any additional signs and symptoms in all neonates (Table 2, Appendix 1). The diagnosis and the treatment were at the discretion of the attending clinician.

Symptom or sign	Definition
History from mother/caretaker	
Diarrhoea	More than three watery stools in 24 hours
Vomiting	More than three episodes of vomiting in 24 hours
Abnormal behaviour	Mother reports baby is more irritable or more sleepy than normal
Feeding Difficulty	Mother reports baby unable to feed as well as usual
Convulsions	Mother reports baby has had a convulsion
Fever	Mother reports baby has felt hot to touch
Cold	Mother reports baby has felt cold to touch
Clinician assessment	
Jaundice	Jaundice visibly detected
Cyanosis	Central cyanosis of gums or tongue
Pallor	Palmar pallor
Fast breathing	Respiratory rate >60 breaths per minute
Chest indrawing	Presence of lower chest wall indrawing / sub-costal recession
Lethargy	Difficult to arouse with stimulation
Seizures	Witnessed seizure in the neonate
Trismus	Unable to insert finger into the neonate's mouth
Omphalitis	Pus and/or erythema in umbilical area

Table 2: Definitions of symptoms and signs used

All nursing and medical staff working on the NNU at MRRH were trained to diagnose pSBI using local neonatal guidelines based on the WHO Young Infant Study. In addition, three research Medical Officers (MO) were trained in basic neonatal care for a minimum of three months before the study

commenced. A study MO would review each case of pSBI admitted to MRRH-NNU and identify those that fulfilled the study entrance criteria for recruitment.

INCLUSION CRITERIA

The following inclusion criteria were used:

- Neonates weighing over 2000g at admission
- Neonates fulfilling the case definition of pSBI
- Maternal age of at least 18 years

EXCLUSION CRITERIA

The following were exclusion criteria for the study:

- Any neonate with congenital abnormalities
- Use of parenteral antibiotics for 24 hours or more prior to recruitment
- History of perinatal asphyxia, that is an Apgar score of less than 6 at 5 minutes after birth or failure to cry at birth
- Neonates of mothers who were unable to speak English, Luganda, Lumasaba, Ateso or Lugwere well enough to provide informed consent

ENROLMENT AND CONSENT

Every morning, prior to identification of eligible patients, all the mothers and caregivers on the NNU were informed of the ongoing study by a member of the study team. When the study team identified an eligible patient, parental permission to be involved in the study was sought by one of the study team members. In cases where emergency management was required, this was

initiated immediately by the receiving healthcare worker. In order not to delay urgent care for such neonates, verbal consent was taken by a study staff from the mother prior to the removal of samples (Molyneux et al., 2013). In these cases, full written informed consent was then gained from the mother after investigations and treatment had been initiated and/or she had recovered from the delivery when she was better able to receive, evaluate and discuss the information.

TREATMENT AND MANAGEMENT

A case report form (CRF) was used to record demographic details, maternal, antenatal and perinatal history. A full neonatal history and examination was undertaken by a member of the study team and recorded onto the CRF.

All neonates were routinely treated according to local neonatal protocols, based on WHO guidelines. Neonates with pSBI and no clinical signs of meningitis were begun empirically on parenteral broad-spectrum antibiotics including intravenous ampicillin (50mg/kg twice daily) and gentamicin (5mg/kg once daily) for 7 days. If there was no clinical improvement within 48 hours, the neonate was changed to cefotaxime (50mg/kg twice daily) or ceftriaxone (50mg/kg once daily) together with gentamicin (5mg/kg once daily). Similarly, if there was no clinical response following 48 hours of parenteral cefotaxime/ceftriaxone and gentamicin, the neonate was changed to cefotaxime (50mg/kg twice daily) or ceftriaxone (50mg/kg once daily) and amikacin (15mg/kg once daily).

If there were clinical or biochemical signs of meningitis the neonates was begun on cefotaxime (50mg/kg twice daily) or ceftriaxone (80mg/kg once daily) and gentamicin for 14 days. Similarly, if was no clinical improvement within 48 hours, the neonate was changed to cefotaxime (50mg/kg twice daily) or ceftriaxone (80mg/kg once daily) together with amikacin (15mg/kg once daily).

Neonates with oxygen saturations less than 90% were given oxygen therapy until they were able to maintain their oxygen saturations above 90% in room air as recommended by WHO. In cases of severe respiratory distress, neonates were given bubble continuous positive airways pressure (bCPAP) if a machine was available. If present, seizures were treated with intravenous phenobarbitone as first line, loading with up to 40mg/kg and maintaining at 5mg/kg once daily. For those neonates whose seizures were not controlled on phenobarbitone alone, intravenous phenytoin (20mg/kg) was added as second line and maintained at 2.5mg/kg twice daily. Infants with clinical jaundice were treated with phototherapy if available and if not by sunshine therapy twice daily.

PATHOGEN DETECTION

In order to identify the infectious agents responsible, both blood and CSF were sampled. Blood was removed for routine microbiological cultures and thick and thin blood films were performed to inspect for malaria parasites. In order to minimize contamination, standard operating procedures were created for blood sampling and CSF sampling (Appendix 3 and 4). Venous blood for blood culture was collected using an aseptic technique. Sterile gloves were worn,

cleaning the skin once thoroughly with an alcohol swab and cleaning a second time with a betadine swab. A second pair of sterile gloves was then worn for a final clean with an alcohol swab before collecting blood using a cannula. Blood was collected using a syringe and was introduced directly into an empty cryovial (0.5ml), a preservative filled cryovial (1ml) and a BacTec/ALERT PF blood culture bottle (500-1000μL). The remaining blood was used to prepare a thick and thin smear (100μL) and to undertake an HIV antibody test (100μL, SD Bioline HIV 1/2 3.0).

The blood culture was mixed and stored at room temperature until transfer to the microbiology laboratory within 3 hours of collection. Blood cultures were incubated for five days using a BioMerieux BacTec/Alert system and cultured organisms were identified using standard bacteriological techniques. Antibiotic sensitivities of organisms were assessed using disc diffusion antibiotic sensitivity testing.

The two cryovials were inverted and placed immediately into liquid nitrogen. They were transferred weekly to -80°C storage for future testing using advanced molecular techniques to identify bacterial and viral DNA and viral RNA.

A lumbar puncture was performed on all neonates when not contraindicated. In neonates that were considered to be too sick for a lumbar puncture to be performed, such as those with active convulsions, severe respiratory distress or apnoea, it was deferred until such a time that the neonate was stable and

able to tolerate the procedure safely. CSF was collected using an aseptic technique. Sterile gloves were worn, first the skin was cleaned once thoroughly with alcohol swab, cleaning a second time with a betadine swab. A second pair of sterile gloves was then worn for a final clean with an alcohol swab before collecting blood using a spinal needle (25g BD Spinal Needle). CSF was collected into an empty cryovial (0.5ml), a preservative filled cryovial (1ml), a sterile universal container for culture, protein analysis and cell count (1ml) and a fluoride tube for glucose analysis (300µL). The two cryovials were inverted and placed immediately into liquid nitrogen. The CSF culture and analysis samples were stored at room temperature until transfer to the microbiology laboratory as soon as possible and always within 3 hours of collection. A positive CSF was defined as one in which an organism was identified by gram stain or culture. The upper bound values for a normal protein concentration and normal WBC count were; 127mg/dl and 15cells/mm^3 respectively (Thomson et al., 2018). The lower bound value for glucose concentration was 25 mg/dL (1.4mmol/L). The preservative-filled cryovials and fresh frozen cryovials were stored at 80°C for future testing to identify bacterial and viral DNA, viral RNA.

For each neonate enrolled in the study, maternal venous blood samples were also collected. Thick and thin smears were prepared (100µL) for identification of malaria parasites and an HIV antibody test (100µL, SD Bioline HIV 1/2 3.0) was performed. For those mothers who were HIV positive a CD4 count was performed.

IMAGING STUDIES

To assess the presence of intracranial pathology, a cranial ultrasound (cUS) examination was performed on all neonates on the day of presentation (day 1). Additional scans were performed 3, 7 and 28 days after presentation, and additionally if acute events intervened or death was imminent.

All scans were performed by one of five trained clinicians. Intensive training in cUS for the five clinicians was provided through in-person training over a one-week period by a single expatriate neonatologist specialising in perinatal neurology. The same neonatologist ran a repeat one-week training course 6 months after the start of the study to assess, refresh and improve skills in cUS.

For each infant, a standard set of coronal and sagittal images, as described in chapter 2, were acquired via the anterior fontanelle using a Sonosite M-Turbo ultrasound machine and a Cx11 probe. Using the Doppler setting in the midline sagittal view, the resistive index (RI) was measured across the anterior cerebral artery. In order to assess the cortex in greater detail, a second set of coronal and sagittal images were then acquired using the same ultrasound machine with a linear probe (SLAX). A colour Doppler assessment in both the sagittal and coronal planes allowed the superior sagittal sinus (SSS) blood flow to be assessed.

The cUS images were interpreted and reported in real-time by the clinician performing the cUS to provide clinical feedback to the medical team caring for the patient. Scans were anonymised at the point of acquisition, stored as

DICOM images in a password protected cloud storage system and centrally read by two consultant neonatologists with extensive experience in neonatal neuroimaging. The cUS images were viewed by the two assessors using OsiriX Lite v.8.0.2. Assessors were blinded to all clinical details of the neonate and images from each infant were grouped together.

Each cUS was assessed for any abnormalities of the basal ganglia, thalami, cortex, white matter, ventricles, the presence of abnormalities suggestive of CNS infection and complications of these infections such as post-infectious hydrocephalus as described in chapter 2.

FOLLOW-UP

The participants were given follow-up cards at discharge from MRRH-NNU with details of their four follow-up appointment dates. They were also reminded of their scheduled appointment three to four days before the appointment through phone calls and/or through SMS by a member of the study team.

The study follow-up clinic was run alongside the well-established and well-attended weekly hospital neonatal clinic to improve attendance rates. Transport was reimbursed to study participants for the follow-up appointments. If a patient failed to attend their follow-up appointment or was not contactable by phone, then a member of the study team would undertake a community visit to locate and assess the infant. Where infants could not be traced by phone, the village chairperson or by a community visit, the infant was declared lost to follow-up.

GROWTH, NEUROLOGICAL AND NEURODEVELOPMENTAL OUTCOME

The infants were invited to attend follow-up at 2, 6 and 12 months of age (Figure 19). At these appointments, their growth was assessed through measuring their weight, length and head circumference. Standard operating procedures were followed for all three anthropometric measurements. Nude weights were recorded using an electronic weighing scale (SECA 354), length was taken using a measuring mat (SECA 210) and head circumference was recorded using a standard tape measure. All measurements were plotted on the WHO weight-for-age, length-for-age and head circumference-for-age charts. The weight-for-length z-score calculated, and in cases where the weight-for-length z-score was less than 3 standard deviations, the infant was referred to MRRH Malnutrition Unit for further assessment.

The developmental of the infants was determined at each of the follow-up visits using Bayley Scales of Infant Development-3rd edition (BSID-III). The BSID-III is recognised as a comprehensive tool to assess the development of infants. BSID-III assesses the following domains:

- Psychomotor development including fine and gross motor assessment
- Cognitive development
- Language development including receptive and expressive language

Four members of the study team were trained by a qualified local BSID-III trainer to perform developmental assessments using the BSID-III. This

involved three days of theoretical and practical training before the study commenced. In addition, refresher training was provided by the same local trainer at the start of the 6-month and 12-month follow-up periods. All assessments during the study were performed by the BSID-III trainer or one of the 4 trained assessors. To assess the agreement between the four assessors, all 4 assessors administered the BSID-III assessment on 12 infants, each scoring the test independently, and one serving as the primary examiner for three tests and the observer for the other 8 tests. The Intraclass Correlation Coefficient for the raw scores was 0.96 (95% CI: 0.94-0.98).

Developmental assessment requires comparison to age-corrected norms by converting raw scores to scaled scores. Normative samples are usually cross-sectional, drawn from healthy children in the target population. Local normative data was not available, so we used the BSID-III normative data to create our scaled scores. The assessors undertook all the first conversions to scaled scores and the site investigator undertook a second conversion and the double data were compared and amended as required.

At the follow-up appointments, each infant was assessed for clinical signs of hydrocephalus. A clinical diagnosis of hydrocephalus was made if the head circumference was over the 97th centile for age, there was a large or tense fontanelle, sun-setting eye, distended veins over the forehead or open sagittal or coronal sutures. Infants with clinical signs of hydrocephalus were referred to CURE Children's Neurosurgical Hospital for further assessment. Children

with seizures or neurological impairment were referred to the MRRH Paediatric Outpatient clinic.

Although this study undertook a thorough developmental assessment at 2, 6 and 12 months of age, it formed part of a large multi-centre study, which did not include formal assessments for cerebral palsy, hearing impairment or visual impairment in the protocol.

EXPOSURES

- Laboratory-confirmed sepsis by positive blood culture
- Laboratory-confirmed meningitis by positive CSF culture and/or raised protein level and/or elevated white cell count

MARKERS OF INFECTION

- Cranial ultrasound abnormalities at presentation and on day 3, day 7 and day 28 after presentation.

OUTCOMES

The following outcomes were measured:

- Inpatient, neonatal and infant mortality
- Developmental impairment at 2, 6 and 12 months of age using BSID-III
- Post-infectious hydrocephalus

SURVIVAL OUTCOMES

If when contacted for follow-up, the child was reported to be dead then the date, place and likely cause of death were recorded. Neonatal mortality rates

(death before 28 days of age) and infant mortality rates (death before 12 months of age) were reported.

SAMPLE SIZE

This thesis was part of a large multi-site study, the CONSHA study, which aimed to accurately estimate the prevalence of meningitis among neonates with suspected sepsis. The sample size for the CONSHA study was calculated using the formula of N= (Z2 x P(1 / P))/e2, where N = the required sample size, z = 1.96 (the value from the standard normal curve corresponding to the 95%), p = the estimated proportion of patients with meningitis, and d = precision (5%). Setting p = 15%, which is anticipated from existing literature, calculations indicated that a sample size of 196 patients would be sufficient to estimate the percentage of patients having meningitis with a 95% confidence interval no wider than ±5%.

In addition, variations in the incidence of neonatal meningitis and post-infectious hydrocephalus have been documented in relation to the seasons, specifically the rainfall in Uganda. In order to maximise the chances of detecting these potential aetiological variations and relationships to rainfall, the CONSHA study aimed to recruit neonates over a two-year period.

For this thesis, a 2-year study period was chosen for convenience. It also ensured an adequate opportunity for both recruitment over a 12-month period and follow-up of these patients until 12 months of age. At MRRH-NNU, approximately 600 neonates with pSBI are admitted each year. It was

therefore anticipated that 196 patients could be recruited over a one-year period to accurately estimate the incidence of meningitis and also cover two rainy seasons.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM® SPSS® Statistics Version 19 for Macintosh. Normally distributed continuous variables were expressed as a mean and standard deviation (SD) whilst all other continuous variables were expressed as a median with an inter-quartile range (IQR). All categorical variables were expressed as a percentage. Comparisons between groups were made using Mann-Whitney's *U,* Student's *t,* Fisher's exact and χ^2 tests as appropriate. Two-tailed *P*-values <0.05 were considered statistically significant.

ETHICS

This study was granted ethical approval by the following Research Ethics Committees (RECs); Mbarara University of Science and Technology REC, Uganda National Council for Science and Technology REC, Penn State University REC, University of Liverpool REC.

CHAPTER 5 - SERIOUS BACTERIAL INFECTIONS AMONG UGANDAN NEONATES: CLINICAL FEATURES, AETIOLOGY AND NEONATAL OUTCOMES.

BACKGROUND

SSA bears a disproportionate burden of neonatal mortality (Seale et al., 2014). It is estimated that serious bacterial infections account for 21% of neonatal deaths worldwide and that in SSA there are up to 2.6 million cases of pSBI every year, leading to an estimated 250,000 deaths (Seale et al., 2014, Liu et al., 2016, Health, 2008b). In Uganda, the NMR remains high at 28/1000 live births and has not changed over 2 decades (UBoSUa, 2017). Neonatal infections are one of the leading causes of neonatal deaths in Uganda.

DEFINING NEONATAL SERIOUS BACTERIAL INFECTIONS

Neonatal sepsis is classically defined as the presence of symptoms or signs of systemic infection within 28 days of birth accompanied by bacteriological isolation of a pathogen from the blood or CSF (Qazi and Stoll, 2009). As discussed in Chapter 1, numerous challenges persist in the diagnosis of serious bacterial infections. Not only are the signs and symptoms non-specific, but bacterial isolation is infrequent. In low-resource settings, laboratory facilities are frequently limited, therefore the diagnosis of serious bacterial infection typically relies on clinical algorithms. PSBI is a clinical syndrome used in the Integrated Management of Neonatal and Childhood Illness (IMNCI) to identify sick neonates and to direct their management (Young Infants Clinical

Signs Study, 2008). This pragmatic approach ensures high sensitivity rather than specificity in the detection of what can be a devastating disease and is the approach that continues used in many LICs like Uganda.

DIAGNOSIS OF NEONATAL INFECTIONS

The 'gold-standard' for diagnosis of neonatal sepsis is blood-culture, and the isolation of bacteria from the blood of a neonate with a clinical diagnosis of sepsis has a high specificity. As described in more detail in Chapter 2, their sensitivity is however low, and pathogens are rarely recovered (Vergnano et al., 2011). A large study using blood cultures, carried out in multiple LMICs, isolated bacteria from fewer than 10% of cases of young infants with symptoms and/or signs of systemic infection (sepsis, meningitis or pneumonia), leaving the aetiology of the majority of cases unknown (Hamer et al., 2015). One reason for the low sensitivity, is that insufficient volumes of blood are drawn and neonates become symptomatic at low levels of bacteraemia meaning that the number of colony-forming units inoculated into the culture media may not be enough for automated blood culture systems to detect bacterial growth (Schelonka et al., 1996, Brown et al., 1995, Kellogg et al., 1997). The sensitivity of blood cultures in low-resource settings, is further hindered by the use of intrapartum antibiotics or prior over-the-counter antibiotic use in the community, which is common in Uganda (Kiwanuka et al., 2013). It is possible that cases of pSBI with negative blood cultures, may actually be due to viral or parasitic pathogens or even bacteria that are not easily grown using traditional microbiological techniques. Furthermore, the clinical syndrome of pSBI is non-specific and clinical features overlap with other non-infectious pathologies

such as prematurity, cardiac failure, and neonatal encephalopathy. Blood cultures also have a minimum reporting time of 48 hours, meaning that neonates may succumb to their infection before the results are available. A diagnosis of neonatal sepsis can be supported by the presence of raised inflammatory markers such as C-reactive protein (CRP) and procalcitonin. These tests are rarely available in low-resource settings and have limited sensitivity (Brown et al., 2019, Vouloumanou et al., 2011).

MENINGITIS

An early complication of neonatal sepsis is meningitis, an acute inflammation of the meninges, subarachnoid space and vasculature (Barichello et al., 2013). It is a devastating illness that not only has a higher mortality than neonatal sepsis but a higher risk of neurodevelopmental disability. Unfortunately, the presentation of neonatal meningitis can be subtle as typical clinical features such as bulging fontanelle, opisthotonus and seizures are not always present (Mwaniki et al., 2011). The exact incidence of meningitis in low-resource settings, is hard to evaluate as many cases of neonatal sepsis do not undergo a lumbar puncture for CSF culture or analysis. Many cases of neonatal meningitis are therefore missed or inadequately treated leading to high mortality and morbidity.

Even when facilities exist, only a minority of pathogens are identified by culture, however abnormal CSF white blood cell (WBC) count, protein concentration and glucose concentration can support a diagnosis of meningitis (Ahmed et al., 1996, Martin-Ancel et al., 2006, Nascimento-Carvalho and

Moreno-Carvalho, 1998, Byington et al., 2011, Kestenbaum et al., 2010, Shah et al., 2011, Laving et al., 2003, Thomson et al., 2018). Two studies in East Africa that undertook lumbar punctures in neonates presenting with clinical sepsis reported a prevalence of meningitis of 3-18% (Laving et al., 2003, Talbert et al., 2010). In these studies, the CSF cultures were positive in less than 4% of cases and the diagnosis of meningitis relied on other tests including the presence of bacteria on Gram stain, positive CSF antigen test for one of; *Haemophilus influenzae* type b, GBS, *E. coli* or *Streptococcus pneumoniae*, or a raised white cell count ≥50 cells/μl.

AETIOLOGY OF SEPSIS

Traditionally, EONS is believed to be of maternal origin, from before or during delivery from the maternal genital tract (vertical transmission) and presents within the first 48 h after birth (Newton and English, 2007). Chorioamnionitis, intrapartum fever, prolonged rupture of membranes and prematurity all increase the risk of EONS (Puopolo et al., 2011). Conversely, LONS is more likely to be acquired after delivery – either from a hospital or community source and conventionally presents after 48 hours from birth. In HICs, these differences lead to differences in the pathogens detected, however in LICs, these differences are less apparent (Group, 1999c, Waters et al., 2011, Hamer et al., 2015, Zaidi et al., 2009). In facility-based deliveries, unclean delivery practices and poor infection prevention during delivery and the postnatal period lead to an increased number of early nosocomial infections (Zaidi et al., 2005). A high rate of home deliveries can contributes to neonatal infections with community-acquired pathogens in the early neonatal period (Zaidi et al.,

2005). Meta-analyses of neonatal sepsis in LMICs have found the leading pathogens in EONS and LONS to be almost identical (Hamer et al., 2015, Waters et al., 2011, Zaidi et al., 2009). Meta-analyses of studies of community-acquired sepsis in SSA report *S. Aureus, E. coli, Klebsiella,* GBS and *Streptococcus pyogenes* to be the most commonly detected pathogens (Waters et al., 2011, Zaidi et al., 2009) Whilst studies in SSA that have focused on hospital-acquired neonatal infections, have also predominantly cultured similar pathogens, namely *Escherichia coli, Klebsiella* and *Staphylococcus aureus* (8-22%) (Zaidi et al., 2005, Zaidi et al., 2009, Downie et al., 2013, Stoll et al., 2011). These findings suggest that the timing of sepsis in such settings is less significant, and that the source, community versus hospital, might be more important, especially when considering the antibiotic sensitivity patterns of pathogens.

AETIOLOGY OF MENINGITIS

Similar to blood cultures, many studies report a high rate of culture-negative cases of suspected neonatal meningitis. The majority of data on the aetiology of neonatal meningitis are from HICs, where the leading causes are reportedly GBS and *E. coli* (Kimberlin, 2002, de Louvois et al., 1991). Data on the aetiology of neonatal meningitis in LMICs are limited, however studies using CSF culture show that *Klebsiella* species are common in LMICs (Kasirye-Bainda and Musoke, 1992, Musoke and Malenga, 1984). A more recent Kenyan study used both CSF culture and latex particle agglutination assay, reported *E. coli.,* GBS and *Klebsiella pneumoniae* to be the most common isolates (Laving et al., 2003). Another Malawian study of culture positive

isolates from CSF of neonates <7 days with clinically suspected found the most common pathogens to be GBS (45%), *Strep. Pneumoniae* (22%) and nontyphoidal *Salmonella enterica* (12%) (Swann et al., 2014).

IMPROVING PATHOGEN DETECTION IN NEONATAL INFECTIONS

Significant advances in molecular diagnostics have been made including quantitative PCR (qPCR) to specific microorganisms, 16S rDNA amplicon sequencing, and sequencing of bulk DNA or RNA. Unlike culture, these techniques allow detection of both viable and non-viable bacteria, and they also help to detect difficult-to-culture pathogens. A recent large study of pSBI in infants up to 59 days after birth in Bangladesh performed blood culture and species-specific PCR for 15 bacterial and 13 viral pathogens on blood and respiratory samples (Saha et al., 2018). Using this approach, they identified pathogens in up to 28% of pSBI episodes with 16% being bacterial and 12% viral. The most commonly detected pathogen was respiratory syncytial virus (RSV), followed by *ureaplasma* sp (Saha et al., 2018). These are pathogens that are not normally detected using blood culture.

Real-time PCR has also been used to test culture-negative CSF in cases of suspected meningitis based on CSF analysis of protein, white cell and glucose levels. In adults, such techniques have detected pathogens in up to 90% of culture-negative samples (Khater and Elabd, 2016). A Japanese study reported the CSF results of 150 neonates and children with meningitis (Chiba et al., 2009). Pathogens were detected in only 48% of their samples by culture

however real-time PCR for eight common pathogens increased the detection rate to 72%.

Even with this combined approach, the majority of cases of pSBI still have no detectable pathogen. Firstly, this suggests that a substantial proportion of pSBI may be due to alternative or novel pathogens. Despite the highly sensitive and specific nature of qPCR, it will only ever detect those pathogens that are anticipated and therefore novel pathogens will still be missed. Secondly, it is possible that many cases of pSBI may actually not be due to a bacterial or a viral pathogen. The highly sensitive clinical algorithm for pSBI may actually include many non-infectious aetiologies.

In the absence of a rapid, sensitive and specific test for severe bacterial infections in neonates, clinicians continue to rely on a combination of clinical and laboratory findings (Zea-Vera and Ochoa, 2015). In low-resource settings, where little or no laboratory support exists, the diagnosis continues to rely almost entirely on clinical findings (Young Infants Clinical Signs Study, 2008).

CLINICAL PREDICTORS

Clinical signs and history can be used to identify neonates with severe infection (Gupta et al., 2000). Studies have also looked at the signs associated with death and severe disease (including hypoxaemia, lung consolidation, positive blood culture, positive CSF culture) from infections in young infants (Bang et al., 2005, Duke et al., 2005, Weber et al., 2003, English et al., 2004). Together, these studies have found a history of difficulty feeding, no spontaneous

movements, being weak or unconscious, difficulty breathing, fast breathing (>60 breaths per minute), chest indrawing, cyanosis, apnoea, hypothermia (cold to touch, <35.5ºC), fever (>38ºC), history of convulsions, abdominal distension and history of change in activity to be valuable signs in identifying severe illness in young infants (Bang et al., 2005, Gupta et al., 2000, Duke et al., 2005, Weber et al., 2003, English et al., 2004). All these signs have a high sensitivity (87-97%) for identifying serious neonatal infections, but a lower specificity (51-59%). Utilising the presence of a single sign has the best sensitivity. If more stringent combinations are used, sensitivity is reduced. Despite multiple attempts to refine prediction through clinical signs alone, the accuracy of prediction that is achievable using this method is limited.

The challenge of all these studies is that there is still no gold standard for the diagnosis of neonatal sepsis. Although these clinical signs were associated with severe illness and death, it is still very possible that these cases may still have been due to another aetiology.

SUMMARY

Despite pSBI being a leading cause of global neonatal mortality, key challenges remain in the prevention, diagnosis and management of neonatal sepsis, especially in low-resource settings. To improve outcomes requires a better understanding of the aetiology of pSBI, be it infectious or otherwise, so that more effective preventative and treatment options can be explored. Due to the lack of specificity of the clinical diagnosis and the challenges in pathogen detection, the causes of most cases of neonatal pSBI remain undefined. This

restricts the success of prevention and treatment policies and limits progress on reducing infection-related deaths (Kiwanuka et al., 2013),(Liu et al., 2015).

AIMS

This study sought to describe the clinical features, aetiology as identified on microbiological culture of blood or CSF and neonatal outcomes in term infants admitted with pSBI to a neonatal unit in eastern Uganda over a 12-month period.

METHODS

STUDY DESIGN

This was an observational study of term infants presenting with pSBI over a 12-month period to Mbale Regional Referral Hospital Neonatal Unit (MRRH-NNU).

SETTING

MRRH serves a population of 4.5 million people and has a dedicated neonatal unit (NNU) that admits over 2500 neonates a year (Burgoine et al., 2018). Neonates are admitted directly from the labour ward, referred from surrounding health facilities and, due to a high rate of home deliveries, some neonates are brought in directly from home (UBoSUa, 2017). As a government hospital, medical care and treatment are provided free of charge to the patient, however not all antibiotics are routinely available in our healthcare system.

PARTICIPANTS

Over 12-month period, from 9th December 2016 until 8th December 2017, any caregiver of a neonate (less than 28 days of age) who presented to MRRH-NNU with pSBI (Figure 20) and who fulfilled inclusion and exclusion criteria (described below) was invited to enrol their neonate in the study. Inclusion criteria included weight >2000g, maternal age ≥18 years and ability of the mother to provide informed consent. Exclusion criteria included neonates with congenital abnormalities, use of parenteral antibiotics for 24 hours or more prior to recruitment, history of perinatal asphyxia (Apgar score <6 at 5 minutes after birth or failure to cry) and inability of the mother to speak one of the local languages (English, Luganda, Lumasaba, Ateso or Lugwere) well enough to provide informed consent, were excluded. Infants were recruited Monday to Friday due to laboratory limitations. Up to a maximum of three sequential infants were recruited each day.

CASE DEFINITION

The clinical features of pSBI can overlap with a number of different aetiologies, the most common of which are complications of prematurity and neonatal encephalopathy due to perinatal asphyxia. Uganda has a high incidence of preterm births and preterm infants often suffer from respiratory distress syndrome, environmental hypothermia, apnoea of prematurity, poor feeding and jaundice, all of these signs can also be present due to infection (McGuire et al., 2004). In this study we chose to exclude those with a birthweight <2000g in order to be more confident that these clinical signs were secondary to infection and not prematurity. There is also a high incidence of perinatal

asphyxia and neonatal encephalopathy (NE) in Uganda and similar settings (Tann et al., 2014). Although infection is an important risk factor for developing NE, we wanted to exclude those with NE and focus on those with neonatal infection alone (Tann et al., 2014). We only included those who had a normal Apgar score at birth and/or did not require significant resuscitation at birth. In addition, in order to define a group that unequivocally required hospital-based treatment and had severe disease that was most likely due to infection, we defined pSBI using a combination of signs and symptoms from Young Infant study as shown in Figure 20.(Group, 1999b)

The presence of one of the following three combinations of symptoms and signs in a neonate:

a) Fever (>37.5°C), lethargy and poor feeding
b) Hypothermia (< 35.5°C) , lethargy and poor feeding
c) Full fontanelle and/or seizures, fever, poor feeding

Figure 20: Case definition of possible severe bacterial infection (pSBI)

CSF ANALYSIS

There are limited data on CSF reference values for neonates (Ahmed et al., 1996, Martin-Ancel et al., 2006, Nascimento-Carvalho and Moreno-Carvalho, 1998, Byington et al., 2011, Kestenbaum et al., 2010, Shah et al., 2011). A recent large multicentre study of presumptively uninfected neonates has established age-specific reference values for CSF in neonates (Thomson et al., 2018). The upper bound values for protein concentration and WBC count

are; 127mg/dl and 15cells/mm^3 respectively. The lower bound value for glucose concentration was 25 mg/dL (1.4mmol/L). These are the values that have been adopted for interpretation of this study.

DATA COLLECTION

Mothers who gave informed consent to participate in the study, were interviewed by a member of the research team. Data regarding their demographics, social situation, pregnancy and birth were recorded on a specially designed case-report form (Appendix 1). Wherever possible, information was also collected from maternal records from those born in MRRH and from the referral letter for those born at different health-facilities.

In order to identify the infectious agents responsible, all neonates had venous blood sampled for culture. In order to minimize contamination, standard operating procedures were created for blood sampling (Appendix 3). Venous blood was collected using an aseptic technique, using sterile gloves, cleaning the skin once thoroughly with alcohol swab, cleaning a second time with a betadine swab and lastly with an alcohol swab before collecting blood using a cannula. Blood was collected using a syringe and was introduced directly into a BacTec/ALERT PF blood culture bottle (500-1000μL). The remaining blood was used to prepare a thick and thin smear (100μL) and to undertake an HIV antibody test (100μL, SD Bioline HIV 1/2 3.0). The blood culture was stored at room temperature until transfer to the microbiology laboratory within 3 hours of collection. Blood cultures were incubated for five days using a BioMerieux BacTec/Alert system and cultured organisms were identified using standard

bacteriological techniques. Antibiotic sensitivities of organisms were assessed using disc diffusion antibiotic sensitivity testing.

A lumbar puncture was performed on all neonates when not contraindicated. For those neonates with active convulsions, severe respiratory distress or apnoea, lumbar puncture was delayed until it was safe to perform. CSF was collected using an aseptic technique, sterile gloves were worn, cleaning the skin once thoroughly with an alcohol swab, cleaning a second time with a betadine swab and lastly with an alcohol swab again before collecting blood using a spinal needle (25g BD Spinal Needle). CSF was collected into a sterile universal container for culture, protein analysis and cell count (1ml) and a fluoride tube for glucose analysis (300μL). The CSF culture and analysis samples were stored at room temperature until transfer to the microbiology laboratory as soon as possible and always within 3 hours of collection.

ADJUDICATION OF POSITIVE CULTURE RESULTS

Medical records were reviewed for cases with positive cultures: antibiotic coverage was evaluated for effectiveness against the cultured organism and database outcomes were confirmed. Cultured organisms were then classified as definite pathogens, possible pathogens and probable contaminants. The organism was considered to be a definite pathogen if: 1) the antibiotics received by the infant had a spectrum which appropriately treated the cultured organism and clinical improvement was noted after initiation of treatment, or 2) the antibiotics did not cover the cultured organism and the infant failed to improve clinically. Organisms were considered to represent possible

pathogens if: 1) The antibiotics covered the cultured organism, but the infant did not improve or improved slowly, or 2) the antibiotics did not cover the organism and the infant improved slowly. Organisms were considered to be probable contaminants if the antibiotics did not cover the organism but the neonate rapidly improved.

OUTCOMES

Data on the antibiotics given, the duration of antibiotic therapy, final diagnosis (Figure 21) and both in-patient and neonatal outcomes (Figure 22) were recorded.

- **Sepsis**: clinical diagnosis fulfilling the definition of pSBI outlined above in the absence of respiratory and meningeal signs together with a normal CSF if performed.
- **Meningitis**: positive CSF culture, organism on Gram stain and/or raised WBC count and/or protein concentration and/or low glucose concentration.*
- **Pneumonia**: clinical diagnosis defined as pSBI + signs of respiratory distress (sub-costal recession, sternal recession, grunting, hypoxia and cyanosis).
- **Neonatal tetanus**: defined clinically as per World Health Organisation (WHO), as an illness occurring in a neonate who has the normal ability to suck and cry in the first 2 days of life, but who loses this ability between days 3 and 28 of life and becomes rigid or has spasms.

*Limits used include: Protein concentration >127mg/dl, WBC count >15cells/mm^3 and glucose concentration <25 mg/dL.

Figure 21: Definitions of the final diagnoses

Final inpatient outcome

- Discharged alive with no known sequelae
- Discharged alive with known sequelae including abnormal tone, seizures, post-infectious hydrocephalus, inability to breastfeed
- Inpatient death
- Discharged against medical advice

Neonatal outcome (within 28 days of birth)

- Alive with no known sequelae
- Alive with sequelae including seizures, post-infectious hydrocephalus
- Neonatal death

Figure 22: Definitions of inpatient and neonatal outcomes

STUDY SIZE AND STATISTICAL ANALYSIS

Convenience sampling was used. The sample size reflects a one-year time period. Data were analysed using IBM SPSS Statistics Version 25. Categorical variables were examined using Chi-squared test and Fisher's exact test as appropriate for sample size. Continuous variables were examined using student's T-test and the Mann-Whitney U test according to normality. Infants with missing data were excluded from the relevant analysis.

ETHICS

The Institutional Review Board of Mbarara University of Science and Technology, Uganda, the Uganda Council for Science and Technology and Penn State University approved the study.

RESULTS

RECRUITMENT

During the 12-month study period, 2251 neonates were admitted to MRRH-NNU (Figure 23). Of the 585 neonates admitted with pSBI, 216 were recruited to the study. Parental consent was later withdrawn for two of the 216 recruited neonates and they were excluded from the analysis.

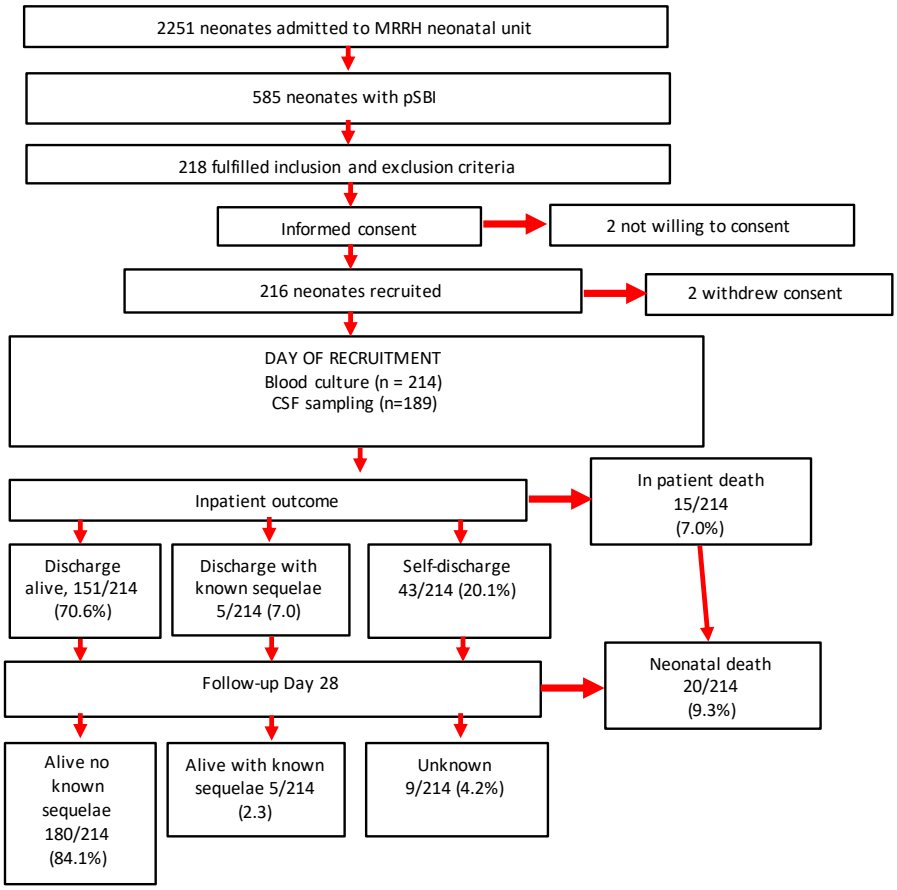


Figure 23: Flow chart of the study consent, recruitment, sampling and follow-up

POPULATION CHARACTERISTICS

The majority of cases presented within the first 48 hours after birth (130/214, 60.7%) as shown in Figure 24, and the median age at presentation was 2.0

days of age (IQR 2.0, 4.0, range 1 to 27 days of age). The majority of cases were male (129/214, 60.3%), and for those mothers with a known last normal menstrual period, the majority of neonates were born at term, defined as 37 completed weeks of gestation (127/136, 93.4%) as shown in Figure 25.

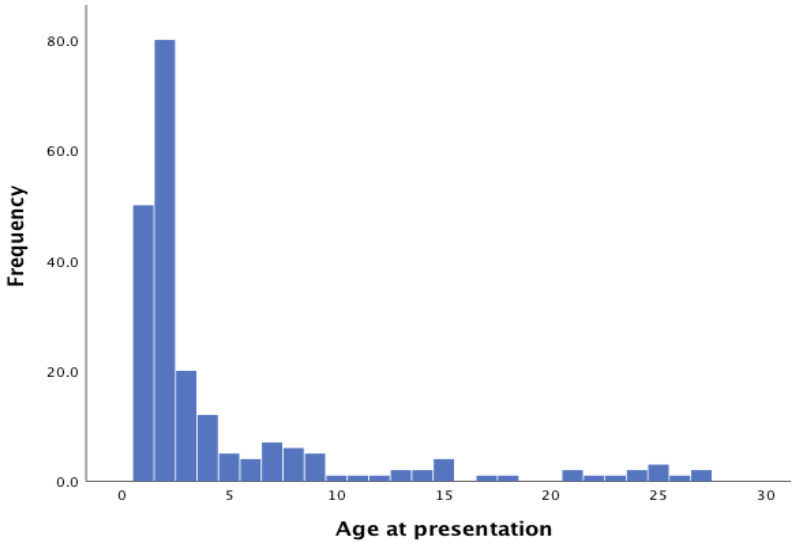


Figure 24: The age of neonates at presentation to the neonatal unit with a possible serious bacterial infection (pSBI)

Almost all of the infants were born in a health-facility and the majority of births were attended by a healthcare worker (Table 3 and Figure 26). As shown in Figure 26, two-thirds of the neonates were born by spontaneous vaginal delivery (67.3%) and one-third by caesarean section (31.8%) (Table 4).

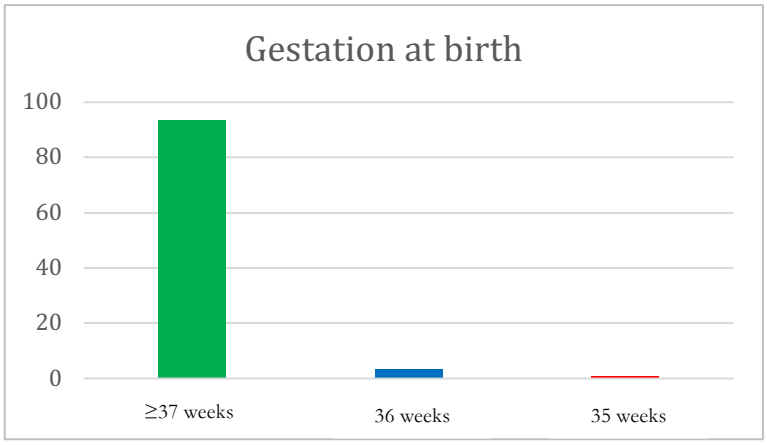


Figure 25: Gestational age at birth of neonates who presented to the neonatal unit with a possible serious bacterial infection (pSBI)

Characteristic	Overall (n=214)
Age of neonate at presentation (days)	
- Mean (SD)	4.6 (5.9)
- Median (IQR)	2.0 (2.0, 4.0)
Early or late presentation, Freq (%)	
- Presentation <48 hours after birth (early)	130 (60.7)
- Presentation ≥48 hours after birth (late)	84 (39.3)
Sex Freq (%)	
- Male	129 (60.3)
- Female	85 (39.7)
Gestation at birth (n=136)	
- Median (IQR)	40 (39, 41)
- >37 weeks	127/136 (93.4)
- 36 weeks	7/136 (3.3)
- 35 weeks	2/136 (0.9)
Neonatal resuscitation, Freq (%)	
- Need for resuscitation	38/214 (17.8)
- Suction/stimulation only	19/214 (8.9)
- Bag-mask ventilation	13/214 (6.1)
- Apgar score at 5 minutes (n=187)	9.6 (0.8) Mean (SD) Range 7-10
Cord care, Freq (%)	
- Water	36 (16.8)
- Water and baby powder	3 (1.4)
- Saltwater	123 (57.5)
- Saltwater and baby powder	7 (3.3)
- Baby powder	1 (0.5)
- Chlorhexidine	2 (0.9)
- Bat faeces	1 (0.5)
- Herbs	1 (0.5)
- Soap	1 (0.5)
- Nil	17 (7.9)
- Unknown	22 (10.3)
Surgery, Freq (%)	
- Circumcision	1 (0.5)
Feeding, Freq (%)	
- Breastmilk	212 (98.6)
- Formula	0 (0)
- Mixed (breastmilk and cow's milk)	2 (0.9)
Antimicrobial therapy initiated on admission	
- Ampicillin and Gentamicin	107/214 (50.0)
- Ceftriaxone/Cefotaxime and Gentamicin	107/214 (50.0)
Duration of hospitalisation (days)	
- Median (IQR)	7.0 (5.0, 8.0)
- Range	1 – 26

Table 3: Characteristics of the neonates with possible serious bacterial infection (pSBI)

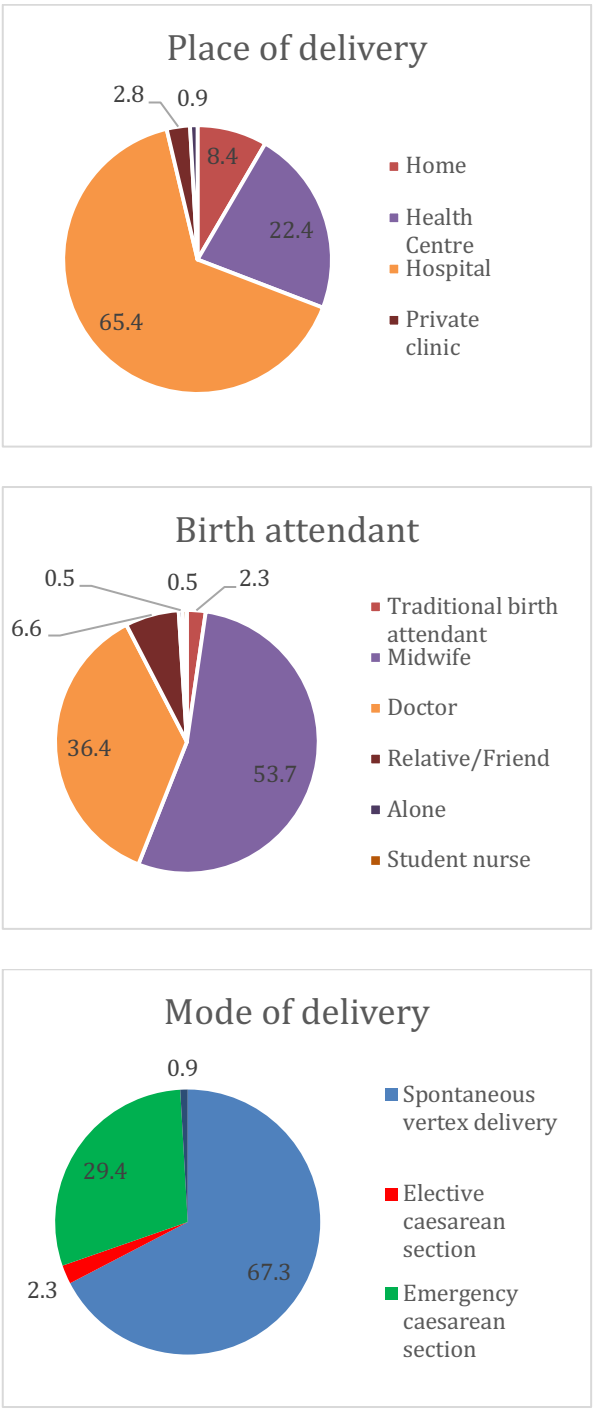


Figure 26: Place of delivery, birth attendant present and mode of delivery of neonates presenting with a possible serious bacterial infection (pSBI)

As shown in Table 4, the median duration of labour was 15.0 hours (IQR 10, 24) and 10.7% (18/168) of mothers reported a prolonged rupture of membranes over 18 hours, 3.3% (7/214) reported foul-smelling liquor and 5.1% (11/214) reported meconium stained liquor.

Characteristic	Overall (n=214)
Place of delivery, Freq (%)	
- Home	18 (8.4)
- Health centre	48 (22.4)
- Hospital	140 (65.4)
- Private clinic	6 (2.8)
- On the way	2 (0.9)
Type of birth attendant, Freq (%)	
- Traditional birth attendant	5 (2.3)
- Midwife	115 (53.7)
- Doctor	78 (36.4)
- Relative/Friend	14 (6.6)
- Alone	1 (0.5)
- Student nurse	1 (0.5)
Mode of delivery, Freq (%)	
- Spontaneous vertex delivery	144 (67.3)
- Elective caesarean section	5 (2.3)
- Emergency caesarean section	63 (29.4)
- Operative vaginal birth	2 (0.9)
- Breech delivery	0 (0)
Maternal fever during labour, Freq (%)	
- Yes	90 (42.1)
- No	
Maternal Abx during labour, Freq (%)	
- Yes	98 (45.8)
- No	116 (54.2)
Rupture of membranes, Freq (%)	
- ≥18 hours (n=168)	15/168 (8.9)
- Spontaneous	184 (86.0)
- Manual	30 (14.0)
Duration of labour (n=197)	
- Hours, Median (IQR)	15.0 (10, 24)
Liquor, Freq (%)	
- Clear	142 (66.4)
- Meconium	11 (5.1)
- Foul smelling	7 (3.3)
- Unknown	54 (25.2)

Table 4: Peripartum history of neonates with possible serious bacterial infection (pSBI)

As described in the methods, this study was designed to exclude neonates with perinatal asphyxia. The Apgar score was known for 187/214 neonates and in all these cases the Apgar score at 5 minutes was >6 (Table 3). For those 27 neonates with no known Apgar score: fourteen reportedly cried

immediately and required no resuscitation, seven required suction/stimulation, two required basic resuscitation with bag mask ventilation but had a good response and four had no details about resuscitation available but the neonates had no signs of encephalopathy at or during their admission.

For the 209 mothers whose education level was known, only 2.9% had no schooling at all (Table 5). As shown in Figure 27, the others were similarly distributed with approximately one third having completed primary schooling (37.3%), secondary schooling (36.4%) and tertiary education (23.4%). Although most mothers were primarily housewives (35.5%), one-quarter were farmers (25.2%), 20.1% were service or sales workers, and 14.5% were professionals. This wide spectrum of education and employment likely reflects that the study was carried out at a regional referral hospital, which is the only specialist neonatal unit in the region.

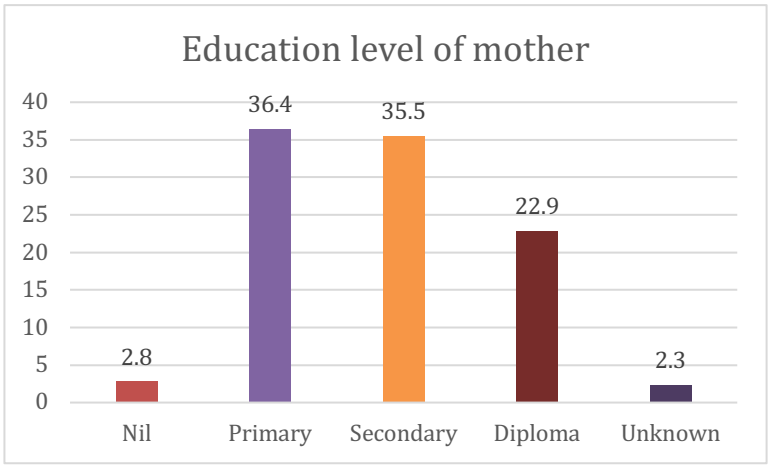


Figure 27: The educational level of the mothers of neonates presenting with possible serious bacterial infection (pSBI).

As shown in Table 5 and Figure 28, the majority of mothers were primigravida (36.9%) and the median parity was 2 (IQR 1, 4). The range extended to a parity of twelve. At presentation, 20/214 (9.3%) of mothers did not know their HIV sero-status but were confirmed to be sero-negative at recruitment; 6/214 (2.8%) were known to be HIV sero-positive and were already on anti-retroviral therapy.

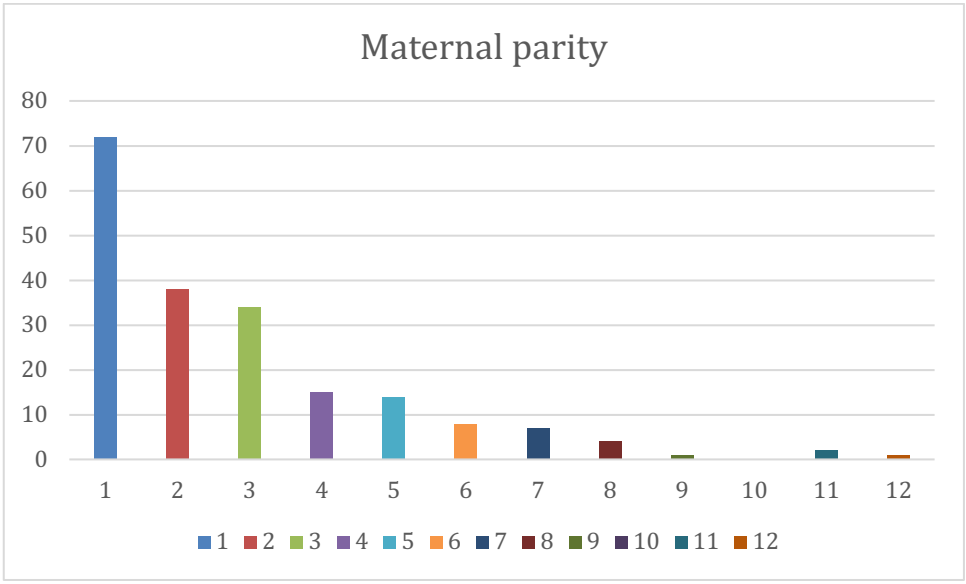


Figure 28: The maternal parity of neonates presenting with a possible serious bacterial infection (pSBI)

Only 36.0% of mothers reported *not* to have experienced a febrile episode during their pregnancy (Figure 29 and Table 5), with 42.5% experiencing two or more episodes. A fever during labour was reported by almost half of mothers (42.1%) and just under half of the mothers (46.7%) received antibiotic therapy during the intrapartum period. The most common antibiotics prescribed to the mothers were amoxicillin (42.0%), ceftriaxone (16.0%) and erythromycin (17%).

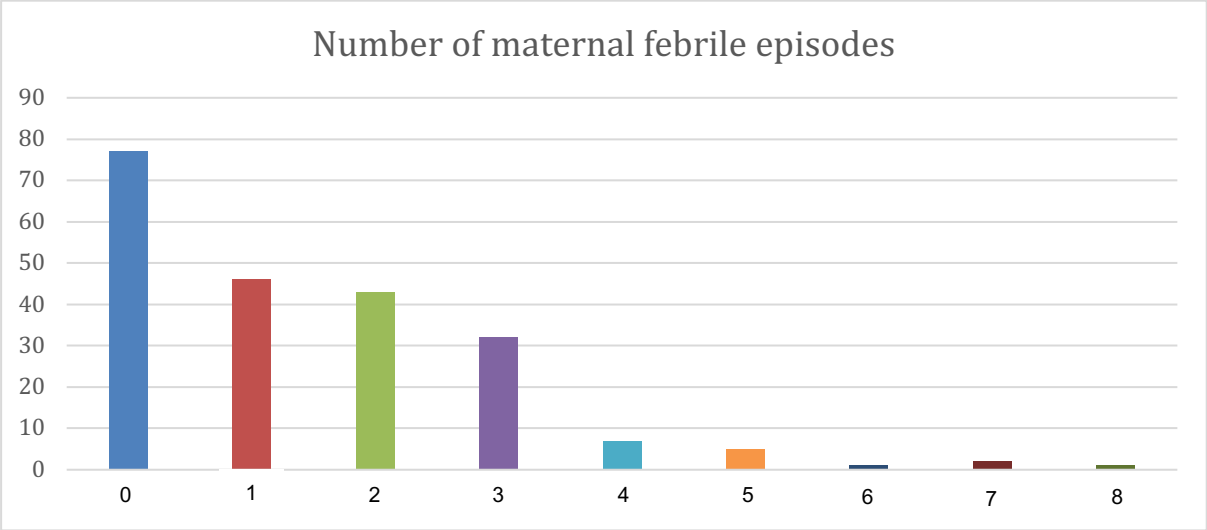


Figure 29: The number of maternal febrile episodes experienced during the pregnancy of neonates presenting with possible serious bacterial infection (pSBI)

Characteristic	Overall (n=214)
Maternal age at delivery (years)	
- Median (IQR)	24.0 (21.0, 29.0)
Maternal fever during pregnancy, Freq (%)	
- 0 episode	77 (36.0)
- 1 episode	46 (21.5)
- 2 or more episodes	91 (42.5)
Prenatal HIV status, Freq (%)	
- Positive	6 (2.8)
- Negative	188 (87.9)
- Unknown	20 (9.3)
Maternal Parity	
- Primigravida, Freq (%)	79 (36.9)
- Median parity	2.0 (1.0, 4.0)
- Range	1 - 12
Maternal malaria smear, Freq (%)	
- P. falciparum	6/214 (2.8)
Education, Freq (%)	
- Nil	6 (2.8)
- Primary	78 (36.4)
- Secondary	76 (35.5)
- Diploma	49 (22.9)
- Unknown	5 (2.3)
Employment, Freq (%)	
- Professionals (accountant, engineer, teacher, healthcare worker, manager)	31(14.5)
- Clerical support worker	2(0.9)
- Service and sales worker (police, security guard, caterer, hairdresser, sales workers)	43(20.1)
- Skilled forestry, agricultural, fisheries worker	54(25.2)
- Craft worker (tailor)	5(2.3)
- Others (student)	1(0.5)
- Housewife	76(35.5)
- Unknown	2(0.9)

Data n (%) unless otherwise stated.

Table 5: Baseline characteristics of the mothers

CLINICAL CHARACTERISTICS

As shown in Table 6 and Figure 30, the most frequently reported clinical features at presentation were fever (98.1%), poor feeding (98.1%), lethargy (93.9%) and excess crying (89.7%). Other common symptoms were seizures, which were reported in 40 (18.7%) cases, jaundice in 83 (38.8%) cases and difficulty in breathing in 44 (20.6%) cases.

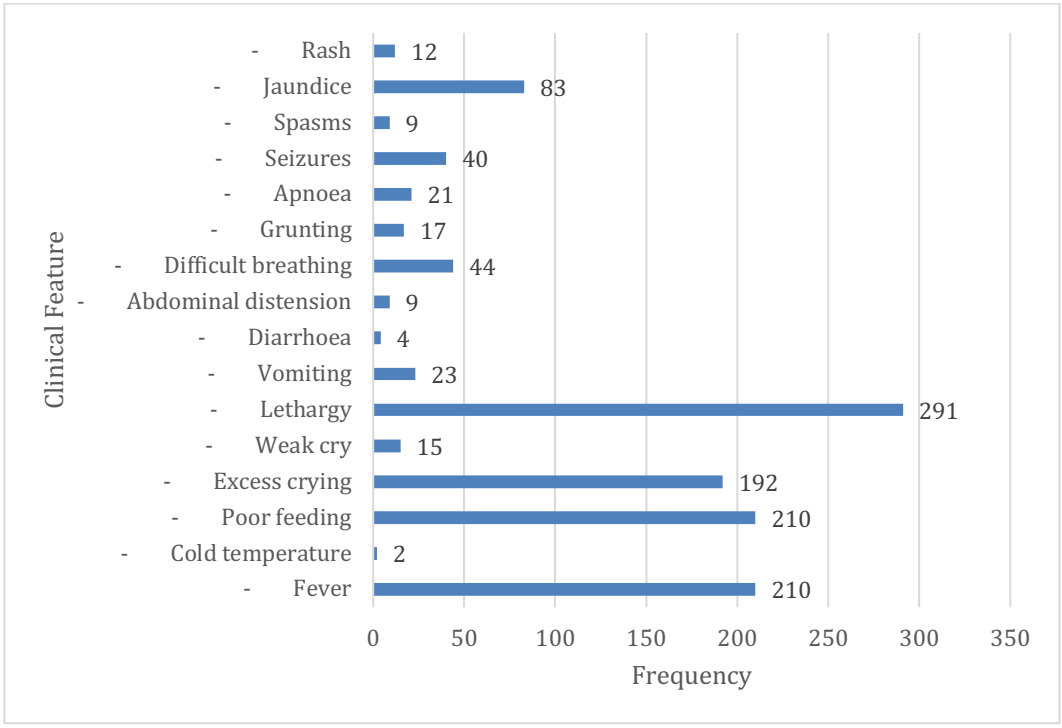


Figure 30: The frequency of clinical symptoms reported by the mother or caregiver in neonates presenting with a possible serious bacterial infection (pSBI)

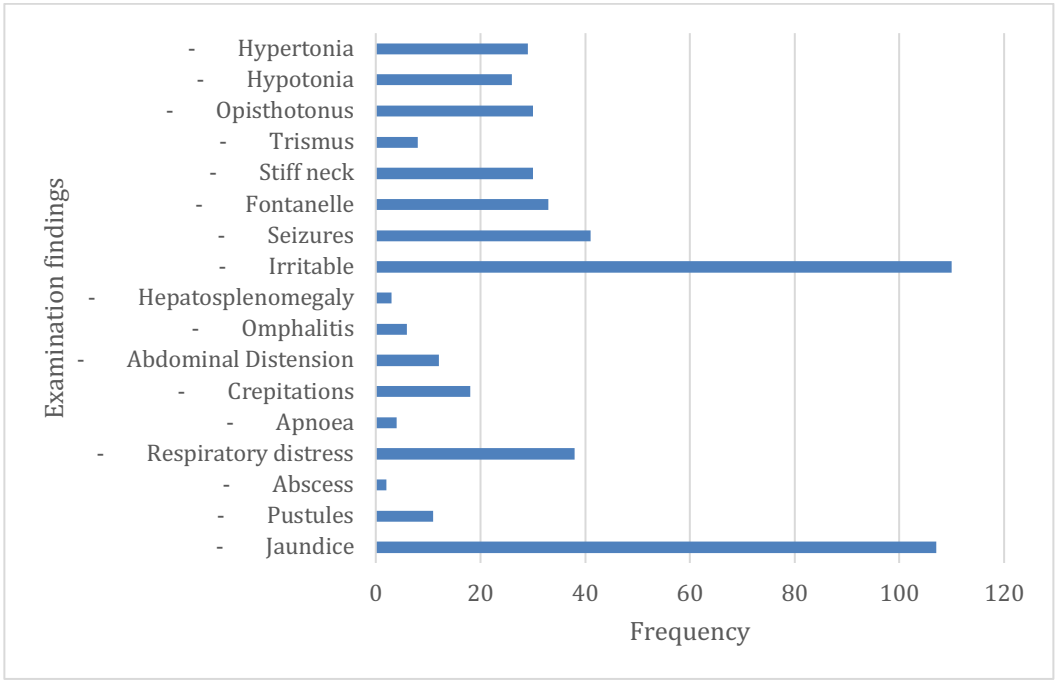


Figure 31: The frequency of clinical signs on examination of neonates presenting with a possible serious bacterial infection (pSBI)

Clinical feature	N=214
Symptom, Freq (%)	
- Fever	210 (98.1%)
- Cold temperature	2 (0.9%)
- Poor feeding	210 (98.1%)
- Excess crying	192 (89.7%)
- Weak cry	15 (7.0%)
- Lethargy	201 (93.9%)
- Vomiting	23 (10.7%)
- Diarrhoea	4 (1.9%)
- Abdominal distension	9 (4.2%)
- Difficult breathing	44 (20.6%)
- Grunting	17 (7.9%)
- Apnoea	21 (9.8%)
- Seizures	40 (18.7%)
- Spasms	9 (4.2%)
- Jaundice	83 (38.8%)
- Rash	12 (5.6%)
Examination findings, Freq (%)	
- Jaundice	107 (50.0%)
- Pustules	11 (5.1%)
- Abscess	2 (0.9%)
- Respiratory distress	38 (17.8%)
- Apnoea	4 (1.9%)
- Crepitations	18 (8.4%)
- Abdominal Distension	12 (5.6%)
- Omphalitis	6 (2.8%)
- Hepatosplenomegaly	3 (1.4%)
- Irritable	110 (51.4%)
- Seizures	41 (19.2%)
- Fontanelle	33 (15.4%)
- Stiff neck	30 (14.0%)
- Trismus	8 (3.7%)
- Opisthotonus	30 (14.0%)
- Hypotonia	26 (12.1%)
- Hypertonia	29 (13.6%)

Data are n (%).

Table 6: Prevalence of individual clinical signs for infants presenting with pSBI

As shown in Table 7, the mean admission weight was 2.996 ± 0.540kg, ranging from 2.0 to 4.7kg. Mean admission temperature was 38.7±1.8°C ranging from 33.8°C to 41.8°C. Only one infant was hypothermic (<36.0°C) on admission, however 86.4% infants were febrile. Nearly half of infants (47.3%) were tachypnoeic (>60 breaths per minute), a quarter (27.7%) were tachycardic (>160 beats per minute), and 7.9% had a heart rate >180 beats per minute.

Measurement	N=214
Weight (kg) - Mean (SD) - Range	 2.996 (0.540) 2.000 - 4.700
Head circumference (cm) - Mean (SD) - Range	 35.87 (1.5) 30 – 39.5
Length (cm) - Mean (SD)	 50.4 (7.3)
Admission temperature - Mean (SD) - Number >38 - Number <35.5	 38.7 (1.8) 185 (86.4) 1 (0.5)
Admission respiratory rate per minute - Mean (SD) - Tachypnoeic >60	 61 (22) 97/205 (47.3)
Admission heart rate per minute - Mean (SD) - Tachycardic >160	 145 (27) 59/214 (27.7)

Table 7: Vital signs and anthropometric measurements of cases of pSBI at admission

TREATMENT

The mean duration of antibiotic treatment was 7.6 days (±4.4) ranging from 1-26 days. Half of the infants received first-line treatment of ampicillin and gentamicin (50.0%). And half (50.0%) were given ceftriaxone/cefotaxime and gentamicin, either because of a diagnosis of neonatal meningitis or lack of response to ampicillin and gentamicin. Only one infant in this study received amikacin.

FINAL CLINICAL DIAGNOSIS

Of the 214 pSBI cases enrolled, a final diagnosis of sepsis was given to 76.6% (164/214) of cases, pneumonia to 6.5% (14/214) of cases, tetanus to 4.2% (9/214) of cases and meningitis to 12.6% (27/214) (Table 8 and Figure 32). For 20/27 of the cases of meningitis the CSF parameters were suggestive of neonatal meningitis, however in 7/27 cases a lumbar puncture was either

contraindicated or failed, but clinical suspicion was high due to signs of encephalopathy, opisthotonus or seizures.

One neonate was subsequently diagnosed with cyanotic heart disease, from which he later died. Jaundice was present in 33/214 neonates, of whom four were diagnosed with acute bilirubin encephalopathy (severe hyperbilirubinaemia with one or more of abnormal tone, opisthotonus and/or seizures) and two were discharged with abnormal neurology. One infant presented with an extensive pustular skin infection with gangrenous limbs.

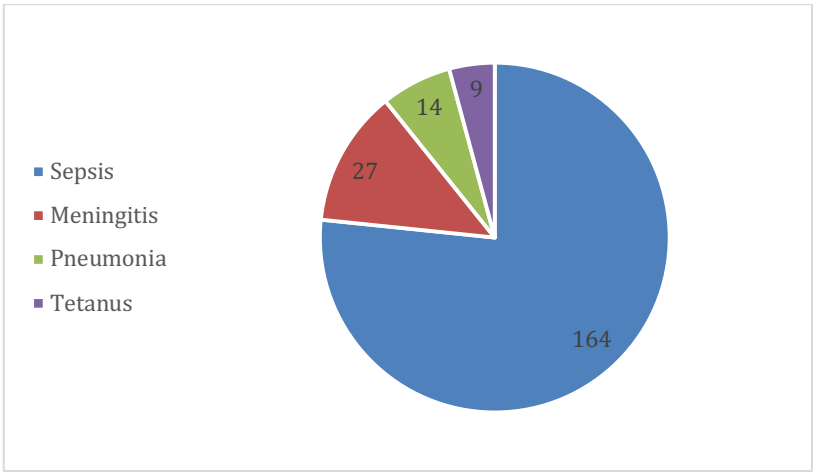


Figure 32: The final diagnosis at discharge or death of neonates admitted with a possible serious bacterial infection (pSBI).

Characteristic	Overall (n=214)
Final diagnosis at discharge or death, Freq (%)	
- Sepsis	164 (76.6)
- Meningitis	27 (12.6)
- Pneumonia	14 (6.5)
- Tetanus	9 (4.2)
Final inpatient outcome	
- Discharged alive with no sequelae	151/214 (70.6)
- Discharged alive with known sequelae	5/214 (2.3)
- Inpatient death	15/214 (7.0)
- Self-discharged before completion of treatment	43/214 (20.1)
Neonatal outcome (within 28 days)	
- Alive with no known sequelae	180/214 (84.1)
- Alive with known sequelae	5/214 (2.3)
- Died	20/214 (9.3)
- Unknown	9/214 (4.2)

Data are n (%).

Table 8: Neonatal outcomes

IN-PATIENT OUTCOMES

The majority of infants were discharged alive and well (151/214, 70.6%). Unfortunately, 43/214 (20.1%) of infants were discharged against medical advice before completion of their treatment. Five infants were noted to already have abnormal neurology or had developed post-infectious hydrocephalus by the time of discharge from NNU. All five of these infants had had seizures and bulging fontanelles at presentation, and 2/5 had presented with opisthotonus. Although one of these infants was too sick for a lumbar puncture, the other four had normal CSF parameters, including two who had acute bilirubin encephalopathy, which would likely account for the abnormal neurology.

Aetiology of clinical sepsis

All cases had a blood culture performed and bacteria were isolated in 12.1% (26/214). Cultured organisms were then classified as definite pathogens, possible pathogens and probable contaminants as described in the methods. Using this classification, 5.6% (12/214) grew a definite or probable pathogen as described in Table 9. Growth of multiple organisms in a blood culture is a

strong sign of contamination, however in this study, no infants had multiple pathogens detected by culture. Staphylococcus aureus was the most common bacteria detected (11/214, 5.1%), however only nine of these were found to be a probable pathogen upon review. Other gram-positive bacteria detected were coagulase negative staphylococcus (CoNS), corynebacter, bacillus, micrococcus, all bacteria that are commonly associated with contamination. One of the cases with CoNS in the blood culture died, although the CoNS may not have been the cause of death. Gram-negative bacteria found to be definite pathogens were detected in 3 infants; 2 cases of *Klebsiella* and 1 case of *E. coli.* All three of these infants were hospital-born and all presented within 48 hours of birth (Table 10).

Pathogen	Frequency, n (%)	Classification	Percentage of pathogens (n=12)
Gram positive			
Staphylococcus aureus	9 (4.2)	Possible pathogen	9 (75.0)
	2 (0.9)	Probable contaminant	-
Micrococcus	4 (1.9)	Probable contaminant	-
Coagulase negative staphylococcus	2 (0.9)	Probable contaminant	-
Corynebacter	4 (1.9)	Probable contaminant	-
Bacillus	2 (0.9)	Probable contaminant	
Gram negative			
Klebsiella	2 (0.9)	Definite pathogen	2 (16.7)
E. coli	1 (0.5)	Definite pathogen	1 (8.3)
Total, n=214	26 (12.1%)		

Table 9: Frequency and classification of bacterial isolates detected using blood culture

Pathogen	<48h N = 18	Case fatality rate N (%)	>48h N = 8	Case fatality rate N (%)	Total N = 26	Case fatality rate N (%)
Gram positive						
Staphylococcus aureus	8	1/8(12.5)	3	0/3	11	1/11 (9.1)
Micrococcus	2	0/2	2	0/2	4	0/4
Coagulase negative staphylococcus	1	1/1 (100)	1	0/1	2	1/2 (50.0)
Corynebacter	3	0/3	1	0/1	4	0/4
Bacillus	1	0/1	1	0/1	2	0/2
Gram negative						
Klebsiella	2	2/2	0	0/0	2	2/2 (100)
E. coli	1	0/1	0	0/0	1	0/1

Table 10: Blood culture results stratified by early and late onset illness with fatality rates

The antibiotic sensitivity of these pathogens is described in Table 11. All three gram-negative isolates were multidrug resistant, including to the common medications used to treat neonatal sepsis in this setting; gentamicin, ampicillin and ceftriaxone. The two infants with *Klebsiella* sepsis succumbed before the results of the blood culture were available and therefore never received amikacin to which the Klebsiella was sensitive. The infant with *E. Coli* sepsis received and responded well to amikacin.

None of the *S. aureus* isolates were sensitive to penicillin and only three *isolates were sensitive to oxacillin* suggesting a high rate of methicillin-resistant *S. aureus* in this setting. 64% were sensitive to gentamicin and all were sensitive to vancomycin. It is possible therefore that, although not ideal, some of these infants responded to gentamicin monotherapy.

Antibiotic	S. Aureus N = 9	Klebsiella N = 2	E. Coli N = 1
Amikacin	Not tested	2/2	1/1
Imipenem	Not tested	2/2	Not tested
Ciprofloxacin	7/9	2/2	1/1
Ceftazidime	Not tested	0/2	0/1
Ampicillin	**Not tested**	**0/2**	**0/1**
Amoxi-Clav	Not tested	0/2	1/1
Ceftriaxone	**Not tested**	**0/2**	**0/1**
Cefuroxime	Not tested	0/2	0/1
Chloramphenicol	9/9	0/2	0/1
Gentamicin	**7/9**	**0/2**	**0/1**
Co-trimoxazole	1/9	0/2	0/1
Vancomycin	9/9	Not tested	Not tested
Erythromycin	6/9	Not tested	Not tested
Oxacillin	3/9	Not tested	Not tested
Penicillin	0/9	Not tested	Not tested
Tetracycline	7/9	Not tested	Not tested
Meropenem	Not tested	Not tested	1/1

Table 11: The antibiotic sensitivity of cultured pathogens

Malaria

All mothers and babies enrolled in the study had a blood film examined for malaria parasites. In the maternal blood smears, 5/214 (2.3%) were diagnosed with *p. falciparum*. The neonates of all these mothers had negative blood smears for malaria. In the neonates, only 1/214 (0.5%) infant was diagnosed with *p. falciparum* on blood smear. The mother of this case had a negative blood smear and of note the neonatal blood culture was positive for *S. aureus.*

Tetanus

A diagnosis of neonatal tetanus was made in 4.2% (9/214) of infants. 6/9 of these cases died in the NNU, two were discharged against medical advice before completion of treatment and one was discharged alive.

CSF results

Some infants did not undergo a lumbar puncture due to clinical contraindications; in others the procedure was unsuccessful. A successful lumbar puncture was achieved in 189/214 infants. None of the infants in the study had a positive CSF culture or Gram stain. 20/189 (10.6%) had abnormal CSF analysis (Table 12) suggestive of possible CNS infection. Of the 20 infants with abnormal CSF, suggestive of meningitis, 8/20 (40.0%), had no symptoms or signs suggestive of meningitis.

Elevated WBC counts ranged from 25-175cells/mm^3, elevated protein ranged from 130-730mg/dl and low glucose concentration ranged from 5-24mg/dL.

Those who had only a reduced glucose level, but normal protein levels and WBC counts were not considered abnormal. One limitation of this study was the inability to measure simultaneous blood glucose levels, therefore these hypoglycaemic CSF samples could have been secondary to hypoglycaemia.

Abnormal CSF parameters*, N=189	Frequency, n (%)
Combinations of abnormal parameters	
Elevated WBC only	7
Elevated WBC and protein	2
Elevated WBC and reduced glucose	1
Elevated WBC and protein, reduced glucose	2
Elevated Protein only	7
Elevated Protein and reduced glucose	1
Total	20/189 (10.6%)

*Limits used include: Protein concentration > 127mg/dl, WBC count >15cells/mm^3 and glucose concentration <25 mg/dL.

Table 12: Frequency of abnormal CSF analysis

Seizures

Seizures were reported or observed at presentation in 40/214 (18.7%) infants and of these 4/40 died (10.0%). Of those with seizures, 34/40 (85.0%) had a lumbar puncture performed when the seizures had been controlled. Of those who had a lumbar puncture, 7/34 had abnormal CSF parameters consistent with meningitis. For the remaining 6 infants with seizures, two had lumbar punctures which failed, and four infants were too sick for lumbar puncture: 3/6 subsequently died and 1/6 developed post-infectious hydrocephalus.

Neonatal mortality

Overall in-patient mortality on the NNU during the study period was 16.7% (377/2251). The overall in-patient mortality from neonatal sepsis during the study period was 7.8% (46/585). The in-patient mortality of those infants recruited into the study was 7.0% (15/214). An additional 5/43 (11.6%) infants who were discharged against medical advice from the NNU before completing

treatment, were later confirmed to have died at home within the neonatal period. The overall neonatal mortality was therefore 9.3% (20/214). There were no recorded maternal deaths.

For those with a diagnosis of neonatal meningitis, 4/27(14.8%) died within 48 hours of admission to NNU and 2/27 developed post-infectious hydrocephalus prior to discharge. 9/27 (33.3%) self-discharged within 48 hours of admission, of these 2 went on to die at home and 1 developed post-infectious hydrocephalus. The overall neonatal mortality from neonatal meningitis was 22.2% (6/27) and 11.1% (3/27) of these infants developed post-infectious hydrocephalus.

For those with definite or possible pathogens isolated on blood culture, the associated neonatal mortality was higher 3/12 (25.0%, OR 3.63, 95%CI 0.89, 14.68). Two of these cases had multi-drug resistant *Klebsiella* isolated and the third had neonatal tetanus in addition to isolation of *Staphylococcus Aureus*. The neonatal mortality of infants with neonatal tetanus was 66.7% (6/9).

DISCUSSION

MENINGITIS

This study is one of few studies in SSA that has routinely undertaken CSF culture and CSF analysis in infants presenting with pSBI. Our lack of positive CSF cultures was unexpected, however a similar study of clinical neonatal sepsis in Kenya also reported a low yield of CSF culture (4.8%) (Laving et al., 2003). They were able to detect a further 13% of cases by adding a latex

particle agglutination assay (LPA). Another study of neonatal sepsis from Kenya, reported 60 neonates with a CSF white cell count ≥50 cells/mm^3 but negative culture and gram stain (Talbert et al., 2010). This is likely due to the high rate of maternal antibiotic use and commencement of antibiotics in neonates prior to lumbar puncture.

Studies of neonatal meningitis in SSA have reported a wide variety of bacterial pathogens, including *E. coli, Klebsiella,* GBS, *Enterobacter spp., Haemophilus influenzae, S. Aureus*, non-typhoidal *Salmonella enterica, Acinetobacter* spp., and *Strep. Pneumoniae* (Group, 1999a) *(Laving et al., 2003, Gebremariam, 1998, Reta and Zeleke, 2016)* (Airede, 1993) (Campagne et al., 1999) (Longe et al., 1984) (Nathoo et al., 1991) (Swann et al., 2014).

In the absence of a positive CSF culture, CSF white cell count, protein level and glucose level can act as useful guides as to the presence of meningitis. In this study we detected abnormal CSF parameters suggestive of meningitis in 20/189 (10.6%) of infants. An additional 7 neonates had a high clinical suspicion of meningitis due to signs of encephalopathy or seizures, but a lumbar puncture was either contraindicated or was not successful. Overall 12.6% (27/214) had a final diagnosis of neonatal meningitis. This is comparable to other studies of neonatal sepsis in SSA reporting rates of neonatal meningitis from 3-18% (Laving et al., 2003, Talbert et al., 2010).

It is therefore apparent, that there is need to improve CSF diagnostics, particularly in low-resource settings. Culture-independent techniques may hold

the key to the identification of more causative organisms in neonatal meningitis. In addition, the aetiology of neonatal meningitis varies with time and geographical location. Together, these elements represent huge challenges to the prevention and treatment of neonatal meningitis.

AETIOLOGY

This study undertook blood cultures from all recruited infants, stringent aseptic techniques were utilised during sampling and a high-quality microbiological laboratory processed our blood cultures. In addition, our adjudication of positive blood culture results allowed us to increase our certainty of those infants with either a definite and possible pathogen. Despite being the gold-standard for the diagnosis of neonatal sepsis, we were still only able to identify confirmed or probable pathogens in 5.6% of our infants with pSBI. Our low culture positivity rates are not dissimilar to other studies in SSA. A study of young infants hospitalised with pSBI in Kilifi, Kenya isolated pathogens in only 12.8% (Berkley et al., 2005). Whilst a study of community-acquired pSBI at the same location identified isolates in only 9% (Brent et al., 2006). The lower-frequency of culture-confirmed pSBI cases in this study may in part be due to our stringent aseptic procedures to minimise contamination and the careful clinical review and exclusion of contaminants.

This study still found *Staphylococcus aureus* to be the predominant isolate followed by *Klebsiella* and *E. coli;* similar to another two studies in Uganda (Mugalu et al., 2006, Tumuhamye et al., 2020). Similarly, two studies of neonatal sepsis from Tanzania found *E.coli, Klebsiella* and *Staphylococcus*

infections to be predominant isolates (Blomberg et al., 2005, Mhada et al., 2012). In infants up to 7 days of age, they found *Klebsiella* spp. (33% and 32%), *E coli* (19%, 11%) and *S. aureus* (29%, 11%) and in infants 7-28 days of age, the findings were similar, *Klebsiella* spp. (23%, 23%), *E coli* (18%, 10%) and *S. aureus* (55%, 16%). These observations are also comparable to the results from a meta-analysis of 27 studies of community-acquired neonatal sepsis in LMICs and another of 63 studies focusing on community-acquired neonatal sepsis in LMICs, both of which identified these as the three major pathogens (Waters et al., 2011, Zaidi et al., 2009). This study and those studies highlighted above are different from a study in Malawi, which found the commonest causes of neonatal sepsis to be GBS and non-typhoidal Salmonella (Milledge et al., 2005).

Due to challenges in isolating certain pathogens such as *Haemophilus influenzae, S. pneumoniae* and *group B streptococcus*, it is possible that their prevalence was underestimated in this study (Blaschke, 2011). Similarly, it is possible that the contribution of less fastidious organisms such as *S. aureus* might have been overestimated (Nickerson et al., 2009). GBS was not identified in this study, however it is thought to be a frequent causative pathogen. It is possible that neonates with GBS infections were less likely to be included in this study, because of the fatal nature of this disease (Seale et al., 2017, Airede, 1992, Quiambao et al., 2007). The challenge of sampling babies before death, particularly those who are born in the community is high. In addition, almost half (45.8%) of our mothers received intrapartum antibiotics,

which are reported to be associated with lower rates of GBS infection (Edmond et al., 2012).

Although this study included home-born and facility born neonates and included early and late onset sepsis, we only recruited 20 home-born neonates. Only one of these 20 home-born infants presented before 48 hours of age. This suggests that cases of EONS in infants born at home do not seek medical attention and therefore likely die before reaching a facility. It is also possible that there is a strong association between EONS and facility-based deliveries due to poor infection control measures and the risk of EONS might actually be higher in facility-born infants. In this study, all three cases of gram-negative sepsis were facility-deliveries, and all presented within 48 hours of birth. The hospital environment was likely to be the source of these infections, due to poor intrapartum and postnatal infection control practices (Zaidi et al., 2009, Zaidi et al., 2005). Almost half of pathogens isolated in cases of EONS are due to gram-negative pathogens including *Klebsiella, Pseudomonas,* and *Acinetobacter* sp, which thrive in multi-use containers such as liquid soap and disinfectants and poorly cleaned equipment, which can lead to common-source outbreaks (Moffet et al., 1967). Similarly, the main source of *S aureus* is the hands of healthcare workers. All of this is exacerbated by limited hand-washing facilities, an unreliable water supply, broken taps and lack of sterile gloves for examinations of mothers during labour in this and similar settings. Driven by these poor infection-control practices and inappropriate use of antibiotics, alarming rates of antimicrobial resistance have been reported globally in bacterial isolates from neonatal infections (Zaidi et al., 2005). This

means that improved hygiene at birth through maternal, community and healthcare worker education could have a significant impact on the incidence of neonatal sepsis.

Many other studies do not report inclusion or exclusion criteria relating to prior antibiotic therapy. Many of our neonates had received at least one dose of antibiotics prior to inclusion, but any neonate who had received more than 24 hours of intravenous antibiotic therapy was excluded. Even so, this may have changed the microbiological results and have biased the data. Often it is not possible to exclude neonates with previous antibiotics from research studies as they are often administered before referral and need for investigation should never preclude the need for treatment in a sick neonate. It is not an uncommon practice in Uganda and other LMICs for parents to self-treat using one of the widely-available over-the-counter antibiotics before admission, which contributes to a poor sensitivity of microbiological cultures (Vergnano et al., 2005). In this study, when infants presented outside of study hours i.e. night duty, treatment was initiated, and samples were taken when a study member was available the following day.

In HICs, Coagulase-negative *Staphylococcus (CoNS)* is associated with in-patient neonatal sepsis and is associated with long-line placement. In LMICs, CoNS is often considered as a potential contaminant. It should however be considered that given the importance of nosocomial infections in hospital-born neonates, CoNS may actually be pathogenic. In fact, one neonate who died from pSBI had a growth of CoNS on blood culture. It is possible that the true

aetiology of the pSBI was not identified, however CoNS may in fact be a cause of neonatal sepsis even in settings without long-lines, especially when infection control procedures are not as flawless as they should be.

Culture-independent techniques such as PCR and antigen detection could provide a more accurate evaluation of the true aetiology (Saha et al., 2018). In the ANISA study, the causal organisms identified in blood culture were again *E coli, Klebsiella* sp, *Staphylococcus aureus,* with the addition of PCR, *ureaplasma* sp was identified as the leading cause and was associated with EONS and with neonatal mortality (Saha et al., 2018). *Ureaplasma* is an atypical bacterium that does not grow under conventional culture methods, so it is not surprising that it was not detected in this study using only traditional culture. Previous associations of *ureaplasma* with chorioamnionitis, preterm delivery, neonatal sepsis and neonatal meningitis, especially in preterm infants, have been reported (Waites et al., 1993, Yoon et al., 2003, Waites et al., 2005, Viscardi, 2014). If the pathogenic role of *ureaplasma* sp in neonatal infections in SSA is to be properly evaluated, then culture-independent methods, such as PCR will be needed.

ANTIBIOTIC RESISTANCE

Clinicians have a low threshold for treating sick neonates with antibiotics. WHO recommends empirical treatment with ampicillin or penicillin plus gentamicin for pSBI. Antibiotic resistance is a true threat to the improvement in neonatal sepsis outcomes and existing neonatal sepsis guidelines may no longer be appropriate.

In this study there was a 100% resistance to ampicillin/penicillin and only a moderate sensitivity to gentamicin (50%). Similar findings are reported from a study in the Ugandan National Referral hospital (Mugalu et al., 2006). The number of *E.coli, Klebsiella* and *Staphylococcus* infections in neonates, which are resistant to these antibiotics are increasing (Waters et al., 2011, Hamer et al., 2015). A meta-analysis of 19 studies in LICs with over 4000 blood cultures found *E. coli, Klebsiella spp.* and *Staphylococcus aureus* accounted for 55% (39-70%) of culture positive neonatal sepsis (Downie et al., 2013). Worryingly, isolates were only 57% sensitive to penicillin/gentamicin and 56% sensitive to third-generation cephalosporins. This study showed only 50% of isolates to be sensitive to gentamicin and in addition, none of the *E. coli* and *Klebsiella spp.* isolates were sensitive to third-generation cephalosporins. In fact, the gram-negative bacteria in this study were only sensitive to amikacin, imipenem and meropenem, drugs that are not routinely available in our healthcare system. Amikacin can be bought in Uganda and some patients can afford to do so. Imipenem and meropenem are both available to buy, but in reality, the high cost of these drugs means they are not obtainable by almost all patients. The aetiology of neonatal sepsis and antimicrobial sensitivity may vary over time and by geographic location and this study highlights the importance of ongoing reliable local microbiological data to address the challenge of antibiotic resistance in neonatal sepsis. One potential reason for this emerging antibiotic resistance is the wide availability of over-the-counter antibiotics in Uganda and other LMICs (Vergnano et al., 2005). The high level of antimicrobial resistance seen in this study is also likely due to the fact than many of these infants were

hospital-born where levels of resistance are higher than for community-acquired infection.

TETANUS

MRRH-NNU had developed a successful neonatal tetanus protocol in the preceding year (Burgoine et al., 2019). During the study-period the availability of tetanus immunoglobulin in Uganda was limited and therefore the mortality due to neonatal tetanus was higher than expected. Until it is eradicated, neonatal tetanus should continue to be considered as a possible aetiology of pSBI in LMICs. Improved training and education in the recognition and management of neonatal tetanus have the potential to have a huge impact on these deaths (Burgoine et al., 2019).

HIV EXPOSURE

6/214 (2.8%) of mothers were known to be HIV sero-positive and were already on anti-retroviral therapy. This was unexpected as it is lower than the reported prevalence of HIV among females aged 15 to 49 years in Uganda Population-Based HIV Impact Assessment (UPHIA) 2017, which is 7.5%.

MORTALITY

Reported case fatality rates (CFRs) for pSBI in LICs are variable, depending on the level and quality of the health facility, ranging from 5% up to as high as 48% (Waters et al., 2011, Duke et al., 2000, English et al., 2003, Group, 1999a, English et al., 2004, Burgoine et al., 2018, Milledge et al., 2005, Mugalu et al.,

2006, Swann et al., 2014, Tumuhamye et al., 2020). The CFRs for neonates 1-7 days old are reportedly higher than for 8-28 days (English et al., 2004). Higher CFRs are often anticipated in referral hospitals because sicker infants will be referred. If referral is challenging or there are limited skills and resources in lower level health facilities, the mortality may also be high. This study was in a referral hospital; however, the CFR was only 9.3%, similar to the CFR reported in the Ugandan National Referral Hospital (9.5%), suggesting the level of neonatal care was relatively good (Tumuhamye et al., 2020). This is likely due to significant improvements in the standard of care at MRRH-NNU, resulting in decreased neonatal mortality, following implementation of a two-tiered hospital-based neonatal care package and bCPAP (Burgoine et al., 2018, Okello et al., 2019). Improved pathogen detection would likely help identify those neonates with pathogens that are often multi-drug resistant and help trigger early escalation of antimicrobial cover. Obviously, this relies on continued surveillance of aetiology and antibiotic sensitivities to keep management guidelines up to date.

The CFR for neonatal meningitis in Africa is high, varying from 33-67% (Laving et al., 2003, Airede, 1993, Milledge et al., 2005, Campagne et al., 1999, Longe et al., 1984, Nathoo et al., 1991). In this study, CRF for meningitis was 22.2% (6/27), which was more than double that of neonates with pSBI. Similar to this study, mortality from neonatal meningitis in Kenya was more than double that of neonates with sepsis, 67% v. 32% respectively.(Laving et al., 2003)

CONCLUSION

This study demonstrates that even with careful aseptic sampling of blood and CSF and high-quality microbiological analysis of these samples, the aetiology of the majority of cases of pSBI still remain undefined. Despite negative CSF cultures, the CSF analyses still suggested a relatively high rate of neonatal meningitis in this setting, confirming the ongoing need to consider meningitis in infants presenting with pSBI. The neonatal mortality of pSBI in this low-resource setting was relatively high and the neonatal mortality associated with meningitis was more than double that of pSBI. Early post-infectious hydrocephalus developed in a significant number of survivors. Improving our understanding of the specific aetiologies and risk factors associated with mortality and the development of post-infectious hydrocephalus, is necessary to inform prevention strategies and treatment approaches.

CHAPTER 6 - CRANIAL ULTRASOUND FINDINGS ON ADMISSION AMONG TERM NEONATES PRESENTING WITH POSSIBLE SERIOUS BACTERIAL INFECTION (PSBI) IN UGANDA

BACKGROUND

It is estimated that in SSA there are up to 2.6 million episodes of serious neonatal infections every year, leading to an estimated 250,000 deaths (Seale et al., 2014). Neonatal meningitis is one such infection (Barichello et al., 2013). Neonates have the highest incidence of bacterial meningitis, it being more common during the neonatal period than at any other time in life. Although studies from high-income countries have reported an incidence of up to 0.2 to 0.5 cases per 1000 live births, there is limited data on the incidence of neonatal meningitis in LICs (de Louvois et al., 2005, Holt et al., 2001, Talbert et al., 2010).

Meningitis can severely damage the developing neonatal brain (Berman and Banker, 1966). Neonatal meningitis can occur de novo, but it is usually the result of bacteraemia, with initial seeding of the CNS via the choroid plexus (Yikilmaz and Taylor, 2008). The infection then spreads into the cerebral spinal fluid (CSF) causing inflammation of the ventricles (ventriculitis) and the meninges (meningitis) (Berman and Banker, 1966). Meningeal inflammation can extend to the brain parenchyma (cerebritis) and cause cerebral oedema through the disruption of the blood-brain barrier. In addition, it can cause thrombophlebitis and vascular occlusion leading to ischaemic infarcts (Berman and Banker, 1966, DiNubile et al., 1990). Purulent exudate can also obstruct the normal flow of CSF through the aqueduct or foramina of the

fourth ventricle leading to hydrocephalus (Perlman et al., 1992). Hydrocephalus can also result from impaired resorption by inflamed arachnoid channels.

Neonatal meningitis can be a devastating illness with a higher mortality than neonatal sepsis and a much higher risk of subsequent neurodevelopmental and neurological disability (Stevens et al., 2003). The mortality from neonatal meningitis in LICs is estimated to be up to 58%, almost 6 times higher than in high-income countries, and up to two-thirds of survivors will have serious neurological sequelae such as hearing impairment, cerebral palsy, post-infectious hydrocephalus or neurodevelopmental delay (Furyk et al., 2011). A relatively high incidence of post-infectious hydrocephalus has been observed in East Africa, and these cases often have a prior history of neonatal sepsis and evidence of bacterial infection in the CSF at the time of neurosurgery (Warf, 2005, Li et al., 2011). Incorrect diagnoses and insufficient duration of antimicrobial therapy for neonatal CNS infections are likely major factors in the development of post-infectious hydrocephalus in such settings.

The high burden of morbidity and mortality associated with neonatal meningitis can be reduced by early recognition and diagnosis (Ku, et al, 2015). Unfortunately, many neonates with meningitis present with non-specific symptoms and signs such as poor feeding, lethargy, apnoea and vomiting. Typical signs of meningitis such as bulging fontanelle, seizures, opisthotonus and irritability are not always present and therefore meningitis can often not be diagnosed clinically (Mwaniki et al., 2011). Even neonates with brain abscesses may initially exhibit no neurological symptoms except irritability (Algubaisi et al., 2015). Neonates with signs of sepsis should therefore be thoroughly investigated including blood culture, CSF culture and CSF analysis (Laving et al.,

2003). Unfortunately, even when facilities exist, only a minority of pathogens are identified by culture. This is especially true in cases where CSF cultures are obtained after antibiotics have been started in the neonate or if their mothers received intrapartum antibiotics (Garges et al., 2006). Abnormal CSF white blood cell (WBC) count, protein concentration and glucose concentration can also support a diagnosis of meningitis (Ahmed et al., 1996, Byington et al., 2011, Kestenbaum et al., 2010, Laving et al., 2003, Martin-Ancel et al., 2006, Nascimento-Carvalho and Moreno-Carvalho, 1998, Shah et al., 2011, Thomson et al., 2018). There are also data to suggest that meningitis can still occur in the presence of normal CSF WBC, glucose and protein levels (Garges et al., 2006).

In low-resource settings, multiple limitations mean that lumbar punctures and CSF sampling are often not routinely performed in cases of neonatal sepsis. These limitations include a lack of the equipment required to perform safe and sterile lumbar puncture, insufficient skilled medical personnel who routinely perform lumbar puncture and limited laboratory support to analyse the CSF. So frequently, these infants go without the accurate diagnosis of meningitis, and therefore do not receive the more prolonged antimicrobial treatment regimens appropriate for meningitis that optimize outcomes.

In high-resource settings, imaging of the CNS is part of the routine assessment of sick and preterm neonates in neonatal units. The major advantages of cranial ultrasound (cUS) are the rapidity with which imaging can be performed on admission, the relatively short time period needed to obtain the images, safety due to lack of ionizing radiation, portability of equipment allowing bedside examinations, relative affordability

and lack of need for sedation during the procedure. Bedside cUS allows the early detection of CNS pathology such as intraventricular haemorrhage (IVH), ventricular dilatation, cysts, calcifications and also white matter (WM), central grey matter (basal ganglia/thalami) and cortical changes, and cerebellar abnormalities. For neonates with meningitis in high-resource settings, the first brain imaging modality used is usually cUS. A cUS performed at presentation establishes the presence of normal anatomy, evidence of longstanding injuries, the presence of sub-ependymal cysts or lenticulostriate vasculopathy, as well as evidence of any acute evolving injuries due to haemorrhage or ischaemia (Algubaisi et al., 2015). Sequential scans can monitor the development and progression of ischaemic, inflammatory and haemorrhagic injuries and they can also help to monitor brain growth and to screen for complications such as abscess formation and post-infectious hydrocephalus (Algubaisi et al., 2015).

CRANIAL ULTRASOUND FINDINGS ASSOCIATED WITH CNS INFECTION

To date, many of the data published on cUS in neonatal infections have focused on infants with proven CNS infections, with a focus on specific pathologies such as abscesses or specific pathogens such as *E. coli* and GBS (de Vries et al., 2004, Lequin et al., 2005, Renier et al., 1988, Shah et al., 2005, Verboon-Maciolek et al., 2006). In studies where they have considered cUS findings in neonatal sepsis they have focused on preterm neonates only.

The most common and earliest sign of meningitis on cUS is echogenic widening of the brain sulci, or meningeal thickening, seen in up to 83% of neonates with meningitis, as shown in Figure 33 and Figure 34 (Arrumugham et al., 1994, Han et al., 1985, Kapoor et al., 1989, Littwin et al., 2018, Mahajan et al., 1995, Raju et al., 1995,

Yikilmaz and Taylor, 2008). In normal neonates, the pia-arachnoid membrane is seen as an echogenic line over the surface of the brain. Accumulation of inflammatory exudate within the sulci leads to widening and increased echogenicity.

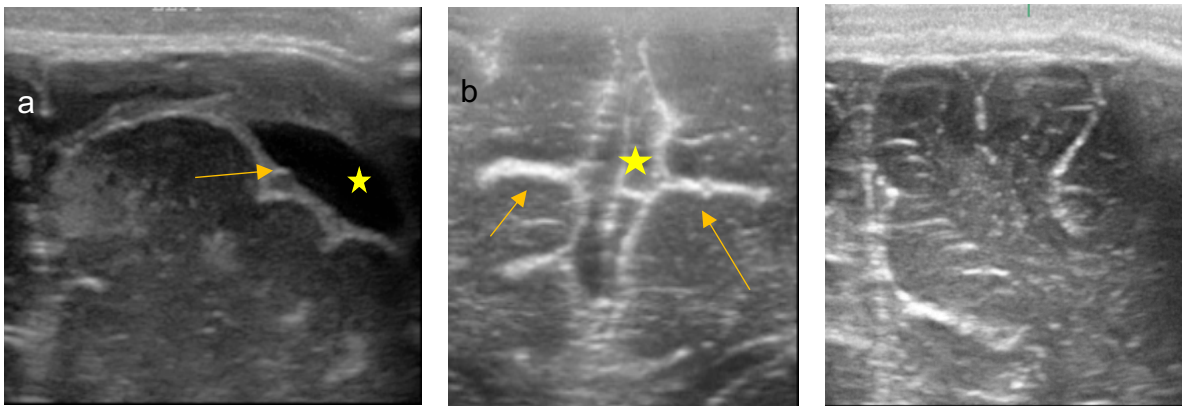


Figure 33: Coronal images using a linear probe showing a) meningeal thickening (arrow) and prominent extra-axial spaces (star) are shown with associated abnormal cortex and subcortical white matter, b) echogenic thickened meninges (arrows) with associated widened interhemispheric fissure (star) and c) a corresponding normal scan of the cortex (Credit K Burgoine)

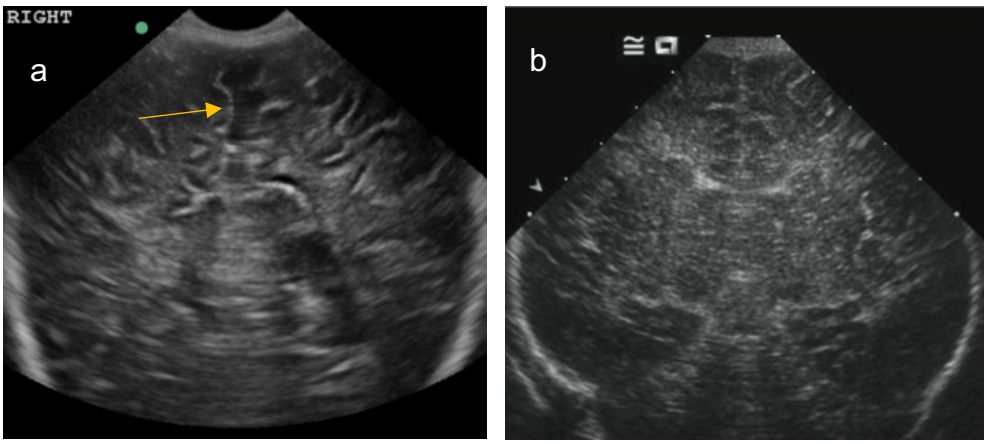


Figure 34: Coronal images using the curvi-linear probe showing a) widened interhemispheric fissure (arrow) and b) a corresponding normal scan (Credit K Burgoine)

Intraventricular debris, intraventricular strands and irregular and echogenic ependyma are all highly suggestive of ventriculitis as seen in Figure 35 (Lequin et al., 2005, Littwin et al., 2018, Soni et al., 1994, Yikilmaz and Taylor, 2008). In addition, ventriculomegaly (Figure 36, Figure 37) can be seen, particularly as the disease progresses, which may be suggestive of early post-infectious hydrocephalus (Agrawal and Mahapatra, 2005, Li et al., 2011, Renier et al., 1988, Warf, 2005).

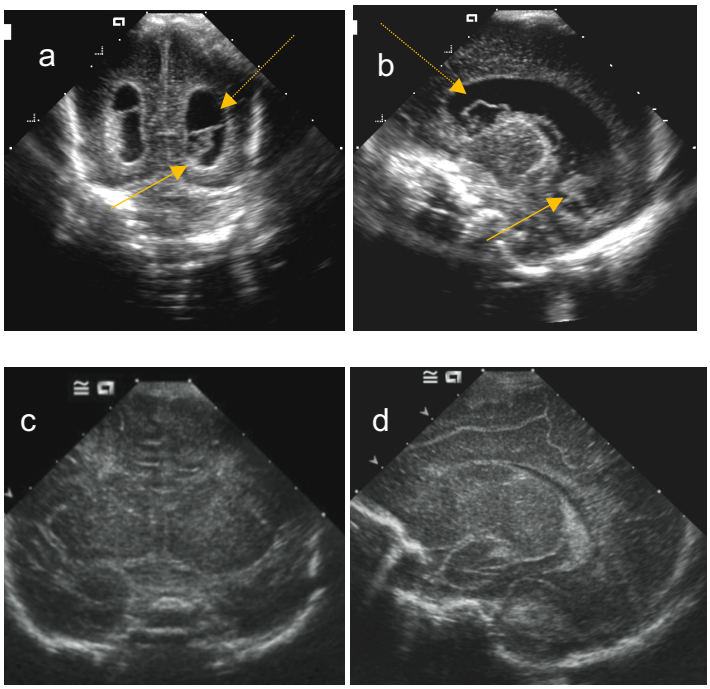


Figure 35: A coronal image (a) and sagittal image (b) showing ventriculomegaly of the lateral ventricles with strands (dashed arrow) and debris (solid arrow) in both ventricles. (Credit C Hagmann) Corresponding normal images (c and d)

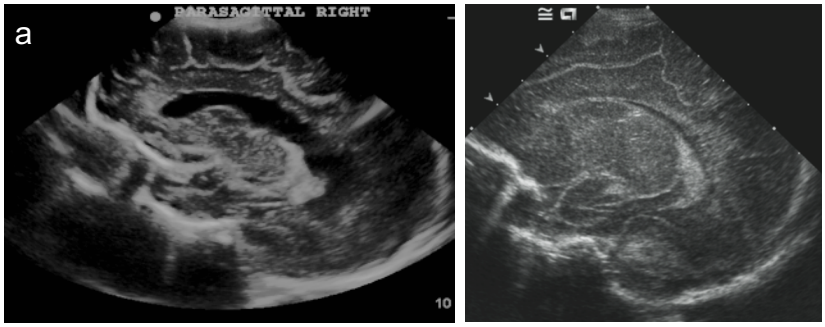


Figure 36: A parasagittal image of ventriculomegaly of the right lateral ventricle (a). In addition, the ependyma is both echogenic and irregular. A corresponding normal scan (b). (Credit K Burgoine)

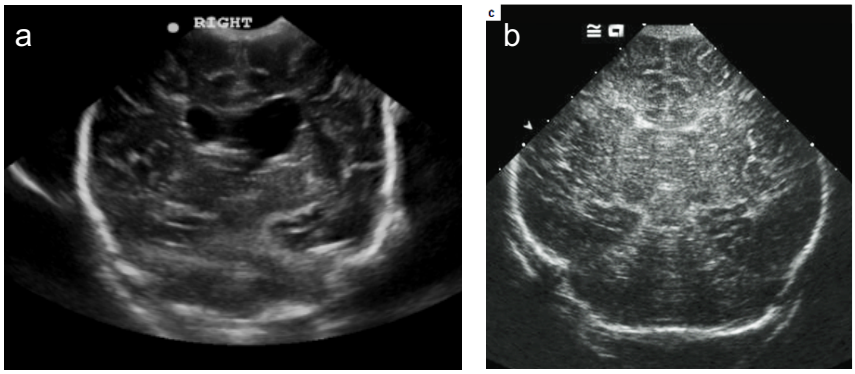


Figure 37: A coronal image of ventriculomegaly of the lateral ventricles with associated echogenic ependyma (a). A corresponding normal scan (b). (Credit K Burgoine)

Abnormal WM echogenicity can be focal or diffuse and can be due to cerebritis, infarction, haemorrhage or abscess (Han et al., 1985, Mahajan et al., 1995). Figure 38 shows widespread increased WM echogenicity in the fronto-parietal region. Figure 39 similarly shows increased WM echogenicity, which is brighter and more extensive and suggestive of haemorrhage within damaged WM.

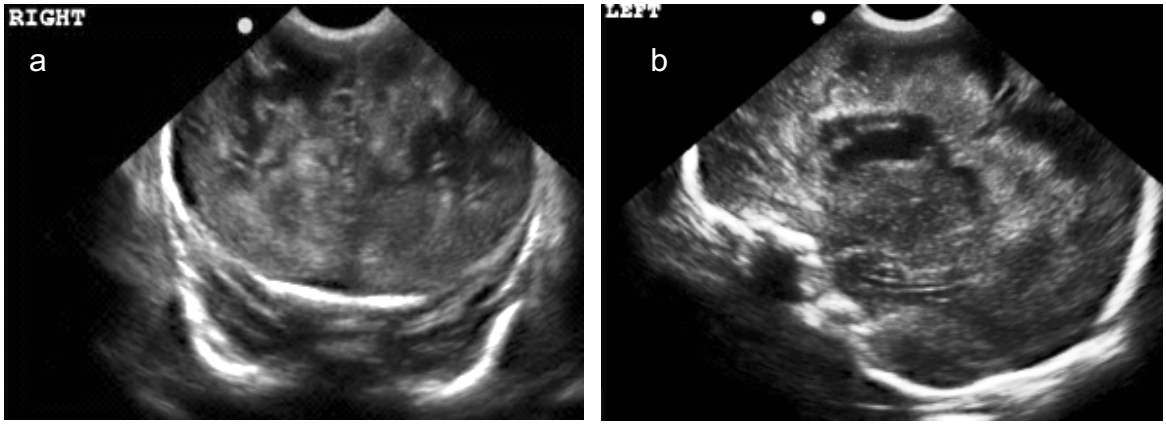


Figure 38: Severely abnormal scan showing widespread white matter echogenicity in the fronto-parietal region on a coronal image (a) and a parasagittal sagittal image (b). (Credit K Burgoine)

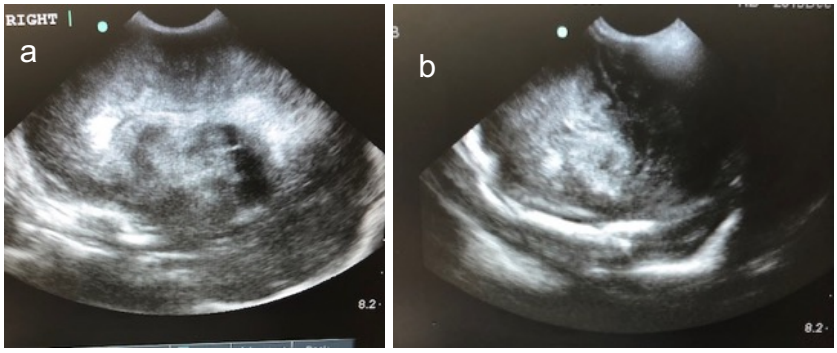


Figure 39: Examples of extensive and markedly increased echogenicity likely due to parenchymal haemorrhage in the right fronto-parietal region (a), and right frontal lobe with midline shift (b) (Credit K Burgoine)

Inflammation from bacterial meningitis can also lead to vasculopathy and prothrombotic states resulting in infarction, this is especially prominent with group B *Streptococcus* (GBS) (Chang et al., 2003, Fitzgerald and Golomb, 2007). Two distinct patterns have been reported in neonatal GBS meningitis (Figure 40): firstly deep arterial ischaemic strokes within the territories of small perforating arteries affecting deep grey matter including thalami and basal ganglia and also periventricular white

matter (Hernandez et al., 2011, Tibussek et al., 2015). Secondly focal cortical infarctions can be seen (Figure 40).

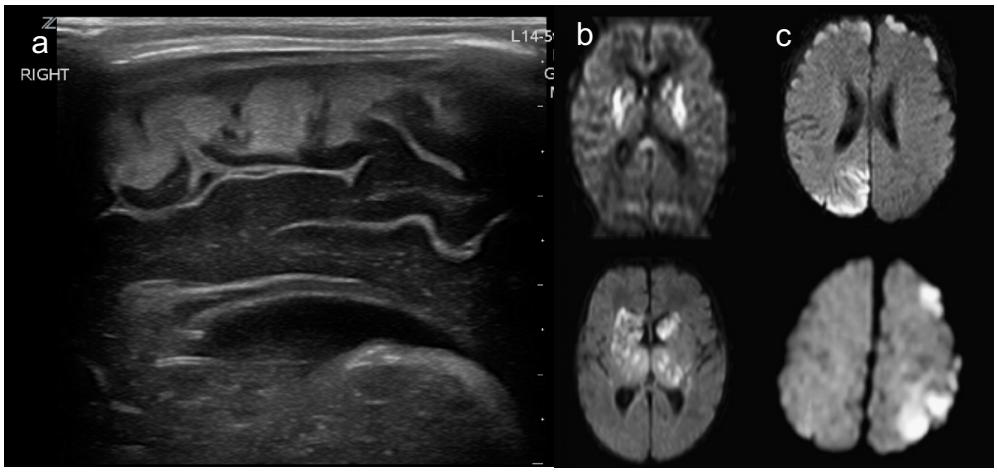


Figure 40: A cranial ultrasound (a) performed using a linear probe in the sagittal plane showing a) focal increased cortical echogenicity suggestive of cortical infarction (credit C Hagmann). Diffusion weighted MRI showing b) a deep arterial ischaemic stroke in GBS meningitis and c) multiple cortical infarctions in GBS meningitis (Algubaisi et al., 2015, Hernandez et al., 2011).

A bacterial brain abscess is usually a well-circumscribed collection of purulent fluid within the brain parenchyma. Neonatal cerebral abscesses are thankfully rare (Figure 41), but have been reportedly associated with gram-negative bacteria, including *Acinetobacter, Salmonella, Serratia, Proteus, Pseudomonas* and *Citrobacter* (Agrawal and Mahapatra, 2005, Algubaisi et al., 2015, Doran, 1999, Etuwewe et al., 2009, Renier et al., 1988, Rodrigues et al., 2014, Vaz Marecos et al., 2012). Neonates with brain abscess may initially exhibit no neurological symptoms except irritability but can develop focal seizures later (Algubaisi et al., 2015).

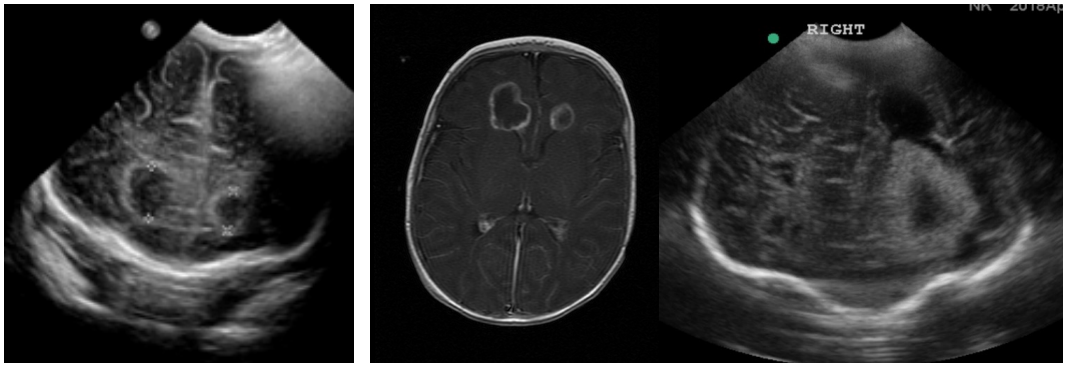


Figure 41: Two cerebral abscesses in the frontal region seen as two fluid filled cavities (*) on cranial ultrasound (a), confirmed on MRI (b) (Algubaisi et al., 2015). A large left frontal lobe abscess on cranial ultrasound with central echolucency, surrounded by echogenic rim (c) (Credit K Burgoine).

Thrombosis of the deep cerebral veins can also cause destruction of the white matter, deep grey matter, basal ganglia and thalami. Sagittal sinus thrombosis may also be observed, which can be detected by reduced or absent venous flow on doppler of the superior sagittal sinus (Figure 42). Additional complications can include cerebral oedema, white matter injury, necrotizing meningoencephalitis, cerebritis and empyema (Mahajan et al., 1995, Shah et al., 2005).

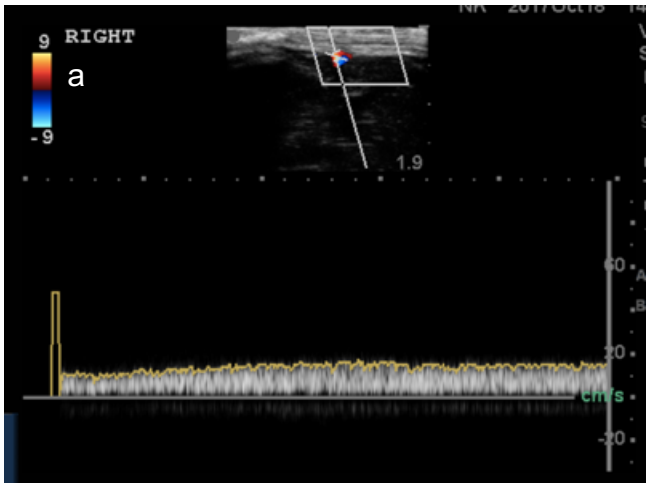


Figure 42: Normal venous flow in superior sagittal sinus

A large retrospective study of 96 neonates admitted to a neonatal unit in the Netherlands with either a viral, fungal or bacterial CNS infection showed a wide diversity of abnormalities (de Vries et al., 2006). Areas of increased echogenicity in the periventricular and deep white matter were common, likely suggestive of haemorrhagic necrosis, as suggested by post mortem of some of these cases. Ventricular dilatation, echogenic ependymal lining of the ventricles, strands and debris within the ventricles and parenchymal abscesses were reported in bacterial cases. Ventricular dilatation, periventricular calcification, lenticulostriate vasculopathy and germinolytic cysts were associated with congenital CMV infection.

Later pathological findings due to complications of meningitis may include cerebral cortical and white matter atrophy, post-infectious hydrocephalus, multicystic encephalomalacia and porencephaly, examples of which are described below (Berman and Banker, 1966, Han et al., 1985, Kalsbeck et al., 1980, Rodrigues et al., 2014).

In severe infections, extensive white matter injury can lead to white matter atrophy, volume loss and subsequent compensatory enlargement of the CSF spaces, known as ex-vacuo dilatation or hydrocephalus ex-vacuo, which is characterised by enlarged ventricles and subarachnoid spaces. Figure 43 shows the brain of a neonate who suffered widespread and severe white matter injury that led to subsequent compensatory enlargement of the CSF-spaces in response to loss of the brain parenchyma.

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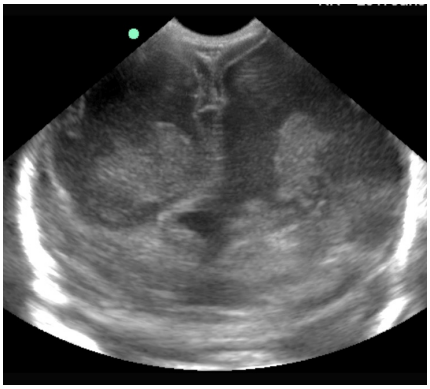


Figure 43: An example of ex-vacuo dilatation following severe white matter injury (Credit: K Burgoine)

The purulent exudate present within the ventricles in ventriculitis can obstruct the circulation of CSF through the aqueduct or foramina of the 4th ventricle. If this occurs, ventriculomegaly will follow and if it progresses will result in post-infectious hydrocephalus as shown in Figure 44 (Warf, 2005).

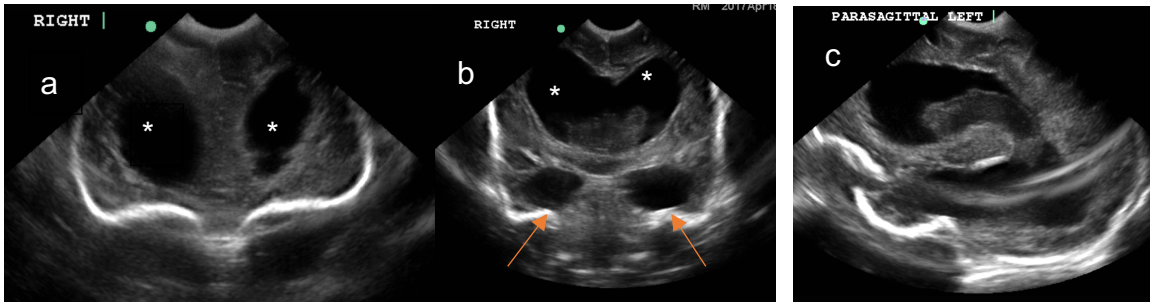


Figure 44: Cranial ultrasound images of neonate with post-infectious hydrocephalus taken on day 28: a) Coronal image of the frontal lobes showing hugely dilated anterior horns of both lateral ventricle (*), b) Coronal image taken more posteriorly demonstrating the dilated anterior horns (*) and occipital horns (arrows) of both lateral ventricles, in addition debris can be seen within the ventricles, c) corresponding sagittal view showing hugely dilated left lateral ventricle with bright irregular ependyma and debris within the ventricle (Credit: K Burgoine)

Porencephaly is characterised by the presence of cysts or cavities within the brain. They can occur at any location and their causes are multiple, both antenatal and

postnatal. They can be found following a severe intraventricular haemorrhage in a preterm infant or after hypoxic ischaemic encephalopathy (Figure 45). They have also been reported in cases of neonatal encephalitis and meningitis.

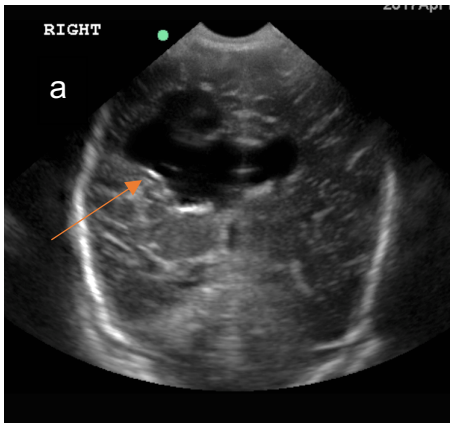


Figure 45: Example of a porencephalic cyst: a coronal ultrasound image of a large right parietal porencephalic cyst (arrow) extending from the lateral ventricle following a grade IV intraventricular haemorrhage in a preterm infant (Credit: K Burgoine)

In cases of confirmed neonatal meningitis, studies report imaging abnormalities in up to three-quarters of cases. These imaging studies used earlier ultrasound machines and did not use linear probes to examine the cortex, therefore it is possible that abnormalities were missed and that the incidence of abnormalities is actually much higher. The lack of blinding and/or the retrospective nature of these studies also has the potential to be a confounder. A prospective study of 44 infants with confirmed meningitis in India, reported 35.4% of cUS examinations to be normal (Soni et al., 1994). In another Indian study of 61 infants with bacterial meningitis, abnormalities were found on cUS examination of all neonates with clinical evidence of complications, such as persistent seizures or abnormal neurology (Mahajan et al., 1995). A retrospective study in neonatal unit in the Netherlands identified 42 cases of bacterial

and fungal meningitis/encephalitis who had a cUS examination immediately following admission (de Vries et al., 2006). They reported only one-quarter (10/42, 23.8%) of the cUS examinations to be normal at presentation. Despite the possible limitations of these earlier studies, it is clear that even in the absence of an abnormal neurological examination and signs of seizures, imaging abnormalities may be present in at least two-thirds of neonates with meningitis confirmed on CSF culture or analysis. It must also be remembered that abnormal imaging does not necessarily mean abnormal outcome. Understanding the longer-terms consequences of abnormalities on neuroimaging in these infants is also important, as will be discussed in Chapter 9.

CRANIAL ULTRASOUND FINDINGS IN CLINICALLY WELL FULL-TERM NEONATES

It is important to remember that abnormalities, particularly minor findings, can also be seen on cUS images of apparently healthy term neonates. Previous studies in high-income settings report a wide variation, 0.26-20%, in the incidence of abnormalities on cUS in healthy term neonates. The majority of these abnormalities are minor such as anatomical variants, minor haemorrhages, choroid plexus cysts and subependymal cysts. In a large German study, cUS scans were undertaken at 3 days of age in 1000 clinically well term neonates (Heibel et al., 1993). They reported abnormal examinations in 90/1000 (9%) of the neonates, including 35 cases of intracranial haemorrhage, 34 cases of subependymal cysts and choroid plexus cysts and 21 cases of anatomical variants including enlarged cavum vergae, absent cavum septum pellucidum and enlarged cisterna magna. In another study of 177 well full-term neonates carried out in the UK, the incidence cUS abnormalities was much higher with abnormalities reported in 35/177 (20%) before 48 hours of age (Mercuri et al., 1998). The abnormalities reported included periventricular echogenicities (7%), unilateral

thalamic echogenicities (2%), haemorrhage (6%), asymmetrical ventricular dilatation (3%), choroid plexus cysts (2%) and bulky choroid (8%). A larger study of 2309 clinically normal term infants in Taiwan found major abnormalities on only 6/2309 (0.26%) of cUS examinations performed within 3 days of birth (Wang et al., 2004). The major abnormalities included intracerebral haemorrhage, agenesis of corpus callosum and an infarct. More recently a large Italian retrospective study of 6771 full-term neonates, found abnormalities on cUS in only 114/6771 (1.7%) (Ballardini et al., 2017). The abnormalities were minor in the majority of cases, including subependymal cysts or choroid plexus cysts, enlarged cisterna magna, mild ventriculomegaly. Similar to Wang et al, 0.19% had major abnormalities including agenesis of corpus callosum, calcifications, infarct, hydrocephalus, ventriculomegaly and hypoxic ischaemic encephalopathy. All of the neonates with major abnormalities had either microcephaly or abnormal neurological examination.

The differences in the rates of abnormalities reported may be due to the size of the studies, the larger studies by Wang et al. and Ballardini et al. found similarly low incidences of abnormalities. It is also possible that there are differences in the techniques used for cUS including the probes and fontanelles, the experience of the operator or the population being examined. Wang et al. and Ballardini et al, both used similar transducers and the anterior fontanelle to obtain standard coronal and sagittal images and are comparable to the techniques used in this study. There may also be variations in interpretation of the images, such as judging the severity of white matter echogenicity or mistaking prominent choroid plexus for intraventricular haemorrhage.

Data that is potentially more comparable to our population comes from a Ugandan observational study of 112 apparently healthy term infants considered well enough to be on the postnatal ward (Hagmann et al., 2010). They also used the anterior fontanelle to obtain standard sagittal and coronal images. They found the incidence of cUS abnormalities to be much higher than those discussed above, with only 57/112 (51%) of cUS studies reported to be normal. Focal grey matter echogenicity typically in the central grey matter was seen in 6.5%, increased white matter echogenicity typically in the parieto-occipital region was seen in 7.5%. Intracranial haemorrhage was seen in one infant and a middle-cerebral artery infarct in another. Developmental cysts related to the ventricles were common with subependymal cysts seen in 19.6%, choroid plexus cysts seen in 19.6% and both types seen in 4.5%.

SUMMARY

Point-of-care tests are urgently needed to optimise the detection of neonatal CNS infections as this has implications for dosage and duration of empirical antimicrobial therapy. cUS is a relatively inexpensive, portable, safe and reliable imaging modality. It is possible that in settings where lumbar puncture and CSF culture and analysis are challenging or impractical, the patterns of pathology seen on cUS can help determine whether there is a CNS infection or not in neonates presenting with pSBI.

HYPOTHESES

- Neonates with CSF analysis and/or culture results suggestive of CNS infection will have pathological findings on cUS at presentation

- A cUS will detect CNS involvement more reliably than CSF analysis and culture alone

AIMS

This study sought to characterise the abnormalities on cUS examination at admission in neonates presenting with pSBI, and to correlate these imaging findings with CSF culture, CSF analysis and neonatal outcome.

METHODS

STUDY DESIGN

As described in Chapter 5, this was an observational study of term neonates (>2000g) presenting with pSBI to Mbale Regional Referral Hospital (MRRH) neonatal unit (NNU) in eastern Uganda over a 1-year period (See Chapter 4 for more detailed information on recruitment and sampling). The diagnosis of pSBI was defined as one of the three following combinations: 1) Axillary temperature >37.5°C, lethargy and poor feeding: 2) Axillary temperature <35.5°C, lethargy and poor feeding: 3) Full fontanelle and/or seizures, axillary temperature >37.5°C and poor feeding.

In addition, a contemporaneous control group of 50 well neonates with a birthweight of >2000g with no history of perinatal asphyxia or signs of infection were recruited.

SETTING

MRRH serves a population of 4.5 million people and has a dedicated neonatal unit (NNU) that admits over 2500 neonates a year. Neonates are admitted directly from the labour ward, referred from surrounding health facilities and, due to a high rate of home deliveries, some neonates are brought directly from home.

PARTICIPANTS

Between 7th December 2016 and 6th December 2017, neonates with pSBI were screened for eligibility. Maternal written informed consent was obtained by one of the study team able to communicate in the mother's language. Inclusion criteria were: (1) weight over 2kg at time of recruitment; (2) age at recruitment ≤28 days; (3) clinical signs and symptoms fulfilling the case definition of pSBI; (4) maternal age at least 18 years. Exclusion criteria were: (1) neonatal congenital abnormalities; (2) use of parenteral antibiotics for 24 hours or more prior to recruitment; (3) history of perinatal asphyxia; (4) neonates of mothers who were unable to speak English, Luganda, Lumasaba, Ateso or Lugwere well enough to provide informed consent. Infants were recruited 8am-5pm Monday to Friday.

An additional 50 well neonates with normal vital signs were recruited from the postnatal ward. Inclusion criteria were: (1) weight over 2kg when recruited; (2) no history of perinatal asphyxia; (3) no suspicion of neonatal infection; (4) no evidence of congenital abnormality; (5) maternal age at least 18 years; (6) mother able to speak English, Luganda, Lumasaba, Ateso or Lugwere well enough to provide informed consent, (7) recruitment possible between 8am-5pm Monday to Friday.

For all cases, vital signs at admission were recorded by the admitting healthcare worker. For controls, vital signs were recorded at recruitment. All neonates were examined, and anthropometric measurements taken by one of the four study doctors at recruitment (KB, RM, EE, KN). For all neonates the maternal medical history and demographics, antenatal events and birth history were collected from a structured interview and the obstetric medical records where available (Appendix 1). For cases, neonatal clinical data were extracted from the medical records at discharge or death.

A lumbar puncture was performed on all cases when not contraindicated. For those neonates with active convulsions, severe respiratory distress or apnoea, the lumbar puncture was delayed until it was safe to perform. CSF was collected using an aseptic technique, sterile gloves were worn, cleaning the skin once thoroughly with alcohol swab, cleaning a second time with a betadine swab and lastly with an alcohol swab before collecting CSF using a spinal needle (25g BD Spinal Needle). CSF was collected into a sterile universal container for culture, protein analysis and cell count (1ml) and a fluoride tube for glucose analysis (300µL). The CSF culture and analysis samples were stored at room temperature until transfer to the microbiology laboratory as soon as possible and always within 3 hours of collection. Meningitis was defined as a positive CSF culture and/or raised protein concentration above 127mg/dl and/or white blood cell count above 15cells/mm^3 (Thomson et al., 2018). The lower bound value for a normal glucose concentration was defined as 25 mg/dL (1.4mmol/L).

All cases underwent a cUS within 24 hours of their admission to the NNU. All controls underwent a cUS on the day of recruitment. All cUS examinations were performed with a portable ultrasound machine (Sonosite M-Turbo®). A C11x curved probe

(Frequency 8-5 MHz) and a linear probe (Frequency 13-6 MHz) were used in all infants. The probe was cleaned with alcohol between infants. The examinations were performed by KB, KN, EE, RM and JI. The cUS protocol is described in detail in Chapter 1. In brief, the protocol included a minimum of 5 coronal images using the curved probe via the anterior fontanelle, one midline sagittal, two left and two right parasagittal views. The Doppler resistive index from blood flow in the anterior cerebral artery was also recorded. The linear probe was then used via the anterior fontanelle to image the cortex in the coronal and sagittal planes in addition to taking colour Doppler images from the sagittal superior sinus (SSS).

All clinicians performing the scans were trained in brain anatomy and cUS by a consultant neonatologist (CH). A one-day theoretical training in cUS was provided by CH for all study doctors (KB, RM, KN, EE, JI). One-on-one bedside teaching of approximately 9 hours was provided by CH to each study doctor. The study protocols for cUS scanning were available on the ward for reference. Ongoing feedback on the cUS quality and advice on improvements was given by the two consultant neonatologists to the clinicians performing the scans.

The cUS scans were interpreted at the time of scanning by the physician or principal investigator to help with directing clinical management. The cUS data were also downloaded and stored digitally as DICOM images. The images were analysed by FC and CH, blinded to the neonate's clinical data and outcome, using OsiriX 10.0.5 software. Initially, 50 scans were read together and then 50 were read independently by both readers and then compared to ensure good inter-observer agreement. The remaining scans were read independently by each examiner.

Each scan was assessed systematically as detailed in Figure 46. The scans were assessed for normal anatomy as listed in Figure 46. The lateral ventricles were examined for signs suggestive of ventriculitis including shape, dilatation, presence of strands or debris, bright or irregular ventricular margin and presence of intraventricular haemorrhage. The cortex was examined for cortical highlighting (echogenic sulci with hypoechogenic cortex), increased cortical echogenicity, cystic change and focal lesions. The WM was examined for the presence and severity of echogenicity and changes suggestive of haemorrhage. The basal ganglia and thalami were examined for abnormal echogenicity and the site of the posterior limb of the internal capsule for abnormal echolucency sometimes seen in neonates with asphyxia. The presence and site of subependymal cysts and choroid plexus cysts were recorded. Evidence of calcification in general and lenticulostriate vasculopathy in particular was noted. The cerebellum was examined for the presence of haemorrhage or cystic changes, and the posterior fossa also for enlarged cisterna magna, tectorial angle and haemorrhage. The presence of thrombosis was evaluated using Doppler imaging of the superior sagittal sinus. The extracerebral space was examined for enlargement and increased echogenicity.

Key abnormalities
Anatomical variants - Agenesis of corpus callosum - Absent cavum septum pellucidum - Enlarged cavum vergae - Abnormal cortical folding - Abnormal cortical maturation - Cerebellar abnormality
Ventricular findings - Abnormal shape of lateral ventricles - Prominent 3rd ventricle - Prominent choroid in roof of the 3rd ventricle - Strands and/or debris within the ventricles - Ventricular dilatation - Bright ventricular margin
Ventricular Score (combining findings from 3rd and lateral) - 0 normal ventricles - 1 ventricular finding - 2 or more ventricular findings
Intraventricular haemorrhage (IVH) - Grade I-III - Parenchymal haemorrhagic infarction (Grade IV) - Unilateral or bilateral
Cortical echogenicity - Normal cortex - Cortical highlighting - Increased echogenicity - Cystic changes - Focal Lesion
White matter - Normal or mild echogenicity - Moderate echogenicity - Severe echogenicity - Haemorrhage
Basal ganglia, thalamus, posterior limb of internal capsule - Abnormal thalamic echogenicity - Abnormal basal ganglia echogenicity - Posterior limb of internal capsule present - Echogenicity typical of hypoxic ischaemic encephalopathy (
Cerebellum - Haemorrhage
Cysts - Sub-ependymal - Caudothalamic - Choroid plexus
Calcifications
Lenticulostriate vasculopathy
Thrombosis
Extracerebral space - Increased echogenicity - Enlarged extracerebral space

Figure 46: Key abnormalities considered on examination of cranial ultrasound scans

STUDY SIZE AND STATISTICAL ANALYSIS

Convenience sampling was used, and the sample size reflects this one-year time period. Data were analysed using IBM SPSS Statistics Version 25. Categorical variables were examined using Chi-squared test and Fisher's exact test as appropriate for sample size. Continuous variables were examined using student's T-test and the Mann-Whitney U test according to normality.

ETHICS

The Institutional Review Board of Mbarara University of Science and Technology, Uganda, the Uganda Council for Science and Technology and Penn State University approved the study.

RESULTS

POPULATION

A cUS examination within 24 hours of presentation was performed and assessed for 196/214 neonates presenting with pSBI over the 1-year period. Baseline demographic and clinical characteristics of the 196 infants are shown in Table 13, and were not significantly different to the cohort of 214 (Appendix 2). Maternal fever during labour was reported in 40.8% of mothers and 9.1% reported prolonged rupture of membranes >18 hours. Only 3.1% were known to be HIV sero-positive. The majority (91.9%) of neonates were born in a health facility and the majority (91.3%) were delivered by a trained health worker (midwife, nurse, doctor).

Maternal characteristic	Overall (N=196)
Maternal age at delivery, Mean (SD) in years	25.5 (5.5)
Prenatal HIV status, (%)	
- Positive	6 (3.1)
- Negative	172 (87.8)
- Unknown	18 (9.2)
Primigravida (%)	72 (36.7)
Median parity (range)	2.0 (1-12)
Education, Freq (%)	
- No education	5 (2.6)
- Primary	70(35.7)
- Secondary	69 (35.2)
- Diploma	48 (24.5)
- Unknown	4 (2.0)
Employment, Freq (%)	
- Professionals (accountant, engineer, teacher, healthcare worker manager)	27 (13.8)
- Clerical support worker	2 (1.0)
- Service and sales worker (police, security guard, caterer, hairdresser, sales workers)	42 (21.4)
- Skilled forestry, agricultural, fisheries worker	46 (23.5)
- Craft worker (tailor)	5 (2.6)
- Others (student)	1 (0.5)
- Housewife	71 (36.2)
- Unknown	2 (1.0)
Maternal fever during labour (%)	80 (40.8)
Rupture of membranes >18 hours, n=154, (%)	14 (9.1)
State of liquor (%)	
- Clear	129 (65.8)
- Meconium	11 (5.6)
- Foul smelling	6 (3.1)
- Unknown	50 (25.5)
Place of delivery (%)	
- Home or on the way to health facility	16 (8.1)
- Health facility	180 (91.9)
Type of birth attendant (%)	
- Traditional birth attendant (TBA)	5 (2.6)
- Trained healthcare worker	179 (91.3)
- Relative/Friend/Alone	12 (6.1)
Mode of delivery (%)	
- Spontaneous vertex delivery	133 (67.9)
- Elective caesarean section	5 (2.6)
- Emergency caesarean section	56 (28.6)
- Operative vaginal birth	2 (1.0)

Data are n (%).

Table 13: Baseline characteristics of mothers

The mean weight at admission was 2.998 kg (±0.540kg) and the median age at presentation was 2.0 days, with almost two-thirds (62.8%) presenting within 48 hours of birth (Table 14).

Characteristic	Overall (N=196)
Sex, male (%)	117 (59.7)
Admission weight mean (SD) in g	2998 (540)
Early presentation <48 hours (%)	123 (62.8)
Age at presentation in days, median (range)	2.0 (1-27)
Head circumference Mean (SD) in cm	35.6 (1.5)
Gestation at birth (n=123), mean (SD) in weeks	39.7 (2.1)
Neonatal resuscitation (%)	
- Need for resuscitation	36 (18.4)
- Suction/stimulation only	24 (12.2)
- Bag-mask ventilation	12 (6.1)
Apgar score at 5-minute, n=173	
- Mean (SD)	9.6 (0.8)
- Range	6 - 10
Final diagnosis at discharge or death, Freq (%)	
- Sepsis	150 (76.5)
- Meningitis	26 (13.3)
- Pneumonia	13 (6.6)
- Tetanus	7 (3.6)
Final inpatient outcome	
- Discharged alive	147 (75.0)
- Inpatient death	12 (6.1)
- Discharged against medical advice	37 (18.9)
Neonatal outcome (28 days)	
- Alive and well	174 (88.7)
- Alive with neurological sequelae	5 (2.6)
- Neonatal death	17 (8.7)

Data are n (%).

Table 14: Baseline characteristics of the neonates with possible serious bacterial infection (pSBI)

FINDINGS ON CSF CULTURE AND ANALYSIS

A successful lumbar puncture was achieved in 176 of the 196 infants who were scanned on admission. A lumbar puncture was not done in 14 infants due to clinical contraindications and it was unsuccessful in 6 infants. None of these infants had a positive CSF culture or Gram stain. 20/176 (11.4%) had an abnormal CSF analysis suggestive of possible CNS infection as shown in Table 15 (Thomson et al., 2018).

Elevated WBC counts ranged from 25-175cells/mm^3 (normal <15cells/mm^3), elevated protein ranged from 130-730mg/dl (normal ≤127mg/dl) and low glucose concentration ranged from 5-24mg/dL (normal >25 mg/dl).

A reduced glucose level alone, with normal protein levels and WBC counts was not considered abnormal. One limitation of this study was the inability to measure simultaneous blood glucose levels, therefore hypoglycaemic CSF samples could likely have been secondary to systemic hypoglycaemia.

Abnormal CSF parameters*, N=176	Frequency, N=176
Combinations of abnormal parameters	
Elevated WBC only	7 (4.0)
Elevated WBC and protein only	2 (1.1)
Elevated WBC and reduced glucose only	1 (0.6)
Elevated WBC and protein, reduced glucose	3 (1.7)
Elevated protein only	6 (3.4)
Elevated protein and reduced glucose only	1 (0.6)
Reduced glucose only	5 (2.8)
Total (excluding reduced glucose only)	20/176 (11.4%)

Data are n (%). *Limits used include: Protein concentration > 127mg/dl, WBC count >15cells/mm^3 and glucose concentration <25 mg/dL or 1.4 mmol/L .

Table 15: Frequency of abnormal CSF analysis

All the infants with abnormal CSF parameters had at least one abnormality on their cUS examination at presentation (Table 16). Only 12.9% (20/155) of infants with normal CSF parameters also had a normal cUS examination at presentation.

	Abnormal CSF parameters N=20	Normal CSF parameters N=155
cUS>10, one or more abnormality, n (%)	20 (100)	135 (87.1)
cUS = 10, normal cUS, n (%)	0 (0)	20 (12.9)

Table 16: Comparison of infants with abnormal CSF analysis and abnormal cranial ultrasound

OVERALL FINDINGS ON CRANIAL ULTRASOUND EXAMINATIONS AT ADMISSION

A wide range of imaging abnormalities was observed in the cUS examinations undertaken at admission to the neonatal unit in infants with pSBI. A normal cUS was reported in 20.0% (39/196) of infants. At least one abnormal finding was reported in 80.0% of infants and the overall frequency of these abnormalities are shown in Table 17.

Finding	All (N=196)
Anatomical variants	
- Partial agenesis of corpus callosum	2 (1.0)
- Absent cavum septum pellucidum	0 (0.0)
- Enlarged cavum vergae	4 (2.0)
- Abnormal cortical folding	4 (2.0)
- Abnormal cortical maturation	7 (3.6)
Ventricular findings	
- Abnormal shape ventricle	5 (2.6)
- Prominent choroid 3rd ventricle	50 (25.5)
- Strands and/or debris	2 (1.0)
- Ventricular dilatation	5 (2.6)
- Bright ventricular margin	10 (5.1)
Ventricular Score (3rd and lateral ventricles)	
- 0 normal ventricles	136 (69.4)
- 1 abnormality	51 (26.0)
- 2 or more abnormalities	9 (4.6)
Intraventricular haemorrhage (IVH)	
- Bilateral grade 2	1 (0.5)
- Unilateral grade 1	1 (0.5)
Cortical echogenicity	
- Normal cortex	179 (91.4)
- Cortical highlighting	11 (5.6)
- Increased echogenicity	4 (2.0)
- Subcortical cystic	1 (0.5)
- Focal Lesion	1 (0.5)
White matter	
- Focal haemorrhage	3 (1.5)
- Normal or mild echogenicity	180 (92.3)
- Moderate echogenicity	10 (5.1)
- Severe echogenicity	6 (3.1)
Basal ganglia, thalamus, posterior limb of internal capsule	
- Abnormal basal ganglia	21 (10.7)
- Abnormal thalamus	13 (6.6)
- Posterior limb of internal capsule present	2 (1.0)
- Echogenicity typical of hypoxic ischaemic encephalopathy	12 (6.1)
Cerebellum	
- Haemorrhage	2 (1.0)
Cysts	
- Subependymal	4 (2.0)
- Caudothalamic	29 (14.8)
- Choroid plexus	19 (9.7)
Calcification	4 (2.0)
Lenticulostriate vasculopathy	47 (24.0)
Thrombosis in superior sagittal sinus	1 (0.5)
Extracerebral space	
- Increased echogenicity	14 (7.1)
- Enlarged	21 (10.7)
Resistive Index (RI) of anterior cerebral artery	
- Mean (SD)	0.65 (0.10)
- Range	0.34, 0.90

Data are n (%).

Table 17: Abnormalities seen on cranial ultrasound within 24 hours of admission to NNU

VENTRICULAR FINDINGS

Overall, 2.6% (5/196) of the infants had abnormally shaped ventricles. Two of these infants had other features of ventriculitis but in three infants it was an isolated finding and therefore more likely to be developmental in origin (Table 17). Ventricular strands and/or debris were present in 1% infants, ventricular dilatation in 2.6% and a bright ventricular margin in 5.1%. These findings are all suggestive of ventriculitis. The most common finding was a prominent choroid in the roof of the 3rd ventricle (25.5%) an example is shown in Figure 47. One neonate had a grade I IVH and another had bilateral grade II IVH. The majority of ventricular findings were solitary, however 7/196 had two abnormal ventricular findings, 1/196 had three ventricular abnormalities and one infant had five ventricular abnormalities (Figure 48).

CORTICAL FINDINGS

Overall 5.6% neonates had cortical highlighting at presentation. This finding may represent cortical inflammation due to vasculitis, which can progress to cortical infarction. Another 2.0% had increased cortical echogenicity (Figure 49), which is more suggestive of cortical infarction. One case had a focal lesion and 1 had cystic change (Figure 50).

WHITE MATTER FINDINGS

The majority of neonates had normal WM or only mild echogenicity (91.8%). WM haemorrhage was seen in 1.5% neonates and 8.2% of neonates had moderate or severe WM echogenicity (Figure 51). No abscesses were noted on any of the day 1 scans.

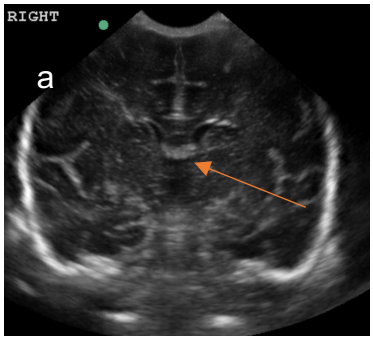


Figure 47: An example of bright choroid in the roof of the 3rd ventricle on cUS in the coronal view.

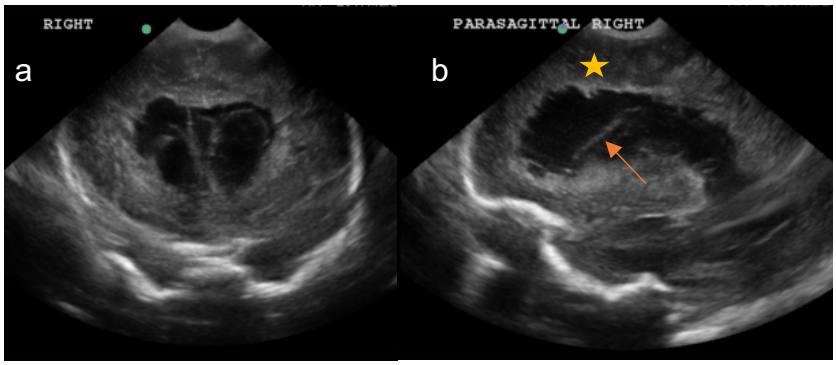


Figure 48: A coronal image (a) and right parasagittal image (b) showing multiple ventricular findings: ventricular debris, strands (arrow), dilatation, bright and irregular ependymal margin (star) and abnormal shape

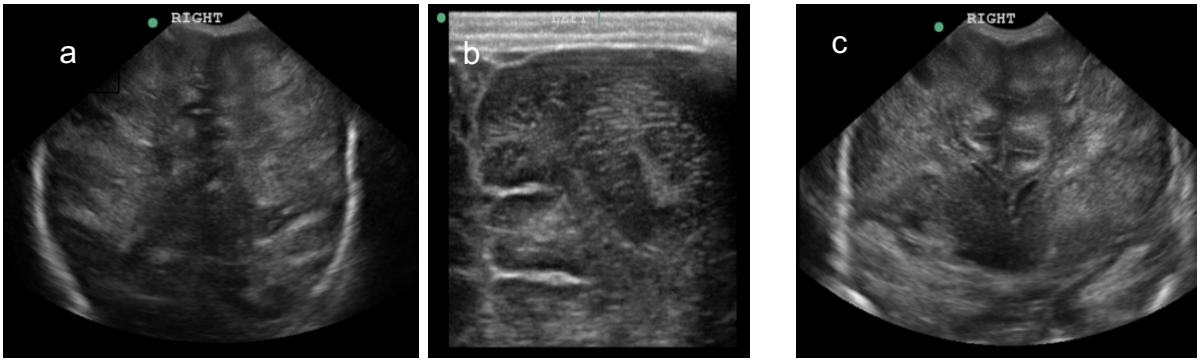


Figure 49: Examples of cortical changes on cUS examination: Widespread increased echogenicity of white matter and cortex on coronal view (a); Cortical echogenicity seen using linear probe (b); Widespread cortical and white matter hyper echogenicity on coronal view (c).

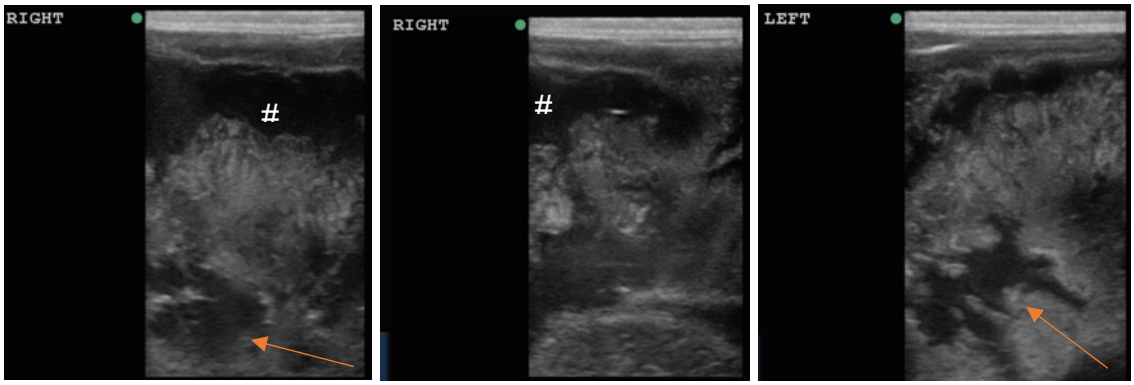


Figure 50: Images made using the linear probe demonstrating increased cortical echogenicity associated with white matter hyper echogenicity and cystic subcortical white matter (arrow) and volume loss (#). Images taken on the day of admission in a 10-day old neonate.

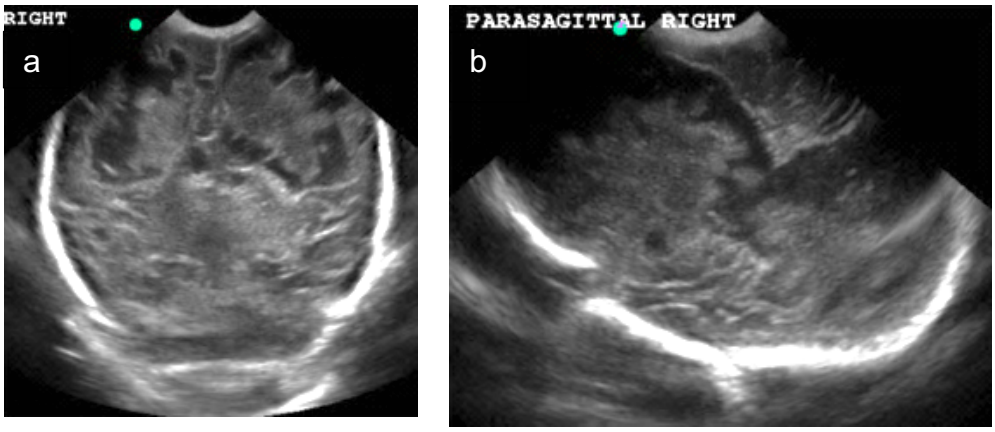


Figure 51: Examples of white matter abnormalities: a) Severe white matter echogenicity with associated encephalomalacia of parietal lobes on coronal scan, b) corresponding right parasagittal image demonstrating severe white matter echogenicity with associated encephalomalacia

BASAL GANGLIA AND THALAMUS FINDINGS

19.9% (39/196) of infants had increased echogenicity of the basal ganglia and/or thalamus. Unexpectedly, in 12 of these infants the posterior limb of the internal capsule was also echolucent, an appearance that is typically described in neonates with hypoxic ischaemic injury. 10/12 of these infants had a documented Apgar score of 8-

10 at 5 minutes of age. The other two infants were reported to need stimulation and suction only and had no signs of encephalopathy either at or during admission.

CYSTS

Subependymal, caudothalamic and choroid plexus cysts were observed in 2.0%, 14.8% and 9.7% of neonates respectively (Figure 52). Six infants had cysts in two locations, three having choroid plexus and caudothalamic cysts and three having caudothalamic and subependymal cysts.

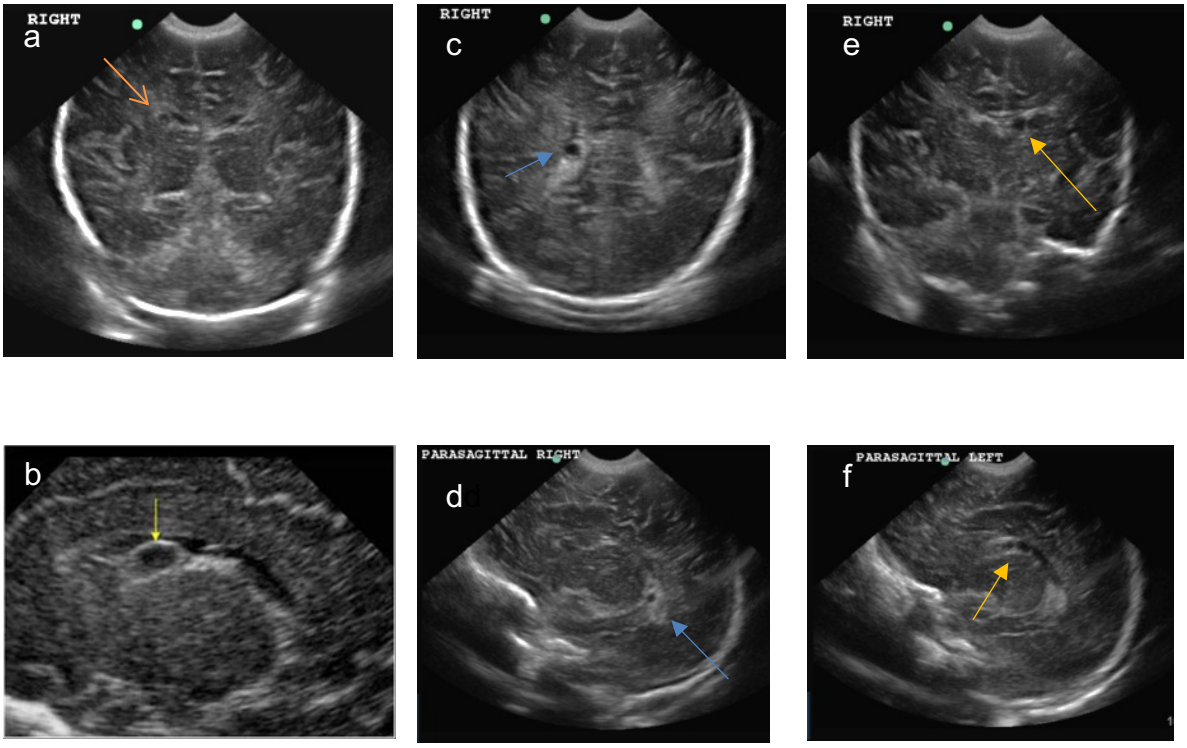


Figure 52: Examples of cysts, subependymal cyst (a,b), choroid plexus cyst (c, d), caudothalamic notch cyst (e,f) (arrows)

INCREASED EXTRACEREBRAL SPACE

An increased extracerebral space was a common finding and was seen in 10.7% of infants of pSBI (Figure 53). This finding can be due to parenchymal volume loss or accumulation of fluid. One-third of these infants (33.3%, 7/21) also had increased echogenicity in the extracerebral space. A further 7 infants had increased echogenicity without enlargement of the extracerebral space. Only three (14.3%) of the infants with increased extracerebral space had associated dilated ventricles.

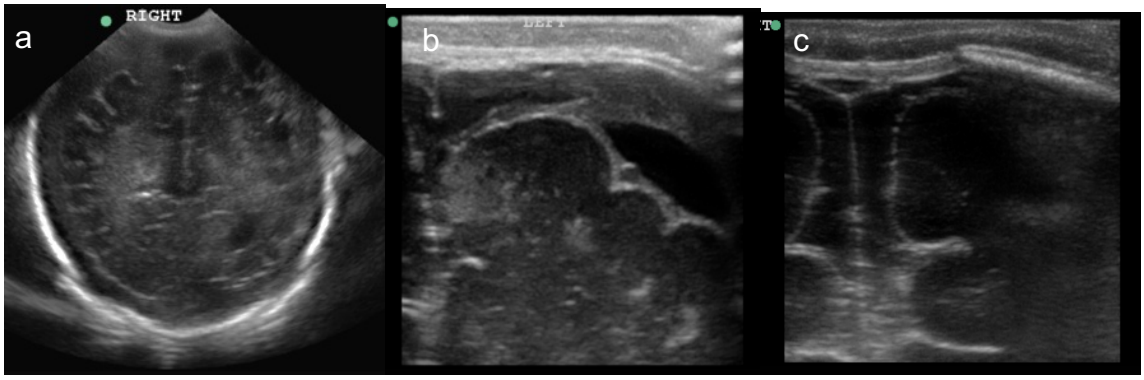


Figure 53: Examples of abnormal extracerebral space: a) Increased hyperechogenic extracerebral space suggestive of subdural empyema, b) image with the linear probe of the same patient showing increased hyperechogenic extracerebral space and, thickened meninges, cystic change of the subcortical white matter is also evident, and c) an image with the linear probe shows a widened interhemispheric fissure with increased extracerebral space and normal echogenicity within the extracerebral space

COEXISTING CRANIAL ULTRASOUND FINDINGS

There were 9 infants with 2 or more ventricular abnormalities. Two of these infants also had moderately or severely abnormal cortical appearances as well (Table 18). One infant also had severe WM echogenicity. Of the 17 and 16 infants with cortical and WM echogenicity respectively, 10 (62.5%) had moderately or severely increased echogenicity in both locations.

	2 or more ventricular abnormalities (n=9)	Cortex moderate to severe abnormality (n=17)	White Matter moderate to severe abnormality (n=16)	Basal ganglia and/or thalamus abnormal (n=39)	Cysts (choroid, subependymal, caudothalamic) (n=51)	Extracerebral space increased or echogenic (n=33)
2 or more ventricular abnormalities		2	1	3	3	3
Cortex abnormal	2		10	10	2	4
White Matter abnormal	1	10		12	2	4
Basal ganglia and/or thalamus abnormal	3	10	12		9	10
Cysts (choroid, subependymal, caudothalamic)	3	2	2	9		11
Extracerebral space increased or echogenic	3	4	4	10	11	

Table 18: Co-existing pathologies on cranial ultrasound on day 1 of presentation

FINDINGS IN EARLY AND LATE PRESENTATION

Infants were eligible for recruitment up to 28 days after birth and the age range at recruitment was 1-27 days. The majority (62.8%) of neonates presented within 48 hours of birth, termed early-onset presentation. cUS scan findings were compared between those with early-onset and late-onset presentations. Those presenting after 48 hours were significantly more likely to have abnormally shaped ventricles (p=0.007) and there was a trend towards more ventricular dilatation in those presenting later (Table 19). In addition, those with late-onset presentation were significantly more likely to have cortical echogenicity (p=0.001), and WM echogenicity (p=0.013).

Finding on examination	Early presentation <48h of age (N = 123)	Late presentation >48h of age (N = 73)	P value
Ventricular findings			
- Ventricular dilatation	1 (0.8)	4 (5.5)	0.065
- Abnormal shape	0 (0)	5 (6.8)	**0.007**
- Prominent choroid 3rd ventricle	32 (26.0)	18 (24.7)	0.867
- Strands and/or debris	1 (0.8)	1 (1.4)	1.000
- Bright ventricular margin	5 (4.1)	5 (6.8)	0.504
Ventricular Score			
- 0 normal	88 (71.5)	48 (65.8)	0.425
- 1 abnormality	31 (25.2)	20 (27.4)	
- 2 or more abnormalities	4 (3.3)	5 (6.8)	
Intraventricular haemorrhage			
- Bilateral grade II IVH	1 (0.8)	0 (0)	1.000
- Unilateral grade I IVH	1 (0.8)	0 (0)	
Cortical echogenicity			
- Normal	119 (96.7)	60 (82.2)	**0.001**
- More than 50% highlighting	3 (2.4)	8 (11.0)	
- Increased echogenicity	1 (0.8)	3 (4.1)	
- Subcortical cystic	0 (0)	1 (1.4)	
- Focal Lesion	0 (0)	1 (1.4)	
White matter			
- Normal or mild echogenicity (1/2)	118 (95.9)	62 (84.9)	**0.013**
- Moderate echogenicity (3)	4 (3.3)	6 (8.2)	
- Severe echogenicity (4/5)	1 (0.8)	5 (6.8)	
- Haemorrhage	0	0	
Basal ganglia, thalamus, posterior limb of internal capsule			
• Abnormal basal ganglia	10 (8.1)	9	0.4543
• Abnormal thalamus	19 (15.4)	14	0.5555
• Posterior limb of internal capsule present	2 (1.6)	2	0.6295
• Echogenicity typical of hypoxic ischaemic encephalopathy	9 (7.3)	9	0.3069
Cysts			
- Subependymal	3 (2.4)	1 (1.4)	1.000
- Caudothalamic	20 (16.3)	11 (15.1)	1.000
- Choroid plexus	16 (13.0)	6 (8.2)	0.356
Calcification	1 (0.8)	3 (4.1)	0.146
Lenticulostriate vasculopathy	32 (26.0)	19 (26.0)	1.000
Thrombosis	1 (0.8)	0 (0)	1.000
Extracerebral space			
- Increased echogenicity	6 (4.9)	9 (12.3)	0.092
- Enlarged	15 (12.2)	10 (13.7)	0.826

Data are n (%).

Table 19: Comparison of findings on cranial ultrasound examinations performed at presentation in neonates with early onset versus late onset illness

Findings in control infants and comparison with the cases

None of the 44 well term neonates had abnormal anatomical variants on cUS (Table 20). As shown in Figure 54, the ventricles were significantly more abnormal in the cases of pSBI compared to the controls (*P*=0.0007*)*. In the controls there were only two well term neonates with a prominent choroid in the 3rd ventricle, which was significantly fewer than in the cases (*p*=0.004). None of the well term neonates had abnormal ventricular shape, strands, debris, ventricular dilatation and bright ventricular margin. None of the infants with abnormal CSF parameters had a normal cUS, whilst only 20/155 of those with a normal CSF analysis had a normal cUS examination.

Finding	Controls (N = 44)	pSBI cases (N = 196)	P value
Anatomical variants			
- Abnormal corpus callosum	0 (0)	4 (2.0)	1.000
- Large cavum septum pellucidum	0 (0)	0 (0)	-
- Large cavum vergae	0 (0)	4 (2.0)	1.000
- Abnormal cortical folding	0 (0)	4 (2.0)	1.000
- Abnormal cortical maturation	0 (0)	7 (3.4)	0.3551
Ventricular findings			
- Abnormal shape	0 (0)	5 (2.6)	0.587
- Prominent choroid 3rd ventricle	2 (4.5)	50 (25.5)	**0.001**
- Strands and/or debris	0 (0)	2 (1.0)	1.000
- Ventricular dilatation	0 (0)	5 (2.6)	0.587
- Bright ventricular margin	0 (0)	10 (5.1)	0.215
Ventricular Score			
- 0	42 (95.5)	136 (69.3)	**0.0001**
- 1 abnormality	2 (4.5)	51 (26.0)	
- 2 or more abnormalities	0 (0)	9 (4.6)	
Intraventricular haemorrhage			
- Nil	44 (100)	194 (99.0)	1.000
- Bilateral grade 2 IVH (10)	0 (0)	1 (0.5)	
- Unilateral grade 3 IVH (6)	0 (0)	1 (0.5)	

Data are n (%).

Table 20: Comparison of abnormal anatomical findings and ventricular findings on cUS examination between controls and cases

None of the 44 well term neonates had abnormal cortical findings. As shown in Figure 54 and Table 21, there was a trend towards abnormal cortical findings in cases of pSBI (8.7%) compared to the well term neonates (p=0.088). Thus, cortical highlighting, increased cortical echogenicity and cystic changes were seen only in infants with pSBI. A similar trend was seen with WM; none of the well term neonates had abnormal WM on cUS examination compared to 8.2% cases of pSBI with moderate to severe WM echogenicity (P=0.103, Table 21).

There was no significant difference in the presence of subependymal cysts and caudothalamic notch cysts in infants with pSBI and control neonates. Choroid plexus cysts were actually more likely to be found in the control neonates. Lenticulostriate vasculopathy was reported in one-quarter of infants with pSBI and was also significantly more common (p=0.0037) when compared to well term neonates. Increased extracerebral space and hyperechogenic extracerebral space were predominantly observed in pSBI, only one control neonate having these findings.

Finding	Controls (N = 44)	pSBI cases (N = 196)	P value
Cortical echogenicity - Normal - More than 50% highlighting - Increased echogenicity - Subcortical cystic - Focal Lesion	 44 (100) 0 (0) 0 (0) 0 (0) 0 (0)	 179 (91.3) 11 (5.6) 4 (2.0) 1 (0.5) 1 (0.5)	 **0.0479**
White matter - Haemorrhage - Normal or mild echogenicity (1/2) - Moderate echogenicity (3) - Severe echogenicity (4/5)	 0 (0) 44 (100) 0 (0) 0 (0)	 3 (1.5) 180 (91.8) 10 (5.1) 6 (3.1)	 0.999 0.048
Basal ganglia, thalamus, posterior limb of internal capsule • Normal • Abnormal basal ganglia • Abnormal thalamus • Posterior limb of internal capsule present • Echogenicity typical of hypoxic ischaemic encephalopathy	 1 (2.3) 3 (6.8) 0 0	 12 (6.1) 21 (10.7) 2 (1.0) 12 (6.1)	 0.4720 0.5832 1.000 0.13505
Cysts - Subependymal - Caudothalamic - Choroid plexus	 0 (0) 9 (20.4) 12 (27.2)	 4 (2.0) 29 (14.8) 19 (9.7)	 1.000 0.3639 **0.0046**
Calcification	0 (0)	4 (2.0)	0.884
Lenticulostriate vasculopathy	2 (4.5)	51 (26.0)	**0.004**
Thrombosis	0 (0)	0 (0)	
Extracerebral space - Increased echogenicity - Enlarged	 1 (2.3) 1 (2.3)	 15 (7.7) 25 (12.8)	 0.340 **0.057**

Data are n (%).

Table 21: Comparison of cortical, parenchymal and white matter findings on cUS examination between control infants and case infants

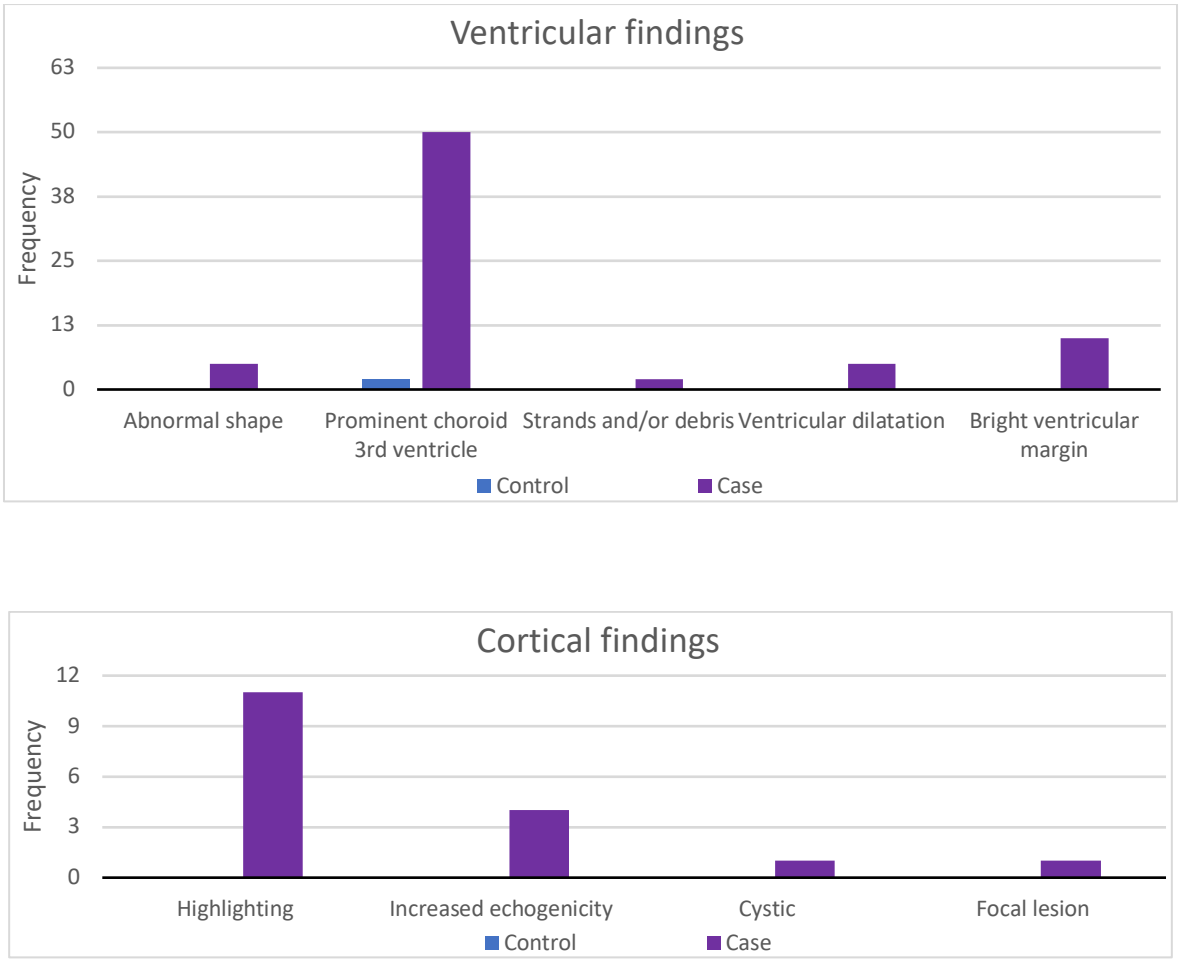


Figure 54: Incidence of ventricular and cortical findings in cases and controls

Outcomes

Overall 8.2% (17/196) of infants died during the neonatal period. Twelve were inpatient deaths and five were infants who discharged against medical advice before completing treatment. The infants who self-discharged received between 3 to 9 days of treatment, one had sepsis, two had meningitis and two had tetanus. The median time to death for the inpatient deaths was 2.0 days (IQR 2.0, 6.75). When comparing the cUS findings at presentation in those who survived compared to those who died (Table 22), the resistive index was significantly lower in those infants who died (0.56 v. 0.66, $p<0.001$). There were no other significant differences in the abnormalities seen.

Finding	Alive at 28d (N = 179)	Neonatal death (N = 17)	P value
Ventricular findings			
- Abnormal shape	5 (2.8)	0 (0)	1.000
- Prominent choroid 3rd ventricle	48 (26.8	2 (11.8)	0.247
- Strands and/or debris	2 (1.1)	0 (0)	1.000
- Ventricular dilatation	4 (2.2)	1 (5.9)	0.367
- Bright ventricular margin	9 (5.0)	1 (5.9)	1.000
Intraventricular haemorrhage			
- Bilateral grade 2 IVH	1 (0.6)	0 (0)	
- Unilateral grade 1 IVH	1 (0.6)	0 (0)	
Cortical echogenicity			
- Normal	165 (92.2)	15 (88.2)	0.634
- More than 50% highlighting	9 (5.0)	2 (11.8)	
- Increased echogenicity	4 (2.2)	0 (0)	
- Subcortical cystic	1 (0.6)	0 (0)	
- Focal Lesion	1 (0.6)	0 (0)	
White matter			
- Haemorrhage	3 (1.7)	0 (0)	1.000
- Normal or mild echogenicity (1/2)	166 (92.7)	14 (82.4)	0.149
- Moderate echogenicity (3)	8 (4.4)	2 (11.8)	
- Severe echogenicity (4/5)	6 (3.3)	0 (0)	
Basal ganglia, thalamus, posterior limb of internal capsule			
- Abnormal basal ganglia	9 (5.0)	3 (17.6)	0.0732
- Abnormal thalamus	17 (9.5)	4 (2.4)	0.0915
- Posterior limb of internal capsule present	2 (1.1)	0 (0)	1.0000
- Echogenicity typical of hypoxic ischaemic encephalopathy	10 (5.6)	2 (11.8)	0.2790
Cerebellum haemorrhage	2 (1.1)	0 (0)	1.000
Cysts			
- Subependymal	4 (2.2)	0 (0)	1.000
- Caudothalamic	29 (16.2)	2 (11.8)	1.000
- Choroid plexus	20 (11.1)	2 (11.8)	1.000
Lenticulostriate vasculopathy Present	48 (26.8)	3 (17.6)	0.566
Thrombosis	1 (0.6)	0 (0)	1.000
Extracerebral space			
- Increased echogenicity	14 (7.8)	1 (5.8)	1.000
- Enlarged	21 (11.7)	4 (23.5)	0.242
Resistive Index (RI) of anterior cerebral artery, Mean (SD)	0.66 (0.10)	0.56 (0.05)	**<0.001**

Data are n (%).

Table 22: Findings on day 1 cranial ultrasound examination in neonates who survived compared with those who died before 28 days (N=196)

When comparing the cUS findings on day 3 in those who survived compared to those who died (Table 22b), the white matter, cortex, basal ganglia and thalami were significantly more likely to be abnormal in infants who died. The extracerebral space was also more likely to be enlarged in those who died (44% v. 14%, p=0.04). The resistive index was significantly lower in those infants who died (0.60 v. 0.68, p=0.02).

Finding	Alive at 28d (N =140)	Neonatal death (N =9)	P value
Ventricular findings			
- Abnormal shape	6 (4.3)	1 (11.1)	0.3595
- Prominent choroid 3rd ventricle	48 (34.3)	0 (0)	0.0583
- Strands and/or debris	5 (3.6)	0 (0)	1.000
- Ventricular dilatation	8 (5.7)	0 (0)	1.000
- Bright ventricular margin	9 (6.4)	0 (0)	1.000
Intraventricular haemorrhage			
- Bilateral grade 2 IVH	1 (0.7)	0 (0)	1.000
- Unilateral grade 1 IVH	1 (0.7)	0 (0)	1.00
Cortical echogenicity			
- Normal	128 (91.4)	5 (55.5)	**0.0082**
- More than 50% highlighting	6 (4.3)	3 (27.2)	**0.0108**
- Increased echogenicity	5 (3.6)	1 (11.1)	0.3165
- Subcortical cystic	1 (0.7)	0 (0)	1.000
- Focal Lesion	0 (0)	0 (0)	1.000
White matter			
- Haemorrhage	3 (2.1)	0 (0)	1.000
- Normal or mild echogenicity (1/2)	132 (94.3)	6 (66.7)	**0.0199**
- Moderate echogenicity (3)	3 (2.1)	2 (22.2)	0.0296
- Severe echogenicity (4/5)	5 (3.6)	1 (11.1)	0.3165
Basal ganglia, thalamus, posterior limb of internal capsule			
- Abnormal basal ganglia	10 (7.1)	3 (33.3)	**0.0324**
- Abnormal thalamus	20 (14.3)	4 (44.4)	**0.0378**
- Posterior limb of internal capsule present	2 (1.4)	0 (0)	1.000
- Echogenicity typical of hypoxic ischaemic encephalopathy	11 (7.8)	2 (22.2)	0.1784
Cerebellum haemorrhage	2 (1.4)	1 (11.1)	0.1716
Cysts			
- Subependymal	4 (2.9)	0 (0)	1.000
- Caudothalamic	15 (10.7)	1 (11.1)	1.000
- Choroid plexus	18 (12.9)	2 (22.2)	0.3462
Lenticulostriate vasculopathy present	51 (36.4)	2 (22.2)	0.4919
Thrombosis	2 (1.4)	0 (0)	1.000
Extracerebral space			
- Increased echogenicity	9 (6.4)	1 (11.1)	0.4745
- Enlarged	20 (14.3)	4 (44.4)	**0.0378**
Resistive Index (RI) of anterior cerebral artery, Mean (SD)	0.68 (0.09)	0.60 (0.10)	**0.02**

Data are n (%).

Table 22b: Findings on day 3 cranial ultrasound examination in neonates who survived compared with those who died before 28 days (N=149)

When comparing the cUS findings on day 7 in those who survived compared to those who died (Table 22c), there were no significant differences observed.

Finding	Alive at 28d (N =101)	Neonatal death (N =10)	P value
Ventricular findings			
- Abnormal shape	5 (5.0)	0 (0)	1.000
- Prominent choroid 3rd ventricle	28 (27.7)	1 (10)	0.4497
- Strands and/or debris	3 (3.0)	0 (0)	1.000
- Ventricular dilatation	9 (8.9)	2 (20)	0.2582
- Bright ventricular margin	8 (7.9)	3 (30)	0.0596
Intraventricular haemorrhage			
- Bilateral grade 2 IVH	1 (1.0)	0 (0)	1.000
- Unilateral grade 1 IVH	1 (1.0)	0 (0)	1.000
Cortical echogenicity			
- Normal	93 (92.1)	10 (100)	1.000
- More than 50% highlighting	2 (2.0)	0 (0)	1.000
- Increased echogenicity	5 (5.0)	0 (0)	1.000
- Subcortical cystic	1 (1.0)	0 (0)	1.000
- Focal Lesion	0 (0)	0 (0)	1.0000
White matter			
- Haemorrhage	2 (2.0)	0 (0)	1.000
- Normal or mild echogenicity (1/2)	91 (90.1)	9 (90)	1.000
- Moderate echogenicity (3)	3 (3.0)	0 (0)	1.000
- Severe echogenicity (4/5)	5 (5.0)	0 (0)	1.000
Basal ganglia, thalamus, posterior limb of internal capsule			
- Abnormal basal ganglia	9 (8.9)	0 (0)	1.000
- Abnormal thalamus	25 (24.8)	0 (0)	0.1129
- Posterior limb of internal capsule present	2 (2.0)	0 (0)	1.000
- Echogenicity typical of hypoxic ischaemic encephalopathy	10 (9.9)	0 (0)	0.5948
Cerebellum haemorrhage	3 (3.0)	0 (0)	1.000
Cysts			
- Subependymal	4 (4.0)	1 (10)	0.3820
- Caudothalamic	14 (13.9)	1 (10)	1.000
- Choroid plexus	10 (9.9)	0 (0)	0.5948
Lenticulostriate vasculopathy Present	37 (36.6)	6 (60)	0.1816
Thrombosis	1 (1.0)	0 (0)	1.000
Extracerebral space			
- Increased echogenicity	13 (12.9)	1 (10)	1.000
- Enlarged	29 (28.7)	2 (20)	0.7230
Resistive Index (RI) of anterior cerebral artery, Mean (SD)	0.71 (0.08)	0.71 (0.05)	0.88

Data are n (%).

Table 22c: Findings on day 7 cranial ultrasound examination in neonates who survived compared with those who died before 28 days (N=111)

CLINICAL ASSOCIATIONS

43/196 (21.9%) neonates had a history of seizures or had seizures observed at presentation. The findings on cUS were significantly different between those neonates with and without seizures. Ventricular pathology, most specifically those with ventricular dilatation, abnormal ventricle shape and bright ventricular lining, was significantly more common in those neonates presenting with seizures (Table 22). It should be noted that four infants with two or more ventricular abnormalities did not have seizures. The presence of cortical echogenicity or highlighting was also associated with seizures. Similarly, increased WM echogenicity, basal ganglia and thalamic echogenicity and posterior limb of the internal capsule hypo echogenicity were significantly more common in infants with seizures.

Finding	No seizures N = 153 (%)	Seizures N = 43 (%)	P value
Ventricular findings			
- Normal ventricles	111 (72.5)	23 (53.4)	**0.036***
- Abnormal shape	1 (0.7)	4 (9.3)	**0.009**
- Prominent choroid 3rd ventricle	38 (24.8)	12 (27.9)	0.695
- Strands and/or debris	0 (0)	3 (7.0)	0.221
- Ventricular dilatation	2 (1.3)	3 (7.0)	0.071
- Bright ventricular margin	5 (3.3)	5 (11.6)	**0.045**
Ventricular Score (0-5)			
- 0 (normal)	111 (72.5)	23 (53.4)	**0.036***
- 1 (1 abnormality)	38 (24.8)	13 (30.2)	
- 2-5 (2 or more abnormalities)	4 (2.6)	5 (11.6)	
Cortical echogenicity			
- Normal cortex	147 (96.1)	32 (74.4)	**0.000***
- More than 50% highlighting	4 (2.6)	7 (16.2)	
- Increased echogenicity	1 (0.7)	3 (7.0)	
- Subcortical cystic	0 (0)	1 (2.3)	
- Focal Lesion	1 (0.7)	0 (0)	
White matter			
- Normal or mild echogenicity (1/2)	149 (97.4)	31 (72.1)	**0.000***
- Moderate echogenicity (3)	3 (2.0)	7 (16.3)	
- Severe echogenicity (4/5)	1 (0.7)	5 (11.6)	
Basal ganglia, thalamus, posterior limb of internal capsule			
- Normal	138 (90.1)	29 (67.4)	**0.0010***
- Abnormal basal ganglia	5 (3.3)	7 (16.2)	**0.0052**
- Abnormal thalamus	10 (6.5)	11 (25.6)	**0.0011**
- Posterior limb of internal capsule present	1 (0.7)	1 (2.3)	0.3915
- Echogenicity typical of hypoxic ischaemic encephalopathy	7 (4.6)	5 (11.6)	0.1407
Cysts			
- Nil present	105 (68.6)	36 (83.7)	0.274*
- Subependymal	2 (1.3)	2 (4.7)	0.148
- Caudothalamic	27 (17.6)	4 (9.3)	0.605
- Choroid plexus	20 (13.1)	2 (4.7)	0.392
Lenticulostriate vasculopathy	40 (26.1)	11 (25.6)	0.628
Thrombosis	1 (0.7)	0 (0)	0.384
Extracerebral space			
- Increased echogenicity	10 (6.5)	5 (11.6)	0.327
- Enlarged	17 (11.1)	8 (18.6)	0.202

Data are n (%). * Comparison of normal findings to any abnormality.

Table 23: Comparison of cranial ultrasound findings on day 1 of presentation in those neonates with and without seizures

Overall 79 neonates presented with signs suggestive of encephalopathy or meningism including seizures, hypotonia, reduced consciousness, opisthotonus and bulging fontanelle. Table 24 shows the comparison of cUS findings between neonates with any signs of encephalopathy or meningism

versus those without. Neonates with encephalopathy or meningism were significantly more likely to have increased cortical or white matter echogenicities, compared to those neonates without. The ventricular findings were not significantly different between these two groups.

Finding	Normal neurological examination (N = 115)	Abnormal neurological examination (N = 79)	P value
Ventricular findings			
- Normal	80 (69.6)	56 (70.9)	0.294*
- Abnormal shape	0 (0)	5 (6.3)	**0.010**
- Prominent choroid 3rd ventricle	34 (29.6)	15 (18.9)	0.130
- Strands and/or debris	0 (0)	3 (3.8)	0.163
- Ventricular dilatation	1 (0.9)	4 (5.1)	0.160
- Bright ventricular margin	4 (3.5)	6 (7.6)	0.167
Ventricular score (0-5)			
- 0 (normal)	80 (69.6)	56 (70.9)	0.294*
- 1 (1 abnormality)	33 (28.7)	17 (21.5)	
- 2-5 (2 or more abnormalities)	3 (2.6)	6 (7.6)	
Cortical echogenicity			
- Normal (1)	112 (97.4)	66 (83.5)	**0.001***
- More than 50% highlighting (2)	3 (2.6)	8 (10.1)	
- Increased echogenicity (3)	0 (0)	4 (5.1)	
- Subcortical cystic (4)	0 (0)	1 (1.3)	
- Focal Lesion (5)	1 (0.9)	0 (0)	
White matter			
- Normal or mild echogenicity (1/2)	113 (98.3)	66 (83.5)	**0.003***
- Moderate echogenicity (3)	3 (2.6)	7 (8.9)	
- Severe echogenicity (4/5)	0 (0)	6 (7.6)	
Basal ganglia, thalamus, posterior limb of internal capsule			
- Normal	100 (87.0)	65 (82.3)	0.415*
- Any abnormal echogenicity present	15 (13.0)	14 (17.7)	0.415
- Echogenicity typical of hypoxic ischaemic encephalopathy (HIE)	4 (3.5)	8 (10.1)	**0.011**
Cysts			
- Nil present			
- Subependymal	2 (1.7)	2 (2.5)	0.743
- Caudothalamic	22 (19.1)	9 (11.4)	0.293
- Choroid plexus	16 (13.9)	6 (7.6)	0.158
Lenticulostriate vasculopathy			
- Present	21 (18.3)	17 (21.5)	0.727
Thrombosis			
- Present	1 (0.9)	0 (0)	0.385
Extracerebral space			
- Increased echogenicity	7 (6.1)	8 (10.1)	0.412
- Enlarged	14 (12.2)	10 (12.7)	1.000

Data are n (%). * Comparison of normal findings to any abnormality.

Table 24:Comparison of cranial ultrasound findings on day 1 of presentation in those neonates with and without encephalopathy or meningism

ULTRASOUND FINDINGS COMPARED WITH CEREBROSPINAL FLUID FINDINGS

In Table 25, the cUS findings are compared between infants with and without abnormal CSF analysis. Although there was a significant association between WM and cortical echogenicity and abnormal CSF parameters, many neonates with these changes on cUS had a normal CSF analysis. Importantly, almost all those neonates with abnormal ventricles on cUS, even those with multiple ventricular abnormalities, had normal CSF analysis.

Finding	Normal CSF analysis (N = 156)	Abnormal CSF analysis (N = 20)	P value
Ventricular findings			
- Normal	108 (69.2)	14 (70.0)	1.000*
- Abnormal shape	3 (1.9)	1 (5.0)	0.385
- Prominent choroid 3rd ventricle	41 (26.3)	5 (25.0)	1.000
- Strands and/or debris	1 (1.9)	0 (0)	1.000
- Ventricular dilatation	3 (1.9)	0 (0)	1.000
- Bright ventricular margin	7 (4.5)	1 (5.0)	1.000
Ventricular score (0-5)			
- 0 (normal)	108 (69.2)	14 (70.0)	1.000*
- 1 (1 abnormality)	42 (26.9)	5 (25.0)	
- 2-5 (2 or more abnormalities)	6 (3.8)	1 (5.0)	
Cortical echogenicity			
- Normal (1)	146 (93.5)	15 (75.0)	**0.006***
- More than 50% highlighting (2)	8 (5.1)	2 (10.0)	
- Increased echogenicity (3)	1 (0.6)	2 (10.0)	
- Subcortical cystic (4)	1 (0.6)	0 (0)	
- Focal Lesion (5)	0 (0)	1 (10.0)	
White matter			
- Normal or mild echogenicity (1/2)	146 (93.5)	15 (75.0)	**0.057***
- Moderate echogenicity (3)	7 (4.5)	3 (15.0)	
- Severe echogenicity (4/5)	3 (1.9)	2 (10.0)	
Basal ganglia, thalamus, posterior limb of internal capsule			
- Normal	135 (87.1)	15 (75.0)	0.183*
- Any abnormal echogenicity present	21 (13.5)	5 (25.0)	0.183
- Echogenicity typical of hypoxic ischaemic encephalopathy (HIE)	10 (6.4)	1 (5.0)	0.286
Cysts			
- Subependymal	3 (1.9)	0 (0)	1.000
- Caudothalamic	26 (16.7)	2 (10.0)	0.770
- Choroid plexus	19 (12.2)	2 (10.0)	1.000
Lenticulostriate vasculopathy	43 (27.6)	5 (25.0)	0.845
Thrombosis	1 (0.6)	0 (0)	0.464
Extracerebral space			
- Increased echogenicity	13 (8.3)	1 (5.0)	0.711
- Enlarged	20 (12.8)	2 (10.0)	1.000

Data are n (%). * Comparison of normal findings to any abnormality.

Table 25: Comparison of cranial ultrasound findings on day 1 of presentation in those neonates with and without meningitis on CSF

DISCUSSION

We hypothesised that neonates with a CSF analysis and/or culture results suggestive of CNS infection would have pathological findings on the first cUS performed at presentation, thus making early cUS a reliable alternative for the

diagnosis of CNS infection in low-resource settings. All neonates with an abnormal CSF result, suggestive of meningitis, had at least one abnormality on their cUS examination at presentation. In addition, 135/155 neonates who had a negative CSF culture and normal CSF analysis had at least one abnormality on their cUS examination at presentation.

This study demonstrates that CSF culture and analysis are not sufficient to rule out involvement of the CNS in neonates presenting with clinical features of pSBI. Many neonates had abnormal cUS examinations suggestive of CNS infection, especially ventricular abnormalities, but had normal CSF results, therefore supporting the second hypothesis that the addition of cUS improves the detection rate of neonatal CNS infections, when compared to CSF cultures and analysis alone. It is also possible that the abnormal findings seen on cUS are due to systematic inflammation without the infant necessarily having an infection within the CNS. Lastly, organisms can be present within the brain parenchyma and intraventricular fluid without being detected in the CSF acquired from a lumbar puncture.

The findings in infants with pSBI were very different to those in the control infants. Significantly more cases had abnormal ventricular findings than controls and although not significant, all the cUS scans with abnormal WM and cortical findings were in infants with pSBI.

This imaging study clearly demonstrated that infants presenting with pSBI often have evidence of brain damage. The most common finding in neonates

with pSBI was the prominent choroid in the roof of the 3rd ventricle, present in 25.5% of cases. This is suggestive of inflammation or hyperaemia, and is particularly interesting as it is the choroid plexus that is thought to be the portal of entry for bacteria to the CSF (Yikilmaz and Taylor, 2008). This finding was almost entirely limited to infants with pSBI compared to controls (2/44 v. 50/196, *p*=0.004). Ventriculoscopy in cases of post-infectious hydrocephalus has demonstrated direct observation of choroid plexus scarring consistent with this (Warf, 2005). If this finding, of prominent choroid in the roof of the third ventricle, is indeed due to inflammation present within the choroid plexus, then the rate of CNS involvement in neonates with pSBI could be much higher than previously thought. Given that there was no association between prominent choroid plexus and abnormal CSF analysis, it is possible that effective and early antibiotics prevents the development of ventriculitis and meningitis.

Choroid plexus cysts are located in the choroid plexus itself and are typically the most common type of intraventricular cyst. Subependymal cysts are usually located lateral to the ventricle, most commonly in the anterior horns and caudothalamic cysts are located in the notch between the thalamus and the head of the caudate (Shen and Huang, 1985). Subependymal cysts and choroid plexus cysts were seen in 2.0% and 9.7% of infants with pSBI respectively. Although subependymal cysts were not observed in control infants, choroid plexus cysts were observed in 27.3% of control infants, which was significantly higher than in infants with pSBI (*p*=0.004). A previous study in Uganda also reported a high frequency of both these types of cysts in well term infants (subependymal cysts in 19.6% of neonates and choroid plexus

cysts in 19.6% of infants) (Hagmann et al., 2010). Subependymal cysts have been associated with congenital infections, especially cytomegalovirus and rubella (Herini et al., 2003, Shackelford et al., 1983, Shaw and Alvord, 1974). Choroid plexus cysts can be incidental but are also associated with chromosomal abnormalities, especially if large and bilateral, but have also been associated with cytomegalovirus (de Vries et al., 2006, Fernandez Alvarez et al., 2009, Herini et al., 2003, Oosterom et al., 2015). Both subependymal cysts and caudothalamic notch cysts can also be normal variants, but they are usually antenatal in origin and not related to acute neonatal illness. They can also be associated with in-utero infections, most commonly CMV, especially when large and/or multiple.

There are limited data on the incidence of congenital CMV (cCMV) in Africa, in LICs the reported prevalence of cCMV infection varies substantially but is as high as 14% in some studies (Bates and Brantsaeter, 2016, Madrid et al., 2018). Although we knew the HIV status of all the mothers, screening for maternal CMV is not routine in Uganda. The high prevalence of lenticulostriate vasculopathy (24.0%) and cysts may represent a high rate of cCMV in our population, and deserves further investigation given the long-term risk of neurological problems, particularly sensorineural hearing and neurodevelopmental impairment (Dollard et al., 2007, Fowler, 2013). In addition, recent work in Uganda has demonstrated a significantly higher prevalence of CMV in the CSF of neonates with post-infectious hydrocephalus compared to congenital hydrocephalus (Paulson et al., 2020). It is possible

therefore, that cCMV may play a role in the development of neonatal meningitis and/or subsequent post-infectious hydrocephalus.

As mentioned above lenticulostriate vasculopathy is associated with congenital CMV (cCMV) infection (de Vries et al., 2006). This study found evidence of lenticulostriate vasculopathy, in 24% of neonates with pSBI but only 4.5% of well term-neonates (p=0.0037). Similarly, the previously mentioned Ugandan study of normal neonates, found lenticulostriate vasculopathy in 3.6% of well term-neonates (Hagmann et al., 2010). It is therefore possible that this higher frequency of lenticulostriate vasculopathy in our cases of pSBI compared to our controls and the study by Hagmann et al. could be a vasculopathy due to bacterial CNS infection. That said, there was no association observed between abnormal CSF analyses or signs of meningism and encephalopathy and the observation of lenticulostriate vasculopathy. If the lenticulostriate vasculopathy is indeed due to cCMV, it is also plausible that cCMV infection predisposes to bacterial CNS infection (Paulson et al., 2020). Closer investigation with PCR of the CSF samples from these neonates for the presence cCMV, will be vital to clarify this observation, and explore the relationship between cCMV and neonatal CNS infection. This will be carried out in the future, on the stored samples collected in this study.

Increased echogenicity in the WM can be suggestive of cerebritis or infarction. The majority of neonates had normal WM or only mild echogenicity (180/196). Three infants with pSBI had appearances suggestive of WM haemorrhage and sixteen had moderate or severe WM echogenicity. There was a trend towards

moderately or severely increased echogenicity in the WM at presentation for pSBI compared to controls, present in 8.2% and 0% respectively (p=0.103). Increased WM echogenicity is associated with CNS infections and is one of the most common ultrasound findings in neonatal meningitis (de Vries et al., 2006). This study found increased WM echogenicity to be associated with abnormal CSF analysis, supporting this earlier observation. It should be noted that there were still 10 neonates with pSBI with normal CSF analysis that had moderate or severe WM. It is of course possible that these neonates may have had a viral infection or hypoglycaemia, which can also lead to WM injury. Unfortunately, data on viral pathogens and hypoglycaemia were not available for this study. CSF samples have however been stored for later analysis of viral RNA that will be available for comparison.

39 infants had increased echogenicity in the central grey matter (Basal ganglia and/or thalami). Unexpectedly, in 12 of these infants the , basal ganglia and thalami had appearances which were similar to those described with acute severe hypoxic-ischaemic injury at term infants (Bano et al., 2017, Connolly et al., 1994, Salas et al., 2018, Tann et al., 2016). Eight infants had bilateral basal ganglia and/or thalamic changes and one infant had echolucent posterior limb of the internal capsule bilaterally. 10 of these infants had a normal documented Apgar score of 8-10, at 5 minutes of age. The caregivers of the two infants without a documented Apgar score, reported the need only for stimulation and suction. It is therefore likely that these findings, although typically associated with neonatal hypoxic-ischaemic injury and encephalopathy, were actually related to infection and perhaps associated hyperaemia. Group B

streptococcal meningitis can give imaging findings that overlap with hypoxic-ischaemic injury and future genomic analysis of the CSF of these patients for this fastidious bacteria and other potential pathogens will be imperative to improve our understanding (Hernandez et al., 2011).

The leading causes of increased extracerebral space are brain atrophy and subdural fluid collection secondary to intracranial infections or haemorrhage. Although in older infants, an increased extracerebral space can also be due to benign enlargement of the subarachnoid space (also known as benign external hydrocephalus), benign familial megalencephaly, or achondroplasia, these are not expected to be observed in the neonatal period (Khosroshahi and Nikkhah, 2018). Only one control infant had an increased extracerebral space with increased echogenicity. Increased extracerebral space echogenicity was present in 7.7% of pSBI infants and the frequency of an enlarged extracerebral space was significantly higher in infants with pSBI (12.8%, p=0.08). Changes in the extracerebral space were not associated with signs of encephalopathy, meningism or abnormal CSF analysis, despite their association with pSBI.

Those neonates that presented with features of encephalopathy or meningism were significantly more likely to have abnormalities on their cUS examination, including ventricular pathology and WM, cortical, basal ganglia and/or thalamic echogenicity. As found in previous studies of neonatal meningitis, significant imaging abnormalities were also observed in the absence of specific clinical signs (de Vries et al., 2006, Mahajan et al., 1995, Rosenberg et al., 1983, Soni et al., 1994)

Overall, it appears from this study that there are two distinct early imaging patterns of CNS infection, firstly that limited to the ventricles causing dilatation, inflamed ependyma, ventricular debris and strands and secondly that involving the WM and cortex. These two patterns of CNS involvement were relatively distinct with only 2/9 infants with severely abnormal ventricles (score 2 or more) having additional WM and/or cortical changes at presentation. The lack of association between ventricular findings and abnormal CSF analyses suggests that in these infants the infection is limited to the ventricles and, due to the obstruction of CSF flow, CSF sampling from the lumbar spine does not represent the CSF in the ventricles and therefore can be falsely reassuring. Even infants with very specific and multiple findings in the ventricles suggestive of ventriculitis had normal lumbar CSF. In fact, 48/54 (88.9%) infants with one or more abnormal findings on ventricular examination had normal CSF analyses. When present, meningeal inflammation can extend to the brain parenchyma. Infants in this study with cortical and WM changes consistent with CNS infection, were significantly more likely to have abnormal lumbar CSF analyses. It should also be noted that despite this association between abnormal CSF and WM and cortical echogenicities, many neonates with such cUS abnormalities still had a normal lumbar CSF analysis.

Considering the above findings, a cUS examination should always be considered in pSBI and should always be performed for those infants presenting with seizures, encephalopathy or meningism as these were most strongly associated with cUS abnormalities. The relevance of these initial cUS

findings also need to be further explored. Firstly, a crucial and strategic step will be to undertake PCR on the blood and CSF samples from these infants with pSBI to confirm the presence/absence of viral, fungal or fastidious bacterial pathogens. Secondly, the correlation between the imaging findings and longer-term outcomes, such as neurodevelopmental impairment and post-infectious hydrocephalus, must be established.

Timing of presentation, early v. late

The majority of infants in this study presented within 48 hours of birth; this is likely because this was a hospital-based study and therefore many neonates were identified on labour ward and referred directly to the neonatal unit. Those infants presenting later were more likely to have abnormal findings on cUS including abnormal ventricles and cortical and WM echogenicity. Abnormalities on cUS often take time to appear and may not be present early on in the infective process. The potentially longer exposure of the brain to inflammation and infection in the cases presenting after 48 hours, may explain this finding. It is also possible that these neonates may have a different aetiology to those presenting earlier. Further genomic analysis of the CSF samples from these infants will be vital to help identify any aetiological differences.

Mortality

There were no significant differences in the cUS findings at presentation in those neonates who survived compared to those who died. As described above, it is possible that even if there was CNS involvement, the findings on cUS were not yet established. Six of the deaths (35.0%) were from neonatal

tetanus and almost one-third (29.4%) of the deaths were in infants that presented in septic shock, defined by a temperature >38.0°C and heart rate >180bpm. It is likely that these infants died from overwhelming sepsis and systemic complications rather than brain injury.

Future

The long-term significance of the cUS findings in neonates at presentation with pSBI are important to understand. The analysis of sequential scans on day 3, 7 and 28 of illness is discussed in Chapter 7. Developmental follow-up of this cohort of neonates was undertaken until 12 months of age and the relationship between the cUS findings and developmental impairment is discussed in Chapter 9. Lastly, there is on-going genomic analysis of the CSF of these neonates to establish the presence of fungal, parasitic, viral and bacterial pathogens present in the CSF. These pathogens will then be compared directly to the cUS findings.

CONCLUSION

An unexpectedly high proportion of neonates presenting with pSBI had abnormalities on their cUS at presentation, findings that were not seen in the control cohort. These abnormalities were diverse and affected all anatomical areas of the brain. The findings included ventriculomegaly, ventricular strands and ventricular debris, as well as WM, cortical, basal ganglia and thalamic echogenicity, lenticulostriate vasculopathy, cysts and increased extracerebral space. Surprisingly, it was not possible to predict which neonates would have pathology on cUS from the CSF analysis and clinical signs alone. Even in the

absence of seizures or abnormal neurology, imaging abnormalities on cUS were present. Similarly, in infants with negative CSF culture and normal CSF parameters imaging abnormalities were present. The low-sensitivity of CSF culture and analysis for the diagnosis of CNS involvement in neonates with pSBI, highlights clearly the need for improved tests to detect CNS infections in neonates. Given the frequency of cUS abnormalities observed in this study, it is likely that the incidence of CNS infection is much higher than previously thought. It is also possible that these imaging findings are related to a high incidence of co-existing congenital infections such as cCMV. Given that no pathogens were detected by CSF culture, these cUS findings need to be further correlated with genomic analysis of the CSF to evaluate their correlation with bacterial, parasitic and viral pathogens. In Chapter 7, the cUS examinations performed at presentation in cases of pSBI will be compared to the progressive changes on serial cUS examinations, which may detect additional pathology. In Chapter 9, the significance of these cUS findings in the early childhood outcomes of these infants will be explored.

CHAPTER 7 - PROGRESSIVE CHANGES ON CRANIAL ULTRASOUND AMONG NEONATES ADMITTED WITH POSSIBLE SEVERE BACTERIAL INFECTION (PSBI) IN UGANDA

BACKGROUND

Cranial ultrasound (cUS) is a relatively cheap, safe and portable method of assessing the neonatal CNS. As described in Chapter 6, it is possible that cUS can be used to detect CNS involvement in neonates presenting with pSBI. In contrast to haemorrhage, which is generally seen early on, many imaging changes, particularly those related to ischaemic injury, are known to take time to be seen. It is therefore important to assess if a cUS on the day of presentation is sufficient to detect all pSBI cases with CNS involvement. The use of cUS in neonates suffering from hypoxic-ischaemic encephalopathy (HIE) and prematurity is well established. Although early images can help establish the timing of the injuries, repeating the cUS examinations over time allows the full evolution of the disease process to be appreciated, since abnormalities may become more prominent over subsequent days (van Wezel-Meijler et al., 2010).

There are a growing number of studies demonstrating the relationship between white matter injury and sepsis in preterm infants (Heo et al., 2018, Shah et al., 2008, Graham et al., 2004, Glass et al., 2008, Vermeulen et al., 2001). Although there are no studies reporting brain imaging findings in term infants with sepsis, a study of preterm and term infants with confirmed CNS infections

found a correlation between increased echogenicity and ventricular dilatation and mortality (de Vries et al., 2006). To our knowledge, this study is the first study to systematically undertake serial cUS scans in term infants presenting with pSBI. As described in Chapter 6, we demonstrated a variety of abnormalities on cUS performed at presentation in term neonates with pSBI. In this chapter we consider the evolution of these cUS findings over the subsequent 28 days.

METHODS

STUDY DESIGN

As described in detail in Chapter 5, this was an observational study of term (>2000g) neonates presenting with pSBI to MRRH NNU in eastern Uganda over a 1-year period (See Chapter 4 for more detailed information on recruitment and sampling). The diagnosis of pSBI was defined as one of the three following combinations: 1) Axillary temperature >37.5°C, lethargy and poor feeding: 2) Axillary temperature <35.5°C, lethargy and poor feeding: 3) Full fontanelle and/or seizures, axillary temperature >37.5°C and poor feeding.

All infants underwent a cUS within 24 hours of admission to the NNU. Whenever possible, we repeated a cUS 3, 7 and 28 days after presentation. We examined the cUS images of all infants who underwent all four scheduled cUS examinations. This was not possible when patients were discharged against medical advice or if they failed to attend their scanning appointment, as we were unable to perform cUS scans in the community.

All cUS examinations were performed with a portable ultrasound machine (Sonosite M-Turbo®). A C11x curved probe (Frequency 8-5 MHz) and a linear probe (Frequency 13-6 MHz) were used in all infants. The probe was cleaned with alcohol between infants. The examinations were performed by KB, KN, EE, RM and JI. The cUS protocol is described in detail in Chapter 1. For each cUS examination the entire brain was scanned via the anterior fontanelle, then a minimum of 10 images were taken, including 5 coronal images, one midline sagittal, two left and two right parasagittal views as described in Chapter 1. The Doppler resistive index from blood flow in the anterior cerebral artery was also recorded. The linear probe was then used via the anterior fontanelle to image the cortex in the coronal and sagittal planes in addition to taking colour Doppler images from the sagittal superior sinus (SSS) as described in Chapter 1.

All clinicians performing the scans were trained in brain anatomy and cUS by a consultant neonatologist (CH). A one-day theoretical training in cUS was provided by CH for all study doctors (KB, RM, KN, EE, JI). One to one bedside teaching of approximately 9 hours was provided by CH to each study doctor. The study protocols for cUS scanning were available on the ward for reference. Ongoing feedback on the cUS quality and advice on improvements was given by the two consultant neonatologists to the clinicians performing the scans.

The cUS scans were interpreted at the time of scanning by the physician or principal investigator to help with directing clinical management. The cUS data were also downloaded and stored digitally as DICOM images. As described in

Chapter 6, each scan was assessed systematically. The images were analysed by FC and CH, blinded to the neonate's clinical data and outcome, using OsiriX 10.0.5 software.

Data were analysed using IBM SPSS Statistics Version 25. The prevalence of abnormalities on the cUS examinations performed on day 1 and day 28 were examined using Chi-squared test and Fisher's exact test as appropriate for sample size.

ETHICS

The Institutional Review Board of Mbarara University of Science and Technology, Uganda, the Uganda Council for Science and Technology and Penn State University approved the study.

RESULTS

There were 85 infants with pSBI who underwent a cUS examination on day 1, 3, 7 and 28 of presentation. The frequencies of the findings on the serial cUS scans are described below.

Ventricular findings

The ventricular findings were seen to vary over time as shown in Table 26 with almost all findings increasing over time. The presence of ventricular dilatation increased significantly from 2.4% on day 1 to 11.8% on day 28 (p=0.03). Of the ten infants with ventricular dilatation at day 28; five of them had only mild

ventriculomegaly which was not present at presentation; the other five infants had severely abnormal white matter and/or cortex at presentation (cases 1, 2, 3, 4 and 6 below), which would have generated a repeat scan and prompted closer follow-up.

Although, the three infants that developed post-infectious hydrocephalus requiring endoscopic third ventriculostomy (ETV) all had severely abnormal white matter and/or cortex at presentation (cases 1-3 below), only one of these three infants had ventricular dilatation present on the day 1 scan (case 3).

At presentation only 3.5% of infants had abnormally shaped ventricles but by day 28, this had increased steadily to 8.2%. Only one infant had ventricular debris, which was present from day one (case 3). This patient (case 3) also had strands, ventricular dilatation and bright ventricular margins and later went on to develop post-infectious hydrocephalus as described above.

Although prominent choroid in the roof of the 3rd ventricle was a relatively common abnormal finding at presentation (34.1%), the prevalence did not vary over time, being present in 31.8% at day 28.

Ventricular findings n=85	Day 1	Day 3	Day 7	Day 28	P value*
Ventricular dilatation	2 (2.4)	5 (5.9)	9 (10.6)	10[#] (11.8)	0.03
Abnormal shape	3 (3.5)	4 (4.7)	5 (5.9)	7 (8.2)	0.33
Ventricular debris	1 (1.2)	1(1.2)	1 (1.2)	1 (1.2)	
Ventricular strands	1 (1.2)	3 (3.5)	3 (3.5)	4 (4.7)	0.36
Bright ventricular margin	7 (8.2)	7 (8.2)	8 (9.4)	10 (11.8)	0.61
Prominent choroid in roof of 3rd ventricle	29 (34.1)	30 (35.3)	27 (31.8)	27 (31.8)	0.87

*Comparing frequency of abnormality on day 1 and day 28. [#]Three cases required endoscopic third ventriculostomy.

Table 26: Ventricular findings on serial scan performed on day 1, 3, 7 and 28 of presentation.

Cortical findings

The cortical findings showed little variation over time. For all 5 patients with abnormal cortical echogenicity at 28 days, these abnormalities had been present on the cUS at presentation. There was no evidence of cortical, basal ganglia or thalamic haemorrhage.

White matter findings

Regarding the white matter, bilateral echoes confined to the trigone were common, occurring in 50-60% of scans and considered normal, but about a third of infants also had more widespread echoes in the parietal, occipital or frontal white matter that tended to lessen by the 28 day scan without any cystic change or other abnormal change; only one infant´s white matter progressed to diffuse echogenicity (case 6) (Table 27). Of the three infants with diffuse white matter echogenicity at presentation, one improved to mild fronto-parietal echogenicity. There were 3 infants with globally abnormal white matter at presentation, two went on to develop post-infectious hydrocephalus (case 1

and case 3). There was one infant with multi cystic encephalomalacia at presentation, which persisted until the day 28 scan and this infant developed post-infectious hydrocephalus (case 2).

The number of patients with white matter haemorrhage increased from 2.4% to 4.7% between day 1 and day 28. The two infants with late white matter haemorrhage had normal white matter on their previous three scans.

Findings	Day 1	Day 3	Day 7	Day 28	P value*
Cortical findings					
Normal	78 (91.8)	77 (90.6)	78 (91.8)	80 (94.1)	0.7660
More than 50% highlighting	4 (4.7)	4 (4.7)	2 (2.4)	1 (1.2)	
Increased echogenicity	2 (2.4)	3 (3.5)	4 (4.7)	3 (3.5)	
Subcortical cystic change	1 (1.2) †	1 (1.2)	1 (1.2)	1 (1.2)	
White matter haemorrhage	2 (2.4)	2 (2.4)	3 (3.5)	4 (4.7)	0.6819
White matter findings					
Mild trigonal flare (normal)	47 (55.3)	42 (49.4)	49 (57.7)	58 (68.2)	0.1142
Mild parietal/occipital/frontal echogenicity	31 (36.5)	36 (42.3)	29 (34.1)	20 (23.5)	
Diffuse, patchy or local echogenicity	3 (3.5)	3 (3.5)	3 (3.5)	4 (4.7)	
Globally abnormal	3 (3.5)	3 (3.5)	3 (3.5)	2 (2.4)	
Multicystic encephalomalacia[#]	1 (1.2) ‡	1 (1.2)	1 (1.2)	1 (1.2)	

*Comparing frequency of normal findings on day 1 and day 28 †Abnormal cortical findings includes cases 1-6 and case 8. ‡ Abnormal white matter findings includes cases 1- 6. # Multiple cysts of varying size and shape located bilaterally in the white matter and inner cortex

Table 27: Serial cortical and white matter findings on cranial ultrasound

Of the seven infants with moderately to severely abnormal white matter at 28 days after presentation, three also had abnormal cortical findings and three developed post-infectious hydrocephalus.

The basal ganglia and thalamic findings over time are shown in Table 28. No significant change was seen in the prevalence of abnormalities in the basal ganglia, thalami and the region of the posterior limb of the internal capsule over the serial scans.

Findings	Day 1	Day 3	Day 7	Day 28	P value*
Basal ganglia					
Unilateral abnormality	5 (5.9)	6 (7.1)	7 (8.2)	5 (5.9)	1.000
Bilaterally abnormal	4 (4.7)	4 (4.7)	4 (4.7)	4 (4.7)	
Basal ganglia					
Swollen/focal abnormality	5 (5.9)	6 (7.1)	6(7.1)	5 (5.9)	1.000
Severely abnormal	5 (5.9)	5 (5.9)	6 (7.1)	5 (5.9)	
Thalamus					
Unilateral abnormality	9 (10.6)	12 (14.1)	11 (12.9)	10 (11.8)	1.000
Bilateral abnormal	7 (8.2)	5 (5.9)	9 (10.6)	6 (7.1)	
Thalami					
Swollen/focal abnormality	12 (14.1)	14 (16.5)	18 (21.2)	14 (16.5)	1.000
Severely abnormal	4 (4.7)	3 (3.5)	2 (2.4)	2 (2.4)	
Posterior limb internal capsule					
Unilateral dark	0 (0)	1 (1.2)	1 (1.2)	0 (0)	1.000
Bilateral dark	1 (1.2)	0 (0)	1 (1.2)	1 (1.2)	

*comparing frequency of normal findings on day 1 and day 28

Table 28: Serial basal ganglia and thalamic findings on cranial ultrasound

The presence of subependymal, caudothalamic notch and choroid plexus cysts were recorded. Table 29 shows the prevalence of these cysts in the serial scans. The prevalence of both caudothalamic notch and choroid plexus cysts appear to increase over time. It should be noted that if these cysts are small they can easily be missed in the recorded images.

Findings	Day 1	Day 3	Day 7	Day 28	P value*
Subependymal cysts					
Single	0 (0)	1 (1.2)	1(1.2)	1(1.2)	0.6205
Multiple	1 (1.2)	1 (1.2)	2 (2.4)	2 (2.4)	
Caudothalamic cysts					
Single	8 (9.4)	9 (10.6)	13 (15.3)	12 (14.1)	0.6840
Multiple	5 (5.9)	4 (4.7)	4 (4.7)	4 (4.7)	
Choroid plexus cyst					
Single	8 (9.4)	14 (16.5)	15 (17.6)	15 (17.6)	0.1469
Multiple	2 (2.4)	3 (3.5)	4 (4.7)	3 (3.5)	

*Comparing frequency of normal findings on day 1 and day 28

Table 29: Location and frequency of cysts on serial ultrasounds

A non-significant increase in lenticulostriate vasculopathy was observed over time from 32.9% to 40.0% (Table 30). Only 4 infants had findings suggestive of superior sagittal sinus thrombosis, and these were only seen on their later

scans; none were apparent at presentation. One of these infants (case 3) had a severely abnormal scan at presentation and developed post-infectious hydrocephalus. The other three infants had otherwise normal or mildly abnormal cUS scans throughout (Table 30).

The prevalence of an enlarged extracerebral space and increased echogenicity of the extracerebral space increased with serial scans. By day 28, 48.2% of infants had an enlarged extracerebral space (sinocortical width >3mm) and 24.7% of infants were noted to have increased echogenicity (Table 30). The resistive index increased significantly between day 1 and day 28 ($p<0.0001$), and by day 28 was similar to that observed in the control infants.

Findings	Day 1	Day 3	Day 7	Day 28	* p value
Calcification	4 (4.7)	3 (3.5)	2 (2.4)	2 (2.4)	0.6819
Lenticulostriate vasculopathy	28 (32.9)	33 (38.8)	35 (41.2)	34 (40.0)	0.4258
Evidence suggestive of thrombosis	0 (0)	2 (2.4)	2 (2.4)	4 (4.7)	0.1206
Enlarged extracerebral space	10 (11.8)	15 (17.6)	22 (25.9)	41 (48.2)	0.0001
Increased echogenicity of extracerebral space	11 (12.9)	7 (8.2)	12 (14.1)	21 (24.7)	0.0763
Resistive Index (RI) of anterior cerebral artery, Mean (SD)	0.65 (0.10)	0.68 (0.09)	0.72 (0.09)	0.75 (0.10)	<0.0001

*Comparing frequency of abnormality on day 1 and day 28

Table 30: Calcification, lenticulostriate vasculopathy, thrombosis and extracerebral space findings on serial cranial ultrasound scans in cases of pSBI

In the control cohort, a second scan was repeated between 1 to 4 weeks of age and a third scan at 5 to 8 weeks of age. As shown in Table 31, no evidence of calcification or suggestion of thrombosis were seen in any of the cranial ultrasound examinations. The increased frequency of both enlarged extracerebral space and increased echogenicity of the extracerebral space

seen in the cases, was not observed in the control cohort. No change in the RI was observed over time in the control infants.

Findings	Scan 1, n=44	Scan 2, n=35	Scan 3, n=23
Calcification	0 (0)	0 (0)	0 (0)
Lenticulostriate vasculopathy	6 (13.6)	10 (28.6)	6 (26.1)
Evidence suggestive of thrombosis	0 (0)	0 (0)	0 (0)
Enlarged extracerebral space	1 (2.3)	0 (0)	2 (8.7)
Increased echogenicity of extracerebral space	1 (2.3)	0 (0)	0 (0)
Resistive Index (RI) of anterior cerebral artery, Mean (SD)	0.73 (0.07)	0.73 (0.07)	0.76 (0.06)

Table 31: Calcification, lenticulostriate vasculopathy, thrombosis and extracerebral space findings on serial cranial ultrasound scans in control infants

CASE SERIES

CASES DEVELOPING POST-INFECTIOUS HYDROCEPHALUS

Three infants (case 1-3) in our cohort went on to develop post-infectious hydrocephalus. One infant developed post-infectious hydrocephalus alone, whilst two infants developed post-infectious hydrocephalus together with encephalomalacia. All three of these infants presented with late-onset sepsis, presenting at days 7, 9 and 17 of age respectively. All three cases had a severely abnormal scans on the day of presentation that became progressively worse over time.

Case 1

The first patient was male and born at term, by SVD at home to an HIV negative mother. The delivery was reportedly uneventful, and he cried immediately. He presented to MRRH-NNU on day 7, weighing 2860g with seizures, fever and hypertonia, pulse rate of 131 beats per minute (bpm), temperature of 37.5°C and respiratory rate of 47 breaths per minute. The CSF culture was negative and the CSF white cell count was zero, but the CSF protein level was elevated (230 mg/dl) and the glucose level was low (10.1mg/dl). The blood culture and malaria smear were negative. He was commenced on ceftriaxone and gentamicin and anticonvulsant therapy.

The cUS at presentation showed normal ventricles (Figure 55). There was cortical echogenicity, severe white matter echogenicity bilaterally together with severely abnormal basal ganglia and thalami. There were no cysts, no lenticulostriate vasculopathy, a normal extracerebral space and a normal

resistive index (RI 0.78). The cUS scans on day 3 and day 7 after admission demonstrated increasing white matter echogenicity and early cystic change in the white matter but no ventriculomegaly.

This infant´s mother self-discharged with her son after he had received only 8 days of ceftriaxone and gentamicin. The patient did not attend clinic for the scheduled 28-day cUS but was later found in the community and assessed at 2 months of age when he had severe motor impairment (unable to control head) and mild language impairment (no vocalisation and no response to sound) and cognitive impairment (no reaction to objects or faces). A cUS at this time showed post-infectious hydrocephalus and he was referred for neurosurgical evaluation. Unfortunately, he was subsequently lost to follow-up and did not undergo neurosurgical intervention.

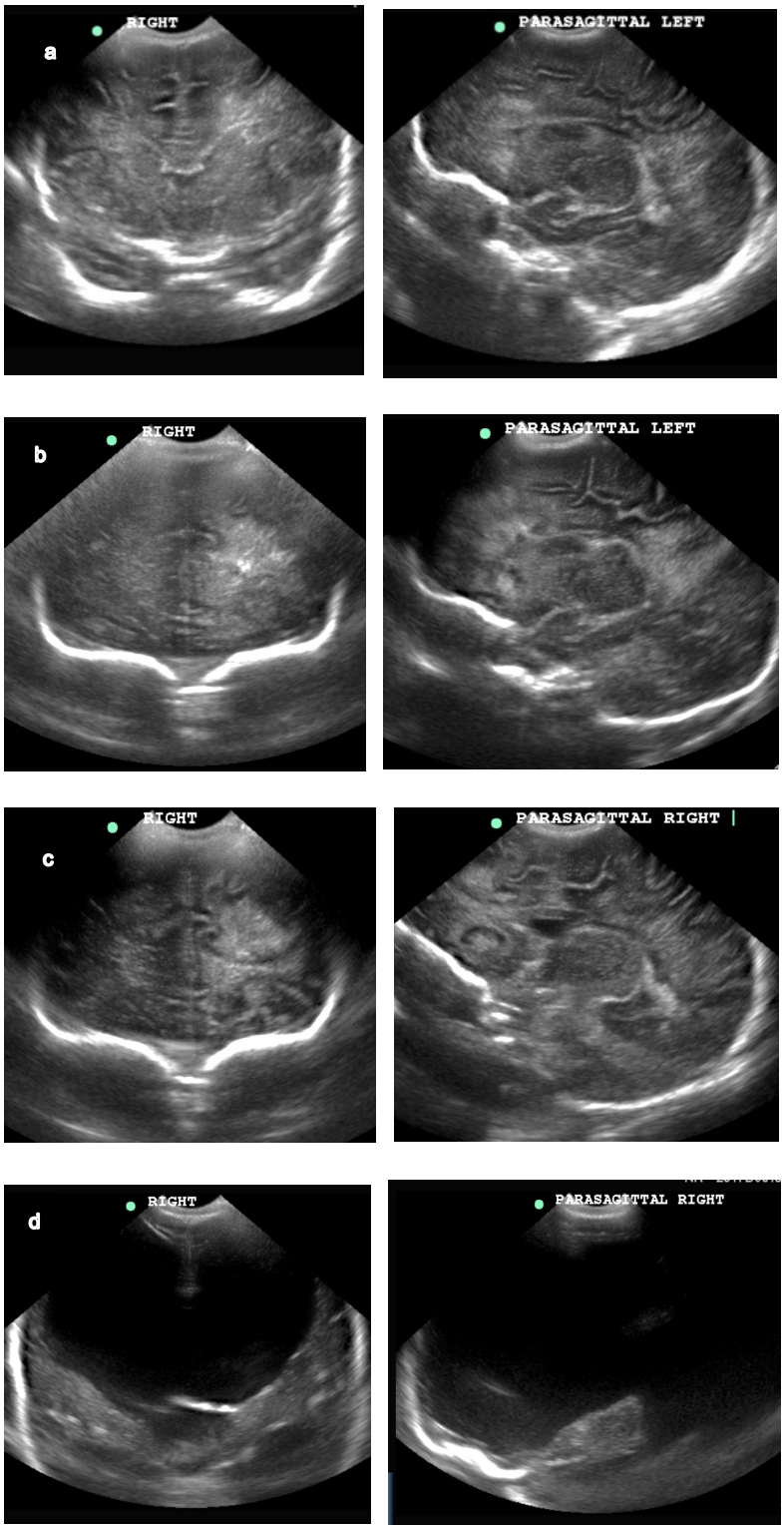


Figure 55: Sequential cUS scans of case 1, showing coronal views (left) and parasagittal views (right): a) scan at presentation on day 7, showing severe white matter echogenicity in the frontal and parietal lobes: b) scan 3 days after presentation showing increasing white matter echogenicity: c) scan 7 days after presentation showing further increase in white matter echogenicity with early cystic change: d) scan at 2 months of age demonstrating hydrocephalus likely post-infectious.

Case 2

The second infant was also a male, delivered at term, by SVD at home, to an HIV negative mother. Again, the reports were that the delivery was uneventful, and he cried immediately. He presented at day 9 of age, weighing 2000g with seizures, fever and a bulging fontanelle. His pulse rate was 125 bpm, he had a temperature of 38.1°C and a respiratory rate of 36 breaths per minute. The CSF culture was negative, white cell count was zero, a protein level was not available, and the glucose level was normal (38.7mg/dl). The blood culture was negative and malaria smear negative. He was commenced on intravenous cefotaxime, gentamicin and anticonvulsant therapy.

The cUS at presentation showed abnormally shaped ventricles, but no dilatation, debris or strands (Figure 56). The cortex was cystic, there was severe white matter echogenicity bilaterally and the basal ganglia and thalami were abnormal bilaterally. There was no lenticulostriate vasculopathy, no cysts and the RI was normal (0.70). The extracerebral space was both enlarged and echogenic. By day 7, there was increasing white matter echogenicity with marked encephalomalacia with enlarging ventricles. Despite completing 14 days of intravenous cefotaxime and gentamicin, the white matter and cortical echogenicity increased, encephalomalacia followed and post-infectious hydrocephalus developed. They were referred for neurosurgical input at discharge, where they underwent an Endoscopic Third Ventriculostomy (ETV). At 12 months of age he had severe language, motor and cognitive developmental impairment.

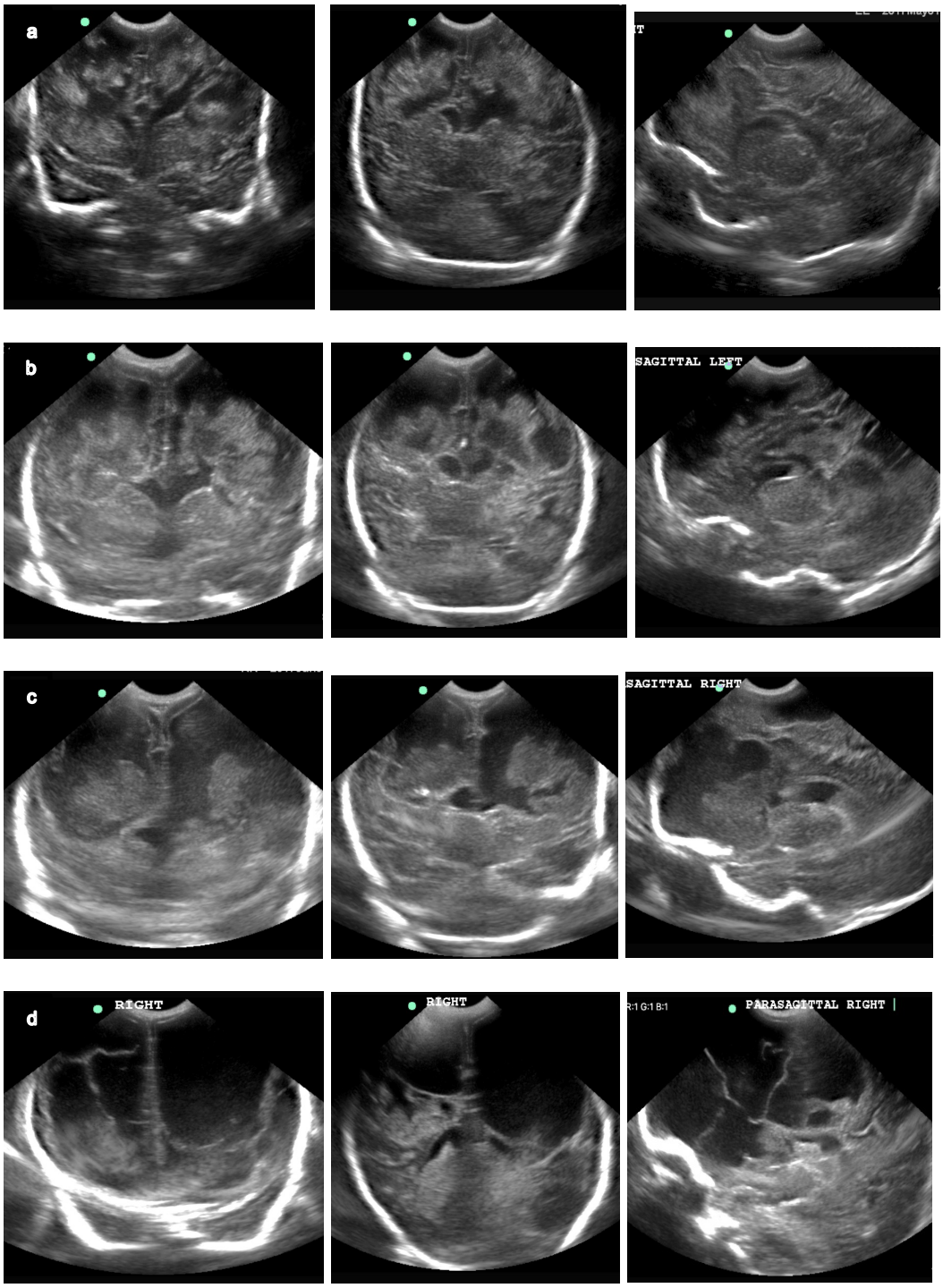


Figure 56: Sequential cUS scans of case 2, showing coronal views (left and middle) and parasagittal views (right): a) scan at presentation on day 9, showing severe white matter echogenicity in the frontal and parietal lobes with cystic change and early encephalomalacia: b) scan 3 days after presentation showing increasing white matter echogenicity and progressive encephalomalacia: c) scan 7 days after presentation showing further increase in the white matter echogenicity with marked encephalomalacia of the frontal and parietal lobes and enlarging

ventricles: d) scan at 28 days of age demonstrating post infectious hydrocephalus with debris and strands clearly visible.

Case 3

The third infant was again male but had been delivered by SVD in hospital by a midwife. He was born with meconium stained liquor, but only required stimulation at birth and was given an Apgar score of 8 at 5 minutes. He presented on day 17, weighing 2880g with seizures, fever and a bulging fontanelle. He had a pulse rate of 135 bpm, temperature of 38.2°C and respiratory rate of 45 breaths per minutes. He was thought to be too sick to tolerate a lumbar puncture and therefore CSF results were not available. The blood culture and malaria smear were negative. He was commenced on intravenous ceftriaxone, gentamicin and anticonvulsant therapy.

The cUS performed at presentation was grossly abnormal (Figure 57). Both lateral ventricles were dilated with strands and debris on the initial scan. There was cortical echogenicity, severe white matter echogenicity bilaterally and abnormal basal ganglia and thalami. There was no lenticulostriate vasculopathy or cysts. The extracerebral space was again enlarged and echogenic. This infant completed 16 days of cefotaxime and gentamicin and were referred for neurosurgical input at discharge and underwent ETV. At 12 months of age he had severe cognitive, language and motor impairment.

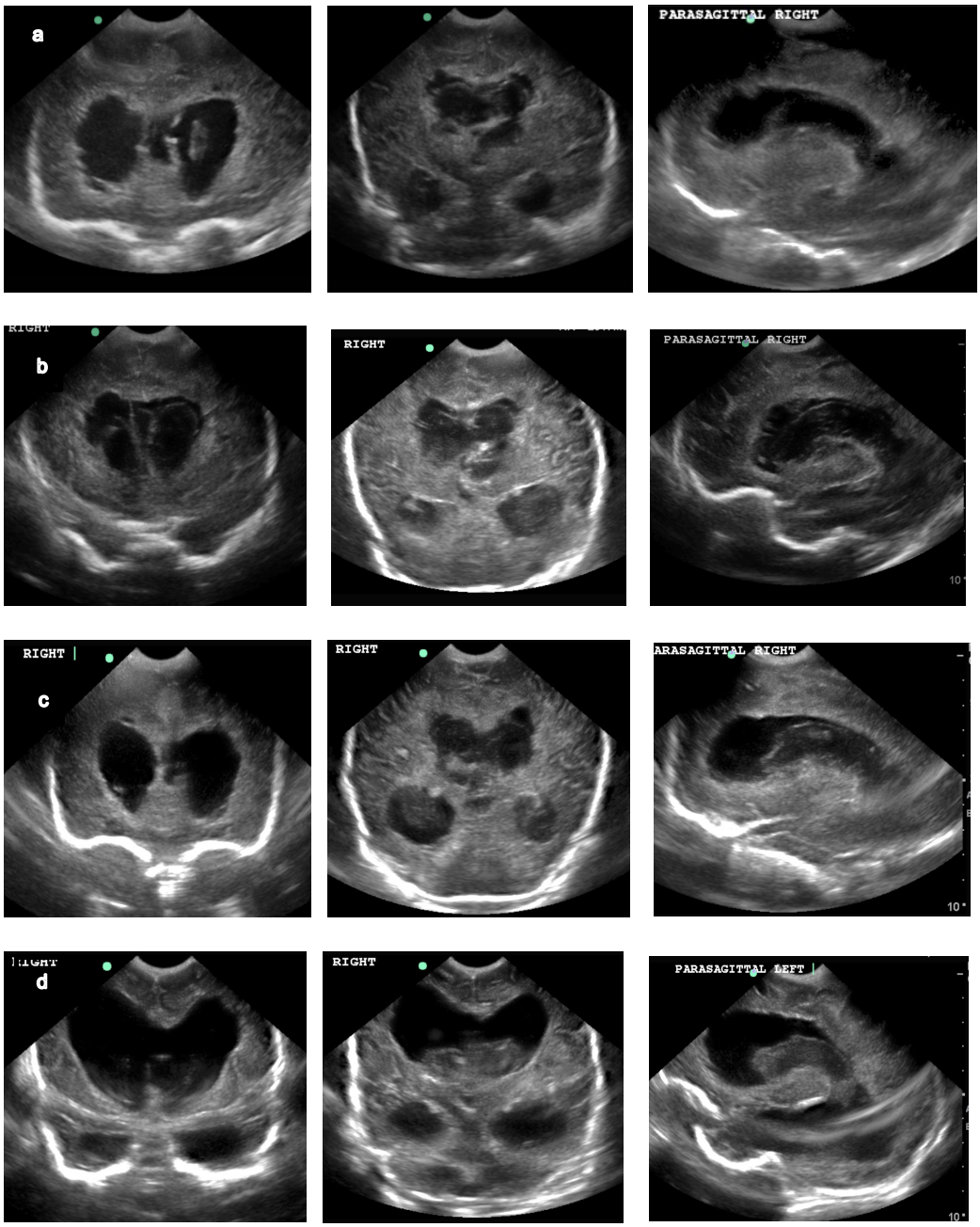


Figure 57: Sequential cUS scans of case 3, showing coronal views on (left and middle) and parasagittal views on the right: a) scan at presentation on day 17, showing severe and widespread white matter hyper echogenicity, cortical hypo echogenicity with dilated and abnormally shaped ventricles with debris and strands: b) scan 3 days after presentation showing increasing white echogenicity and dilated and abnormally shaped ventricles with debris and strands: c) scan 7 days after presentation: d) scan at 28 days of age demonstrating hydrocephalus with debris and strands

CASES WITH BOTH WHITE MATTER AND CORTICAL ABNORMALITIES AT PRESENTATION

There were an additional 3 cases (case 4-6) that had moderate to severely abnormal white matter and cortex at presentation, that did not develop post-infectious hydrocephalus. All 3 of these cases had a poor outcome by 12 months of age, with ongoing seizure disorder and severe developmental impairment.

Case 4

The fourth infant was male and presented on day 3 with a history of fever, poor feeding and seizures. He had been delivered by SVD in a health centre by a midwife and although meconium was present at delivery, the baby only required stimulation at birth. On admission, his weight was 2930g, the temperature was 38.7°C and oxygen saturations were 85% on air. The respiratory rate was 39 breaths per minute and heart rate was elevated at 190bpm. The blood culture and CSF culture were negative, and the CSF analysis was normal (protein 10mg/dL, no white blood cells and glucose 47mg/dL).

At presentation there was cortical highlighting and moderate white matter echogenicity bilaterally on cUS (Figure 58). The infant received 7 days of ampicillin and gentamicin. By day 28, there was severe atrophy, evidenced by the increased extracerebral space, a very abnormal appearance to the cortex with subcortical cystic change. By 12 months of age there was global severe developmental delay and he was taking carbamazepine for ongoing seizures.

There was an associated deceleration in head growth, with the OFC dropping from the 75th centile to the 0.4th centile.

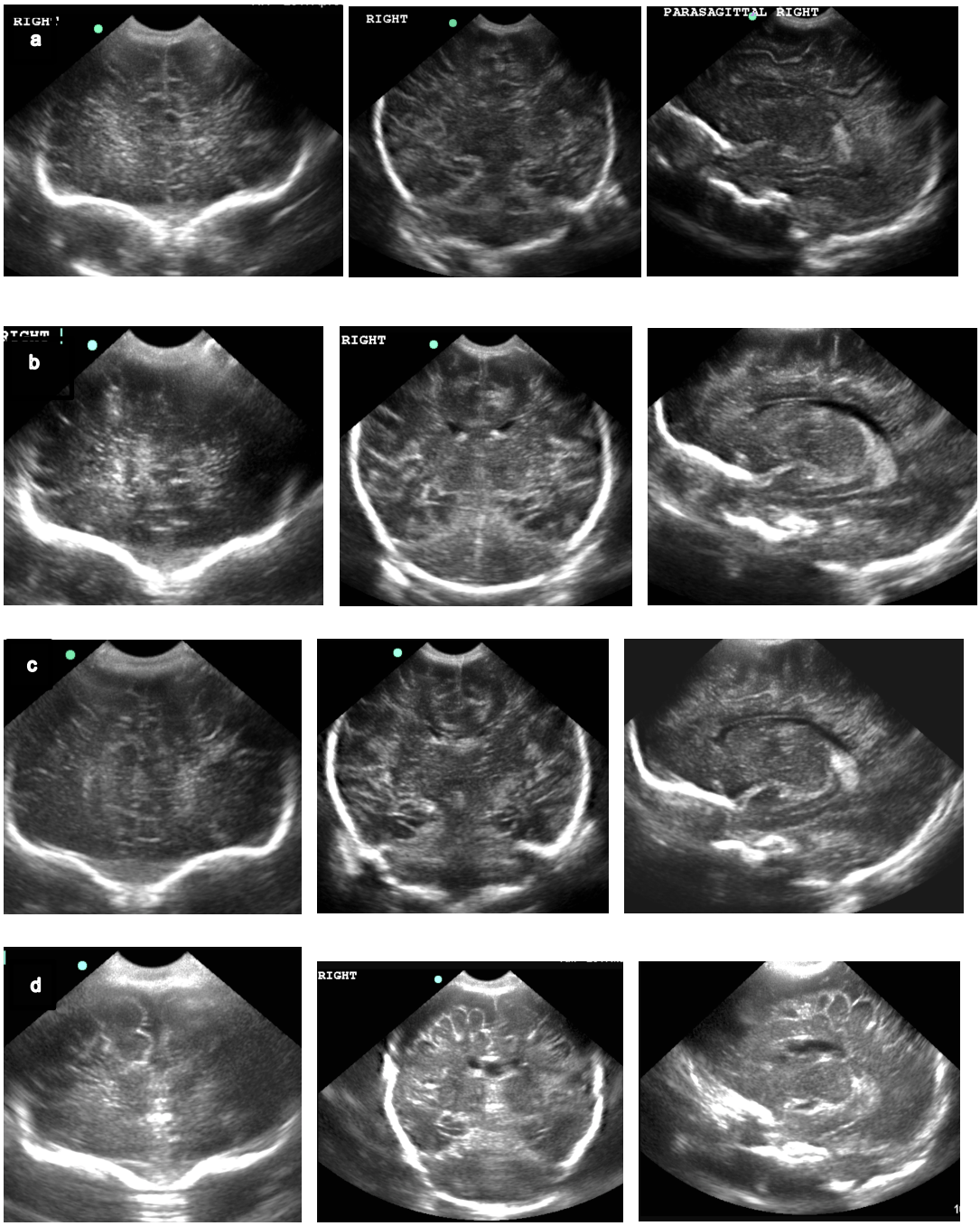


Figure 58: Sequential cUS scans of case 4, showing coronal views (left and middle) and parasagittal views (right): a) scan at presentation on day 3, showing severe and widespread white matter hyper echogenicity and cortical highlighting: b) scan 3 days after presentation showing increasing white echogenicity and some early cystic change in the frontal lobes: c) scan 7 days after presentation: d) scan at 28 days of age demonstrating abnormal cortex and increase in

extracerebral space. This was associated with a deceleration in head growth and therefore suggestive of atrophy

Case 5

Case 5 was a 5-day old male infant delivered by SVD in a hospital by a doctor. He cried immediately at birth and no resuscitation was required. There was a history of fever, poor feeding, difficulty in breathing and seizures. At presentation he weighed 3200g, temperature was 38.3°C, respiratory rate 28 breaths per minute and heart rate 162 bpm. On examination seizures were observed and he was irritable and had a bulging fontanelle. Both the blood culture and CSF culture were negative, and the CSF analysis was normal (protein level 110mg/dl, no white cells seen and glucose 48mg/dL).

His cUS examination at presentation showed cortical highlighting and severe white matter echogenicity, especially in the parietal and occipital areas (Figure 59). In addition, the thalamus and basal ganglia were severely swollen. He received intravenous ceftriaxone and gentamicin for 14 days. By 28 days, there was marked increase in extracerebral space around the occipital lobe with increasing white matter echogenicity in the parieto-occipital lobes. At 12 months of age, he was still experiencing seizures and had severe delay in motor and cognitive domains with moderate delay in language.

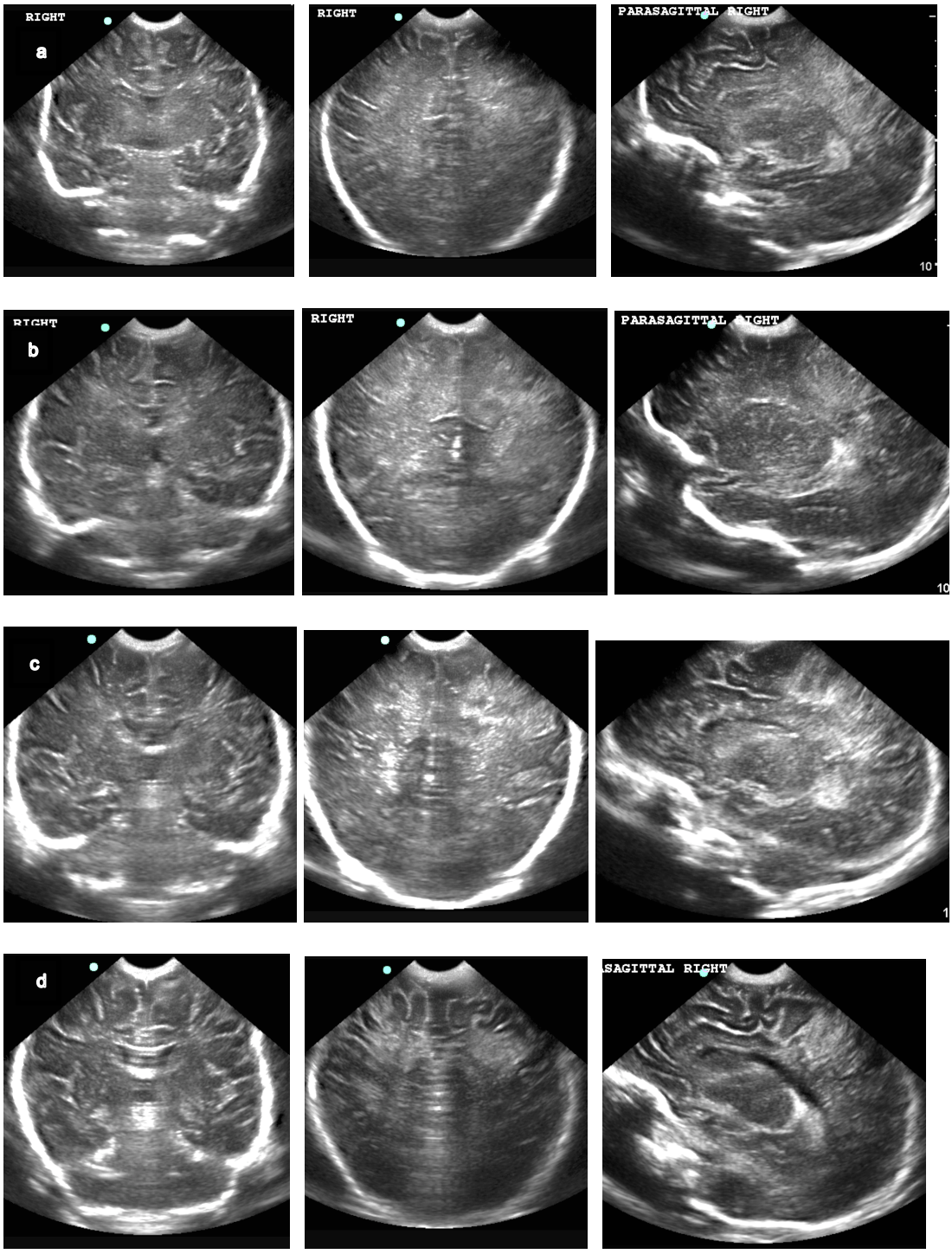


Figure 59: Sequential cUS scans of case 5, showing coronal views (left and middle) and parasagittal views on the right: a) scan at presentation showing severe white matter hyper echogenicity in the parietal and occipital areas and cortical highlighting: b) scan 3 days after presentation showing increasing white matter echogenicity in these areas: c) scan 7 days after presentation: d) scan at 28 days of age demonstrating marked increase in extracerebral space around the occipital lobe suggestive of atrophy

Case 6

The sixth case was a male infant that presented on day 25 with a history of fever, poor feeding, lethargy and seizures. He had been delivered at home to an HIV negative mother by a traditional birth attendant by SVD. At presentation he weighed 2020g, had a fever of 39.0C, an elevated heart rate of 189bpm and a respiratory rate of 58 breaths per minute. He had seizures, opisthotonus, bulging fontanelle and jaundice. His malaria smear, CSF culture and blood culture were negative. The CSF analysis was normal with no white cells, protein of 100mg/dL and a glucose level of 27.9mg/dL.

The cUS scan performed at presentation showed cortical highlighting, mild white matter echogenicity, lenticulostriate vasculopathy, mild ventriculomegaly and bright ventricular margin suggestive of ventriculitis (Figure 60). There was also an increased extracerebral space with increased echogenicity suggestive of a subdural empyema. He was treated with 14 days of intravenous cefotaxime and gentamicin. At 28 days the cUS demonstrated focal echogenic lesions in the cortex and the white matter, the ventriculomegaly and bright ventricular margins persisted and the lenticulostriate vasculopathy was still visible. At 12 months of age he had severe cognitive and motor impairment and moderate language impairment.

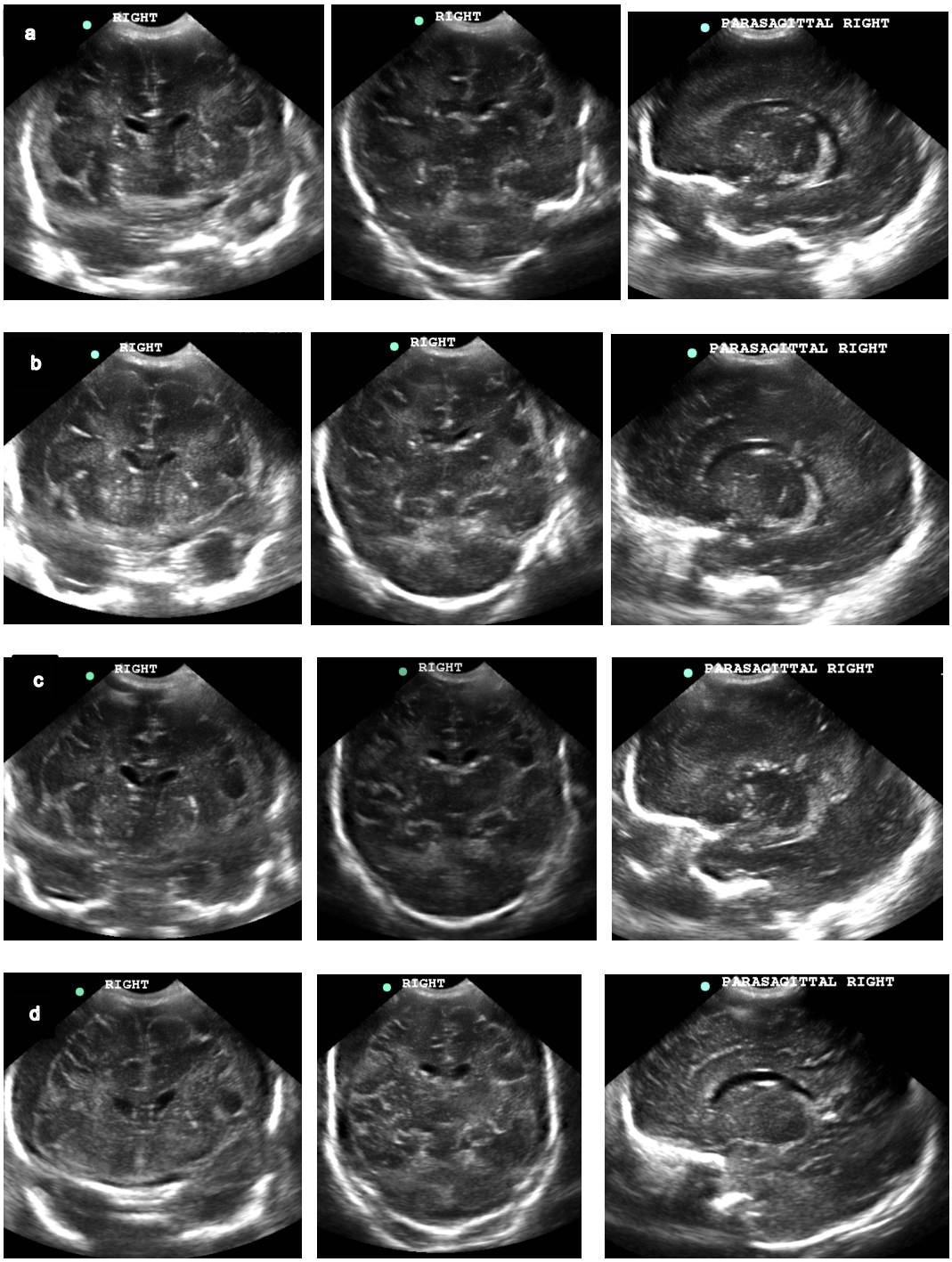


Figure 60: Sequential cUS scans of case 6, showing coronal views (left and middle) and parasagittal views (right): a) scan at presentation showing mild white matter echogenicity, increased and enlarged extracerebral space, bright ventricular lining and mild dilatation and lenticulostriate vasculopathy: b) scan 3 days after presentation showing increasing extracerebral space, with increased echogenicity particularly on the left: c) scan 7 days after presentation shows increased lenticulostriate vasculopathy: d) scan at 28 days of age demonstrating increasing focal white matter and cortical echogenicity.

CASES WITH ABNORMAL WHITE MATTER OR ABNORMAL CORTEX

There was one case, case 7, with only moderately abnormal white matter at presentation. This male infant was born at home, delivered by a relative by SVD and reportedly cried immediately. He presented at 15 days of age with lethargy, poor feeding and fever. He weighed 2280g, his temperature was 39.0C, his heart rate and respiratory rate were elevated at 192 bpm and 77 breaths per minute respectively. His CSF culture was negative with a normal CSF analysis (protein 120mg/dL, no cells and glucose 90mg/dL). His blood culture was also negative. He subsequently developed spasms and was diagnosed with neonatal tetanus. He was treated with ceftriaxone, gentamicin, metronidazole, diazepam and magnesium. By 12 months of age he was well with apparently normal development at this time.

There was one infant, case 8, who had isolated cortical highlighting present on all 4 cUS scans. No other abnormalities were noted on any of the images. He presented at 2 days of age with fever, lethargy and poor feeding. There were no seizures reported or witnessed. He was born in hospital to an HIV negative mother by caesarean section, where only stimulation was needed with a good response and his Apgar scores were 8 at 1 minute and 10 and 5 minutes. His malaria smear, blood culture and CSF culture were all negative, with a normal CSF analysis (protein 30mg/dL, no white cells and glucose 135mg/dL). The infant was followed up until 12 months of age when he was well with apparently normal development across all domains at this time.

DISCUSSION

In this study we observed 58.8% (50/85) of infants with pSBI had either a normal cUS or only mild abnormalities on all 4 of their cUS examinations. A further 11.8% (10/85) of infants had moderate changes on their cUS, which persisted throughout their 4 serial cUS examinations. There were 4 infants with severe abnormalities observed throughout all 4 serial cUS examinations (cases 1-3, case 6). In 23.5% (20/85) of infants the changes progressed over time; 2 infants who had only moderate imaging abnormalities at presentation had severe abnormalities on their cUS by 28 days (case 4 and 5), the other infants progressed from normal/mild abnormalities to moderate abnormalities on cUS examination at day 28. Reassuringly, all of the infants with severe abnormalities at 28 days had a moderately or severely abnormal scan at presentation.

SPECIFIC FINDINGS ON CRANIAL ULTRASOUND

The presence of ventricular dilation was observed to increase in the 28 days after presentation from 2.4% to 11.8% (p=0.03). Half of the infants (5/10) with ventriculomegaly also had severely abnormal white matter and/or cortex on their first scan at presentation. Three of these five infants went on to develop post-infectious hydrocephalus and all five of them had severe developmental impairment at 12 months of age. The other half of these infants (5/10) had mild ventriculomegaly with normal cortex and WM and were found to have normal development at 12 months of age. These 5 infants did not have any abnormality on cUS examination at presentation to trigger a repeat scan to be performed. It is possible that these mild changes are a risk factor for more

subtle developmental impairments, and it will be important to assess these children at an older age to ascertain the implication of this finding.

The cortex was considered abnormal if there was more than 50% highlighting, increased cortical echogenicity or cystic change. All 5 infants with an abnormal cortex at 28 days after presentation had had abnormal cortical findings at presentation. This suggests a cUS at presentation is sufficient to detect all those infants who will develop cortical abnormalities, however the cortical changes in these 5 infants progressed over time. The white matter was considered moderately to severely abnormal if there was diffuse, patchy or local echogenicity, if there was global white matter abnormality or if there was multicystic encephalomalacia. Seven infants had moderate to severe abnormal white matter at 28 days, and 6/7 of these had been identified at presentation. 6/7 of these infants had associated abnormal cortex at presentation and 3/7 developed post-infectious hydrocephalus. This suggests that any infant with abnormal white matter or cortical findings at presentation should undergo a minimum of one repeat cUS after 28 days to evaluate the extent of the brain injury. These infants should undergo close follow-up, with the potential for early intervention to improve outcome.

Those infants with abnormal basal ganglia and thalami were all detected at presentation. Only half of these infants had associated white matter and cortical abnormalities. Thalami can also appear echogenic secondary to WM change and this may be the case in these infants. In Chapter 9 those infants with abnormal basal ganglia or thalami at presentation are shown to have an

increased risk of a poor outcome. The presence of echogenic basal ganglia or thalami, even in isolation, should also prompt additional follow-up.

The presence of choroid plexus, subependymal and caudothalamic notch cysts did not change over time. As shown in Chapter 9, the presence of cysts was not associated with a poor outcome at 12 months of age. Although cysts can be associated with syndromes and infections, especially cytomegalovirus (CMV), the majority are likely to be developmental in origin (de Vries et al., 2004). It is important to remember that abnormalities, particularly minor findings, can also be seen in images of apparently healthy term neonates (Hagmann et al., 2010). A Ugandan study of well term neonates reported the presence of developmental cysts related to the ventricles to be common with subependymal cysts seen in 19.6%, choroid plexus cysts seen in 19.6% and both types seen in 4.5% (Hagmann et al., 2010).

The prevalence of lenticulostriate vasculopathy increased over the 28-day period. Although lenticulostriate vasculopathy is generally considered a benign finding, it is more common in a variety of disorders most notably congenital viral infections including congenital CMV, rubella, toxoplasmosis and HIV (Cantey and Sisman, 2015, Maayan-Metzger et al., 2016, Robinson et al., 2017, Sisman and Rosenfeld, 2015). Our observation may represent a high rate of congenital infections in this population, which needs further future investigation. It is also possible that this observation is secondary to a vasculopathy due to bacterial CNS infection. Examination with PCR of the CSF

samples from these infants, will be vital to investigate the aetiology of lenticulostriate vasculopathy further.

CASE SERIES

In this study, six infants had marked and severe changes observed on the brain imaging. This case series describes the progressive development of brain injury in these six infants presenting with pSBI in eastern Uganda during the neonatal period. We have demonstrated a relatively high incidence of devastating brain injury in these neonates. Six of the 85 infants studied (7.1%) developed hugely destructive white matter and cortical injury, 4 of them had moderate to severe basal ganglia and/or thalamic injury and 3 of them also developed hydrocephalus, which was likely post-infectious in origin. Post-infectious hydrocephalus was not observed in one infant until 2 months later, in another infant, post-infectious hydrocephalus had started on the day 7 scan but was not obvious until day 28 and in the third infant, post-infectious hydrocephalus was not seen at day 7 but was present at day 28. This supports the need for repeat scans in those infants who have moderate to severe abnormalities at presentation.

Five of the six infants in this case series had moderate to severe developmental impairment by 12-months of age, including 3 with globally severe impairment and seizures. The sixth infant was unfortunately lost to follow-up but already had post-infectious hydrocephalus and severe motor impairment at 2 months of age.

There was substantial overlap between the infants with abnormal cortical findings, moderate to severely abnormal white matter and post-infectious hydrocephalus. Of the 85 infants, 8 infants developed either post-infectious hydrocephalus and/or abnormal cortex and/or moderate-severely abnormal white matter within 28 days of presentation. Another 9 infants had moderate/severely abnormal basal ganglia and/or thalami by 28 days. One infant had a small parenchymal haemorrhage in the white matter, which was resolving by day 28. The three infants that developed post-infectious hydrocephalus as discussed in cases 1-3 above, all had abnormal cortex and white matter at presentation and three other infants had both abnormal white matter and cortex. All 6 of these infants had a poor outcome at 12 months.

The three cases that developed post-infectious hydrocephalus were all male, presented after 7 days of age and all presented with fever and seizures. Two of them also had a bulging fontanelle. Malaria smears and blood cultures were negative for all three infants and for the two infants who were well enough for LP, the CSF cultures were negative, although one had elevated protein level and a low glucose level. The three infants with abnormal cortex and white matter were also all male and presented between 3-28 days of age with fever and seizures. All three infants had negative malaria smears and negative blood and CSF cultures with normal CSF analyses.

It is highly likely, based on the clinical presentation including fever and seizures, that all six of these infants would have received a prolonged course of antibiotic therapy usual for meningitis. Without performing a cUS, we would

not have been aware of the extent of the damage to the brain. Performing cUS scans in these infants allowed the early detection of post-infectious hydrocephalus and early referral for neurosurgical intervention (Warf, 2005). Unfortunately, even the 2/3 cases of post-infectious hydrocephalus that completed a prolonged course of antibiotic therapy and underwent an ETV still developed severe developmental impairment by 12 months of age. This is likely in part due to the significant cortical and white matter injuries.

It is also very clear from these six cases, that if the cUS scan is abnormal at presentation, there is the potential for it still to progress significantly despite seemingly adequate antibiotic therapy. In the future it will be vital to understand the pathogen or pathogens responsible for this aggressive, invasive and devastating pathology so that alternative therapies can be explored. Closer investigation with PCR of the CSF samples from these neonates, which will be carried out on the stored samples collected in this study, will be vital to investigate this further. What is however reassuring from these analyses and this case series, is that for all those infants who went on to have a poor early childhood outcome by 12 months of age, including post-infectious hydrocephalus, seizures and moderate to severe developmental impairment, the cUS at presentation was significantly abnormal.

CONCLUSION

This is the first study assessing serial cUS findings in neonates with pSBI. We have demonstrated a relatively high incidence of progressive and devastating brain injury in infants presenting with pSBI in eastern Uganda during the

neonatal period. All the infants with moderately or severely abnormal brain imaging 28 days after presentation had had abnormal scans at presentation and all presented with fever and seizures, sometimes associated with opisthotonus and bulging fontanelle. The brain injuries of these infants progressed significantly despite seemingly adequate antibiotic therapy. These findings suggest that all infants presenting with clinical features of CNS infection in this setting should undergo a cUS at presentation to confirm the presence and extent of the brain injury. For those with moderate or severe abnormalities in the white matter or cortex, a minimum of one follow-up scan after 28 days should be undertaken to evaluate the progression of the injury. This repeat cUS scan will allow identification of infants with post-infectious hydrocephalus who may benefit from neurosurgical intervention and will also allow identification of infants who are at high risk of a poor outcome and would benefit from an early intervention programme. Lastly, it is with great urgency that the aetiology of these devastating and progressive brain injuries is investigated so that improved prevention and treatment strategies can be explored.

CHAPTER 8 - POST-NEONATAL MORTALITY, MORBIDITY AND DEVELOPMENTAL OUTCOME AFTER POSSIBLE SEVERE BACTERIAL INFECTION (PSBI) IN UGANDAN NEONATES: A COHORT STUDY WITH EXTERNAL CONTROLS.

BACKGROUND

In SSA there are believed to be up to 2.6 million cases of pSBI in neonates every year, leading to an estimated 250,000 deaths (Seale et al., 2014). Unfortunately, the burden of these serious bacterial infections is not limited to neonatal mortality alone. Even neonates who receive appropriate and effective treatment for their pSBI are at an increased risk of developmental impairment, post-infectious hydrocephalus, neurological disability and infant mortality, most especially those who were born preterm or experienced neonatal meningitis (Stevens et al., 2003, Seale et al., 2013). Data are still lacking on the incidence and level of these sequelae after sepsis and pneumonia, especially in term neonates.

Neonatal meningitis carries the highest risk of long-term morbidity. Even in HICs, where the mortality from neonatal meningitis has reduced, the complications of this devastating disease have persisted. In fact in HICs, complications such as convulsions, hydrocephalus and neurodevelopmental impairment have been reported in up to one-quarter of cases (Ranjeva et al., 2018, Holt et al., 2001, Furyk et al., 2011). Moderate to severe neurodevelopmental impairment was estimated to occur in 23% of survivors of neonatal meningitis in a recent meta-analysis of 451 cases (Seale et al., 2013). In addition, 12% of survivors were estimated to have mild impairment in a meta-analysis of 311 cases (Seale et al., 2013). Although the majority of studies

included in this meta-analysis were from HICs, the two Nigerian studies included reported moderate to severe impairment in 22% and 29% of neonatal meningitis survivors (Airede, 1993, Airede et al., 2008). An Ethiopian study of 55 cases of neonatal meningitis reported severe impairment (post-infectious hydrocephalus, cerebral palsy and seizures) in 21% of survivors (Gebremariam, 1998). An observational study in Malawi reported neurodevelopmental impairment in 60% of neonatal meningitis survivors at 12 months of age (Dube, 2014).

The brains of preterm infants are particularly susceptible to injury and the relationship between infection and adverse neurological and neurodevelopmental outcomes in preterm infants has been well documented (Stoll et al., 2004b, Schlapbach et al., 2011, Mitha et al., 2013). There are limited data on the longer-term outcomes of term infants surviving neonatal sepsis, especially in low-resource settings where the burden is highest. A large retrospective study from America, that included 2677 infants who suffered from confirmed or suspected neonatal sepsis, reported that cases with confirmed sepsis had 1.7 times the risk of neurodevelopmental impairment in almost all sub-types when compared to controls at 5-years of age (Savioli et al., 2018). Even cases of suspected sepsis had a significantly higher risk of learning delays. In SSA there are a few small studies that evaluate at the longer-term outcomes of neonatal sepsis. A small retrospective study in Kenya of 24 patients who had received a diagnosis of clinical neonatal sepsis reported significant gross and fine motor impairment compared to controls at 18-32 months of age (Gordon et al., 2005). A prospective study of 62 cases of neonatal sepsis in Malawi reported the risk of fine motor and cognitive delay to be 6-times higher when compared to controls at 12 months of age (Dube, 2014). Although these studies are small, it is conceivable that

term infants who survive neonatal sepsis may suffer high rates of neurodevelopmental impairment, cerebral palsy and post-infectious hydrocephalus (Warf, 2005, Dube, 2014). Given that neonatal sepsis is much more common than neonatal meningitis and in settings such as Uganda, the rates of neonatal infection are relatively high, it is vital that we improve our understanding of the interaction between infection, brain injury and adverse neurological and neurodevelopmental outcomes.

HYPOTHESIS

- Neonates, in a low-resource setting in Uganda, who suffer an episode of pSBI will have an increased risk of post-neonatal mortality, developmental impairment, poor growth and post-infectious hydrocephalus at 12 months of age compared to infants who did not suffer from pSBI during the neonatal period.

AIMS

The aim of this study was to assess four specific outcomes – post neonatal survival, morbidity, growth and neurodevelopment – in a hospital-based cohort of neonates with pSBI and compare this to an external cohort of healthy term neonates in eastern Uganda.

METHODS

RECRUITMENT

This was a prospective cohort study with external controls assessing post-neonatal mortality, morbidity, growth and development in infants. The cases were known to have had pSBI during the neonatal period and were recruited at presentation with pSBI to Mbale Regional Referral Hospital Neonatal Unit (MRRH-NNU) (Burgoine et al., 2018, Okello et al., 2019). Neonates were recruited over a 12-month period from 9th December 2016 until 8th December 2017. Further details of recruitment are described in Chapter 5. In addition, a contemporaneous control group of well neonates with a birthweight of >2000g and no history of perinatal asphyxia or infection were recruited (Details described in Chapter 6).

We attempted to follow up all infants who survived the neonatal period until 12 months of age. We aimed to assess all infants at 2, 6 and 12 months of age. All assessments were carried out over a 14-month period from December 2017 until Feb 2019 by a team of 3 research doctors (KN, RM, EE) and two research nurses (GS, JM). Measures were taken to ensure the best rate of follow-up possible, including Short Message Service (SMS) appointment reminders, phone calls a few days prior to the appointment, refunding of transport costs, and rebooking of those who did not attend. Families of infants not successfully contacted by phone were traced in the community by the research team and the infants either assessed at home or given transport costs to attend clinic.

DATA COLLECTION

For all neonates the maternal medical history and demographics, antenatal events and birth history were collected at the time of recruitment using a structured maternal

interview and the obstetric medical records where available (Appendix 1). For cases, neonatal clinical data were extracted from the medical records at discharge or death. To assess survival, we collected information on the date of death, likely cause of death and place of death by interviewing the parent or guardian. mother.

Growth was assessed for each infant by measuring length and nude weight. Weights were recorded using an electronic weighing scale (SECA 354) with reading increments of 10g. Length was taken using a measuring mat (SECA 210) and measured to the nearest 0.5cm using SECA length chart and head circumference was measured using a tape measure to and measured to the nearest 1mm. All growth data were collected by the research doctors and nurses who had been trained prior to the start of the study. Weight-for-corrected-age (WAZ), length-for-age (LAZ), and weight-for-length (WLZ) z-scores were derived using WHO child growth standards (WHO, 2006). The WHO Anthro Software (https://www.who.int/childgrowth/software/en/) was used to create these indices. A cut-off of -2SD for these three indices was used to define infants as wasted (WLZ <-2SD), underweight (WAZ <-2SD), stunted (LAZ <-2SD).

Development was assessed using the Bayley Scales of Infant Development-3rd edition (BSID-III). The BSID-III is recognised as a comprehensive tool to assess the neurodevelopment of infants. BSID-III assesses the following domains: cognitive development, receptive and expressive language development and fine and gross motor development. The three research doctors (KN, RM, EE) were trained by a qualified BSID-III trainer. This involved three days of theoretical and practical training before the study commenced. In addition, refresher training was provided by the same local trainer at the start of the 6-month and 12-month follow-up periods. All

assessments during the study were performed by the BSID-III trainer or one of the 4 trained assessors. To assess the agreement between the four assessors, all 4 assessors administered the BSID-III assessment on 12 infants, each scoring the test independently, and one serving as the primary examiner for three tests and the observer for the other 8 tests. The Intraclass Correlation Coefficient was 0.96 (95% CI: 0.94-0.98). At the completion of each developmental assessment the raw scores for each domain were recorded on the case report form.

Developmental assessment requires comparison to age-corrected norms by converting raw scores to scaled scores. Conversions to scaled scores were done independently by one the three research doctors (KN, RM, EE) and by the site investigator (KB). In cases where the two scaled scores were not in agreement, they were rechecked a third time. Normative samples are usually cross-sectional, drawn from healthy children in the target population, these data are currently not available for Ugandan infants and therefore our scaled scores for both cases and controls were generated from the BSID-III manual based on United States norms. As per the BSID-III, scaled scores were calculated for all subtests from the raw scores, scaled scores range from 1-19, with a mean of 10 and a standard deviation (SD) of 3. Composite scores were derived from the various sums of the subtest scaled scores to create the Composite Cognitive, Composite Language and Composite Motor Scores. Each of these three composite scores were then scaled to a metric with a mean of 100, a SD of 15 and a range of 40 to 160. For each composite score, mild neurodevelopmental delay was defined as score <85, moderate neurodevelopmental delay was defined as a score <70 and severe delay was defined as a score <55.

Poor outcome was defined as a composite of neurodevelopmental impairment, post-infectious hydrocephalus, seizures or death at 12 months.

STATISTICAL ANALYSIS

All data were double entered. Any discrepancies and outlying results were reviewed. Data were analysed using IBM SPSS Statistics Version 25. Analyses of the entire cohort of pSBI and controls were undertaken. Neurodevelopment and growth were compared between cases and controls at each time point for 2, 6 and 12 months. In addition, data within the cases of pSBI, outcomes for those with and without meningitis, as defined in Chapter 3, were compared. Lastly, data between controls, infants with meningitis and those infants who suffered pSBI without meningitis were compared. Categorical variables were examined using a Chi-squared test when the sample size was ≥5 and Fisher's exact test when <5. For continuous variables, normal distribution was assessed using Shapiro-Wilk normality test (normal >0.05). Continuous variables were examined using Student's T-test and the Mann-Whitney U test according to normality. Sub-group analysis was undertaken using the same methods.

RESULTS

NEONATAL AND POST-NEONATAL MORTALITY

A total of 214 neonates with pSBI were recruited over a 12-month period, including 25 (11.7%) neonates who were diagnosed with meningitis as defined in Chapter 5. The overall neonatal mortality of the infants with pSBI was 9.3% (20/214). Of the 194

survivors of pSBI, 3.1% (6/194) were alive at 28 days but their parents withdrew from further follow-up (Figure 61). Of the 188 infants followed-up, 4 infants died between 1 and 12 months of age. One infant died from lower limb gangrene following staphylococcus aureus sepsis after the family discharged themselves before completion of treatment. The other three died from febrile illnesses at 2 months, 5 months and 11 months respectively. Two of the four post-neonatal deaths were in cases of neonatal meningitis. There was therefore an overall infant mortality within our sample of 11.2% (24/214) and 2.1% (4/188) for post-neonatal mortality. Overall, 85.6% (161/188), 82.4% (155/188) and 89.4% (168/188) infants were seen alive at 2 months, 6 months and 12 months of age respectively.

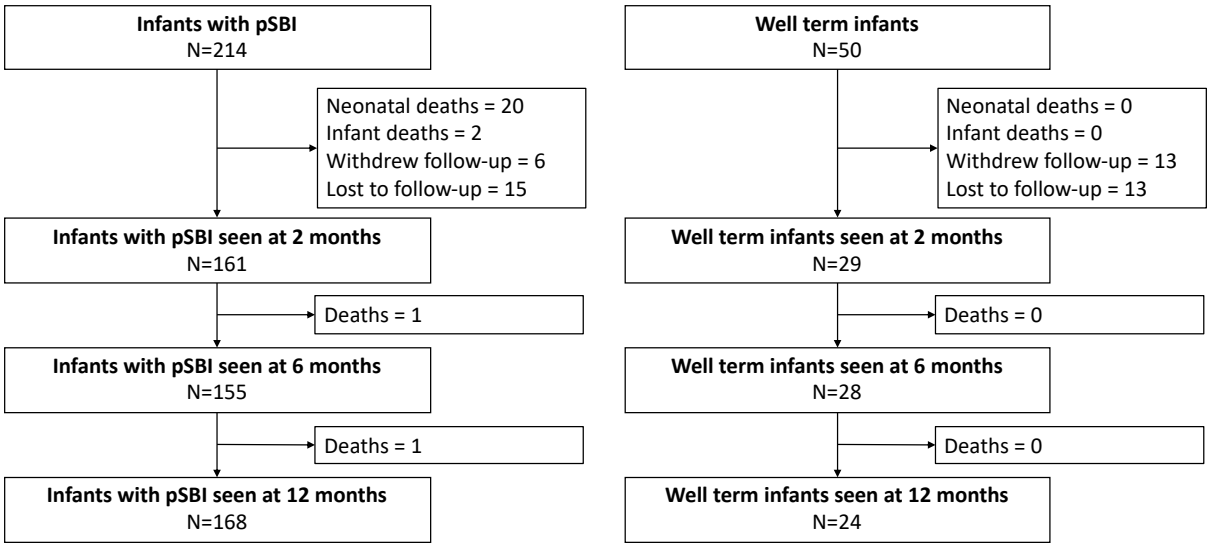


Figure 61: Flow diagram of participants from enrolment until 12 months of age

BASELINE AND CLINICAL CHARACTERISTICS

The baseline characteristics of the cases and controls are described in Table 32. The majority of characteristics were similar between the two groups. As one would expect, the temperature at enrolment was significantly higher in the cases of pSBI compared to the control infants. The rate of emergency caesarean-section was significantly higher in the cases (30.8% vs. 0.0%, p=<0.0001). In addition, the birthweight of the cases was significantly lower than the control infants (3.20kg vs. 3.35kg, p=0.0004).

Characteristic	Cases, n=188	Controls, n=37	P value
Maternal factors			
HIV exposed (%)	4/188 (2.1)	1/37 (2.7)	1.000
Maternal age, median (IQR)	25 (22, 29)	27 (23, 32)	0.082
Primigravida (%)	66/188 (33.8)	9/37 (24.3)	0.2537
Maternal education level ≤primary school	76/185 (41.0)	19/37 (51.4)	0.275
Secondary	63/185 (34.1)	11/37 (29.7)	0.705
Tertiary	46/185 (24.9)	7/37 (18.9)	0.531
Employment (n=185)			
Housewife	63/185 (34.1)	11/37 (29.7)	0.705
Farmer	48/185 (25.9)	14/37 (37.8)	0.158
Service or sales workers	46/185 (24.9)	8/38 (21.6)	0.834
Professionals	28/185 (15.1)	4/37 (10.8)	0.615
Emergency caesarean-section	58/188 (30.8)	0/37 (0)	**<0.0001**
Elective caesarean-section	5/188 (2.7)	0/37 (0)	0.5941
Assisted	2/188 (1.1)	0/37 (0)	0.9999
SVD	123/188 (65.4)	37/37 (100)	**<0.0001**
Neonatal resuscitation, Freq (%)			
- Need for resuscitation	35/188 (18.6)	2/37 (5.4)	0.0525
- Suction/stimulation only	23/188 (12.2)	2/37 (5.4)	0.3881
- Bag-mask ventilation	12/188 (6.4)	0/37 (0)	0.2238
- Apgar score at 5 minutes	9.6 (0.8), mean (SD Range, 7-10 (n=167)	9.8 (0.6) Range 8-1 (n=36)	0.1585
Infant factors			
Male sex	119/188 (63.3)	21/37 (56.8)	0.4635
Age at recruitment, Median (IQR)	2 (1, 4)	0.0 (0, 1)	0.000
Birth weight (Mean ±SD)	3.02±0.53	3.35 ±0.44	**0.0004**
Head circumference, Median (IQR)	35.8 (35.0, 36.5)	35.5 (34.9, 36.0)	0.242
Length mean Median (IQR)	50.0 (48.8, 51.5)	51.0 (49.0, 51.5)	0.413
Temperature (Mean ±SD)	38.7 ±1.9	36.5 ±0.44	**0.000**

Table 32: Maternal and infant characteristics at baseline

COMPLICATIONS

Three of the pSBI survivors (1.6%) developed post-infectious hydrocephalus by 12 months of age, as discussed in Chapter 7, but none of the controls. All three cases

were detected by cranial ultrasound (cUS) before discharge from NNU. Two of these three infants had severe developmental impairment at 12 months of age; one infant was not available for follow-up at 12 months but had severe developmental delay when assessed at 2 months of age.

Three other case infants (1.6%) were reported to suffer from seizures at 12 months; all three had had normal CSF examination at presentation. All three of these infants had severe developmental impairment at 12 months of age. None of the control infants developed post-neonatal seizures.

MORTALITY OUTCOMES

Four infants died post-neonatally. All were pSBI survivors (4/188) and two had had neonatal meningitis. None of the control infants died (RR 2.15, 95%CI 0.12 to 39.3, P=0.60). When the 20 neonatal deaths in cases of pSBI were considered, the overall risk of infant mortality was 11-fold higher in those who suffered pSBI compared to the control group (RR 11.6, 95%CI 0.71 to 180.0, P=0.0841).

NEURODEVELOPMENTAL OUTCOMES AT 2 MONTHS OF AGE

A total of 161 survivors of pSBI had neurodevelopmental assessments performed using BSID-III at the age of 2 months. The mean age at assessment was 2.0 months ± 0.4 months. Neurodevelopmental impairment was present in all five domains (Table 33). The rates of neurodevelopmental impairment ranged from 3.1% in the fine and gross motor domains to 10.6% in the expressive language domain. Similarly, the three composite scores demonstrated neurodevelopmental impairment in between 3.7% to

4.3% of infants. The levels of developmental delay demonstrated by the three composite scores included mild, moderate and severe impairment (Table 34).

Domain	Median raw score (IQR)	Median scaled score (IQR)[#]	Total number of infants with scores <-1SD[$], n (%)	Number with scores -1SD to -2SD[$], n (%)	Number with scores -2SD to -3SD[$], n (%)
Cognitive	13.0 (10.5 15.0)	14.0 (12.0, 15.0)	7 (4.3)	6 (3.7)	1 (0.6)
Receptive language	7.0 (6.0, 8.0)	13.0 (11.0, 13.0)	6 (3.7)	4 (2.5)	2 (1.2)
Expressive language	5.0 (4.0, 6.0)	11.0 (9.0, 13.0)	17 (10.6)	15 (9.4)	2 (1.2)
Gross motor	10.0 (8.0, 12.0)	12.0 (10.0, 13.0)	5 (3.1)	3 (1.9)	2 (1.2)
Fine motor	8.0 (5.0, 9.0)	13.0 (10.0, 14.0)	5 (3.1)	4 (2.5)	1 (0.6)

[#] Scaled scores in relation to BSID-III normative data. [$]Rates of impairment based on BSID-III normative data

Table 33: Individual BSID-III scores and associated rates of neurodevelopmental impairment at 2 months of age for 161 cases of pSBI

Domain	Median composite score[#] (SD)	Total developmental delay <-1SD[$], n (%)	Mild developmental delay -1SD to -2SD[$], n (%)	Moderate delay -2SD to -3SD[$], n (%)	Severe delay <-3SD[$], n (%)
Composite cognitive score	120.0 (110.0, 125.0)	7 (4.3)	6 (3.7)	1 (0.6)	0 (0)
Composite language score	110.5 (103.0, 118.0)	6 (3.7)	2 (1.2)	3 (1.9)	1 (0.6)
Motor composite score	112.0 (100.0, 121.0)	6 (3.7)	4 (2.5)	1 (0.6)	1 (0.6)

[#] Scaled composite scores in relation to BSID-III normative data. [$]Rates of impairment based on BSID-III normative data

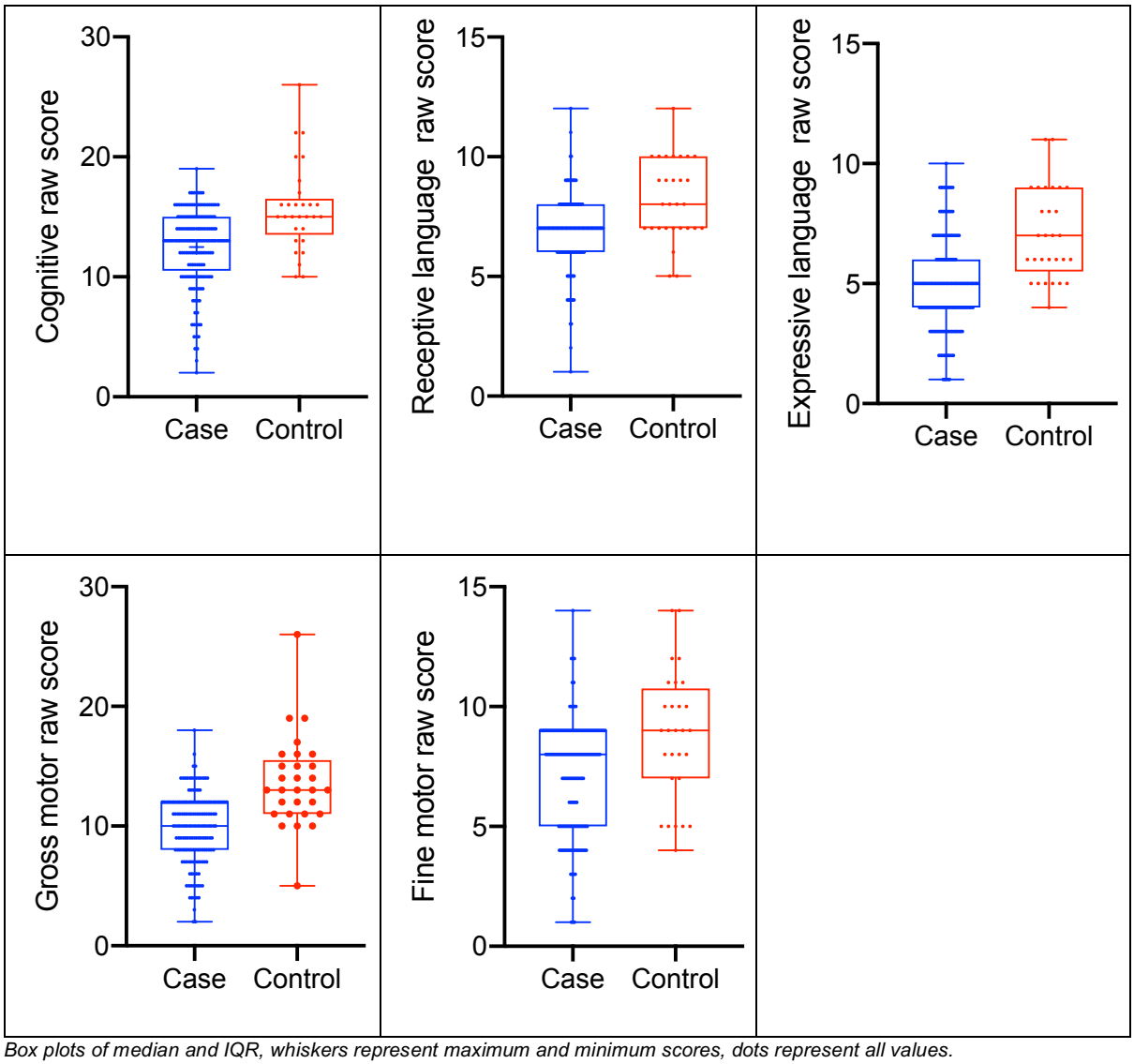
Table 34: Composite BSID-III scores and associated rates of neurodevelopmental impairment at 2 months of age for 161 cases of pSBI

From the control cohort, 29 infants were available for assessment at 2 months. The mean age at assessment was 2.6±0.7months, which was significantly older than the cases (95%CI 0.41 to 0.178, *p*= <0.0001). The raw scores for each of the five domains are compared between the pSBI survivors and the control group in Table 35 and Figure 62. In all five domains, the median raw score was significantly lower in the pSBI survivors than in the cohort of control infants. This may in part be due to the older age of the control infants at assessment.

Domain	pSBI cohort median raw score (IQR) (n=161)	Control cohort median raw score (IQR) 1SD (n=29)	Median difference (95% CI)†	P value‡
Cognitive	13.0 (10.5 15.0)	15.0 (13.5, 16.5)	2.0 (1.0, 4.0)	0.001
Receptive language	7.0 (6.0, 8.0)	8.0 (7.0, 10.0)	1.0 (1.0, 2.0)	0.001
Expressive language	5.0 (4.0, 6.0)	7.0 (5.5, 9.0)	2.0 (1.0, 3.0)	0.000
Gross motor	10.0 (8.0, 12.0)	13.0 (11.0, 15.5)	3.0 (2.0, 5.0)	0.000
Fine motor	8.0 (5.0, 9.0)	9.0 (7.0, 11.0)	2.0 (1.0, 3.0)	0.001

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 35: Comparison of the BSID-III raw scores for each of the five neurocognitive domains at 2 months of age in infants who suffered from pSBI vs. control infants



Box plots of median and IQR, whiskers represent maximum and minimum scores, dots represent all values.

Figure 62: BSID-III *raw scores* in each of the five neurocognitive domains between infants who suffered from pSBI (blue bars) vs. control infants (red bars) at 2 months of age

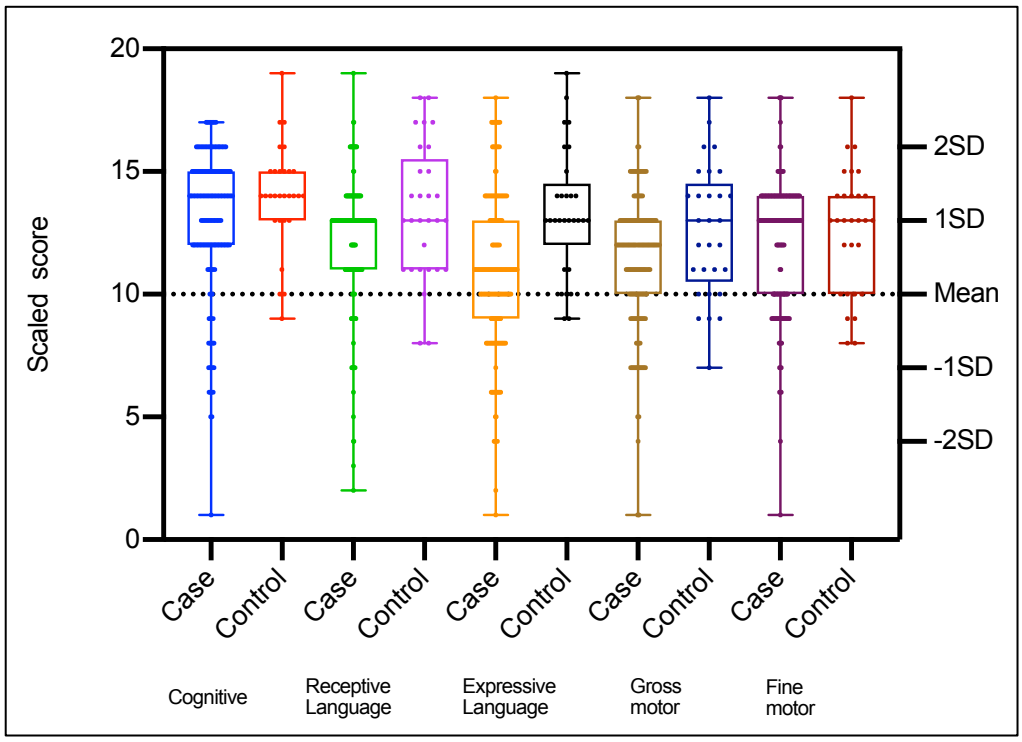
When the scaled scores for the 5 domains at 2 months were compared between the pSBI survivors and the control infants, the pSBI infants only scored significantly lower in the expressive language and gross motor domain. As Figure 63 clearly demonstrates, although the medians are very similar, it is only in survivors of pSBI that score below -1SD. It is also important to note the presence of pSBI survivors who achieved the same high neurodevelopmental scores as the infants in the control

cohort. The median score for all 5 domains for both cases and controls, lies well above the mean value for the US normative population.

Domain	pSBI cohort median scaled score (IQR)	Control cohort, median scaled score (IQR)	Median difference (95% CI)†	P value‡
Cognitive	14.0 (12.0, 15.0)	14 (13,15)	1 (0, 2)	0.194
Receptive language	13.0 (11.0, 13.0)	13 (11, 16)	1 (0,2)	0.079
Expressive language	11.0 (9.0, 13.0)	13 (12, 15)	2 (2, 3)	**0.000**
Gross motor	12.0 (10.0, 13.0)	13 (11, 15)	1 (0,2)	**0.049**
Fine motor	13.0 (10.0, 14.0)	13 (10, 14)	0 (0,1)	0.454

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 36: Comparison of the BSID-III *scaled scores* for each of the five neurocognitive domains at 2 months of age in infants who suffered from pSBI vs. control infants



Box plots of median and IQR, whiskers represent maximum and minimum scores, dots represent all values.

Figure 63: BSID-III scaled scores in each of the five neurocognitive domains between infants who suffered from pSBI vs. control infants at 2 months of age

When the composite cognitive, language and motor scores at 2 months were compared between the pSBI survivors and the control group in Table 37, there was no significant difference between the composite cognitive and motor scores. The composite language score was significantly lower in the pSBI survivors compared to the control infants.

Domain	pSBI cohort, Total developmental delay <-1SD (N=161)	Control cohort, Total developmental delay <-1SD (N=29)	Difference in medians† (95%CI)	P value‡
Composite cognitive score	120 (110, 125)	120 (115, 125)	5 (0,10)	0.194
Composite language score	**111 (103, 118)**	**121 (109, 134)**	**9 (6, 15)**	**0.001**
Motor composite score	112 (100, 121)	112 (109, 126)	3 (0,9)	0.111

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 37: Comparison of the BSID-III composite scores for each of the three domains at 2 months of age in infants who suffered from pSBI vs. control infants

At 2 months of age, all the cases of neurodevelopmental impairment were in the survivors of pSBI (Table 38). Although the relative risk of neurodevelopmental impairment ranged from 2.41 to 2.78 across the three composite scores when compared to the control infants, this trend did not reach statistical significance.

Domain	pSBI cohort, Total developmental delay <-1SD, (n=161)	Control cohort, Total developmental delay <-1SD (n=29)	RR (95%CI)	P value
Composite cognitive score	7 (4.3)	0 (0)	2.78 (0.16, 47.4)	0.48
Composite language score	6 (3.7)	0 (0)	2.41(0.14, 41.6)	0.54
Motor composite score	6 (3.7)	0 (0)	2.41(0.14, 41.6)	0.54

Table 38: Rates of neurodevelopmental impairment at 2 months comparing infants who suffered from pSBI vs. term control infants

NEURODEVELOPMENTAL OUTCOMES AT 6 MONTHS OF AGE

A total of 154 infants who had survived a pSBI underwent developmental assessments using BSID-III at the age of 6 months. The mean age at assessment was 6.12 ±0.48 months. The rates of neurodevelopmental impairment were similar across the five domains, and the rates of impairment had increased from the assessments at 2 months of age (Table 39). At 6 months of age the rates of neurodevelopmental impairment ranged from 8.4% in receptive language up to 14.9% in fine motor. This included cases of both mild and moderate impairment, with the highest rates of moderate neurodevelopmental impairment seen in gross motor (7.1%) and fine motor domains (8.4%).

Domain	Median raw score (IQR)	Median scaled score (IQR)[#]	Total developmental delay <-1SD[$], n (%)	Mild developmental delay -1SD to -2SD[$], n (%)	Moderate delay -2SD to -3SD[$], n (%)
Cognitive	31 (26, 34)	12 (9,14)	14 (9.1)	6 (3.9)	7 (4.5)
Receptive language	11 (7,10)	12 (9,14)	13 (8.4)	5 (3.2)	7 (4.5)
Expressive language	9 (7,10)	11 (9,13)	17 (11.0)	10 (6.5)	7 (4.5)
Gross motor	27 (25, 28)	12 (10,13)	17 (11.0)	4 (2.6)	11 (7.1)
Fine motor	24 (21, 26)	15 (12, 17)	23 (14.9)	9 (5.8)	13 (8.4)

[#] Scaled scores in relation to BSID-III normative data. [$]Rates of impairment based on BSID-III normative data

Table 39: Individual BSID-III scores at 6 months of age and associated rates of neurodevelopmental impairment for 154 cases of pSBI

The composite cognitive, language and motor scores show that cases of developmental impairment were distributed across the spectrum from mild to severe impairment (Table 40). Unsurprisingly, the highest rate of impairment was in the motor composite score (13.6%) and almost half of these cases were severely delayed. In the composite cognitive score and the composite language score, 8.4% and 12.3% of infants had developmental impairment respectively.

Domain	Median composite score[#] (IQR)	Total developmental delay <-1SD[$], n (%)	Mild developmental delay -1SD to -2SD[$], n (%)	Moderate delay -2SD to -3SD[$], n (%)	Severe delay <-3SD[$], N (%)
Composite cognitive score	110 (95, 120)	13 (8.4)	6 (3.9)	7 (4.5)	0 (0)
Composite language score	109 (97, 121)	19 (12.3)	10 (6.5)	6 (3.9)	3 (1.9)
Composite motor score	121 (103, 127)	21 (13.6)	11 (7.1)	1 (0.6)	9 (5.8)

[#] Scaled composite scores in relation to BSID-III normative data. [$]Rates of impairment based on BSID-III normative data

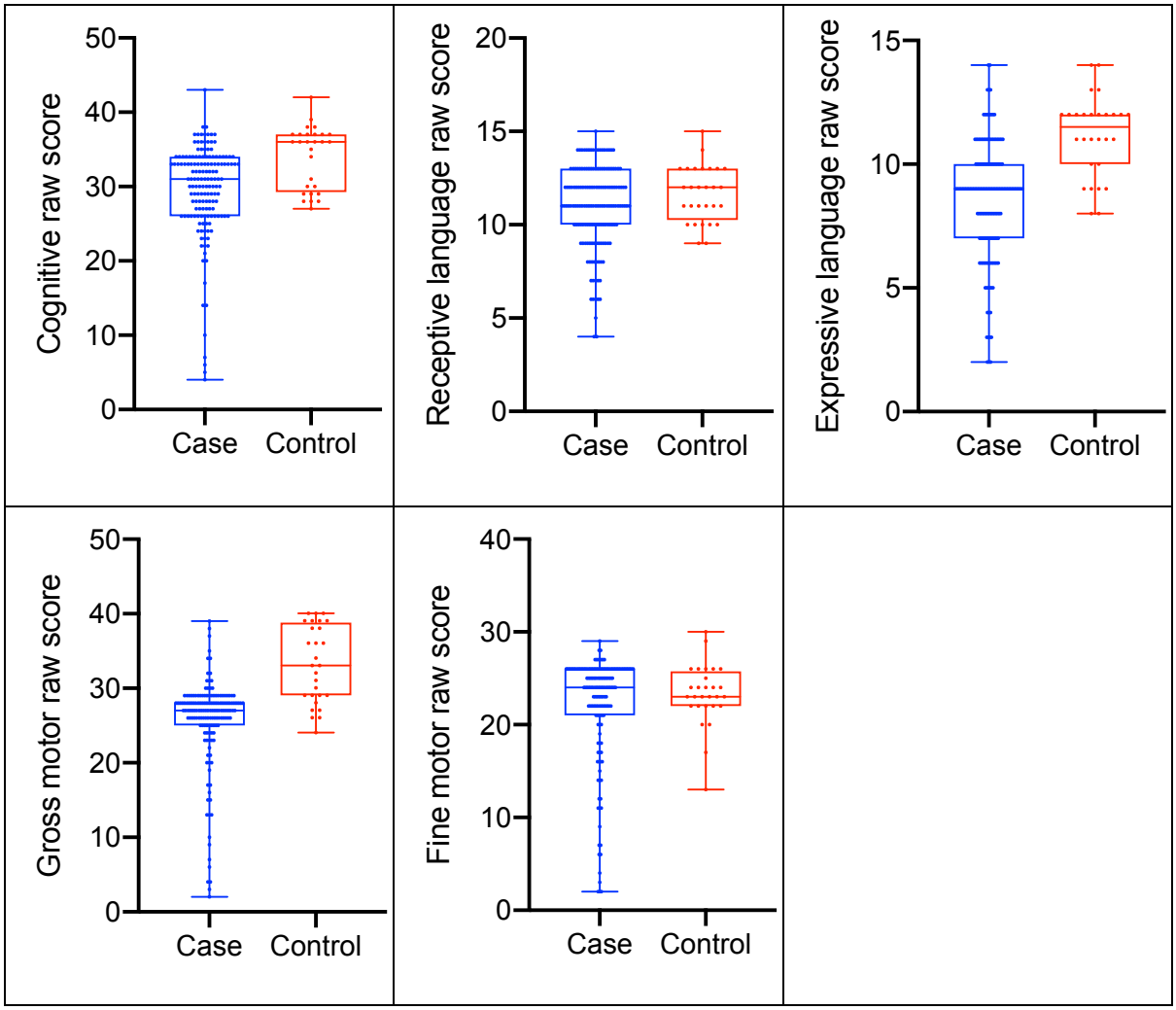
Table 40: Composite BSID-III scores and associated rates of neurodevelopmental impairment at 6 months of age for 154 cases of pSBI

28 control infants were available for assessment at 6 months. The mean age at assessment was 6.1±0.6 months, not significantly different to the cases (p=0.8457). The raw scores for each of the five domains are compared between the pSBI survivors and control group in Table 41 and Figure 64. In the cognitive, expressive language and gross motor domains, the median raw score was significantly lower in the pSBI survivors than the control infant group.

Domain	pSBI cohort median raw score (IQR) (N = 155)	Control cohort median raw score (IQR) 1SD (N = 28)	Median difference (95% CI)†	P value‡
Cognitive	31 (26, 34)	36 (29, 37)	4 (2, 6)	**0.000**
Receptive language	11 (7,10)	12 (10, 13)	0.5 (0, 1.0)	0.220
Expressive language	9 (7,10)	12 (10, 12)	2 (2,3)	**0.000**
Gross motor	27 (25, 28)	33 (29, 39)	7 (4,10)	**0.000**
Fine motor	24 (21, 26)	23 (22, 26)	0 (-1, 1)	0.722

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 41: Comparison of the BSID-III raw scores for each of the five neurocognitive domains at 6 months of age in infants who suffered from pSBI vs. term control infants



Box plots of median and IQR, whiskers represent maximum and minimum scores, dots represent all values.

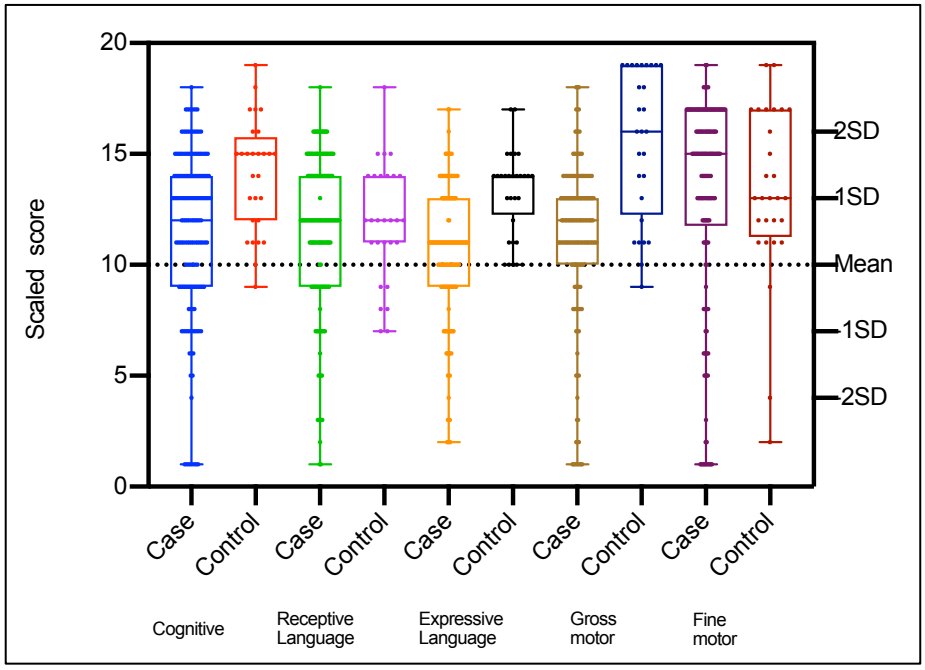
Figure 64: BSID-III raw scores in each of the five neurocognitive domains between infants who suffered from pSBI (blue bars) vs. term control infants (red bars) at 6 months of age

The scaled scores for the 5 domains at 6 months of age were then compared between the pSBI survivors and the control infants as shown in Table 42. Similar to the assessments at 2 months of age the pSBI survivors scored significantly lower in the expressive language and gross motor domains. In addition, they also scored significantly lower than the control term infants in the cognitive domain. Again, as was seen at 2 months of age, it was only survivors of pSBI that scored below -1SD (Table 42 and Figure 65). Again, there continued to be pSBI survivors who achieved the same high developmental scores as the infants in the control cohort. The median score for all 5 domains for both cases and controls continued to lie above the mean value for the normative population.

Domain	pSBI cohort median scaled score (IQR) N = 155	Control cohort, median scaled score (IQR), N = 29	Median difference (95% CI)†	P value‡
Cognitive	12 (9,14)	15 (12, 16)	2 (1, 3)	**0.001**
Receptive language	12 (9,14)	12 (11, 14)	0 (-1, 1)	0.817
Expressive language	11 (9,13)	14 (12, 14)	3 (2, 4)	**0.000**
Gross motor	12 (10,13)	16 (12, 19)	5 (3, 6)	**0.000**
Fine motor	15 (12, 17)	13 (11, 17)	0 (-2, 1)	0.619

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 42: Comparison of the BSID-III *scaled scores* for each of the five neurocognitive domains at 6 months of age in infants who suffered from pSBI vs. term control infants



Box plots of median and IQR, whiskers represent maximum and minimum scores, dots represent all values.

Figure 65: BSID-III *scaled scores* in each of the five neurocognitive domains between infants who suffered from pSBI vs. term control infants at *6 months* of age

The composite cognitive, language and motor scores at 6 months are compared between the pSBI survivors and the control infant group in Table 43. All were significantly lower for the cases of pSBI than the control term infants.

Domain	pSBI cohort median score (IQR)	Control cohort median score (IQR)	Median difference (95% CI) †	P value‡
Composite cognitive score	110 (95, 120)	125 (110, 129)	10 (5, 15)	**0.001**
Composite language score	109 (97, 121)	115, (109, 124)	8 (2, 14)	**0.011**
Motor composite score	121 (103, 127)	132 (113, 136)	9 (3, 17)	**0.002**

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 43: Comparison of the BSID-III composite scores for each of the three domains at 6 months of age in infants who suffered from pSBI vs. term control infants

None of the control infants had a composite score <-1 SD in any domain and therefore none were diagnosed with developmental impairment at 6 months. Conversely, the rates of neurodevelopmental impairment in the cases of pSBI ranged from 8.4% to 14.3% as previously described. Although it was not statistically significant, there was a trend for an increased risk of neurodevelopmental impairment across the 3 composite scores when compared to the control term infants (Table 44). At 6 months of age, pSBI was associated with a 5-fold increase in cognitive delay. There was also a 7-fold increase in the risk of language delay and 8-fold increase in motor delay. These risks of neurodevelopmental impairment were two to four times higher than the increased risks of neurodevelopmental delay reported at the 2-month assessments above.

Domain score (sc)	pSBI cohort, Total developmental delay <-1SD (n=154)	Control cohort, Total developmental delay <-1SD (n=28)	RR (95%CI)*	P value
Composite cognitive sc.	13 (8.4)	0 (0)	5.05 (0.31, 82.6)	0.26
Composite language sc.	19 (12.3)	0 (0)	7.29 (0.45, 117.5)	0.16
Composite motor score	21 (13.6)	0 (0)	8.41 (0.53, 129.9)	0.14

*Relative Risk (RR)

Table 44: Rates of neurodevelopmental impairment at 6 months comparing infants who suffered from pSBI vs. term control infants

NEURODEVELOPMENTAL OUTCOMES AT 12 MONTHS OF AGE

A total of 164 infants who survived pSBI had a neurodevelopmental assessment performed using BSID-III at the age of 12 months. An additional four infants were seen alive but were either malnourished or acutely unwell and although they are considered in the growth analyses, they were unable to undergo BSID-III assessment. There were

4 post-neonatal deaths, thus, outcome data (known death or neurodevelopmental outcome) was available for 89.4% (168/188). The mean age at assessment was 12.4±0.77 months. Of the 164 infants assessed, 134 had had sepsis, 17 meningitis, 1 tetanus and 12 pneumonia.

The rates of developmental impairment are shown in Table 45 and neurodevelopmental impairment was evident across all 5 domains. Compared to the assessments at 6 months of age, the rates of impairment were slightly increased receptive language, expressive language and gross motor domains. Rates of impairment ranged from 7.3% for fine motor impairment to 14.6% for gross motor impairment.

Domain	Median raw score (IQR)	Median scaled score (IQR)[#]	Total developmental delay <-1SD[$], n (%)	Mild developmental delay -1SD to -2SD[$], n (%)	Moderate delay -2SD to -3SD[$], n (%)
Cognitive	49 (45, 55)	15 (12, 17)	13 (7.9)	4	9
Receptive language	14 (13, 16)	9 (8, 12)	18 (11.0)	11	7
Expressive language	15 (12, 17)	10 (8, 12)	23 (14.0)	16	7
Gross motor	42 (40, 46)	10 (8, 13)	24 (14.6)	10	14
Fine motor	31 (29, 32)	12 (10, 14)	12 (7.3)	2	10

[#] Scaled scores in relation to BSID-III normative data. [$]Rates of impairment based on BSID-III normative data

Table 45: BSID-III raw and scaled scores with associated rates of neurodevelopmental impairment at 12 months of age for cases of pSBI

The composite cognitive, language and motor scores show that cases ranged from mild to severe neurodevelopmental impairment (Table 46). The highest rate if impairment was in the language composite score (16.5%).

Domain	Median composite score[#] (IQR)	Total developmental delay <-1SD[$], n (%)	Mild developmental delay -1SD to -2SD[$], n (%)	Moderate delay -2SD to -3SD[$], n (%)	Severe delay <-3SD[$], n (%)
Composite cognitive score	125 (111, 135)	13 (7.9)	4	9	0
Composite language score	97 (89, 109)	27 (16.5)	15	7	5
Composite motor score	110 (94, 118)	20 (12.2)	8	4	8

[#] Scaled composite scores in relation to BSID-III normative data. [$]Rates of impairment based on BSID-III normative data

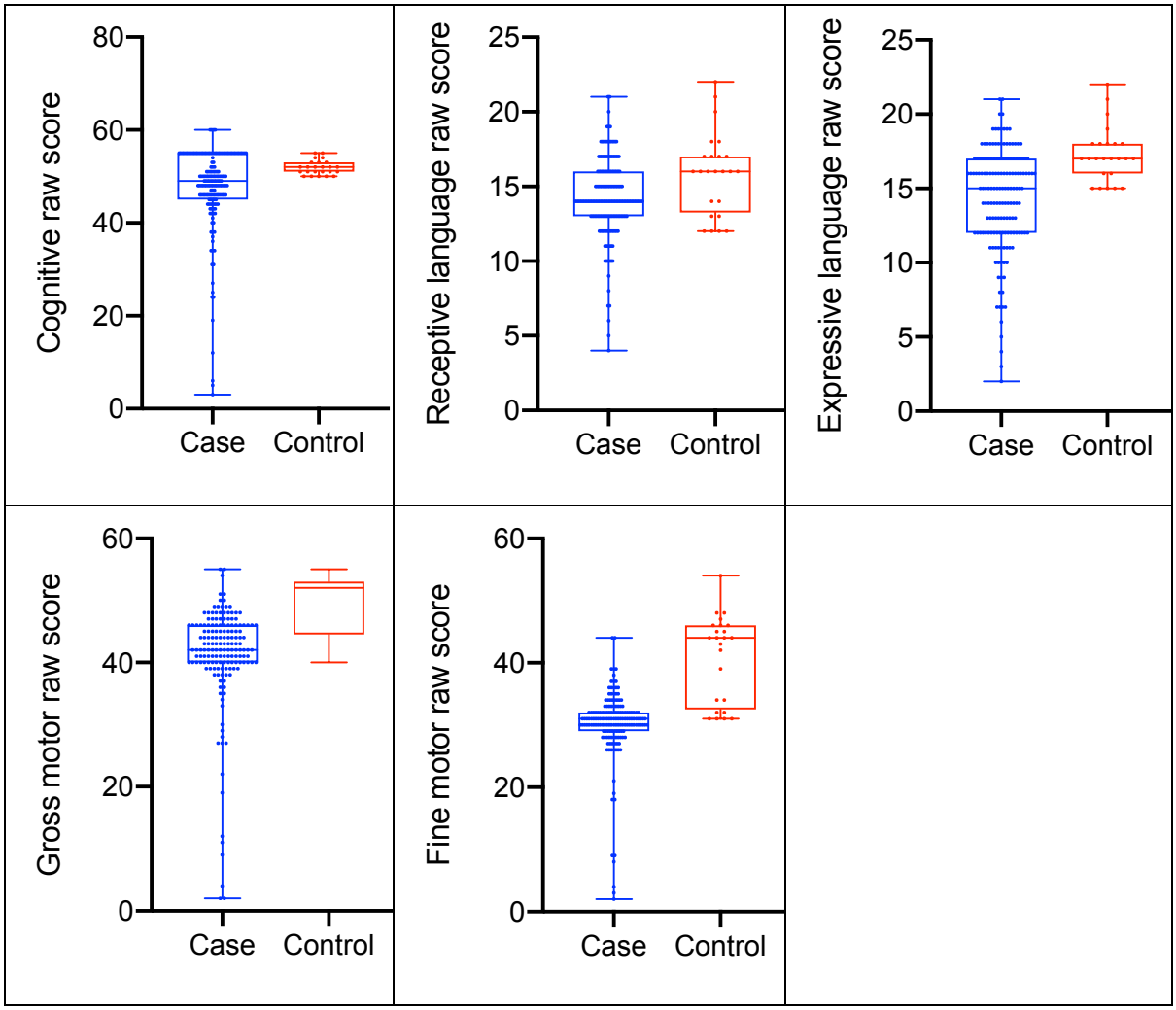
Table 46: Composite BSID-III scores and associated rates of neurodevelopmental impairment at 12 months of age for cases of pSBI

24 control infants were available for assessment at 12 months of age. Their mean age at assessment was 12.0±0.5months, which was significantly younger than the cases (95%CI -0.067 to -0.03, *p* value = 0.0322). The raw scores at 12 months of age are compared between the pSBI survivors and control infant group in Table 47. For all five neurocognitive domains, the median raw score was significantly lower in the pSBI survivors, despite the control group being younger (Figure 66).

Domain	pSBI cohort median raw score (IQR), n= 164	Control cohort median score (IQR), n=24	Median difference (95% CI)[†]	P value[‡]
Cognitive	49 (45, 55)	52 (51, 53)	3 (1,5)	**0.002**
Receptive language	14 (13, 16)	16 (13, 17)	2 (0, 3)	**0.013**
Expressive language	15 (12, 17)	17 (16, 18)	2 (1,4)	**0.000**
Gross motor	42 (40, 46)	52 (45, 53)	8 (5, 10)	**0.000**
Fine motor	31 (29, 32)	44 (33, 46)	12 (9, 14)	**0.000**

[†] Hodges-Lehman Median Difference, [‡]Mann-Whitney U test

Table 47: Comparison of the BSID-III raw scores for each of the five neurocognitive domains at 12 months of age in infants who suffered from pSBI vs. control infants



Box plots of median and IQR, whiskers represent maximum and minimum scores, dots represent all values.

Figure 66: Comparison of BSID-III raw scores in each of the five neurocognitive domains between infants who suffered from pSBI (blue bars) vs. control infants (red bars) at 12 months of age

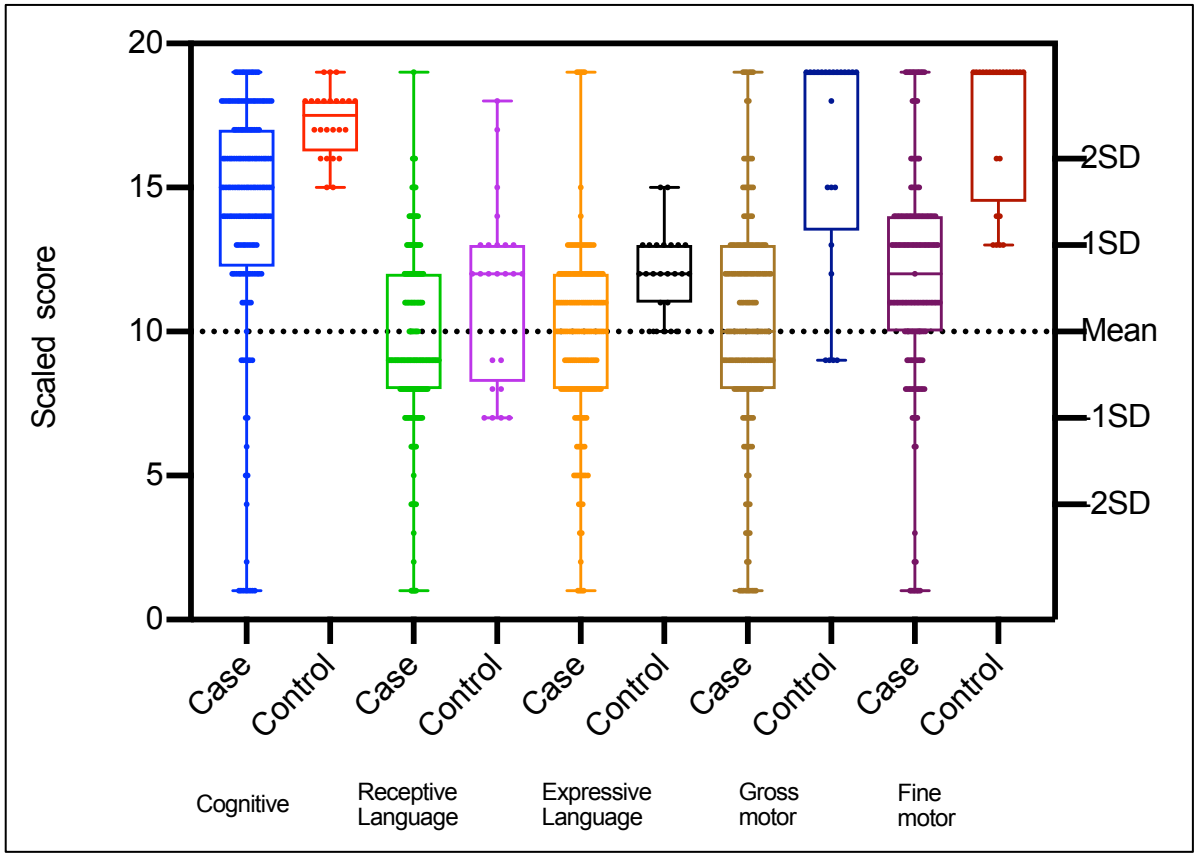
The scaled scores for the 5 domains at 12 months of age were then compared between the pSBI survivors and the control infants as shown in Table 48. Compared to the assessments at 6 months, where the pSBI survivors had significantly lower scaled scores in only 3 of the domains, at 12 months, the scaled scores were significantly lower in all 5 domains for the pSBI survivors compared to the control term infants. Again, as was seen at the earlier assessments, it was only the survivors of

pSBI that scored below -1SD (Figure 67). Some survivors of pSBI still continued to achieve the same high neurodevelopmental scores as the infants in the control cohort. The median score for all 5 domains for both cases and controls lie at or above the mean value for the normative population.

Domain	pSBI cohort median scaled score (IQR) n=164	Control cohort, median scaled score (IQR), n=24	Median difference (95% CI)†	P value‡
Cognitive	15 (12, 17)	18 (26, 28)	2 (1, 3)	**0.000**
Receptive language	9 (8, 12)	23 (8, 13)	2 (0, 3)	**0.012**
Expressive language	10 (8, 12)	12 (11, 13)	2 (1, 3)	**0.000**
Gross motor	10 (8, 13)	19 (14, 19)	7 (5,8)	**0.000**
Fine motor	12 (10, 14)	19 (15, 19)	6 (5, 6)	**0.000**

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 48: Comparison of the BSID-III *scaled scores* for each of the five neurocognitive domains at 12 months of age in infants who suffered from pSBI vs. control infants



Box plots of median and IQR, whiskers represent maximum and minimum scores, dots represent all values.

Figure 67: BSID-III *scaled scores* in each of the five neurocognitive domains between infants who suffered from pSBI vs. control infants at *12 months* of age

The composite cognitive, language and motor scores at 12 months are compared between the pSBI survivors and the control infant group in Table 49. All three composite scores continued to be significantly lower for the cases of pSBI than the control infants.

Domain	pSBI cohort median score (IQR)	Control cohort median score (IQR)	Median difference (95% CI) †	P value‡
Composite cognitive score	125 (111, 135)	138 (131, 140)	10 (5, 15)	**0.000**
Composite language score	97 (89, 109)	112 (99, 118)	12 (6, 18)	**0.000**
Composite motor score	110 (94, 118)	154 (122, 154)	36 (30, 44)	**0.000**

† Hodges-Lehman Median Difference, ‡Mann-Whitney U test

Table 49: Comparison of the BSID-III composite scores for each of the three domains at 12 months of age in infants who suffered from pSBI vs. control infants

At 12 months of age, none of the control infants had a composite score <-1 SD in any domain and therefore none were diagnosed with developmental impairment at 12 months of age (Table 50). Although it was not statistically significantly different, there was a trend for an increased risk of neurodevelopmental impairment across the 3 composite scores when compared to the control infants. At 12 months of age, pSBI was associated with a 4-fold increase in cognitive delay, similar to that seen at 6 months. There was also an 8-fold increase in the risk of language delay, similar to that seen at 6 months. There was a 6-fold increase in motor delay, which was lower than that seen at 6 months, possibly because one case identified with severe motor delay at 6 months had died at 11 months from a severe febrile illness and was therefore not assessed.

Domain	pSBI cohort, Total developmental delay <-1SD (n=164)	Control cohort, Total developmental delay <-1SD (n=24)	RR (95%CI)*	P value
Composite cognitive score	13 (7.9)	0 (0)	4.09	0.32
Composite language score	27 (16.5)	0 (0)	8.33	0.13
Composite motor score	20 (12.2)	0 (0)	6.21	0.19

*Relative Risk (RR)

Table 50: Rates of neurodevelopmental impairment at 12 months comparing infants who suffered from pSBI vs. control infants

SEQUENTIAL RATES OF DEVELOPMENTAL IMPAIRMENT

The rates of developmental impairment were all relatively low at 2 months of age, this is expected as there are limited tests that can be administered to evaluate the development of infants at this age (Figure 68). By 6 months the majority of infants with developmental impairment in all 3 domains were detected, suggesting 6 months is the earliest that those infants with developmental impairment can be identified. It is however important to observed that there are still cases of developmental impairment in all three domains that were not detected until 12 months of age, highlighting the need for not only early evaluation of these infants, especially to detect those with severe impairment, but also the need for repeat evaluations to ensure that all infants are identified.

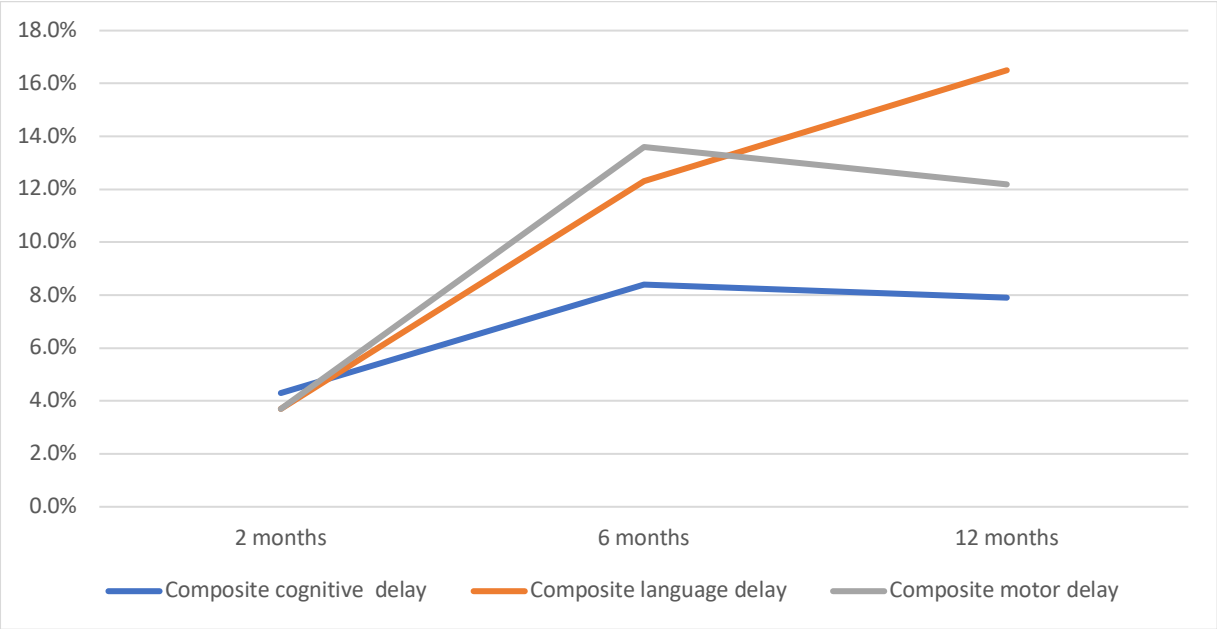


Figure 68: Frequency of developmental delay (<-1SD) at 2, 6 and 12 months in the three domains: motor, language and cognition

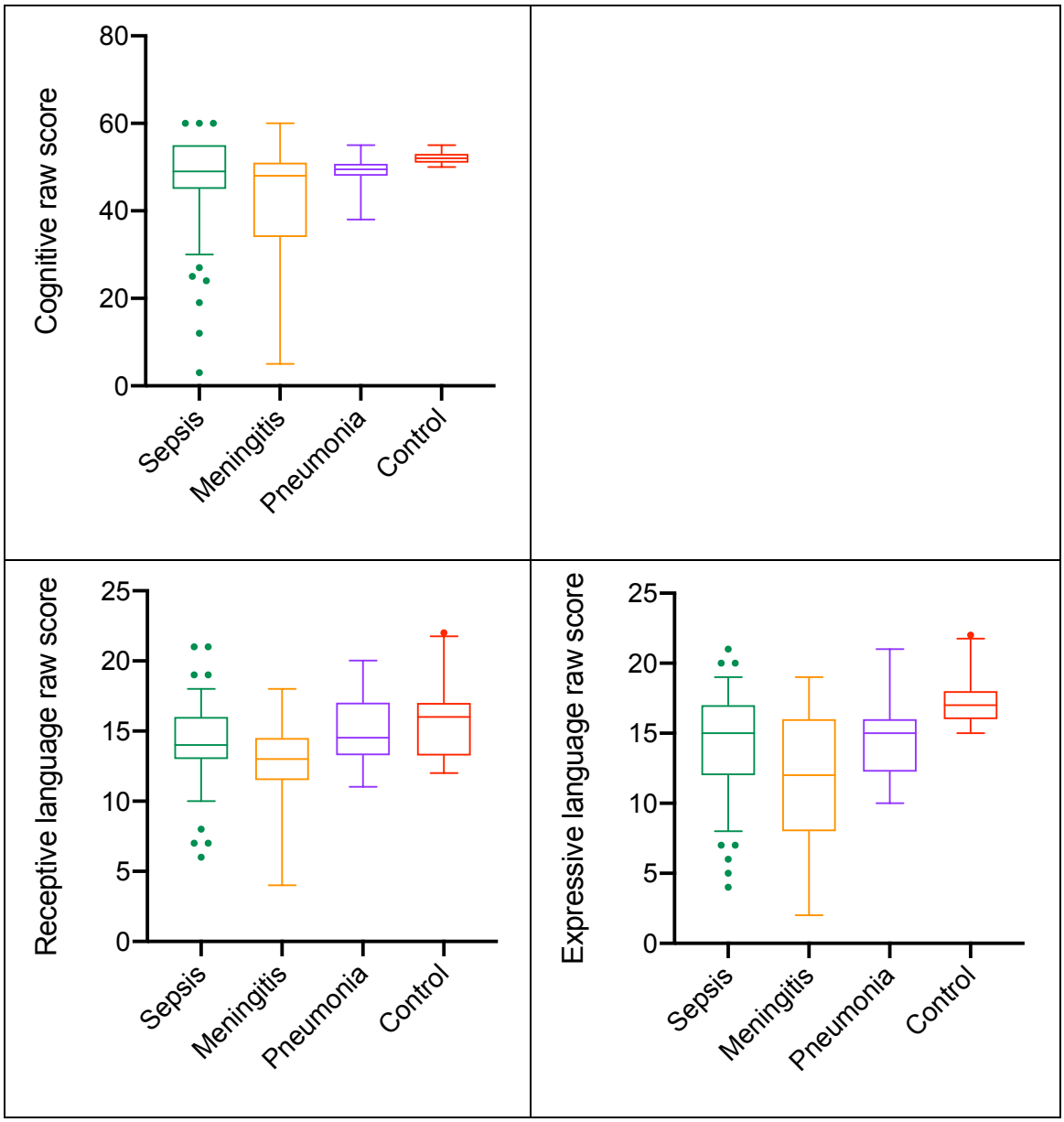
COMPARISON OF NEURODEVELOPMENTAL OUTCOMES BY DIAGNOSIS

In Table 51 and Figure 69, the median raw scores for each of the five neurocognitive domains are compared to the control infants for each diagnosis (sepsis, meningitis, pneumonia). In all five domains, those infants with a diagnosis of neonatal meningitis had the lowest raw scores. For cases of sepsis and meningitis, in all five domains the raw scores were significantly lower when compared to the raw scores in the control infants. For pneumonia, all domains except receptive language were significantly lower when compared to the raw scores in the control infants.

Domain	Control cohort median raw score (IQR), n=24	Sepsis median raw score (IQR), n=134	Meningitis median raw score (IQR), n=17	Pneumonia median raw score (IQR), n=12	P value‡*	P value‡†	P value‡$
Cognitive	52 (51, 53)	49 (45, 55)	48 (34, 51)	50 (48, 51)	**0.006**	**0.002**	**0.004**
Receptive language	16 (13, 17)	14 (13, 16)	13 (12, 15)	15 (12, 16)	**0.012**	**0.011**	0.575
Expressive language	17 (16, 18)	15 (12, 17)	12 (8, 16)	15 (12, 16)	**0.000**	**0.000**	**0.001**
Gross motor	52 (45, 53)	43 (40, 46)	40 (37, 46)	44 (40, 48)	**0.000**	**0.000**	**0.005**
Fine motor	44 (33, 46)	31 (29, 32)	30 (27, 32)	31 (30, 34)	**0.000**	**0.000**	**0.000**

‡Mann-Whitney U test, * sepsis vs. control, †meningitis vs. control, $pneumonia vs. control

Table 51: Comparison of the BSID-III raw scores for each of the five neurocognitive domains at 12 months of age in infants who suffered from sepsis, meningitis and pneumonia vs. control infants


Cognitive raw score
80
60
40
20
0
Sepsis
Meningitis
Pneumonia
Control
Receptive language raw score
25
20
15
10
5
0
Sepsis
Meningitis
Pneumonia
Control
Expressive language raw score
25
20
15
10
5
0
Sepsis
Meningitis
Pneumonia
Control

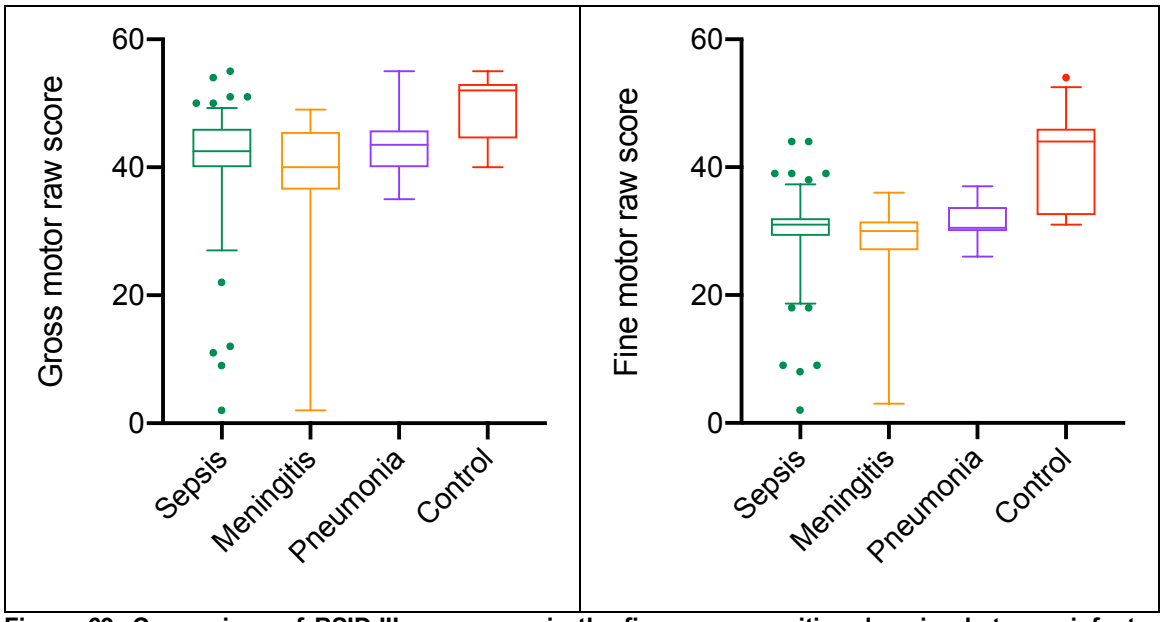


Figure 69: Comparison of BSID-III raw scores in the five neurocognitive domains between infants who suffered sepsis (green), meningitis (orange), pneumonia (purple) vs. control infants (red) at 12 months

In Table 52, the three composite scores for each diagnosis are compared. All three composite scores, for all three diagnoses, were significantly lower compared to the control cohort.

Neurocognitive domain	Controls median score (IQR) n=24	Sepsis median score (IQR), n=134	Meningitis median score (IQR) n=17	Pneumonia median score (IQR) n=12	P value‡*	P value‡†	P value‡$
Composite cognitive score	138 (131, 140)	125 (110, 135)	120 (80, 130)	125 (120, 130)	0.000	0.000	0.000
Composite language score	112 (99, 118)	100 (91, 109)	89 (77, 99)	97 (89, 106)	0.001	0.000	0.009
Motor composite score	154 (122, 154)	110 (94, 118)	94 (84, 117)	112 (98, 118)	0.000	0.000	0.000

‡Mann-Whitney U test, * sepsis vs. control, †meningitis vs. control, $pneumonia vs. control

Table 52: Comparison of the BSID-III composite scores at 12 months of age in infants who suffered from sepsis, meningitis and pneumonia vs. control infants

Survivors of neonatal meningitis had the highest rates of neurodevelopmental impairment, with rates of neurodevelopmental impairment of 24%, 35% and 24% in the cognitive, language and motor domains respectively (Table 53). In survivors of neonatal sepsis only, the rates of neurodevelopmental impairment were much lower at 7%, 11%, 14% in the cognitive, language and motor domains respectively. There were only 12 infants who were diagnosed with severe pneumonia and only one of them had a mild motor impairment. Neonatal meningitis was associated with a statistically significant increased risk of cognitive and language developmental delay compared to those with a diagnosis of pSBI without meningitis (Table 53). The risk of cognitive delay was 3.5-fold higher in those infants diagnosed with meningitis, whilst the risk of language delay was 2.5-fold higher. There was also a non-significant trend toward increased risk of motor delay.

Neurocognitive domain	Sepsis Total developmental delay <-1SD (n=134)	Meningitis, Total developmental delay <-1SD (n=17)	Pneumonia, Total developmental delay <-1SD (n=12)	RR* (95%CI)	P value
Composite cognitive score	9 (6.7)	4 (23.5)	0 (0)	3.50 (1.2, 10.2)	0.0209
Composite language score	19 (14.1)	6 (35.3)	0 (0)	2.49 (1.15, 5.36)	0.0197
Motor composite score	15 (11.2)	4 (23.5)	1 (8.3)	2.10 (0.78, 5.6)	0.1376

*Relative Risk (RR) meningitis vs. sepsis

Table 53：Rates of neurodevelopmental impairment at 12 months for sepsis, meningitis and pneumonia

When compared to controls at 12 months of age, those infants who suffered from neonatal meningitis had a 12 to 18-fold increased risk of neurodevelopmental

impairment across all domains (Table 54). The small numbers of both neonatal meningitis survivors and controls likely contribute to the borderline statistical significance (Table 54). Although there was also a trend towards increased risk of neurodevelopmental impairment across all domains for those neonates who suffered from sepsis only when compared to controls, it did not reach statistical significance. It is highly possible that given the challenges previously highlighted in achieving an accurate diagnosis of meningitis, that some of these infants actually had CNS involvement.

	All cases of pSBI 164		Neonatal meningitis only (n=17)		Neonatal sepsis only (n=134)		Severe pneumonia only (n=12)	
Neurocognitive domain	RR (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value	RR (95%CI)	P value
Composite cognitive score	4.09 (0.25, 66.7)	0.3225	12.50 (0.71, 217.90)	0.0833	3.52 (0.21, 58.5)	0.3805	1.92 (0.04, 91.5)	0.74
Composite language score	8.33 (0.52, 132.34)	0.1329	18.06 (1.08, 300.49)	0.0437	7.22 (0.45, 115.78)	0.1625	1.92 (0.04, 91.5)	0.74
Motor composite score	6.21 (0.39, 99.51)	0.1968	12.50 (0.71, 217.90)	0.0833	5.74 (0.35, 92.9)	0.2185	5.77 (0.25, 131.93)	0.2724

Table 54: Neurodevelopmental impairment outcomes at 12 months comparing survivors of three different diagnoses to controls

GROWTH

There was no significant difference between the LAZ, WAZ and WLZ mean scores between the pSBI survivors and the control infants at 2, 6 or 12 months of age (Table

55). However, the HCAZ was significantly lower in the pSBI survivors than the control infants at both 2 and 12 months of age (Table 55). When the rates of underweight, wasting and low head circumference were considered, the rates between the pSBI survivors and the control cohort were not significantly different from each other (Table 56 and Table 57). In addition, the rates of underweight and wasted infants did not change significantly over the first 12 months. The rates of stunting remained similar in the control cohort from 2 months until 6 months of age, however the rates of stunting in the pSBI survivors increased over time from 11.5% at 2 months to 17.9% at 6 months and 35.2% at 12 months. By 12 months of age the rate of stunting in the pSBI survivors was significantly higher than in the control cohort (35.2% vs. 8.3%, *p*=0.0085).

	pSBI cases Mean LAZ (SD)	Control cohort LAZ (SD)	P value	pSBI cases Mean WAZ (SD)	Control cohort Mean WAZ (SD)	P value	pSBI cases Mean WLZ (SD)	Control cohort Mean WLZ (SD)	P value	pSBI cases Mean HCAZ (SD)	Control cohort Mean HCAZ (SD)	P value
2 months	-0.08 (1.70)	0.44 (1.40)	0.1224	-0.08 (1.56)	-0.16 (1,19)	1.0000	0.18 (2.45)	-0.56 (1.56)	1.0000	0.86 (1.55)	1.54 (1.32)	0.028
6 months	-0.46 (1.59)	-0.48 (0.92)	1.0000	-0.10 (1.38)	-0.39 (0.96)	1.0000	0.34 (1.55)	-0.08 (1.20)	1.0000	0.75 (1.50)	1.17 (1.03)	0.157
12 months	-1.32 (1.79)	-1.08 (1.06)	0.5235	-0.29 (1.27)	-0.27 (1.05)	0.9416	0.49 (1.57)	0.34 (1.07)	1.000	0.40 (1.92)	1.27 (0.74)	0.029

Table 55: Comparison of mean LAZ, WAZ, WLZ and HCAZ scores between control infants and pSBI survivors

Age	pSBI cases low head circumference <-2SD, freq (%)	Control cohort low head circumference <-2SD, freq (%)	P value*
2 months	7/163 (4.3)	0/29 (0)	0.5972
6 months	6/149 (4.0)	0/28 (0)	0.5915
12 months	14/165 (8.5)	0/24 (0)	0.223

*fisher's exact test, two-sided

Table 56: Comparison of frequency of low head circumference between pSBI survivors and control infants

Age	pSBI cases Stunted <-2SD	Control cohort Stunted <-2SD	P value*	pSBI cases underweight <-2SD	Control cohort underweight <-2SD	P value*	pSBI cases Wasted <-2SD	Control cohort Wasted <-2SD	P value*
2 months	18/157 (11.5)	2/29 (6.8)	0.7743	13/158 (8.2)	2/29 (6.8)	0.9999	15/156 (9.6)	4/29 (13.8)	0.5069
6 months	27/151 (17.9)	2/28 (7.1)	0.2614	8/151 (5.3)	2/28 (7.1)	0.6572	9/151 (6.0)	2/28 (7.1)	0.6832
12 months	56/159 (35.2)	2/24 (8.3)	**0.0085**	11/159 (6.9)	3/24 (12.5)	0.4009	8/159 (5.0)	2/24 (8.3)	0.6226

*fisher's exact test, two-sided

Table 57: Comparison of frequency of stunting, underweight and wasting between pSBI survivors and control infants

MORTALITY AND POOR OUTCOME

When considering the composite poor outcome of post-neonatal mortality, neurodevelopmental impairment, post-infectious hydrocephalus and seizures, all infants with poor outcomes were survivors of pSBI. For the five infants with either post-infectious hydrocephalus or seizures with known outcome, all had severe developmental delay at 12 months. There were 4 post-neonatal deaths. There were 24 infants who had neurodevelopmental impairment in one or more of the three

composite scores. Overall 28/188 survivors of pSBI were known to have a poor outcome at 12 months of age compared to none (0/44) of the control infants at the same age. Even infants who survive the acute episode of pSBI have an increased risk of post-neonatal mortality, neurodevelopmental impairment and neurological disability at 12 months of age that is almost 14-fold higher than those who did not experience pSBI (RR 13.6, 95%CI 0.85 to 219.28, P=0.065).

DISCUSSION

It is well recognised that serious bacterial infections are a leading cause of neonatal mortality worldwide (Liu et al., 2016, Liu et al., 2015). In low-resource settings, the diagnosis is challenging due to non-specific signs and symptoms and limited availability of investigations. In such settings, the majority of clinicians rely on a clinical diagnosis of pSBI, therefore our analyses focus initially on survivors of pSBI compared to controls. This is one of the first studies to show, that the burden of pSBI in term neonates in this low-resource setting is not limited to neonatal mortality alone. This study focused on neonates >2000g and found that for term neonates who survive pSBI, there is an increased risk of post-neonatal mortality, developmental impairment and neurological disability at 12 months of age that is almost 14-fold higher than those who did not experience pSBI during the neonatal period.

Many cases of developmental impairment were not detectable at 2 months, this was not surprising as there are limited developmental tests that can be administered at such a young age. In fact, of the 8 infants with severe developmental impairment by 12 months of age, only one was detected at 2 months of age. By 6 months of age, the

majority of infants with developmental impairment were detected; all 8 of those infants with severe motor impairment at 12 months were detected at 6 months, 3/5 of those with severe language impairment were detected at 6 months.

Overall, there was a trend towards an increased risk of neurodevelopmental impairment at 12 months of age in pSBI survivors compared to controls in the cognitive, language and motor domains with a 4-fold, 8-fold and 6-fold increased risk respectively. In the sub-group analyses, the highest risk of neurodevelopmental impairment was in the survivors of neonatal meningitis. The rates of cognitive, language and motor delay were seen in 23.5%, 35.3% and 23.5% respectively. The majority of neonatal meningitis survivors had normal development at 12 months of age. The risk of developmental impairment was up to 18-fold higher than controls at 12 months of age.

Data on neonatal meningitis are primarily from HICs. Despite the reduction in the associated neonatal mortality in HICs, neurodevelopmental impairment is still estimated to occur in up to 35% of survivors, very similar to the rate of 35.3% we observed in this study (Seale et al., 2013). Two Nigerian studies reported moderate to severe impairment in 22% to 29% of survivors of neonatal meningitis and an Ethiopian study in 21% of survivors, all of these studies are very similar to our study where 23.5% of meningitis survivors had moderate to severe impairment (Airede, 1993, Airede et al., 2008, Gebremariam, 1998). An observational study in Malawi has reported neurodevelopmental impairment which was as high as 60% in neonatal meningitis survivors at 12 months of age; this is double what was observed in this study (Dube, 2014). This may be due to the different levels of neonatal care and differences in the

level of protein and white cell counts used to define a positive CSF. Their use of locally generated norms for BSID-III for the analysis of their study, may have increased the observed rates of developmental impairment compared to this study (Cromwell et al., 2014).

This study also demonstrated that although the risk of neurodevelopmental impairment was highest for those who had neonatal meningitis, there was a trend towards an increased risk, 3 to 7 times higher, of developmental impairment in survivors of sepsis alone compared to controls. In HICs, it is well documented in preterm infants, that survivors of neonatal sepsis have an increased risk of neurodevelopmental impairment of neurological disability (Mitha et al., 2013, Schlapbach et al., 2011, Stoll et al., 2004b). Preterm studies report up to a 2-fold increased risk of neurological disability or neurodevelopmental impairment in preterm infants that suffered from neonatal infections compared with uninfected preterm infants. A more recent retrospective study of 2677 term infants who suffered from confirmed or suspected neonatal sepsis, reported 1.7 times increased risk of neurodevelopmental impairment at 5 years of age for those children who suffered from confirmed sepsis (Savioli et al., 2018). Despite our population being >2000g and of term gestation, the risks of neurodevelopmental impairment were higher than those reported in both term and preterm infants in high-resource settings. This may be due to the differences in the level of neonatal care available or due to later presentations leading to neonates being sicker at presentation.

Globally, undernutrition underlies 45% of deaths in children under 5 years of age and linear growth failure, stunting, is the most prevalent form of undernutrition (Black et al.,

2013, UNICEF). This 'stunting syndrome' is associated with increased morbidity and mortality, neurodevelopmental impairment and an increased risk of metabolic disease later in life. Among neonates in LICs, the average length-for-age Z-score (LAZ) is -0.5 and continues to decline after birth, reaching a nadir at 18-24 months of age of -2.0 (Victora et al., 2010). In keeping with this, both our case and control groups a decline in LAZ was seen from 2 months until 12 months. In this study, at 12 months of age, there was a significantly increased risk of stunting in the survivors of pSBI compared to the controls, with 35.2% of pSBI survivors being stunted. Stunting is most responsive to interventions before 2-years of age and this study therefore highlights a high risk of group of children that would benefit from close growth monitoring and nutritional support. Although was not significantly different, it is important to note that all infants with a low head circumference at 12 months of age were survivors of pSBI. It is possible that microcephaly may be a predictor of poor outcome, as has been seen in survivors of hypoxic ischaemic encephalopathy in this setting (Tann et al., 2018). This relationship will be further explored in Chapter 9.

What is clear from this study is that serious bacterial infections during the neonatal period, even without meningitis, carry an increased risk of post-neonatal mortality, neurological disability and developmental impairment. Given the huge burden of neonatal pSBI in SSA, these neonatal infections carry a potentially huge public health and economic burden in SSA (Ranjeva et al., 2018). Further data are needed to evaluate this burden more accurately.

LIMITATIONS

There were limitations to this study. Firstly, participants were only followed-up until 12 months of age, and at this age there are a limited number of items that can be adequately tested. So, although by this age many moderate-severe impairments will be apparent, other developmental challenges that become more apparent late in life will have been missed. The rates of neurodevelopmental impairment in this study are likely to have been underestimated. Although a reasonable number of cases of sepsis were assessed, there were relatively few cases of meningitis and severe pneumonia included, thus the confidence intervals for these diagnoses are wide. In addition, although the outcomes of 90% of cases were known at 12 months of age, in the control group this value fell to only 55% and this may have introduced bias. Caregivers of the control infants were less interested in attending follow-up and despite multiple efforts to communicate with the caregivers and a small financial incentive to attend follow-up, many were reluctant to do so.

The developmental assessment tool that was used in this study, the BSID-III, was developed in the U.S. and there are concerns about the use of a psychometric test in a population that is was not validated in. There are currently no normative data available for the BSID-III assessments in healthy Ugandan children, therefore the scaled scores and composite scores in this study were generated using the BSID-III United States (U.S.) norms. Ideally one would use normative data from the population being studied. The same normative data was applied to our small control group thus allowing us to evaluate the relative differences in neurodevelopmental impairment between the cases and controls using both the raw and scaled scores. This proved to be vital in evaluating the impact of pSBI on developmental outcome since this study

observed mean scaled scores above the US mean in all subsets for both cases and controls at 2 and 6 months of age. By 12 months of age, although the mean scaled scores of the cases were closer to the US mean, the mean scaled scores for the control infants remained well above the US mean. Although this finding was unexpected, similar findings have been reported in Malawi that found relying on U.S. based normative data resulted in the misclassification of development (Cromwell et al., 2014). Crowell et al report only moderate agreement between US and Malawian norms, with higher raw scores for all subtests at younger ages (6-12 months) in the Malawian infants, especially cognitive and language skills. Given the high mean scaled scores in this study, it is possible that we have underestimated the rates of neurodevelopmental impairment, both in the cases and the controls. What remains very clear from this study is the significant differences between the cases and controls.

One must also consider if a developmental testing tool from a HIC is appropriate to use in a LIC in an African setting. Lack of familiarity with test demands, variations in education level and unfamiliar tools and toys in the test kit are some of the challenges. It may be more appropriate in future studies to use a culturally appropriate developmental assessment such as the Malawi Developmental Assessment Tool (MDAT) or the Kilifi Developmental Inventory (KDI) (Gladstone et al., 2010, Abubakar et al., 2008).

Although this study undertook a thorough developmental assessment at 2, 6 and 12 months of age, it formed part of a large multi-centre study, which did not include formal assessments for cerebral palsy, hearing impairment or visual impairment in the protocol. It is possible that children with cerebral palsy will still score well on a

developmental assessment and their neurological disability will go undetected and therefore unreported. Conversely, children with cerebral palsy may perform poorly in other domains because of motor impairments so their developmental impairment may be overestimated. It would be helpful to understand the contribution of these neurological disabilities to the rates of developmental impairment observed. In addition, children with cerebral palsy, hearing impairment and visual impairment can benefit from very specific early interventions.

CONCLUSION

This is one of the first studies to look at the post-neonatal outcomes of neonatal sepsis survivors in SSA and one of few to evaluate the outcomes of neonatal meningitis survivors. This study found that an episode of pSBI during the neonatal period increased the risk of developmental impairment. Although the risk was highest for the survivors of neonatal meningitis, this study demonstrated an increased risk of developmental impairment for those who suffered from neonatal sepsis alone. Although many hospitals provide routine follow-up and neurodevelopmental screening of preterm and meningitis survivors, neonatal sepsis survivors are rarely included. It will therefore be important to consider the incorporation of neurodevelopmental follow-up for survivors of neonatal sepsis into healthcare services so that developmental impairment can be detected early. The huge burden of neonatal sepsis in SSA will make the provision of such services challenging, however the introduction of early intervention programmes could have a huge benefit in reducing the rates impairment and disability.

CHAPTER 9 - CLINICAL, LABORATORY AND IMAGING PREDICTORS FOR POOR EARLY CHILDHOOD OUTCOME AMONG UGANDAN NEONATES WITH POSSIBLE SEVERE BACTERIAL INFECTION (PSBI)

BACKGROUND

Serious bacterial infections, such as sepsis, pneumonia and meningitis, are estimated to be a leading cause of neonatal mortality (Liu et al., 2016, Liu et al., 2015). The diagnoses of both neonatal sepsis and neonatal meningitis are challenging since signs and symptoms are often non-specific and laboratory facilities are limited particularly in low-resource settings. In many settings and in previous studies, the diagnosis of a pSBI relies on the young infant clinical algorithm, which is only 85% for pSBI and also does not apportion one of the three infection syndromes (sepsis, pneumonia and meningitis) (2008, Group, 1999b). Therefore, the outcomes of the individual syndromes are hard to estimate. In SSA there are believed to be up to 2.6 million cases of pSBI every year, leading to an estimated 250,000 deaths (Seale et al., 2014).

Even for neonates who receive appropriate and effective treatment for their pSBI, further estimates suggest survivors of these infections are also at an increased risk of developmental impairment and neurological disability, especially those who were born preterm or experienced neonatal meningitis (Stevens et al., 2003, Seale et al., 2013). The existing data focuses primarily on survivors of neonatal meningitis. In a recent meta-analysis of eight studies

including 451 cases of neonatal meningitis, moderate to severe neurodevelopmental impairment was estimated to occur in 23% of survivors (Seale et al., 2013). In a meta-analysis of five studies including 311 cases of neonatal meningitis, 12% of survivors were estimated to have mild impairment (Seale et al., 2013). Very few studies have conducted detailed developmental assessments of survivors of sepsis and pneumonia, and therefore data are still lacking on the incidence and severity of impairment following these infection syndromes, especially in term neonates.

As described in Chapter 8, this study has shown that in Ugandan term infants diagnosed with pSBI, there is an increased risk of post-neonatal mortality, post-infectious hydrocephalus, seizures and developmental impairment compared to well term infants (RR 13.6, 95%CI 0.85-219.28). At 12-months of age, compared to United States normative data, neurodevelopmental impairment was present across all five neurocognitive domains in survivors of pSBI with neurodevelopmental scores significantly lower in all five domains when compared to contemporarily recruited, well, term control infants. In this study, rates of developmental impairment were highest in those with meningitis, up to 35%, but even in those infants with sepsis alone, developmental impairment was present in up to 14%. This is similar to global estimates and other small studies of survivors of neonatal meningitis in SSA (Airede, 1993, Airede et al., 2008, Dube, 2014, Gebremariam, 1998, Seale et al., 2013). There are limited data on the neurodevelopmental outcome following neonatal sepsis, especially in term infants. Many studies have relatively short follow-up periods, focusing on the inpatient admission period

or the neonatal period only. The few data that are available demonstrate an increased risk of neurodevelopmental impairment following clinical neonatal sepsis, both in high and low-resource settings (Dube, 2014, Gordon et al., 2005, Savioli et al., 2018). More studies that incorporate detailed neurodevelopmental assessments of survivors of neonatal infections, especially neonatal sepsis, are needed to improve our understanding of their contribution to longer term neurodevelopmental impairment. This study was able to look at the early childhood outcomes of survivors of sepsis, meningitis and pneumonia using detailed developmental assessments up to 12 months of age.

Early detection of those neonates at risk of a poor outcome would help in providing effective and appropriate treatment during admission and also assist in the identification of infants who deserve early follow-up and intervention.

Previous studies have focused primarily on risk factors associated with poor outcomes following neonatal meningitis. There are a number of small and relatively old cohort studies from high income settings that have explored these risk factors. Seizures, presence of coma, poor feeding, respiratory distress, shock, elevated CSF protein, low peripheral white cell count and hearing impairment detected during hospitalization have previously been identified as indicators of poor prognosis, including death, post-infectious hydrocephalus or neurological sequelae (Lin et al., 2012, Kornelisse et al., 1995, Tan et al., 2015, Daoud et al., 1996, Klinger et al., 2000). There are few data available on risk factors for survivors of neonatal sepsis, with many studies having very

short follow-up periods. Some studies have looked at the association of clinical signs with very severe disease (death, oxygen saturation <90% on air, positive blood or CSF culture) (Duke et al., 2005, Bang et al., 2005, English et al., 2004). Clinical signs from previous studies that have been shown to be strongly associated with very severe disease include; history of difficult feeding, pallor, cyanosis, apnoea, hypothermia, bulging fontanelle, unconsciousness, lethargy and abnormal movements (Duke et al., 2005, Bang et al., 2005, English et al., 2004). Low peripheral white blood cell count, positive blood culture and CSF culture have also been shown to be risk factors for death (Group, 1999a). More recently, a clinical trial of treatment for pSBI in infants <60 days in Malawi found; weight <2500g on admission, convulsions, inability to feed, low oxygen saturations <90% and positive blood culture to be significantly associated with mortality and neurological sequelae including hearing loss, blindness, developmental delay and hydrocephalus (Molyneux et al., 2017).

In preterm infants, the relationship between infection during the neonatal period and adverse neurodevelopmental outcomes is well documented (Shah et al., 2008, Stoll et al., 2004b, Schlapbach et al., 2011, Mitha et al., 2013). There are a growing number of studies demonstrating a relationship between white matter injury and sepsis in preterm infants (Heo et al., 2018, Shah et al., 2008, Graham et al., 2004, Glass et al., 2008, Vermeulen et al., 2001). It is therefore likely that the effect of infection on the neurodevelopment of preterm infants is partly mediated by white matter injury (Shah et al., 2008). Although there are no studies reporting brain imaging in term infants with sepsis, a study

of term and preterm infants with CNS infection in Holland found a correlation between increased echogenicity in the periventricular and/or deep white matter, ventricular dilatation and mortality (de Vries et al., 2006). Our study demonstrated a variety of abnormalities on cranial ultrasound in neonates who present with pSBI, including increased echogenicity in the cortex, white matter, basal ganglia and thalami as well as ventricular dilatation, strands and debris and bright ventricular lining (Chapter 6). Compared to control neonates, there was significantly more ventricular pathology, lenticulostriate vasculopathy and increased extracerebral space and a trend towards an increased incidence of white matter and cortical hyper echogenicity (Chapter 6). This study is the first study to report the findings on brain imaging in term infants presenting with pSBI, and to explore the relationship between these findings and early childhood outcome.

OBJECTIVE

The aim of this thesis was to explore the clinical, laboratory and imaging risk factors that predict a poor early childhood outcome including mortality, neurological disability and developmental impairment, in a cohort of infants who suffered from an episode of pSBI during the neonatal period in a low-resource setting.

METHODS

SETTING

As previously described in Chapter 5, this study was undertaken at MRRH, which serves a population of 4.5 million people and has a dedicated NNU that admits over 2500 neonates a year (Burgoine et al., 2018, Okello et al., 2019). Neonates are admitted directly from the labour ward, referred from surrounding health facilities and, due to a high rate of home deliveries, some neonates are brought in directly from home (UBoSUa, 2017). Care in MRRH-NNU includes oxygen therapy, bubble continuous positive airways pressure (bCPAP), intravenous fluids, antibiotics and anti-seizure medication.

STUDY DESIGN

This study was a hospital-based prospective cohort study of outcomes at 12 months of age amongst term-born infants who were all known to have suffered an episode of pSBI during the neonatal period and were recruited at presentation with pSBI to MRRH-NNU. Neonates were recruited over a 12-month period from 9th December 2016 until 8th December 2017. Additional details of recruitment are described in Chapter 5.

Inclusion criteria included weight >2000g, maternal age ≥18 years and ability of the mother to provide informed consent. Neonates with congenital abnormalities, use of parenteral antibiotics for 24 hours or more prior to recruitment, history of perinatal asphyxia (Apgar score <6 at 5 minutes after birth or failure to cry) and inability of the mother to speak one of the local

languages (English, Luganda, Lumasaba, Ateso or Lugwere) well enough to provide informed consent, were excluded.

We aimed to assess all infants at 2, 6 and 12 months of age during a 14-month period from December 2017 until Feb 2019. Assessments were done by a team of 3 research doctors and two research nurses. Measures were taken to ensure the best rate of follow-up possible, including Short Message Service (SMS) appointment reminders, phone calls a few days prior to the appointment, refunding of transport costs, and rebooking of those who did not attend. Infants not successfully contacted by phone were traced in the community by the research team and either assessed at home or given transport costs to attend clinic.

DATA COLLECTION

Socio-demographic characteristics, antenatal and perinatal data and neonatal clinical information were gathered at the time of recruitment as described in Chapter 5. At recruitment, each neonate had blood drawn for culture. A lumbar puncture was also performed on all neonates when not contraindicated. CSF was cultured and analysed for white cell count, protein and glucose levels. Meningitis was defined as a positive CSF culture and/or raised protein concentration above 127mg/dl and/or white blood cell count above 15cells/mm^3 (Thomson et al., 2018). The lower bound value for glucose concentration was 25 mg/dL (1.4mmol/L) (Thomson et al., 2018). Further details of these procedures are explained in Chapter 5.

As described in Chapter 6, each neonate had a cranial ultrasound (cUS) examination performed by one of four trained clinicians at recruitment. The cUS protocol is described in detail in Chapter 1 and included a minimum of 5 coronal views, one midline sagittal, two left and two right parasagittal views via the anterior fontanelle. The linear probe was also used to image the cortex and the Doppler resistive index was recorded from the anterior cerebral artery. The cUS data were then downloaded and stored digitally as DICOM images. The images were analysed using OsiriX 10.0.5 software by one of two consultant neonatologists with extensive experience in neuroimaging, who were blinded to the neonate's clinical data and outcome. The images were assessed systematically for abnormalities as defined in Chapter 6. Following a consensus decision between the two consultant neonatologists, a cranial ultrasound scoring system to evaluate the severity of any abnormalities noted in each location was developed (Figure 70). The total cranial ultrasound score was a combination of the individual scores. The minimum score for a cranial ultrasound scan, when all findings were normal, was 10. The maximum score was 34.

Location/abnormality	Description	Score
Ventricle score	Consider each of shape of ventricle, prominent choroid in 3rd ventricle, ventricular debris, ventricular strands, ventricular dilatation, bright ventricular margin. Each abnormality scores 1. Min 1, max 7.	1 = no abnormality 2 = 1 abnormality 3 = 2 abnormalities 4 = 3 abnormalities 5 = 4 abnormalities 6 = 5 abnormalities 7 = 6 abnormalities
Cortical score	Min 1, max 4.	1 = normal 2 = more than 50% highlighting 3 = increased echogenicity 4 = subcortical cystic change
White matter score	Min 1, max 5.	1= mild trigonal flare (normal) 2 = parietal, occipital or frontal echogenicity 3 = diffuse, patchy or locally echogenicity 4 = globally abnormal 5 = multicystic encephalomalacia
Basal Ganglia	Min 1, max 3.	1 = normal 2 = swollen and/or focal abnormality 3 = severely abnormal
Thalami	Min 1, max 3.	1 = normal 2 = swollen and/or focal abnormality 3 = severely abnormal
Posterior limb of the internal capsule	Min 1, max 2.	Absent = 1 Present = 2
Extracerebral space echogenicity	Min 1, max 2.	1 = normal 2 = echogenic
Extracerebral space size	Min 1, max 2.	1 = normal 2 = enlarged
Lenticulostriate vasculopathy	Min 1, max 2.	Absent = 1 Present = 2
Cysts	Min 1, max 4. Choroid plexus, caudothalamic notch, subependymal cysts. Score 1 for each location.	1 = no cysts 2 = one location 3 = two locations 4 = three locations
Total score	Normal Scan, minimum score 10 Maximum score 34	

Figure 70: The scoring protocol for the neonatal cranial ultrasound scans

Development was assessed using the Bayley Scales of Infant Development-3rd edition (BSID-III) (Johnson et al., 2014, Bayley, 2006). The BSID-III is recognised as a comprehensive tool for assessing infant development in the following domains; fine and gross motor skills, cognition and expressive and receptive language. Composite scores were derived from the various sums of the subtest scaled scores to create the Composite Cognitive, Composite Language and Composite Motor Scores. Each of these three composite scores were then scaled to a metric with a mean of 100, a SD of 15 and a range of 40 to 160. For each composite score, mild neurodevelopmental delay was defined as score <85, moderate neurodevelopmental delay was defined

as a score <70 and severe delay was defined as a score <55. Further details of the developmental assessments are described in Chapter 8.

Growth was assessed by measuring the head circumference, length and nude weight at recruitment and at each follow-up visit. Weights were recorded using an electronic weighing scale (SECA 354) with reading increments of 10g. Length was measured using a plastic measuring mat (SECA 210) and measured to the nearest 0.5cm and head circumference was measured using a plastic tape to the nearest 1mm. All growth data were collected by the research doctors and nurses who had been trained prior to the start of the study. Weight-for-corrected-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ) z-scores and head-circumference-for-age (HCAZ) were derived using WHO child growth standards (WHO, 2006). The programme Anthro, available from the WHO website was used to create these indices (https://www.who.int/childgrowth/software/en/). A cut-off of -2SD for these indices was used to define infants as wasted (WLZ <-2SD), underweight (WAZ <-2SD), stunted (LAZ <-2SD) and low head circumference (HCAZ <-2SD).

Statistical analysis

Poor outcome was defined as either death, post-infectious hydrocephalus, post-neonatal seizures or developmental impairment at 12-months of age.

Variables that have previously been associated with poor developmental outcome were considered in the analysis and included: presence of seizures, bulging fontanelle, pallor, cyanosis, apnoea, hypothermia, abdominal

distension, positive blood or CSF culture, maternal education, mode of delivery, place of delivery, male sex, 5-minute Apgar score <7 and need for resuscitation at delivery. For neuroimaging, each of the ten individual scores for the separate locations and abnormalities were considered as normal or abnormal in the analysis.

Data were analysed using IBM SPSS Statistics Version 25. All categorical risk factors for poor outcome were tabulated and are presented as frequencies and percentages for those with and without poor outcome. Categorical variables were examined using Chi-squared test and Fisher's exact test as appropriate for sample size. All continuous risk factors were tabulated and are presented as median and interquartile range or mean and standard deviation according to normality. Comparison of continuous variables between those with and without poor outcome was made with either Mann-Whitney U test or students T-test as appropriate.

Univariate (binary) logistic regression was done to estimate the effect of each factor on dichotomized outcome (poor outcome or well) at 12 months of age. Unadjusted odds ratios with 95% confidence intervals were calculated using logistic regression with poor outcome at 12 months of age as the outcome and risk factors as covariates of interest. Adjusted odds ratios with 95% confidence intervals were reported as the strength of association between the risk factors and poor outcome after adjusting for continuous measures of age at presentation and weight at presentation as well as neonatal sex. Lastly, risk

factors with a p value <0.05 at univariate analysis were entered into a stepwise multivariate logistic regression.

RESULTS

A scan was performed on day 1 of presentation in 196 infants with pSBI. At 12 months of age the outcomes of 90.3% (177/196) were known. Four infants had been withdrawn from the study by their parent/guardian and 15 infants were not traceable in the community.

OUTCOMES

Of those with known outcomes (n=177), the infant mortality was 11.9% (21/177). Developmental delay (<-1SD) in one component was present in 10.2% (18/177) of infants, most commonly language. In 1.1% (2/177) of infants there was delay in two of the three composite scores and in 6.8% (12/177) of infants there was developmental delay in all three composite scores. Three infants (1.6%) were reported to be suffering from seizures at 12 months, all three of these infants had developmental impairment. Three infants developed post-infectious hydrocephalus. Two of these three infants had severe developmental impairment at 12 months of age; one infant was not available for follow-up at 12 months but severe developmental delay was observed when assessed at 2 months of age. Overall, 30.5% (54/177) of pSBI survivors had a poor outcome by 12 months of age.

COMPARISON OF THE RISK FACTORS PRESENT IN THOSE WITH AND WITHOUT POOR OUTCOME

Comparison of the clinical and demographic features of the neonates and their mothers who did and did not have a poor outcome are depicted in Table 58 and Table 59. Maternal-related features were similar between infants with and without a poor outcome (Table 58) including; maternal age, level of education and class of employment. There was a higher rate of mothers who were housewives in the group of infants with a poor outcome (49.1% vs. 30.6%, p=0.0261).

There was a trend towards increased non-facility-based delivery and delivery by a non-skilled birth attendant in those with a poor outcome. Those with poor outcome were also significantly more likely to have had a spontaneous vertex delivery (SVD, 79.6% v. 62.6%, p=0.0354). The Apgar score was known for 63% of infants and for all of these infants the Apgar score at 5 minutes was ≥7.

Variable	Well infant N = 123	Poor outcome N = 54	P value±
Maternal-related			
Maternal age (years)[a]	25 (22, 29)	24 (20, 28)	0.167*
Education, N=173, n (%)			
≤ Primary	48/121 (39.7)	22/52 (42.3)	0.8683
Secondary	39/121 (32.2)	21/52 (40.4)	0.3905
Tertiary	34/121 (28.0)	9/52 (17.3)	0.1315
Employment			
Housewife	38 (30.9)	26 (48.1)	0.0409
Farmer	31 (25.2)	12 (22.2)	0.7083
Service or sales	34 (27.6)	11 (20.4)	0.3524
Professionals	20 (16.3)	6 (11.1)	0.4908
Place of delivery			
Facility (hospital, health centre, clinic)	116 (94.3)	46 (85.2)	0.0743
Home or before arrival	7 (5.7)	8 (14.8)	
Birth attendant			
Skilled birth attendant	115 (93.5)	45 (83.3)	0.0504
Traditional birth attendant, self or relative	8 (6.5)	9 (16.7)	
Mode of delivery			
Emergency caesarean-section	41 (33.3)	9 (16.7)	**0.0291**[b]
Elective caesarean-section	4 (3.3)	1 (1.9)	1.000
Spontaneous vertex delivery	77 (62.6)	43 (79.6)	**0.0354**[b]
Assisted vaginal	1 (0.8)	1 (1.9)	0.5183
Apgar score at 5 minutes (n=112)			
≥7	112/112 (100)	44/44 (100)	
HIV exposed	2 (1.6)	2 (3.7)	0.5865

[a] Continuous variable expressed as median and interquartile range. [b] Significant results.
± χ^2 or Fishers exact test for categorical variables. *Mann-Whitney U test for continuous variable.

Table 58: The maternal-related features of those infants with and without poor outcome by 12 months of age

As shown in Table 59, those with a poor outcome at 12 months of age presented to the neonatal unit at an older age (day 4 vs. day 2, p=0.000). Only 37.0% (20/54) of those with a poor outcome presented within 48 hours of birth compared to 74.8% (92/123) of those who were well at 12 months (p=0.000). The sex distribution, weight and length at presentation were all similar between the two groups. Those neonates with a poor outcome were significantly more likely to have been irritable and have had seizures, opisthotonus and respiratory distress at presentation. They were also more likely to have been less than 2500g and tachycardic (>180 beats per minute).

Variable	Normal outcome N = 123	Poor outcome N = 54	P value [c]
Neonate-related			
Male sex	74 (60.2)	33 (61.6)	1.000
Age at presentation [b]	2.0 (1.0, 3.0)	4.0 (2.0, 7.25)	**0.000**[d]
<48 hours, n(%)	92 (74.8)	20 (37.0)	**0.000**
Admission weight (kg)[a]	3.08 (0.51)	2.91 (0.58)	0.087[e]
Admission weight <2.5kg	14 (11.4)	13 (24.1)	0.041
Admission length (cm)[a]	51.2 (9.2)	49.7 (2.7)	0.250[e]
Admission head circumference (cm) [b]	35.6 (1.4)	36.0 (1.6)	0.111[e]
Temperature (°C)[a]	38.9 (1.0)	38.5 (1.1)	**0.023**[e]
Hypothermia, <36°C at admission	2 (1.6)	1(1.9)	1.000
Respiratory rate (breaths per minute)[a]	62 (24)	57 (21)	0.175[e]
Respiratory rate > 60 breaths per minute	62/118 (52.5)	31/51 (60.8)	0.132
Heart rate (HR, beats per minute)[a]	142 (22)	152 (33)	**0.018**[e]
Heart rate >180 beats per minute	7 (5.7)	11 (20.4)	**0.006**
Pallor	3 (2.4)	3 (5.6)	0.3710
Cyanosis	1 (0.8)	1 (1.9)	0.5183
Skin pustules	4 (3.3)	3 (5.6)	0.4375
Seizures	8 (6.6)	27 (50.0)	**0.000**
Respiratory distress	15 (12.2)	16 (29.6)	**0.013**
Abdominal distension	6 (4.8)	3 (5.6)	1.000
Bulging fontanelle	15 (12.2)	12 (22.2)	0.131
Opisthotonus	6 (4.9)	20 (37.0)	**0.000**
Irritability	66 (53.7)	20 (37.0)	**0.034**
Apnoea	0 (0)	2 (3.7)	0.120
Hypotonic	11 (8.9)	8 (14.8)	0.293
Jaundice	68 (55.3)	23 (42.6)	0.087

[a] Continuous variable expressed as mean ±SD. [b] Continuous variables expressed as median and interquartile range. [c] Fishers exact test or χ^2 for categorical variables. [d] Mann-Whitney U test. [e] student's t-test

Table 59: The neonatal-related features of those infants with and without poor outcome by 12 months of age

There was no difference in the rates of positive blood cultures between the two groups (Table 60). None of the infants had a positive CSF culture but when CSF analysis was considered, those with a poor outcome were more likely to have a raised protein over 127mg/dl, than the well infants (21.4% vs. 2.7%, p=0.001). There was no significant difference between those with raised white cell counts and low glucose levels in their CSF analysis.

Variable	Normal outcome N = 123	Poor outcome N = 54	p
Laboratory-related			
Positive blood culture	13 (10.6)	5 (9.6)	1.000
CSF raised protein over 127mg/dl (n=152)	3/110 (2.7)	9/42 (21.4)	**0.001**
CSF raised white cell >15cells/mm (n=156)	8/114 (7.0)	4/42 (9.5)	0.735
CSF low glucose <1.4mmol/dl (n=157)	5/114 (4.4)	4/43 (9.3)	0.259

[±] Fishers exact test for categorical variables.

Table 60: The laboratory-related features of those infants with and without poor outcome by 12 months of age

Cranial ultrasound scans were undertaken on the day of presentation to the neonatal unit. As shown in Table 61, those infants with poor outcome were significantly more likely to have abnormal findings in the cortex (p=0.0002), white matter (0.0491), thalami (p=0.0004) and basal ganglia (0.0001). Those infants with poor outcome were also more likely to have increased extra-cerebral space (p=0.019). The total cranial ultrasound score was significantly higher in those with poor outcome compared to the well infants (p=0.008). There was no significant difference in the proportion of infants with a normal cranial ultrasound scan (score of 10), between the two groups (p=0.3910). Overall only 4.9% (6/123) infants with a good outcome at 12 months had moderate or severe abnormalities in the WM, cortex, basal ganglia and/or thalami at presentation compared to 48.1% (26/54) of infants with a poor outcome at 12 months (p<0.0001).

Variable	Well infant N = 123	Poor outcome N = 54	P value
Ventricular Score 1-6			
1 = normal	92 (74.8)	34 (63.0)	0.1487
2 = 1 abnormality	26 (21.1)	16 (29.6)	
3 – 2 abnormalities	5 (4.1)	3 (5.6)	
4 – 3 abnormalities	0	0	
5 – 4 abnormalities	0	1 (1.9)	
6 – 5 abnormalities	0	0	
Cortical score: min 1, max 4			
1 = normal	119 (96.0)	42 (79.2)	**0.0002**
2= more than 50% highlighting	4 (3.3)	6 (11.1)	
3 = increased echogenicity	0	4 (7.4)	
4 = subcortical cystic change	0	1 (1.9)	
5 = focal	0	1 (1.9)	
White matter score: min 1, max 5			
1= mild trigonal flare (normal)	75 (60.5)	24 (45.3)	**0.0491**
2 = parieto-occipital or frontal echogenicity	45 (36.3)	17 (32.1)	
3 = diffuse, patchy or locally echogenicity	3 (2.4)	7 (13.2)	
4 = globally abnormal	0 (0)	5 (9.4)	
5 = multicystic encephalomalacia	0 (0)	1 (1.9)	
Basal Ganglia (1-3)			
1 – normal	121 (98.4)	44 (81.5)	**0.0001**
2 – swollen	1 (0.8)	1 (1.9)	
3 – severely abnormal	1 (0.8)	9 (16.7)	
Thalami (1-3)			
1 – normal	117 (95.1)	41 (75.9)	**0.0004**
2 – swollen	2 (2.0)	1 (1.9)	
3 – severely abnormal	4 (3.3)	12 (22.2)	
Posterior limb of the internal capsule (1-2)			
1= absent	123/123 (100)	52/54 (96.3)	0.092
2 = present	0/123 (0)	2/54 (3.7)	
Extracerebral space size (1-2)			
1= normal	114/123 (92.7)	43/54 (79.6)	**0.019**
2 = increased	9/123 (7.3)	11/54 (20.4)	
Extracerebral space echogenicity (1-2)			
1= normal	116/123 (94.3)	48/54 (88.9)	0.220
2 = increased	7/123 (5.7)	6/54 (11.1)	
Lenticulostriate vasculopathy (1-2)			
1= absent	85/123 (69.1)	44/54 (81.5)	0.183
2 = present	38/123 (30.9)	10/54 (18.5)	
Cysts (1-3)			
1= none	85 (69.1)	46 (85.1)	**0.026**
2 = one location	33 (26.8)	8 (14.8)	
3= 2 locations	5 (4.1)	0 (0)	
3 = 4 locations	0	0	
Total cranial ultrasound score			
Median (IQR)	11.0 (11.0, 12.0)	12.0 (11.0, 14.25)	**0.008**
Range	10 to 17	10 to 25	
Normal cranial ultrasound (score = 10)	24/123 (19.5)	7/54 (13.0)	0.3910

± Fishers exact test for categorical variables.

Table 61: Findings on ultrasound for those infants with and without a poor outcome at 12 months of age

A receiver operating curve (ROC) analysis to predict poor outcome by the total cranial ultrasound score was conducted (Figure 71). The area under the curve (AUC) predicting poor outcome was 0.623. The co-ordinates of the ROC for the total ultrasound scores are shown in Table 62. Although a total score ≥10.5

yielded a sensitivity of 87%, the corresponding specificity was only 19.5%. If a total imaging score of ≥13.5 was used to predict poor outcome the sensitivity dropped to 42.6%, however the specificity rose to 77.2%.

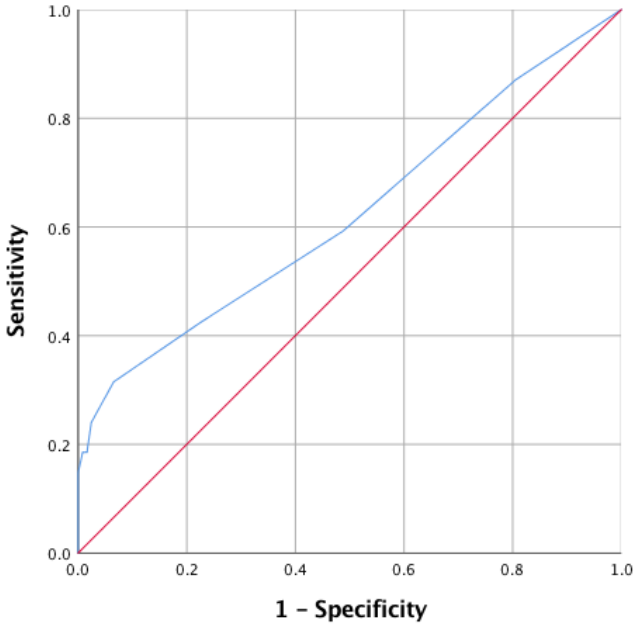


Figure 71: Receiver operating curve for total ultrasound score predicting poor outcomes in neonates with pSBI. Sensitivity is plotted against 1-specificity. The area under the curve was 0.623.

Total ultrasound score	Sensitivity	1 - Specificity
≥10.5	1.000	1.000
≥11.5	0.870	0.805
≥12.5	0.593	0.488
≥13.5	0.426	0.228
≥14.5	0.315	0.065
≥15.5	0.241	0.024
≥16.5	0.185	0.016
≥17.5	0.185	0.008
≥18.5	0.148	0.0
≥19.5	0.111	0.0
≥20.5	0.074	0.0
≥22.5	0.056	0.0
≥24.5	0.037	0.0

Table 62: Co-ordinates of the ROC for the corresponding total ultrasound scores

Growth was assessed by measuring the head circumference, length and nude weight at recruitment and at each follow-up visit. Anthropometric measurements were only available for those infants who were alive at the time

of the appointment. Comparison of the WAZ, WLZ, LAZ, HCAZ of the neonates who did and did not have a poor outcome at 12 months of age are depicted in Table 63 (WHO, 2006). There were significantly more infants with microcephaly amongst infants with a poor outcome (21.2%) compared to those infants who were well at 12 months (4.9%, p=0.0069). There was also at trend towards a higher rate of underweight infants in those with a poor outcome (16.1%), compared to those with a normal outcome (5.1%, p=0.0516).

Variable	Normal outcome	Poor outcome	P value
12 month follow-up			
WAZ <-2SD, underweight	6/118 (5.1)	5/31 (16.1)	0.0516
LAZ <-2SD, stunted	38/118 (32.2)	14/31 (45.2)	0.2063
WLZ <-2SD, wasted	4/117 (3.4)	3/31 (9.7)	0.1600
HCAZ <-2SD, microcephaly	6/123 (4.9)	7/33 (21.2)	**0.0069**

[±] Fishers exact test for categorical variables.

Table 63: Comparison of underweight, stunting, wasting and microcephaly between infants with and without a poor outcome at 12 months of age

UNIVARIATE ANALYSIS OF RISK FACTORS FOR POOR OUTCOME AT 12 MONTHS OF AGE

As shown in Table 64, in the univariate analysis of maternal risk factors associated with poor outcome at 12 months of age, delivery by a skilled birth attendant was associated with a 65% reduction in poor outcome (OR 0.348, 95%CI 0.126-0.958) and spontaneous vertex delivery was associated with a 2-fold increased risk (OR 2.335, 95%CI 1.096-4.975). In the univariate analysis of neonatal factors, early presentation (<48 hours after birth) was associated with an 80% reduction in poor outcome (OR 0.198, 95%CI 0.100-0.394). The presence of neonatal seizures was associated with a 13.9-fold increase in poor outcome, respiratory distress was associated with 2.9-fold increase (95%CI 1.316-6.474) and opisthotonus was associated with a 11.4-fold increase

(95%CI 4.236-30.759). The presence of irritability had a significant risk reduction (OR 0.472, 0244-0.914). Infants who were underweight at 12 months of age were significantly more likely to have a poor outcome (OR 3.58, 95%CI 1.01 – 12.67). Microcephaly (HCAZ<-2SD) at 12 months of age, was associated with a 5.3 fold increase in poor outcome (95%CI 1.62 – 16.92).

	Univariate analysis		
Maternal-related	**OR**	**95% CI**	**p-value[b]**
Maternal age	0.964	0.907 – 1.025	0.242
Education ≤primary	0.897	0.464 – 1.734	0.746
Facility-based delivery	0.347	0.119 – 1.012	0.053
Skilled birth attendant	0.348	0.126 – 0.958	0.041[a]
Spontaneous vertex delivery	2.335	1.096 – 4.975	0.028[a]
HIV exposed	4.692	0.416 – 52.891	0.211
Neonate-related			
Male sex	0.961	0.499 – 1.851	0.905
Early onset disease <48 hours after birth,	0.198	0.100 – 0.394	0.000[a]
Admission weight <2500g	2.469	1.070-5.695	0.034[a]
Admission weight (g),	0.539	0.285 – 1.017	0.056
Admission length (cm)	0.880	0.763 – 1.016	0.081
Admission head circumference (cm)	1.209	0.956 – 1.528	0.112
Temperature, (°C)	0.028	0.509 – 0.962	0.028[a]
Hypothermia, <36°C at admission	1.142	0.101 – 12.864	0.915
Heart rate >180 beats per minute	4.203	1.540-11.542	0.005[a]
Heart rate (HR, beats per minute)	1.015	1.002 – 1.029	0.020[a]
Respiratory rate >60 breaths per minute	0.583	0.299-1.137	0.113
Respiratory rate (RR, breaths per minute)	0.989	0.974 – 1.005	0.177
Seizures	13.875	5.675 – 33.925	0.000[a]
Respiratory distress	2.919	1.316 – 6.474	0.008[a]
Bulging fontanelle	1.981	0.856 – 4.586	0.110
Opisthotonus	11.414	4.236 – 30.759	0.000[a]
Irritability	0.472	0.244 – 0.914	0.026[a]
Hypotonic	1.455	0.137 – 1.781	0.626
Jaundice	0.556	0.290 – 1.066	0.077
Laboratory-related			
Positive blood culture	0.863	0.292 – 2.555	0.791
Raised protein over 127mg/dl (n=152)	9.727	2.487 – 38.039	**0.001[a]**
Raised white cell >15cells/mm (n=156)	1.395	0.397 – 4.898	0.604
Low glucose <1.4mmol/dl (n=157)	2.236	0.571 – 8.753	0.248
Radiology-related			
Ventricular Score >1	1.746	0.879 – 3.467	0.111
Cortical score >1	8.500	2.599 – 27.801	0.000[a]
White matter score >1	1.953	1.022 – 3.732	0.043[a]
BG score >1	13.75	2.898 – 65.231	0.001[a]
Thalamic score>1	6.183	2.206 – 17.330	0.001[a]
Extracerebral space increased	3.240	1.255 – 8.363	0.015[a]
Extracerebral space hyperechogenic	2.071	0.662 – 6.484	0.211
Lenticulostriate vasculopathy	0.508	0.232 – 1.116	0.092
Cysts in one or more location	0.389	0.168 – 0.903	0.028[a]
Growth at 12 months			
WAZ <-2SD, underweight	3.589	1.01 – 12.67	0.047[a]
LAZ <-2SD, stunted	1.734	0.77 – 3.88	0.181
WLZ <-2SD, wasted	3.027	0.64 – 14.30	0.1622
HCAZ <-2SD, microcephaly	5.250	1.62 – 16.92	0.006[a]

[a] Significant result at univariate analysis [b]Univariate logistic regression p value

Table 64: Unadjusted odds ratios for poor outcome at 12 months of age

MULTIVARIATE ANALYSIS OF RISK FACTORS FOR POOR OUTCOME AT 12 MONTHS OF AGE

An adjusted logistic model of the odds for poor outcome at 12 months of age that included neonatal sex, weight at presentation and age at presentation is presented in Table 65. After adjustment, the following factors were associated with poor outcome at 12 months of age: presentation at >48 hours of age, respiratory distress, seizures, opisthotonus, hypotonia, weight <2500g, heart rate >180 beats per minute and raised CSF protein level were all found to increase the odds of having a poor outcome by 12 months of age. The aOR for presentation before 48 hours of age was 0.2 (95%CI 0.1-0.4) and for admission weight <2500g was 2.6 (95%CI 1.1-6.1). The aOR for poor outcome was 2.7(95%CI 1.2-6.2) when respiratory distress was present at admission compared to those without. Neonates with seizures and opisthotonus also had increased odds of poor outcome aOR 13.0 (95%CI 5.2-32.4) and aOR 9.5 (95%CI 3.5-27.0) respectively. Similarly, those with hypotonia at presentation had increased odds of a poor outcome, aOR 3.0 (95%CI1.1-8.3). When considering CSF analysis after adjustment, an elevated protein level increased the odds of having a poor outcome, aOR 9.5 (95%CI 2.3-38.6).

When considering findings on cranial ultrasound examination at presentation, the following findings were significantly associated with a poor outcome by 12 months of age: abnormal cortex (aOR 6.9, 95%CI 2.0-23.5), abnormal white matter (aOR 2.0, 95%CI 1.0-3.9), abnormal basal ganglia (aOR 13.6, 95%CI 2.7-68.2) and abnormal thalami (aOR 5.28, 95%CI 1.8-15.2). The presence of

innocent cysts in one or more location was associated with a reduced risk of poor outcome (aOR 0.3, 95%CI 0.1-0.8).

Infants with poor outcome at 12 months of age, were significantly more likely to have microcephaly (HCAZ<-2SD) than those infants who were well (aOR 5.609, 95%CI 1.612-19.518). The presence of stunting or wasting or being underweight at 12 months of age was not associated with poor outcome.

Maternal-related	**aOR**	**95% CI**	**p-value**
Maternal age	0.972	0.914-1.034	0.376
Education ≤primary	1.051	0.523-2.11	0.888
Facility-based delivery	1.867	0.854-5.971	0.293
Skilled birth attendant	0.533	0.179-1.590	0.533
Spontaneous vertex delivery	1.934	0.881-4.248	0.100
HIV exposed	6.613	0.570-76.721	0.131
Neonate-related			
Male sex	0.971	0.462-1.823	0.805
<48 hours, n(%)	0.181	0.075-0.438	**0.000**[a]
Admission weight <2500g	2.560	1.072-6.113	**0.034**[a]
Admission length (cm)	0.915	0.775-1.080	0.293
Admission head circumference (cm)	1.673	1.216-2.303	**0.002**[a]
Temperature, (°C)	0.756	0.539-1.061	0.105
Hypothermia <36°C	1.127	0.088-14.417	0.927
Heart rate >180 beats per minute	2.972	1.001-8.824	0.050[a]
Heart rate (HR, beats per minute)	1.011	0.998-1.025	0.109
Respiratory rate >60 breaths per minute	0.511	0.250-1.044	0.066
Respiratory rate (RR, breaths per minute)	0.987	0.971-1.004	0.127
Respiratory distress	2.682	1.155-6.229	**0.022**[a]
Seizures	12.978	5.198-32.402	**0.000**[a]
Bulging fontanelle	1.648	0.680-3.995	0.269
Opisthotonus	9.474	3.511-27.060	**0.000**[a]
Irritability	0.468	0.236-0.926	0.029
Hypotonic	2.966	1.056-8.334	0.039[a]
Jaundice	1.954	0.713-5.358	0.193
Laboratory-related			
Positive blood culture	0.924	0.302-2.828	0.890
Raised protein over 127mg/dl (n=152)	9.517	2.346-38.615	**0.002**[a]
Raised white cell >15cells/mm (n=156)	1.372	0.379-4.965	0.629
Low glucose <1.4mmol/dl (n=157)	1.899	0.468-7.696	0.369
Radiology-related			
Ventricular Score >1	1.737	0.850-3.552	0.130
Cortical score >1	6.882	2.020-23.452	**0.002**[a]
White matter score >1	1.984	1.009-3.901	**0.047**
BG score >1	13.609	2.714-68.226	**0.002**
Thalamic score>1	5.283	1.834-15.221	**0.002**
Extracerebral space increased	2.666	0.987-7.202	0.053
Extracerebral space hyperechogenic	1.230	0.346-4.369	0.749
lenticulostriate vasculopathy present	0.443	0.191-1.027	0.058
Cysts in one or more location	0.346	0.144-0.832	**0.018**
Growth at 12 months			
WAZ <-2SD, underweight	2,880	0.748-11.094	0.124
LAZ <-2SD, stunted	1.406	0.601-3.292	0.432
WLZ <-2SD, wasted	2.556	0.486-13.445	0.268
HCAZ <-2SD, microcephaly	5.609	1.612-19.518	**0.007**[a]

[a] Significant result at univariate analysis

Table 65: Odds ratios for poor outcome at 12 months of age after adjustment for neonatal sex, age at presentation and admission weight

A stepwise multivariate logistic regression was undertaken, including all factors with a p value <0.05 at univariate analysis. At multivariate analysis, only two risk factors, the presence of seizures and opisthotonus at presentation, were found to significantly increase the odds of having a poor outcome by 12 months of age. The OR for seizures at presentation was 7.7 (95%CI 1.6-37.5) and for opisthotonus at presentation was 8.6 (95%CI 1.5-49.2).

Maternal-related	**OR**	**95% CI**	**p-value**
Maternal age			
Education ≤primary			
Facility-based delivery			
Skilled birth attendant	8.12	0.49 – 135.64	0.145
Spontaneous vertex delivery	0.79	0.26 – 2.35	0.672
HIV exposed			
Neonate-related			
Male sex			
<48 hours, n(%)	0.791	0.267 – 2.345	0.672
Admission length (cm)			
Admission head circumference (cm)	1.209	0.956 – 1.528	0.112
Temperature, (°C)			
Heart rate (HR, beats per minute)			
Respiratory rate >60 breaths per minute			
Respiratory rate (RR, breaths per minute)			
Respiratory distress	1.324	0.344 – 5.102	0.683
Seizures	**7.747**	**1.600 – 37.504**	**0.011**
Bulging fontanelle			
Opisthotonus	**8.623**	**1.511 – 49.208**	**0.015**
Irritability	0.588	0.216 – 1.600	0.298
Hypotonic			
Jaundice			
Laboratory-related			
Positive blood culture			
Raised protein over 127mg/dl (n=152)	2.986	0.468-19.061	0.247
Raised white cell >15cells/mm (n=156)			
Low glucose <1.4mmol/dl (n=157)			
Radiology-related			
Ventricular Score >1			
Cortical score >1	2.246	0.298-16.921	0.432
White matter score >1	0.711	0.250 – 2.016	0.521
BG score >1	3.350	0.299 – 37.505	0.327
Thalamic score>1	1.787	0.244 – 13.095	0.568
Extracerebral space increased	1.718	0.156 – 1.636	0.511
Extracerebral space hyperechogenic			
Lenticulostriate vasculopathy present			
Cysts in one or more location			

Table 66: Odds ratios for poor outcome at 12 months of age after multivariate logistic regression

DISCUSSION

This is one of the first studies to evaluate the risk factors for early childhood outcome in term neonates with pSBI in SSA. Prior to this study, most studies that have explored risk factors for a poor outcome in pSBI have only considered risk factors for severe disease in the neonatal period (Duke et al., 2005, Bang et al., 2005, English et al., 2004). We were able to identify only one other study of pSBI in infants in SSA which identified risk factors correlating with poor early childhood outcomes (Molyneux et al., 2017). Those studies in SSA that have explored the outcomes following neonatal meningitis are relatively old, have a comparatively limited follow-up period and report limited development assessments (Airede, 1993, Airede et al., 2008, Gebremariam, 1998). None of the studies from SSA have looked at the risk factors for early childhood outcome following neonatal meningitis, although there are a few studies from high income settings that have evaluated risk factors for poor early childhood outcomes following neonatal meningitis (Lin et al., 2012, Kornelisse et al., 1995, Tan et al., 2015, Daoud et al., 1996, Klinger et al., 2000). We were able to identify only one study from SSA looking at the relationship between risk factors and early childhood outcomes in pSBI (Molyneux et al., 2017). This study was able to explore not only clinical, laboratory and microbiological factors, but were also able to consider the neuroimaging findings on cranial ultrasound as risk factors for poor outcome. This study was also followed infants until 12 months of age and undertook full developmental assessments.

In this study, 31% of our cohort of term neonates who suffered from pSBI during the neonatal period had a poor outcome by 12 months of age, including death, post-infectious hydrocephalus, post-neonatal seizures or developmental delay. The clinical risk factors for poor outcome identified by this study are similar to those found in previous cohort studies of neonatal meningitis in term infants in high income settings. This includes; the presence of seizures, coma, respiratory distress and shock (Lin et al., 2012, Kornelisse et al., 1995, Tan et al., 2015, Daoud et al., 1996, Klinger et al., 2000). Half of our patients with poor outcomes had neurological signs during hospitalisation, namely seizures, opisthotonus and hypotonia and all increased the odds of having a poor outcome. In our study, seizures and opisthotonus continued to be significant risk factors for poor outcome at multivariate analysis.

There are few studies which have examined which risk factors predict poor outcomes for infants who suffer from neonatal sepsis with most studies having limited duration of follow-up. Studies of pSBI in SSA have found history of difficulty feeding, pallor, cyanosis, apnoea, hypothermia, bulging fontanelle, unconsciousness, lethargy and abnormal movements to be strongly associated with neonatal mortality (Duke et al., 2005, English et al., 2004). More recently, a clinical trial of treatment for pSBI in infants <60 days in Malawi found weight <2500g on admission, convulsions, inability to feed, low oxygen saturations <90% and positive blood culture, to be significantly associated with mortality and neurological sequelae including hearing loss, blindness, developmental delay and hydrocephalus (Molyneux et al., 2017). Although this study did not measure the oxygen saturations, we did find that weight <2500g

and convulsions at presentation increased the risk of a poor outcome by 12 months of age. We did not find any association between positive blood culture and outcome. This may be related to the low sensitivity of blood cultures in neonates, variations in sampling techniques and laboratory differences.

Currently, a positive CSF culture remains the gold standard for the diagnosis of neonatal bacterial meningitis, however as demonstrated in this study (Chapter 5), it has a very poor sensitivity. Clinicians often rely on the presence of raised white cell count, elevated protein level and low glucose level in the CSF to determine the presence of neonatal meningitis (Garges et al., 2006, Thomson et al., 2018). Presently, no single CSF value can reliably exclude the presence of meningitis in neonates.

When considering CSF in this study, there were no positive CSF cultures and white cell count and glucose level showed no correlation with poor outcome. We found the presence of an elevated protein level significantly increased the odds of having a poor outcome. CSF protein levels are not known to be affected by a systemic inflammatory response in the absence of meningitis (Noureldein et al., 2018). Future proteomic analysis on the cases in this study with elevated protein levels will help distinguish between patterns of proteins associated with infection from proteins associated with inflammation. Elevated protein has however been associated with neonatal death, post-infectious hydrocephalus and neurological sequelae in other studies of culture confirmed neonatal meningitis in high income settings (Kornelisse et al., 1995, Lin et al., 2012, Tan et al., 2015). Low CSF glucose has also previously been

significantly associated with poor outcome, but this was not observed in this study (Klinger et al., 2000, Kornelisse et al., 1995). Although white cells may signify the presence of infection, the presence of raised protein may be more representative of inflammation and injury within the CNS. Given our strong association with raised protein and poor outcome, in settings where lumbar punctures are feasible, even when CSF cultures are negative, it is pertinent to consider prescribing the prolonged antibiotic therapy that is given for meningitis in the presence of a raised CSF protein. In addition, follow up should be undertaken, wherever possible, for all neonates with an elevated CSF protein. In the future genomic analyses of the stored CSF of these infants, it will be important to identify how many of those with a raised protein have evidence of infection.

Preterm infants are known to be at increased risk of neurodevelopmental impairment, and this risk increases if they have had neonatal sepsis. It is likely that the effect of infection on the neurodevelopment of preterm infants is partly mediated by white matter injury (Heo et al., 2018, Shah et al., 2008, Graham et al., 2004, Glass et al., 2008, Vermeulen et al., 2001). We believe this is the first study to explore the relationship between cranial ultrasound findings in a cohort of term infants with clinical sepsis and their early childhood outcome. Although we observed cases with ventricular dilatation, strands and debris and bright ventricular lining, consistent with acute ventriculitis, these findings were not found to be related to poor outcome by 12-months. The presence of increased echogenicity in the cortex, white matter, basal ganglia and thalami at presentation increased the risk of poor outcome significantly in our cohort.

Infants with a poor outcome at 12 months were significantly more likely to have had moderate or severe abnormalities in the WM, cortex, basal ganglia and/or thalami at presentation compared to those with a good outcome, 48.1% v. 4.9% respectively ($p<0.0001$). This study suggests that brain imaging at presentation is a good predictor of outcome at 12 months. It is possible that the imaging abnormalities are related to acute infection but may represent abnormalities related to an in utero infection, which are either unrelated to the acute presentation or have predisposed the infant to a pSBI. The relationship between sepsis, brain injury and outcome in term infants therefore deserves further exploration.

At multivariate analysis, when all significant (p value <0.05) clinical, laboratory and imaging risk factors were considered, only seizures and opisthotonus at presentation remained significant predictors of poor outcome at 12 months of age. Therefore, although brain imaging and CSF analysis can detect the extent of the abnormality and aid in the management of neonates with pSBI, it is clear that simple clinical signs remain the best indicator of those at risk of poor outcome. Therefore, neonates presenting with seizures and/or opisthotonus should always undergo close neurodevelopmental follow-up.

At 12 months of age, those infants with a low head circumference (<-2SD), were significantly more likely to have a poor outcome. This finding is similar to survivors of hypoxic ischaemic encephalopathy (HIE) in this setting, where a HCAZ <-2SD at 1 year of age, significantly increased the risk of neurodevelopmental impairment in survivors by 4.9-fold (Tann et al., 2018).

This simple clinical predictor of outcome therefore has the potential to be a useful screening tool to support targeted neurodevelopmental follow-up of survivors of both pSBI and HIE.

STRENGTHS AND LIMITATIONS

This study has a number of strengths as well as limitations. The prospective design of this study meant that both selection bias and recall bias were minimized in this study. In addition, at recruitment, a wide variety of data were recorded including clinical, laboratory and cranial ultrasound findings allowing a wider exploration of potential risk factors for poor outcome. Although this study was carried out in an urban setting, due to the large catchment area we were able to include neonates born both in hospital and at home.

Severe developmental impairment that would be seen by 12 months of age is normally associated with perinatal factors such as prematurity and perinatal asphyxia (Namazzi et al., 2019, Tann et al., 2018). Sociodemographic variables such as poverty, maternal parity, maternal education and sex have all been associated with developmental impairment (Namazzi et al., 2019, Tann et al., 2018). These factors seem to have the greatest effects on mild educational disabilities, which would not be detectable at 12 months of age. While we maintained a good follow-up rate of our cohort, with known outcomes for over 90% of cases of pSBI, this study only undertook follow-up until 12 months of age. This means severe impairments are unlikely to have been missed, however mild and moderate levels of impairment would not necessarily have been detected. Extending the follow-up period of this cohort

will be important to understand the incidence of minor neurological deficits, learning impairments and pervasive developmental disorders.

This study was part of a larger multicentre cohort study which focused primarily on exploring the aetiology of pSBI in Uganda and therefore funding and ethical approval were not available to formally evaluate sensorineural hearing loss (SNHL) or visual impairment, both of which may have contributed to developmental impairment. The protocol for the multicentre study did not include formal neurological examinations, the addition of which would have enabled us to make a more accurate assessment of motor function and therefore to diagnose and classify cases of cerebral palsy (Novak et al., 2017). The addition of hearing, visual and motor evaluations will be important to consider in the extended follow-up of our cohort.

CONCLUSION

In a low-resource setting, like Uganda, where lumbar punctures and cranial ultrasounds are not always readily available, this study suggests that even clinical signs alone are critical in the identification of high-risk neonates suffering from pSBI. When lumbar punctures are feasible, if a neonate with pSBI has an elevated protein level on CSF analysis, then a poor outcome becomes more likely, even in the absence of an elevated white cell count or positive CSF culture. Lastly cUS has the potential to be a low-cost, safe and sustainable method to help identify those with cortical, white matter, basal ganglia and thalamic changes that significantly increase the risk of poor outcome.

Depending on the availability of the tests, those neonates with pSBI presenting with any of these clinical, laboratory or radiological findings deserve increased vigilance during their inpatient admission due to their higher risk of neonatal mortality. They also merit a strong consideration for a prolonged course of antibiotic therapy. Early intervention programs are known to benefit both cognitive and motor delay in infancy, so these neonates also warrant more cautious and targeted follow-up to enable early detection of impairment, with the potential for earlier intervention.

CHAPTER 10 - DISCUSSION

The aim of this thesis was to describe the aetiology, imaging findings and one-year outcomes among neonates with pSBI in Uganda. In Uganda, the NMR has not changed over two decades, remaining high at 28/1000 live births (UBoSUa., 2017). This remains far from the UNSDG of reducing the NMR to 12/1000 live births by 2030 (Nations). Serious bacterial infections are one of the leading causes of these neonatal deaths in SSA and have previously been reported as the leading cause of neonatal mortality in Uganda (Liu et al., 2016, Health, 2008b).

In many resource-limited settings, laboratory facilities are limited, and the diagnosis of neonatal sepsis relies on clinical symptoms and signs (Young Infants Clinical Signs Study, 2008). Many of these symptoms and signs are non-specific and can be observed due to conditions other than infection, primarily neonatal encephalopathy, prematurity, congenital heart disease and inborn errors of metabolism. With this knowledge, in this thesis, infants <2000g were excluded and stricter definition of pSBI was used: the presence of a combination of these features including a) fever, lethargy and poor feeding b) hypothermia, lethargy and poor feeding c) full fontanelle and/or seizures, fever, poor feeding. Infants who required resuscitation or had Apgar scores less than 6 at 5 minutes were also excluded. It is possible that some infants included may have still had hypoxic ischaemic encephalopathy as we relied on the information available from the referral letter or from the mother in the case of home delivery. One infant had signs suggestive of cyanotic heart disease,

which was later confirmed by echocardiography and the infant was excluded. Many of the infants presented with jaundice and it was not possible to measure their serum bilirubin levels and it is possible that hyperbilirubinaemia may have contributed to their outcomes.

AETIOLOGY

The 'gold-standard' for the diagnosis of neonatal sepsis is the isolation of bacteria from blood culture, however despite their high specificity, their low sensitivity remains a challenge (Vergnano et al., 2011). The blood specimens in this thesis were removed using strict aseptic procedures to minimise contamination. In addition, a panel of experts reviewed the results of those infants with positive blood cultures together with the medical records to determine those that were possible pathogens, definite pathogens and likely contaminants. Definite or possible pathogens were identified in 5.6% (12/214) of blood cultures. This is similar to research performed in other LMICs (Hamer et al., 2015). Confirming, that even with careful aseptic sampling and high-quality microbiological analysis, the aetiology of the vast majority of cases of pSBI remain undefined. Similar to other hospital and community-based studies published from SSA, the commonest pathogens identified in this thesis were *Staphylococcus Aureus, Escherichia Coli* and *Klebsiella* (Waters et al., 2011, Zaidi et al., 2009, Downie et al., 2013, Stoll et al., 2011, Zaidi et al., 2005). In this thesis all cases of gram-negative sepsis were in facility-delivered infants and all presented within 48 hours of birth. Poor intrapartum and postnatal infection control practices, coupled with poorly cleaned multi-use equipment are the likely source of these severe and often fatal infections (Zaidi et al.,

2005, Zaidi et al., 2009). *S. aureus* is normally spread through direct contact and therefore improved infection control both at home and in hospital is vital to reduce these infections. Improved access to and training in hand hygiene around the time of delivery and in the neonatal period, either with soap and water or alcohol-based hand rub, has the potential to do this.

Due to the challenges of isolating certain bacteria, the prevalence of pathogens such as *Haemophilus influenzae, Streptococcus pneumoniae* and *group B streptococcus*, may have been underestimated in this thesis (Blaschke, 2011). The frequency of aetiological diagnosis can be improved with the addition of molecular diagnostic testing of the blood and CSF. A large study of pSBI that used qPCR in Bangladesh was able to improve pathogen detection in up to 28% of cases, and respiratory syncytial virus and *ureaplasma* sp, which would not be detected by traditional blood culture, were actually the two most commonly detected pathogens (Saha et al., 2018). The majority of cases still had no identifiable pathogen detected, which may be due to the presence of novel pathogens not detected by predefined qPCR or the possibility that many cases of pSBI are in fact not infectious in origin. In the future, stored samples from the patients in this thesis will be used to undertake broad-range 16s rDNA PCR and whole genome sequencing to identify both known and novel pathogens. In addition, qPCR for known viral and fungal pathogens will be performed on the samples. Until the aetiology of pSBI can be better defined, the success of both prevention and treatment policies will be restricted and our progress in reducing infection-related deaths in neonates will be limited.

There are reports of growing antibiotic resistance in neonatal sepsis both in Uganda and across LMICs (Mugalu et al., 2006, Hamer et al., 2015, Waters et al., 2011, Downie et al., 2013). This thesis found none of the isolates to be sensitive to ampicillin/penicillin, only 50% of isolates to be sensitive to gentamicin, and none of the *E. coli* and *Klebsiella spp.* to be sensitive to third-generation cephalosporins. These gram-negative bacteria were only sensitive to medications that are not routinely available in our setting; meropenem, imipenem and amikacin. These high rates of antibiotic resistance may represent the relatively high number of hospital deliveries included in this thesis, but this is of extreme concern as we continue to encourage women to deliver in health facilities. Rising antibiotic resistance is a growing threat to the reduction of the mortality and morbidity associated with neonatal sepsis. Whilst blood cultures may have limited sensitivity especially in neonates, their use in providing local microbiological data to guide empirical antimicrobial therapy remains paramount.

Neonatal sepsis can lead to infection of the CNS causing meningitis and ventriculitis (Barichello et al., 2013). Neonatal meningitis is known to be a devastating illness with a higher risk of mortality than neonatal sepsis. Unfortunately, it is not always possible to diagnose neonatal meningitis clinically and although the ‘gold standard’ diagnosis is the isolation of bacteria form the CSF, again like blood cultures, only the minority of pathogens are identified by culture. In this thesis, neonatal meningitis was defined as the presence of a positive CSF culture, and/or a raised white cell count and/or a

raised protein level (Laving et al., 2003, Thomson et al., 2018). There are limited data on the aetiology of neonatal meningitis in SSA, although it appears that *Klebsiella, E. coli, Streptococcus pneumoniae* and Group B *Streptococcus* are the most commonly isolated pathogens (Kasirye-Bainda and Musoke, 1992, Laving et al., 2003, Musoke and Malenga, 1984, Swann et al., 2014). Regarding the CSF in this thesis, 88% of the infants had a CSF sample removed, but no pathogens were isolated. Despite this, significant findings in the CSF analysis suggestive of meningitis were seen in 10.6% (20/189). This is similar to other studies in East Africa where lumbar punctures were performed in neonates presenting with pSBI (Laving et al., 2003, Talbert et al., 2010). As described above, the clinical features of neonatal meningitis can be non-specific and, in this thesis, only 55% (11/20) of those infants with abnormal CSF analyses had clinical features suggestive of meningitis, including seizures, opisthotonus, bulging fontanelle and hypotonia. There were a further 7 neonates, where a lumbar puncture was either contraindicated or failed, but clinical suspicion of neonatal meningitis was high due to signs of encephalopathy or seizures. In many low-resource settings, neonates presenting with pSBI do not undergo a lumbar puncture, CSF analysis or CSF culture and therefore a diagnosis of neonatal meningitis can be overlooked. The relatively high rate of neonatal meningitis in this thesis confirms the ongoing need to consider neonatal meningitis in neonates presenting with pSBI, even when clinical features of meningitis are absent. Although only one of 214 (0.5%) neonates was diagnosed with *p. falciparum* on blood smear, it also highlights the need to consider congenital and neonatal malaria in infants

presenting with pSBI who are living in malaria endemic areas (Olupot-Olupot et al., 2018).

IMAGING

The challenges of using traditional blood culture and CSF culture to diagnose neonatal sepsis, especially in a low-resource setting, have been described above. It is therefore very clear that supplementary or alternative tests are urgently needed to optimize the diagnosis of neonatal sepsis and neonatal meningitis. In low-resource settings like Uganda, such tests should be affordable and feasible and ideally suitable to be undertaken at the bedside. Cranial ultrasound is a relatively affordable, safe, and portable method of imaging the neonatal CNS, that is already widely used in neonatal care in high-resource settings. This thesis hypothesised that bedside cUS examination of neonates presenting with pSBI could help detect whether the brain has been affected in infants presenting with pSBI. Previous studies reporting the use of cUS in neonatal infection have focused primarily on those with confirmed CNS infections or preterm infants with sepsis. This thesis is one of the first studies to report the use of routine cUS examination in term infants presenting with pSBI. Cranial ultrasound scans were also available for 44 well term infants with no signs of infection or encephalopathy for comparison.

In this thesis, cUS scans were performed at presentation for 91.6% (196/214) infants, a standard imaging protocol was followed, and all cUS scans were undertaken by one of four trained clinicians. The images were interpreted by one of two experts blinded to the clinical details of the infant. A wide range of

imaging abnormalities were observed in the cUS examinations performed at presentation in term infants with pSBI and at least one abnormal finding was reported in 80.0% of these infants.

Although no association between cUS findings at presentation and neonatal mortality was observed, term neonates with pSBI frequently had abnormalities on cranial ultrasound at presentation that were not seen in the control neonates. Infants with pSBI were significantly more likely to have ventricular dilatation, strands, debris, bright margins and prominent choroid in the roof of the third ventricle when compared to control infants ($p<0.001$). There was also a trend towards increased white matter (WM) echogenicity and increased cortical echogenicity in those infants with pSBI compared to controls. Lastly, the prevalence of lenticulostriate vasculopathy, increased extracerebral space and hyperechogenic extracerebral space were all significantly higher in infants with pSBI than in controls.

85% (17/20) of those infants with abnormal CSF analysis had one or more abnormalities on their cranial ultrasound. Moderate and severe cortical and/or white matter echogenicity were significantly associated with abnormal CSF analysis. There were 17 infants with moderately or severely echogenic cortex, 16 infants with moderately or severely echogenic white matter and 10 of these infants had moderate/severe echogenicity in both locations. There were 9 infants with 2 or more ventricular abnormalities, but only one-third (3/9) had associated moderate/severe cortical or white matter echogenicity. It therefore appears that in this population, CNS infections affect primarily either the

ventricles or the white matter and cortex. Future genomic work on the CSF samples will be vital to explore differences in the aetiology of these distinct imaging patterns.

In this thesis, 21.9% (43/196) of infants presented with seizures or a history of seizures. The findings on cranial ultrasound in these infants were significantly different to those infants without seizures. The prevalence of ventricular, cortical and white matter pathologies were significantly higher, thus suggesting that any infant with pSBI presenting with neonatal seizures should always undergo a cUS examination at presentation to evaluate the nature and the extent of a possible brain injury.

In this thesis imaging abnormalities were also observed on cUS in infants who had negative CSF culture, normal CSF analysis, no seizures and normal neurology. This suggests that the CNS may be involved in more infants with pSBI than is suggested by the CSF findings alone and that CSF culture and analysis are not sufficient to rule out involvement of the CNS in neonates presenting with pSBI. As shown in this thesis, CSF culture often has a low sensitivity, and in addition neonatal meningitis can occur in the presence of normal CSF parameters (Laving et al., 2003, Garges et al., 2006) Studies that have used qPCR in cases of suspected meningitis with culture-negative CSF have detected pathogens in 72-90% of samples (Chiba et al., 2009, Khater and Elabd, 2016). It will be vital to consider the imaging findings in this thesis together with the genomic analysis of the stored CSF that will be undertaken in the future to establish the presence of fungal, viral and bacterial pathogens.

If a correlation is seen between the CSF bacterial, viral and fungal genomic analyses and the cUS findings, then it would suggest that cUS improves the detection rate of CNS infections when compared to CSF culture and analysis alone.

Although early ultrasound images can help establish the timing of injuries, repeating cUS examinations can allow the full evolution of a disease process to be appreciated, as some abnormalities may become more apparent over the subsequent days. In this thesis 85 infants underwent serial cranial ultrasound scans on day 1, 3, 7 and 28 of presentation.

The cranial ultrasounds performed at presentation detected all 5 infants who had an abnormal cortex at 28 days, however the cortical changes progressed in severity during that same time period. For the 7 infants with moderately to severely abnormal white matter at 28 days, 6/7 of them had had abnormal white matter at presentation. Although the presence of ventricular dilatation increased in the 28 days after presentation, it was only those infants in which it was observed at presentation or who had associated abnormalities in the cortex or white matter that had a poor outcome at 12 months of age. The five infants who developed isolated mild ventriculomegaly after presentation, had normal development at 12 months. Those infants with abnormal basal ganglia and thalami all had these abnormalities detected at presentation. Only half of these infants had associated white matter and cortical abnormalities. No significant change in the prevalence of basal ganglia and thalamic findings was observed over time. Infants with abnormal basal ganglia or thalami at

presentation had an increased risk of a poor outcome. Regarding the three infants who developed post-infectious hydrocephalus, two of the infants had globally abnormal white matter at presentation and one had multi cystic encephalomalacia, all findings which would have prompted a repeat cUS examination. Together these findings suggest that any infant with ventricular dilatation, moderately to severely abnormal white matter or cortex at presentation or abnormal basal ganglia or thalami, should have a minimum of one repeat scan, one to two months after presentation, to evaluate the progression of the brain injury. Such an approach would facilitate the identification of infants who are at high risk of a poor outcome.

This thesis also clearly demonstrates that even if CNS involvement is detected at presentation by the use of cUS and seemingly adequate antibiotic therapy is promptly administered, there is still potential for significant and devastating progression of the brain injury. This thesis describes a relatively high incidence of devastating brain injury, with 7.1% (6/85) of infants developing hugely destructive white matter and cortical injury by day 28 of presentation. Half of these infants (3/6) also developed associated post-infectious hydrocephalus. Although all six of these infants presented with fever and seizures suggestive of meningitis, without performing a cUS the extent of the brain injury would not have been known. The pathogen or pathogens responsible for these aggressive and invasive brain injuries need to be urgently identified to allow the development of appropriate prevention and treatment strategies. Genomic analysis of stored CSF samples from these cases with destructive brain injury will hope to elucidate this further. In addition, it will be important to assess this

cohort of children at an older age to detect any subtler developmental impairments that may be associated with the mild abnormalities on cranial ultrasound such as mild ventriculomegaly.

OUTCOMES

The reported case fatality rates (CFRs) for pSBI in low-resources settings are highly variable, depending on the quality of care available, ranging from 5% up to 48% (Burgoine et al., 2018, Duke et al., 2000, English et al., 2003, English et al., 2004, Milledge et al., 2005, Mugalu et al., 2006). In this thesis, the NMR was 9.3% (20/214), suggesting that with improved neonatal care, a relatively low mortality from pSBI can be achieved in a low-resource setting (Burgoine et al., 2018). In those infants where a potential pathogen was detected in the blood, the NMR increased to 25.5% (3/12). The CFR rate for neonatal meningitis is known to be higher than sepsis alone, with studies from SSA reporting CFRs from 33-67% (Airede, 1993, Campagne et al., 1999, Longe et al., 1984, Milledge et al., 2005, Nathoo et al., 1991). In this thesis, the neonatal mortality in those with meningitis was 22.2% (6/27), almost double that of those infants with a diagnosis of sepsis.

In addition to the estimated 250,000 neonatal deaths due to pSBI in SSA every year, survivors of pSBI are at an increased risk of developmental impairment and neurological disability, most especially those who were born preterm or experienced meningitis(Seale et al., 2014, Seale et al., 2013). There are limited data on the incidence and level of these sequelae in survivors of meningitis, however data on the outcomes of neonates who survive neonatal

sepsis, especially term infants, are still lacking. This thesis has assessed the developmental outcome of term infants who experienced pSBI, including sepsis, pneumonia and meningitis, during the neonatal period in a low-resource setting in SSA.

In this thesis, survival, growth and development were evaluated at 2, 6 and 12 months. A composite poor outcome was defined as post-neonatal mortality, developmental impairment, post-infectious hydrocephalus and seizures. The developmental assessments were performed using BSID-III and developmental impairment was defined as a scaled-score <-1SD below the mean. At 12 months of age, 87.7% of survivors were available for assessment and 2.1% (4/188) were known to have died during the post-neonatal period. One died from complications of the primary admission after the family discharged themselves, whilst the other three infants died from a later febrile illness. As described above, 3 infants developed post-infectious hydrocephalus, which was confirmed by cranial ultrasound. Overall 14.9% (28/188) survivors of pSBI were known to have a poor outcome at 12 months of age compared to none (0/24) of the control infants seen at the same age (RR 13.6, 95%CI 0.85 to 219.28, P=0.065).

This thesis demonstrated developmental impairment across all 5 domains of the BSID-III in the survivors of pSBI. At 12 months of age, expressive language and gross motor domains had the highest rates of impairment, 14.0% and 14.6% respectively. The rates of impairment in the cognitive, receptive language and fine motor domains ranged from 7.3 – 11.0%. When compared

to the control infants at 12 months, the raw and scaled scores for all five neurocognitive domains were significantly lower in the survivors of pSBI.

Neonatal meningitis is known to have the highest risk of long-term morbidity, and even in HICs complications seizures, hydrocephalus and neurodevelopmental impairment are reported in up to 35% of survivors (Furyk et al., 2011, Holt et al., 2001, Ranjeva et al., 2018, Seale et al., 2013). As was anticipated, when considering the final diagnoses, the rates of impairment were much higher in survivors of neonatal meningitis compared to neonatal sepsis and pneumonia. This thesis found that there was a 12 to 18-fold increased risk of developmental impairment in survivors of neonatal meningitis, as defined above, compared to control infants with the highest rates of developmental impairment being 24%, 35% and 24% in cognitive, language and motor domains respectively. This is similar to findings from some other studies of survivors of neonatal meningitis in SSA (Airede, 1993, Airede et al., 2008, Gebremariam, 1998). It is much lower than the rate of neurodevelopmental impairment of 60%, that was reported in survivors of neonatal meningitis in Malawi at 12 months of age (Dube, 2014). These observed differences may be due to differences in the level of neonatal care and the use in Malawi of locally generated norms for BSID-III.

Sepsis in premature infants is known to increase the risk of adverse neurological and neurodevelopmental outcomes by up to 2-fold compared to uninfected preterm infants (Mitha et al., 2013, Schlapbach et al., 2011, Stoll et al., 2004b). There are limited corresponding data on the outcomes of term

infants who survive sepsis, although a recent large retrospective study from the United States found a 1.7 times increased risk of neurodevelopmental impairment at 5 years of age in term infants with confirmed neonatal sepsis compared to uninfected term infants (Savioli et al., 2018). This thesis observed significantly lower raw and scaled scores in survivors of neonatal sepsis compared to control infants. There was a trend towards increased risk, 3 to 7 times higher, of developmental impairment across all domains in survivors of sepsis compared to controls. This higher risk may be due to the differences in neonatal care in our low-resource setting, differences in aetiology, later presentations leading to sicker infants at presentation and the possibility that some of these infants had meningitis that was not detected by CSF culture and analysis. It is clear from this thesis, that serious bacterial infections during the neonatal period, even without meningitis, may have a substantial public health and economic burden in settings such as Uganda. More larger studies are therefore needed to confirm this observation, especially in low-resource settings where the burden of sepsis is highest.

The BSID-III, the developmental assessment tool that was used in this thesis, was developed in the U.S. and there are concerns about the use of a psychometric tests in a population that is was not validated in. One of the key challenges is the use of U.S. normative data to scale the developmental assessment scores. There are currently no normative data available for the BSID-III assessments in healthy Ugandan children, therefore the scaled scores and composite scores in this thesis were generated using the BSID-III United States (U.S.) norms. Ideally one would use normative data from the

population being studied. This thesis observed mean scaled scores above the US mean in all subsets for both cases and controls at 2 and 6 months of age. By 12 months of age, although the mean scaled scores of the cases were closer to the US mean, the mean scaled scores for the control infants remained well above the US mean. Although this finding was unexpected, similar findings were reported in a previous study in Malawi that found relying on the U.S. based normative data resulted in the misclassification of developmental impairment (Cromwell et al., 2014). Cromwell et al., report only moderate agreement between US and Malawian norms, with higher raw scores for all subtests at younger ages (6-12 months) in the Malawian infants, especially cognitive and language skills. Given the high mean scaled scores in this thesis, it is possible that we have underestimated the rates of neurodevelopmental impairment, both in the cases and the controls. What remains very clear from this thesis is the significant differences between the cases and controls.

At 12 months of age, many moderate and severe developmental impairments will be apparent. In this thesis, it was only possible to evaluate the infants up to 12 months of age and therefore mild impairments that become more apparent later in life are likely to be have been missed at such a young age. It is therefore again possible that the rates of developmental impairment have been underestimated in this thesis. It will be important to repeat the developmental assessments at older ages to ensure that mild developmental impairment is detected. In addition, it will be important to consider using a culturally relevant developmental assessment tool with proven reliability,

validity and sensitivity for identification of children with neurodisabilities in these later assessments and in any future studies.

Lastly, it is possible that children with cerebral palsy will still score well on a developmental assessment such as BSID-III. In this thesis, although we undertook a thorough developmental assessment at 2, 6 and 12 months of age, it formed part of a large multi-centre study, which did not include formal assessments for cerebral palsy, hearing or vision in the protocol. Infants with neurological disability, hearing impairment or visual impairment may therefore not have been detected. In later assessments of this cohort and in other future studies, neurological examinations together with assessment of hearing and vision, should form part of the evaluation.

PREDICTORS OF POOR OUTCOME

As described above, this thesis demonstrated that term infants who suffer from both neonatal sepsis and neonatal meningitis have an increased risk of neurodevelopmental impairment. The huge burden of neonatal infections in SSA makes the prospect of offering neurodevelopmental follow-up to all survivors of pSBI impossible. Early recognition of neonates at risk of poor outcome, has the potential to improve the provision of prompt and effective treatment and facilitate targeted follow-up and early intervention of these infants. This thesis was a prospective cohort study, which allowed diverse data to be collected at presentation with pSBI including microbiological, imaging, clinical and demographic data. These data could all be considered as factors contributing to a poor outcome at 12 months of age.

Many of the data available on the predictors of poor outcome of neonatal infections have focused on survivors of neonatal meningitis. Previous studies of survivors of neonatal meningitis have highlighted seizures, coma, poor feeding, respiratory distress, shock, elevated CSF protein, low peripheral white cell count as indicators of poor outcome (Daoud et al., 1996, Klinger et al., 2000, Kornelisse et al., 1995, Tan et al., 2015). There are few data on the indicators of poor outcome following neonatal sepsis and the data that are available focus primarily on the neonatal period (Bang et al., 2005, Duke et al., 2005, English et al., 2004). Similar to the data on meningitis, one Malawian study of young infants with pSBI identified weight <2500g, seizures, poor feeding, low oxygen saturations and positive blood culture to be risk factors for poor outcome (Molyneux et al., 2017). This thesis is one of the first studies in SSA to report risk factors for poor early childhood outcome following neonatal sepsis, meningitis and pneumonia. It is the first study to report data on the relationship between cUS findings in term neonates with pSBI and early childhood outcomes.

In this thesis, poor outcome was defined as either death, post-infectious hydrocephalus, post-neonatal seizures or developmental impairment (scaled-score <-1SD below the mean). By 12 months of age 30.5% (54/177) of infants had a poor outcome: 11.9% (21/177) of infants had died, 3 had developed post-infectious hydrocephalus with associated developmental impairment, 3 infants were suffering from seizures and had developmental impairment and 27 had developmental impairment.

This thesis identified the following clinical and laboratory factors to increase the risk of poor outcome by 12 months of age: age <48 hours at presentation, respiratory distress, neonatal seizures, opisthotonus, hypotonia and raised CSF protein. Also, all infants who had a poor outcome at 12 months of age had had a moderate to severely abnormal ultrasound at presentation. This thesis identified a wide variety of abnormalities on ultrasound in neonates presenting with pSBI. Imaging findings at presentation that were significantly associated with poor outcome were abnormal cortex, abnormal white matter, abnormal basal ganglia and abnormal thalami.

CHAPTER 11 - CONCLUSION

It is clear from this thesis that without improved and novel diagnostics, the aetiology of the majority of cases of pSBI will remain unknown. The relatively low CFR of pSBI in this thesis, demonstrates that will good neonatal care, the high CFR of pSBI in a low resource setting can be substantially lower than is reported from many similar settings. Even with comparatively good neonatal care, access to good quality laboratory testing and administration of appropriate antimicrobial therapy, serious bacterial infections during the neonatal period, even without confirmation of neonatal meningitis on CSF culture or analysis, carry an increased risk of post-neonatal mortality, neurological disability and developmental impairment. The association of poor outcome with seizures and abnormal ultrasound findings suggests that many more infants may be experiencing CNS involvement than is detected by CSF

culture and CSF analysis alone. Cranial ultrasound therefore deserves further exploration as a point of care diagnostic test, the first step in this will be the comparison of the imaging findings in this thesis to the genomic analysis of the corresponding CSF samples. Secondly, any infant presenting with seizures or who has an abnormal cUS examination at presentation, deserves not only a minimum of one repeat cUS to evaluate the progression and severity of the injury but deserves closer follow-up. This would allow early detection of developmental impairment and intervention programmes, which could have a huge benefit in reducing the rates of impairment and disability.

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APPENDICES

APPENDIX 1

CONSHA Study

Control of the Neonatal Septisome and Hydrocephalus in sub-Saharan Africa (CONSHA)

Case Report Form

STUDY NUMBER ☐☐☐☐

MOTHER'S INITIALS ☐☐

MOTHERS HOSPITAL ID ☐☐☐☐☐☐☐☐

1. Date of enrolment: (dd/mm/yy):

   |__|__| day |__|__| month |__|__| year (not applic$^{88/88/88}$/unknown$^{99/99/99}$) **DATENR**

2. Infant's hospital number: |__|__|__|__|__|__|__| **INFNUM**

3. Mother's initials: |__|__| (Unknown^{99}) **INFINI**

4. Date of birth: (dd/mm/yy): |__|__| day |__|__| month |__|__| year

   (not applic$^{88/88/88}$/unknown$^{99/99/99}$) **DATBIR**

5. Age on admission (days): |__|__| (Unknown^{99}) **AGEDAY**

6. Current Residence:

   a. (Village) Cell:_____

      ADDVIL

   b. Parish: _____

      ADDPAR

   c. Sub-county: :_____

      ADDSUB

   d. County: : _____

      ADDCOU

   e. District:_____

      ADDDIS

   f. Telephone number: |__|__|__|__|__|__|__|__|__|__| **PATMOB**

   g. Alternative phone number: |__|__|__|__|__|__|__|__|__|__| **ALTMOB**

7. Description of how to find home (eg nearest shop/bar/hotel):

   _____

   _____ **ADDLOC**

8. Name of LC village chairman:_____ **LCCHAIR**

9. LC chairman mobile phone number: |__|__|__|__|__|__|__|__|__|__| **LCCMOB**

Maternal details

10. Mothers initials: |__|__| (Unknown[99]) **MATINI**
11. Mother's age (years): |__|__| (Unknown[99]) **MATAGE**
12. Mother's highest education level attained *(select one):* |__| **MATEDU**

    No schooling [0] / Primary school [1] / Secondary school [2] / Tertiary education [3] / Unknown[9]

13. Mother's occupation:

    _____ **MATOCC**

14. Mother's gravidity: |__|__| (Unknown[99]) **MATGRA**
15. Mother's parity: |__|__| (Unknown[99]) **MATPAR**
16. HIV status in prenatal period *(select one)*: |__| **MATHIV**

    Negative [0] / Positive [1] / Unknown [9]

If answer is "negative" or "unknown" skip to question 19

17. ART received in pregnancy/delivery? |__| **MATARV**

    No [0] / Yes [1] / Unknown [9]

18. ART regimen received in pregnancy or delivery *(select one)*: |__| **ARVTPY**

    HAART [1] / Sd-NVP [2] / 6 weeks-NVP [3] / Dual therapy [4] / Other [5] / Unknown [9]

    If other please specify:

    _____

19. Number of maternal febrile episodes during pregnancy? |__|__|

    (Unknown[99]) **FEBEPI**

20. Febrile during labour? No [0] / Yes [1] / Unknown [9] |__| **FEBLAB**
21. Abnormal vaginal discharge during pregnancy? No [0] / Yes [1] / Unknown [9] |__|

    VAGDIS

22. Antibiotic therapy received by mother in perinatal period? No [0] / Yes [1] / Unknown [9] |__| ABXPER

If Yes, give details:

_____

Birth history

23. Last normal menstrual period (LNMP):

|__|__| day |__|__| month |__|__| year (not applic[88/88/88]/unknown[99/99/99]) DATELNMP

24. Gestation age at birth (weeks): |__|__| (Unknown[99]) GESTWK

25. Place of delivery: |__| PLCDEL

Hospital [1] / Health centre [2] / Private clinic [3] / Home [4] / Other [5] / Unknown [9]

If other please specify:

_____

26. Birth attended by: |__| BIRATT

Doctor [1] / Midwife [2] / Traditional birth attendant [3] / Family member [4] / None [0] / Unknown [9]

27. Mode of delivery: |__| MODDEL

SVD [1] / Assisted vaginal [2] / Elective C-section [3] / Emergency C-section [4] / Unknown [9]

If Emergency C-section give indication: _____

28. Duration of labour (hours): |__|__|(Unknown[99]) LABHRS

29. Mode of rupture of membranes: |__| MODMEM

Spontaneous [1] / Manual [2] / Unknown [9]

30. State of liquor at rupture of membranes (*select one*): |__| STALIQ

Clear [1] / Meconium-stained [1] / Foul-smelling [2] / Purulent [3] / Unknown [9]

31. Time duration from rupture of membranes to delivery (hours):

    |__|__| (Unknown[99]) **ROMHRS**

32. Apgar at 5 minutes: |__|__|(Unknown[99]) **APG5M**

33. Apgar at 10 minutes: |__|__| (Unknown[99]) **APG10M**

34. Condition of baby at birth: |__| **CONBIR**

    Cried immediately [1] / Delayed to cry [2] / Never cried [3] / Unknown [9]

35. Any resuscitation done after birth

    No [0] / Yes [1] / Unknown [9] |__| **RESBIR**

If "No" continue to Question 35, if "Yes" specify:

a. Suction: No [0] / Yes [1] / Unknown [9] |__| **SUCBIR**

b. Bag/mask ventilation: No [0] / Yes [1] / Unknown [9] |__| **VENBIR**

36. Birth weight (kg): |__| . |__|__|__| (Unknown[9.999]) **BIRWHT**

Postnatal care

37. Cord care:

a. Antiseptic No [0] / Yes [1] / Unknown [9] |__| **ANTCOR**

b. Saline No [0] / Yes [1] / Unknown [9] |__| **SALCOR**

c. Water No [0] / Yes [1] / Unknown [9] |__| **WATCOR**

38. Any other substance applied to cord stump:

a. Saliva No [0] / Yes [1] / Unknown [9] |__| **SVACOR**

b. Dung No [0] / Yes [1] / Unknown [9] |__| **DUNCOR**

c. Earth/soil No [0] / Yes [1] / Unknown [9] |__| **ETHCOR**

d. Ash No [0] / Yes [1] / Unknown [9] |__| **ASHCOR**

e. Powder No [0] / Yes [1] / Unknown [9] |__| **POWCOR**

f. Vaseline No [0] / Yes [1] / Unknown [9] |__| **VASCOR**

g. Herbs No [0] / Yes [1] / Unknown [9] |__| HERCOR

h. Other No [0] / Yes [1] / Unknown [9] |__| OTHCOR

If other please specify: _____

39. Any therapeutic/surgical procedures done so far:

a. Circumcision No [0] / Yes [1] / Unknown [9] |__| CIRPRO

b. Tooth extraction No [0] / Yes [1] / Unknown [9] |__| TTHPRO

c. Skin cuts/burns No [0] / Yes [1] / Unknown [9] |__| CUTPRO

d. Other No [0] / Yes [1] / Unknown [9] |__| OTHPRO

If other please specify: _____

40. Mode of feeding *(select one):* |__| MODFED

Exclusive breastfeeding [1] / Replacement feeding [2] / Mixed feeding [3] / Unknown [9]

41. Any antibiotic treatment received since birth: |__| ABXPER

No [0] / Yes [1] / Unknown [9]

If Yes, give details: _____

Symptoms

		Present (No [0] / Yes [1])	Code
42.	Fever	|__|	**FEVDY**
43.	Cold body	|__|	**CLDDY**
44.	Poor feeding	|__|	**PFEDDY**
45.	Crying excessively	|__|	**XCRYDY**
46.	Weak cry	|__|	**WCRYDY**
47.	Lethargy	|__|	**LETHDY**
48.	Vomiting	|__|	**VOMDY**
49.	Diarrhoea	|__|	**DIARDY**
50.	Abdominal distension	|__|	**ABDODY**
51.	Difficulty Breathing	|__|	**DIBDY**
52.	Grunting	|__|	**GRNTDY**
53.	Apnoeic episodes	|__|	**APNDY**
54.	Seizures	|__|	**SEIZDY**
55.	Jaundice	|__|	**JAUNDY**
56.	Skin rash	|__|	**SKINDY**
57.	Others (specify)	 |__| |__| |__|	 **OTHSYM1** **OTHSYM2** **OTHSYM3**

Physical Signs

Sign		Data	Code
58.	Current weight (kg)	|__|.|__|__|__|kg (Unknown$^{9.999}$)	ADMWGT
59.	Temperature (°C)	|__|__| . |__| °C (Unknown$^{99.9}$)	ADMTMP
60.	Head circumference (cm)	|__|__| . |__| cm (Unknown$^{99.9}$)	ADMHC
61.	Respiratory rate (/min)	|__|__|__| / min (Unknown^{999})	ADMRR
62.	Heart Rate (/min)	|__|__|__| / min (Unknown^{999})	ADMHR

		Present (No ^{0} / Yes ^{1})	Code
63.	Lethargy / Reduced activity	|__|	
64.	Jaundice	|__|	
65.	Anaemia	|__|	
66.	Cyanosis	|__|	
67.	Skin pustules	|__|	
68.	Other skin rash	|__|	
69.	Deep skin sepsis (cellulitis, abscess)	|__|	
70.	Palpable lymph nodes	|__|	
71.	Nasal flaring	|__|	
72.	Sub-costal recession	|__|	
73.	Sternal recession	|__|	
74.	Grunting	|__|	
75.	Apnoea	|__|	
76.	Chest sounds (crackles, rhonchi)	|__|	
77.	Abdominal distension	|__|	
78.	Umbilical flare	|__|	
79.	Umbilical discharge	|__|	
80.	Hepatosplenomegaly	|__|	
81.	Irritability	|__|	
82.	Seizures	|__|	
83.	Fontanel bulging/tense	|__|	
84.	Neck stiff	|__|	
85.	Opisthotonus	|__|	
86.	Hypotonic / floppy	|__|	

87. Diagnosis: List all current diagnoses made on this admission

I. _____ |__|__| DIAG1

II. _____ |__|__| DIAG2

III. _____ |__|__| DIAG3

88. Treatments given: List all

I. _____ |__|__| TRT1

II. _____ |__|__| TRT2

III. _____ |__|__| TRT3

IV. _____ |__|__| TRT4

V. _____ |__|__| TRT5

89. Outcome of admission to neonatal unit: |__| OUTCOME

Survived with no sequelae [0] / Survived with sequelae [1] / Died [3] / Discharged against medical advice [4] / Unknown [9]

If survived with sequelae, give details:

i. ..... |__| SEQ1

ii. ..... |__| SEQ2

iii. ..... |__| SEQ3

90. Duration of hospitalization (dd): |__||__| days (unknown[99]) DURADM

91. Any other general comments

APPENDIX 2

Maternal characteristic	Complete cohort (N=214)	cUS cohort (N=196)	P value
Maternal age at delivery, Mean (SD) in years	24.0	25.5 (5.5)	0.006
Prenatal HIV status, (%)			
- Positive	6 (2.8)	6 (3.1)	1.000
- Negative	188 (87.9)	172 (87.8)	1.000
- Unknown	20 (9.3)	18 (9.2)	1.000
Primigravida (%)	79 (36.9)	72 (36.7)	1.000
Education, Freq (%)			
- No education	6 (2.8)	5 (2.6)	1.000
- Primary	78 (36.4)	70(35.7)	0.918
- Secondary	76 (35.5)	69 (35.2)	1.000
- Diploma	49 (22.9)	48 (24.5)	0.728
- Unknown	5 (2.3)	4 (2.0)	1.000
Employment, Freq (%)			
- Professionals (accountant, engineer, teacher, healthcare worker manager)	31(14.5)	27 (13.8)	0.888
- Clerical support worker	2(0.9)	2 (1.0)	1.000
- Service and sales worker (police, security guard, caterer, hairdresser, sales workers)	43(20.1)	42 (21.4)	0.808
- Skilled forestry, agricultural, fisheries worker	54(25.2)	46 (23.5)	0.730
- Craft worker (tailor)	5(2.3)	5 (2.6)	1.000
- Others (student)	1(0.5)	1 (0.5)	1.000
- Housewife	76(35.5)	71 (36.2)	0.918
- Unknown	2(0.9)	2 (1.0)	1.000
Maternal fever during labour (%)	90 (42.1)	80 (40.8)	0.841
Rupture of membranes >18 hours (%)	15/168 (8.9)	14/154 (9.1)	1.000
State of liquor (%)			
- Clear	142 (66.4)	129 (65.8)	0.917
- Meconium	11 (5.1)	11 (5.6)	0.831
- Foul smelling	7 (3.3)	6 (3.1)	1.000
- Unknown	54 (25.2)	50 (25.5)	1.000
Place of delivery (%)			
- Home or on the way to health facility	20	16 (8.1)	0.729
- Health facility	194	180 (91.9)	0.729
Type of birth attendant (%)			
- Traditional birth attendant (TBA)	5 (2.3)	5 (2.6)	1.000
- Trained healthcare worker	193	179 (91.3)	0.735
- Relative/Friend/Alone	16	12 (6.1)	0.696
Mode of delivery (%)			
- Spontaneous vertex delivery	144 (67.3)	133 (67.9)	0.916
- Elective caesarean section	5 (2.3)	5 (2.6)	1.000
- Emergency caesarean section	63 (29.4)	56 (28.6)	0.913
- Operative vaginal birth	2 (0.9)	2 (1.0)	1.000

Data are n (%). cUS – cranial ultrasound.

Baseline characteristics of mothers in the complete cohort compared to those who had a cranial ultrasound scan performed within 24 hours of presentation

APPENDIX 3

Purpose/Objective

This standard operating procedure (SOP) describes how to collect venous blood from neonates.

Scope

This SOP applies to the sampling of blood in the CONSHA study for investigations including: blood culture and molecular diagnostics.

Responsibilities

1. The CONSHA study principal investigators and co-investigators at each site are responsible for the implementation of this procedure and for ensuring that all staff performing blood sampling are trained.
2. All staff working on the CONSHA study are responsible for reading and understanding this SOP prior to performing the procedures described.

Materials and Equipment

- New sterile gloves
- Soap and water OR alcohol hand wash
- 3 x alcohol skin wipe (one for blood culture bottle, two for the skin preparation)
- 1 x betadine swabs
- 2 x sterile 24g yellow neonatal cannula
- 2 x 2ml sterile syringe and needle
- 1x paediatric BacTec blood culture bottle
- 1 x 2ml cryovial with 1ml of 2x concentration liquid **preservative**
- 1 x 2ml or 1ml **empty** cryovial
- 1 glass slides for malaria smears
- Coverslip for smearing thin malaria blood smears
- 1 x HIV rapid test
- 1 x bottle of buffer for HIV rapid test
- 1x sharps container
- Dry cotton and tape

References

Maiwald M, Chan ESY. The Forgotten Role of Alcohol: A Systematic Review and Meta-Analysis of the Clinical Efficacy and Perceived Role of Chlorhexidine in Skin Antisepsis. Khan AU, ed. *PLoS ONE*. 2012;7(9):e44277.

Procedure

Preparation

1. Record the barcode numbers from the cryovials onto the case report form.

2. Label the cryovials:
   a. "**BP" plus study number** for cryovial with liquid preservative e.g. **BP 3001**
   b. "**BF" plus study number for** empty cryovial e.g. **BF 3001**

3. Label the malaria slide with **"N" plus the study number** on the frosted edge e.g. **N 3001**

4. Ensure that the neonate is in a safe and comfortable position on the procedure table.

5. Identify appropriate vein in **either the neonate's hand or foot.** Preferably a peripheral vein should be used dependent on the doctor withdrawing the blood.

6. Wash hands thoroughly with soap and water.

7. Remove the cap from the culture bottle and clean the top of the bottle with an alcohol swab and allow to air dry.

8. Open a pack of sterile gloves

9. Open and empty two 2ml syringes with needles into the sterile gloves packaging. Take care not to touch the needle or end of the syringe.

10. Open a yellow cannula into the sterile gloves packaging. Take care not to touch the needle or end of the syringe.

11. Have the preservative cryovial, empty cryovial, culture bottle, HIV rapid test and malaria slide within reach on a clean surface.

12. Put on the clean pair of sterile examination gloves.

13. Clean the skin thoroughly and vigorously with an alcohol swab in a circle approximately 5cm in diameter for **30 seconds**.

14. Starting in the centre of the circle, apply betadine in widening circles until the entire circle is saturated with betadine.

15. Leave the betadine on the venipuncture site to act for **60 seconds**.

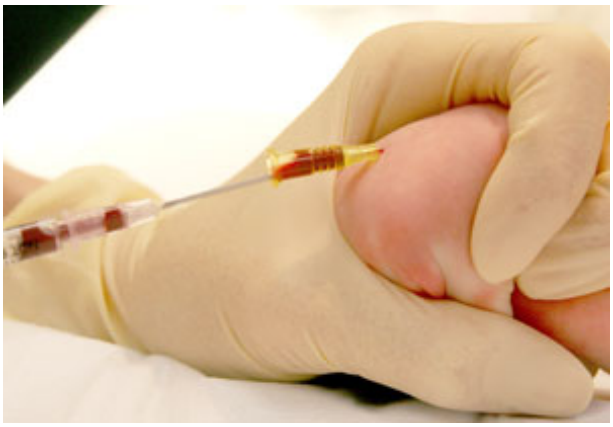
16. Finally wipe the area where the needle will go once with an alcohol swab.

17. Do not touch the venipuncture site after preparation.

Sampling

18. Insert the cannula into the vein without touching the venipuncture site and remove the needle from the cannula

19. Once blood begins to flow back into the hub of the cannula, remove the cover from the first 2ml syringe and without touching either the needle or the hub of the cannula withdraw at least 1.0ml of blood into the syringe, if possible withdraw 1.5ml. Place the syringe back in the sterile field.



20. Remove the cover from the second 2ml syringe and without touching either the needle or the hub of the cannula withdraw at least 0.5ml of blood into the syringe. Place the syringe back in your sterile field
21.
22. Once blood collection is complete, secure and flush the cannula ready for use.

Failed or challenging samples

23. No individual is to attempt more than 3 times without consulting the PI.
24. If you have difficulty in getting specimens inform PI and the PI can attempt if appropriate.
25. Take into account the individual situation, if you, the mother or staff feel uncomfortable contact the PI for advice.
26. You are not alone, the PI is responsible and will help when needed

Sample Tubes

27. Remove the caps from the two cryovials.

28. If sufficient volume has been collected place 0.5ml in the **empty cryovial**

29. Next, take the **preservative cryovial** and place the needle under the surface of the preservative. Place 0.5ml - 1.0ml of blood slowly into the preservative, taking care not to splash or bubble the preservative.

30. Take the syringe with 0.5ml blood.

31. Without touching the needle on the test, drop 0.1ml blood onto the HIV rapid test

32. Without touching the needle on the slide, drop1 small drop of blood onto one end of a ***glass slide*** for a thick malaria blood smear, near the frosted part where the participant ID has been written.

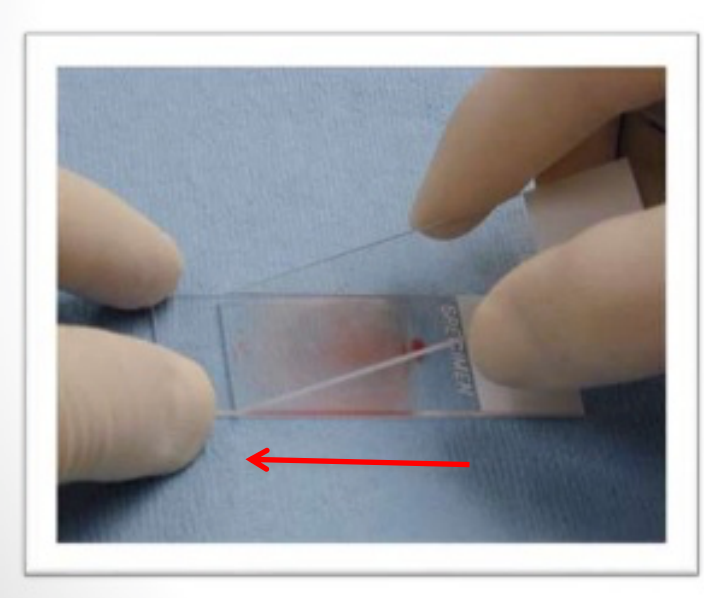
33. Without touching the needle on the test, drop 1 more small drop of blood on the ***glass slide next to your first drop,***

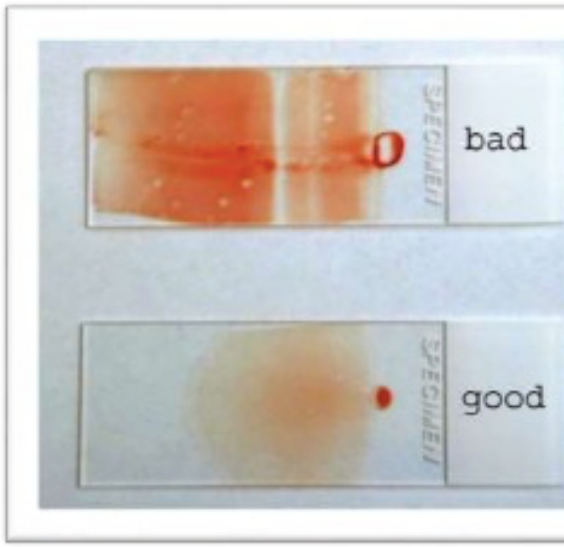
34. Without touching the top of the blood culture bottle, place the remaining 0.3ml of blood into the blood culture bottle by inserting the needle carefully into the top of the culture bottle.

35. Now prepare your malaria slide.

a. Use the corner of a glass coverslip/malaria slide to spread the drop for the thick smear around slightly. Allow to dry.

b. Next use the second slide to SMEAR the second drop towards the non-frosted end of the slide for a thin malaria blood smear. Allow to dry.





36. Add the buffer to the HIV test.

37. Place the needles and syringes carefully in sharps container. Gently shake all samples to mix blood

38. Place other clinical waste in appropriate bin provided including used gloves.

39. Place the PCR sample in liquid nitrogen storage/freezer as per site arrangement as soon as possible, keeping the corresponding lab request in the PCR file.

40. Label blood culture bottle with: study number, hospital ID number, date and time of collection.

22. Clean hands with gel hand wash or soap and water.

23. Record samples taken onto the **Clinical Report Form**.

24. Record all samples taken in the **Sample Logbook.**

25. Complete the lab request form for blood culture, malaria smear,

26. Take the blood culture and form to microbiology laboratory as soon as possible.

27. Send the malaria smear to the laboratory for reading

28. Record the result of the HIV test on the **clinical report form** and **Sample Logbook**

Potential hazards

1. Risk of sharps injury.
2. Contact with bodily fluids including blood.

Personal protective equipment

1. Sterile gloves should be worn throughout procedure.

Procedural controls

1. Observe universal precautions including appropriate disposal of sharps in bins supplied.
2. Sampling of blood should be done in an appropriate space on a clean surface.

Accident procedures

1. In the event of a needle-stick injury, management will be in accordance with the hospital guidelines.

APPENDIX 4

Purpose/Objective
This standard operating procedure (SOP) describes how to collect cerebrospinal fluid (CSF) from neonates.

Scope

This SOP applies to the sampling of CSF in the CONSHA study for downstream CSF culture and molecular diagnostics.

Responsibilities

3. The CONSHA study principal investigators and co-investigators at each site are responsible for the implementation of this procedure and for ensuring that all staff performing lumbar punctures are properly trained.
4. All staff working on the CONSHA study are responsible for reading and understanding this SOP prior to performing the procedures described.

Materials and Equipment

- Trained assistant to hold the neonate
- Sterile gloves – 2 pairs
- 1(one) sterile drape
- Soap and water OR alcohol hand wash
- 2 (two) alcohol skin swabs
- 1 (one) betadine swab
- 1 (one)22g 1-1.5inch spinal needle
- 1 (one) CSF culture bottle labeled with study number i.e**. 3 - 218**
- 1 (one) cryovial tube containing 2x concentration liquid preservative (RNA/DNA Shield) for molecular diagnostics labeled with CP and study nuber i.e. **CP 3 - 218**
- 1 (one) empty 1ml cryo vial labeled with CF and study nuber i.e. **CF 3 - 218**
- 1 (one) fluoride tube labeled with study number i.e**. 3 - 218**
- 1 (one) serum tube labeled with study number i.e**. 3 - 218**
- 1 (one) sharps container
- Dry cotton and tape

Labelling and recording

41. Ensure each tube is labeled as described above
42. Label the cryovials:

a. “**CP” plus study number** for cryovial with liquid preservative e.g. **CP 3218**

b. “**CF” plus study number for** empty cryovial e.g. **CF 3001**

43. Record the barcodes for the CF and CP tubes on the case report form **AND** in the study logbook

Procedure

Contraindications

- Seizures
- Severe respiratory distress
- Apnoeas
- Local infection at the site of the LP

Preparation

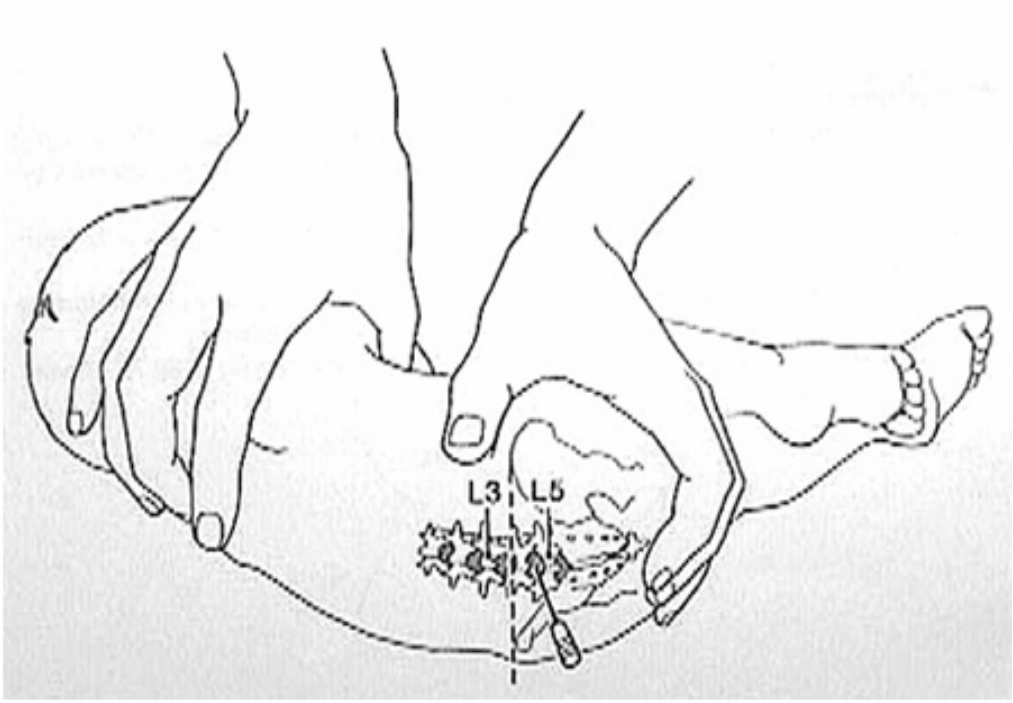
The most important determinant of a successful lumbar puncture is a strong, calm, experienced assistant to hold the patient. Position of the patient is critical**.**

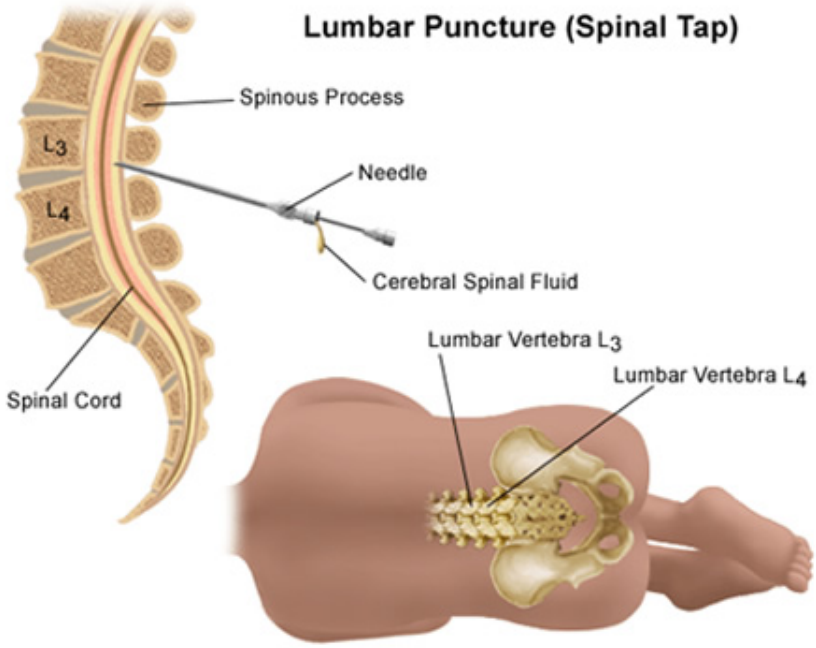
1. Ensure that the neonate is in a safe and comfortable position on the procedure table.

2. A reliable assistant should restrain the infant in a lateral decubitus position. Avoid overflexing the neck as this may cause respiratory compromise.

3. Ensure the plane of the back is exactly at 90 degrees to the bed (i.e. not leaning towards or away from you).

4. Palpate the iliac crests and imagine a line between the top of the iliac crests. Slide your finger centrally toward the L4 vertebral body. Identify the L3-4 or L4-5 interspace.





5. Wash hands thoroughly with soap and water or clean thoroughly with alcohol gel wash.

6. Prepare your spinal needle and swabs by opening them into a sterile area.

7. Aseptically put on two clean pairs of sterile examination gloves (double glove).

8. Clean the skin thoroughly and vigorously with an alcohol swab in a circle approximately 5cm in diameter for 30 seconds.

9. Starting in the centre of the circle, apply betadine in widening circles until the entire circle is saturated with betadine.

10. Leave the betadine on the site to act for 60 seconds.

11. Finally, wipe the area where the needle will be inserted once with an alcohol swab.

12. Do not touch the site after preparation.

Sampling

13. Remove your outer pair of sterile gloves

14. Palpate again to find the correct interspace and insert the needle midline and angled towards the umbilicus

15. Advance the needle slowly and then remove the stylet every few millimeters to check for the appearance of CSF

16. Once CSF appears, first collect 0.5ml of CSF into the cryovial **WITHOUT** liquid preservative by allowing the fluid to drip into the tube. Screw or have an assistant immediately screw the lid on tightly in a sterile fashion as it is essential to prevent contamination.

17. Next, take the **preservative cryovial** and carefully drip 1.0ml of CSF slowly into the preservative tube, taking care not to splash or bubble the preservative.

18. Next collect 200µL of CSF into the culture tube by allowing the fluid to drip into the tube. Again, have an assistant close the tube in a sterile fashion.

19. If applicable next collect 300µL of CSF into a fluoride tube for glucose level and 300µL of CSF into an empty serum tube for protein and cell count.

20. Replace the stylet and withdraw the needle.

21. Maintain pressure on the area and then cover with a small plaster.

22. Place the needle carefully in sharps container and all other equipment in the clinical waste.

Sample Tubes

23. Place or have an assistant place the two cryovial samples in a liquid nitrogen dewer within 5 minutes of collection.

21. Place other clinical waste, including used gloves, in appropriate bin provided.

22. Clean hands with gel hand wash or soap and water.

29. Record samples taken onto the **Clinical Report Form**.

30. Record all samples taken in the **Sample Logbook.**

31. Complete the two lab request forms; CSF culture, PCR.

32. Take the CSF culture and form to microbiology laboratory as soon as possible.

Failed or challenging procedure

1. No individual is to attempt an LP more than 3 times without consulting the PI.
If you have difficulty in getting specimens inform PI and the PI can attempt if appropriate.

2. Take into account the individual situation, if you, the mother or staff feel uncomfortable contact the PI for advice.

3. You are not alone, the PI is responsible and will help when needed.

Potential hazards

3. Risk of sharps injury.
4. Contact with bodily fluids including blood and CSF.

Personal protective equipment

2. Sterile gloves should be worn throughout procedure.

Procedural controls

3. Observe universal precautions including appropriate disposal of sharps in bins supplied.
4. The LP should be done in an appropriate space on a clean surface.

Accident procedures

2. In the event of a needle-stick injury, management will be in accordance with the hospital guidelines.