AETIOLOGY AND OUTCOME OF NEONATAL SEPSIS AND MENINGITIS IN MALAWI

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By

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CERTIFICATE OF APPROVAL

The Thesis of Queen Dube is approved by the Thesis Examination Committee

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Declaration of work done

The study which was funded by MLW was done at 2 sites QECH and Zingwangwa health centre. The study was done in collaboration with the Ceftriaxone vs Benzyl penicillin in the treatment of neonatal sepsis and meningitis study at Queen Elizabeth Central hospital in Blantyre Malawi. I was involved in both studies but mainly in the Aetiology and Outcome of Neonatal Sepsis and Meningitis in Malawi.

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This work has not previously been accepted in substance for any degree and is not being currently submitted in candidature for any degree

Queen Dube

Dedication

I would like to dedicate the thesis to God, my dad and mum, my husband Andrew, and my three children Esther, Joshua and Chipokwero. Many thanks to you all for your encouragements, love and patience.

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ABSTRACT

AETIOLOGY AND OUTCOME OF NEONATAL SEPSIS AND MENINGITIS IN MALAWI.

Introduction

In Malawi there has been significant progress in reducing post-neonatal and under-5 deaths over the past decade but very little progress in reducing neonatal deaths. The major causes of neonatal deaths in Malawi are prematurity, infections and birth asphyxia. Neonatal sepsis has been shown to have long term complications ranging from motor deficits to cognitive impairment, epilepsy and behavioural disorders in preterm very low birth weight infants in the developed setting. Contrary to the epidemiology in the developed setting where neonatal sepsis is predominantly seen in preterm low birth weight infants, in the developing setting neonatal sepsis is also common among term babies. However, very little is known on the long term outcome of neonatal sepsis in the resource restrained setting. In this thesis the aetiology and outcome of neonatal sepsis and meningitis is investigated.

Methodology

This was study had 2 components; a cross sectional arm and a prospective cohort arm. The cross sectional study was looking at the aetiology, resistance pattern and in hospital outcome of severe neonatal infection cases presenting at QECH in Blantyre. The prospective cohort arm involved participants who were recruited in the cross sectional arm at QECH and were residing within Blantyre urban and infants that never had an episode of severe neonatal infection were recruited from Zingwangwa health Centre. The infants from Zingwangwa acted as controls. The participants in the prospective cohort arm were followed up to the age of 1 year where neurodevelopmental outcomes were assessed using the Bayley's assessment tool. These participants also had detailed neurologic examination during the follow

up visits at 6 and 12 months of age. A comparison between the cases and controls was made to ascertain the impact of neonatal infection outcome.

Results

During the study a total of 412 cases were enrolled in the cross sectional arm. 75% of the cases had late onset disease. GBS was the commonest organism grown in blood culture 17/42(40%) and CSF culture 16/33(48%). 44% had abnormal serum sodium levels on admission and hypernatraemia was independently associated with an increased risk of dying in hospital (8.34[95% CI 1.95-35.7]). 51% of the gram negative organisms were multidrug resistant. In the long term outcome neonatal sepsis without overt meningitis was associated with an up to 6.6 –fold {95% CI (2.38-18.4} increased risk of developmental delay at 1 year of age. Meningitis was associated with a 17-fold {95% CI 4.89- 61.7} increased risk of developmental delay at 1 year of age. Positive blood or CSF culture and being HIV exposed were independent predictors of delay at I year of age.

Conclusion

GBS is a significant cause of neonatal infections in Malawi. The magnitude of developmental delay observed in infants who had neonatal sepsis without meningitis is worrying up to 35% of these infants were delayed. It is therefore important to employ measures that can prevent neonatal infections. Follow up is recommended in infants who had an episode of severe neonatal infection.

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Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
APGAR	Appearance, Pulse, Grimace, Activity, Respiration
ART	Antiretroviral Therapy
ARV	Antiretroviral
AVPU	Alert Voice Pain Unresponsive
BMP	Blantyre Malaria project
CI	Confidence interval
CNS	Central Nervous System
CoM	College of Medicine
CRP	C- reactive protein
CSF	Cerebrospinal Fluid
DHS	Demographic and health survey
DNA	Deoxyribonucleic Acis

DWI	Diffusion weighted imaging
ETAT	Emergency Triage Assessment and Treatment
FLAIR	Fluid attenuated inversion recovery imaging
GBS	Group B Streptococcus
GM- CSF	GM –Colony stimulating factor
HAART	Highly Active Antiretroviral Treatment
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
IFN	Interferon gamma
IGg	Immunoglobulin G
IMF	International monetary fund
IMR	Infant mortality rate
MDI	Mental Development Index
MLW	Malawi Liverpool Wellcome Trust
MRI	Magnetic resonance imaging
MTCT	Mother to Child Transmission (of HIV)
NCD	Non communicable diseases

NMR	Neonatal Mortality rate
PCR	Polymerase Chain Reaction
PDI	Psychomotor development Index
PMTCT	Prevention of Mother to Child Transmission (of HIV)
QECH	Queen Elizabeth Central Hospital
RNA	Ribonucleic Acid
TB	Tuberculosis
TLR	Toll like receptors
TNF	Tumour necrosis factor
UNAIDS	United Nations Programme on HIV/AIDS
UNDP	United Nations Development Programme
UNICEF	United Nations Children Education Fund
VDRL	Venereal disease research Laboratory
WHO	World Health Organization

CHAPTER ONE

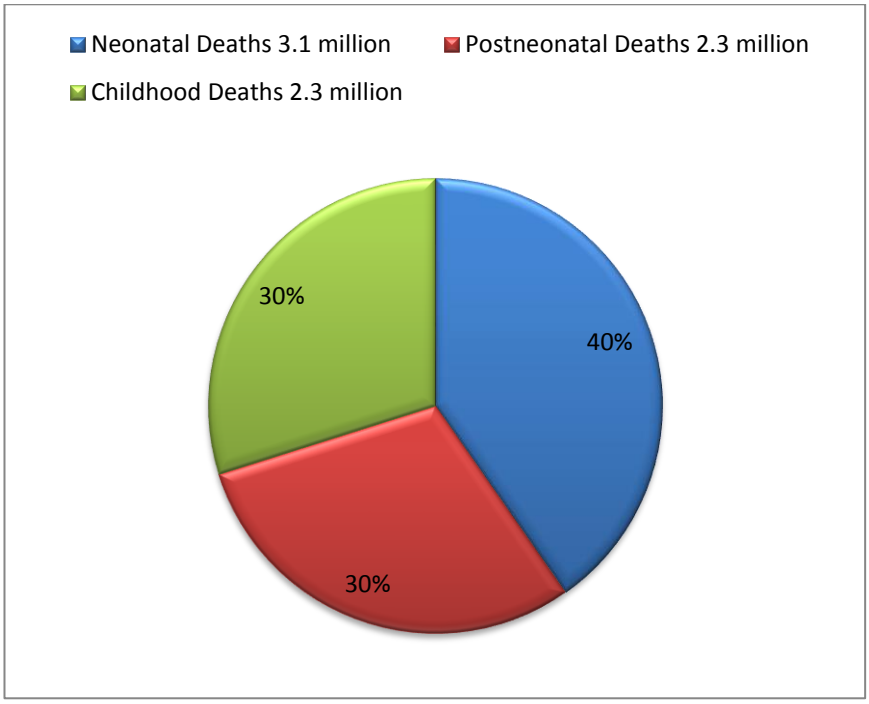
INTRODUCTION

1.1 Epidemiology of Neonatal Mortality

1.1.1 Global picture

Every year 130 million babies are born globally and 3.1 million die in the first 4 weeks of life{Lawn J.E, H. Blencowe et al (2013)}. Most of the neonatal deaths (75%) occur in the first week of life, the highest risk of death is on the first day of life{Zupan J and E Aahman (2005)}. A similar number of babies are still born {Lawn J.E, H. Blencowe et al (2013}. Perinatal deaths are responsible for almost 7% of the total global burden of disease a proportion which exceeds that caused by vaccine preventable diseases and malaria together{WHO Global burden of disease report (2004)}. Worldwide mortality in children younger than 5 years has dropped from 11·9 million deaths in 1990 to 7·7 million deaths in 2010, consisting of 3·1 million neonatal deaths, 2·3 million post neonatal deaths, and 2·3 million childhood deaths see Figure 1.1{RajaratnamJ, J.Marcus et al (2008)}. Neonatal deaths contribute 40% of all the deaths in children less than 5 years of age hence reducing neonatal deaths is an important target for the eventual reduction of childhood deaths overall{RajaratnamJ, J.Marcus et al (2008)}.

Figure 1.1 Deaths of Children Under the Age of five {RajaratnamJ, J.Marcus et al (2008)}



The neonatal mortality rate is widely used as an indicator of public health, quality of health services, distribution of wealth and the general standard of living in a society {Lawn J.E, S.Cousens et al (2005)}.

As many as 99% of the 3.1 million neonatal deaths that occur each year take place in the poorest countries of the world making newborn health as one of the most striking examples of health inequality { Lawn J.E, S.Cousens et al (2005)}. The largest number of neonatal deaths occurs in South East Asia but this region has seen a dramatic drop in neonatal deaths as a result of several initiatives aimed at improving newborn health {WHO Global burden of disease report (2004)}. On the other hand the actual number of neonatal deaths has increased in Africa especially

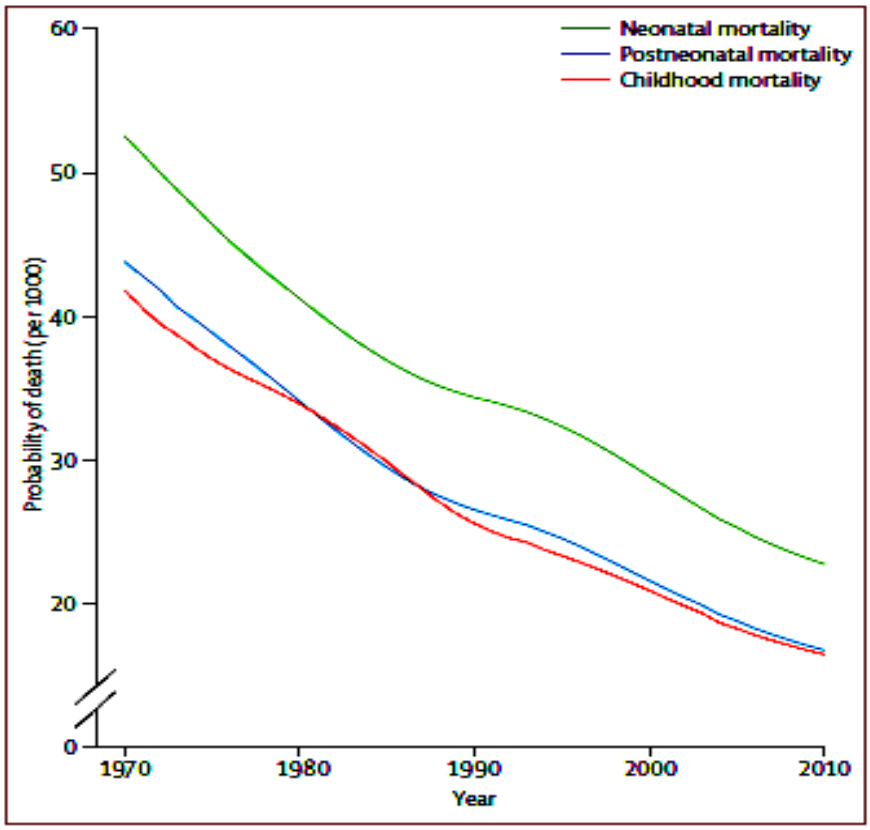
the Sub-Saharan region accounting for almost 30% of neonatal deaths worldwide{ WHO Global burden of disease report (2004)}. Most of the countries (80%) with the highest neonatal mortality rates are in Sub-Saharan Africa{ WHO Global burden of disease report (2004)}.The neonatal mortality rate for high income countries is 4 per 1000 live births whereas in low-income and middle income countries it is estimated to be 33 per 1,000 live births {Zupan J, E. Aahman (2005)}.

1.1.2 Trends in Neonatal Mortality

Across 21 regions of the world, rates of neonatal, post neonatal, and childhood mortality are declining.

Over the last 30 years, the reduction in neonatal mortality rates has been slower, compared to both the under-5 and post neonatal mortality rates{Rajaratnam J, J Marcus et al (2008)}. Even though the reduction in neonatal mortality rates has been slow globally there has been a significant decline in neonatal mortality rates in the developed world (figure 1. 2) { Rajaratnam J, J Marcus et al (2008)}.

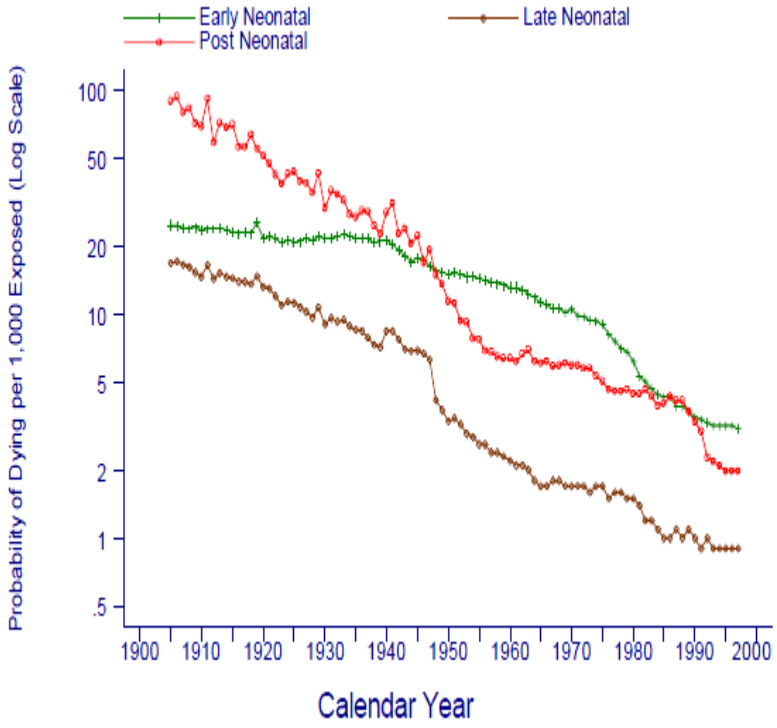
Figure 1.2 Worldwide neonatal postneonatal and childhood mortality from 1970 to 2010{RajaratnamJ, J.Marcus et al (2008)}



The United Kingdom and United States of America are some of the countries in the developed world that have shown success stories in the reduction of neonatal mortality. Early in the 20th century, neonatal mortality was 40-50 per 1,000 live birth for both the United Kingdom and the United States of America, and by 1997 it had been reduced to 4-5 per 1,000 live births{ Child health research project special report (1999),Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik

Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}. The trends in England and Wales are shown in Figure 1.3{ Hill K, C. Yoonjoung et al (2005)}.

Figure 1.3 : Early neonatal, late neonatal and postneonatal mortality rates for: England and Wales, 1905 to 1997{ Hill K, C. Yoonjoung et al (2005)}

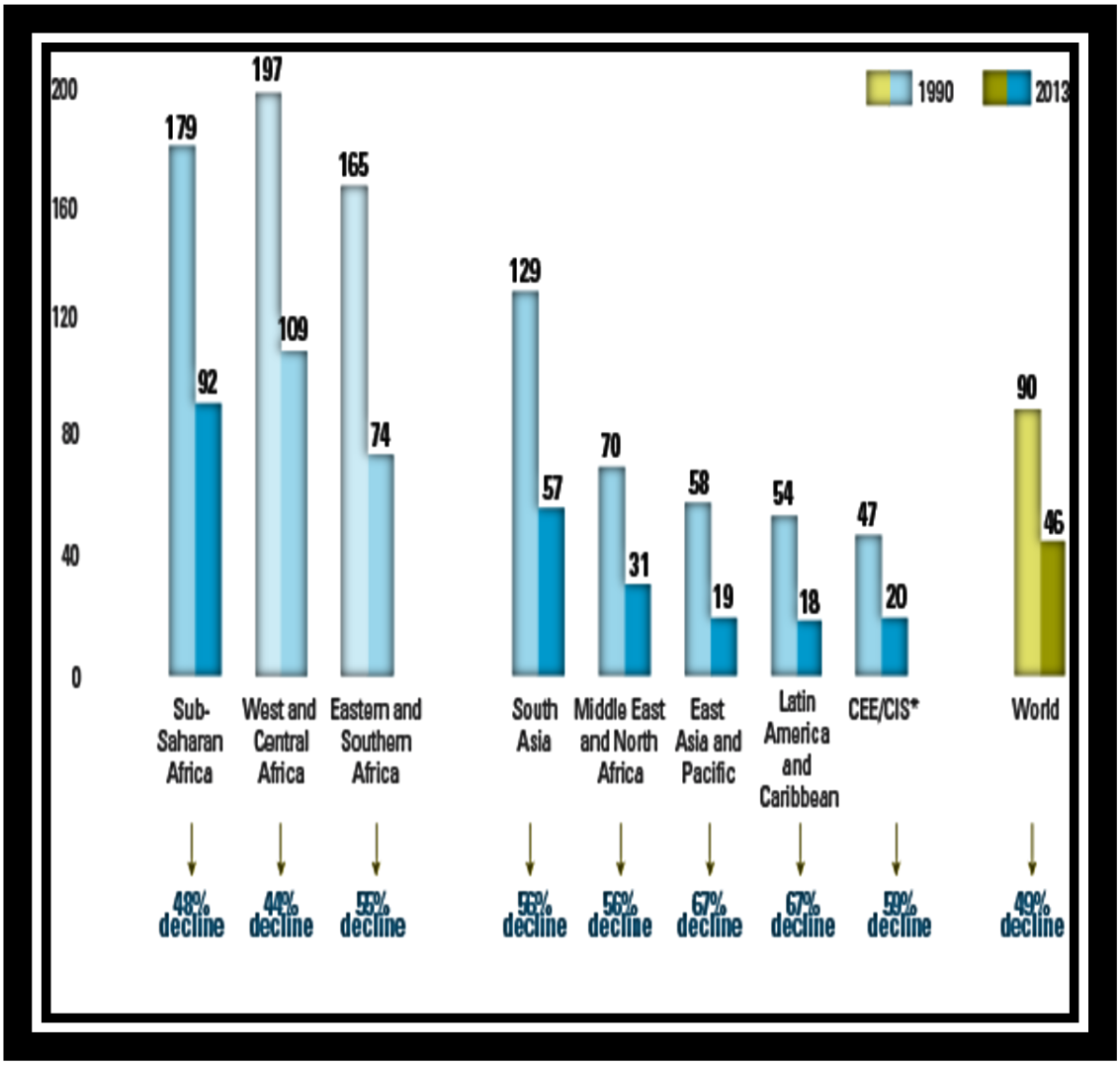


These improvements in neonatal mortality have been largely due to changes in obstetric care, maternal health, introduction of neonatology and better infant nutrition. Neonatology has evolved over the last century from being a simple, empirical care to modern, evidence-based medicine{Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Heath P.T, N.K Nik Yusoff et al (2003)}.The pattern of decline in the early neonatal deaths(0-7 days of age) was different from the late neonatal deaths(7-28 days of age).There was a very slow decline in the early neonatal deaths up to about 1950 but then there was faster decline to about 1975

and a sharply faster decline since 1975 {Hill K. C. Yoonjoung et al(2005)}.This has beeen attributed to changing patterns in reproductive health, socioeconiomic progress and improvements in the quality of obstetric and neonatal facilities in the UK {Seale A.C, M Mwaniki et al (2009), Hill K. C. Yoonjoung et al(2005) }. In the 1950's, the medical care of newborn infants was transferred from the obstetricians to the paediatricians and in the 1960's, the speciality of neonatology was developed. There were also significant improvements in neonatal care from the 1950's with the introduction of surfactant replacement therapy for the management of respiratory distress syndrome, antenatal corticosteroids and respiratory support including oxygen therapy and mechanical ventilators {Milestones in neonatology (2005)}. The earlier decline in the late neonatal deaths could possibly be attributed to the introduction of penicillin in 1944 which markedly reduced mortality from severe neonatal sepsis as infection is one of the major contributors of late neonatal deaths { Milestones in neonatology (2005}.This pattern has been noted throughout the developed world.

On the other hand the decline in neonatal mortality in the developing world has varied widely between regions with Sub-Saharan Africa showing the least rate of decline (see figure 1.4) {A promise renewed-Progress report 2014, UNICEF, New York 2014 Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Hill K., Yoonjoung choi et al (2005), Osrin D, S. Vergnano et al (2004),Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}.

Figure1. 4 Changes in neonatal mortality rates between 1990 and 2013 {A promise renewed-Progress report 2014, UNICEF, New York 2014}

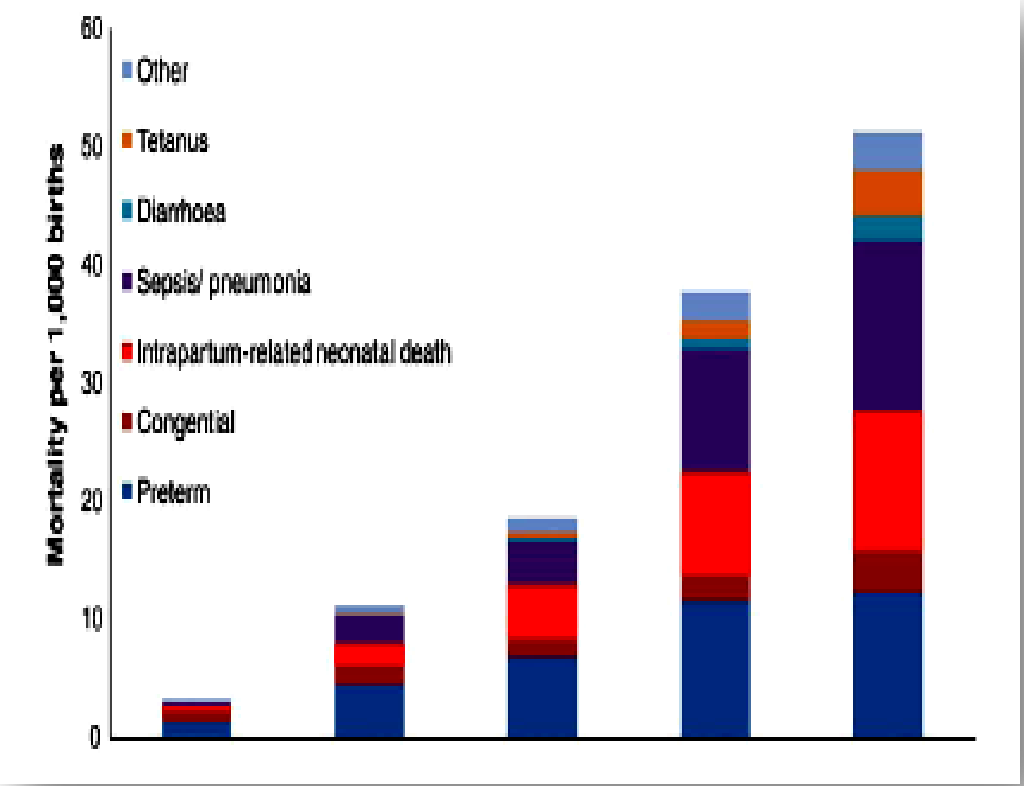


This trend in Africa especially in the Sub-Saharan region is worrying and needs exploring the possible contributing factors. Much of the progress in neonatal survival in the developing countries in Africa and South Asia has been in the late neonatal period with little improvement in the first week of life where most of the neonatal deaths occur {World Heath report (2005)}.

1.1.3 Causes of neonatal deaths

Global estimates for the direct causes of neonatal deaths are prematurity [26%], severe infections [36%], complications of asphyxia [23%] and deaths related to congenital abnormalities [7%]{Lawn J.E, S. Cousens et al (2005)}. However infection is one of the known risk factors for preterm labour and these preterm infants have reduced immunity rendering them prone to infections early on in life{Klinger G, L. Lerner-Geva et al (2009), Graham P. L,E. Larson et al(2006), Romero R, J.C Hobbins et al (1998), Minkoff H, W.M. McCormack et al (1984)} . In most of the developing settings birth asphyxia is a clinical diagnosis and laboratory tests like umbilical cord gasses are not done but most of the clinical symptoms and signs of birth asphyxia are the same as of neonatal infections and as such there is a possibility of mislabelling neonatal infection cases as birth asphyxia{Neonatal handbook internet (cited July 2013)} . The distribution of the causes of neonatal deaths varies between countries with infections causing most of the neonatal deaths in very high mortality settings like the Sub Saharan region see fig 1.5 {Lawn J.E,A.Lee etal (2009)}.

Figure 1.5. The distribution of the causes of neonatal deaths in relationship to NMR {Lawn J.E, A.Lee etal (2009)}



The top 3 causes of neonatal deaths ; infections, asphyxia and prematurity are closely related to obstetric care and as such neonatal health cannot be separated from obstetric care{World health report (2005), Neonatal and Perinatal mortality (2006)}. In the case of neonatal infections there are known risk factors which include prolonged rupture of membranes in the mother, prematurity, low birth weight, maternal group B streptococcal carriage and maternal HIV status{Tausch W.H, R.A.Ballard et al (1998), Andreson-Berry A., L Bellig (2010)}. Haematogenous and transplacental dissemination of maternal infection occurs in the transmission of certain viral (e.g rubella, cytomegalovirus, HIV, parvovirus and varicella zoster), protozoal (e.gToxoplasma gondii) and treponemal (e.gTreponema pallidum) pathogens. A few bacterial pathogens (e.g Listeria monocytogenes, Mycobacterium

tuberculosis) may reach the fetus transplacentally, but most are acquired by the ascending route in utero or as the fetus passes through the colonized birth canal {Tausch W.H, R.A.Ballard et al (1998), Andreson-Berry A., L Bellig (2010)}. It therefore comes as no surprise to see that interventions that have been put in place to try and reduce neonatal deaths have put an emphasis on obstetric care {Neonatal and Perinatal mortality (2006}.These interventions include improvements in antenatal care, better management of hypertensive disorders of pregnancy, use of corticosteroids in preterm labour, better management of maternal carriage of Group B steptococci, use of antibiotics in prolonged premature rupture of membranes { Andreson-Berry A., L Bellig (2010}.

The significance of good obstetric care can be seen in the association between skilled attendance at birth and the neonatal mortality rate. It has been shown that countries with low coverage of skilled attendants at birth have much higher neonatal mortality rates compared to countries with higher coverage see Table 1.1 below{ Lawn J.E,A.Lee etal (2009)}.

Table 1.1 Association between level of Skilled Attendance at Birth and Neonatal mortality Rate { Lawn J.E,A.Lee etal (2009)}

Neonatal mortality rate(NMR)	Level of skilled birth attendant
Very low NMR (<5)	100%
Low NMR (6-15)	99%
Moderate NMR (16-30)	88%
High NMR (31-45)	52%
Very high NMR (>45)	45%

In developing countries, nearly 50% of the women still have unskilled attendants at birth signaling the need for community based interventions that would lead into a reduction in neonatal deaths{Count down to 2015, the 2008 report, Count down to 2015 the decade report}. The lack of skilled attendants at birth could encourage the use of harmful practices like application of cow dung to the umbilical stump of the neonate {Ayaz S, S. Efe et al (2008), Girma T, H. Nida et al (2008)}. These practices could lead to introduction of infection like tetanus in the newborn.

Recently there have been several new interventions aimed at community participation in resource restrained settings that have led to a reduction in neonatal deaths. These interventions have included use of women groups, volunteer female health workers and community management of neonatal infections{ Bang A.T, R.A Bang et al (1999), Bhutta Z.A, S. Ali et al (2005), Bang A.T, R.A Bang et al (2005), Bhutta Z. A, G.L Darmstadt et al (2005)}. These interventions led to an improvement in the health seeking behaviour of the mothers/caregivers. The women who were

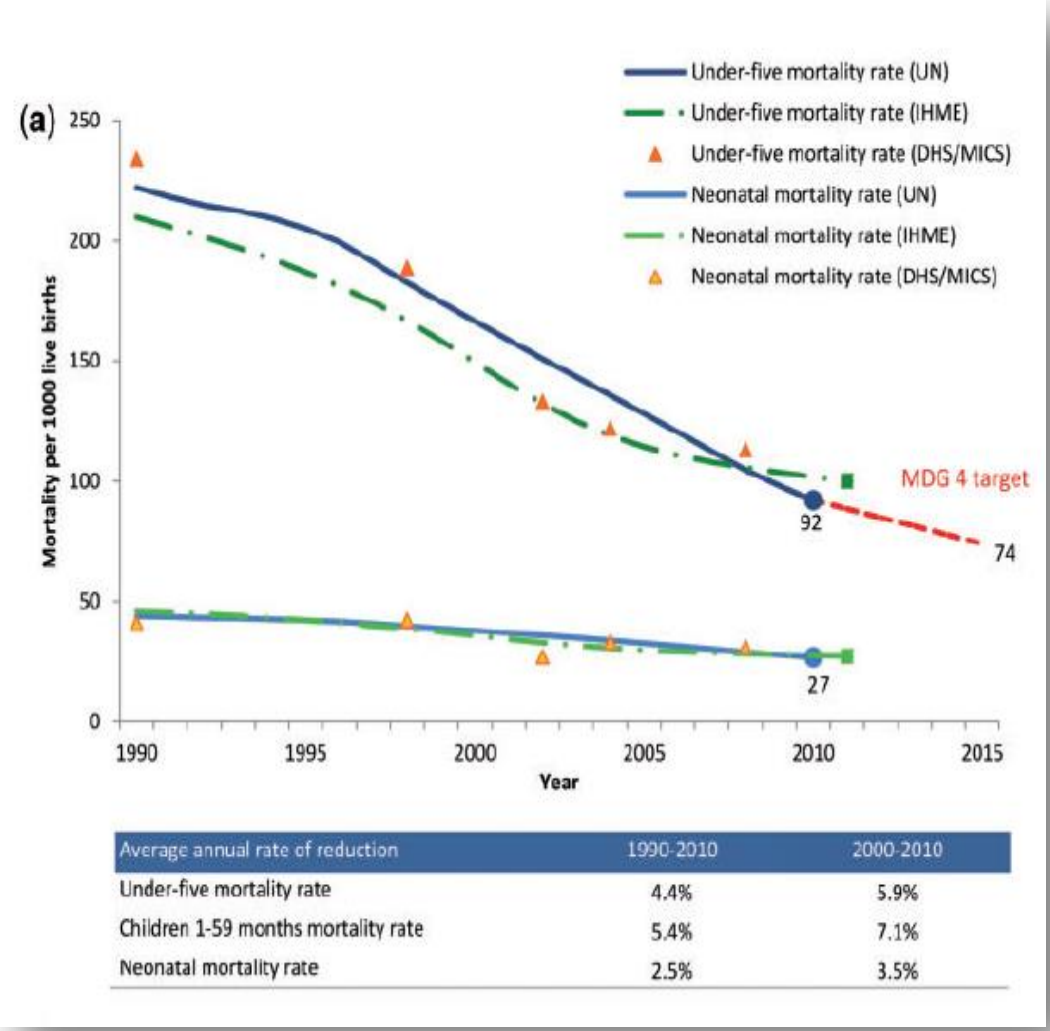
involved in the women groups were much more likely to seek medical attention in case of a sick neonate and attend antenatal clinics. The use of female health workers helped in identifying a sick neonate through home visits as early as possible and either referring them to the nearest facility for proper management or treating them at home with antibiotics if it was not possible to refer the neonate. This therefore ensured that the sick newborn was promptly identified and treated. These interventions have been tried in Asia and it would be interesting to see if these interventions would have the same impact in Sub-Saharan africa. The African WHO community management of severe neonatal sepsis study (AFRINEST) is currently underway {WHO (2013)}.

1.1.4 Epidemiology of Neonatal Mortality in Malawi

Malawi is a sub-Saharan African country of 16,777,547 people with annual births of 599,000, and a neonatal mortality rate of 29 per thousand live births {Count down to 2015 decade report, Liu L, H.L Johnson et al (2012), Zimba E. M.V Kinney et al (2012)}. Malawi is one of the few countries which has achieved millenium development goal number 4 which aims to reduce the 1990 reported childhood deaths by two thirds by 2015{UNICEF Malawi report 2013}. Despite this progress, the neonatal mortality rate has not fallen –Fig 1.6 { Count down to 2015 decade report, Liu L, H.L Johnson et al (2012), Zimba E. M.V Kinney et al (2012)}. The reported neonatal mortality rate for 2004 was 26 per 1,000 live births and 29 per 1,000 live births in 2008{Count down to 2015 decade report, Liu L, H.L Johnson et al (2012), Zimba E. M.V Kinney et al (2012)}. Neonatal deaths account for 31% of all the under-5 deaths in Malawi and nearly 50% of infant deaths. The top 3 causes of

neonatal deaths in Malawi are similar to the global ones namely; prematurity 32%, asphyxia 26% and infections 28% {, Liu L, H.L Johnson et al (2012), Zimba E. M.V Kinney et al (2012)}. It therefore follows that efforts aimed at reducing neonatal deaths would go along way to reduce childhood deaths {Liu L, H.L Johnson et al (2012), Zimba E. M.V Kinney et al (2012)}.

Fig 1.6 Malawi's progress towards MDG 4 from 1990 {Zimba E. M.V Kinney et al (2012)}.



Average annual rate of reduction	1990-2010	2000-2010
Under-five mortality rate	4.4%	5.9%
Children 1-59 months mortality rate	5.4%	7.1%
Neonatal mortality rate	2.5%	3.5%

1.2 Diagnosis of severe neonatal sepsis and meningitis

1.2.1 Clinical diagnosis of severe neonatal sepsis and meningitis

Severe neonatal sepsis is defined as sepsis within the first 28 days of life {Lawn J. E, S Cousens et al (2005)}.In the literature; severe neonatal sepsis may be defined both clinically and/or microbiologically, by positive blood and/or cerebrospinal fluid cultures. However, for the purposes of this review and in line with current thinking, sepsis will be regarded as a clinically defined syndrome where there is a systemic response to a possible infection {Critical care Medicine Consensus conference (1992)}.

This systemic response is characterized by two or more of the following: fever or hypothermia, tachycardia, tachypnoea or hyperventilation and abnormal white blood cells or increase in immature forms {Critical care Medicine Consensus conference (1992)}. However there are other conditions in a neonate which can mimic severe neonatal sepsis. These include congenital cardiac anomalies, birth asphyxia and inborn errors of metabolism. This makes confirmation of the diagnosis of severe neonatal sepsis challenging {Anderson-Berry A., L.Bellig et al (2010)}.

Currently, criteria for severe neonatal sepsis usually include documentation of infection in a new-born infant with a serious systemic illness in which non-infectious explanations for the abnormal pathophysiologic state are excluded or unlikely {Claudio C, A. Panero et al (2004)}.

1.2.2 Laboratory Diagnosis of Severe neonatal sepsis

Diagnosis of severe neonatal sepsis still poses a major challenge to both the clinician and the laboratory {Mishra U.K, S.E Jacobs et al (2006)}.The current gold standard for the diagnosis of severe neonatal sepsis is a positive blood culture which in itself has challenges {Mishra U.K, S.E Jacobs et al (2006}. The drawback with blood cultures include the 24-48 hour assay time and the low yield of positive cultures {Squire E, B Favara et al (1979)}. Cultures are not free from error because they can be falsely sterile, as suggested by blood cultures taken post-mortem {Squire E, B Favara et al (1979)}. In this study babies who had died from presumed severe neonatal sepsis and had initial blood cultures negative had repeat cultures after death which subsequently were positive. The low yield from blood cultures may also be caused by insufficient sample volumes, intermittent or low-density bacteraemia, or suppression of bacterial growth by earlier antibiotic administration {Claudio C, A. Panero et al (2004)}.

It would be useful to have a cheap bedside test with good sensitivity and specificity for the diagnosis of severe neonatal sepsis. This would ensure that appropriate treatment is instituted early and that unnecessary use of antibiotics is avoided.

A test with a rapid turnaround time with 100% sensitivity, rather than high specificity, which allows accurate diagnosis and appropriate antimicrobial treatment or which allows antibiotics to be withheld in non-infected infants, is desirable especially in this era of multidrug resistance{ Mishra U.K, S.E Jacobs et al (2006}. There are newer diagnostic tests which in combination will ensure greater diagnostic accuracy for severe neonatal sepsis but they are not routinely available

to the laboratory { Mishra U.K, S.E Jacobs et al (2006}.The newer diagnostic tests are grouped into acute phase reactants, cell surface markers, granulocyte colony stimulating factor, cytokines, molecular genetics and molecular and cellular proteomics{Tripathi S, G.K Malik et al (2010)}. These will be discussed below.

Acute phase reactants

These endogenous peptides are produced by the liver as part of an immediate response to infection or tissue injury. These reactants include C-reactive protein (CRP), procalcitonin, fibronectin, haptoglobin, lactoferrin, neopterin and oromucosoid{ Tripathi S, G.K Malik et al (2010)}. CRP has been most extensively investigated {Fowlie P.W, B. Schmidt et al (1998)} but there has been more recent interest in procalcitonin as a marker of severe neonatal sepsis.

CRP is synthesized by the liver within six to eight hours of exposure to an infective process or tissue damage, with a half life of 19 hours, and may increase more than 1000 fold during an acute phase response{Vigush D.M, M.B Pepys et al (1993)}. Serial measurements of CRP are therefore important{ Tripathi S, G.K Malik et al (2010)}. The ranges of sensitivity and specificity for diagnosis of early onset severe neonatal sepsis range between 43-90% and 70-78% respectively across more than 10 studies{ Fowlie P.W, B. Schmidt et al (1998)} }. Specificity and positive predictive value of CRP for late onset sepsis increases from 93% to 100% respectively making it a more reliable marker of late neonatal infection{Ng P.C, S.H.Chenget al (1997)}. CRP as a diagnostic marker in neonates has higher sensitivity and specificity than total neutrophil count and immature to total neutrophil ratio{Da Silva O., A. Ohlsson et al (1995)}. In the majority of published reports, upper limits for CRP during the

neonatal period have been obtained from symptomatic uninfected patients with only few studies having obtained the upper limit in healthy newborns{Mathers N.J, F Pohlandt et al (1987)Pourcyrous M, H.S Bada et al (1991), Shine B, J.Gould et al (1985)}. Most of the studies on healthy newborns were cross-sectional with small sample sizes ranging from 16 to 69 and the sampling times were not specified {Chiesa C, Signore F et al (2001)}. The upper limits for CRP in the studies ranged from 5mg/litre to 10mg/litre. It is widely accepted that a CRP of > 10mg/litre is suggestive of infection in a newborn{Van den Hoogen A, L.J Gerards et al (2009)}.

Procalcitonin is another important acute phase reactant produced by monocytes and hepatocytes. It begins to rise four hours after exposure to bacterial endotoxin, peaking at six to eight hours, and remaining raised for at least 24 hours with a half life of 25 to 30 hours {Dandona P, D. Nix et al (1994)}.The sensitivity of procalcitonin at birth is low ranging from 70-80% but increases thereafter {Chiesa C, G Pelligrini et al (2003)} making serial measurements useful as is the case with CRP { Dandona P, D. Nix et al (1994}.

One needs to be aware of the natural fluctuation that procalcitonin presents in the immediate postnatal period that necessitates very careful adjustments in the normal ranges {Assuma M, F Signore et al (2000).The fluctuation may explain the conflicting cut-off points for abnormal values that have been reported for procalciton{Franz A.R, M Kron et al (1999)}. It may be superior to other acute phase reactants with sensitivity and specificity ranging from 87% to 100% but it is not a readily available diagnostic assay in most institutions in developed countries as it is expensive {Ballot D E, O Perovic et al (2004)}.

Cell surface markers

There has been advances in flow cytometric technology which have opened up new ways of detecting cell surface antigens on blood cells.This technology is better than the conventional immunological assay methods for localising the activated markers to a specific cell type {Simms H H, R. D'Amico et al (1995)}. Assessing the cellular response to the cytokines may be a better way of identifying early immunological response to bacterial invasion than circulating concentrations of cytokines which may not necessarily reflect their biological activities {Lehr H A, F. Krombach et al (1995) }. Cell surface markers CD11b and CD64 appear to be promising markers for the diagnosis of early and late onset infections {Weirich E, R.L Rabin et al (1998)}. CD11b is a subunit of the 2 intergrin adhesion molecule which is normally expressed at a very low concentration on the surface of non-activated neutrophils {Weirich E, R.L Rabin et al (1998). There is a 2-4 fold increase in neutrophil CD11b expression in infants with blood culture positive sepsis {{Weirich E, R.L Rabin et al (1998)}. The sensitivity and specificity of CD11b for diagnosing early onset severe neonatal sepsis are 86.3-100% and 100% respectively {Nupponen I, S. Andersson(2001) et al}. However CD11b has been noted to increase in preterm infants with respiratory distress syndrome who are mechanically ventilated {Turunen R, I Nupponen I et al (2006)}. Mechanical ventilation is thought to induce the rise making CD11b not a good marker for severe neonatal sepsis in these preterm ventilated babies { Turunen R, I Nupponen I et al (2006)}. On the other hand CD64 which is also a neutrophil cell surface marker which when compared with CD11b and two lymphocyte surface markers (CD25, CD45RO) has a better sensitivity 97%, specificity 90% and negative predictive value 99% as a diagnostic marker of early onset

neonatal infection both at the onset of infection and 24 hours later { Turunen R, I Nupponen I et al (2006)}.These promising white cell markers require flowcytometry and are not readily available diagnostically { Turunen R, I Nupponen I et al (2006)}.

Granulocyte colony stimulating factor

Granulocyte colony stimulating factor (GCSF), a mediator produced by the bone marrow, facilitates proliferation and differentiation of neutrophils, and has been proposed to be a reliable infection marker for early diagnosis of severe neonatal sepsis {Mishra U.K, S.E Jacobs et al (2006)}. A concentration of > 200 pg/ml has a high sensitivity (95%) and negative predictive value (99%) for predicting early onset neonatal bacterial and fungal infections {Fowlie P.W, B Schmidt et al (1998)}.

Cytokines

Neonates initially depend on innate (natural, non specific) immunity as antigen specific immunity develops later on at 2 years of age for encapsulated bacteria{ Fowlie P.W, B Schmidt et al (1998)}. This innate immunity includes phagocytosis (by monocytes, tissue macrophages and neutrophils),natural killer cells, and humoral mediators (CRP, complement and maternal antibodies) {Mishra U.K, S.E Jacobs et al (2006)}.

In response to antigens such as bacterial endotoxins {Schultz C, C Rott et al (2002)} activated tissue macrophages produce TNF and IL1. These proinflammatory cytokines stimulate endothelial cells to express receptors for intercellular adhesion

molecule on white blood cells. This initiates the cytokine cascade towards increased production of IL6, IL8, and chemokines {Berner R, Niemeyer C.M et al (1998)}. Some bacteria activate epithelial cells directly to produce inflammatory cytokines.

Newborn infants display a higher percentage of IL6 and IL8 positive cells than do adults {Berner R, Niemeyer C.M et al (1998}.There is a sharp rise in IL6 concentration on exposure to bacterial products, which precedes the increase in CRP. Umbilical cord blood IL6 has been consistently shown to be a sensitive marker for diagnosing early onset severe neonatal sepsis, with sensitivities of 87–100% and negative predictive values of 93–100% {Smulian J.C, A.M. Vintzileos et al (1999), Ng P.C, S.H Cheng et al (1997)}. IL6 has the highest sensitivity (89%) and negative predictive value (91%) at the onset of infection compared with other biochemical markers, including CRP, IL1β, TNFα, but sensitivity is reduced at 24 and 48 hours (67% and 58% respectively) because IL6 concentrations fall rapidly and become undetectable after 24 hours{Buck C, Bundschu J et al (1994)}. The combined measurement of IL6 (early and sensitive) with CRP (late and specific) in the first 48 hours of presumed septic episodes improves the sensitivity compared with either marker alone {Buck C, Bundschu J et al (1994)}.

IL8 is a proinflammatory cytokine that is predominantly produced by monocytes, macrophages, and endothelial cells, {Baggiolini M, A, Walz et al (1989)} with similar kinetics to IL6 {Buck C, Bundschu J et al (1994)}. It is produced in response to various stimuli such as LPS and TNF {Baggiolini M, A Walz et al (1989)}. IL8 is considered to be a highly accurate marker with sensitivities ranging from 80% to 91% and specificities from 76% to 100%. IL8 and IL8 mRNA concentrations are substantially higher in infected than non-infected newborns {Buck C, Bundschu J et al (1994)}.}.

The simultaneous measurement of either CRP {Franz A.R, G Steinbach et al (2001), Franz A.R, G Steinbach et al (1999)} or neutrophil cell surface marker CD11b with IL8 further enhances the diagnostic value in the diagnosis of severe neonatal sepsis. A recent multicentre randomized controlled trial of 1291 clinically stable infants with clinical signs or obstetric risk factors suggesting early onset severe neonatal sepsis reported that the combination of IL8 >70 pg/ml and/or CRP >10 mg/l significantly reduced antibiotic therapy from 49.6% to 36.1% ($p<0.0001$) without missing infections; sensitivity was 80%, specificity 87%, positive predictive value 68%, and negative predictive value 93%{ Franz A.R, Bauer et al (2004)}.

Another group of proinflammatory cytokines often linked with sepsis is the IL1 family, including IL1α, IL1β, and IL1 receptor antagonist (IL1ra). The diagnostic usefulness of IL1β is minimal given conflicting reports of both increasing {89, Atici A, M Satar et al (1996), Buck C, Bundschu J et al (1994)} and decreasing {Atici A, M Satar et al (1996)} concentrations associated with sepsis. In contrast, concentrations of IL1ra have been shown to be consistently increased in septic patients with concentrations of 6–30 μg/l {Kuster H, M. Weiss et al (1998} compared with lower concentrations in uninfected neonates of 2–3 μg/l. IL1ra is therefore a better marker than IL1 β. TNFα is a proinflammatory cytokine that stimulates IL6 production and has a broad spectrum of biological actions on several types of target cell, both immune and non-immune. Newborns developing early onset infection are born with higher TNFα concentrations than non-infected infants { Kowalik K, M.B. Czeszynska et al (2003), Zilow E.P, W Hauck et al 1997}.

Molecular detection of bacteria

There have been an increasing number of reports on the use of nucleic acid amplification techniques such as PCR in the detection of bacterial genomes in blood cultures {Tripathi S. and G.K Malik (2010), Maiwald M (2004)}. Polymerase chain reaction relies on the fact that bacteria specific 16srRNA gene is highly conserved in all bacterial genomes {43 Maiwald M (2004)}. Amplification targeting of this 16srRNA gene is a potentially valuable clinical tool in samples with low copy numbers of bacterial DNA. PCR can also be targeted for species specific detection of bacteria in clinical samples as the gene has divergent regions nested within it {{Maiwald M et al (2004)}.

These newer tests are promising but at the moment most of these diagnostic tests are available in a few industrialised countries where the burden of neonatal infections is least. These tests have to be used in combination making the laboratory work up too expensive and unaffordable to many.

1.2.3 Diagnosis of Severe neonatal sepsis in the developing world

In the low and middle income countries most of the diagnosis is based on clinical signs and symptoms as laboratory confirmation is frequently unavailable. There have been a number of studies that have attempted to develop an algorithm of clinical symptoms and signs that is sensitive enough for the diagnosis of severe neonatal sepsis in the absence of laboratory confirmation. In other studies the presence of two or three categories {by organ system} of clinical signs of infection in the infant has been taken to strongly support a diagnosis of sepsis {Buck C, Bundschu J et al (1994), Messer J, D. Eyer et al (1996)}. A multi-centre prospective study developed an algorithm that includes seven signs and symptoms: history of

difficulty feeding, movement only when stimulated, temperature below 35.5 C or 37.5 C or more, respiratory rate over 60 breaths per minute, severe chest in drawing, and history of convulsions – to predict the need for hospitalisation in young infants presenting to health facilities, particularly the first week of life {Young Infants study group (2008)}. The presence of any one sign had high sensitivity (87%) and specificity (74%). Even though this algorithm is very helpful in resource restrained countries where diagnostic tests are scarce other cases of severe neonatal sepsis would be missed. On the other hand the symptoms and signs are not pathognomic of severe neonatal sepsis as such one would also run into problems of over treatment {Kliegman R.M, R.E. Behrman et al (2011)}. It is therefore important that studies continue to explore affordable sensitive and specific laboratory tests that can aid in the diagnosis of severe neonatal sepsis.

1.3. Aetiology of severe neonatal sepsis

Severe neonatal sepsis is divided into early and late onset. Differentiation is important as early onset is more likely to reflect vertically acquired infection whereas late onset reflects community acquired or nosocomial infections { Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003), Bennet R, S.Berdahl et al (1989)}.

The outcome also differs between early onset and late onset severe neonatal sepsis with mortality being high in early onset severe neonatal sepsis. Few studies in sub Saharan Africa differentiate between early and late onset severe neonatal sepsis.

{Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003), Bennet R, S.Berdahl et al (1989)}.

1.3.1 Early Onset Severe neonatal sepsis and Meningitis

Early-onset sepsis usually results from organisms acquired intrapartum. Transplacental infection or an ascending infection from the cervix may be caused by organisms that colonize in the mother's genitourinary tract, with acquisition of the microbe by passage through a colonized birth canal at delivery {Klinger G., I Levy et al (2009), American academy of paediatrics (2003), Seaward P.G, M.E Hannah et al (1998)}.

In the industrialized countries infectious agents associated with severe neonatal sepsis have changed over the past 50 years. *Staph aureus* and *E. coli* were the most common bacterial pathogens causing early onset severe neonatal sepsis during the 1950s in the United States {Anderson-Berry A., L. Bellig et al (2010)}. Over the ensuing decades *Group B streptococcus* (GBS) and gram-negative enteric organisms (predominantly *E. coli*) have accounted for most cases of early onset severe neonatal sepsis in the developed countries{ Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al

(1989)}.Trends in the epidemiology of early onset sepsis show a decreasing incidence of GBS sepsis.

Other organisms that have been known to cause early onset severe neonatal sepsis include other gram negative enteric bacilli e.g. *Klebsiella* spp, gram-positive organisms like *Listeria monocytogenes, enterococci (e.g. Enterococcus faecalis, Enterococcus faecium), group D streptococci* (e.g., *Streptococcus bovis*), *alpha hemolytic streptococci, Streptococcus pneumoniae and Staphylococci* {Seale A.C, Mwaniki M et al (2009), Tausch W.H and R.A Ballard (1998) ,Anderson-Berry A, L.Bellig et al (2010), Chan K.Y, H.S Lam et al (2009), Byington C.L, F.R Enriquez et al (2004)}.

1.3.2 Late Onset Severe neonatal sepsis and Meningitis

Late onset sepsis is usually acquired from the environment either in the hospital (nosocomial) or more commonly in the community. The neonate's skin, respiratory tract, conjunctivae, GI tract and Umbilicus may become colonized from the environment, leading to the possibility of late onset sepsis from invasive microorganisms, Indwelling lines, vascular or Urinary catheters or contact from caregivers with bacterial colonization can act as vectors for the colonization { Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996) , Tausch W.H and R.A Ballard (1998) ,Anderson-Berry A, L.Bellig et al (2010) American Academy of paediatrics(2003)}.

Coagulase negative staphylococci, *S. aureus* and *group B streptococci* (*S. agalactiae*) are the dominant causes of late onset severe neonatal sepsis in the developed countries {11}. Other organisms that can causes late onset severe neonatal sepsis are *E coli, Klebsiella, Pseudomonas, Enterobacter, Candida, Serratia,Acinetobacter* and anaerobes {Tausch W.H and R.A Ballard (1998) ,Anderson-Berry A, L.Bellig et al (2010), Osrin D, S. Vergnano et al (2004), Heath P.T, N.K Nik Yusoff et al (2003)}.

The pathogens most often implicated in severe neonatal sepsis in developing countries differ from those seen in developed countries. Gram negative organisms are more common and mainly include *Klebsiella* sp., *Escherichia coli, Pseudomonas sp*. and *Salmonella.* Gram positive organisms that are most commonly isolated include *Staphylococcus aureus, coagulase negative staphylococci, Streptococcus pneumoniae* and *Streptococcus pyogenes* { Osrin D, S. Vergnano et al (2004), Heath P.T, N.K Nik Yusoff et al (2003)}.

Coagulase negative staphylococci are important causes of septicaemia in patients with compromised host defences such as newborn infants, and especially in the premature babies receiving invasive procedures. In addition to being a cause of severe neonatal sepsis, the ubiquitous nature of coagulase-negative staphylococcus as part of the normal skin flora makes it a frequent contaminant of blood cultures. Thus a blood or CSF culture growing coagulase negative staphylococcus may represent a contaminated sample rather than true coagulase negative staphylococcal septicaemia. The clinical setting (high risk in preterm babies undergoing invasive procedures), colony counts and presence of polymorphonuclear (PMN) cells on gram stain of the submitted specimen often help

to differentiate true infection and positive culture from a false-positive or contaminated specimen {Shet A, Ferrieri et al (2004), Anderson-Berry A, L Bellig et al(2010)}.

GBS was infrequently reported in the developing world until recently {Shet A, Ferrieri et al (2004)}. A multicentre study of the bacterial aetiology of serious infections in infants of <3 months of age by World Health Organisation reported a striking absence of GBS {The Young infant study group (1999)}. This was particularly surprising because the prevalence of maternal carriage of GBS in developing countries, including sub Saharan Africa is similar to that identified in populations in the United States. However recent studies from Kenya {Berkley J.A, B.S Mwangi et al (2005)}, South Africa {Bomela HN, D.E Ballot et al 2001;}, Zimbabwe{ Nathoo K.J, P.R Mason et al (1990} and Malawi {Milledge J, J.C Callis et al (2005),Gray K.J (2007)} suggest that GBS is emerging as an important cause of severe neonatal sepsis in Africa.

The reason for the differences in the pathogens causing severe neonatal sepsis across the world are not well known These could perhaps reflect an epidemiological transition in some countries or it could reflect an epidemiologic bias linked to the fact that most early onset sepsis babies in the developing world die at home before reaching the health facilities and they do not appear in the statistics and the causative organisms are never known {Shet A, Ferrieri et al (2004)}. The differences in the definition of early onset severe neonatal sepsis, inability to culture certain organisms e.g. *Listeria monocytogenes* and differences in surveillance periods could

also have an impact on the wide variety of bacteria described in different countries {Tausch W.H and R.A Ballard (1998), Shet A, P Ferrieri et al (2004)}.

1.3.3 Non-bacterial Causes of Severe neonatal sepsis and Meningitis

Severe neonatal sepsis and meningoencephalitis can also be caused by a variety of viruses, including herpes simplex virus, enterovirus, adenovirus, cytomegalovirus, HIV, respiratory syncytial virus, and rubella. Other sexually transmitted diseases have also been implicated in neonatal infections. These include syphilis and Trichomonas vaginalis. Toxoplasmosis and candida can also cause neonatal infections {Tausch W.H and R.A Ballard (1998), Anderson-Berry A, L. Bellig et al (2010)}.

In malaria endemic areas, malaria has also been found in neonates masquerading as septicaemia {Van den Hoogen A, L.I Gerards et al (2009), Klinger G., I Levy et al (2009)}.

1.4 Treatment of Severe neonatal sepsis and Meningitis

It is important to initiate treatment promptly once there is suspicion of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis as the neonate is a relatively immunocompromised host { Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}.Treatment consists of intravenous antibiotics with supportive treatment tailored as per the neonate's needs. The duration of antimicrobial therapy for neonatal meningitis is 14-21 days whereas 7-10 days may be appropriate for severe

neonatal sepsis (Tausch W.H and R.A Ballard (1998), Anderson-Berry A, L. Bellig et al (2010)}.

The antibiotic combination prescribed in most countries for the treatment of severe neonatal sepsis is penicillin together with an aminoglycoside. The biggest challenge to this combination is the development of resistance {Tausch W.H and R.A Ballard (1998) Anderson-Berry A, L. Bellig et al (2010), Red Book (2003)}. There are an increasing number of reports of multi-resistant bacteria causing severe neonatal sepsis in developing countries, particularly in intensive care settings. There are few studies which compare antibiotic susceptibility over time in the same unit, but where data are available they show increasing resistance to commonly used antibiotics. Most gram negative bacteria are now resistant to gentamicin. The emergence of a reduction in the susceptibility to third generation cephalosporins and quinolones is worrying {American academy of paediatrics (2003) Tausch W.H and R.A Ballard(1998), Anderson-Berry A., L Bellig et al (2010)}.

There have been other therapies investigated in the treatment of severe neonatal sepsis; however no substantial clinical trials have shown that these treatments are beneficial. These therapies include granulocyte transfusion, intravenous immune globulin (IVIG) replacement, exchange transfusion, and the use of recombinant cytokines {Anderson-Berry A., L Bellig et al (2010)}.

Granulocyte transfusion has been shown to be suitable for infants with significant depletion of the storage neutrophil pool; however documentation of a depleted storage pool requires a bone marrow aspiration, which is invasive. The granulocyte transfusion has to be administered quickly to be beneficial. The transfusion has

potential side effects including graft versus host disease, transmission of CMV or hepatitis B and pulmonary sequestration is considerable. This therapy is still experimental {Anderson-Berry A., L. Bellig et al (2010)}.

IVIG infusion has been studied as a possible therapy for severe neonatal sepsis to provide type specific antibodies to improve opsonisation and phagocytosis of bacterial organisms and improve complement activation and chemotaxis of neonatal neutrophils. However the effect has been transient and clinically available IVIG solutions do not contain type specific antibody and adverse effects associated with the infusion of any blood product can follow. At present, the data do not support the routine use of IVIG in severe neonatal sepsis {Anderson-Berry A., L. Bellig et al (2010)}.

Recombinant human cytokine administration to stimulate granulocyte progenitor cells has been studied as an adjunct to antibiotic therapy. This has shown promise in animal models especially for GBS sepsis but require pre-treatment or immediate treatment to demonstrate efficacy. The use of granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) has been studied in clinical trials but their use in clinical neonatology remains experimental {Anderson-Berry A., L. Bellig et al, Moraes-Pinto M.I, F. Verhoeff et al (1998)}

1.5 Impact of maternal HIV on newborn immunity

The neonate can be regarded as an immune compromised host especially when premature and are therefore at a higher risk of catching infections. Neonates have

extremely low immunoglobulin (Ig) levels except for IgG to specific maternal antigens transferred passively across the placenta during the last trimester of pregnancy {Stiehm E.R, H.H. Fudenberg et al (1966), Levy O (2007), Moraes-Pinto M.I, F. Verhoeff et al (1998)}. T cell function is relatively unimpaired but complement activity is half that of healthy adults {Stiehm E.R, H.H. Fudenberg et al (1966), Moraes-Pinto M.I et al (1998), Kemp A.S, D.E, Campbell et al (1996)}. Neonates have a low neutrophil storage pool, and their existing neutrophils have impaired capacity to migrate from the blood to sites of infection {Levy O, S. Martin et al (1999)}. The basal expression of Toll-like receptors (TLRs, receptors that detect the presence of microbes) is similar in the neonate and adult {Kemp A.S et al (1996)}. However, innate immune responses of neonatal mononuclear cells are characterised by markedly reduced release of the proinflammatory Th1-polarizing cytokines tumour necrosis factor-alpha (TNF-) and interferon-gamma (IFN-).

Several clinical conditions can interfere with the materno-fetal transport of specific IgG antibodies across the human placenta. These include prematurity, maternal hypergammaglobulinemia, maternal HIV infection and placental malaria {Moraes-Pinto M.I, F. Verhoeff et al (1998)}. It has been noted that among South African infants, antenatal HIV exposure was associated with lower specific antibody responses in exposed, uninfected infants compared with unexposed infants at birth. These HIV exposed uninfected infants had lower levels of specific antibodies to Haemophilus influenza b, pertussis, pneumococcus and tetanus {Kemp A.S, D.E Campbell et al (1996), Jones E.C, S Naidoo et al (2011)}. One would therefore expect that HIV exposed non-infected babies would be at a greater risk of developing severe neonatal sepsis compared to babies born from HIV non-infected mothers

and perhaps have a worse clinical course leading to a poorer outcome. This has yet to be fully established.

In infants who acquire HIV in utero and around the time of delivery, disease progression occurs very rapidly in the first few months of life often leading to death {Meyers T., H. Moultrie et al (2007), Newell M.L, H Coovadia et al (2004}. One would expect that the risk of severe neonatal sepsis will be considerably increased in these HIV infected babies. There is need for more data on the outcome of neonatal infections in HIV exposed babies.

1.6 Outcome of severe neonatal sepsis and meningitis

1.6.1 Outcome of Severe neonatal sepsis

Term infants are not likely to experience long term complications of severe neonatal sepsis if the diagnosis is made early enough and appropriate treatment instituted {Anderson-Berry A., L Bellig et al (2010)}. However if early signs and or risk factors are missed the mortality rate increases. Severe neonatal sepsis (especially early onset) has been shown to carry a high mortality risk {Anderson-Berry A, L. Bellig et al (2010)}. In the developing world where the majority of neonatal infections are the mortality rate ranges from 30 to 50% { Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}.The mortality rate is much higher in cases of neonatal meningitis with rates above 50%.

Severe neonatal sepsis has been shown to have long term effects on neurodevelopment especially in preterm and very low birth weight infants { Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}. The developing brain, particularly the periventricular white matter, is vulnerable to cytotoxic and hypoxic/ischaemic injury, which places these infants at increased risk for abnormal cognitive and motor functioning. Recent studies have linked infection associated with chorioamnionitis, sepsis and necrotizing enterocolitis with adverse neurodevelopmental outcome and impaired growth in preterm infants {Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}.

An association between infection and brain injury including severe intraventricular haemorrhage and periventricular leukomalacia has been shown by other investigators { Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}.

It has been postulated that exposure of the preterm brain to inflammatory mediators during infectious episodes contributes to brain injury and poor developmental outcome { Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}.

1.6.2 Outcome of neonatal meningitis

Meningitis in neonates can progress rapidly to serious complications {Anderson-Berry A, L. Bellig et al (2010)}. These include cerebral oedema, hydrocephalus, haemorrhage, ventriculitis, abscess formation and cerebral infarction. Cerebral oedema, hydrocephalus and haemorrhage each may cause increased intracranial pressure, with potential for secondary ischemic injury to the brain { Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}.

Cerebral oedema can result from vasogenic changes, cytotoxic cell injury and inappropriate antidiuretic hormone secretion {Anderson-Berry A., L Bellig et al (2010)}.

Hydrocephalus which develops as a result of debris obstructing CSF flow through the ventricular system or dysfunction of the arachnoid villi has been reported to occur in as many as 24% of neonates with bacterial meningitis {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}.

Meningitis has been shown to be associated with 1.6% of all cases of neonatal arterial stroke and 7.7% of venous infarcts {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001), Fitzgerald K.C, M.R Golomb et al (2007), Ment L.R, R.A Ehrenkranz et al (1986)}.

Survivors of neonatal meningitis are at a significant risk for moderate to severe disability; 25-50% have significant problems with language, motor function, hearing, vision and cognition and five to twenty percent have future epilepsy { Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}.

Survivors of neonatal meningitis are also more likely to have more subtle problems, including visual deficits, middle ear disease and behavioural problems { Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}. Recent data suggests that as high as 20% of children who were being identified as normal at 5 year follow-up may have significant educational difficulties lasting into late adolescence {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}.

There are poor prognostic factors known to predispose to the development of complications from neonatal meningitis. These include low birth weight, prematurity, significant leucopenia or neutropenia, high CSF protein, delayed sterilization of the CSF, seizures lasting for more than 72 hours and coma {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001) , Volpe J.J (2008) }.

The use of acyclovir has reduced morbidity and mortality from herpes simplex virus meningitis yet 50% of the survivors will have neurological sequelae {Kimberlin D. (2004)}.

There is an existing gap on the long term outcome of severe neonatal sepsis and meningitis in sub-Saharan Africa. It is important to establish what happens to the babies who have been discharged with a diagnosis of severe neonatal sepsis. Important questions to answer include what sequelae do they develop? What happens to their development? Are they still alive a few months down the line? If they die what is the cause of death? Are they at a higher risk of catching infections during the first year of life?

1.7 Prevention of Severe neonatal sepsis and Meningitis

The bulk of the 4 million annual global neonatal deaths (99%) occur in developing countries and approximately 36% are attributed to infections. In communities with high neonatal mortality rates, infections account for approximately half of all newborn deaths A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}. Continued efforts are required to describe optimal community-based delivery of proven interventions and to identify new, affordable, efficacious, and safe interventions to prevent infections in low-resource settings.

Chlorhexidine is a broad-spectrum antiseptic that has been used extensively for many decades in hospital and other clinical settings. It has also been given as maternal vaginal lavage, full-body newborn skin cleansing, and/or umbilical cord cleansing to prevent infection in neonates {Mullany L.C, G.L Darmstadt et al (2006), Tielsch J.M, G.L Darmstadt et al (2005) Bakr A.F, T karkour et al (2005), Taha T.E, R.J. Biggar et al (1997)}. Recent evidence suggests that these chlorhexidine interventions may have significant public health impact on the burden of neonatal infection and mortality in developing countries. There is evidence that delivery of chlorhexidine solutions by vaginal lavage during labour and delivery, full-body newborn skin cleansing, and/or umbilical cord cleansing reduces neonatal bacterial colonization, infection and mortality {Mullany L.C, G.L Darmstadt et al (2006), Tielsch J.M, G.L Darmstadt et al (2005) Bakr A.F, T Karkour et al (2005), Taha T.E, R.J. Biggar et al (1997)}. Research on chlorhexidine use in newborns in developed countries has focused mainly on antisepsis of central venous catheters, as well as prevention of vertical transfer of microorganisms, especially group B streptococcus (GBS), from mother to newborn at the time of delivery {Adriaanse A.H, L.A.A. Kollee et al (1995), Burman L.G, P. Christensen et al (1992)}. In developing countries, investigators have examined the potential of chlorhexidine vaginal cleansing to reduce vertical transmission of HIV and prevent neonatal morbidity and mortality {Taha T.E, R.J Biggar et al (1997), Bakr A.F, T Karkour et al (2005)}. The evidence for impact of intrapartum vaginal cleansing with chlorhexidine on vertical transmission of HIV, neonatal colonization and infection with GBS, and on other infections has been reviewed by Cochrane meta-analyses {Denton G.W (2001), Stade B, V Shah et

al (2004), Lumbiganon P et al (2004)}. On the whole studies have shown a positive impact on the reduction of neonatal infections.

Although the potential impact of chlorhexidine-based interventions has been demonstrated in a number of studies discussed above, there have been few well-designed studies in low-resource settings. Given the burden of neonatal mortality in communities of developing countries, there is a need for simple interventions to reduce this burden {Mullany L, G. Darmstadt et al (2006)}.

Group B streptococcus remains one of the top causes of early onset severe neonatal sepsis. Increased screening and intrapartum antibiotic treatment of GBS positive mothers has led to a substantial reduction in the contribution of GBS to early onset severe neonatal sepsis {Anderson-Berry A, L. Bellig et al (2010), Gordon A.L, M. English et al (2005}. This has mainly been done in the developed world and is yet to be routinely implemented in the developing world where the burden of the disease is. Intrapartum antibiotic use has not been shown to have an impact on late onset GBS disease (See Figure 1. 7 and 1. 8 below):

Figure 1.7 Incidence of Early-Onset Invasive Group B Streptococcal Disease in Black Neonates and White Neonates in Four Active Surveillance Areas (California, Georgia, Tennessee, and Maryland), 1993 through 1998 {Schrag S.J, L. Lefkowitz et al (2000)}.

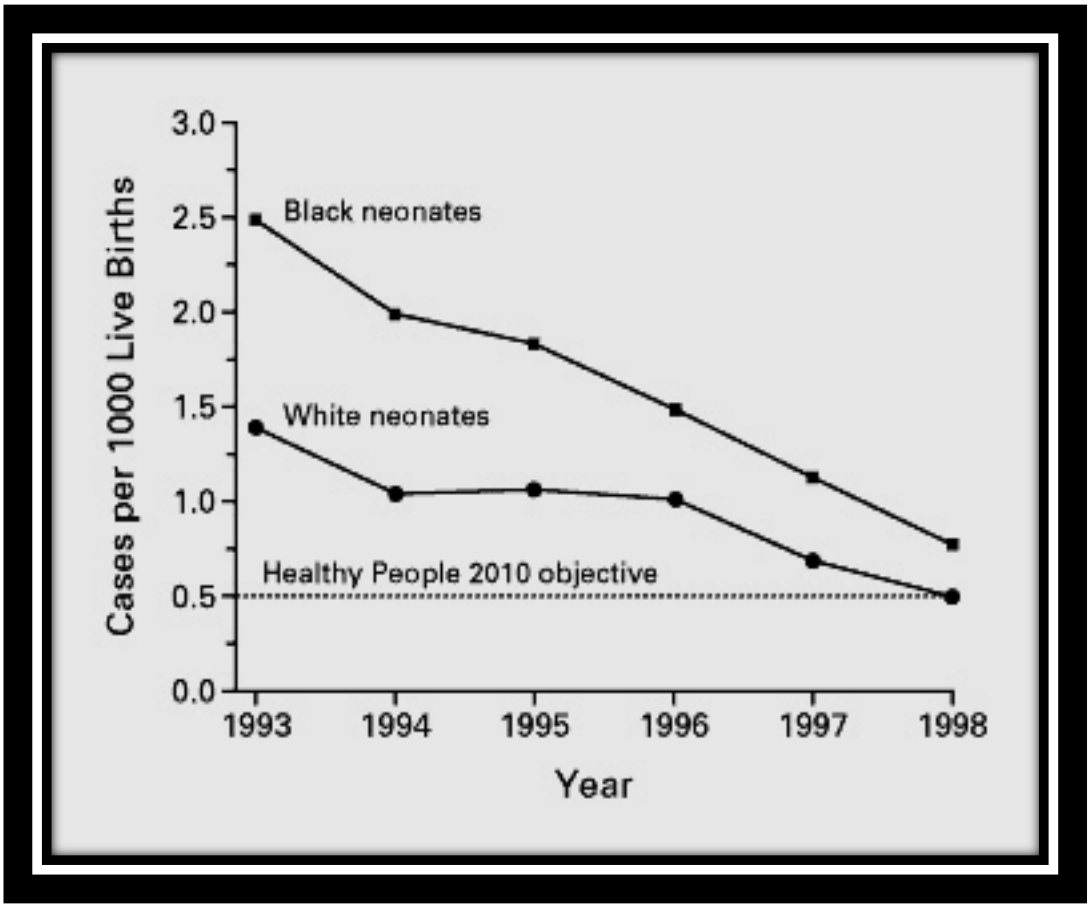
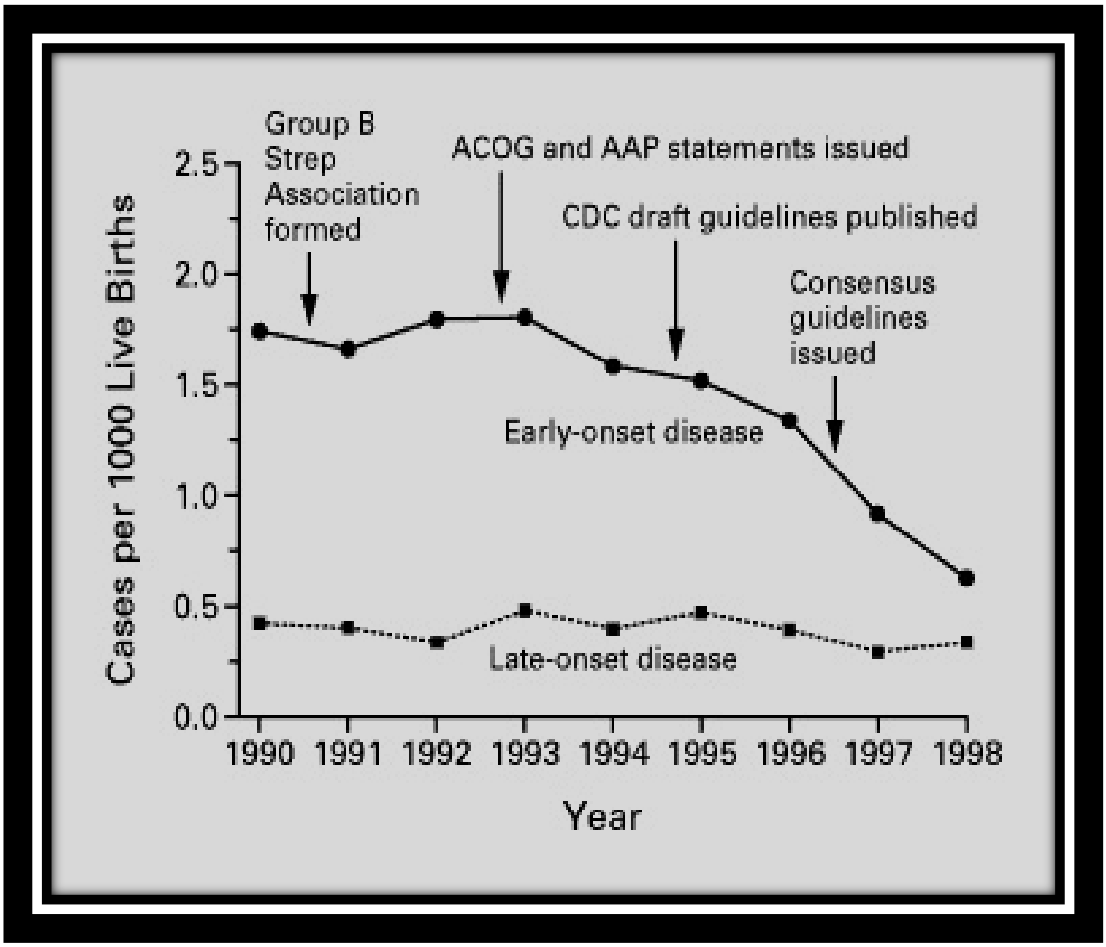


Figure 1.8 Incidence of Early- and Late-Onset Invasive Group B Streptococcal Disease in Three Active Surveillance Areas (California, Georgia, and Tennessee), 1990 through 1998, and Activities for the Prevention of Group B Streptococcal Disease {Schrag S.J, L. Lefkowitz et al(2000)}.



In spite of universal screening, and the great progress that has been made, GBS-early onset disease continues to occur and the GBS burden remains a significant public health issue {Anderson-Berry A., L. Bellig et al (2010), Schrag, S Zywicki et al (2000)}. Continuous efforts to improve screening for GBS status continue to be important and may be able to take advantage of new rapid diagnostic technologies.

The current screening-based strategy for prevention is highly effective but imperfect. Given the challenges, limitations and potential complications of maternal intrapartum prophylaxis, a new approach is still needed. Maternal immunization against GBS is an attractive alternative for the prevention of not only neonatal diseases but potentially also stillbirths and maternal diseases {Schrag, S Zywicki et al (2000)}. Vaccines against GBS may become the most effective and sustainable long-term preventive strategy.

1.8 Research question

It is very clear that neonatal infections are a major contributor to neonatal deaths. Proper management of these infections is important in the reduction of neonatal mortality. In trying to address the problem of neonatal infections in Malawi we looked at the "***Aetiology, and Outcome of Severe neonatal sepsis and Meningitis in Malawi***".

1.9 Study hypothesis and objectives

1.9.1 Hypothesis

The risk of neurodevelopmental delay is increased by 10% at one year of age in infants who had an episode of severe neonatal infection compared to those who never had severe neonatal infection

1.9.2 Study Objectives

The following were the study aims:

- To determine the aetiology, resistance pattern and outcome of bacterial severe neonatal sepsis, severe neonatal pneumonia and meningitis at Queen Elizabeth central hospital (QECH) in Malawi between June 2010 and June 2013.

- To determine inpatient mortality outcomes of bacterial severe neonatal sepsis, severe neonatal pneumonia and meningitis cases at Queen Elizabeth central hospital (QECH) in Malawi between June 2010 and June 2013.
- To determine the mortality, neurodevelopmental and neurological outcomes at 6 and12 months of age of neonatal meningitis, severe neonatal pneumonia and severe neonatal sepsis cases discharged from QECH between June 2010 and June 2013 and residing within Blantyre urban.
- To determine the impact of maternal HIV infection on the aetiology, mortality, neurodevelopmental and neurological outcomes at 6 and12 months of age in neonatal meningitis, severe neonatal pneumonia and severe neonatal sepsis cases at QECH between June 2010 and June 2013.

We aimed to better define the organisms that cause neonatal infections in Malawi and their resistance pattern. We also aimed to better define the outcome of severe neonatal sepsis both in the short term and long-term looking at mortality, and morbidity in the first year of life. We also described the neurological sequelae that these babies end up with and also their neurocognitive development.

The information on aetiology will help in coming up with better treatment packages for severe neonatal sepsis and perhaps more lobbying for vaccines like the group B streptococcal vaccine and pneumococcal vaccine .

1.10 Thesis Plan

Within this thesis all the methods will be summarised in chapter 2. The results will be set out in chapters 3 to 6 that cover the following;

- Chapter 3- The clinical features and in hospital outcome of severe neonatal infection in Malawi in a prospective cross-sectional study
- Chapter 4 The long term outcome of severe neonatal infection in Malawi in a longitdunial prospective cohort study with the recruitment of controls from a nearby health centre.
- Chapter 5 Predictors of poor neurodevelopmental outcomes in severe neonatal infection cases at 12 months of age in this cohort.
- Chapter 6 Case series of MRI brain findings in severe neonatal infection cases at QECH in Malawi derived from the cross-sectional cohort.

In chapter 7, the overall results will be summarised and put in the context of the current literature. Further work will then be proposed.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study Location

Malawi is a landlocked country within the sub-Saharan region. It is located south of the equator. It shares boundaries with Zambia in the west and North West, Mozambique in the east, south and southwest and Tanzania in the north and north east (Fig. 2.1). Malawi is 901 kilometres long and has a width that ranges from 80 to 161 kilometres. Malawi has an area of 118,484 km^2 of which 94,276 km^2 is land and the remaining area is mostly composed of Lake Malawi, which is about 475 kilometres long and delineates Malawi's eastern boundary with Mozambique. Malawi's climate is generally tropical continental with some maritime influences. Rainfall and temperature vary depending on altitude and proximity to the lake. There are 3 seasons in a year. A rainy season runs from November to April. The weather becomes cool from May to August and hot from September to November. The country is divided into three administrative regions, namely the northern, central and southern regions. The regions are further divided into 28 districts with 6 districts in the northern region, 9 and 13 districts in the central and southern regions respectively. The study was conducted in the southern region in Blantyre district the commercial city of Malawi.

Fig 2.1 Map of Malawi

Malawi is one of the least developed countries in the world {CIA World Fact Book (2010)}. Malawi is currently ranked number 177 out of 184 countries on the Human Development Index by UNDP {UNDP Human Development reports 2013}. Over the past two decades the human development index for Malawi has been showing some improvement (Fig 2.2).

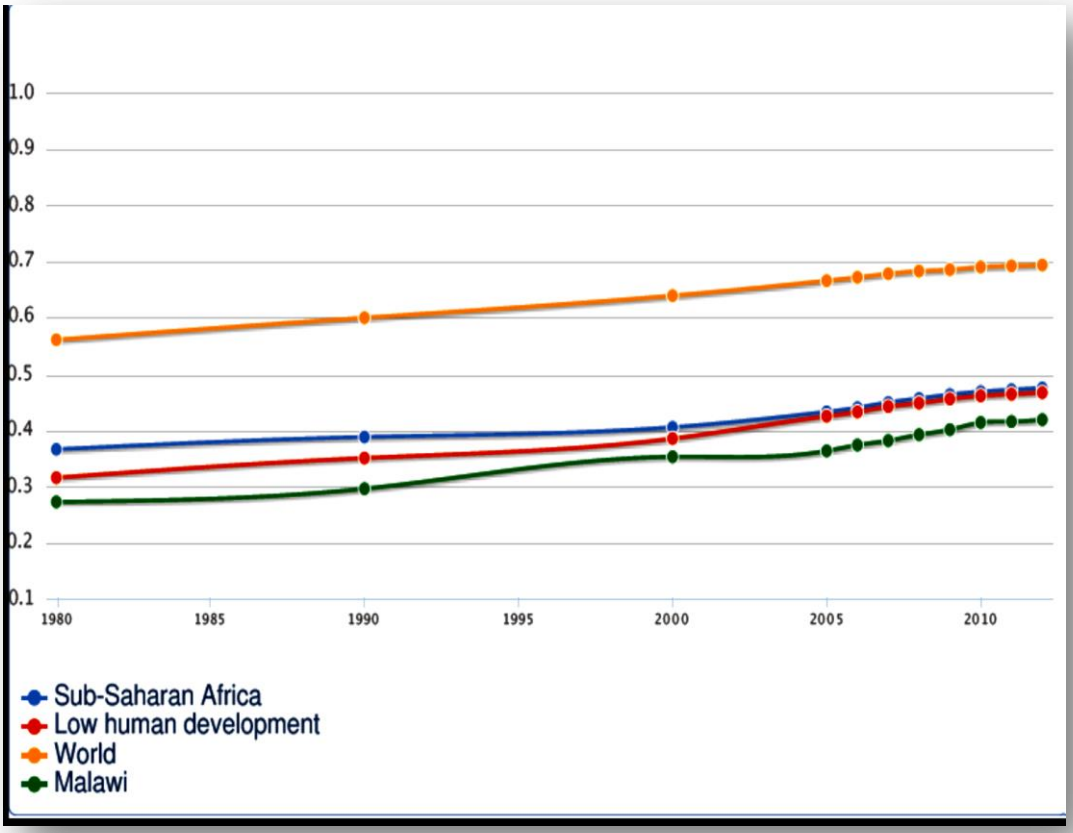


Fig 2.2 Human Development index graph for Malawi compared to the rest of the world

The majority (80%) of the population live in the rural areas {CIA World Fact Book (2010), Republic of Malawi and World Bank (2006)}. Malawi's economy heavily relies on agriculture {Republic of Malawi, Growth and Development strategy (2007),

Republic of Malawi and World Bank (2006)}. The agricultural sector accounts for 35% of the GDP, industry for 19% and services for the remaining 46%.The agricultural sector also accounts for more than 80% of export earnings and it supports more than 85% of the population.

Tobacco has remained the most important export crop accounting for 70% of all export revenues from the agricultural sector {Republic of Malawi and World Bank (2006), Republic of Malawi, Growth and Development strategy (2007)}. There has been an increased pressure from the international community to limit tobacco production and this has led to a decline in the world prices {Republic of Malawi and World Bank (2006)}. This is likely going to place a heavy burden on the country's economy.

The country also relies heavily on tea, sugarcane and coffee, these three plus tobacco make up more than 90% of Malawi's export revenue {Malawi Wikipedia, Republic of Malawi and World Bank (2006), Republic of Malawi, Growth and Development strategy (2007)}. Other crops include cotton, corn, potatoes, sorghum, cattle and goats.

Malawi has few exploitable mineral resources like uranium, bauxite {Republic of Malawi, Growth and Development strategy (2007)}.

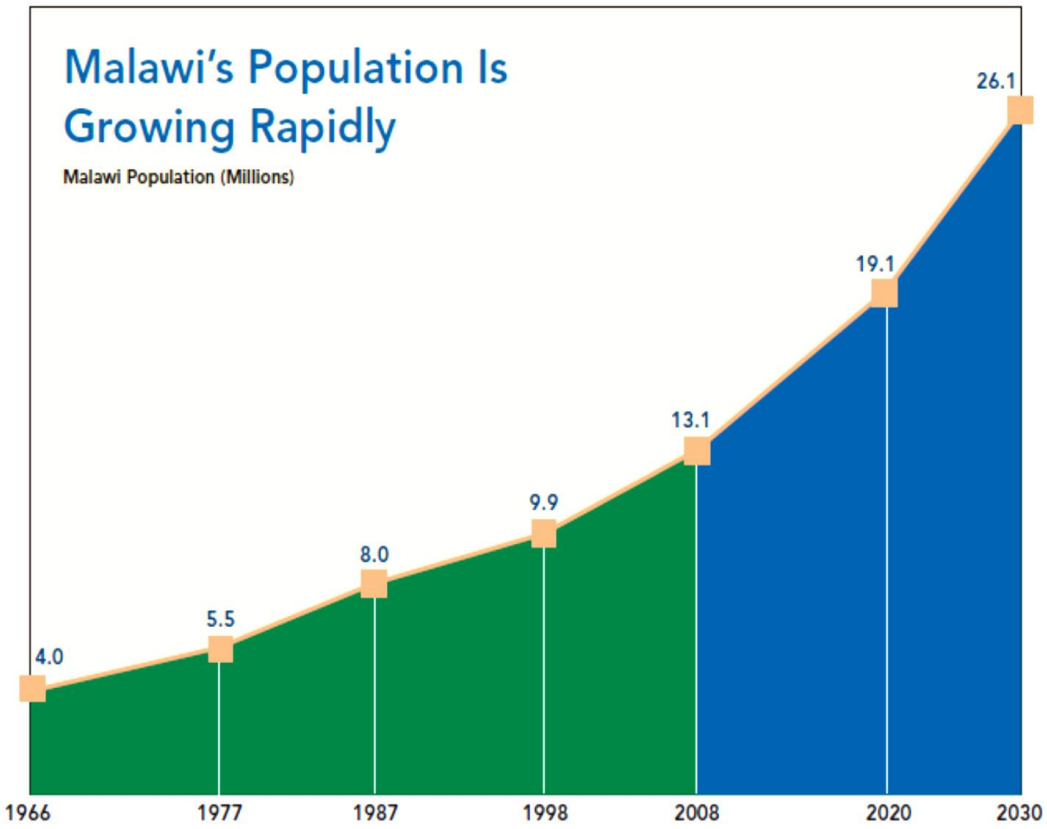
The economy is also dependent on substantial economic aid from the World Bank, International Monetary Fund and other donor countries {Republic of Malawi and World Bank (2006)} .

Table 2.1 Malawi Demographic Indicators from the 1996-2008 Malawi Population and Housing Census (Malawi DHS 2010)

Indicator	Census 1966	Census 1977	Census 1987	Census 1998	Census 2008
Population	4,039,583	5,547,460	7,988,507	9,933,868	13,077,160
Intercensal growth rate	3.3	2.9	3.7	2.0	2.8
Density(pop/sq.km)	43	59	85	105	139
Women of child bearing age as a percentage of female population	47.6	45.1	44.2	47.2	44.4
Sex-ratio (males/100 females)	90.0	93.0	94.0	96.0	94.7
Crude birth rate	N/A	48.3	41.2	37.9	39.5
Crude death rate	N/A	25.0	14.1	21.1	10.4

The Malawi population and housing census is conducted once every ten years however the estimated population for 2013 stands at 16,777,547. This shows that over the past twenty years the population in Malawi has more than doubled see Fig 2.3 below.

Fig 2.3 Malawi Population from the 1966 to 2008, and then extrapolated based on population growth estimates to 2030



Malawi's population is growing rapidly, and in just over 40 years has increased from 4 million people in 1966 to 13.1 million in 2008. Given the high number of births per woman (currently on average 5.7), the population will continue to increase steadily. Even if the total fertility rate declines from the 2010 level of 5.7 to 4.6 by 2020, the population will still grow to 26 million in 2030.

Malawi Population Data Sheet 2012 1

2.1.1 Blantyre

Blantyre, the commercial and industrial capital of Malawi, is in the Shire Highlands and geographical centre of the Southern Region of the country and lies at 35° east of Greenwich Meridian and 15° 42" south of the Equator.

The climate of Blantyre is influenced by its location in the tropical zone and altitude. Blantyre stands at an altitude of 1,039 m (3,409ft) above sea level. The climate in Blantyre is classified as humid subtropical-highland. Blantyre experiences the tropical continental climate with two distinct seasons in the year. The rainy season is from November to April, with continuing light cold showers from end of May to July. The dry season is from May to October.

Blantyre had a population of 732,518 inhabitants in 2008 but it is now estimated to be over 1 million. The majority of the population are located in the urban area. The study took place at Queen Elizabeth Central Hospital (QECH) and Zingwangwa health centre in Blantyre City with full support and sponsorship from the Malawi Liverpool Wellcome Trust Clinical Research Programme (section 2.1.1.1).

2.1.1.1 The Malawi Liverpool Wellcome Trust Clinical Research Programme.

The Malawi Liverpool Wellcome Trust (MLW) clinical research programme {https://www.mlw.medcol.mw} was established in 1995 and became a Wellcome Trust Major Overseas programme in 2003. It is situated within the Queen Elizabeth Central Hospital campus and is in close proximity to the University of Malawi, College of Medicine (CoM) to which it is affiliated. MLW has strong collaborative links with several clinical (Medicine, Paediatrics, Obstetrics & Gynaecology, Surgery, Community Medicine) and laboratory/ basic science CoM departments (Biochemistry, Microbiology, Pathology, Pharmacy/ Pharmacology). It also provides vital diagnostic laboratory services for patients who are seen at QECH. This includes parasitology, haematology, chemistry and microbiology services. This unique

arrangement has a major advantage to QECH which at the moment is the only public hospital in Malawi with access to this wide array of laboratory services.

The vision of MLW is to be an internationally leading laboratory-based health research institution led by Malawian & International Scientists, improving the health of people in Malawi and elsewhere in the Region.

MLW aims to:

(1) Conduct internationally leading, cutting edge biomedical research focusing on health problems with a high disease burden in Africa in a place where these problems occur. There is an emphasis on increasing both the quantity and quality of research outputs, and ensuring that MLW research informs policy & practice

(2) Provide excellence in research training for clinical and laboratory scientists both from the host country and abroad, generating a multidisciplinary team of Research Leaders, supported by a strong research infrastructure

(3) Further strengthen the capacity of CoM to pursue research and research leadership at the highest level. As a Research Centre within CoM, MLW will provide mentorship and access to research infrastructure.

MLW has established itself as an internationally recognised centre for research excellence and research training. MLW has an outstanding translational research portfolio that links a state-of-the–art laboratory research base to strong hospital and community-based research teams. Research is focused into four themes:

- **Malaria**: the basic biology, genetics, immunology and clinical manifestations of severe malaria. The clinical programs of phase III and IV studies focus on the optimization of case management program ranging from novel interventions for severe malarial anaemia, to programmatic use of first-line drugs. The mechanisms of antimalarial resistance and side-effects. The operational research focus is on the development of facility and community-based methods for the monitoring & evaluation of malaria control progress.

- **TB & HIV**: The assessment of novel diagnostics for TB & HIV. The development and large-scale testing of novel strategies to reduce the dual burden of these epidemics. HIV & TB pharmacology and systems biology. Improving access to TB & HIV care. Adolescent HIV. Assessing health seeking behaviour & treatment acceptability

- **Non-Communicable Diseases (NCDs)**: the origins, epidemiology and mechanisms of NCDs in Malawi. The development of affordable preventative and therapeutic interventions focusing on diabetes, hypertension, lung health, epilepsy and stroke

- **Microbes, Immunity and Vaccines**: surveillance for respiratory pathogens and invasive bacterial disease. The molecular epidemiology, pathogenesis and management of bacteraemia, pneumonia and meningitis. Carriage studies. Immune responses to bacterial and viral pathogens that infect via the mucosa; influence of HIV on naturally acquired and vaccine induced immune responses; effects of clinical and subclinical infections on vaccine-induced

immunity; novel strategies for vaccine prevention. Assessing health seeking behaviour & treatment acceptability.

This study was conducted under the Microbes, immunity and Vaccines theme.

MLW in collaboration with the Blantyre Malaria Project (BMP) and the Department of Paediatrics and Child Health runs a paediatric research ward where the study participants were admitted and followed up. It generally acts as an inpatient facility for most of the paediatric research studies taking place at QECH. The ward provides high quality nursing care with an average nursing to patient ratio of 1:3 compared with 1:350 on the general wards.

2.1.2 Study site

The study took place at QECH and Zingwangwa health centre in Blantyre. QECH acts as both a tertiary and secondary level facility. Patients self-refer and are referred from surrounding district health centres and district hospitals in the southern region of Malawi. QECH is the only public district hospital in Blantyre which offers free health services and covers both the rural and urban settings of Blantyre. It also serves as a teaching hospital for the University of Malawi, College of Medicine. QECH has a separate paediatric accidents and emergency unit which caters for all children presenting to the hospital. The unit sees 96,000 children annually out of which 23,000 are admitted. These large numbers make QECH a suitable site for the study of a relatively rare disease such as neonatal meningitis and sepsis. Zingwangwa health centre on the other hand is a primary health care facility that serves a population of 147,679. It is situated about 7 km away from QECH. Its close

proximity to QECH compared to other health centres in Blantyre made this a suitable site where healthy children could be enrolled. Its catchment area has similar characteristics to the other urban health centres in Blantyre. The study was staffed by one medical officer, two nurses and the principal investigator (specialist paediatrician).

2.1.3 Study population

This study recruited infants less than 2 months who presented to the paediatric Accident and Emergency unit at QECH with a clinical suspicion of severe sepsis or meningitis (cases)**;** and young infants 3 months of age (controls) attending the immunisation clinic at Zingwangwa Health Centre.

2.1.4 Study design

2.1.4.1 Design

This study had two components, a hospital-based cross-sectional study and a prospective cohort study. The cross sectional study addressed the in-hospital microbiological aetiology and antimicrobial resistance pattern of severe neonatal sepsis and meningitis at QECH. The prospective cohort study addressed the outcome of severe neonatal sepsis and meningitis, inpatient and post discharge from QECH compared with control infants recruited at Zingwangwa health centre.

2.1.4.2 Study duration

The study was conducted over a 3 year period from June 2010 to June 2013.

2.1.4.3 Areas of potential Bias

2.1.4.3.1 Representability of the study population

QECH is a district general and tertiary hospital. It caters for both the urban and rural settings and as such has the benefit of its data representing both the rural and urban setting. However this might not be wholly representative of other districts that have a predominantly rural population.

Zingwangwa Health Centre is a large busy health Centre which serves QECH catchment's area and has similar socio-demographic characteristics to most urban Health Centres in Blantyre.

2.1.4.3.2 Loss to follow up

In order to minimise loss to follow-up only participants who resided within Blantyre urban were recruited in the prospective cohort arm upon discharge from hospital. The participants were given follow up cards and they were reminded of their scheduled appointments a week before the appointment date through phone calls to all who had phones. All the participants who had failed to turn up on their scheduled date were followed at home after 2 weeks from their failed scheduled visit. Reasons for loss-to-follow up were documented where possible.

The proximity of Zingwangwa Health Centre to QECH made it easier to follow up control participants recruited from this site.

2.1.4.4 Sample size

2.1.4.4.1 Disease outcome for severe neonatal sepsis and meningitis

A sample size was calculated that was able to detect a 10% difference in the prevalence of neuro developmental delay between the cases and controls.

There is no baseline data for the prevalence of neuro-developmental delay and neurological sequelae in Malawian children. It was however estimated that the prevalence of developmental delay would be 8% in the general Malawian paediatric population. This estimate was arrived at after looking at the prevalence of developmental delay in some African settings namely; Uganda 6% and Rwanda (before the ethnic war) 5% for one year olds {Msellati P, P. Lepaje et al (1993), and Drotar D., K. Olness et al (1997)}. We postulated that the prevalence of developmental delay would be higher in Malawi than the 2 countries due to the high prevalence of HIV and poor socio-economic status compared to the 2 countries.

A sample size of 235 severe neonatal infection cases and 235 controls was required in order to detect the 10% difference between the cases and controls. In order to achieve this, the following steps were taken;

Severe neonatal infection cases: In order to get the 235 severe neonatal infection cases we needed to recruit 1,000 eligible cases presenting at QECH. Mortality from these cases was postulated at 48%. This was based on a retrospective study done in Malawi on the aetiology of severe neonatal sepsis that found that in-hospital case fatality rates from severe neonatal sepsis was 48% {Milledge J, J. C Callis et al (2005)}. This meant that 520 babies would survive from their illness and around 400

of these would be coming from Blantyre urban. It was therefore planned that upon discharge a total of 235 babies from the 400 with neonatal meningitis and sepsis would be actively followed up till the age of one year.

Controls: A total of 235 healthy babies were recruited at 3 months of age and actively followed for up to one year of age.

This prospective cohort study had 80% power to detect a 10% difference between neuro-developmental delay and neurological sequelae in the cases and controls at 1 year of age with 95% confidence, assuming a baseline prevalence of 8% in the control arm, and taking into account a 15% loss to follow up.

2.1.4.4.2 Impact of maternal HIV

The national HIV prevalence rate for Malawi is 11% but it is twice this figure for antenatal mothers (Gray K.J, G. Kafulafula (2011)}. We therefore expected that almost 20% of our mothers in this cohort would be HIV positive translating to 200 HIV exposed babies.

2.1.5 Study procedures

2.1.5.1 Selection and withdrawal of study participants

2.1.5.1.1 Cross sectional arm (Aetiology and drug-resistance)

The sick neonates were recruited from the paediatric Accidents and Emergency department and the neonatal nursery at QECH. Study participants were admitted on the neonatal nursery and the paediatric research ward. Neonates from birth up

to 6 days of age were admitted to the neonatal nursery. These sick neonates who were admitted on the neonatal nursery came as referrals from QECH labour ward and postnatal ward or were referred from surrounding health centres. On the other hand neonates who were discharged well after delivery from QECH or surrounding health centres but who then developed symptoms suggestive of severe neonatal sepsis and meningitis were admitted to the paediatric research ward.

2.1.5.1.2 Prospective cohort arm (Disease outcome for severe neonatal sepsis and meningitis)

Selection of exposed group:

The neonates who came from Blantyre urban were invited into the disease outcome study upon discharge. The first child to be discharged for the day and fulfilled the inclusion/ exclusion criteria were invited into the study. If they declined, the next child was approached.

Selection of non-exposed group: Healthy infants were recruited from the vaccination clinic at Zingwangwa Health Centre. Healthy babies aged 3 months with no history of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis were invited to join the study if they fulfilled the inclusion criteria. The mothers or guardians were asked if their baby had a history of a febrile illness within the first 4 weeks of life that required hospitalisation or use of injectable antibiotics and the young infants hand held health record was also checked.

2.1.5.2 Subject screening

2.1.5.2.1 Aetiology, Resistance and Short term outcome

Babies less than 2 months of age who presented to the Accidents and Emergency unit and the neonatal unit at QECH were assessed for symptoms and signs of severe neonatal sepsis, severe neonatal pneumonia and meningitis using the clinical definition (section 2.1.5.4). Standard investigations outlined below (section 2.1.5.5.1) were done. Children who fulfilled the inclusion criteria were invited to join the study.

2.1.5.2.2 Long-term outcome

Subject screening exposed: children who were admitted with the diagnosis of severe neonatal sepsis, severe neonatal pneumonia or meningitis and were recruited in the cross sectional study were invited into the long term outcome study provided they came from Blantyre urban.

Subject screening non-exposed: All the babies who were enrolled at Zingwangwa Health centre were recruited in the prospective cohort arm

2.1.5.3 Subject enrolment

The mothers were informed of the study by one of the study team members from QECH and Zingwangwa Health centre (Refer to patient information sheet in the appendix). Parental permission to be involved in the study was sought by the study team members after the mothers had been fully informed about the study.

2.1.5.3.1 Inclusion and exclusion criteria

Inclusion criteria severe neonatal sepsis severe neonatal pneumonia or meningitis (cases).

- Babies less than 2 months of age admitted to Queen Elizabeth Central Hospital in whom a clinical suspicion of severe neonatal sepsis, severe neonatal pneumonia or meningitis (as defined in section 2.1.5.4) was made.

- Babies less than 2 months of age admitted to QECH with a clinical suspicion of severe neonatal sepsis severe neonatal pneumonia, or meningitis that came from Blantyre urban setting for the disease outcome arm of the study.

Exclusion criteria severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis (cases)

- Known to have a neurological problem like neural tube defects, microcephaly and hydrocephalus prior to the onset of the study.

- Prior history of birth asphyxia. Birth asphyxia was a clinical diagnosis made in babies who had APGAR scores of less than 6 at 5 minutes after birth. Birth asphyxia impacts neurodevelopment negatively and would have been a significant confounder in this study since neurodevelopment was one of the main outcomes of the study.

- Nosocomial infection, i.e babies who had been inpatients for some other reason and then develop severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis after 72 hours of in hospital stay. Co-

morbid conditions that had led to the neonate's admission in hospital were deemed to be potential confounders.

- Other major congenital abnormalities like Trisomy 21.
- Prematurity less than 32 weeks of gestation or very low birth weight of less than 1500g. Prematurity was estimated using maternal expected date of delivery recorded in her health passport and birth weight of less than 1500g. Prematurity especially less than 32 weeks is also known to negatively impact on neurodevelopment.
- Parenteral antibiotics for at least 24 hours prior to admission to QECH. This would have affected blood culture growth of bacterial organisms.
- A previous history of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis (documented in the health passport or by maternal history).

Inclusion criteria controls

- Healthy babies 3 months of age with no previous history of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis and reside within Blantyre urban.

Exclusion criteria controls

- Known to have a neurological problem like neural tube defects and hydrocephalus prior to the onset of the study.
- Prior history of birth asphyxia (as above).

- Other major congenital abnormalities.
- Prematurity less than 32 weeks of gestation or very low birth weight of less than 1500g (as above).

2.1.5.4 Clinical diagnosis of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis

A clinical diagnosis of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis was considered if a baby presented with any of the following:

- Fever or hypothermia
- Tachycardia and/or capillary refill time of </= 2 seconds
- Tachypnoea, apnoea or hyperventilation, marked chest recessions
- Irritability and convulsions
- Difficulty feeding
- Poor colour e.g. very pale, dusky or grey.
- Bulging or tense fontanelle
- Umbilical redness extending at least 1cm to the skin.

All these cases were treated for at least 5 days with parental antibiotics.

2.1.5.5 Participant management at QECH during hospitalisation

A Performa (appendix) was used to record demographic details, maternal, antenatal and delivery history and findings of a full history and examination undertaken. All babies who were enrolled in the study with a diagnosis of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis were started on parenteral broad spectrum antibiotics- Penicillin and gentamicin as first line and ceftriaxone as

second line therapy according to QECH standard guidelines. The penicillin dose for severe neonatal sepsis was 50,000 international units per kilogramme body weight twice daily during the first week of life and 25,000 international units per kilogramme body weight every 6 hours beyond the first week of life. Gentamicin was given at a dose of 5mg per kilogramme once daily. Ceftriaxone was given at a dose of 50 mg per kilogramme body weight once daily. Penicillin and ceftriaxone doses were doubled in neonates with meningitis. Convulsions were treated with intramuscular phenobarbitone 20mg per kilogramme body weight as the first line with phenytoin being the second line.

Monitoring and supportive care was standardized and carried out according to the paediatric unit at QECH and the paediatric research ward protocols (see appendix). Care was provided by study staff, nurses and clinicians working on the units.

2.1.5.5.1 Laboratory Investigations

Study participants had the following laboratory investigations; blood culture, Full Blood Count, random blood glucose, Packed Cell Volume, C-Reactive Protein, malaria parasite thick film, urea and electrolytes, infant VDRL if there was a suspicion of congenital syphilis and cerebro-spinal fluid (CSF) analysis. All the samples were taken on admission prior to commencing antibiotics. In neonates that were deemed to be too sick lumbar puncture was deferred. CSF samples were stored for future molecular diagnostics. PCR analysis on the CSF samples would further increase the yield of positive cultures and thereby improve the confidence in the final diagnosis of these neonates. It would help pick out the ones that would be falsely labelled as not having meningitis just based on routine CSF culture which

is not as sensitive as PCR. All these investigations were done by the MLW Core Laboratory except for VDRL which was done by the QECH laboratory according to standard protocols.

Standard operating procedures were put in place for blood culture sampling to minimise contamination rate.

A positive CSF was defined as one in which an organism was identified on a gram stain or by culture and/ or the white cell count was >30 cells/microml in a low birth weight baby and >20/microml in a full term neonate {Neonatal Handbook}. Biochemistry tests on the CSF included protein and glucose. An abnormal CSF glucose was considered at a level that is less than 2/3 the blood glucose level at the time the lumbar puncture was performed. An abnormal CSF protein was considered at a level of more than 0.7g/litre. A positive CRP was considered at a value of greater than 5mg/L {Neonatal Handbook}.

The mothers were tested for HIV infection using 2 rapid tests for HIV 1 and HIV 2 namely; Determine and Unigold rapid tests (Abbot and Biotech laboratories). In case of discrepancy between the 2 (Determine and Unigold), the SD Bioline HIV Ag-Ab combo rapid test was used as a tie breaker. The seropositive (i.e. HIV exposed) babies had an HIV DNA PCR (Roche Amplicor) done at 6 weeks according to national guidelines. HIV testing was only done if the guardians/parents had accepted testing after pre-counselling. All the study nurses were trained counsellors and they offered post-test counselling to all. All HIV tests were done by the MLW Core Laboratory according to standard protocols.

2.1.5.5.2 Imaging Studies

Two imaging studies were undertaken on selected neonatal meningitis cases. These included cranial ultra sound scan and magnetic resonance imaging (MRI) of the brain. These cranial ultra sound scans were done by the QECH radiology and paediatric department whilst the MRI scans were done at the QECH MRI facility run by the Blantyre Malaria Project. The MRI scans were reported by a specialist neuroradiologist. These scans were done in patients whose neurological condition were not improving or had deteriorated whilst on the ward. These mostly included patients who had refractory seizures or remained comatose after several days of treatment

2.1.5.6 Subject follow up - long-term outcome component

2.1.5.6.1 Follow-up of cases and controls

Neonates who upon discharge were recruited in the disease outcome study were followed up at 6 and 12 months of age at QECH. The control infants were also followed up at 6 and 12 months of age. At each follow up visit each infant had a neuro-developmental assessment, hearing screen and a full neurological exam by the study physician. Details of any admissions were also recorded.

2.1.5.6.2 Neurodevelopmental assessment using the Bayley's assessment tool

The neurodevelopmental assessment was done using the Bayley scales of infant development III. The Bayley Scales of Infant Development (Bayley-III) are recognised internationally as one of the most comprehensive tools to assess children from as young as one month old and has been found to be highly effective in this

population. With Bayley-III, it is possible to obtain detailed information even from non-verbal children as to their functioning {Bayley Nancy (2006)}. It was for this reason that this tool was chosen in this study. It includes the following domains:

- **Psychomotor development**: includes fine and gross motor assessment. Scores (raw and standardized) and classification (normal, moderate, severe delay) of the psychomotor section of the Bayley scales of infant development III were used
- **Mental (cognitive) development**: Scores (raw and standardized) and classification (normal, moderate, severe delay) of the mental section of the Bayley scales of infant development III were used
- **Language development**: Includes both receptive and expressive language. Scores (raw and standardized) and classification (normal, moderate, severe delay) of the language section of the Bayley scales of infant development III were used.

A score of less than -2SD in any of the domains is indicative of developmental delay.

All study team members were trained in neurodevelopmental assessments using the BSID III tool and developmental tips to be given to caregivers a week prior to the initiation of the study. The training involved both theoretical and practical sessions. The first 3 months of the study neurodevelopmental assessments on one participant were being done by 2 study team members and agreements were made on the scoring of each item. Areas of disagreement were referred to the principal investigator who would reassess the participant. During the rest of the study period the assessments were not done in pairs but by one individual with the principal

investigator doing spot checks on a biweekly basis. Refresher Bayley's trainings were done on the last Saturday of every month during the study period.

The Bayley's assessment tool was validated for use in Malawian children in the HIV encephalopathy study in 2008 and in 2013 using data from this study we constructed BSID-III norms for cognitive, fine motor (FM), gross motor (GM), expressive communication (EC) and receptive communication (RC) subtests using 5173 tests scores in 167 healthy Malawian children age range 6 weeks to 30 months {Cromwell E., Q. Dube et al (2014)}. Norms were generated using Generalized Additive Models for location, scale and shape, with age modelled continuously. Standard z-scores were used to classify neurodevelopmental delay. Weighted kappa statistics were used to compare the classification of neurological development using US-based and Malawian norms {Cromwell E., Q. Dube et al (2014)}. The generated Bayley's norms for Malawian children were then used in the analysis of the cases recruited in this study.

2.1.5.6.3 Neurological sequelae outcomes

The following neurologic sequelae were assessed at 6 and 12 months of age; epilepsy, hydrocephalus, visual loss and hearing loss. All other anomalies in the neurological exam like cranial nerve palsies, motor neurone lesions paresis were also documented.

A diagnosis of hydrocephalus was made using both clinical (head circumference above 2SD the normal or sun setting eyes) and cranial ultrasound features (dilated ventricles)..

A diagnosis of epilepsy was made clinically with a history of recurrent seizures. All presumed febrile seizures were not regarded as epilepsy.

Visual assessments were done as part of a full neurological exam and all cases of suspected visual impairment were then referred for a full ophthalmology examination and where a diagnosis of visual loss was made with formal testing by the following ; response to light, pupillary response, ability to follow a target, cover and uncover test and visually evoked response testing.

Hearing screens were done using auto acoustic emission and all infants who failed the test had a repeat test within a fortnight and if they failed the repeat they were referred for specilaist ENT review from where a definitive diagnosis of hearing loss was made with formal testing by otoacoustic emissions (OAEs) and auditory brain stem response (ABR).

2.1.5.7 Subject withdrawal

Reasons for loss-to-follow up were documented as much as possible. Caretakers were free to withdraw their children from the study at any time point if they wished to do so. If a subject misses their study visit and upon being followed up at home did not turn for their visit within a fortnight were considered as lost to follow-up.

2.1.6 Data collection and management

All data were collected using standard data collection instruments. The study participants were identified by study ID code numbers. All documents containing participant data and forms linking patient personal information to study ID code

numbers were kept in securely locked filling cabinets. Only the principal and co-investigators had access to these files. The cabinet was kept in a lockable room.

A study specific database was constructed to capture and link all clinical, diagnostics and laboratory data. The database was password protected and only accessible to the principal investigator and members of the data team involved in data processing, data management and analysis. Descriptive statistical analyses were utilized for accuracy assessment of variables, detecting out of range, implausible values and outliers.

2.1.7 Statistical analysis

Statistical analysis was done using STATA version 12 (Statacorp, USA) and Graph pad prism software (GraphPad Software Inc.,USA).

Baseline socio-demographic, clinical, biological and anthropometric characteristics of the study participants were described using standard descriptive statistics. Appropriate summary statistics i.e. mean and standard deviation, median and inter-Quartile Range (IQR), were used for continuous data. For categorical data proportions or percentage of participants within a selected category has been reported.

2.1.7.1 Descriptive analysis of severe neonatal sepsis and meningitis cases

Clinical variables for the severe neonatal sepsis and meningitis cases including fever, cough, poor feeding, convulsions, vomiting, irritability, duration of symptoms, gestation at birth, mode of delivery, pulse rate, respiratory rate, capillary refill time, level of consciousness using the AVPU score(A-alert, V-responding to voice, P-

responding to pain and U- unresponsive), head circumference, blood pressure, oxygen saturation, lactate, malaria film, haemoglobin, WBC, HIV status, serum sodium and potassium, blood glucose on admission, duration of hospital stay, final outcome in hospital, CRP, bacterial organisms grown in blood culture and their resistance pattern. The severe neonatal sepsis and meningitis cases have been divided into 2 categories:

- Early onset (< 7 days)
- Late onset (7days-60 days)

The severe neonatal sepsis and meningitis cases were analysed as separate categories. For each of the categories descriptive statistics has been provided.

A comparison between severe neonatal sepsis and meningitis on the same variables was also done.

Appropriate statistics were done for both continuous and categorical data as above. Significance tests were performed on the following to assess whether there is a significant difference between those with early onset and those with late onset disease and those with severe neonatal sepsis and meningitis: duration of symptoms, bacterial organisms grown, and duration of hospital stay, HIV status, sex and final outcome in hospital. Two sample t-tests were used to test normally distributed continuous variables, while Mann-Whitney-U tests were used to test non-Normally distributed continuous variables; for binary variables, Fisher's exact tests were used. Significance was set at 5% and confidence interval at 95%. The combined effects of all variables on outcome were assessed using logistic regression methods.

Neonatal meningitis cases

The following CSF variables were summarised for the meningitis cases; WBC, percentage lymphocytes, percentage polymorphs, glucose, protein levels, culture results and resistance pattern of the organisms. Other clinical variables including MRI head scans were recorded. Descriptive statistics as provided above have been done.

2.1.7.2 Follow-up data and losses to follow-up

The number and percentage of study participants attending scheduled follow-up visits at 1 month post discharge, 6 months and 1 year of age for the severe neonatal sepsis and meningitis cases was recorded and was compared with number (percentage) of controls at 6 months and 1 year of age. The reasons where known, have been documented. This has been presented as a flow diagram (see Chapter 4).

2.1.7.3 Analysis of long term outcomes

There were 3 outcome measures namely;

i. Mortality within the first year of life.
ii. Neurodevelopment scores (Bayley's III) within the first year of life.
iii. Neurological sequalae within the first year of life(defined as motor deficits, hydrocephalus, visual loss or deafness)

For these outcomes a significance level of 0.05 (5% level) was used to declare statistical significance

2.1.7.3.1 Comparison of first outcome between the groups

The number of deaths within each group was reported as numbers (and percentages). The interval (in months) from enrolment to death has been summarized by Kaplan-Meier curves overall and then for important sub-groups separately (with comparisons by the log rank test). The Cox proportional hazards regression model was then fitted, to identify risk factors for death. Three different models were used:

a) Including the neonatal infection effect only using the infection indicator variables.

b) Including the neonatal infection effect together with design factors namely sex and gestation age

c) Adjusting for design factors as well as post design baseline factors like; HIV status.

Hazards ratios (and 95% CIs) have been summarized for all risk factors and confounding variables. Multiple logistic model-odds ratio was also done.

2.1.7.3.2 Comparison of second outcome

There are 3 outcome measures for the second outcome namely: psychomotor, mental (cognitive), language development scores. These have been considered as separate outcomes at different time points 6 and 12 months of age and logistic regression analysis has been performed. Covariates were evaluated for effect modifying and confounding and appropriate multivariate backward elimination models were constructed to evaluate the measure of association between exposure

and outcome. The covariates include gender, timing of infection, and maternal HIV status.

2.1.7.3.3 Comparison of third outcome

The number of neurological sequelae has been reported as numbers and percentages. Differences between the 2 groups were analysed using Fischer exact tests.

2.1.8 Ethical approval

The study was granted approval by the College of Medicine Research and Ethics Committee in August 2010.

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CHAPTER THREE

CLINICAL FEATURES AND IN-HOSPITAL OUTCOME OF SEVERE NEONATAL SEPSIS AND MENINGITIS IN MALAWI – A HOSPITAL-BASED CROSS SECTIONAL STUDY

3.1 Introduction

It has been discussed in chapter one that severe neonatal infection causes nearly 25% of all deaths within the first 4 weeks of life in Malawi {Zimba, E., M.V Kinney et al. (2012)}. It is important that prevention and proper management of severe neonatal infection cases is done so as to reduce deaths that result from infection.

Proper management of neonatal infection relies on early identification of the cases, investigations and prompt treatment {Lawn J, A. Lee et al (2009)}. The current gold standard for the diagnosis of severe neonatal sepsis is a positive blood or cerebral spinal fluid (CSF) culture which in itself has challenges {Claudio C, A., Panero et al (2004), Squire E, B. Favara et al (1979)}.

In most of the developing world blood and CSF cultures are not routinely done and the diagnosis is largely clinical and as such having clinical signs and symptoms as developed by "The Young infants Clinical Signs Study group" that better predict illness in this age group is useful in these settings {The Young infants Clinical Signs

Study group, (2008), Vergnano S., M. Sharland et al (2005)}. These signs and symptoms include: history of difficulty feeding, movement only when stimulated, temperature below 35.5 C or 37.5 C or more, respiratory rate over 60 breaths per minute, severe chest in drawing, and history of convulsions. These signs and symptoms were used in this study.

However even though these signs and symptoms are good predictors of severe illness in this age group knowledge of the aetiological organisms is of paramount importance as it will guide the type and duration of antibiotics to be used when treating these neonates.

There have been differences reported in the aetiology of severe neonatal infections in the developed and developing world with gram positives being the main cause in the developed world and gram negatives being the predominant cause in the developing setting {Vergnano S., M. Sharland et al (2005), Ganatra H.A., B.J., Stoll et al (2010)} . In Malawi the antenatal carriage of GBS has been reported to be 21% which is similar to the USA and as such one would expect a high burden of GBS disease in the neonatal period which has not been the case {Gray K.J, G. Kafulafula et al (2011), Milledge J., J.C.J Calis et al (2005)}. The data was however retrospectively collected and as such it would be important to prospectively collect data so as to better describe the aetiology of severe neonatal sepsis and meningitis in Malawi {Milledge J., J.C.J Calis et al (2005)}.

It is also important to monitor the microbial sensitivity pattern of the organisms grown in the cultures especially at a time where there is emerging resistance to the standard antibiotics {Blackburn R.M, N.Q Verlander et al, (2009)}. It is difficult to

compare antibiotic resistance between countries because the epidemiology of neonatal sepsis is extremely variable {Vergnano S., M. Sharland et al (2005)}. However in Malawi resistance to first line antibiotic therapy amongst neonatal infection cases was reported at 22% between 1996 and 2001 {Milledge J., J.C.J Calis et al (2005)}. Further retrospective audit in Malawi between 2002 and 2007 did show an overall resistance to first line therapy of 28% {Gwee A., E. Molyneux et al (2012)}. Another audit in bacterial meningitis amongst young infants less than 2 months of age in Malawi showed that more isolates were susceptible to ceftriaxone than to the combination of penicillin and gentamicin (99.1% vs. 91.8%, Fisher's exact test *P* = 0.006). In particular, Gram negative isolates were significantly more susceptible to ceftriaxone than to gentamicin (97.3% vs. 85.1%, Fisher's exact test *P* = 0.020). Penicillin and gentamicin provided less coverage for Gram-negative than Gram- positive isolates (86.0% vs. 95.1%,*P* = 0.012) {Swann O, E.M Molyneux et al (2014)}.

The resistance pattern trend in Malawi indicates an increase as such closely monitoring the resistance pattern is of importance in Malawi and across the globe.

3.2 Aims of the Study

The study was set out to describe the clinical features, microbiological aetiology, antimicrobial resistance pattern and in hospital outcome of severe neonatal sepsis, severe neonatal pneumonia and neonatal meningitis cases at Queen Elizabeth Central Hospital (QECH) in Malawi between June 2010 and June 2013.

3.3 Materials and methods

These are set out in Chapter 2. In brief, this cross sectional study took place at QECH in Blantyre which is the largest tertiary unit in Malawi. Infants less than 2 months were recruited who presented to the paediatric Accident and Emergency unit and the neonatal unit at QECH with a clinical suspicion of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis and had met the inclusion criteria. The study was conducted over a 3 year period from June 2010 to June 2013.

The study aimed to include 1,000 eligible babies less than 2 months who were admitted during weekdays and during working hours with a diagnosis of severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis at QECH between June 2010 and June 2013 (see chapter 2).

3.4 Results

3.4.1 Study demographics

During the study period from May 2010 to June 2013, a total of 1,200 cases of neonatal infections were screened and 420 (49%) were eligible for the study.

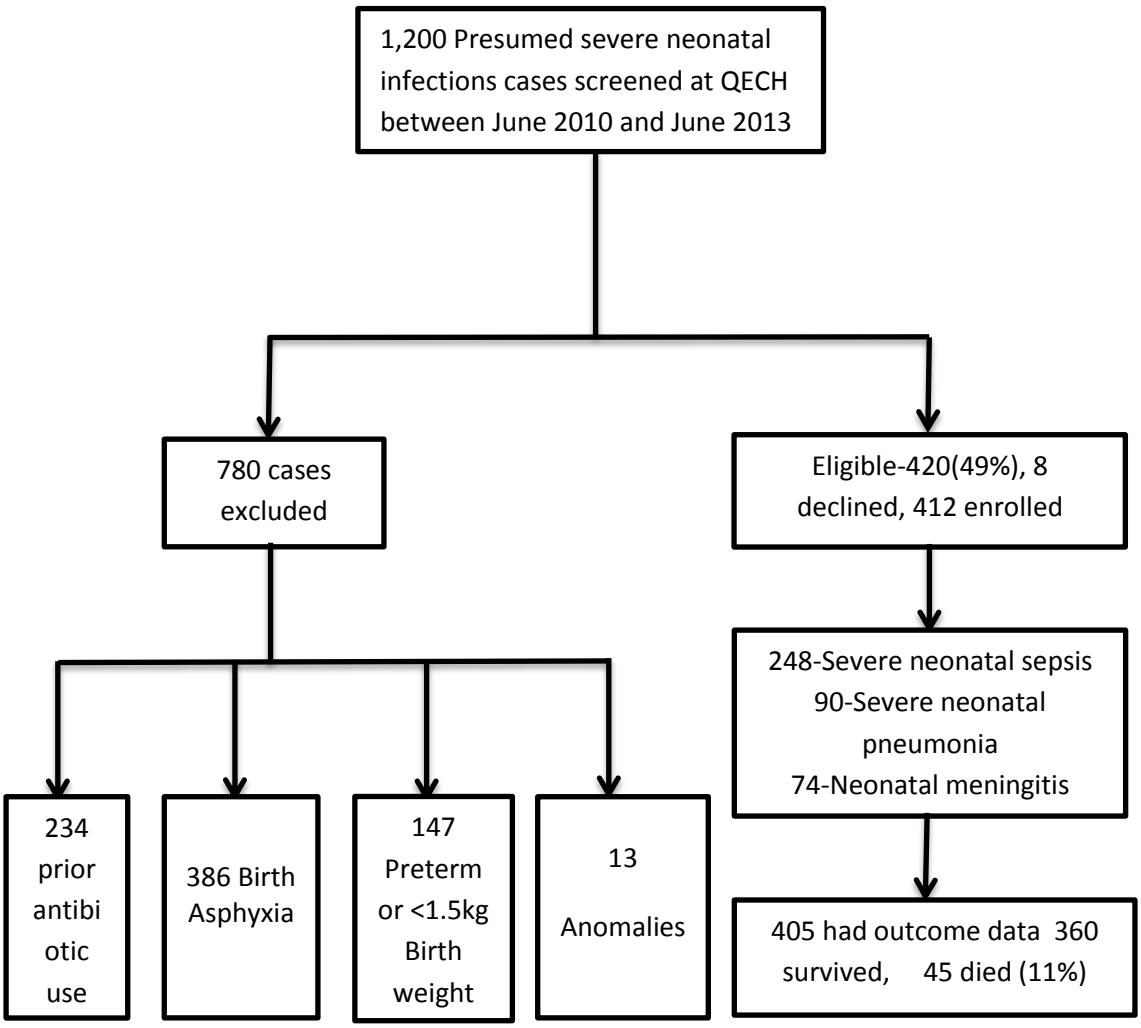


Fig 3.1 Study flow chart Aetiology and Outcome of Severe neonatal sepsis and meningitis

The top 2 causes of ineligibility were prior antibiotic use and co-morbid conditions mostly birth asphyxia. A total of 412 children were recruited in the study out of which 74(18%) had neonatal meningitis, 247(60%) had severe neonatal sepsis, 90(22%) had severe pneumonia. Severe pneumonia was a clinical diagnosis made in participants who had tachypnoea, and significant chest findings like crepitations, bronchial breath sounds. The age range was 1 to 60 days old with a median of 15 days and an interquartile range of 8 to 29 days. The majority of the cases 315(76%) were late onset and 221(54%) were males. Gestation at birth was available for 371 cases out of which 317(85%) were born at term. Place of delivery was available for 380 cases. The majority of the cases 345(91%) were born at a health facility, 4(1%) were born on the way to the hospital and 31(8%) were either born at home or at a traditional birth attendant. 374 cases had mode of delivery recorded, with 330(88%) having been delivered through spontaneous vertex delivery, 38(10%) were born through caesarean section and 6(2%) were either breech deliveries or vacuum extraction. The majority 350 (94%) were singleton pregnancies and the remaining 24(6%) were twin. Only one set of twins was enrolled. 40 severe neonatal infection cases were treated with less than 5 days of parental antibiotics most of whom died early on in their hospital admission, 120 had 5 days,194 had between 6 and 14 days of antibiotics and 58 had between 14 and 28 days of parenteral antibiotics. 150/412 (37%) participants were in hospital for more than 7 days.

Table 3.1 Baseline characteristics for all neonatal infection cases admitted to QECH (n=412)

Parameter	
Age(days) at recruitment (n=412)	Median 15 {IQR 8-29, range 1-60}
Early onset (≤7days)	97/412 (24%)
Late onset (>7 days)	315/412 (76%)
Sex (n=412)	
Male	221/412 (54%)
HIV status (n=366)	
HIV negative unexposed	235/366 (64%)
HIV negative exposed	60/366 (17%)
HIV exposed indeterminate	56/366 (15%)
HIV positive	15/366 (4%)
Gestation at birth (n=371)	
Preterm (32-37 weeks gestation)	54/372 (15%)
Term	318/372 (85%)
Place of delivery (n=380)	
Health facility	345/380 (91%)
Home	23/380 (6%)
Traditional Birth Attendant	7/380 (2%)
Born on the way to hospital	4/380 (1%)
Mode of delivery	
Spontaneous Vertex Delivery	330/374 (88%)
Caesarean section	38/374 (10%)
Breech	4/374 (1%)
Vacuum Extraction	2/374 (1%)
Singleton	350/375 (93%)
Twin	25/375 (7%)
Final diagnosis(n=412)	
Severe neonatal sepsis	247/412 (60%)
Neonatal Meningitis	74/412 (18%)
Severe pneumonia	90/412 (22%)

3.4.2 Clinical features of neonatal infection cases

The commonest symptom on admission was fever (379/412 (92%)). Thirty-eight (10%) of these babies had been given an antipyretic before their arrival at Queen Elizabeth Central Hospital. Temperature was recorded in 375 cases on admission: 238 (63%) were febrile, 104(28%) normothermic and 33(9%) were hypothermic. The median temperature on admission was 38^{o}C (range 32^{o}C to 42.6^{o}C; IQR 36.9^{o}C to 38.8^{o}C). Four babies had unrecordable low temperatures. Difficulty in breathing was the second commonest symptom occurring in 201(49%) of the cases. Respiratory rate was measured in 343 cases on admission and 165 (48%) were tachypnoeic with a rate greater than 60 cycles per minute. 113 (33%) were hypoxic on admission defined as an oxygen saturation of less than 90%. Poor feeding was reported in 103(28%), vomiting in 87(23%), convulsions in 45(12%) and diarrhoea in 34(9%) of the cases. Sixty five cases (17%) were comatose defined as a score of P (response to pain) and below on the AVPU score on admission. One hundred and sixty six cases had blood pressure measurements on admission and the median systolic blood pressure was 72 (range of 30 to 124; IQR 63-88). Two hundred and one (57%) cases were tachycardic on admission defined as a pulse rate above 160 beats per minute.

Table 3.2 Clinical Features of neonatal infection cases on admission

Symptoms on admission	**Percentage affected**
Fever	379/412 (92%)
Poor feeding	115/412 (28%)
Difficulties in breathing	201/412 (49%)
Cough	136/412 (33%)
Vomiting	87/378 (23%)
Jaundice	46/275 (17%)
Convulsions	45/378 (12%)
Diarrhoea	34/377 (9%)
Signs on admission	
Comatose *(AVPU=P and U)*	65/379 (17%)
Blood Pressure (systolic) n= 166 *Median(IQR, range)*	72 (63-88; 30-124)
Pulse rate n= 354, *Median(IQR, range)*	165 (148-180;13-254)
Respiratory rate n= 343, *Median(IQR, range)*	60 (48-72;10-99)
Temperature (^{0} c) n= 375, *Median(IQR, range)*	38 (36.9-38.8;32-42.6)
SaO_2 n=350, *Median(IQR, range)*	93 (87-96; unrecordable-100)

3.4.3 Haematology and Biochemistry Results for all Neonatal infection cases

Sixty one cases (18%) had haemoglobin of less than 10g/dl. The median haemoglobin level was 12.7g/dl (range 1.1 to 27.8 g/dl IQR 10.7 to 15.5g/dl).The prevalence of anaemia (haemoglobin of less than 10g/dl) was highest in the severe pneumonia group (24/81 cases (30%)) the meningitis group came second (12/56 {21%}) and lastly the sepsis group 25/200 (12.5%). The difference in the prevalence of anaemia amongst the 3 groups was statistically significant with a chi square p value of 0.019). Forty of the sixty one cases of anaemia had their HIV exposure status determined and 20 (50%) were HIV exposed. The prevalence of anaemia was higher in the HIV exposed group (20/120 {17%}) compared to the HIV non-exposed group (20/206 {9%}) but the difference was not statistically significant (p= 0.065). The median white cell count was 11.4 (range 0.5-76, IQR 7.8-16.3). The median platelet count was 283 (range 5.2-910, IQR 192- 406). The median CRP was 13.1 (range 1-435, IQR 3.5 to 84.3) CRP value of more than 5mg/dl was regarded as raised and 162 out of 239 cases (68%) had a raised CRP. There was a difference in the prevalence of raised CRP between severe neonatal sepsis, meningitis and severe pneumonia. 34/41 cases of neonatal meningitis (83%) had a raised CRP compared to 94/146 (64%) for severe neonatal sepsis and 34/52 (65%) for pneumonia but the difference between the 3 groups was not statistically significant (p=0.068). The CRPs were not run in real time and so repeat samples were not collected. Serum sodium levels were determined in 330 (80%) cases and 74 cases (22%) were hyponatraemic (sodium < 135 mmol/l) and 67 (20%) were hypernatraemic (sodium >145mmol/l). Of the one hundred and ninety four cases of severe neonatal sepsis who had their

serum sodium analysed 34 (17.5%) were hyponatraemic and 51(26%) were hypernatraemic. Fifteen (28%) of fifty four cases of meningitis were hyponatraemic and 10 (19%) were hypernatremic, whereas for severe pneumonia 25 (30%) of 82 cases were hyponatraemic and 6 (7%) were hypernatremic.

Table 3.3 Haematology and biochemistry results of severe neonatal infection cases (n=337)

Parameter	***Median (IQR, Range)***
Haemoglobin g/dl (n=337)	12.7 (10.7-15.5, 1.1-27.8)
WBC $x10^6$/L (n=335)	11.4 (7.8-16.3, 0.5-76)
Platelets $x10^9$/L (n=335)	283 (192-406, 7-775)
CRP mg/dl (n=239)	13.1 (3.5-84.3, 1-432)
Sodium mmol/L (n=330)	138 (135-144, 120-206)
Urea mg/dl (n=324)	4.8 (3.4-8.9, 1-150)
Lactate mmol/L (n=323)	2.4 (1.7-4 ,0.2-17.4)

Table 3.4 Prevalence of abnormal Haematology, biochemistry results amongst severe neonatal sepsis, severe neonatal pneumonia and neonatal meningitis cases (n=337)

Parameter	Severe neonatal sepsis	Neonatal meningitis	Severe pneumonia	P value(chi square test)
Haemoglobin <10g/dl	25/200(12.5%)	12/56(21%)	24/81(30%)	0.020
Hypernatraemia (Sodium >145mmol/l)	51/194(26%)	10/54(19%)	6/82(7%)	0.003
CRP >5 mg/dl	93/146(64%)	34/41(83%)	34/52(65%)	0.070

3.4.4 Aetiology of Neonatal Infections

As shown in table 3.5, a total of 368 blood cultures were done out of which 42(11%) grew a significant organism. Culture positivity rate was higher in the late onset group 37/277 (13%) versus 5/89 (6%) in the early onset group. Group B streptococci (GBS) were the commonest bacteria grown in both blood and cerebral spinal fluid(CSF) culture representing 40% and 48% respectively of significant organisms grown (table 3.5). All but one of the GBS grown in blood or CSF culture was in late onset disease group. Staphylococcus aureus was the second commonest organism grown in blood culture (24%), followed by *Streptococcus pneumoniae* (12%) which was also the second commonest CSF isolate (30%). The one case of Haemophilus influenza type b (Hib) was a six week old baby who had not been immunised against

Hib. Contaminants grew in 86/368 (23%) and the commonest organism was coagulase negative staphylococci.

Table 3.5 Distribution of bacterial isolates in Blood and CSF culture in severe neonatal infection cases (n=366)

Bacterial Organism	Total	Early onset	Late onset
Blood culture	366	89/366 (24%)	277/366 (76%)
Significant organisms	42/366 (11%)	5/89 (6%)	37/277 (13%)
Group B streptococci	17/42 (40%)	0	17/37 (46%)
Group D streptococci	1/42 (2%)	1/5 (20%)	0
Streptococcus pneumoniae	5/42 (12%)	0	5/37 (8%)
Staphylococcus aureus	10/42 (24%)	1/5(20%)	9/37(24%)
Acinetobacter baumannii	2/42 (5%)	0	2/37 (5%)
Escherichia coli	4/42 (10%)	3/5 (60%)	1/37 (3%)
Enterobacter cloacae	1/42 (2%)	0	1/37 (3%)
Salmonella typhimurium	2/42 (5%)	0	2/37 (5%)
Contaminants	85/366 (23%)	18/89 (20%)	67 (24%)
No growth	239/366 (65%)	66/89 (74%)	173/277(62%)
CSF culture	323	79/323 (24%)	244/323 (76%)
Significant organisms	33/323 (10%)	4 (5%)	29/244(12%)
Group B Streptococci	16/33 (48%)	1/4 (25%)	15/29(52%)
Streptococcus pneumoniae	10/33 (30%)	0	10/29 (35%)
Acinetobacter woffii	1/33(3%)	0	1/29(3%)
Acinetobacter baumannii	1/33(3%)	1/4 (25%)	0
Escherichia. coli	2/33(6%)	2/4 (50%)	0
Enterobacter cloacae	1/33(3%)	0	1/29 (3%)
Haemophilus influenza b	1/33(3%)	0	1/29 (3%)
Salmonella typhimurium	1/33(3%)	0	1/29 (3%)
Contaminants	19/323 (6%)	6/79 (8%)	13/244 (5%)
No growth	271/323 (84%)	69/79 (87%)	202/244 (83%)

Antimicrobial sensitivity for isolates grown in blood and CSF culture

Group B streptococci grown in both blood and CSF cultures were sensitive to penicillin and ceftriaxone however 20% of the Streptococcus pneumoniae were resistant to penicillin. On the other hand gram-negative isolates were multidrug resistant especially to the standard first line penicillin and gentamicin (Table 3.6-3.7).

Table 3.6 Antimicrobial Sensitivity pattern for organisms grown in Blood culture from severe neonatal infection cases (n=42)

Antimicrobial	GroupB Streptococcus (n=13)	GroupD Streptococcus (n=1)	Streptococcus pneumonia (n=5)	Staphylococcus aureus (n=9)	Enterobacter cloacae (n=2)	Salmonella typhinurium (n=1)	Escherichia coli (n=4)
Penicillin	13/13 (100)	Not tested	4/5 (80)	Not tested	Not tested	Not tested	Not tested
Erythromycin	11/13 (85%)	0(0)	4/5 (80)	1/9 (11)	Not tested	Not tested	Not tested
Ampicillin	Not tested	0(0)	Not Tested	Not tested	0	0	1/4(25)
Chloramphenicol	Not tested	1/1(100)	4/5 (80)	8/9(89)	1/2(50)	0	1/4(25)
Gentamicin	Not tested	Not tested	Not tested	7/9(77)	1/2(50)	0	1/4(25)
Tetracycline	1/13(77)	0(0)	2/5(40)	0(0)	Not tested	Not tested	Not tested
Cotrimoxazole	4/13(31)	0(0)	1/5(20)	3/9(33)	1/2(50)	0	0
Ceftriaxone	13/13(100)	Not tested	5/5(100)	5/9(56)	1/2(50)	1(100)	2/4(25)
Ciprofloxacillin	Not tested	1/1(100)	Not tested	Not tested	1/2(50)	1(100)	2/4(25)
Cloxacillin	Not tested	Not tested	Not tested	9/9(100)	Not tested	Not tested	Not tested
Amikacin	Not tested	Not tested	Not tested	Not tested	2/2(100)	Not tested	Not tested

*Figures in parenthesis are percentages.

Table 3.7 Antimicrobial Sensitivity pattern for organisms grown in cerebral spinal fluid culture from neonatal meningitis cases (n=33)

Antimicrobial	GroupB Streptococcus (n=14)	Streptococcus pneumonia (n=11)	Escherichia coli (n=2)	Haemophilus Influenzea B (n=1)	Acinetobacter (n=2)
Penicillin	14/14 (100)	11/11(100)	Not tested	Not tested	Not tested
Erythromycin	13/14 (93)	9/11 (82)	Not tested	Not tested	Not tested
Ampicillin	Not tested	Not Tested	1/2(50)	0	1/2(50)
Chloramphenicol	13/14(93)	8/11 (73)	1/2(50)	0	1/2(50)
Gentamicin	Not tested	Not tested	1/2(50)	0	1/2(50)
Tetracycline	13/14(93)	8/11(73)	Not tested	0	Not tested
Cotrimoxazole	4/14(29)	2/11(18)	0	0	Not tested
Ceftriaxone	14/14(100)	Not tested	1/2(50)	1(100)	1/2(50)
Ciprofloxacillin	Not tested	Not tested	1/2(50)	Not tested	1/2(50)

*Figures in parenthesis are percentages

Malaria parasitology

All the cases enrolled in the study had a blood film for malaria parasites but none of the cases had a positive film for malaria.

3.4.5 Morbidity and mortality outcomes of severe neonatal sepsis severe neonatal pneumonia and meningitis

Four hundred and five (98%) of 412 babies with severe neonatal sepsis, severe neonatal pneumonia or neonatal meningitis had outcome data available. Five mothers had absconded with their babies from hospital and two files had gone missing.

Three hundred and sixty cases (89%) survived to discharge. 320 (79%) of the survivors had no neurological sequelae on discharge and 40 (11%) had sequelae. The sequelae included seizures, motor deficits, hearing loss, visual loss and hydrocephalus. The prevalence of neurological sequelae was higher in the meningitis group compared to the sepsis group (39% vs. 5%) p value <0.001. There were no cases with neurological sequelae in the pneumonia group on discharge.

Overall mortality was 45/405 (11%). It was higher for meningitis: 15/74(20%) than severe neonatal pneumonia 8/90 (9%) and severe neonatal sepsis 22/247(9%).

Logistic regression univariate analysis on factors associated with mortality

In the univariate analysis using logistic regression, term gestation showed a significant risk reduction of mortality by 62%. Normal haemoglobin and fever were associated with a non-significant reduced risk of mortality in hospital by 44% and

38% respectively. Thrombocytopenia, hypothermia, hypernatraemia, hypoxia and significant growth in blood or cerebral spinal fluid culture significantly increased the risk of in hospital mortality. On the other hand presence of convulsions was associated with a 2 fold non-significant risk of mortality, meningitis a 1.86 fold risk and HIV exposure 1.75 fold-risk.

Table 3.8 Logistic regression univariate analysis on factors associated with mortality (n=405)

Parameter	OR (95% CI)	*P value
Hypoxia	3.83(1.91-7.69)	0.000
Convulsions	2(0.86-4.68)	0.107
Normal haemoglobin	0.56(0.23-1.37)	0.203
Hyponatraemia (sodium <135mmol/l)	1.15(0.45-2.93)	0.770
Hypernatraemia (sodium > 145mmol/l)	2.38(1.06-5.33)	0.036
HIV exposed	1.75(0.85-3.60)	0.127
Meningitis	1.86(0.70-4.91)	0.212
Severe neonatal sepsis	1(0.42-2.34)	0.990
Positive blood culture	4.19(1.87-9.38)	0.000
Positive cerebral spinal fluid culture	4.02(1.6-10.07)	0.003
Age at onset(Late onset)	1.27(0.56-2.87)	0.560
Term gestation	0.38(0.17-0.81)	0.012
Hypothermia	4.83(1.88-12.43)	0.001
Fever	0.62(0.28-1.39)	0.250
Thrombocytopenia (Platelets <150)	3.53(1.66-7.50)	0.001

*Univariate Logistic regression p value

Table 3.9 Univariate logistic regression analysis on factors associated with neurological sequelae (n=405).

Parameter	OR (95% CI)	*P value
Hypoxia	0.85(0.38-1.90)	0.691
Convulsions	4.6(2.04-10.4)	<0.001
Normal haemoglobin	0.83(0.27-2.54)	0.740
Hyponatraemia (sodium <135mmol/l)	1.36(0.55-3.35)	0.504
Hypernatraemia (sodium > 145mmol/l)	0.37(0.83-1.68)	0.198
HIV exposed	0.69(0.32-1.48)	0.341
Meningitis	14.64(6.57-32.60)	<0.001
Positive blood culture	4.13(1.60-10.62)	0.003
Positive cerebral spinal fluid culture	12.43(4.94-31.27)	<0.001
Age at onset(Late onset)	1.13(0.49-2.58)	0.780
Term gestation	1.12(0.37-3.35)	0.843
Hypothermia	0.94(0.24-3.62)	0.929
Fever	0.48(0.23-1.02)	0.056
Thrombocytopenia (Platelets <150)	1.74(0.66-4.58)	0.260

*Univariate Logistic regression p value

In the univariate analysis using logistic regression, convulsions were associated with a significantly increased risk of neurological sequelae by 4.6, meningitis 14.64, significant growth in blood culture 4.13 and significant growth in cerebral spinal

fluid 12.43. There were no factors that were shown to significantly reduce the risk of developing neurological sequelae in hospital.

Multivariate analysis

In the multivariate analysis for the odds of death all variables that were univariately significantly associated with mortality were included. These variables included hypoxia, hypernatraemia, positive blood culture, positive CSF culture, term gestation, hypothermia and thrombocytopenia. We also included convulsions and a diagnosis of meningitis that are clinically known to increase the risk of dying in these severe neonatal infection cases.

In the multivariate analysis for the odds of neurological sequelae all variables that were univariately significantly associated with neurological sequelae were included. These variables included positive blood culture, positive CSF culture, convulsions and diagnosis of meningitis. We also included hypothermia, hypoxia, prematurity, and a diagnosis of severe neonatal sepsis that are clinically known to increase the risk of neurological sequelae in these severe neonatal infection cases.

Table 3.10 Adjusted logistic model of the odds of death (n=405)

Parameter	OR (95% CI)	*P value
Hypoxia	6.75(1.96-23.32)	0.003
Convulsions	0.94 (0.19-4.57)	0.940
Meningitis	2.92(0.20-42.93)	0.434
Severe neonatal sepsis	1.59 (0.33-7.63)	0.561
Significant blood culture	6.34(1.35-29.8)	0.020
Significant CSF culture	5.58(0.38-81.88)	0.210
Term gestation	0.12(0.027-0.53)	0.005
Hypothermia	0.77(0.13-4.66)	0.770
Fever	0.28(0.07-1.1)	0.070
Thrombocytopenia	1.12(0.30-4.14)	0.860
Hypernatraemia	8.34(1.95-35.70)	0.004

***Adjusted logistic regression p value**

In all adjustments hypoxia, and significant blood culture growth increased the odds of dying in hospital. The highest risk was from hypernatraemia with a significant odds ratio of 8.34, hypoxia had an odds ratio of 6.75 and significant blood culture growth had an odds ratio of 3.89. There was a reduction in the odds of death by 88% if the baby was born at term.

Table 3.11 Adjusted logistic model of the odds of neurologic sequelae (n=405)

Parameter	OR (95% CI)	*P value
Hypoxia	3.55(0.82-15.44)	0.091
Convulsions	0.45(0.08-2.52)	0.363
Severe neonatal sepsis	0.17(0.024-1.12)	0.066
Positive blood culture	2 (0.31-12.85)	0.467
Positive CSF culture	2.36(0.36-15.57)	0.371
Term gestation	1.98(0.35-11.01)	0.441
Hypothermia	0.15(0.007-3.12)	0.220
Thrombocytopenia	6.08 (1.08-34.12)	0.040

***Adjusted logistic regression p value**

In all adjustments babies that had a prolonged hospital stay had an increased risk of having neurological sequelae on discharge with an odds ratio of 10.88. Thrombocytopenia was also noted to be a risk factor with an odds ratio of 6.08 however fever seemed to reduce the odds of sequelae at discharge by 79%.

3.5 Discussion

3.5.1 Clinical features of severe neonatal sepsis and meningitis

This chapter describes the aetiology and outcome of severe neonatal sepsis and meningitis in participants recruited in the study. A total of 412 participants were recruited over the 3 year period out of which 75% had late onset disease which is different from reports in the literature {Osrin D., S., Vergnano et al, (2004), Seale A.C, M. Mwaniki et al (2009)}. The reported prevalence of late onset disease ranges between 25 to 50 % {{Osrin D., S., Vergnano et al, (2004), Seale A.C, M. Mwaniki et al (2009)} } in the developing setting. The low rate of early onset disease observed in this study could be explained by several factors namely;

Poor referral systems; QECH being a referral facility receives patients from a much wider catchment area however there are challenges with transportation of referred patients from the peripheral health centres and many times the guardians have to fend transport on their own. This results in delays in seeking care and since the mortality rate from early onset disease is high some of these babies can end up dying at home. In Malawi a recent survey in health centres in Blantyre district revealed that 60% of sick children who were referred to QECH did not end up there (unpublished ETAT MLW survey 2013). This is in keeping with findings from WHO Africa multicentre Community management of severe neonatal sepsis where up to 60% of young infants who were referred from primary health centres did not end up at the secondary facility { Unpublished report}.

Beliefs in other alternative forms of therapy further complicate the challenges within the referral system. In a study done on knowledge, attitude and treatment preferences for neonatal sepsis in Ntchisi district, Malawi by Kakhobwe T. {Kakhobwe T et al (MMED thesis, (2010)} revealed that 83% of the mothers preferred to take their sick new born to a traditional healer before seeking help at a health facility {unpublished report}. This could have affected the number of neonates that presented at QECH. Cultural beliefs could have impounded this further.

In this study neonates who had a history of birth asphyxia were excluded. The diagnosis of birth asphyxia was solely based on low APGAR score. It is widely accepted that the diagnosis of birth asphyxia also known as hypoxic ischaemic encephalopathy should include profound metabolic or mixed acidemia (pH < 7) in an umbilical artery blood sample, multi organ involvement and neonatal neurologic sequelae apart from low APGAR scores. This could have led to mislabelling of some cases thereby leading to ineligibility for the study. The low APGAR score could have been a sign of a sick septic newborn and as these cases were excluded from the study it could have further led to reduced numbers of early onset neonatal sepsis cases.

In this study 91% of the participants were born at a health facility which is higher that the reported 71% for Blantyre (Malawi DHS 2010). Home deliveries pose a high risk to new-born infections and these babies would present with early onset disease. There is a possibility that the health seeking of the women who deliver at home is different from those who deliver at home. The hypothesized difference

could explain the unexpected high rates of health facility deliveries in this cohort. It would be important to explore the health seeking behaviour of mothers who deliver at home as this could have an impact on the type of care these women seek for their sick neonates. It could be postulated that these mothers who deliver at home are less likely to bring their babies to a health facility if they were unwell. A community based study would better answer this question.

GBS was the commonest cause of severe neonatal infections in this cohort. These findings in this study could be a true reflection of the true epidemiology of GBS disease in Malawi. If that was the case then measures that have been known to reduce GBS early onset disease in the developed world cannot work in Malawi as they have not been proven to reduce late onset GBS disease. The only hope would be a GBS vaccine and currently there are GBS vaccine trials that are taking place in Southern Africa {Clinical Trials.gov: NCT01412801}.

The culture positivity for this study was rather low and future work on molecular diagnostics is planned on stored samples from the participants to try and better understand the aetiology of severe neonatal infections in Malawi.

The HIV exposure rate in the participants was much higher than the reported HIV exposure status observed in pregnant women in some centres in Blantyre (36% vs 17%) {Dube Q. A. Vanrie et al (2012)}. Babies born from HIV infected mothers have a higher risk of infections even if they do not acquire HIV {Le Doare K., R.Bland et al (2012)}.

3.5.2 Predictors of poor inpatient outcome in severe neonatal infection cases

In all adjustments hypernatraemia, prematurity, hypoxia and significant blood culture growth were independently associated with an increased risk of in hospital mortality. The observation was in keeping with other studies {Weseem R., A.A., Shah et al (2005)}. It should be noted however that in most resource restrained settings biochemistry and microbiological testing is not available and as such the ability to pick up abnormalities in these parameters is limited. Governments need to invest more in health care so as to better manage these illnesses.

3.6 Conclusion

Group B Streptococcus has been identified as the main cause of severe neonatal infections in Malawi. However the majority of the infections were in late onset disease and further studies need to be done to better understand this change in the epidemiology of GBS disease. Clinical and laboratory parameters can be used to predict the outcome of severe neonatal infections in hospital.

CHAPTER FOUR

4. LONG TERM OUTCOMES OF SEVERE NEONATAL SEPSIS AND MENINGITIS IN MALAWI - A PROSPECTIVE COHORT STUDY

4.1 Introduction

Severe neonatal infections have been shown to have long term neurological impact on the survivors {Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Gordon A.L, M. English et al (2005), Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}. It is known that during the neonatal period the infant is prone to insults on the rapidly growing brain {Shane A.L, Stoll B.J et al}.

Most of the studies on the long term impact of neonatal sepsis and meningitis have been done in the developed setting where the bulk of the cases are preterm low birth weight infants {Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}. The sepsis rate in premature infants has significantly risen in the last three decades because of increased survival of very low birth weight (VLBW) infants, prolonged stay in neonatal intensive care units where there is frequent exposure to protracted instrumentation, such as intravascular lines and endotracheal tubes {Klinger G, I Levy et al (2009)}. However, in the

developing setting the epidemiology of neonatal infections is different from the developed setting {Ganatra H.A, Stoll B.J et al (2010), Vergnano S., M. Sharland et al (2005)}. Term infants in the developing setting are as equally vulnerable to acquire infections as the preterm babies. This is as a result of the high rate of home deliveries, poor prenatal care, poor health systems, high HIV prevalence, harmful cultural practices on the new born like putting cow dung on the umbilical cord {Seale A.C, M Mwaniki et al (2009), Osrin D, S. Vergnano et al (2004)}. The low socio-economic status of these settings compounds the picture even more {Waseen R., A. A. and Shah (2005)}. In this study 85% of the severe neonatal infection cases were born at term (Chapter 3).

It is noteworthy that even though the mortality rate from neonatal meningitis has improved over the last 3 decades, the morbidity rates have remained high with up to 50% of survivors of neonatal meningitis known to have complications {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M. Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}. On the other hand neonatal sepsis in the absence of overt meningitis has been shown to increase the risk of developmental delay in very low birth weight, preterm babies in the developed setting {Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}.

There is however lack of data in resource-poor settings on the long term outcome of severe neonatal infections. Malawi is one of a number of countries within Sub Saharan Africa with a high HIV prevalence (12%) that continues to struggle with a

high new born mortality {Zimba, E., M.V Kinney et al. (2012), Unicef Malawi report (2013)}. Over the past decade the neonatal mortality rate in Malawi has remained static despite promising changes in the outcome of older children {Zimba, E., M.V Kinney et al. (2012)}.

4.2 Aim of the Study

The study set out to describe the long term outcome of neonatal sepsis and meningitis in Malawi

4.3 Materials and methods

This study took place at Queen Elizabeth Central hospital (QECH) and Zingwangwa health centre. The study was conducted from April 2010 to April 2013. Ethical approval was obtained from the College of Medicine Research and Ethics Committee (Protocol number P.11/09/835).

Young infants coming from Blantyre urban who were recruited in the hospital based cross sectional study (Chapter 2 and 3) were invited to join the prospective study upon discharge from QECH. The study also recruited young infants aged 3 months who had no episode of severe neonatal infection in the past from the immunisation clinic at Zingwangwa health centre. The infants from Zingwangwa health centre acted as controls. All the study participants were followed up to the age of 12 months.

The study aimed to recruit a total of 470 participants; 235 young infants aged 3 months from Zingwangwa health centre and 235 severe neonatal infection cases from QECH. The study aimed to detect a 10% difference between neurodevelopmental delay in the severe neonatal infection cases and the controls

at 12 months of age (Chapter 2). Neurodevelopmental assessments were done at 6 and 12 months of age using the Bayley scales of infant development III [56]

All study participants had a detailed neurological examination and head circumference measurements at 6 and 12 months of age. Neonatal meningitis cases had hearing screens done at 6 months of age and if failed they were repeated 2 weeks later.

Statistical analysis was done using STATA version 12 (Statacorp, USA) and Graphpad prism6 software**.**

4.4 Results

4.4.1 Study Demographics

During the study period from May 2010 to June 2013 a total of 472 young infants were recruited and followed up. These infants comprised 234 neonatal sepsis and meningitis cases and 238 control babies. Figure 4.1 below is the study flow diagram

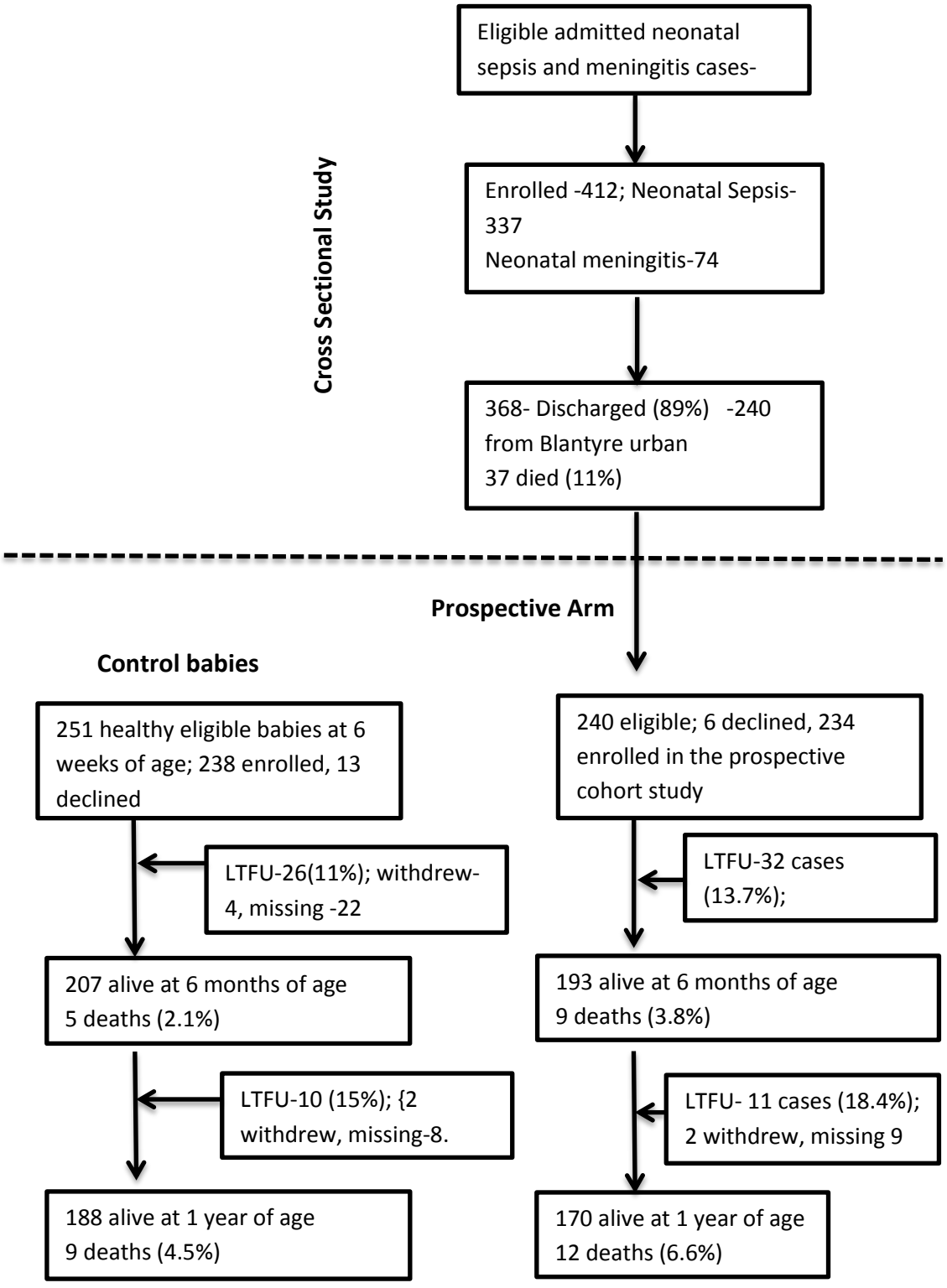


Fig 4.1 Long term outcome of neonatal sepsis and meningitis study flow diagram

4.4.1.1 Neonatal sepsis and meningitis cases

A total of 234 cases were enrolled upon discharge from hospital in the prospective study over a period of 3 years. 193 out of the 234 babies (82.5%) were seen alive at 6 months of age and 9 had died representing a mortality rate of 3.8%. 32 infants were lost to follow-up representing a loss to follow up rate of 13.7% at 6 months of age.

170 out of the 193 infants (83%) reviewed at 6 months were alive at 1 year of age. 160 out of the 170 were reviewed but 10 had information about their survival obtained over the phone. There were 12 deaths between 6 and 12 months of age representing a mortality rate of 6.6% during this 6 months period. 11 infants were lost to follow up representing a rate of 9.4%.

Overall, there were a total of 42 losses to follow up cases (18%) on this group over the 1 year period. 19 out of 42 (45%) loss to follow up cases were as a result of transfers to other districts as such they had to be withdrawn from the study. 8 out of the 42 (19%) had withdrawn from the study on their own accord.

4.4.1.2 Otherwise healthy infants (control group)

A total of 238 babies who had no episode of neonatal sepsis or meningitis were enrolled in this group. 207 out of 238 babies (87%) were seen alive at 6 months of age and 5 had died representing a mortality rate of 2.1%. 26 infants were lost to follow up representing a rate of 10.9% at 6 months of age.

188 out of the 207 infants (91%) were seen alive at 12 months of age. There were 9 deaths between 6 and 12 months of age representing a rate of 4.5%. 10 babies were lost to follow up representing a rate of 15%.

Overall, at the end of the study period there were a total of 36 losses to follow up in this group. 28 mothers had voluntarily withdrawn from this group representing 78% of all the losses to follow-up.

4.4.2 Baseline Characteristics

Gender distribution was similar in the 2 groups with males contributing 54% in the cases and 55% in the controls (table 4.1). There was a difference in HIV exposure status between the 2 groups with 36% of the participants in the neonatal sepsis and meningitis group being HIV exposed compared to 15.5% in the control group. The mothers in the sepsis and meningitis group were younger compared to the controls and 37% of them this was their first child compared to 29% of the controls (see table 4.1).

Table 4.1 Baseline characteristics of all study participants (n=473)

Characteristic	Neonatal infection cases	Controls
Sex		
Male	126/235 (54%)	130/238 (55%)
HIV status		
HIV negative unexposed	150/234 (64%)	201/238 (84.5%)
HIV positive	9 /234 (4 %)	12/238 (5.0%)
HIV negative exposed	37/234 (16%)	32 /238 (10.5%)
HIV exposed indeterminate	38/234 (16%)	0/238
Maternal age (years)	Median 24 (range 12-42) IQR 20-28	Median-30 (range 15-47) IQR 26-34
Maternal Parity		
1	87/234 (37%)	68/238 (28.6%)
2	61/234 (26%)	71/238 (29.8%)
3	42/234 (18%)	57/238 (24%)
4	26/234 (11%)	28/238 (11.8%)
5	9/234 (4%)	12/238 (5%)
6	7/234 (3%)	2/238 (0.8%)
7	2/234 (1%)	0/238

4.4.2.1 Socio Demographics profile

There were no major differences in the socio-demographic profile of the neonatal sepsis and meningitis group and the control group.

The marital statuses of the mothers in both arms were similar with the majority of them being married; 88% in the neonatal sepsis and meningitis group and 87% in the control group. Most of the mothers had attained primary level education but only 9% of the cases and 12% of the controls were employed. Family income data was available in 52% of the cases (122/234) and 22% of the controls (53/238). 64 out of 122 families (52%) and 26 out of 53 families (49%) of the control group were

earning less than 30 US dollars per month. The rest of the socio-economic data; toilet facilities, source of drinking water, wall and roofing material were all indicative of low socio-economic status in both groups (see table 4.2).

Table 4.2 Socio-demographic profile of all study participants (n=473)

	Severe neonatal infection cases	Controls
Marital status(maternal)		
Single	24/234 (10%)	19/238 (8%)
Married	206/234(88%)	207/238 (87%)
Separated	4/234 (2%)	12/238 (5%)
Maternal education		
None	7/234 (3%)	12/238 (5%)
primary	129/234(55%)	109/238(46%)
Some secondary	91/234 (39%)	102/238(43%)
Tertiary	7/234(3%)	12/238(5%)
Employed		
Yes	22/234 (9%)	28/238 (12%)
No	212/234 (91%)	210/238 (88%)
Employment Type		
Skilled	5/22 (23%)	4/28 (14.3%)
Semiskilled	3/22 (14%)	11/28 (39.3%)
Unskilled	14/22 (63%)	13/28 (46.4%)
Monthly family income(US dollars)		
< 10	8/122 (7%)	5/53 (9%)
10-15	14/122 (11%)	5/53 (9%)
15-30	42/122 (34%)	16/53 (30%)
30-60	37/122 (30%)	9/53 (17%)
>60	21/122 (17%)	18/53 (34%)
Toilet Facilities		
Indoor flush toilet	16/234 (7%)	20/238 (8%)
Pit latrine	218/234 (93%)	217/238 (91%)
No toilet	2/234 (1%)	1/238 (0.5%)
Source of drinking water		
Tap in residence	26/234 (11%)	50/238 (21%)
Communal tap	171/234 (73%)	167/238 (70%)
Other(well, river)	37/234 (16%)	21/238 (9%)

Number of rooms in the house	89/234 (38%)	76/238 (32%)
1	94/234 (40%)	117/238 (49%)
2	37/234 (16%)	38/238 (16%)
3	14/234 (6%)	7/238 (3%)
4 and above		
Wall material for the house		
Poles and mud	2/234 (1%)	2/238 (1%)
Mud	42/234 (18%)	3/238 (1%)
Mud bricks	73/234 (31%)	129/238 (54%)
Fired brick	115/234 (49%)	102/238 (43%)
Other	1/234 (0.5%)	2/242 (1%)
Roofing material for the house		
Grass	37/234 (16%)	24/238 (10%)
Iron sheets	197/234 (84%)	212/242 (89%)
Other	0	2/242 (1%)

4.4.3 Long term impact of severe neonatal infections

There were more cases of hydrocephalus observed in the infants that had severe neonatal infection (12/193[6%] participants followed up to the age of 6 months) compared to control infants (1/207 participants [0.005%]). All the hydrocephalus cases were evident by age 6 months. The prevalence of visual loss by age 6 months was also higher in the infection group (5/193 [0.03%]) as compared to control infants where none had visual loss. The prevalence of epilepsy at 1 year of age was 9/162 (5.6%) in the cases (see table 4.3), compared with none in the control group. All the hydrocephalus, epilepsy and visual loss cases in the severe neonatal infections group were associated with meningitis. There were 68 cases of neonatal meningitis out of which 17.6% had hydrocephalus, 7.3% had visual loss and 13.2% had epilepsy. Hearing tests were done in 52 neonatal meningitis cases out of which 11 (21%) had bilateral hearing loss and 6 (12%) had unilateral hearing loss at 6 months of age.

4.4.3.1 Neurocognitive Outcomes at 6 Months of age

A total of 301 infants had neurocognitive assessments done using the Bayley Scales of Infant and Toddler Development (BSID-III) tool at the age of 6 months, comprising 129(67%) infants who had severe neonatal infection and 171(83%) controls. We were unable to fully administer Bayley's in 22 cases in the severe neonatal infection group and 8 in the controls. Bayley's was not administered in 12 cases (6.2%) in the severe neonatal infection group and 2(1%) in the controls mainly because of very severe developmental delay that made it impossible to administer any of the items. Out of the 107 neonatal infection cases that had full Bayley's assessment 64 had neonatal sepsis, 23 had severe pneumonia and 20 had meningitis. The rate of neurodevelopmental delay at 6 months was higher in the group of infants that had an episode of severe neonatal infection ranging from 11% in the receptive language domain to 29% in the fine motor domain compared to 3% in the receptive language domain to 10% in the cognitive domain in infants that never had an episode of neonatal infections (see Table 4.3 below). Amongst the severe neonatal infection group infants those who had an episode of neonatal meningitis had the highest rate of developmental delay ranging from 26% in the receptive language domain to 47% in the fine motor domain. Neonatal sepsis followed the meningitis group with rates ranging from 6% in the expressive language domain to 25% in the fine motor domain and lastly the severe pneumonia group ranged from 0% in the receptive language domain to 24% in the fine motor domain (see Table 4.3).

Table 4.3 Rates of Neurodevelopmental delay in the control infants and severe neonatal infection cases at 6 months of age (n=305)

Neurocognitive Domain	Control Infants	All Neonatal Infection	Neonatal meningitis	Neonatal Sepsis	Severe pneumonia
Gross motor	9/164(5%)	18/102(18%)	7/19(37%)	9/62(15%)	2/21(9.5%)
Fine Motor	15/163(9%)	30/102(29%)	9/19(47%)	16/62(25%)	5/21(24%)
Cognitive	17/164(10%)	17/107(16%)	8/20(40%)	8/57(14%)	1/22(4.5%)
Receptive Language	5/164(3%)	11/101(11%)	5/19(26%)	6/60(10%)	0
Expressive language	6/163(4%)	12/103(18%)	6/19(32%)	4/62(6%)	2/22(9%)

4.4.3.1.1 Logistic regression univariate analysis on the association between severe neonatal infection and developmental delay at 6 months of age

In the univariate logistic regression analysis of the risk of neurodevelopmental delay at 6 months of age, neonatal infection was associated with a 3.7-fold increase in the risk of delay in gross motor skills. There was also a 4.1-fold increase in the risk of delay in fine motor skills 3.5-fold increase in expressive language delay and 3.9-fold increase in receptive language skills delay. There was a non-significant trend towards an increased risk of delay in the cognitive domain of 1.63-fold (95% CI 0.79-3.36). (see Table 4.4).

Neonatal meningitis was associated with a statistically significant increased risk of developmental delay in all the domains compared to controls. There was a 12-fold increase in the risk of having delay in expressive language (P value of <0.0001) and cognitive development at 6 months of age (P value of <0.0001) , 11-fold increase in the risk of receptive language delay (P value of <0.0001), 10-fold increased risk of gross motor delay (P value of <0.0001) and nearly 9-fold risk of fine motor delay at 6 months of age (P value of <0.0001) (Table 4.4).

Neonatal sepsis was associated with a statistically significant increased risk of delay in fine motor skills of 3.4-fold (P value of <0.002) , receptive language of 3.53-fold (P value of <0.04)and gross motor of 2.92-fold(P value of <0.03) compared to infants that never had an episode of neonatal infection. There was a non-significant increased risk of delay in expressive language (1.8-fold; (p= 0.37) and cognitive skills (1.21-fold P=0.67) (see Table 4.4)

Neonatal severe pneumonia cases had no statistically significantly increased risk of delay in any of the domains compared to controls (Table 4.4). There was a trend towards increased risk of delay in the gross motor, fine motor and expressive language skills and a reduced risk of delay in the cognitive and receptive language domains. There was no evidence of delay at 6 months in receptive language among infants who had an episode of neonatal severe pneumonia.

Table 4.4 Logistic regression univariate analysis on the association between severe neonatal infection and developmental delay at 6 months of age

	All neonatal infections		Neonatal meningitis only		Neonatal sepsis only		Severe pneumonia only	
Neurocognitive domain	**OR (95%CI)**	***P value**	**OR (95%CI)**	***P value**	**OR (95%CI)**	***P value**	**OR (95%CI)**	***P value**
Gross motor	3.7(1.59-8.57)	0.002	10.1(3.18-31.7)	<0.001	2.9(1.1-7.8)	0.030	1.8(0.36-9.01)	0.470
Fine motor	4.1(2.08-8.12)	<0.001	8.9(3.12-25.3)	<0.001	3.4(1.58-7.5)	<0.002	3.1(0.99-9.6)	0.050
Expressive language	3.5(1.25-9.5)	0.020	12.1(3.41-42.8)	<0.001	1.8(0.49-6.6)	0.370	2.6(0.49-13.9)	0.260
Receptive language	3.9(1.31-11.5)	0.010	11.4(2.93-44)	<0.001	3.5(1.04-12)	0.04		
Cognitive	1.6(0.79-3.36)	0.180	12.1(3.41-42.8)	<0.001	1.2(0.50-2.97)	0.670	0.4(0.05-3.3)	0.4

***logistic regression univariate analysis p value**

4.4.3.2 Neurocognitive Outcomes at 12 Months of Age

There was an increase in the rates of developmental delay in all the groups at 12 months of age compared to age 6 months. The range of delay in the controls was from 4% in the expressive language domain to 15 % in the gross motor domain. This is in contrast to the range at 6 months of age which was from 3% in the receptive language domain to 10% in the cognitive domain.

All cases that had severe neonatal infections had a range from 26% in the fine motor domain to 35% in the gross motor skills at 12 months of age. This was higher compared to a range of 11% in the receptive language domain to 29% in the fine motor domain at 6 months of age.

The rate of delay in the meningitis group ranged from 44% in the cognitive domain to 60% in the gross motor skills. This again was higher compared to the range of 26% in the receptive language to 47% in the fine motor skills at 6 months of age.

In the neonatal sepsis group the rate of delay at 12 months of age was from 25% in the fine motor domain to 31% in the cognitive domain. This was higher than the rates at 6 months which ranged from 6% in the expressive language domain to 25% in the fine motor skills. The same trend was noted in the severe pneumonia group with the range of delay having increased from 0 (receptive language) to 24% (fine motor) at 6 months of age to 13% (fine motor) to 35% (gross motor) at 12 months of age(see Tables 4.3 and 4.5)

Table 4.5 Rates of neurodevelopmental delay in the control infants and severe neonatal infection cases at 12 months of age (n=298)

Neurocognitive Domain	Control Infants	All Neonatal Infection	Neonatal meningitis	Neonatal Sepsis	Severe pneumonia
Gross motor	22/147(15%)	28/80(35%)	9/15(60%)	11/42(26%)	8/23(35%)
Fine motor	7/147(5%)	21/82(26%)	7/15(47%)	11/44(25%)	3/23(13%)
Cognitive	18/147(12%)	29/91(32%)	7/16(44%)	16/51(31%)	6/24(25%)
Receptive language	80/147(5%)	26/82(32%)	8/15(53%)	12/44(27%)	6/23(26%)
Expressive language	62/146(4%)	26/80(33%)	7/15(47%)	12/43(28%)	7/22(32%)

4.4.3.2.1 Logistic regression univariate analysis on the association between severe neonatal infection and developmental delay at 12 months of age

In the univariate logistic regression analysis for the risk of neurodevelopmental delay in the neonatal infection cases there was a statistically significant increased risk of delay of 3.1-fold (p value 0.001), 6.8-fold (p value <0.0001) and 3.4-fold (p value <0.0001) in gross motor, fine motor and cognitive skills respectively at 12 months of age compared to controls. There was however a risk reduction of 61% (p value 0.001) in the severe neonatal infection cases developing receptive language delay at 12 months of age compared to controls and this was statistically significant (Table 4.9). There was also a risk reduction of 30% in expressive language delay but this was not statistically significant (p=0.143). There was however a difference in the risks reported at 6 months of age with gross motor 3.7- fold (P-value of 0.002) and fine motor- 4.1-fold (p value <0.0001) continuing to show a statistically increased risk at 12 months of age gross motor 3.1-fold (p value 0.001), and fine motor 6.8-fold (p value <0.0001), cognitive skills showing a statistically significant risk at 12 months of age 3.4-fold (p value <0.0001) compared to a statistically non-significant increased risk at 6 months of age 1.6 fold (p value 0.18). There was a reverse on the risk of delay in receptive language skills with an improvement at 12 months of age from an increased risk of 3.9 fold (p value 0.01) at 6 months of age to a risk reduction of 61% (p value 0.001) at 12 months of age (see Table 4.4 and table 4.6)

In the logistic regression analysis on the odds of delay at 12 months in the neonatal meningitis cases there was a statistically increased risk of delay in the gross motor domain of 8.5-fold (p value <0.0001) , fine motor of 17.4-fold (P value <0.0001) and cognitive skills of 5.6-fold (p value 0.002). However there were no statistically significant differences in the risk of delay in the expressive 1.2-fold (95% CI 0.41-3.44) and receptive language 0.96(95% CI 0.33-2.78) domains of the neonatal meningitis cases compared to controls. This is in contrast to what was observed at 6 months of age with the meningitis cases having an increased risk of delay in all the developmental domains(Table 4.4 and 4.6).

In the univariate logistic regression analysis of the odds of developmental delay in the neonatal sepsis cases at 12 months of age there was a statistically significant increased risk of delay in the fine motor domain of 6.6-fold (p value <0.0001) and cognitive skills of 3.3-fold (p value 0.003). However there was a non-significant increase of delay in the gross motor domain of 2-fold (95% CI 0.88-4.54) (Table 4.6). On the other hand there was an improvement in the receptive language skills at 12 months of age with a statistically significant risk reduction of 69% (p value 0.002) compared to an increased risk of 3.5-fold at 6 months of age (p value 0.04) (Table 4.4 and 4.6)

In the logistic regression analysis of the odds of developmental delay at 12 months of age there was a statistically significant 3 fold (p value 0.025) increased risk of delay in gross motor skills in infants that had an episode of severe pneumonia compared to controls. There was also an increased risk of 3 fold (p

value 0.14) and 2.4fold (p value 0.1) in the acquisition of fine motor and cognitive skills but these were not statistically significant. On the other hand there was a risk reduction of 70% (p value 0.02) in receptive language delay at 12 months of age. (Table 4.4 and 4.6)

Table 4.6 Logistic regression univariate analysis on the association between severe neonatal infection and developmental delay at 12 months of age

	All neonatal infections		Neonatal meningitis only		Neonatal sepsis only		Severe pneumonia only	
Neurocognitive domain	**OR (95%CI)**	***P value**	**OR (95%CI)**	***P value**	**OR (95%CI)**	***P value**	**OR (95%CI)**	***P value**
Gross motor	3.1(1.6-5.8)	0.001	8.5(2.76-26.3)	<0.001	2(0.88-4.54)	0.090	3(1.15-8.0)	0.030
Fine motor	6.8(2.76-16.93)	<0.001	17.4(4.89-61.7)	<0.001	6.62(2.38-18.4)	<0.001	3(0.71-12.46	0.140
Expressive language	0.7(0.37-1.16)	0.140	1.2(0.41-3.44)	0.750	0.52(0.25-1.10)	0.090	0.6(0.24-1.64)	0.350
Receptive language	0.4(0.22-0.69)	0.001	0.96(0.33-2.78)	0.940	0.31(0.15-0.66)	0.002	0.3(0.11-0.79)	0.020
Cognitive	3.4(1.73-6.50)	<0.001	5.6(1.85-16.81)	0.002	3.28(1.52-7.08)	0.003	2.4(0.84-6.80)	0.100

***Logistic regression univariate analysis p value**

4.4.3.3 Neurodevelopmental outcomes for neonatal infection infants and control infants - a comparison of scaled BSID-III scores

There was a difference in the scaled scores (chapter 2) between the control babies and the neonatal infection cases at both 6 months and 12 months of age (see Fig 4.2 and 4.3). The control babies had statistically significant higher scores than the neonatal infection cases in all the domains.

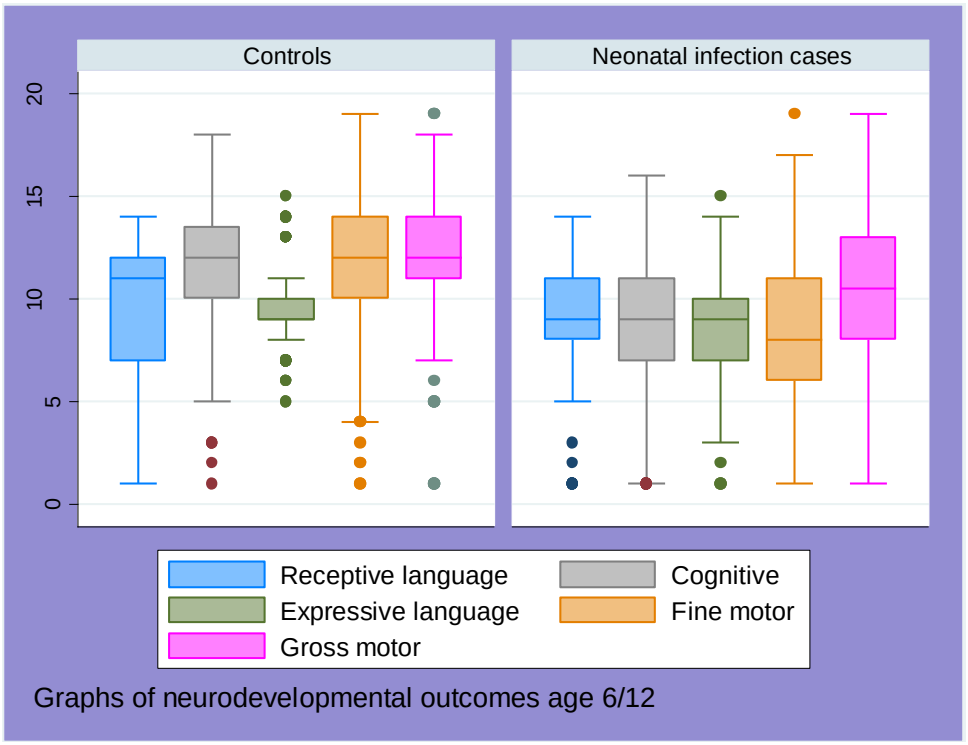


Fig 4.2 Scaled scores of neurodevelopmental outcomes at 6 months of age comparing severe neonatal infection and Control infants

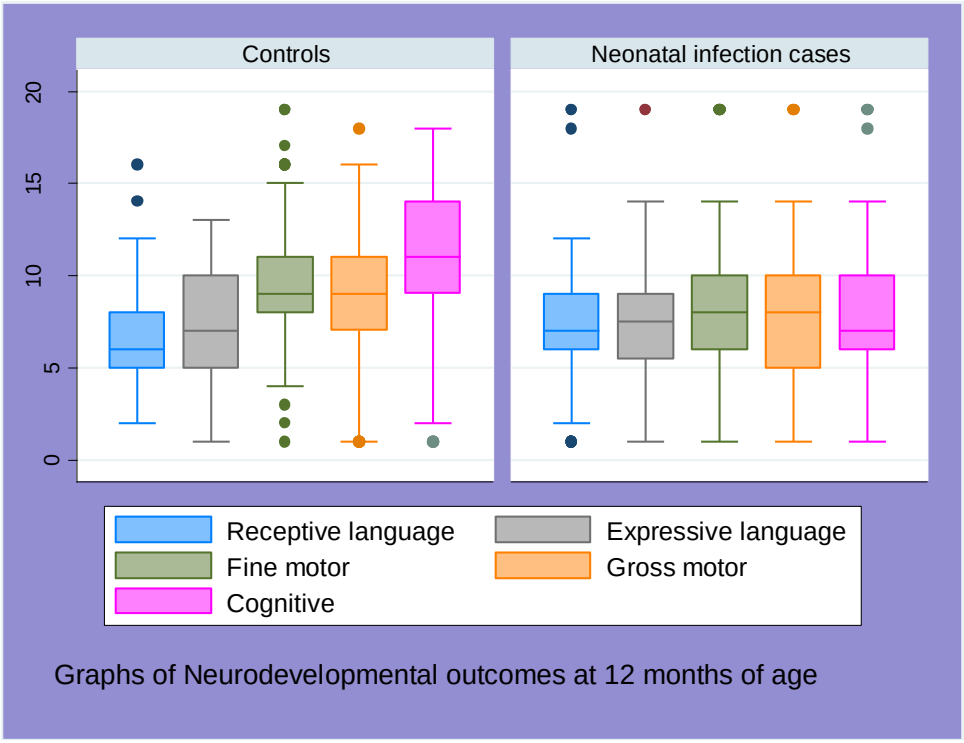


Fig 4.3 Scaled scores of neurodevelopmental outcomes at 12 months of age comparing severe neonatal infection and control infants

4.4.3.3.1 Neurodevelopmental outcomes for neonatal sepsis, meningitis, severe pneumonia infants and control infants- a comparison of scaled BSID-III scores

In a subgroup analysis of the severe neonatal infection group; the control infants had statistically significant higher scaled scores in the gross motor, fine motor, expressive language and cognitive domains at 6 months of age compared to those who had neonatal sepsis. However, the control infants had statistically significant higher scores in all the neurocognitive domains than the meningitis cases (see Table 4.9). On the other hand the control infants had statistically significant higher scaled scores in the fine motor and gross motor domains than the severe pneumonia group.

The severe pneumonia group had statistically significant lower scores in the cognitive domain when compared to the control babies (see Table 4.10).

The scores overall at age 6 months were higher than at 12 months of age (see fig 4.4-4.8)

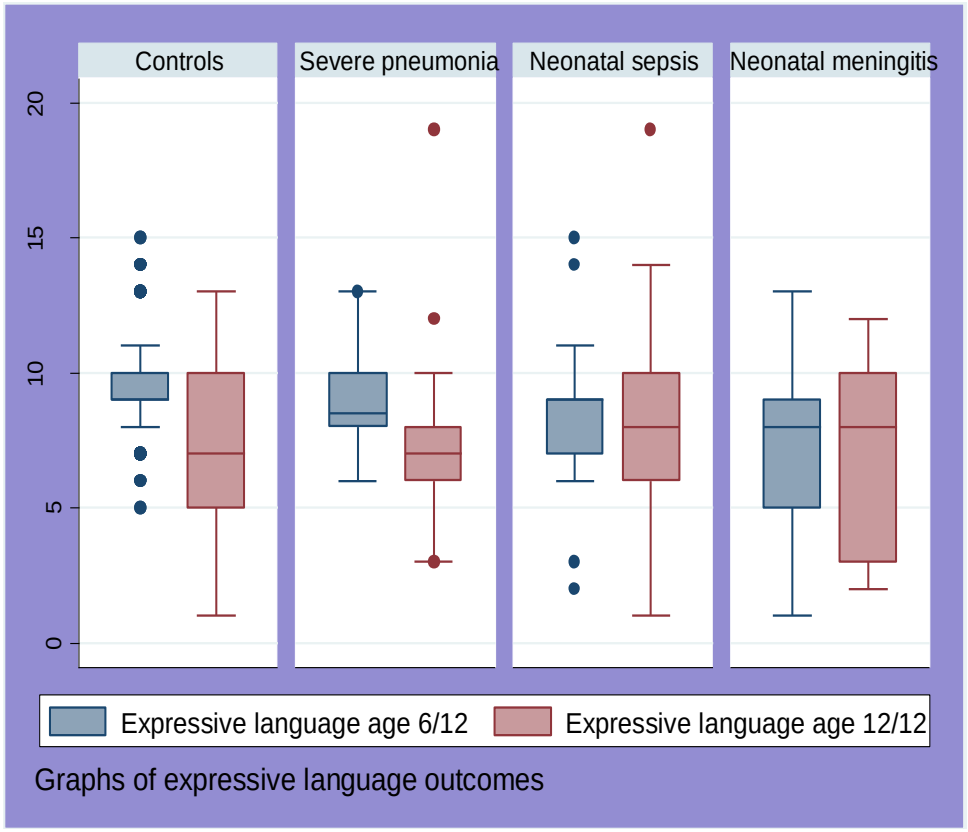


Fig 4.4 Expressive language outcomes- BSID-III scaled scores

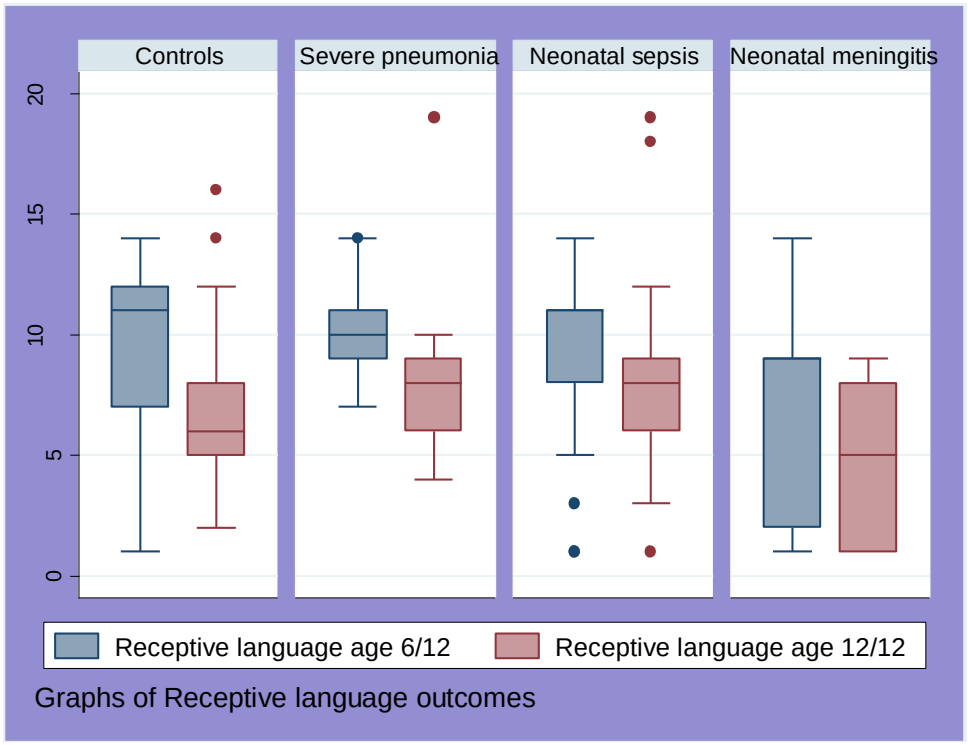


Fig 4.5 Receptive language outcomes- BSID-III scaled scores

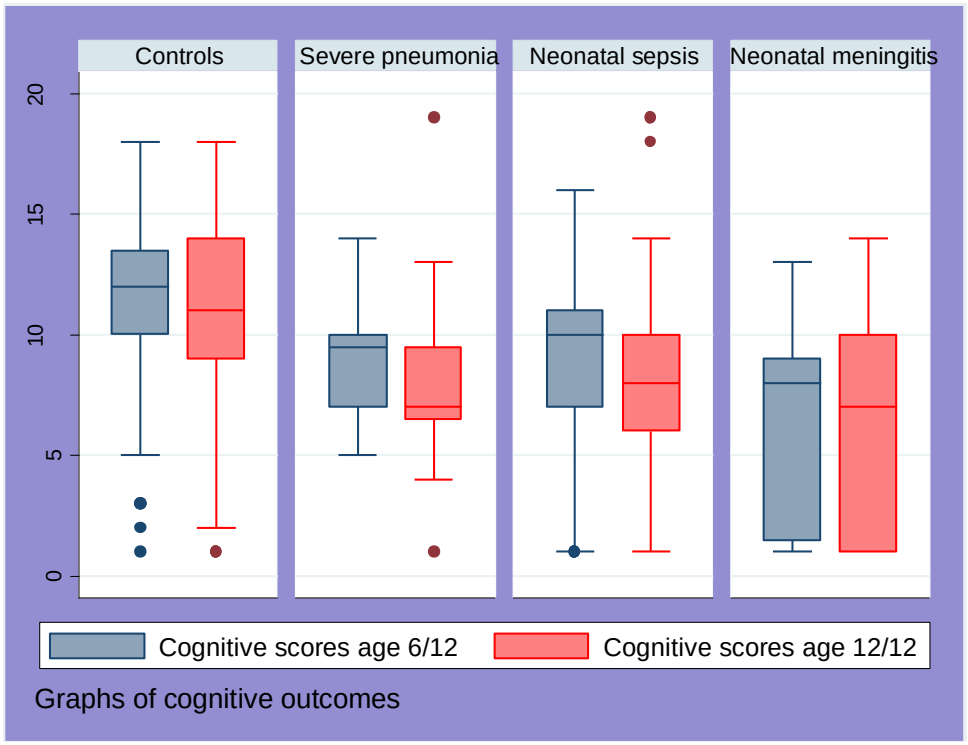


Fig 4.6 Cognitive outcomes- BSID-III scaled scores

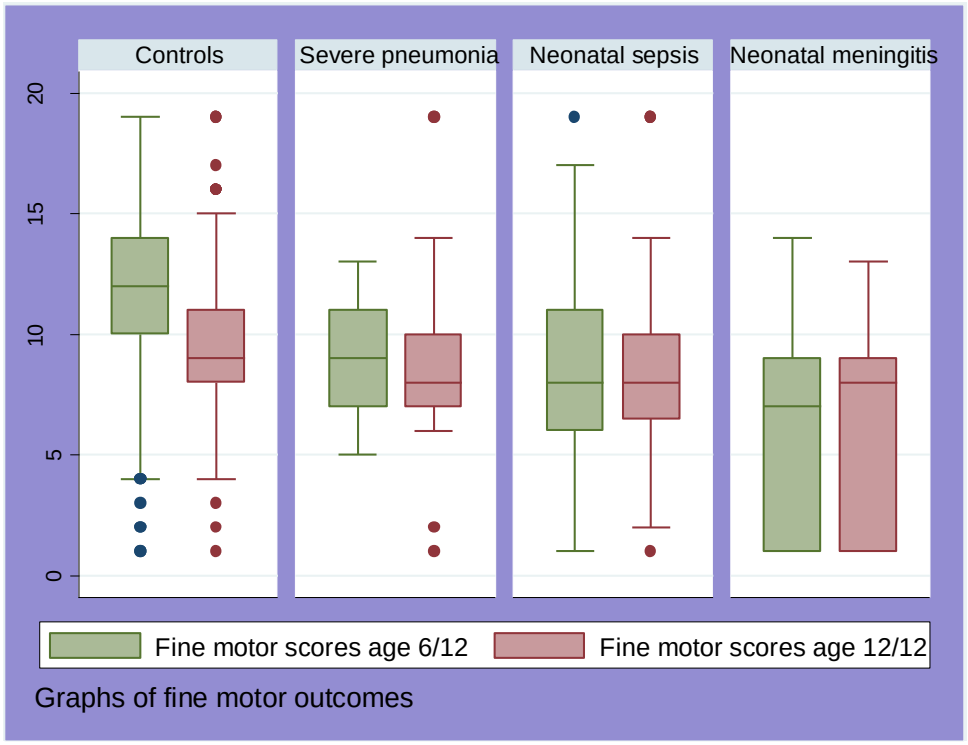


Fig 4.7 Fine motor outcomes- BSID-III scaled scores

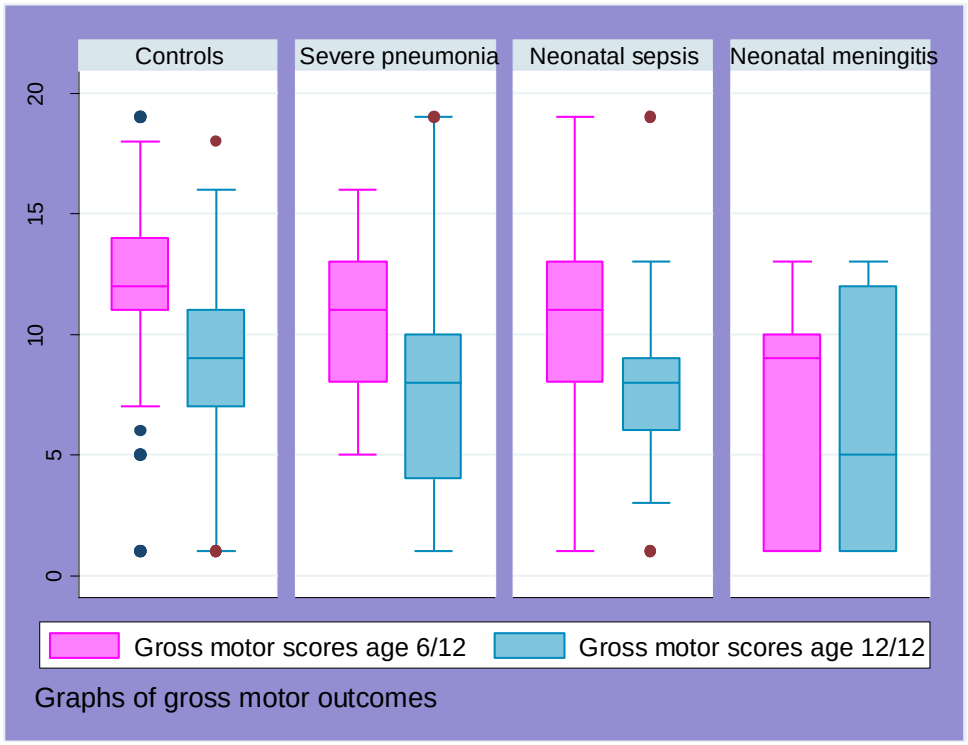


Fig 4.8 Gross motor outcomes- BSID-III scaled scores

4.4.3.4 Mortality Outcomes at 12 months of age.

There were a total of 21 deaths at the end of the follow up period in the severe neonatal infection group and 14 in the control infants group.

In the survival analysis at age 12 months survival chances were higher in the control infants group compared to the severe neonatal infection group (See Fig 4.9). However in the cox regression model analysis for mortality, the hazards ratio for death in the severe neonatal infection group was 1.35 (95% CI 0.68-2.65; P=0.39). In the severe neonatal infection subgroup survival analysis, the highest risk of mortality was in the meningitis group 2.45-fold followed by the severe pneumonia group 1.66-fold (see Fig 4.10). The hazards ratios in the subgroups of severe neonatal infection namely; neonatal meningitis, neonatal sepsis and severe pneumonia were also not statistically significant (see Table 4.7).

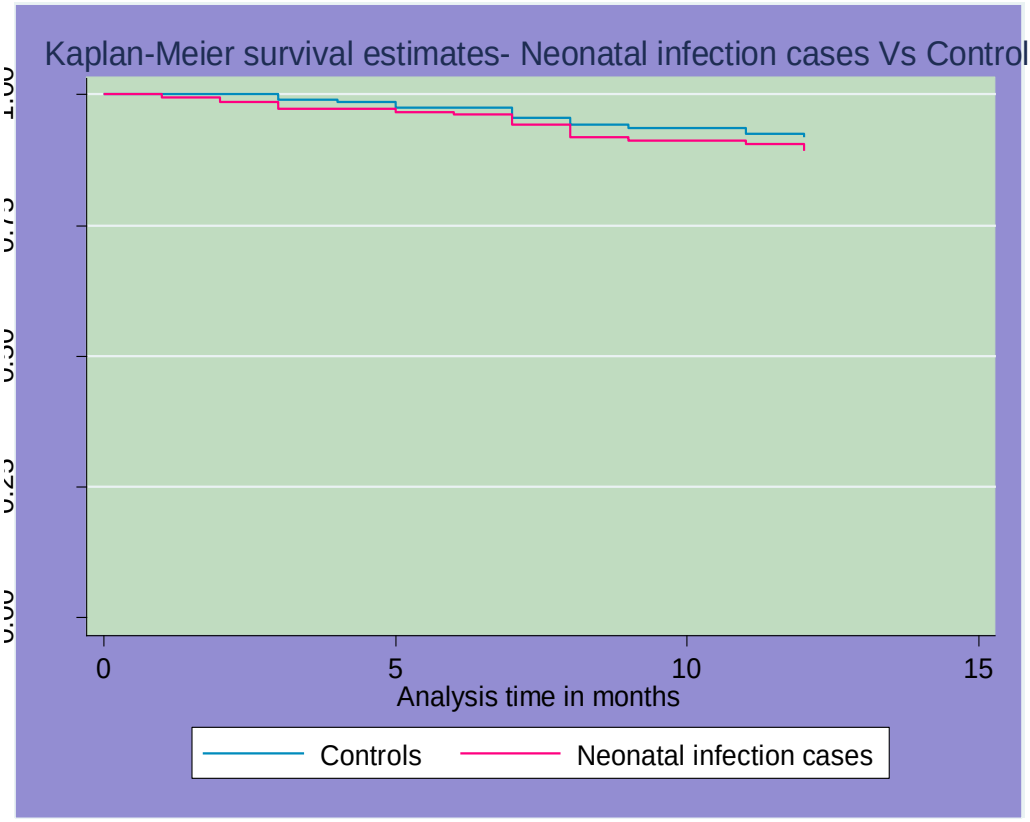


Fig 4.9 Survival analysis comparing severe neonatal infection cases and control infants age 12 months

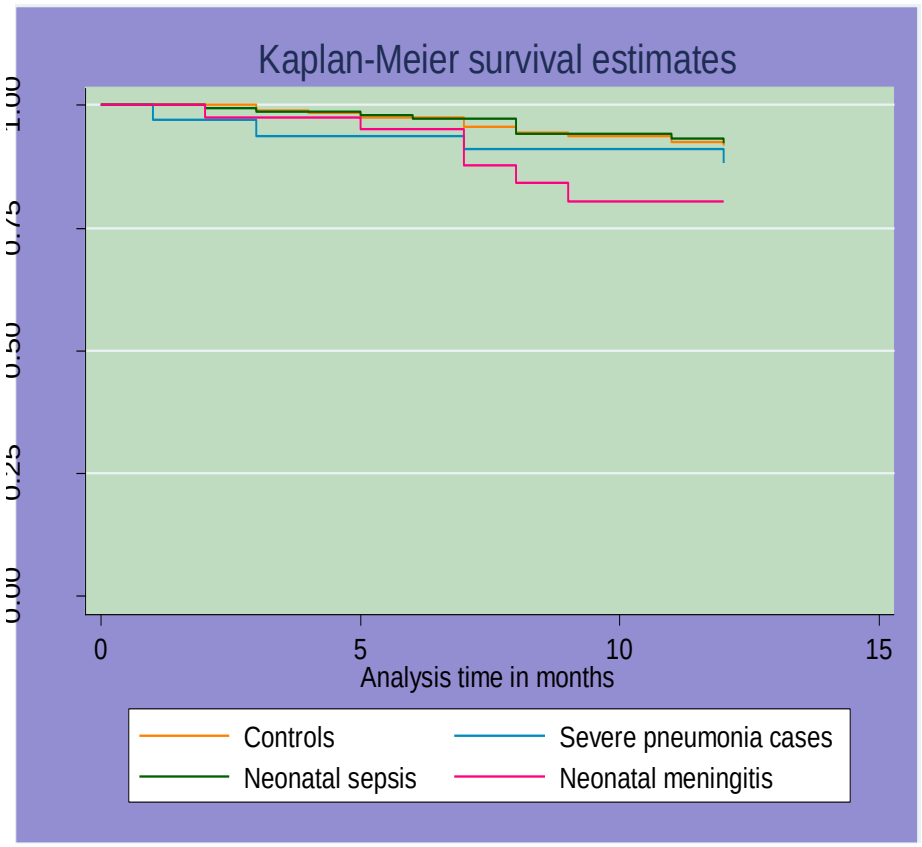


Fig 4.10 Survival analysis comparing subgroups of severe neonatal infection cases and control infants age 12 month

Table4.7 Cox regression analysis on the risk of dying

Parameter	Hazards ratio(95% CI)	P value
Severe neonatal infection	1.35(0.68-2.65)	0.390
Final diagnosis		
Neonatal meningitis	2.45(0.94-6.39)	0.067
Neonatal sepsis	0.96(0.41-2.21)	0.916
Severe pneumonia	1.66(0.64-4.34)	0.298

4.5 Discussion

This study has shown that severe neonatal infections are a risk factor for poor neurodevelopment at 1 year of age. The findings are in keeping with what has been observed in the developed world {Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Seale A.C, M Mwaniki et al (2009), Gordon A.L, M. English et al (2005), Osrin D, S. Vergnano et al (2004)Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989), }. However in this study the majority of the severe neonatal infection cases were born at term (85%) which is in sharp contrast to the predominantly preterm cases in the developed setting. In resource restrained settings there are several factors that impact on neurodevelopment in a child. The factors include HIV infection, low socio-economic status and maternal education {Waseen R., A. A. and Shah (2005)}. In this study the socio demographic profile and baseline characteristics of the severe neonatal infection and the control infants groups were similar except for HIV exposure status which unsurprisingly was higher in the infection group than the control infants group (36% compared to 15.5% respectively). The impact of HIV exposure on the long term outcome of severe neonatal infections has been described in chapter 5. The balance in the baseline characteristics between the 2 groups made it possible for us to associate the neurological outcome at 12 months of age in these infants to the episode of severe neonatal infection.

In this study having had severe neonatal infection was associated with an up to 4-fold increased risk of developmental delay at 6 months of age and up to 6-fold at 12 months of age. However, in the sub-group analysis of the severe neonatal infection cases the highest

risk was from neonatal meningitis up to 17-fold at 12 months of age. It was surprising to note that neonatal sepsis cases (without overt meningitis) had an increased risk of developmental delay of up to 6 fold in the fine motor domain at 12 months of age. The findings in this study were similar from published reports that put the risk at 2-6-fold for neurodevelopmental delay{Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}.}. The majority of the studies were done in preterm or very low birth weight babies and as such one would expect a much higher risk of developmental delay in these groups of patients than term babies. In this study 86% of the neonatal sepsis cases were full term with birth weights of equal to or above 2.5 kg. It has been reported that inflammatory cytokines are associated with an increased risk of neurodevelopmental delay in preterm neonatal sepsis cases {Volpe J.J(2008), Shah D.K, L.W. Doyle et al (2008), Volpe J. J,(2003), Duncan J.R, M.L Cock (2002)} It is therefore important to try and explore possible mechanisms of developmental delay in these term babies with neonatal sepsis. It would be important to explore the role of inflammatory markers in these septic neonates' brains and look at the immunology of these septic newborns.

It is important to note that routinely young infants who have had an episode of neonatal sepsis (without overt meningitis) are not followed up in Malawi and the findings in this study underscore the importance of follow up in this group of infants. However looking at the burden of neonatal sepsis in Malawi it would be a challenge to follow up all the cases and as such identifying those septic infants who have a much higher risk of developmental delay would help in focusing on the type of infants who would benefit from follow up. In

chapter 5 we discuss the predictors of poor long term outcome in the severe neonatal infection cases.

In this study it has been shown that 60% of the survivors of neonatal meningitis had complications at the age of 1 year. The rates of complications in this study are higher than figures reported elsewhere {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M. Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}. It is however important to note that in most of the reported studies on long term outcome of neonatal meningitis the participants were followed up for a duration longer than 1 year {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M., Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}. In this study the infants were followed up to the age of 1 year and this could have led to underestimation of complications arising from neonatal meningitis. It is also a known fact that most of the behavioural problems cannot be picked up in infancy the same is true with learning difficulties {Holt DE, S., Halket (2001)}. A longer period of follow up preferably up to the age of 6 years could have picked up most of the complications arising from neonatal meningitis. However it is important to note that even within this study the rate of neurodevelopmental delay was higher overall at 12 months than at 6 months of age which could be an indicator of higher rates of complications later on in life.

The rates of other neurological sequelae were higher in the neonatal meningitis group with 17.6% of the survivors having hydrocephalus, 7.3% blind, 13.2% had epilepsy and 33% with hearing loss. The rate of hydrocephalus was lower in this study compared to other settings where 24% of neonatal meningitis cases will have hydrocephalus. However the rate of

hearing loss was similar in this study to what has been reported elsewhere i.e. 30% { }. The number of hydrocephalus cases reported in this study could have been lower due to logistical reasons. All the neonatal meningitis cases did not have follow up cranial ultrasound scans as explained in chapter 6. This could have led to some cases of hydrocephalus being missed.

It is therefore important to strengthen the prevention, diagnosis; management and long term follow up of these infants. Group B streptococci were the commonest cause of neonatal meningitis {see Chapter 3). It has been shown in the developed world that better prenatal care can reduce the incidence of GBS infections in the neonate {Lawn J.E, H. Blencowe et al (2013), Ganatra H.A, Stoll B.J et al (2010), Vergnano S., M. Sharland et al (2005), Seale A.C, M., Mwaniki (2009) }. In Malawi the quality of prenatal care is poor {Malawi, New born Health analysis 2013} and there is need to improve the quality of prenatal care. It is important to introduce routine follow up of neonatal meningitis cases in developing countries such as Malawi where routine follow up of these cases is non-existent. Hearing screens also need to be introduced as part of the routine follow up of these patients. In this study all the hydrocephalus, blindness, epilepsy and hearing loss cases occurred in infants who had neonatal meningitis except for I infant in the control group who had hydrocephalus.

In this study the severe pneumonia group had the lowest risk of developmental delay both at 6 months and 12 months at age. As indicated in the literature review set out in Chapter 1, there was paucity of data on the long term impact of neonatal severe pneumonia {Sazawal S, R. E Black et al (2003), Bang A.T, R.A Bang et al (1993) .

In this study it was also noted that the lowest scores were in the expressive and receptive language domains in both the severe neonatal infection and control infants groups. A study done in Malawian children by Cromwell et al {Cromwell E., Q. Dube et al (2014)} demonstrated that population reference curves for the BSID-III, a neurodevelopmental tool developed for and commonly used in the USA, differed depending on the origin of the reference population. Reliance on US norm-based standardized scores can therefore result in misclassification of the neurological development of Malawian children, with the greatest potential for bias in the measurement of cognitive and language skills {Cromwell E., Q. Dube et al (2014)}. This finding could explain the low scores noted in the language domains but not in the motor and cognitive domains.

In the survival analysis there was no overall statistical difference in mortality between the severe neonatal infection and control infants group at 1 year. The same was true for the neonatal meningitis, sepsis and severe pneumonia group.

Limitations of the study

Mortality was not the primary outcome in this study and as such the sample size calculation was not based on mortality but on neurodevelopmental outcomes. One would need a much bigger sample size to better demonstrate differences in survival between the severe neonatal infection and control groups. The survival analysis results from this study should therefore be interpreted with caution.

Participants were followed up to the age of 1 year and as discussed above other developmental challenges that become apparent in later life would have been seen. This

could lead to an under estimation of morbidity outcomes associated with severe neonatal infections.

4.6 Conclusion

In summary, the study found that severe neonatal infections were associated with a higher risk of neurodevelopmental delay. The risk was greatest in the neonatal meningitis group with up to 60% of the meningitis cases being delayed developmentally in at least one neurocognitive domain. The study has also shown an increased risk of neurodevelopmental delay in neonatal sepsis cases (without overt meningitis). It is important therefore to introduce routine monitoring and follow up in all cases of severe neonatal infections. It is important to do neurodevelopmental screening assessments particularly in the first year of life in these young infants who have had severe neonatal infections even in the absence of meningitis. Some of the developmental delay can improve if picked up earlier and appropriate interventions made {Grantham-McGregor S, Y.B., Cheung et al (2007), Engle P.L, M.M., Black et al (2007)}.

CHAPTER FIVE

PREDICTORS OF POOR NEURODEVELOPMENTAL OUTCOMES IN SEVERE NEONATAL INFECTION CASES AT 12 MONTHS OF AGE IN MALAWI

5.1 Introduction

Severe neonatal infections have been shown to have an increased risk of developmental delay in this study (chapter 4). This finding is in keeping with studies in other settings {Seale A.C, H. Blencowe et al (2013), Lawn J.E, H. Blencowe et al (2013), Blencowe H, T.Vos et al (2013), Gordon A.L, M. English et al (2005), Heath P.T, N.K Nik Yusoff et al (2003)}, Bennet R, S.Berdahl et al (1989)}.

However, most of the studies on the long term impact of neonatal sepsis on neurodevelopment have been done in premature and low birth weight babies in the developed setting {Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996)}. Prematurity on its own is a known risk factor for developmental delay {Alshaikh B., K. Yusuf et al (2013), Beaino G., B. Khoshnood et al (2010), Woodward L.J., H.G. Taylor (2003), Volpe J.J (2003), Hack M., D Wilson-Costello (2000) }. It has been shown that preterm babies who have severe neonatal infection have an even higher risk of developmental delay { Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J,

T. Gordon et al (1996)}. There is a paucity of data on the long term impact of either neonatal meningitis or sepsis (without overt meningitis) in term babies within the developing setting. In this study the majority of the cases (85%) were born at term (chapter 3) and up to 60% of neonatal meningitis cases and up to 31% of the neonatal sepsis cases without overt meningitis had developmental delay at 12 months of age (chapter 4). The prevalence of developmental delay was higher in the neonatal sepsis cases without overt meningitis than what was expected (chapter 2). It is important therefore to explore possible risk factors that could have led to such a high prevalence of developmental delay.

This chapter therefore seeks to explore the risk factors associated with poor developmental outcomes in neonates with severe neonatal infection.

5.2 Materials and methods

As previously described, this study took place at Queen Elizabeth Central hospital and included all severe neonatal infection cases that were recruited in the prospective cohort arm (chapter 4). Developmental assessments were done at 6 and 12 months of age using the Bayley Scale of Infant Development III. The assessments were divided into 5 domains; gross motor, fine motor, cognitive, expressive language and receptive language.

Univariate logistic regression analysis was done to identify risk factors associated with developmental delay in each of the domains at 12 months of age. All significant identified risk factors in the univariate analysis were evaluated for effect modifying and confounding and appropriate multivariate backward elimination models were constructed to evaluate the measure of association between exposure and outcome- being developmental delay in

each of the domains. All known clinical risk factors were also evaluated for even though they showed no significance in the univariate analysis.

In this study three different models were used namely;

I Including the Severe neonatal infection effect only using the infection indicator variables like positive blood or CSF culture and raised CRP

ii Including the neonatal infection effect together with design factors namely gestation age

iii Adjusting for design factors as well as post design baseline factors like; HIV status, age at onset of disease and serum sodium results.

5.3 Results

A total of 102 neurocognitive assessments were done in the severe neonatal infection cases at 12 months of age.

5.3.1 Gross motor outcomes at 12 months of age

As shown in Table 5.1, in the univariate analysis of risk factors associated with gross motor delay at 12 months of age a significant CSF culture was associated with a 2.15-fold increased risk of gross motor delay (95% CI 1.02-4.55), hypothermia was associated with an 18-fold increase (95% CI 1.79-181) and HIV exposure was associated with a 3.79-fold increase (95% CI 1.39-10.37). A non-significant increased risk trend for gross motor delay was observed in infants with a significant growth in blood culture(2.1 fold [95% CI 1-4.43], infants who had anaemia (1.3-fold [95% CI 0.37-4.4], hyponatraemia (2.5-fold [95% CI 0.79-7.97], hypoxia (1.38-fold [95% CI 0.5-3.8], prematurity (2.5-fold[95% CI 0.75-8.36], convulsions (1.57-fold [95%CI 0.38-6.38] and early onset disease (1.3-fold [95%CI 0.41-4.13]).

On the other hand a non- significant risk reduction was observed with hypernatraemia (0.32[95% CI 0.04-2.86]), raised CRP (0.58[0.16-2.05]), and thrombocytopenia (0.24 [95% CI 0.03-2.04]) (Table 5.1).

Table 5.1 Univariate logistic regression analysis on factors associated with gross motor delay at 12 months of age

Parameter	OR(95% CI)	*P value
Positive blood culture	2.1 (1.0-4.43)	0.050
Positive CSF culture	2.15 (1.02-4.55)	0.045
HIV exposure	3.79 (1.39-10.37)	0.009
Anaemia	1.3 (0.37-4.4)	0.700
Hyponatraemia	2.5 (0.79-7.97)	0.120
Hypernatraemia	0.32 (0.04-2.86)	0.310
Hypoxia	1.38 (0.5-3.8)	0.530
Prematurity	2.5(0.75-8.36)	0.140
Raised CRP	0.58(0.16-2.05)	0.400
Hypothermia	18 (1.79-181)	0.010
Thrombocytopenia	0.24(0.03-2.04)	0.190
Early onset disease	1.3(0.41-4.13)	0.650
Convulsions	1.57(0.38-6.38)	0.530

*Univariate logistic regression p value

5.3.1.1 Multivariate analysis of risk factors for gross motor delay at 12 months of age

An adjusted logistic model of the odds of gross motor delay at 12 months of age that included positive CSF culture, hypothermia, positive blood culture, convulsions, prematurity and hyponatraemia was formulated as described in section 5.2 above.

Table 5.2 Adjusted logistic model of the odds of gross motor delay at 12 months of age

Parameter	OR (95% CI)	*P value
Positive CSF culture	2.7 (1.22-5.86)	0.014
Positive blood culture	2.3 (0.79-6.76)	0.130
Hypothermia	12.5 (1.13-140)	0.040
Prematurity	2.8 (0.7-11.7)	0.160
Hyponatraemia	2.3 (0.7-7.4)	0.170
HIV exposed	5.04 (1.49-17)	0.009
Convulsions	1.17(0.16-8.52)	0.880

*Adjusted logistic regression p value

In all adjustments infants who had significant growth in their CSF had a 2.7-fold [95% CI 1.22-5.86] increased risk of developmental delay in the gross motor domain (see table 5.2). However after adjustment for hypothermia, prematurity and hyponatremia, a significant blood culture growth was associated with a 2.3-fold [95% CI 0.79-6.76] increased risk of gross motor delay. Infants born from HIV infected mothers were found to have an increased risk of 5-fold [95% CI 1.49-17] in gross motor delay after adjusting for all the factors above (table 5.2). In all adjustments prematurity, convulsions and hyponatremia were associated with a non-significant risk of developmental delay of 2.8-fold [95% CI 0.7-11.7], 1.17-fold [95% CI 0.16-8.52] and 2.3-fold [95% CI 0.7-11.7] respectively.

5.3.2 Risk factors for poor fine motor outcomes at 12 months of age

In the univariate analysis, significant growth in blood or CSF culture was associated with an increased risk of fine motor delay at 12 months of age. A positive blood culture had a 3-fold risk [95% CI 1.38- 6.85] and positive CSF culture had a 2.67-fold risk [95% CI 1.24- 5.7]. A non-significant increased risk of fine motor delay was observed in HIV exposed infants of 2.47 (95% CI 0.87-7), anaemia of 1.5 (95% CI 0.41-5.6), convulsions of 2.64-fold, hyponatraemia of 1.64 (95% CI 0.49-5.47), hypothermia of 1.8 (95% CI 0.33-9.89), prematurity of 1.33 (95% CI 0.36- 4.89) and early onset disease of 2.04 (95% CI 0.64-6.54) (Table 5.3). On the other hand as with gross motor delay hypernatraemia (0.43 [95% CI 0.05-3.88]), fever 0.87[95% CI 0.28-2.72] and raised CRP (0.8 [95% CI 0.21-3.03]) were associated with a non-significant risk reduction of fine motor delay (Table 5.3).

Table 5.3 Univariate logistic regression analysis on factors associated with fine motor delay at 12 months of age

Parameter	OR(95% CI)	*P value
Positive blood culture	3.08(1.38-6.85)	0.006
Positive CSF culture	2.67(1.24-5.7)	0.012
HIV exposed	2.47(0.87-7)	0.090
Anaemia	1.5(0.41-5.6)	0.540
Hyponatraemia	1.64(0.49-5.47)	0.420
Hypernatraemia	0.43(0.05-3.88)	0.450
Hypoxia	1.01(0.33-3.04)	0.980
Hypothermia	1.8(0.33-9.89)	0.500
Fever	0.87(0.28-2.72)	0.800
Prematurity	1.33(0.36-4.89)	0.660
Raised CRP	0.8(.21-3.03)	0.740
Early onset disease	2.04(0.64-6.54)	0.230
Convulsions	2.64(0.65-10)	0.180

*Univariate logistic regression p value

5.3.2.1 Multivariate analysis of risk factors associated with fine motor delay

The adjusted logistic model of the odds of fine motor delay at 12 months of age below includes positive CSF culture, hypothermia, positive blood culture, convulsions, prematurity and hyponatraemia.

In all adjustments a significant growth in either CSF or blood culture is associated with a 4.5-fold [95% CI 1.48-13.8] and 3.7-fold [95% CI 1.27-10.76] increased risk respectively of fine motor delay at 12 months of age. However when adjusted for prematurity, hypothermia, positive CSF or blood culture growth and hyponatraemia, HIV exposure was associated a non-significant increased risk of 2.03 -fold (95%CI 0.56-7.32) in fine motor delay.

Table 5.4 Adjusted logistic model of the odds of fine motor delay at 12 months of age

Parameter	OR (95% CI)	*P value
Positive CSF culture	4.5 (1.48-13.8)	0.008
Positive blood culture	3.7 (1.27- 10.76)	0.020
Hypothermia	3.01 (0.35-26.12)	0.320
Prematurity	5.07 (0.6- 42.54)	0.140
Hyponatraemia	1.79 (0.41-7.8)	0.440
HIV exposed	2.03 (0.56-7.32)	0.280
Convulsions	3.2 (0.43-23.98)	0.260

*Adjusted logistic regression p value

5.3.3 Risk factors for poor neurocognitive outcomes at 12 months of age

Even though in this study several factors had been shown to be associated with an increased risk of neurocognitive delay the only significant risk factor was being HIV exposed. Infants that were born from HIV infected mothers had a 2.7-fold increased risk of neurocognitive delay at 12 months of age (95% CI 1.05-6.86). Positive CSF culture (1.55[95% CI 0.78-3.05]), blood culture (1.44 [95% CI 0.71-2.92]), anaemia (1.58 [95% CI 0.46-5.38]), hyponatraemia (1.06 [95% CI 0.33-3.36], hypoxia (1.23 [95% CI 0.46-3.23]), hypothermia (1.36 [95% CI 0.29-6.38]), convulsions (3.78[95% CI 0.98-14.7], prematurity (1.8[0.56-5.82]) and early

onset disease (1.48 [95% CI 0.51-4.3]) were all associated with a non-significant increased risk of neurocognitive delay (Table 5.5).However in keeping with the fine and gross motor domains hypernatraemia (0.55[95% CI 0.1-2.97]), fever (0.55[95% CI 0.1-2.97]) and raised CRP (0.43[95% CI 0.13- 1.4]) were all associated with a non-significant risk reduction on the odds of neurocognitive delay in these infants (Table 5.5).

Table 5.5 Univariate logistic regression analysis on factors associated with neurocognitive delay at 12 months of age

Parameter	OR(95% CI)	*P value
Positive blood culture	1.44(0.71-2.92)	0.310
Positive CSF culture	1.55(0.78-3.05)	0.210
HIV exposed	2.69(1.05-6.86)	0.040
Anaemia	1.58(0.46-5.38)	0.470
Hyponatraemia	1.06(0.33-3.36)	0.920
Hypernatraemia	0.55(0.1-2.97)	0.500
Hypoxia	1.23(0.46-3.23)	0.680
Hypothermia	1.36(0.29-6.28)	0.690
Fever	0.55(0.20-1.5)	0.250
Thrombocytopenia	0.28(0.32-2.39)	0.240
Prematurity	1.8(0.56-5.82)	0.320
Raised CRP	0.43(0.13-1.4)	0.160
Early onset disease	1.48(0.51-4.3)	0.480
Convulsions	3.78(0.98-14.7)	0.054

***Univariate logistic regression p value**

5.3.3.1 Multivariate analysis of risk factors associated with neurocognitive delay

A logistic regression model on the odds of neurocognitive delay at 1 year of age included hyponatraemia, HIV exposure, gestation, convulsions, positive CSF or blood culture and hypothermia. In the all adjustments HIV remained the only significant risk factor for neurocognitive delay at 12 months of age with a risk of 2.9-fold (95% CI 1.12-7.64) (Table 5.8). However hyponatraemia (1.04 [95% CI 0.32-3.35]), hypothermia (1.2[95% CI 0.2-7.16]), prematurity (2.76 [95% CI 0.48-15.95]) and positive CSF (1.63 [95% CI 0.75-3.53]) or blood culture (1.23 [95% CI 0.53-2.85]) all continued to show an increased risk in all adjustments but it was non-significant (Table 5.8).

Table 5.6 Adjusted logistic model of the odds of neurocognitive delay at 12 months of age

Parameter	OR (95% CI)	*P value
Positive CSF culture	1.63 (0.75-3.53)	0.220
Positive blood culture	1.23 (0.53-2.85)	0.640
Hypothermia	1.2 (0.2-7.16)	0.840
Prematurity	2.76 (0.48-15.95)	0.260
Hyponatraemia	1.04 (0.32-3.35)	0.940
HIV exposed	2.92 (1.12-7.64)	0.030
Convulsions	4.28 (0.53-34.4)	0.170

***Adjusted logistic regression p value**

5.3.4 Risk factors for poor expressive language outcomes at 12 months of age

In the univariate analysis for risk factors associated with poor expressive language outcomes at 1 year of age there was no significant risk factor that was identified. However positive blood (1.18 [95% CI 0.56-2.46]) or CSF culture (1.85 [95% CI 0.89-3.82]), HIV exposure (1.5[(95% CI 0.56-4.05]), convulsions (2.98 [95% CI 0.73-12.2]), anaemia (1.48[95% CI 0.43-5.11]), hyponatraemia (1.78[95% CI 0.56-5.6]), hypoxia (1.47[95% CI 0.54-4.06]), hypothermia (1.44 [95% CI 0.28-7.34]) and prematurity (1.97 [95% CI 0.59-6.61]) were all associated with a non-significant increased risk of expressive language delay. On the other hand hypernatraemia (0.29 [95% CI 0.03-2.56] , fever (0.47[95% CI 0.28-7.34]) thrombocytopenia (0.26 [95% CI 0.03-2.23]) and raised CRP (0.27[0.07-1.03]) were all associated with a non-significant reduction in the risk of developmental delay at 1 year of age.

Table 5.7 Univariate logistic regression analysis on factors associated with expressive language delay at 12 months of age

Parameter	OR(95% CI)	*P value
Positive blood culture	1.18(0.56-2.46)	0.660
Positive CSF culture	1.85(0.89-3.82)	0.100
HIV exposed	1.5(0.56-4.05)	0.420
Anaemia	1.48(0.43-5.11)	0.540
Hyponatraemia	1.78(0.56-5.6)	0.330
Hypernatraemia	0.29(0.03-2.56)	0.260
Hypoxia	1.47(0.54-4.06)	0.450
Hypothermia	1.44(0.28-7.34)	0.660
Fever	0.47(0.28-7.34)	0.660
Thrombocytopenia	0.26(0.03-2.23)	0.221
Prematurity	1.97(0.59-6.61)	0.272
Raised CRP	0.27(0.07-1.03)	0.063
Early onset disease	0.93(0.29-3.03)	0.910
Convulsions	2.98 (0.73-12.2)	0.130

***Univariate logistic regression p value**

5.3.4.1 Multivariate analysis of risk factors associated with expressive language delay

The adjusted logistic regression model included the same factors as in the other developmental domains.

In all adjustments there was no significant risk factor identified however positive CSF (3.37 [95% CI 0.91-12.5]) or blood culture (1.53 [95% CI 0.61-3.84]) , prematurity (2.49[95% CI

0.37-16.87]) , HIV exposure (1.04 [95% CI 0.35-3.1]) and hyponatraemia (1.69 [95% CI 0.52-5.46]) were associated with a non-significant increased risk of expressive language delay (Table 5.8) . Hypothermia on the other hand was associated with a 7% (0.93 [95% CI 0.14-6.19] risk reduction for expressive language delay when adjusted for HIV exposure, positive blood or CSF culture, prematurity and hyponatraemia but this was not statistically significant (95% CI 0.14-6.19).

Table 5.8 Adjusted logistic model of the odds of expressive language delay at 12 months of age

Parameter	OR (95% CI)	*P value
Positive CSF culture	3.37(0.91-12.5)	0.070
Positive blood culture	1.53 (0.61-3.84)	0.360
Hypothermia	0.93 (0.14-6.19)	0.940
Prematurity	2.49 (0.37-16.87)	0.351
Hyponatraemia	1.69 (0.52-5.46)	0.384
HIV exposed	1.04 (0.35-3.1)	0.941
Convulsions	1.12(0.16-7.96)	0.910

***Adjusted logistic regression p value**

5.3.5 Risk factors for poor receptive language outcomes at 12 months of age

In the univariate analysis of risk factors associated with receptive language delay at 1 year of age positive CSF growth was associated with a statistically significant risk of 2.4 –fold (95% CI 1.13-5.1). A positive blood culture was associated with a 1.41- fold increased risk but this was not statistically significant (95% CI 0.68-2.93). Other factors that demonstrated an increased risk of delay though not statistically significant included; HIV exposure 1.87-fold

(95% CI 0.7-4.98), hyponatraemia 1.45 (95% CI 0.44-4.81), hypothermia 1.4-fold (95% CI 0.28-6.98), convulsions 1.85-fold (95% CI 0.45-7.57) and prematurity 1.4-fold (95% CI 0.41-4.79). Anaemia (0.39 [95% CI 0.08-1.92]), hypernatraemia (0.89 [95% CI.16-5.03]), hypoxia (0.9 [95% CI 0.32-2.55]), fever (0.41 [95% CI 0.14-1.16]), thrombocytopenia (0.57 [95% CI 0.11-3.03]), raised CRP (0.42 [95% CI 0.11-1.61]) were all associated with a non-significant risk reduction in receptive language delay (Table 5.9).

Table 5.9 Univariate logistic regression analysis on factors associated with expressive language delay at 12 months of age

Parameter	OR(95% CI)	*P value
Positive blood culture	1.41(0.68-2.93)	0.362
Positive CSF culture	2.4(1.13-5.1)	0.020
HIV exposed	1.87(0.7-4.98)	0.212
Anaemia	0.39(0.08-1.92)	0.251
Hyponatraemia	1.45(0.44-4.81)	0.569
Hypernatraemia	0.89(0.16-5.03)	0.893
Hypoxia	0.9(0.32-2.55)	0.840
Hypothermia	1.4(0.28-6.98)	0.681
Fever	0.41(0.14-1.16)	0.093
Thrombocytopenia	0.57(0.11-3.03)	0.511
Prematurity	1.4(0.41-4.79)	0.590
Raised CRP	0.42(0.11-1.61)	0.210
Early onset disease	0.97(0.3-3.16)	0.970
Convulsions	1.85 (0.45-7.57)	0.536

***Univariate logistic regression p value**

5.3.5.1 Multivariate analysis of risk factors associated with receptive language delay at 1 year of age

Positive CSF culture remained the only significant risk factor with a 2.82-fold risk of receptive language delay (95% CI 1.2-6.73) after being adjusted for hypothermia, prematurity, hyponatraemia and HIV exposure (Table 5.10).

Table 5.10 Adjusted logistic model of the odds of receptive language delay at 12 months of age

Parameter	OR (95% CI)	*P value
Positive CSF culture	2.82 (1.18-6.73)	0.021
Positive blood culture	1.92 (0.72-5.09)	0.198
Hypothermia	2.48 (0.23-26.47)	0.453
Prematurity	1.04 (0.17-6.3)	0.973
Hyponatraemia	1.58 (0.38-6.49)	0.531
HIV exposed	1.92 (0.56-6.58)	0.312
Convulsions	2.26(0.38-13.5)	0.370

***Adjusted logistic regression p value**

5.3.6 Summary of findings

Positive CSF culture, hypothermia and being HIV exposed were associated with an increased risk of gross motor delay at 1 year of age. Positive CSF or blood culture was associated with an increased risk of fine motor delay at 1 year of age. In the neurocognitive domain being HIV exposed was the only identifiable risk factor and a positive CSF culture was the sole significant risk factor in the receptive language domain. There was no significant risk factor

identified in the expressive language domain. Below is table 5.11 with a summary of all the significant findings from the multivariate analysis.

Table 5.11 Summary of significant risk factors in all the domains following multivariate analysis

Neurodevelopmental domain	Risk factor	OR(95% CI)	*P value
Gross motor	Positive CSF culture	2.7(1.22-5.86)	0.014
	Hypothermia	12.5 (1.13- 140)	0.041
	HIV exposed	5.04(1.49-17)	0.009
Fine motor	Positive CSF culture	4.5 (1.48-13.8)	0.008
	Positive blood culture	3.7 (1.27-10.76)	0.021
Neurocognitive	HIV exposed	2.92 (1.12-7.64)	0.031
Receptive language	Positive CSF culture	2.82(1.18-6.73)	0.020
Expressive language	Nil		

***Adjusted logistic regression p value**

5.4 Discussion

In this study being born to an HIV infected mother was associated with an increased risk of gross motor and neurocognitive delay at 1 year of age. HIV exposed non-infected children have been shown to have developmental delay in other poor settings whereas in the developed world they have been shown to have normal neurodevelopment {Afran L. M., Garcia Knight et al (2014), Le Doare K, R. Bland et al (2012), Chase C, J. Ware et al (2000), Levenson R.L jr. C.A., Mellins (1992) }. A number of factors could possibly lead to the increased risk of developmental delay in these HIV exposed un-infected infants. There is a possibility that the mothers could be unwell themselves either physically or mentally which may impact on their ability to care for and stimulate their infants {Le Doare K, R. Bland et al (2012)}. In this study mothers to the participants were not screened for depression and HIV disease staging was not done and impact from these factors could not be measured. There are added influences, such as poverty, early infant malnutrition and growth that combine to give a more complicated picture of developmental challenges in this environment {Le Doare K, R. Bland et al (2012), Seale A.C, M Mwaniki et al (2009), Osrin D, S. Vergnano et al (2004)}. The majority of the families in this study were poor (chapter 4). It is therefore not surprising that in this study being born to an HIV infected mother was independently associated with developmental delay. It is important to note that even if Malawi has made strides in PMTCT, there has been an increase in the rate of new HIV infections amongst adolescents {UNICEF Malawi report 2013}. It is also known that a significant proportion of pregnant women in Malawi are adolescents {Malawi DHS 2010}. This will likely have an impact on morbidity outcomes in infants as the number of HIV exposed infants is likely to increase. Efforts should

not just be directed at PMTCT but prevention of new infections in all age groups otherwise the gains from PMTCT might be diluted.

Significant growth in blood culture was also independently associated with fine motor delay in the severe neonatal infection cases. This finding is in keeping with other studies in preterm and low birth weight infants with neonatal sepsis { Shah D.K, L.W., Doyle et al (2008), Adams-Chapman I, B.J., Stoll et all (2006), Stoll B.J., N. Hansen et al (2002), Hack M.D, D. Wilson-Costello et al (2000), Stoll B.J, T. Gordon et al (1996}. It has been postulated that in neonatal infection cases the presence of a bacteraemia is probably associated with a systemic inflammatory response resulting into cytokine and free radical activation. This would subsequently cause white matter damage eventually leading to developmental delay { Volpe J.J(2008), Shah D.K, L.W. Doyle et al (2008), Volpe J. J,(2003), Duncan J.R, M.L Cock (2002)}. Blood culture positivity rate has been reported to be low in neonatal sepsis {Seale A.C, M Mwaniki et al (2009), Osrin D, S. Vergnano et al (2004), Mishra U.K, S.E. Jacobs et al (2006) Claudio C., A Panero et al (2004),}. In this study the culture positivity rate was 11% (chapter 3).It is therefore important to try and explore affordable ways of increasing the yield from cultures as positive cultures are associated with poor developmental outcomes. The molecular diagnostics available in the developed settings can improve the yield but are too expensive for a developing setting like Malawi.

Neonatal meningitis with a significant growth in CSF has also been shown to be independently associated with developmental delay in these participants. This again is in keeping with other studies { Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M. Eames

et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}.

5.5 Limitations of the study

The major challenge in this study was the small sample size of 102. It was challenging to do most of the sub group analysis as the numbers were further reduced. The small sample size could explain the wide confidence intervals observed in this study.

In view of the high prevalence of developmental delay observed in this study further work is needed to better understand the risk factors associated with poor outcomes in these patients.

5.6 Conclusion

Positive blood culture, Neonatal meningitis with a positive CSF culture and being born to an HIV infected mother were predictors of poor developmental outcomes at 1 year of age in this study.

Severe neonatal infection cases that are HIV exposed or have a positive blood or CSF culture need to be screened for developmental delay in the first year of life. The burden arising from these infections is large and therefore measures that are known to prevent neonatal infections should be undertaken so as to avoid the complications. There is need to invest in better antenatal, antepartum and postnatal care all of which have been shown to improve neonatal outcomes.

HIV exposure as shown in other studies remains a risk of poor developmental outcomes in childhood. More needs to be done to try and reduce the prevalence of HIV in the general population of Malawi.

CHAPTER SIX

6. A CASE SERIES OF MRI BRAIN FINDINGS IN NEONATAL SEPSIS AND MENINGITIS AT QECH, MALAWI.

6.1 Introduction

Although the overall numbers are relatively small when compared to meningitis in older children, the greatest risk of acquiring meningitis is in the neonatal period {Heath P.T, N.K., Nik Yusoff et al (2003), Stevens J.P., M. Eames et al (2003), Bedford H, J. Delouvois et al (2001), Holt D.E, Halket et al (2001), Delouvois J., T. Blackburn et al (2001)}. The effects of infection on the rapidly developing brain, which continually evolves in its susceptibility to damage, and in the context of a rapidly evolving immune system, lead to complex patterns of pathology that are different from those seen in an older child affected by a similar infectious agent {Wintermark P.(2011)}. In the previous chapters (chapter 3 and 4), it has been shown that the mortality associated with neonatal meningitis presenting to QECH is 20% and amongst those that survive up to 60% are impaired at 1 year follow-up. Even in the developed setting, up to 50% of neonatal meningitis cases are known to have complications {Heath P.T, N.K., Nik Yusoff et al (2003)}.

In older children in particular, neuroimaging has been used to identify and monitor structural focal lesions, hydrocephalus, subdural effusion, empyema and ventriculitis some of which require prompt neurosurgical intervention {Jarenko J.L A.S Moon (2010), Srinivasan L. and M.A Rutherford (2008), Maalouf E.F, P.J. Duggan (1999), Levene M.I, A Whitelow (1982)}. Differences have been reported in the imaging and pathologic appearances

amongst the various infectious agents causing meningitis in a neonate {Wintermark P. (2011), Jarenko J.L A.S Moon et al (2010)}. For example, neonatal meningitis due to *Citrobacter koseri* and *Enterobacter sakazakii* is associated with the development of brain abscesses {de Vries L.S., Verboon-Maciolek M.A et al (2006), Kline M.W (1988), Willis J., J.E., Robinson et al (1988), Graham D.R, J.D. Band et al (1981)}. Therefore to better understand the pathology associated with the high mortality and morbidity associated with meningitis in Malawi, the neuroimaging data available from this cohort was reviewed.

Non-invasive trans-fontanelle ultrasonography is available at QECH but although able to provide information on ventricular size and presence of haemorrhage, ultrasonography is heavily operator-dependent and experience is needed to demonstrate the meningeal and parenchymal findings of bacterial meningitis {de Vries L.S., Verboon-Maciolek M.A (2006)}. Neonates in this cohort were not systematically scanned and the data was not recorded. Until recently, CT scans of the brain were available at QECH but although useful in detecting cerebral abscesses, cerebritis, effusions, hydrocephalus and encephalomalacia {Kanamalla U.S, R.A Ibarra et al (2000), Karampekios S, J.Hesselink et al (2005), Castillo M (2005), Harris T.M, Edwards M.K et al (1991)}, the quality of the images at QECH was poor and none of the cohort underwent CT imaging. Fortunately, as part of an ongoing collaborative severe malaria study between Blantyre Malaria Project (Michigan State University) and MLW, a MRI facility (permanent magnet-based Signa Ovation 0.35-T MR scanner, GE Healthcare, Waukesha, WI, USA) was established at QECH which is also available for routine clinical care. This is the only such facility in Malawi and one of the few MR scanners in the Region. Even in industrialised countries, neonates with meningitis are not routinely imaged. MRI is thought

to be superior to CT scans in that it can better detect leptomeningial enhancement and distension of the subarachnoid space with widening of the interhemispheric fissure {Jarenko J.L, A.S Moon (2010), Srinivasan L. and M.A Rutherford (2008), Karampekios S, J.Hesselink et al (2005), Castillo M (2005), Kanamalla U.S, R.A Ibarra et al (2000), Maalouf E.F, P.J. Duggan (1999), Levene M.I, A Whitelow (1982) }.

This chapter therefore reports 5 cases of neonatal meningitis and 2 cases of neonatal sepsis enrolled in the study where MRI scans of the brain were performed as part of routine clinical care.

6.2 Methodology

The recruitment and outcome of this neonatal meningitis and sepsis cohort is described in chapter 2. Brain MRI was done in 5 cases of meningitis and 2 of sepsis as part of routine clinical care, where there were repeated seizures for at least 2 days so as to rule out complications of meningitis. The MRI scans were done on the 0.375T magnet using multiple planes and conventional T1 and T2 weighted images were included. Sagittal T1 FLAIR (fluid attenuated inversion recovery), axial T2 FRFSE, axial T2 FLAIR, axial DWI (diffusion weighted images) and coronal T2 FRFSE sequences were taken. The scans were re-reviewed retrospectively with a specialist neuroradiologist (Dr S Kampondeni). None of the MRIs were done with contrast enhancement (unavailable in the government sector). Additional scans as part of this research study were not possible for financial reasons.

6.3 Case Reports

There were 4 boys and 3 girls who had MRI scans of the brain (age range 6 to 56 days old). All the MRI scans except one were done after 72 hours of inpatient admission.

Case 1

A 13 day old girl presented to the QECH with a day's history of fever, irritability difficulties with breathing and poor feeding. She was born at term to an HIV negative mother at an urban health centre but had a low birth weight of 2.4kg. The delivery was uneventful. On arrival to QECH she was fully conscious and well perfused, had a sunken fontanelle, a pulse rate of 168 beats per minute, temperature of 36.6^{o}C and respiratory rate of 64 cycles per minute. The peripheral white cell count was 1.6 X10^{9}/L (differential not done), haemoglobin of 10.4g/dl and platelets of 228 X10^{9}/L. She had normal serum electrolytes but the CRP was elevated to 58mg/L. Her CSF was xanthrochromic with a white cell count of 12/mm^{3} and protein of 2.58mg/dl. The CSF culture grew group B streptococcus which was sensitive to penicillin and ceftriaxone. There was no growth on the blood culture. She was treated with parenteral ceftriaxone. She rapidly deteriorated over the next 24 hours with repeated generalised seizures lasting at least twenty minutes that spanned the first 2 days of admission. She required IV phenytoin infusions to control the seizures. EEG was done on day 2 of admission at the MLW/ BMP Paediatric Research Ward facility that showed focal epileptiform activity on the left. An MRI brain was done on day 2 of admission and showed diffusion abnormality along the cerebral cortices sparing the right parietal region. There was diffuse swelling in the cerebral hemispheres (see fig 6.1). At the age of 1 year this girl had delayed cognitive and fine motor development and had bilateral hearing loss. She had no reported seizures at this age.

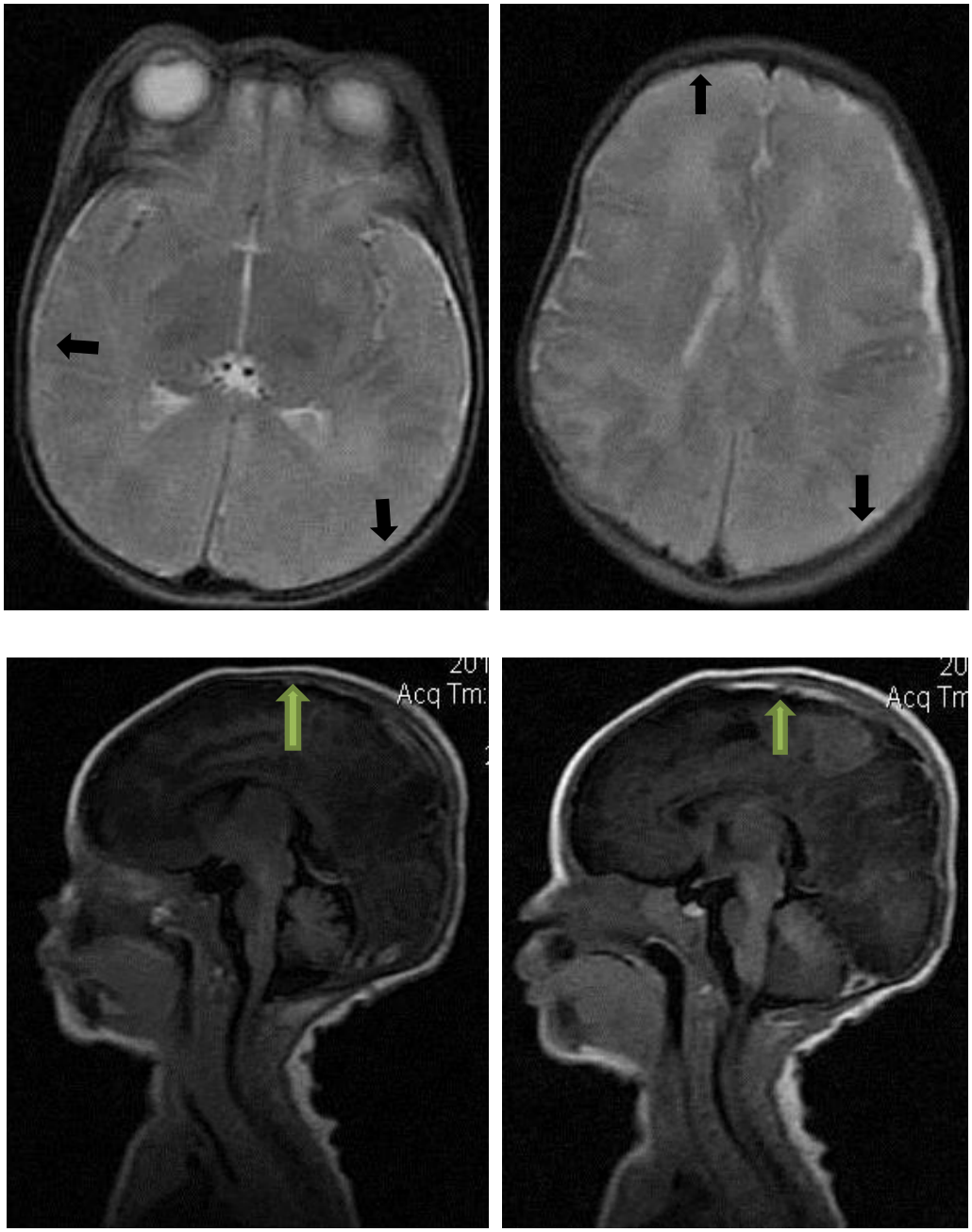


Fig 6.1 Axial and Sagittal Brain MRI images of a 13 day old girl with GBS meningitis showing loss of sulci as a result of brain oedema

Case 2

A 6 day old HIV unexposed boy born at home at term with no complications during delivery presented to QECH with a day's history of fever, poor feeding and irritability. He was lethargic on arrival, tachycardic (pulse rate of 180/min) and febrile (temperature of 40.5^{o}C.). His respiratory rate was 44cycles /minute and had an oxygen saturation of 97% on room air. He had a generalised convulsion on arrival. He had a peripheral white cell count of 14.4 X10^{9}/L, haemoglobin of 10.4g/dl and platelets of 199 X10^{9}/L. He had a raised CRP of 127mg/dl. His CSF was hazy with a raised white cell count of 400/ mm^{3} of which 65% were polymorphs and 35% lymphocytes. CSF protein was 2.16mg/dl and CSF glucose 5.4mmol/dl. The blood glucose at the time of the lumbar puncture was 10.2mmols/dl. There was no significant growth from either the CSF and blood culture. She had repeated focal and generalized seizures on the ward that were difficult to control with parenteral phenytoin. An MRI brain was done on day 11 of admission which showed diffuse heterogeneous high T2 signal and abnormal architecture of the cerebral hemispheres. There was high T2 signal in the basal ganglia (see fig 6.2).

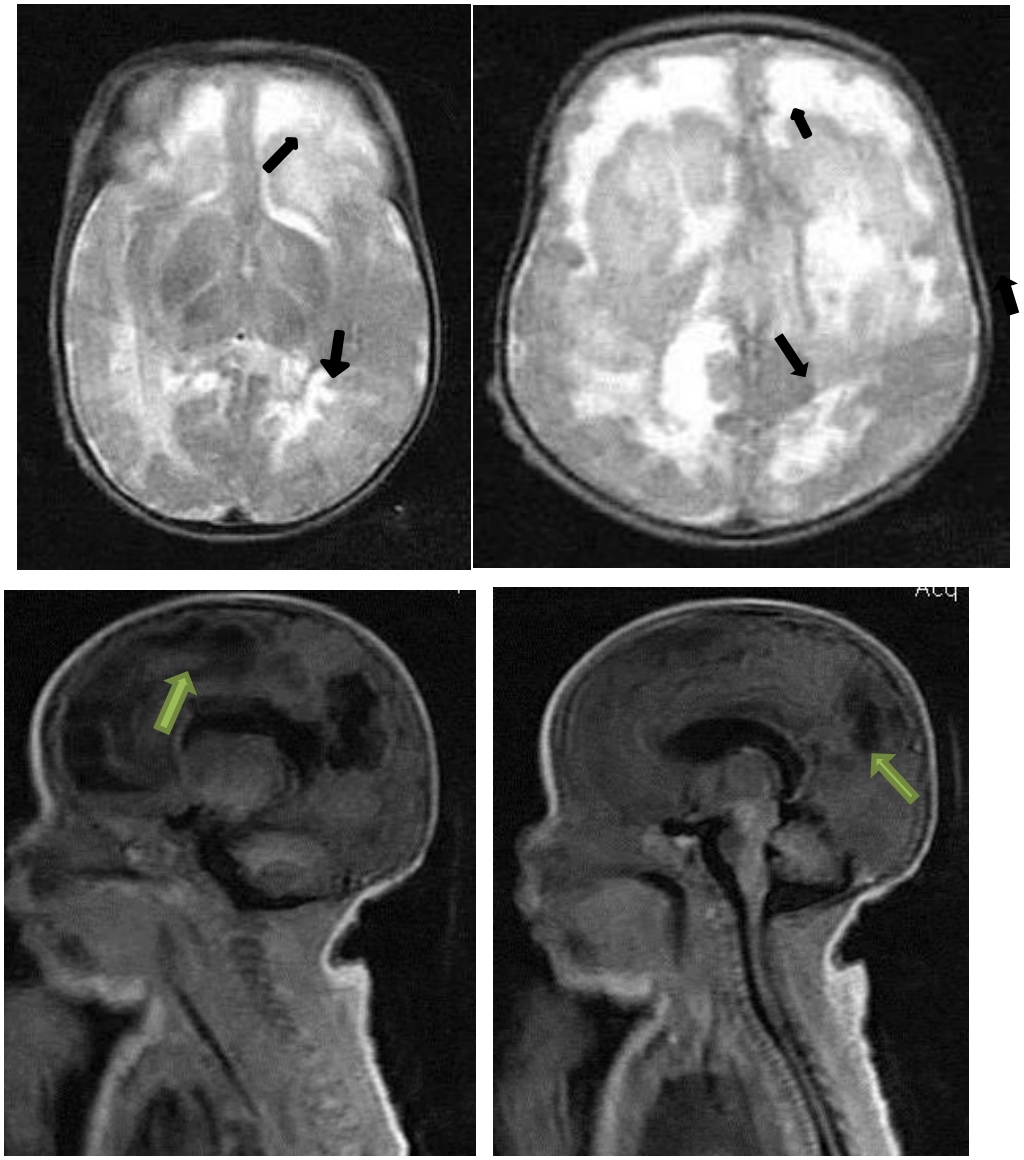
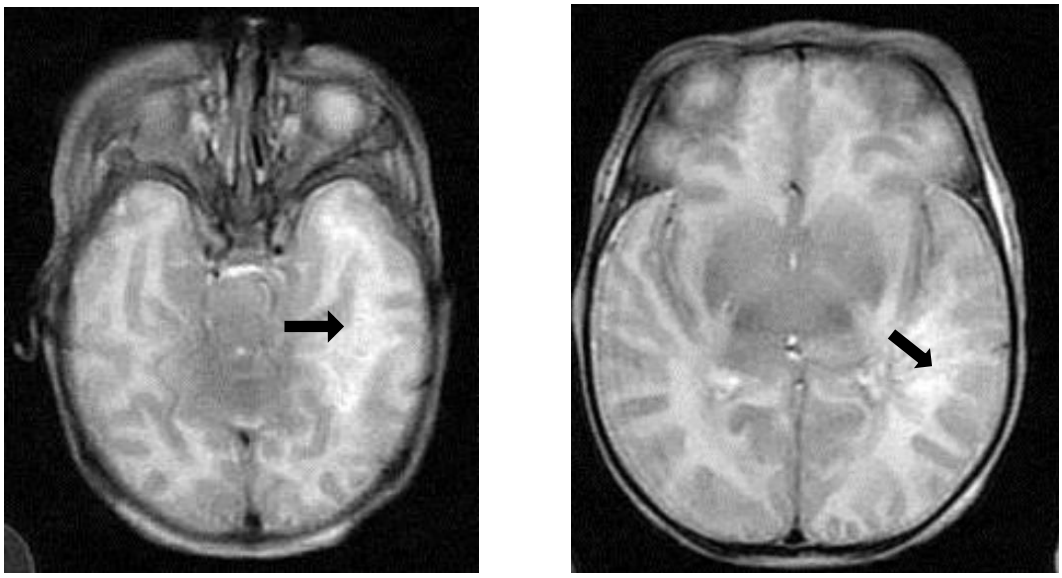


Fig 6.2 Axial and Sagittal Brain MRI Images of a 6 day old boy with bacterial meningitis the arrows are showing abnormal architecture of the cerebral hemisphere.

At the age of 1 year the boy had gross global developmental delay in all the domains, bilateral hearing loss and had developed epilepsy.

Case 3

An 18 day old boy born at an urban health centre at term uneventful delivery to an HIV negative mother presented to QECH with poor feeding and difficulties with breathing for 4 days. On arrival the infant was lethargic, floppy, hypothermic with a temperature of 35^0C, bradycardia (pulse rate of 74 beats per minute), hypoxic (oxygen saturation of 79% in room air) and was having apnoeas. He was noted to have lost 22% of his birth weight. He had a peripheral white cell count of 14.4 $X10^9$/L with 37% neutrophils, 59% lymphocytes and 3% monocytes. His platelet count was 75 $X10^9$/L. He was hypernatraemic with sodium of 179 mmol/L, urea of 77.8 mmol/L, potassium of 5 mmol/L and chloride of 134 mmol/L. His CSF was clear with no cells and had normal protein and glucose, blood and CSF cultures were sterile. He was initially treated with high dose parenteral penicillin and gentamicin but was switched to parenteral ceftriaxone after 24 hours. This boy had repeated focal seizures over 6 days. On day 6 of admission an MRI brain was done which showed prominent gyri and high T2 in the left temporal lobe suggesting ischemia (see Fig 6.3).



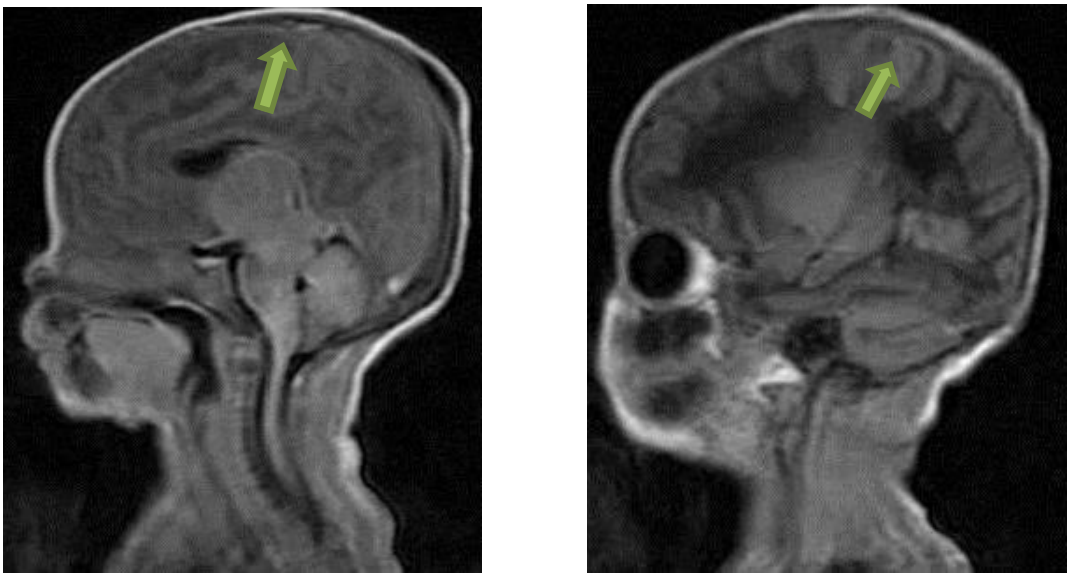


Fig 6.3 Axial and Sagittal Brain MRI images of an 18 day old boy with neonatal sepsis the arrows are showing left temporal lobe ischaemia- top axial images and prominent gyri in the sagittal images.

At 1 year of age he had a left sided convergent squint and had mild delay in language and cognitive development. He had normal hearing at 1 year of age and was seizure free.

Case 4

A 36 day old girl born at term via caesarean section at QECH to an HIV negative mother presented from home with a 2 day history of fever, jaundice, difficulties in breathing and vomiting. She had generalised convulsions on the day of admission. She was unconscious on arrival and had epistaxis. She had a pulse rate of 144 beats per minute, respiratory rate of 44 cycles per minute, temperature of 36.9^0C and oxygen saturation of 99%. She had a bulging fontanelle and was hypertonic. She had a hepatomegaly of 5 cm below the costal margin. She had a peripheral white cell count of 34.8 $X10^9$/L of which 79% were neutrophils, haemoglobin of 5.6g/dl and platelet count of 430 $X10^9$/L. She had a serum total bilirubin of

12.9 mg/dl. Her CSF had a white cell count of 10/mm^3, protein of 1.43mg/dl and glucose of 1.39mmols/l. The random blood sugar at the time of the lumbar puncture was 5.3mmol/l. The CSF and blood cultures were sterile. She had repeated generalized convulsions for 4 days which were controlled with parenteral phenobarbitone and phenytoin. An MRI was done on day 20 of admission which showed a diffuse high T2 signal throughout the left cerebral hemisphere (Fig 6.4). There was an 11mm wide left occipital subdural effusion exerting mass effect on the occipital lobe. A smaller 6mm left cerebellar subdural effusion was also noted. There was mild communicating hydrocephalus. A porencephalic cyst was noted in the right parietal region connecting with the right trigone. There was high T2 and DWI signal in the right frontal lobe, suggesting infarction. She was treated with ceftriaxone for the presumed meningitis. She had gross global developmental delay at 6 months of age and bilateral hearing loss. There were no reported seizures at home. She died at home at 8 months of age of unknown cause.

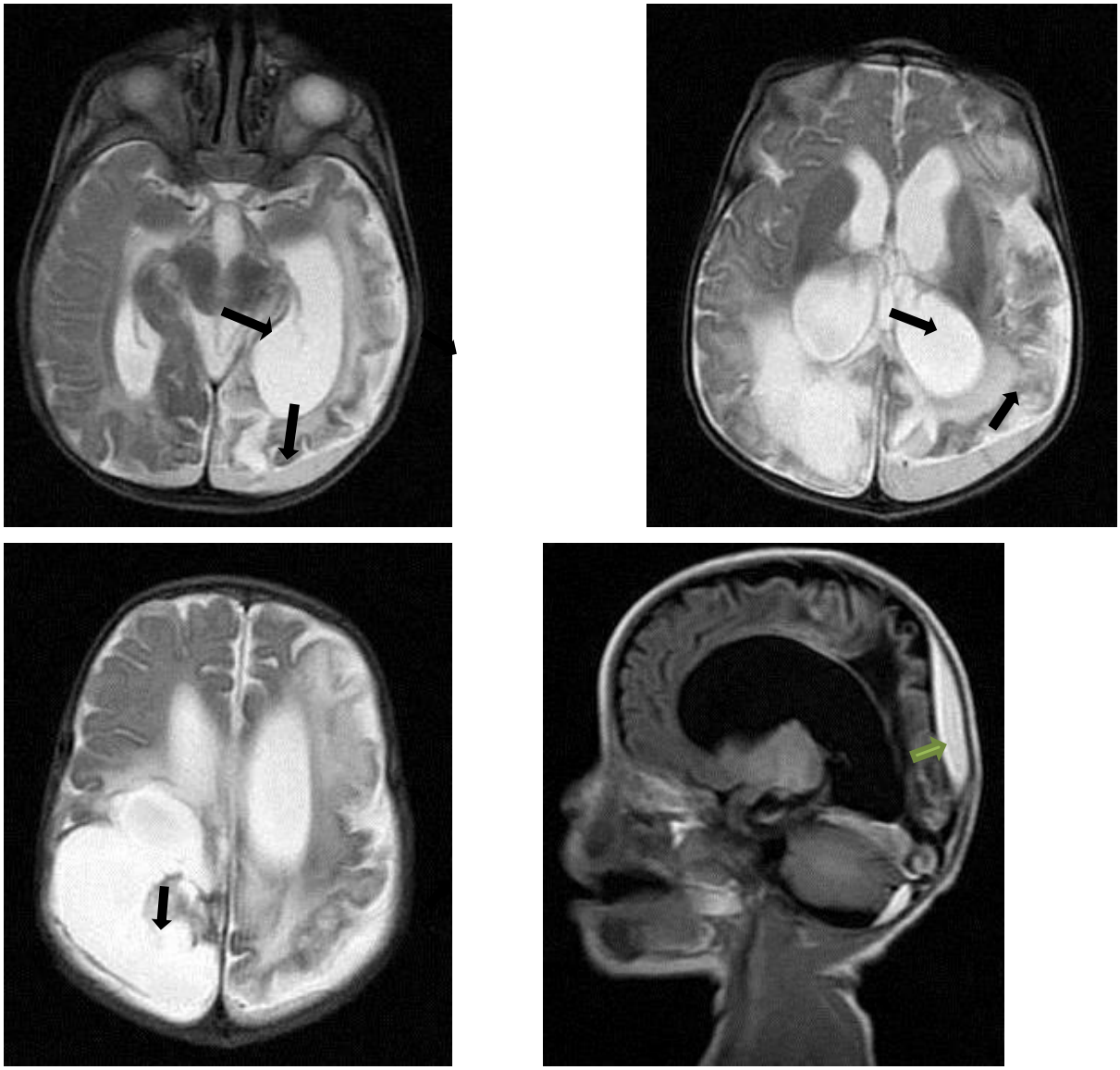


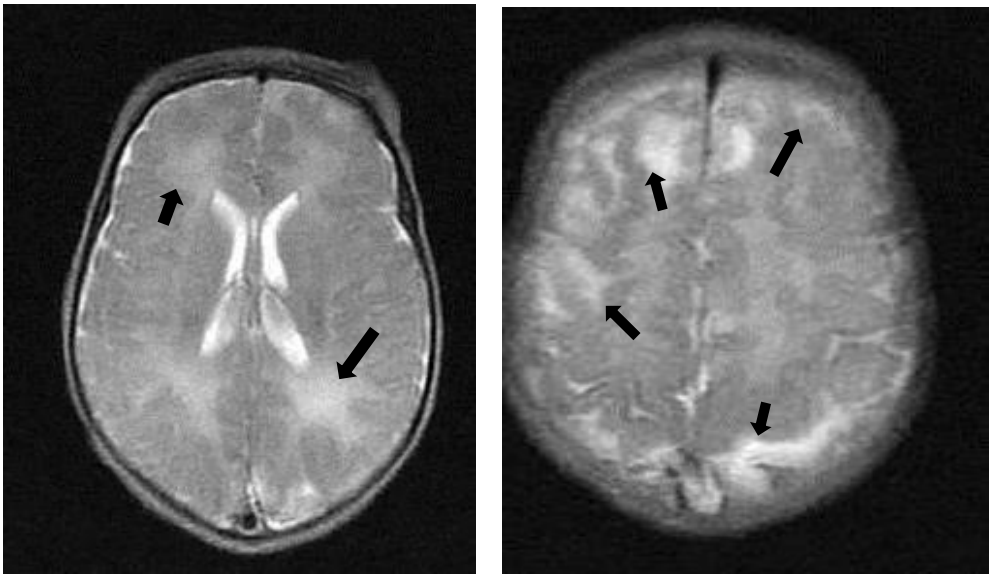
Fig 6.4 Axial and Sagittal Brain MRI images of a 36 day old girl with presumed meningitis showing multiple complications of meningitis- Right Anterior Cerebral Artery infarct, subdural effusion, communicating hydrocephalus, porencephalic cyst and left cerebral gliosis

Case 5

A 23 day old HIV non-exposed girl born at term at an urban health centre, HIV non-exposed presented to QECH with a day's history of fever, difficulties in breathing and irritability. On

arrival she was hypoxic (oxygen saturation 88% on room air), unconscious, tachycardic (pulse rate of 193/minute), febrile with a temperature of 39.5 ^{0}C and had a bulging fontanelle. She had a peripheral white cell count of 4.9 X10^{9}/L with a predominance of lymphocytes 80.8%, haemoglobin was 14.2g/dl and the platelet count was 237 X10^{9}/L. Her electrolytes were normal. The CSF and blood culture both grew Group B streptococcus. She was treated with parenteral ceftriaxone. She had recurrent generalised and focal seizures the first 6 days of admission and an MRI scan of the brain was done on day 5 of admission. The MRI scan showed diffuse cortical high T2 and high DWI signal in the right frontal lobe and both occipital lobes (see Fig 6.5).

She was seen at 6 and 12 months of age and had normal development. Her hearing was normal and was seizure free.



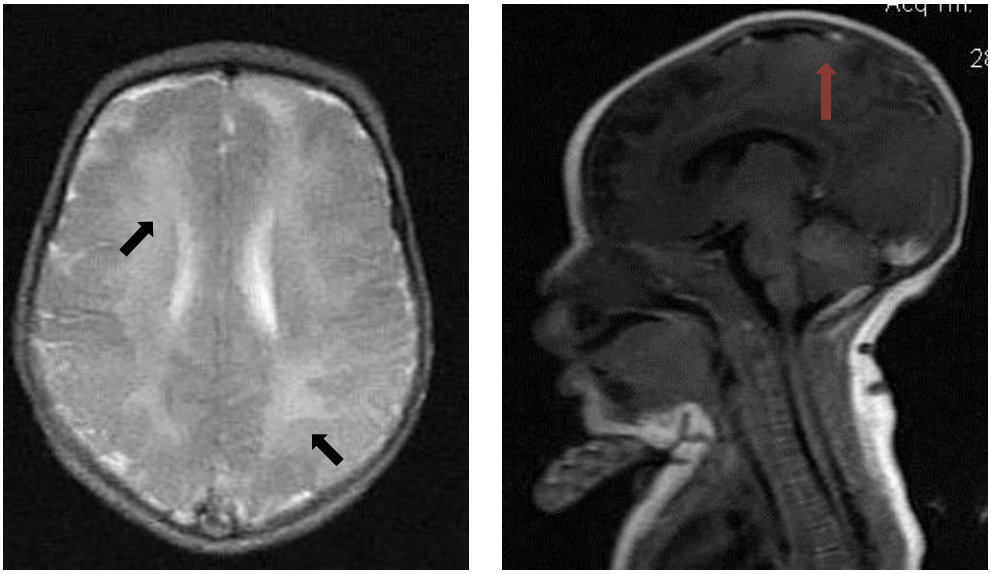


Fig 6.5: Axial and Sagittal MRI brain images of a 23 day old girl with GBS meningitis arrows showing diffuse cortical high T2 signal in the right frontal and Occipital lobes

Case 6

A 56 days old boy HIV exposed born at term at an urban health centre presented to QECH with a 3 days history of cough and a day's history of vomiting, irritability and difficulties in breathing. On arrival he was in shock with a prolonged capillary refill time (no.) and tachycardic (pulse rate of 196 beats/minute). He was tachypnoeic (respiratory rate of 82 cycles/minute) but had normal oxygen saturations of 94% and had oral thrush. His peripheral white cell count was 22 $X10^9$/L which was 62% neutrophils, haemoglobin of 7.7g/dl and platelet count of 227 $X10^9$/L. His urea and electrolytes were normal and she had a CRP count of 15.3 mg/dl. He had a negative HIV DNA PCR at 2 months of age. His CSF had no white cells and the protein was 0.88 mg/dl and glucose of 12.33mmol/l. A random blood sugar was not done at the time of the lumbar puncture. The CSF and blood cultures were sterile. He had repeated focal seizures for the first 6 days of admission. He was treated with parenteral benzyl penicillin and gentamicin and the seizures were controlled with parenteral

phenobarbitone and phenytoin. On day 7 of admission he had an MRI brain done which was normal (Fig 6.6).

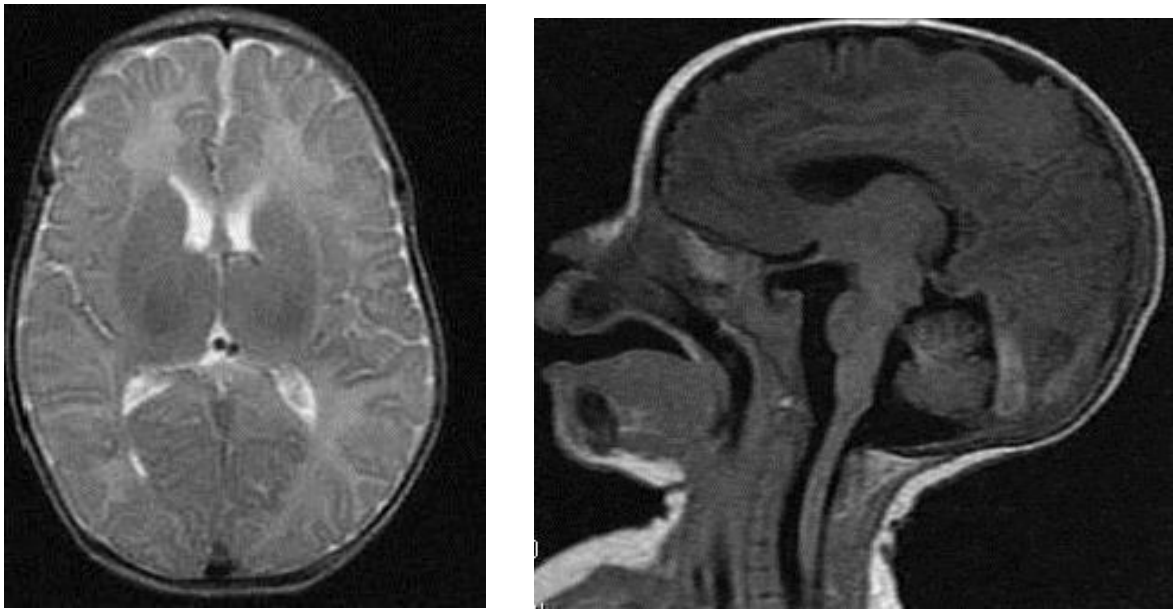


Fig 6.6 Normal axial and sagittal MRI Images of the brain of a 56 day old boy with neonatal sepsis

He was seen at 6 and 12 months of age seizure free with normal development.

Case 7

A 33 day old boy born at term at an urban health centre from an HIV negative mother presented with a 2 days history of a fever and a day's history of poor feeding and difficulties with breathing. She had focal seizures on the day of presentation. On arrival he was afebrile with a temperature of 37.2^0C, tachypnoeic (respiratory rate of 68cycles/minute), had a pulse rate of 171 beats per minute and had normal oxygen saturations of 92%. He was hypotonic with a bulging fontanelle. His peripheral white cell count was 17.8 $X10^9$/L, haemoglobin of 9.6g/dl and a platelet count of 910 $X10^9$/L. His urea and electrolytes were normal but he had a raised CRP of 215 mg/dl. The CSF consisted of pus and so a cell count could not be given,

however there was no growth from both blood and CSF culture. He had repeated focal seizures for the first 3 days of admission which were controlled with parenteral phenobarbitone and phenytoin. He remained in coma over the first 3 days of admission. He was treated with parenteral ceftriaxone for the meningitis. He had an MRI scan of the brain on day 3 of admission which showed severe diffuse oedema of the cerebral hemispheres with loss of grey/white definition and total effacement of sulcal markings. The cerebellum was also diffusely swollen see Fig 6.7 below.

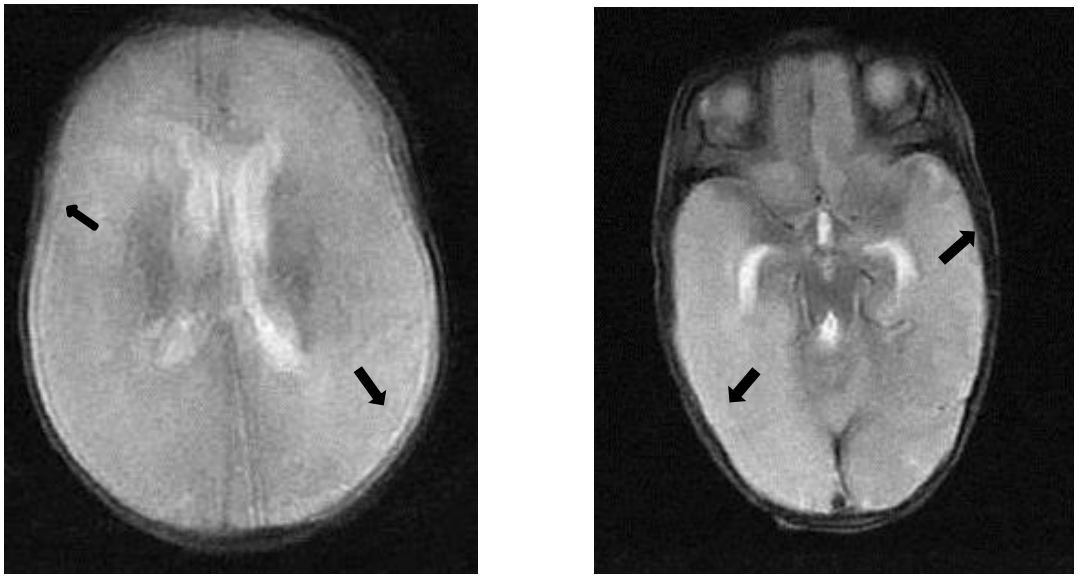


Fig 6.7 Axial MRI brain images of a 33 day old boy with meningitis showing massive brain swelling.

He was seen at 6 months of age seizure free but had bilateral hearing loss and gross global developmental delay.

Table 6.1 Summary of MRI brain findings in neonates with meningitis and sepsis

Age (days)	Diagnosis	MRI findings	Hearing Loss	Developmental Delay	Epilepsy
13	GBS meningitis	Brain oedema	Yes	Yes	No
6	Meningitis	Abnormal cerebral architecture	Yes	Yes	Yes
18	Neonatal sepsis	Temporal lobe ischaemia	No	Yes	No
36	Meningitis	Hydrocephalus, Infarcts, subdural effusions, porencephalic cyst and cerebral gliosis	Yes	Yes	No
23	GBS meningitis	Brain oedema	No	No	No
56	Neonatal sepsis	Normal	No	No	No
33	Meningitis	Brain oedema	Yes	Yes	No

6.4 Discussion

This MRI brain case series describes the extent of complications caused by neonatal meningitis and sepsis. The abnormalities described in these series included brain oedema, abnormal architecture of the cerebral hemispheres, ischaemia, subdural effusion, hydrocephalus, porencephalic cyst and infarction are in keeping with what has been reported in other studies { Jarenko J.L A.S Moon (2010), Srinivasan L. and M.A Rutherford (2008), Kanamalla U.S, R.A Ibarra et al (2000), Karampekios S, J.Hesselink et al (2005), Castillo M. (2005), Harris T.M, Edwards M.K et al (1991)Maalouf E.F, P.J. Duggan (1999), Levene M.I, A Whitelow (1982) }.

In this case series the infant that had temporal lobe ischaemia was a neonatal sepsis case without overt meningitis. We postulate that this 18 day old boy could have had the ischaemia as a result of venous thrombosis secondary to severe dehydration. He had lost 22% of his birth weight and his urea was high on admission 77.8mmols/l. He also had high sodium of 179mmols/l which could have led to the seizures. There are no similar reported cases of neonatal sepsis without overt meningitis with similar findings in the literature.

Because of the logistical challenges, all these infants did not have follow up MRI scans so as to ascertain whether the abnormalities had resolved.

MRI scans are not without risk in children particularly from sedation. All the infants that had MRI scans in this series were critically ill with difficult to control seizures. These infants were on either phenytoin or phenobarbitone for seizure control and these anticonvulsants are known to cause respiratory depression {Wintermark P. (2011)}. The risk of hypoxia or even

apnoea was therefore high in these patients however the benefits of ruling out a potentially amenable surgical cause like a cerebral abscess was judged to outweigh the risk. These infants were being accompanied by a nurse and a medical officer who monitored their vital signs throughout the scanning period.

It was clear from these few cases that were done that MRI has a potentially significant role in the understanding and management of neonatal meningitis. In 4 out of the 7 cases with persistent seizures their CSF parameters on admission were normal with negative cultures after 48 hours. Repeat CSF in all of them at 48 hours was sterile. However the MRI brain findings were strongly suggestive of brain pathology.

Based on this small sample, a larger study is required to fully understand the pathological basis of the poor outcome that was observed

6.5 Limitations

We were not able to do MRI scans in all the participants who had the standard indications namely; poor clinical response after 48 hours of treatment, seizures and lack of CSF sterility after 48 hours of antibiotic administration due to lack of resources. Therefore this was a highly selected group. All the scans were not enhanced by contrast and they were done on different days of admission ranging from day 2 to day 20 of admission. This could have affected the type of abnormalities seen on the scans. Routine contrast- enhanced brain MRI is known to be the most sensitive modality particularly early on in the disease {Wintermark P. (2011), Srinivasan L. and M.A Rutherford (2008), de Vries L.S, M.A Verboon-Maciolek (2006)}. It has been shown that non-enhanced MRI studies performed in patients with uncomplicated meningitis may appear to be normal but enhanced studies in the same

patient can show abnormalities { Wintermark P. (2011), Srinivasan L. and M.A Rutherford (2008), de Vries L.S, M.A Verboon-Maciolek (2006)}. Most of the patients that had MRI scans done 86% (6 out of 7 participants) did show abnormalities on their scans. However there is a possibility that subtle abnormalities might have been missed in the non-enhanced films.

The MRI scanner used on these participants was a 0.375 magnet whereas today, most centres of excellence are using 1.5-3 magnet size MRI scanners. The machine used in this study has a much smaller magnet than standard more modern machines and that could have affected the quality of the images. However, these machines require a level of servicing and consumable supply (liquid nitrogen) not available in Malawi.

6.6 Conclusion

This case series has demonstrated a wide range of complications that arise in neonatal meningitis and sepsis. The findings underscore the importance of MRI scan of the brain in the diagnosis and management of severe neonatal infections. MRI scans are however expensive and out of reach in most resource restrained settings and as such cheaper modalities of neuroimaging like ultra sound scans should be explored in such settings.

CHAPTER SEVEN

7. DISCUSSION

This thesis set out to examine the aetiology and outcome of neonatal sepsis in Malawi, a country where the neonatal mortality rate has remained static over the past two decades {Zimba, E., M.V Kinney et al. (2012). Neonatal sepsis has been reported as a cause of nearly 30% of all neonatal deaths in Malawi {Liu L, H.L. Johnson et al. (2012)}. It has been shown from this thesis that group B streptococci and *Streptococcus pneumoniae* are important causes of neonatal sepsis and meningitis in Malawi (40% of all organisms grown in neonatal blood culture and 48% in CSF culture were group B streptococcus, 12% and 30% were *S. pneumoniae* respectively). This finding has further strengthened the evidence showing the importance of GBS as a cause of neonatal sepsis and meningitis in Malawi and probably in similar countries around sub-Saharan Africa. This thesis has also shown the poor long term outcome associated with neonatal sepsis and meningitis. Importantly, the data show that not only is neonatal meningitis associated with poor neurodevelopmental outcome but also neonatal sepsis without evidence of CNS inflammation on lumbar puncture also poses an increased risk of neurodevelopmental delay.

The thesis literature review started by describing the global epidemiology of neonatal mortality with the developing setting contributing 99% of all neonatal deaths {Zupan, J and Aahman E., (2005) } and Sub-Saharan Africa contributing 30% of all global neonatal deaths {World Health report 2013}. The top 3 causes of neonatal mortality remain prematurity, birth asphyxia and neonatal sepsis {Lawn, J., A., Lee et al (2009)}. It was noted that the

distribution of the causes of neonatal deaths varied between countries, with infections causing most of the neonatal deaths in very high mortality settings like the Sub Saharan region { Lawn, J., A., Lee et al (2009), Lawn, J., S.,Cousens et al (2005)}. It was also noted that although there has been tremendous progress in the reduction of neonatal mortality in the developed setting since the 1970's, there has been minimal progress in the developing setting { Lawn, J., A., Lee et al (2009), Rajaratnam, J., J.,Marcus et al (2008), Lawn, J., S.,Cousens et al (2005)}. These improvements in neonatal mortality have been largely due to changes in obstetric care, maternal health, introduction of neonatology and better infant nutrition{ Lawn, J., S.,Cousens et al (2005), Child Health Research Project Special Report (1999) }. It is therefore imperative that all efforts should be made to try and accelerate the reduction of neonatal mortality in the developing setting.

The literature review then described the aetiology of neonatal sepsis in both the developed and developing settings with GBS being the commonest cause of neonatal sepsis in the developed setting and based on studies of varying quality, gram negative organisms being the commonest cause in the developing world{Zaidi A. K, D. Thaver et al (2009), Vergnano, S., P.T Heath et al (2005)}. It was postulated that the difference in bacterial aetiology was as a result of more unhygienic home deliveries in the developing setting that exposes the newborn to infections by gram negative organisms {Vergnano, S., P.T Heath et al (2005)}. This is in sharp contrast to the developed setting where almost all the deliveries are conducted by a skilled birth attendant in a hospital environment. The thesis also looked at the carriage of GBS by pregnant women in both the developed and developing settings concluding that the carriage rates are similar between these 2 settings – up to 30%{ Gray K.J, G. Kafulafula et al (2011)}. It would be expected therefore that GBS should also be a

significant pathogen in developing countries. The fewer cases of GBS seen in the developing settings raises questions on the quality of data reported from these settings{ Vergnano, S., P.T Heath et al (2005)} as robust microbiology culture facilities are often lacking . There has been a decline in the burden of GBS disease in many developed countries as a result of active screening of pregnant women and properly treating those known to carry GBS with antibiotics in labour{Schrag S.J and A Schuchat (2004)}. However screening of pregnant women for GBS is routinely not done in most developing countries. There are prospects of a GBS vaccine given to pregnant women so as to prevent disease in the newborn {Clinical trials.gov}. The vaccine would further reduce the burden of neonatal GBS disease and would be easier to implement in a developing setting if the price was within reach for most countries.

The long term outcome of neonatal sepsis and meningitis in both the developed and developing settings was also reviewed. There has been a decline in mortality from neonatal meningitis in the developed setting but the progress in the developing setting has been slow{Heath P.T, O.I Okike et al (2011), Puopolo K. M., L.C Madoff et al (2005), Heath P.T, N.K. Yusoff (2003)}. However the major challenge has been lack of progress on the burden of complications as a result of neonatal meningitis with up to 50% of the survivors ending up with sequelae {de Louvois J,S. Halket et al (2007), Krebs VLJ, G.A.M Costa et al (2007), Stevens JP, M.Earmes et al (2003), Bedford H, J. de Louvois et al (2001) } . The thesis has gone further to review the reported long term outcome of neonatal sepsis particularly in preterm infants { Adams-Chapman I (2012), Schlapbach L. J., M. Aebischer (2011), Stoll B. J, Hansen N.I (2004) }. In these studies neonatal sepsis has been shown to increase the risk of neurological complications in surviving preterm babies. However, there is paucity of data on

long term neurological outcomes of neonatal sepsis in term babies from both the developing and developed settings. Understanding the neurological outcomes in neonatal sepsis cases can help in the acute management and follow-up of these cases. The potential long-term burden of these surving but affected children on socities is potentially considerable.

7.1 Review of the methodologies employed

To undertake a study of this nature, it is critically important that the appropriate clinical, developmental and laboratory methods are employed to ensure that this approach can be duplicated in other settings and that the data generated is generalisable. In the development of the methods, there was a focus on three main areas: clinical definitions; laboratory analysis and neurodevlopment assessment.

7.1.1 Clinical Methods

In most developing settings the diagnosis of neonatal sepsis is mainly based on clinical signs and symptoms as laboratory confirmation for neonatal sepsis and meningitis are frequently unavailable. A clinical definition of neonatal sepsis was based on the WHO recommended criteria which lists 7 clinical signs as predictors of neonatal infection {The Young Infants Study group (2008)}. These include history of difficulty feeding, history of convulsions, movement only when stimulated, respiratory rate of 60 breaths per minute or more, severe chest in drawing and temperature of 37·5°C or more or below 35·5°C. Any neonate who presented with any of these signs was included in the study. Neonates who were also cyanosed, lethargic or shocked were also included in the study. Neonatal meningitis was defined based on positive laboratory findings on top of the clinical signs alluded to above.

These laboratory findings included a raised white cell count above 25 in the CSF, positive CSF gram stain and positive microbiological growth in the CSF culture {Squire E, B. Favara et al (1979)}.

The clinical definition of neonatal sepsis that was used in this study has its own challenges. Most of the clinical signs outlined above are not restricted to infection but other conditions can present in a similar fashion in a neonate {Kliegman R.M, R.E Behrman et al (2011) }}. These conditions include complex congenital heart disease, inborn errors of metabolism, hypoxic ischaemic encephalopathy and non-accidental injury. This would lead to a misdiagnosis of neonatal sepsis which might compromise the interpretation of the results in this study. In this study all infants that had APGAR scores of less than 6 at five minutes after birth were excluded so as to avoid enrolling infants that could have had hypoxic ischaemic encephalopathy. As discussed in Chapter 3 the diagnosis of hypoxic ischaemic encephalopathy was purely clinical without any laboratory investigations. It is therefore possible that some neonates who were septic at birth could have been wrongly misclassified as hypoxic ischaemic encephalopathy cases. These would have presented as early onset neonatal sepsis and this could partly explain the low number of early onset neonatal sepsis (25%). Infants that had other signs strongly suggestive of complex congenital heart disease had echocardiography done and in this study 2 infants who had been recruited were found to have complex congenital heart disease. They were excluded in the analysis. It was however logistically challenging to rule out inborn errors of metabolism in the study.

7.1.2 Laboratory analysis

7.1.2.1 Blood culture Analysis

In this study blood specimens were cultured in BacT/ALERT PF plus (BIOMÉRIEUX, INC, Durham, N.C.USA) automated systems and bacterial identification was performed with standard bacteriological techniques {Murray B and Pfaller T (1999)}. Antimicrobial susceptibility testing was done by using the modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute/National Committee on Clinical Laboratory Standards (CLSI/NCCLS). The culture positivity rate for significant bacterial organisms in this study was 11% which is lower than the reported 20% in other studies {Claudio C, A. Panero et al (2004), Maiwald M (2004), Squire E., B. Favara et al (1979)}. The BacT/ALERT PF plus requires up to 4mls of blood to be added to the 30ml volume of culture media. Getting adequate volumes of blood from small babies is challenging and this could have affected the culture positivity rate in this study. It has been shown that increasing the volume of blood drawn for culture in young children improves the culture positivity rate {Sarkar S., I Bhagat et al (2006)}. The low yield from blood cultures in this study could have had an impact on the management of the severe neonatal infection cases as duration and type of treatment is frequently guided by the aetiological organism.{Blackburn R.M, N.Q Verlander et al (2013), Van den Hoogen A, L.J. Gerards et al (2009)}. We found that nearly half of the gram negative organisms in this study were resistant to the standard first line treatment for severe neonatal infections in Malawi. If the organisms were not known in these infants they would have been put on the wrong choice of drugs .This in turn could have had an impact on both the short and long term outcome. In this study the impact of having had a significant positive growth in blood culture on neurodevelopmental outcome

at 12 months of age was analysed and the small number of organisms grown could have affected the analysis.

The frequency of an aetiological diagnosis would have been increased if molecular diagnostics had been done in this study. PCR which does not require such large volume of specimen has been shown to increase the yield from both blood and CSF cultures {Maiwald M (2004), Fowlie P.W and B Schmidt (1998)}. PCR could also have been used to detect viral aetiologies such as Herpes simplex enterovirus, coxsackie and adenovirus which, have been reported as important causes of neonatal sepsis elsewhere [Simonsen KA, A.L. Anderson-Berry et al (2014)]. Viral molecular diagnostics work on samples stored from the study is planned.

7.1.2.2 Cerebral Spinal Fluid Analysis

In this study 330/412(80%) participants had their CSF analysed and 323/330 (98%) had their CSF cultured. Significant findings from CSF analysis were defined by a raised white cell count of at least 25 cells/mm^3, positive gram stain or positive CSF culture. Bacterial identification was performed with standard bacteriological techniques {Murray B and Pfaller T (1999)}. Antimicrobial susceptibility testing was done by using the modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute/National Committee on Clinical Laboratory Standards (CLSI/NCCLS). The normal white cell count in neonatal CSF ranges from below 20 cells to below 30 cells. In this study we chose to use the midway point between 20 and 30. In this study 8 out of the 86 cases (9.3%) of culture-proven meningitis had a normal white cell count below 10 cells in their CSF. In other words 8 out of the 33(24%) positive CSF culture cases had a normal CSF white cell count and if

cultures had not been done they would have been missed. In most developing settings CSF cultures are not available and at best, the diagnosis of neonatal meningitis is mainly clinical aided by a raised white cell count in the CSF. This study underscores the importance of CSF cultures in the diagnosis and management of neonatal meningitis.

7.1.2.3 HIV testing

HIV exposure status was determined through serological testing of the mothers for HIV infection using 2 rapid tests for HIV 1 and 2 namely; Determine and Unigold rapid tests (Abbot and Biotech laboratories). A third test SD Bioline HIV Ag-Ab combo was used as a tie breaker in cases of a discrepancy.

All HIV exposed babies had HIV DNA PCR (Roche Amplicor) testing to ascertain on whether they had acquired the infection or not. HIV DNA PCR testing was done once during enrolment. It was therefore difficult to ascertain whether there had been any change in the HIV status of the participants during follow/up. Most of these HIV exposed infants were still being breastfed and as such they continued to be at risk of acquiring HIV from their mothers {Quintanilla K (1996)}. Therefore results from the impact of HIV should therefore be interpreted with this potential confounder in mind.

7.1.3 Bayley assessment of neurodevelopment

In this study neurodevelopmental assessments were done using the Bayley scales of infant development III (BSID-III; Bayley 2006). The BSID-III assesses developmental functioning in children aged between 1 month and 42 months. Several authors have raised concerns about the use of a psychometric tool in a population other than the one in which the tool was developed as the BSID-III was developed in the USA {Gladstone M., G. A. Lancaster (2010),

Nampijja M, B. Apule (2010), Hambleton R.K (1996), Poortinga Y.H (1995), Geisinger K.F., F.J.R. Van de Vijver (1994) }. In another study by Cromwell *et al* conducted in Malawi reliance on US norm-based standardized scores resulted in misclassification of the neurological development of Malawian children, with the greatest potential for bias in the measurement of cognitive and language skills {Cromwell E, Q. Dube et al (2014)}. However Cromwell *et a*l were able to create the norm for the Bayley in Malawi which made it possible for us in this study to interpret the Bayley results. The other challenge with the Bayley-III is its full use in children with significant sensory impairments such as blindness, deafness and severe spinal cord injuries. Standardized administration is difficult in these groups of children and as such other domains may not be tested {Kolobe T. (2010)}. In this study we were unable to fully administer Bayley in 32 participants.

Mellissa Gladstone developed the Malawi Development Assessment tool (MDAT), a culturally relevant developmental assessment tool which showed good reliability, validity, and sensitivity for identification of children with neurodisabilities in rural Malawi {Gladstone, M., G.A. Lancaster et al. (2010)}. We were unable to use the MDAT in this study because the MDAT had not been validated for use in young infants in the urban setting at the time the study was commencing.

7.2 Aetiology of neonatal sepsis and meningitis

As in many developed countries, the commonest aetiological organism identified in this study was group B streptococcus (GBS). GBS is known to cause most of the neonatal infections in the developed setting {Vergnano, S., P.T Heath et al (2005)}.

7.2.1 Group B streptococcus

In this study GBS comprised 17 out of 42 (40%) organisms grown in blood culture. This is higher than rates reported in previous studies in Malawi {Gray K. J., S.L. Bennett (2007), Milledge J, J. C Callis et al (2005)}. In a retrospective study from 1996-2001 in Malawi GBS was reported to have contributed 17 % of all neonatal pathogens grown in blood culture {Milledge J, J. C Callis et al (2005)}. In 2004-2005 the rate had increased to 22% {Gray K. J., S.L. Bennett et al (2007)}. The increase in the rate of GBS is likely to be a reflection of the true burden of the disease in our community and is unlikely to be due to an ascertainment bias or enhanced surveillance. The carriage rates for GBS in pregnant women in Malawi range from 17 to 21% which is higher than the rates in the USA reported at 14% {Gray K.J., G.Kafulafula et al (2011)}. GBS comprised 48% of all organisms grown in CSF culture. It is worth noting that all except one of the GBS grown in both blood and CSF culture were in late onset disease. This is contrary to reports from the developed setting that have reported that 75% of GBS infection in the neonatal period will occur in the first week of life {Vergnano, S., P.T Heath et al (2005). Other studies in Malawi have reported that 45-50% of GBS infection occurs as early onset disease {Milledge J, J. C Callis et al (2005)}. The predominance of late onset disease observed in this study was therefore surprising. It is noteworthy that, regardless of the culture result, 76% of all severe neonatal infection cases were late onset. Given that this was a general trend, it is therefore possible that since mortality rates in early onset disease are reported to be as high as 50% in this setting { Milledge J, J. C Callis et al (2005)}, a significant proportion of newborns with sepsis or meningitis, particularly those born at home, did not reach a health-care facility. In a study done by Kakhobwe *et al* in Ntchisi district in Malawi on the knowledge, attitude and

treatment preferences for neonatal sepsis, 83% of the mothers preferred home treatment using traditional medicine compared to treatment at a health facility {unpublished work}. It would therefore be important to try and understand the health seeking behaviour of the mothers within the catchment area of the current study. On the other hand this change in the balance between late onset and early onset disease could be a genuine change in the epidemiology of neonatal infections in Malawi. To further investigate this observation, a community-based study is currently underway in Blantyre.

In developed countries, screening pregnant women for GBS and administration of prophylactic antibiotics in labour to those women at risk has led to a dramatic reduction of early onset GBS disease but has had less of an effect on late onset disease { Schrag S.J and A Schuchat (2004)}. In Malawi, screening pregnant women for GBS is not routinely done and it would be challenging given the laboratory facilities available. There are no immediate plans to introduce this measure by the Ministry of Health. Should the data in this study be correct that there is predominance of late onset disease, it would be unlikely for intrapartum antibiotics to result in much benefit. There are, however, phase II and III GBS vaccine trials in pregnant women being carried out currently {Clinical Trials.gov}. The Novartis sponsored phase II study has been looking at safety and immunogenicity of a Group B Streptococcus vaccine in non-pregnant and pregnant women 18-40 years of age In Malawi and South Africa. Preliminary results suggest that the vaccine is (ESPID 2014) generally well-tolerated and immunogenic but that vaccine induced antibody concentrations at delivery are higher among HIV negative mothers and their infants, than HIV positive mothers and their infants. The phase III studies have been looking at prevention of GBS colonization using single GBS vaccination. If the GBS vaccine is proven to work it may provide a potentially affordable

route to achieve reduction of GBS infection in Malawi. The major challenge however with new vaccines is the cost that is unreachable without outside donor funding such as the GAVI Alliance (http://www.gavialliance.org/).

7.2.2 *Staphylococcus aureus*

In this study, *Staphylococcus aureus* was the second commonest organism grown in blood culture 10/42(24%). In a number of studies conducted within sub-Saharan Africa *S. aureus* has been reported to be a predominant cause of neonatal sepsis {van den Hoogen A., L.J. Gerards et al (2009)}. It is also known to cause predominantly late onset disease and the findings in this study are in keeping with this literature (90% were late onset).

S. aureus is generally spread through human to human contact and therefore emphasis on infection control both at home and in the hospitals is vital in the prevention of infection. Hand hygiene is a simple infection control measure that has been proven to work but is not well adhered to in our settings {Nerby J.M., R.Gorwitz et al (2011), von Eiff .C, K.Beckeret al (2001),}.

Biological control especially in health workers which are the main source in nosocomial infections might be a new possible way to control Staphylococcus aureus in body surfaces and this need to be explored further. It has been shown that colonization of body surfaces especially in the nose by Staphylococcus epidermidis- inhibitory strain JK16 impairs the establishment of Staphylococcus aureus {Iwase T., Y Uehara et al (2010), Otto M., Submuth R. et al (1999)} . It is understood that these 2 microorganisms have an amensalistic relationship which is an association between organisms of 2 different species in which one is

inhibited or destroyed and the other is unaffected. This could help prevent infection in the few cases that were possibly exposed to *S. aureus* whilst still in hospital.

7.2.3 *Streptococcus pneumoniae*

In this study, *Streptococcus pneumoniae* was the second commonest aetiological agent in CSF cultures 10/33 (30%). *S. pneumoniae* rarely causes neonatal infections in the developed setting but has been shown to be a common cause of infections in several resource-poor settings {Osrin D, S. Vergnano et al (2004), Downie L., Duke T et al (2012)}. In Malawi and elsewhere in sub-Saharan Africa, children carry *S. pneumoniae* in their nasopharynx from a very early age, probably acquired from their siblings {Marchisio P, S. Esposito et al (2011), Sanae Dashti A., Abdinia et al (2012)}. Exposure at this early stage could easily lead to an invasive infection. In addition, it has been shown that pneumococcal carriage amongst HIV infected adults is high {Janoff E.N, J, O'brien J et al (1993)}}. In this study, out of the 366 young infants with severe infections, where HIV exposure status was determined, 131(36%) were HIV exposed. Therefore, these infants were being looked after by adults who were more likely to be carrying *S. pneumoniae* and therefore at risk of acquiring the pneumococcus from their parents as well as their siblings.

Since November 2011, Malawi introduced the 13 valent pneumococcal vaccine {Ministry of Health, EPI programme}and it is hoped that as the cohort of children who have received the vaccine increases, herd immunity will build up and pneumococcal infections in neonates will go down. Preliminary MLW surveillance data for invasive pneumococcal disease suggests that vaccine-serotype disease is decreasing in children but is too early to predict the impact on neonates {Bar Zeev N., D. Everett et al ISPPD (2014)}. A study by French et al in HIV infected adults in Malawi showed that vaccination with the pneumococcal conjugate

vaccine resulted in a reduction of invasive pneumococcal disease in these adults {French N., S.B Gordon et al (2010), Hammitt L.L., Bruden D.L et al (2006) }. It is therefore important to evaluate the potential impact of offering pneumococcal vaccination to all HIV infected adults as a way of reducing pneumococcal infections in neonates.

7.2.4 Gram negative organisms

Gram negative organisms comprised 17% of all organisms grown in blood culture and 22% of all organisms grown in CSF culture. In an earlier retrospective study conducted in Malawi gram negative organisms comprised 32% of all significant organisms grown in neonatal sepsis cases {Milledge J., J.C.J Callis (2005)}. In this current study the gram negative organisms were resistant to penicillin, erythromycin, ampicillin and chloramphenicol. Fifty percent of the gram negative organisms were resistant to ceftriaxone, gentamicin and ciprofloxacillin. The multidrug resistance observed in this study is in keeping with findings from other studies {Blackburn R.M, N.Q Verlander et al (2013), Mehar V., D Yadav et al (2013), Vergnano S., M. Sharland et al (2005)}. Enterobacter cloacae were only sensitive to amikacin, a drug that is not generally available in Malawi and that has unproven penetration into the CSF. The multidrug resistance exhibited by these gram negative bacteria is worrying in Malawi whose standard antibiotic treatment for severe neonatal infection is parenteral benzyl penicillin and gentamicin. This regimen would not adequately treat the gram negative organisms. Ceftriaxone is the drug of choice for patients who fail on the second line but again it is worrying that up to 50% of these gram negatives exhibit high resistance to ceftriaxone-. This leaves these vulnerable neonates few alternatives as amikacin is not freely available in Malawi. It is therefore not surprising that mortality rates in those young infants that had grown gram negative organisms were high at around 51%.

7.2.5 Coagulase negative staphylococci

In this study 85/366 (23%) of the participants grew coagulase negative *Staphylococcus* in their blood cultures. Coagulase negative *Staphylococcus* has been increasingly seen as a cause of nosocomial or late-onset neonatal infections especially in premature infants in neonatal intensive care settings. It is mostly seen in preterm low birth weight infants who have indwelling catheters. Coagulase negative *Staphylococcus* has several intrinsic properties that allow it to readily adhere to the plastic mediums found in intravascular catheters and intraventricular shunts. The adherence creates a capsule between the microbe and catheter, preventing C3 deposition and phagocytosis thereby making coagulase negative *Staphylococcus* more difficult to treat {Anderson-Berry A.L, T. Rosenkrantz et al (2014), Healy C.M, Baker C.J et al (2013)}. Coagulase negative Staphylococcus is ubiquitous as part of the normal skin flora and as such has been reported as a contaminant of blood and CSF cultures.

It is was a challenge to establish the significance of coagulase negative Staphylococcus growth in this study however none of the cases were nursed in a NICU nor had intravenous catheters or intraventricular shunts. We excluded infants that were at least 32 weeks of gestation and the majority of the cases (85%) were term, normal birth weight babies. We further looked at a number of factors that could possibly indicate a higher chance of a disease rather than colonisation with coagulase negative *Staphylococci* and compared with cases that had grown other significant organisms. Most of the CRP values of the coagulase negative Staphylococci infants were normal (80% vs 25% for coagulase negative *Staphylococci vs.* other significant organisms respectively).The white cell counts for these cases were largely normal (85%). We however did not do colony counts for the coagulase

negative Staphylococci which have been shown to help in the differentiation between true infection and a contaminated specimen {Anderson-Berry A.L, T. Rosenkrantz et al (2014)}. On balance we therefore concluded based on the clinical setting, the gestation of these infants and some of the laboratory parameters that the coagulase negative Staphylococcus grown in blood culture in this study was as a result of contamination.

In Malawi routine blood or CSF cultures are not done except at QECH where this study was carried out. Considering the important role that gram negative organisms play in neonatal infections, it is important for Malawi to strengthen its microbiological laboratory testing so as to better manage neonatal infection cases and monitor sensitivity patterns of the aetiological agents.

7.3 In hospital mortality outcome of severe neonatal infections

In this study the in hospital mortality rate was 11% in all the neonatal infection cases (Chapter 3). An earlier study by MIlledge et al conducted in Malawi reported a much higher neonatal sepsis case fatality rate of 48% [Milledge J., J.C.J Calis et al (2005)]. A number of factors could have contributed to the discrepancy. Firstly, the majority of the research participants (90%) were admitted on a jointly run department of Paediatrics/ MLW/ Blantyre Malaria Project research ward at QECH which is better equipped both with human resource and supplies. Secondly, the majority of the cases (76%) in this study were late onset whereas in the earlier studies at least 50% of the cases were early onset. Mortality is known to be higher in early onset disease than late onset {Klinger G., I Levy et al (2009)}. Earlier presentation could also have helped reduce mortality in this study with up to 60% of

the cases having presented within the first 24 hours of the symptoms developing. The majority of the cases in this study were born at term (85%). It is known that mortality from neonatal sepsis is higher in preterm babies that those born at term. We found that, as in keeping with other studies, term gestation was associated with a significant risk reduction of 88% (p=0.005) in mortality {81, 82, 83 and 84}.

In this study in the univariate logistic regression analysis; thrombocytopenia, hypothermia, hypernatraemia, hypoxia and a significant growth in either blood or CSF culture were associated with a significantly increased risk of in hospital mortality. All these factors have been shown to be predictors of poor outcome in severe neonatal infections in other settings {Wasen R., A.A Fraga JM, R.C. Gracia (1998)}). However in all adjustments hypoxia, significant blood or CSF culture and hypernatraemia independently increased the risk of in hospital mortality.

It was surprising to note that 141/330 (43%) of the severe neonatal infection cases had abnormal serum sodium levels on admission, 67/141 (48%) were hypernatremic. The rates of hypernatraemia were higher in neonatal sepsis cases without overt meningitis and these babies also had high serum urea levels which could reflect a high frequency of dehydration. The rates of hyponatraemia were higher in the neonatal meningitis and severe pneumonia group, which could be as a result of syndrome of inappropriate ADH secretion and is in keeping with what has been reported elsewhere {Wasen R., A.A Fraga JM, R.C. Gracia (1998)}. In many resource constrained settings, electrolytes are not routinely available even for severe neonatal infection cases. The high rate of electrolyte imbalance observed in this

study and the subsequent impact on mortality would justify routine monitoring so as to better manage these cases.

7.4 Long Term impact of Severe Neonatal infections

7.4.1 Mortality at 1 year of age

It was surprising to note that the post neonatal mortality rate at 1 year of age was 4.5% amongst the controls. The post neonatal mortality rates for Malawi are currently estimated at 22/1,000 live births (2.2%) {Zimba, E., M.V Kinney et al. (2012), Liu L, H.L. Johnson et al. (2012)} and the higher rates observed in this study are worrying. Several factors have been known to increase the risk of childhood mortality and these include; poverty, malnutrition, infections, maternal education and urbanization. All the participants enrolled in the prospective cohort study were from Blantyre urban but nearly half of their families were living on less than one US dollar per day. The majority of the mothers (90%) were unemployed and only 42% of them had some secondary level education. Even though these families were residing in Blantyre urban, 93% of them were using pit latrines. All these complex factors would have further increased the risk of dying in infancy regardless of them not having had an episode of severe neonatal infection.

Overall in this study the post neonatal mortality rate at 1 year of age was higher in the severe neonatal infection group than the controls (6.6% vs 4.5%). The difference between the 2 groups was however not statistically significant. This study was not powered to look at differences in mortality and a bigger sample size will be required to better describe the impact of severe neonatal infections on mortality.

In this study HIV status showed no impact on mortality or morbidity in children who have recovered from neonatal infection at 1 year of age. This is contrary to findings from other resource restrained settings where mortality has been shown to be higher in infants born from HIV infected mothers. These results should again be interpreted with caution as the study was not adequately powered to look at the impact of HIV.

7.4.2 Neurodevelopmental outcomes at 1 year of age

In this study it was surprising to note that the rate of neurodevelopmental delay was up to 15% in the controls. It was much higher than the 8% that was postulated at the beginning of the study (see chapter 2). There are several factors that have been known to impact neurodevelopment in children namely nutritional status, low socio-economic status, congenital anomalies, brain insults, infections, HIV, neglect, maternal education and mental status {Amouzou A. and Hill K (2004)}. In Malawi, one of the poorest countries in the world, the rates of stunting which is a marker of chronic malnutrition are very high (thought to be 50%) {UNICEF 2012}. Both cases and controls came from poor families (chapter 4) and this could have compounded the effects of malnutrition. The mental status of the mothers was not analysed and therefore its impact on this cohort cannot be analysed. It is also difficult to rule out any brain insult from other causes ranging from intrauterine infections to environmental causes {Mwaniki M.K, M. Atieno et al (2012)}.More research is required to better understand the risk factors associated with the considerable burden of developmental delay observed in the general Malawian paediatric population so as to develop better prevention and management strategies.

It was found that the rates of neurodevelopmental delay occurred in up to 35% of severe neonatal infection cases and again this was much higher than what was postulated at the beginning of the study. The rates of delay were much higher in the meningitis group (60%) compared to the neonatal sepsis (31%) and severe pneumonia groups (35%). It was surprising to note that neonatal sepsis without overt meningitis was associated with a higher risk of developmental delay at 1 year of age of up to 6-fold in the fine motor domain. Most studies have reported an increased risk of neurodevelopmental delay in preterm, low birth weight infants with neonatal sepsis. In this study as pointed out earlier the majority of the cases were infants born at term and as such one would not have expected such a high magnitude of developmental delay {Alshaikh B., W. Yee et al (2014), Alshaikh B., K Yusuf 2013, Chapman I.A (2012)}. There is paucity of data on the long term outcome of neonatal sepsis (without overt meningitis) in term babies in both the developed and developing settings. This study has shown the high risk of developmental delay associated with neonatal sepsis. In preterm babies with neonatal sepsis inflammatory cytokines have been postulated to cause white matter damage thereby leading to neurodevelopmental delay {Divyen K, D.K. Shah et al (2008)}. The brain of preterm babies is more vulnerable to insults than term babies and as such further studies are required to explore the possible mechanisms leading to delay in these infants with neonatal sepsis.

7.4.3 Neonatal meningitis

60% of Infants who had neonatal meningitis had complications at 1 year of age and this is higher than the 50% reported in other studies {Stevens J.P, M.Eames et al (2003), P.T Heath, N.K.,Nik (2003)}. The complications included visual loss, hydrocephalus, epilepsy and

hearing loss. The presence of other factors like poverty as outlined in section 7.2.4.2 would have further increased the risk of sequelae arising from neonatal meningitis.

7.4.4 Predictors of poor neurodevelopmental outcomes at 1 year of age

Positive growth in either blood or CSF culture was independently associated with an increased risk of developmental delay (chapter 5). This finding is in keeping with other reports {Wasen R., A.A Fraga JM, R.C. Gracia (1998)}. Inflammatory cytokines have been known to be associated with poor neurodevelopmental outcomes in preterm infants with neonatal sepsis. We postulate that the presence of bacteria in the blood stream or CSF could increase the surge cytokines produced thereby causing a greater insult to the rapidly growing brain. Being born to a HIV infected mother was independently associated with an increased risk of developmental delay. Studies have shown that HIV exposed non-infected infants have a high risk of developmental delay in resource restrained settings but those from the developed setting have normal development. Several factors have been shown to contribute towards such an increased risk in the resource restrained setting namely; poor maternal health leading to poor bonding and lack of stimulation of the infants, malnutrition and poverty { Amouzou A. and Hill K (2004)}

7.5 Limitations and gaps in the study

The severe infection cases were recruited from QECH a referral facility and therefore could have missed cases that went to a private hospital (very few inpatient beds) or went to other facilities and were treated as an outpatient or went to traditional healers or dies in the community (there are no other government paediatric inpatient facilities in Blantyre). In one study in a rural setting in Ntchisi, Malawi, 83% of the mothers had opted to seek help from

traditional healers first before visiting a health facility with their sick neonate {Kakhobwe T et al (thesis -2010)}. Although not formally quantified the degree of bias in this urban setting is thought to be small. A community based study is currently planned in Blantyre to determine what would better tackle this problem.

Neonatal sepsis is predominately a clinical diagnosis supported by positive microbiology if available. As such there is the possibility patients were included with non-infectious causes of sepsis {Kliegman R.M, R.E Behrman et al (2011)} } including metabolic causes such as inborn errors of metabolism. This could affect interpretation of the data as not all the neonatal sepsis cases would have been truly septic. However in a setting like Malawi it is difficult to rule out some of these non-infectious causes due to diagnostic challenges. In addition some infants ascribed to the neonatal sepsis group could have had meningitis but it would seem unlikely that this would have sufficed to affect the observations of poor outcome in this group. Some infants presenting with sepsis or meningitis soon after birth may have been mis-labelled as birth asphyxia and therefore excluded, however, it is unlikely that this represents a significant bias.

Changes in clinical practice during the course of the study could have impacted on the results and generalizability. However, the Department of Paediatrics at QECH uses standard protocols that did not change during the course of the study. In particular, changes in clinical practice resulting from the “FEAST Trial” {Maitland K, D.M Gibb et al (2011)} occurred after the cross-sectional component of the study was completed. Similarly, the overall rate of HIV infection was low in the infant cohort and very few mothers were receiving full ART as part of option B+ {Ministry of Health, Malawi (2011)}.

As discussed previously, the culture positivity rate for the study was low at 11% and the yield could have been improved if molecular diagnostics were used. Multiplex PCR has been shown to increase the yield for viruses and bacteria in samples. The low yield could have affected some of the analysis on aetiological organisms as the numbers were small. This is being address in a molecular diagnostic study currently underway.

In this study the participants were followed up to the age of 1 year. Some developmental delay issues become apparent as the child gets older and particularly as they reach school going years {Alshaikh B, K. Yusuf et al (2013). Behavioural problems are more likely to manifest in the school going years. Due to the short duration of follow up this study could have missed some of these developmental problems. It was also clear from the study that the rates of developmental delay had increased by age 12 months (Chapter 4). This again reinforces the fact that longer duration of follow up was likely to pick up more cases of developmental delay. A longer duration of follow up would also have allowed us to better describe the progress amongst the participants particularly those who had delay. It would have been desirable to be in a position to describe as to how many of them improved or retrogressed overtime.

The long term follow up only included infants residing within the urban setting and would raise a question on generalizability. However it was noted that the socio-demographic profile of the participants was similar to the rural setting in Malawi. It can be said therefore that the participants though leaving in an urban setting had the same levels of poverty and living standards as most rural settings in Malawi {Malawi DHS 2010}. The study results can therefore be generalised to the rest of Malawi and to similar settings in sub-Saharan Africa.

7.6 Future work

We found that 15% of the control children had developmental delay at 1 year of age and this is almost double that which was postulated. There should be underlying factors leading to such a high rate of developmental delay. We hypothesize that early insults to the brain whether in utero or very early on in infancy coupled with malnutrition and poor socio economic status could be the causative factors of developmental delay in Malawian children. We would propose intervention studies tackling these factors and measuring their effectiveness on neurodevelopment. Interventions could include provision of cash transfers to these poor families or nutritional supplementation to infants born in these poor families. UNICEF is currently partnering the Malawi government on early childhood development and would find results from such studies useful.

There was an increased risk of developmental delay in the neonatal sepsis cases (without overt meningitis) of up to 6-fold in the fine motor domain. Most of the studies have looked at neurodevelopmental outcomes in preterm or low birth weight babies with neonatal sepsis. We propose to undertake further studies exploring the factors that cause developmental delay in term babies with neonatal sepsis in a resource poor setting. We would want to understand the role of both blood and CNS cytokines in causing developmental delay in these settings. Understanding the role of cytokines can help in the development of treatment therapies that can potentially block the effects of the cytokines {Shah D.K, L.W, Doyle et al (2008)}.

To better understand the aetiology and burden of neonatal infections in Malawi a community based study is currently underway. We are prospectively following up pregnant

women and their babies for the first month of life to see who amongst them would develop neonatal infection and the risk factors associated.

7.7 Conclusion

Group B streptococcus is a significant cause of severe neonatal infections in Malawi. Screening pregnant women for GBS and treating the mothers carrying GBS has been proven to reduce early onset GBS disease in neonates and is not so effective in preventing late onset disease. In this study most of the GBS was late onset and as such screening which logistically would be very challenging in Malawi would not be appropriate. However a GBS vaccine though still in being evaluated in clinical trials would be the most appropriate.

The background rates of developmental delay amongst Malawian children are high and all efforts should be made to better understand factors involved and interventions that can help reduce it.

Significant growth in either CSF or blood culture have been shown as predictors of poor developmental outcomes in Malawi. Microbiology plays an important role in the diagnosis and management of these neonates both in the acute and long term. Malawi's health system needs to improve on the availability of microbiology services in its hospitals so as to better manage severe neonatal infection cases and prevent complications.

Neonatal sepsis is associated with poor developmental outcomes at 1 year of age in Malawi. There is need to better understand the mechanism of delay in this group of patients. Most of the participants recruited in this study were term babies with normal birth weights and according to my knowledge this is the first large prospective study looking at the long term outcome of severe neonatal infections in infants in a resource restrained setting.

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University of Malawi, College of Medicine and Malawi-Liverpool-Wellcome trust
Parental Permission for a Minor Child to participate in the Aetiology and Outcome of Neonatal Sepsis and Meningitis in Malawi Research Study

Consent form version date: September 29, 2009

Title of Study: Aetiology and Outcome of Neonatal Sepsis in Malawi.

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Funding Source: Malawi-Liverpool Wellcome trust

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General Things you and your child should know about research studies

You are being asked to allow your child to take part in a research study. Research studies are designed to obtain new knowledge that may help other people in the future.

Enrolling your child in this study is voluntary. You may refuse to give permission or you may withdraw your child at any time and for any reason. Your child does not have to be in this research study in order to receive treatment. If you choose not to enroll your child in this study, your child can still receive care and treatment.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about your child being in this research

study. You will be given a copy of this form. You should ask the researchers, or staff members who may assist them, any questions you have about this study at any time.

Purpose of this study

The purpose of this study is to describe the complications that may arise in babies that have had a serious infection requiring admission in the first 2 months of their lives. We will look at how the infection affects how the child learns how to move, learn and speak as they grow up.

We will also check whether the infection affects the child's hearing.

We expect to have 470 children in this study and we will compare a group of children who have had the infection and a group who have not. And that the comparison will focus on the first year of life.

How long will your child's part in this study last?

Your child is expected to be in the study up until the age of one year. Your child will be asked to come to the clinic 4 times during the duration of the study. This will be at 3months, 6 months, 9 months and 12 months of age.

The study visit evaluation will take one hour at the maximum.

What will happen if your child takes part in the study?

You will be asked to answer general questions about the child's family's living condition, and about the health of your child, your partner and yourself.

Your child will be examined by a team of nurses and doctors to determine how your child has developed in moving, learning and speech since birth. This will not hurt the child as most of these are observations.

The study nurse will also test your child for hearing and this does not hurt.

We will also check your child for HIV infection at beginning of the study. At each follow up visit your child will be fully examined. There is no specific intervention in this study (no new drugs). Children will receive the standard care.

Possible Benefits from being in the study.

The benefits of your child from being in the study will be that they will be closely followed up and if any problems are detected will be referred to an appropriate institution for early intervention and treatment.

The nurse will also give you information at each visit on age appropriate ways that you can interact and play with your child to stimulate their development.

Your child will have a chance of being tested for HIV and therefore benefit from early referral for treatment if they are HIV infected.

The knowledge gained from this study will teach us how to better follow-up babies with an initial episode of severe infection and the services that they need.

Possible Risks from being in the study

There are minimal risks for your child to participate in this study.

Protection of your child's privacy

All information about your child including the name and date of birth will remain confidential. This means that only researchers will know the information about your child and they will keep it a secret. Your child's name will not be included in any report about this study.

What if you want to stop before your child's part in the study is complete?

Your child can stop participating in this study at any time without penalty. If you choose to withdraw your child from this study, you and your child can still receive care at this hospital.

Information collected on your child before you decide to stop your child's participation will still be used in this study

Cost of involvement in the study

It will not cost anything to be in the study.

What if you have questions about this study?

If you have any questions during the course of the study you can contact the researchers listed on the first page of this form.

Parent's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily give permission to allow my child to participate in this research study.

……
Name of Research Subject (child)

…… ……..
Signature of Parent Date

……..
Name of Parent

…….. ……
Signature of person obtaining permission Date

……..
Name of person obtaining permission

Clinical Form

Administered to: All Children at all Visits

CLINICAL HISTORY:

Visit Type: ☐ Enrollment ☐ 1Month Visit ☐ 3Months Visit ☐ 6 months Visit ☐ 9 Months Visit ☐ 12 Months Visit

Since the birth disharge or last study visit, has your child had any of the following for which you did not go to any health care worker (nurse, doctor, etc.)?

Symptom	Yes	No	If yes, specify when, severity , diagnosis made
1. Skin problems	☐	☐	1a.
2. Cough	☐	☐	2a.
3. Oral Thrush	☐	☐	3a.
4. Ear Infection	☐	☐	4a.
5. Conjunctivitis	☐	☐	5a.
6. Fever	☐	☐	6a.
7. Vomiting	☐	☐	7a.
8. Measles	☐	☐	8a.
9. Meningitis	☐	☐	9a.
10. Malaria	☐	☐	10a.
11. Diarrhea	☐	☐	11a.
12. Other	☐	☐	12a.

Note – If child has experienced a severe adverse event, follow appropriate reporting procedures

13. Has your child visited a clinic, other than the study clinic, since birth / the last visit?

☐ Yes ☐ No

If Yes, 13a. Date (dd/mm/yy): |__|__| / |__|__| / |__|__|

13b. Reason_____

13c. Date (dd/mm/yy): |__|__| / |__|__| / |__|__|

13d. Reason_____

14. Has your child been hospitalized since birth / the last visit? ☐ Yes ☐ No

If Yes, 14a. Date (dd/mm/yy): |__|__|/ |__|__| / |__|__| 14b. Duration: |___|___| days

14c. Reason_____

14d. Date (dd/mm/yy): |__|__| / |__|__| / |__|__| 14e. Duration: |___|___| days

14f. Reason_____

15. Has your child received a blood transfusion since birth/ the last visit? ☐ Yes ☐ No

If Yes, 15a. Reason_____

15b. Date (dd/mm/yy): |__|__| / |__|__| / |__|__|

16. What type of feeding does your child usually receive?

☐ (1) Breast milk only; no solid foods, no liquids (no water) (exclusive breastfeeding)
☐ (2) Breast milk plus water (mixed feeding)
☐ (3) Breast milk plus water and/or other liquid and solid food (mixed feeding)
☐ (4) Formula only
☐ (5) Liquid and solid foods only
☐ (6) Other (specify)_____

CLINICAL EXAMINATION

17. Temperature: |___|___| . |___| ^{o}C

18. Weight: |___|___| . |___| kg

19. Height or length: |___|___|___|. |___| cm
19a. ☐ Height (1) ☐ Length (0)

20. Head circumf.|___|___|. |___| cm

21. Mid-arm circumference:|___|___| . |___| cm

	Normal (1)	Abnormal (0)	**If 'ABNORMAL'** a. Findings
22. General	☐	☐	22a.
23. Skin	☐	☐	23a.
24. Mouth	☐	☐	24a.
25. ENT	☐	☐	25a.
26. Neck	☐	☐	26a.
27. Cardiovascular	☐	☐	27a.
28. Lungs	☐	☐	28a.
29. Abdomen	☐	☐	29a.
30. Extremities	☐	☐	30a.
32. Genitourinary	☐	☐	32a.
33. Lymph nodes	☐	☐	33a.
34. Other findings/notes:			

Use **page 3** to document neurological exam in **all children**

CRANIAL NERVES

Vision	Looks at person for a few seconds	❑			
	Follows across midline	❑			
	Grabs red colored cube	❑			
	Not done	❑			
Nystagmus	Absent	❑			
	Eyes beat to the right	❑			
	Eyes beat to the left	❑			
	Eyes beat in both directions	❑			
	Eyes beat up and down	❑			
	Not done	❑			
Eye movements	Eye movement full	Left	❑	Right	❑
	If no: Eye fails to abduct	Left	❑	Right	❑
	Eye fails to adduct	Left	❑	Right	❑
	Not done	❑			
Facial paralysis	Eyes closed equally	❑			
	If not: eye closed less	Left	❑	Right	❑
	Mouth angle symmetric	❑			
	If not: angle deviates to	Left	❑	Right	❑
	Not done	❑			

CEREBELLAR

Tremor	Absent	❑			
	Present	❑			
	Not done	❑			

TONUS

Elbow extension	Normal	Left	❑	Right	❑
	Rigid / spastic	Left	❑	Right	❑
	Hypotonia	Left	❑	Right	❑
	Not done	❑			
Knee extension	Normal	Left	❑	Right	❑
	Rigid / spastic	Left	❑	Right	❑
	Hypotonia	Left	❑	Right	❑
	Not done	❑			

REFLEXES

Upgoing toe	Absent	Left	❑	Right	❑
	Present	Left	❑	Right	❑
	Not done	❑			
Ankle clonus	Absent	Left	❑	Right	❑
	Transient (< 5 beats)	Left	❑	Right	❑
	Sustained (≥ 5 beats)	Left	❑	Right	❑
	Spontaneous	Left	❑	Right	❑
	Not done	❑			

Biceps reflex	No reflex	Left ❑	Right ❑
	Muscle contraction only	Left ❑	Right ❑
	Limb displacement	Left ❑	Right ❑
	Clonus	Left ❑	Right ❑
	Not done	❑	
Patellar relex	No reflex	Left ❑	Right ❑
	Muscle contraction only	Left ❑	Right ❑
	Limb displacement	Left ❑	Right ❑
	Clonus	Left ❑	Right ❑
	Not done	❑	

Neurologic findings: ☐**Normal** ☐**Abnormal**

Project ID number: |___|___|___|___|

Date of visit (dd/mm/yy): |__|__| / |__|__| / |__|__|

_____Signature _____ Date __/___/_____

PAEDIATRIC ADMISSION SHEET Hosp No:

Name ..... Age ..... Date of Birth ..... Date of Admission .....
Weight .....kg %Weight for Age.....

HISTORY

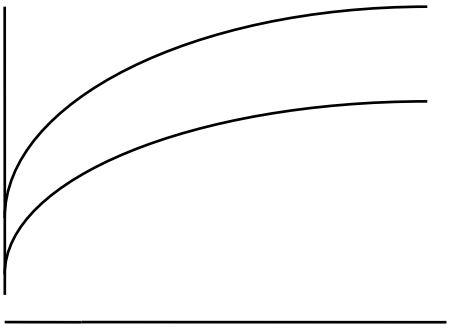
Fever	Y / N		How long for .....
Convulsions	Y / N		How long for .....
Pallor/Jaundice	Y / N		How long for .....
Vomiting	Y / N		How long for .....
Diarrhoea	Y / N		How long for .....
Cough	Y / N		How long for .....
Rash	Y / N		How long for .....
Difficulty breathing	Y / N		How long for .....
Eating / Sucking	Y / N	If N	How long for .....
Oedema	Y / N		How long for ...
Other (Please specify)			.....

Any previous admissions? Y / N (Please give details) Diagnosis. and Dates ....
.....
HIV-test done? Y / N If Y: Date Result: R / NR ON ARV's? Y / N
Mother tested Y/N/NK CoT prophylaxis? ARTs?
FAMILY HISTORY Any history of TB contact Y / N, Epilepsy Y / N, Diabetes Y / N, Allergy Y / N
Other Y /N (detail please).....

Mother well Y / N (details please).....
Father well Y / N (details please).....
Parents separated Y / N when? .....
Number of Siblings Alive ..... Died ..... .
Siblings Well Y / N (details including age) .....
.....

Birth History ..... Vaccinations .....
Previous blood transfusion Y / N when..... Previous drugs in last 2 weeks .....
Other.....

under -fives chart

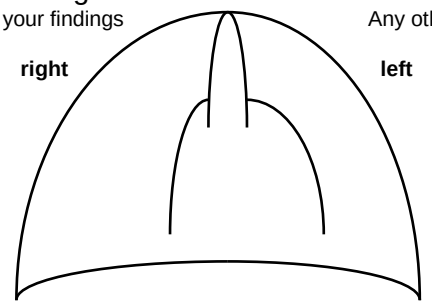


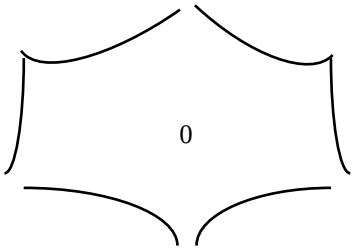
EXAMINATION
Nutritional status good / fair / poor
Blantyre Coma Score (BCS)=

Pallor	0	+	++	+++
Jaundice	0	+	++	+++
Oedema	0	+	++	+++
Rash	0	+	++	+++

Neck stiffness Y / N
Oral thrush/ sores Y / N
Lymphadenopathy Y / N
Respiratory signs Y / N
Finger Clubbing Y / N
Hepatomegaly Y / N .....cm
Splenomegaly Y / N ..... cm
Cardiac signs Y / N
Ear signs Y / N
Neurological signs Y / N
Any other masses Y / N where.....

please draw your findings





Diagnosis.....

NEONATAL INFECTION STUDY

Date of admission……Name……Study number……

Date of Birth: **Age in days:** **Sex F/M**

Place of delivery: **Home** **TBA** **HC** **Hospital** **Other (please specifiy)…….**

Type of birth: **FTND** **Breech** **Forceps** **LSCS (if so give reason for CS)……..**

Preterm/ Full term **Singleton/Twin**

Mother HIV Pos/neg/Unknown **Infant received PMTCT?** **Y/N**

Birth weight **gms** **Immunisations** **Pentavalent 1 2 Polio 0. 1. 2** **BCG**

Mother well Y/N if no what is the problem……..
Father well Y/N if no what s the problem……..
Siblings well Y/N if no what is the problem……
How many siblings?.. **Alive .....** **Died .....**

History of present complaint:

Fever?	**Y/N**	**If yes for how long…….**
Convulsions?	**Y/N**	**If yes for how long……. If yes focal or generalised**
Irritable	**y/n**	**If yes for how long…….**
Cough?	**Y/N**	**If yes for how long…….**
Bulging fontanelle?	**Y/N**	**If yes for how long…….**
Stiff neck/body?	**Y/N**	**If yes for how long…….**
Diarrhoea	**Y/N**	**If yes for how long…….**
Vomiting	**Y/N**	**If yes for how long……**
Sucking?	**Y/N**	**If yes for how long…….**
Difficulty breathing?	**Y/N**	**If yes for how long…….**
Passing urine?	**Y/N**	**If yes for how long…….**
Pallor?	**Y/N**	**If yes for how long……**
Jaundiced	**Y/N**	**If yes for how long…….**
Rash	**Y/N**	**If yes for how long…….**
Oedema	**Y/N**	**If yes for how long…….**

Other **Y/N** **Please say what and for how long……**

Received any medications before arrival at hospital Y/N if yes describe ……. For how long……...
When……

EXAMINATION

Admission weight **Admission length:** **head circumference:** **Temp ….R/A**

Well nourished/fair/poor **Bilirubin level…….**
BCS……
Seizures **Y/N** **Focal / General?** **Cap refill time……sec**

Pale	**+**	**++**	**+++**
Jaundices	**+**	**++**	**+++**
Rash (Purpuric Y/N)	**+**	**++**	**+++**
Oedema	**+**	**++**	**+++**
Oral thrush	**+**	**++**	**+++**
Nappy rash	**+**	**++**	**+++**
Gen LNs Y/N	**+**	**++**	**+++**

Chest signs **Y/N** **…… RR /min SaO2…….**

H sounds Normal **Y/N ……..PR /min**

Umbilicus **Clean/dirty/septic**
Nasal discharge **Y/N** **Tests done LP, BC, FBC, ELISA. MP. PCV. BSugar. U and E**
Ears infected **Y/N** **(*please circle if done)***
Liver size…….. cm
Spleen …… Cm **Other tests……..**