THE BURDEN OF EARLY ONSET SEPSIS IN NEONATES WITH NEONATAL ENCEPHALOPATHY

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Masters of Medicine in Paediatrics

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DECLARATION

I, Kathleen Car declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Masters of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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Kathleen Car

17th day of September 2020 in Johannesburg

DEDICATION

I would like to dedicate this research report to my mother, Patricia and my late father, Frederick Car. If not for all their hard work, sacrifices, love and support I would not be the person or doctor I am today. I would also like to extend my gratitude to my fiancé Nicholas and all my family who have supported me throughout this research process.

PRESENTATIONS ARISING FROM THIS STUDY

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LIST OF ABBREVIATIONS

aORs:	Adjusted odds ratios
BSID:	Bailey Scale of Infant development
CHBAH:	Chris Hani Baragwanath Academic Hospital
CI:	Confidence Interval
CFR:	Case Fatality Rate
CP:	Cerebral Palsy
CRP:	C Reactive Protein
CSF:	Cerebrospinal Fluid
DQ:	Developmental Quotient
EOS:	Early Onset Sepsis
FBC:	Full Blood Count
GBS:	Group B Streptococcus [*Streptococcus Agalactiae*]
GMDS:	Griffiths Scales of Mental Development
HIE:	Hypoxic Ischaemic Encephalopathy
HREC:	Human Research Ethics Committee
ICD-10:	International Classification of Diseases, Tenth revision
IQR:	Interquartile Range
I:T ratio:	Immature to Total Neutrophil Ratio
LMIC:	Low Middle Income Country
NDI:	Neurodevelopmental Impairment
NNDC:	Neonatal Neurodevelopmental Clinic
PCR:	Polymerase Chain Reaction
TOBY:	Total Body Hypothermia Study
RMPRU:	Respiratory and Meningeal Pathogens Unit

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PREFACE

Neonatal encephalopathy contributes to a large burden of deaths and disability worldwide. Studies have shown that Early Onset Sepsis (EOS) (first 72 hours of life) is an independent risk factor for neonatal encephalopathy, and when both are present, the risk of cerebral palsy is dramatically increased. Despite a high burden of neonatal encephalopathy in South Africa, the prevalence of EOS in newborns with neonatal encephalopathy is poorly defined. Furthermore, there is a paucity of data on medium-term outcomes of term newborns with EOS and neonatal encephalopathy. We therefore sought to describe the burden of EOS among newborns born with neonatal encephalopathy in a large secondary-tertiary care hospital and the medium-term neurodevelopmental outcomes of these affected newborns. In order to achieve the above objectives, two manuscripts have been prepared for submission to two different journals.

Manuscript 1 is entitled: "The burden of early onset sepsis in neonates with neonatal encephalopathy" and is formatted for submission to "*Pediatrics".* Author guidelines for this manuscript are available at: https://www.aappublications.org/content/pediatrics-author-guidelines#formatting_requirements. The authors for this manuscript are: Kathleen P. Car, Firdose Nakwa, Fatima Solomon, Sithembiso C. Velaphi, Cally J. Tann, Alane Izu, Sanjay G Lala, Shabir A. Madhi, Ziyaad Dangor.

KPC conceptualized and designed the study, carried out data cleaning and the analyses, and drafted the initial manuscript. FN and ZD were the supervisors. FS, AI and SAM maintain the RMPRU database used in the study. All co-authors critically reviewed the manuscript for important intellectual content.

Manuscript 2 is entitled “Neurodevelopmental Impairment at 1-2 years of age in children born with Neonatal Encephalopathy and Early Onset Sepsis” and is formatted for submission to the *South African Journal of Child Health*. Author guidelines for this manuscript are available at: http://www.sajch.org.za/index.php/SAJCH/announcement/view/1. The authors for this manuscript are: Kathleen P. Car, Ziyaad Dangor, Firdose Nakwa.

KPC conceptualized and designed the study, carried out data cleaning and the analyses, and drafted the initial manuscript. FN and ZD were the supervisors.

The research report is therefore being submitted in the ‘Submissible Format’ in line with the University of the Witwatersrand Faculty of Health Sciences accepted formats for a research report. The formatting for both manuscripts differ slightly; for readability, we have formatted both manuscripts in UK English using double line spacing and 12-point Times New Roman font.

We have also attached the plagiarism declaration and the Turnitin report which has a similarity index of 12% (Appendix 1), the Human Research Ethics Committee approval certificate (Appendix 2) and the postgraduate committee approved protocol (Appendix 3)

MANUSCRIPT 1

THE BURDEN OF EARLY ONSET SEPSIS IN NEONATES WITH NEONATAL ENCEPHALOPATHY

Abstract

Objective: Early-onset sepsis (EOS) is a risk factor for neonatal encephalopathy, a leading cause of neonatal deaths. We evaluated the association of EOS among newborns with neonatal encephalopathy in a low-middle income setting in South Africa; and evaluated for predictors of death in newborns with EOS and neonatal encephalopathy.

Methods: We undertook a retrospective study in newborns born from 1st January 2016 to 30th June 2018 with gestational age ≥35 weeks and/or birth weight ≥2,500 grams, who were diagnosed with neonatal encephalopathy by the attending physician. Overall, EOS (confirmed + probable) was defined as either culture-confirmed sepsis on blood and/or cerebrospinal fluid within 72 hours of birth; or in the absence of culture confirmation, a CRP > 32mg/L or an immature to total neutrophil ratio (I:T) ≥ 0.3 (i.e. probable sepsis).

R**esults:** Of 10,182 hospitalized newborns, 1,027 (10.1%) were diagnosed with neonatal encephalopathy. One-hundred and eighty-one (17.6%) neonatal encephalopathy cases had EOS (confirmed + probable), including 52 (5.1%) that were culture-confirmed sepsis and 129 (12.5%) with probable sepsis. The incidence (per 1,000 live births) of EOS (confirmed + probable) in newborns with neonatal encephalopathy was 2.3 (95% CI: 2.0-2.7); including 0.22, 0.13 and 0.06 for culture-confirmed, Group B *streptococcus, Klebsiella pneumoniae* and *Escherichia coli*, respectively. The case fatality risk (CFR) of

EOS (confirmed + probable) in newborns with neonatal encephalopathy was 19.3% (95% CI: 13.9-25.9). Predictors of fatal outcome in newborns with EOS (confirmed + probable) and neonatal encephalopathy included moderate or severe neonatal encephalopathy (aOR 6.79), seizures (aOR 3.46) and in-utero HIV-exposure (aOR 3.72; $p<0.05$ for all predictors).

Conclusion: In this low-middle income African setting, EOS (confirmed + probable) was prevalent in 17.6% of neonatal encephalopathy cases. Our study highlights the need for preventative strategies against EOS as a strategy to reduce the burden of neonatal encephalopathy.

Introduction

The year-on-year decline in neonatal mortality rate from year 2000 to 2015 has lagged behind reduction in death rates in children 1-59 months age, resulting in newborns now contributing to 45% of all under-5 childhood deaths[1]. In South Africa, the estimated neonatal mortality rate was reported as 12 per 1,000 live births, and accounted for 32% of the under-five mortality rate[2,3]. Using post-mortem minimally invasive tissue sampling coupled with ante-mortem clinical information, we recently reported complications of prematurity (52.9%), complications of intrapartum events (15.0%) and infection (9.8%) being the leading causes of neonatal deaths in our setting[2].

Neonatal encephalopathy is characterized by disturbed neurologic function, manifesting as reduced level of consciousness, seizures, difficulty with initiating and maintaining respiration, and depression of tone and reflexes in an infant born at or beyond 35 weeks of gestation[4]. Globally, the incidence of neonatal encephalopathy is estimated at 3.0 (95%CI: 2.7-3.3) per 1,000 live births[5], being in the causal pathway of approximately 23% of neonatal deaths (99% occurring in low and middle income countries; LMICs)[6]. Although the pathogenesis of neonatal encephalopathy is multifactorial, it is commonly attributed to hypoxic ischemic encephalopathy (HIE), which has an incidence of 9-13 per 1,000 live births in South Africa[7]. Early-onset sepsis (EOS) is also implicated in the causal pathway to developing neonatal encephalopathy in low, middle and high income settings[8–10]. Intra-uterine infection and placental inflammation/ infection are associated with an increased risk of neonatal encephalopathy[8,11–14], possibly by sensitizing the immature brain to perinatal events[15–17]. In one study from Uganda, infants with neonatal encephalopathy had eight times the odds of neonatal bacteraemia when compared to those without[8].

Group B *streptococcus* (GBS) is the commonest cause of neonatal sepsis and meningitis in high-income countries and South Africa[18,19]. In our setting where the overall incidence of EOS was 39.3 per 1,000 live births using whole-blood PCR techniques and blood culture, GBS was attributed as the cause of 4.8% (95%CI: 4.1–5.8) of EOS[19]. In a recent systematic review of only few published studies, GBS was found to be associated with 0.58% (95% CI: 0.18 - 0.98) of neonatal encephalopathy cases[20].

We therefore investigated the burden of EOS in newborns diagnosed with neonatal encephalopathy in a low-middle income setting in South Africa, and analysed for predictors of death in newborns with EOS and neonatal encephalopathy.

Methods

A retrospective descriptive study was undertaken from 1st January 2016 to 30th June 2018 at the neonatal unit of Chris Hani Baragwanath Academic Hospital (CHBAH), a public secondary-tertiary care hospital in Soweto, South Africa. Although South Africa is classified as an upper-middle income country, Soweto is a low-middle income setting where access to health care for pregnant women and children <6 years of age is free of charge[21]. The neonatal unit admits approximately 4,000 newborns annually, from a birth cohort of approximately 28,000 live births (20,000 at CHBAH and 8,000 at surrounding community health centres). It is also a referral centre for newborns requiring critical care, including whole body cooling, from a local district hospital (Bheki Malangeni District Hospital) where approximately 3,000 births occur annually. Newborns at CHBAH with neonatal encephalopathy are graded using Sarnat staging; stage-1 as mild, stage-2 as moderate, and stage-3 as severe neonatal encephalopathy[22]. Criteria for therapeutic hypothermia are based on the modified TOBY criteria[23] and at the discretion of the attending-physician, as well as based on available resources at CHBAH. In all newborns with neonatal encephalopathy, a full blood count and blood culture are done within the first 24 hours of life; and C-reactive protein (CRP) and cerebrospinal fluid (CSF) culture between 24 and 72 hours of life.

Newborns with physician-diagnosed neonatal encephalopathy were identified from an electronic database (administered by the Respiratory and Meningeal Pathogens Research Unit; RMPRU) using ICD-10 codes pertaining to neonatal encephalopathy, HIE, birth asphyxia, and intrauterine hypoxia (Supplementary Table 1). We then excluded newborns that did not strictly meet the case definition of neonatal encephalopathy (<35 weeks gestation age)[4]. In children among whom gestational age was not recorded in the database, birth weight ≥2500 grams was used as a proxy for gestational age ≥35 weeks. Laboratory results were

sourced from the RMPRU database or the National Health Laboratory Service (NHLS) Trakcare® system.

"EOS (confirmed + probable)" was defined as neonatal encephalopathy cases with either culture-confirmed or probable sepsis. Culture-confirmed sepsis was defined as culture of pathogenic bacteria (excluding contaminants) on blood and/or CSF within 72 hours of life. Bacteria considered as contaminants included: *Coagulase-negative staphylococci* (CoNS), *Micrococcus* species, *Bacillus* species, *Corynebacterium* species and *Streptococcus viridans*. The inclusion of "probable sepsis" was based on the low sensitivity (~8%) of blood culture in newborns with invasive bacterial disease[20]. We defined probable sepsis as a CRP >32 mg/L (based on a Ugandan study) and/or an immature to total neutrophil ratio ≥0.3[8,24]. We chose this high CRP threshold to be predictive of probable sepsis because a CRP value can be elevated in newborns with neonatal encephalopathy in the absence of sepsis, and further altered by the use of therapeutic hypothermia[25–29]. Importantly, there is no consensus on which CRP value is predictive of sepsis in newborns with encephalopathy[26,28–30].

Statistical Analysis

Incidence was calculated as the number of cases per 1,000 live births (based on the District Health Information System) for Soweto and surrounding areas. Neonatal encephalopathy cases were stratified into overall EOS (confirmed + probable), culture-confirmed sepsis, probable sepsis, and no sepsis. Categorical variables were reported as frequencies and proportions, and comparisons done using the Chi-squared or Fisher's exact test. Continuous variables were reported as medians and compared using the Mann-Whitney U test. To determine predictors of mortality in cases with EOS (confirmed + probable) and neonatal encephalopathy, we undertook a multivariable logistic regression analysis reporting adjusted

odds ratios (aORs) with 95% confidence intervals; we included variables with a p-value <0.2 in the univariate analysis.

Data were analysed using STATA version 13.1 and differences with p-value <0.05 were considered statistically significant. The study was approved by the University of Witwatersrand Human Research Ethics Committee (HREC number: M181058)

Results

During the observation-period, 1,027 (10.1%) of 10,182 hospitalized newborns were diagnosed with neonatal encephalopathy, including 497 (48.4%), 422 (41.1%) and 82 (8.0%) with mild, moderate and severe neonatal encephalopathy (Figure 1). Of those diagnosed with neonatal encephalopathy, 181 (17.6%) had EOS (confirmed + probable); including 52 (5.1%) with culture-confirmed sepsis and 129 (12.5%) with probable sepsis (Table 1). The overall incidence (per 1,000 live births) of neonatal encephalopathy was 13.0 (95% CI: 12.2-13.8). The incidence of neonatal encephalopathy with culture-confirmed and probable sepsis was of 0.7 (95% CI: 0.5-0.9) and 1.6 (95% CI: 1.4-1.9), respectively (Supplementary Table 2).

Of the 52 culture-confirmed cases with neonatal encephalopathy, the bacteria was cultured from blood only, CSF only, and both blood and CSF in 39 (75%), 7 (13.5%) and 6 (11.5%) cases, respectively. Newborns with culture-confirmed sepsis and neonatal encephalopathy compared to newborns without sepsis were more likely to have moderate or severe neonatal encephalopathy (62.8% vs. 47.9, p= 0.040) and a higher CFR (30.8% vs. 10.5%, p<0.001) (Table 1). Overall, neonatal encephalopathy with Gram-negative culture-confirmed sepsis was associated with a higher CFR (54.6%; 95% CI: 32.2-75.6) than those associated with Gram-positive culture-confirmed sepsis (13.3%; 95% CI: 3.8-30.7; p=0.001). Group B streptococcus was the most commonly identified organism (n=17, 32.7%), for an overall incidence of 0.22 (95% CI: 0.13-0.35) per 1,000 live births (Figure 2, Supplementary Table 2). The most commonly cultured gram-negative organisms associated with neonatal encephalopathy were *Klebsiella pneumoniae* (n=10, 19.2%) and *E. coli* (n=5, 9.6%), with an incidence of 0.13 (95% CI: 0.04-0.23) and 0.06 (95% CI: 0.02-0.15) per 1,000 live births, respectively (Figure 2, Supplementary Table 2).

Newborns with EOS (confirmed + probable) and neonatal encephalopathy compared to those without sepsis were also more likely to have moderate or severe neonatal encephalopathy (61.6% vs. 47.9%, p=0.001), and a higher CFR (19.3% vs. 10.5%, p<0.001) (Table 1).

In a sensitivity analysis, we also compared clinical and laboratory characteristics between culture-confirmed and probable sepsis - except for a higher CFR in newborns with culture-confirmed sepsis compared to probable sepsis (30.8% vs. 14.7%; p=0.013), there were no other differences (Table 1).

In a multivariate regression analysis comparing survivors of EOS (confirmed + probable) with neonatal encephalopathy to those that demised; moderate or severe neonatal encephalopathy (aOR 6.79, 95% CI: 1.15-40.04, p=0.034), seizures (aOR 3.46 95% CI: 1.11-10.74, p=0.032) and in-utero HIV-exposure (aOR 3.72; 95%CI: 1.24-11.16, p=0.019) were associated with an increased risk of death (Table 2).

Discussion

In this low to middle income setting, we report a higher burden of EOS in newborns with neonatal encephalopathy than previously described[8,12,31]. In addition to a four-fold greater incidence (per 1,000 live births) of neonatal encephalopathy in this population (13.0; 95% CI: 12.2-13.8) than global estimates[5], the incidence of EOS (confirmed + probable) with neonatal encephalopathy was also high (2.3; 95% CI: 2.0-2.7). Furthermore, newborns that had EOS (confirmed + probable) with neonatal encephalopathy had an increased risk of moderate or severe neonatal encephalopathy and an increased risk of death. A third of culture-confirmed cases were caused by GBS, followed by *Klebsiella pneumoniae* and *E. coli*.

Our reported prevalence of EOS (confirmed and probable) in term babies with neonatal encephalopathy was 17.6% (95%CI: 15.3-20.1), which is significantly higher than reported in high-income settings like the Netherlands (3.9%) and California (11.3%)[12,31]. Furthermore, our prevalence of culture-confirmed sepsis with neonatal encephalopathy of 5.1% (95% CI: 3.8-6.6%) was three-fold higher than in the Netherlands (1.3%) and California (1.5%) but similar to that reported in Uganda (3.5%)[11,12,31]. Importantly, we used the same case definition of neonatal encephalopathy to the above-mentioned studies and have therefore highlighted the increased prevalence of EOS with neonatal encephalopathy in a low-middle income setting where both sepsis and neonatal encephalopathy rates are generally high.

There is lack of consensus among studies trying to ascertain the true pathogens associated with neonatal encephalopathy. In the absence of molecular techniques, blood cultures are used to detect pathogenic bacteria but have low sensitivity[19]. In a recent study, the use of molecular PCR techniques resulted in a 70% increase in the incidence of early-onset GBS disease (1.91 per 1,000 live births) compared to blood culture-only based incidence (1.12 per

1,000 live births)[19]. Nonetheless, the finding of GBS as the most commonly identified organism in neonatal encephalopathy is not surprising as we have previously reported amongst the highest incidence of GBS globally[32]. The incidence of GBS-associated neonatal encephalopathy in our study (0.22; 95%CI; 0.13-0.35) was 12-times higher than reported in the UK (0.019; 95% CI: 0.019-0.02)[20]. Furthermore, our overall prevalence of GBS-associated neonatal encephalopathy (1.7%; 95% CI: 1.0-2.6) was higher than the global estimate (0.58%; 95% CI: 0.18-0.98%) published in a recent meta-analysis[20]. These findings further emphasise the need for GBS preventative strategies – a maternal GBS vaccine may decrease the burden of neonatal encephalopathy by reducing the risk of in-utero GBS infection that may drive the pathogenesis of neonatal encephalopathy.

Our CFR in newborns with culture-confirmed sepsis and neonatal encephalopathy was lower than reported by Tann et al. in Uganda (44.4%)[8]. This higher CFR in Uganda compared to our setting could be explained by the limited health care services available to mothers and children, with no therapeutic hypothermia or mechanical ventilation offered[8]. Our study was not designed to look at the benefits of therapeutic hypothermia and we found no association between therapeutic hypothermia and the risk of death (aOR 0.62, 95%CI: 0.20-1.95). Therapeutic hypothermia has however been reported to confer neuroprotection with good outcomes in 60% of newborns with perinatal asphyxia and sepsis[31], and in rat pups modelling gram-positive intrauterine infections with perinatal asphyxia[33].

This was a retrospective study and had a number of limitations. We used a database in which physician diagnosed neonatal encephalopathy was identified and therefore some cases may have been misclassified. We are also unable to account for newborns with severe structural brain abnormalities. Furthermore, diagnosing culture-confirmed sepsis in newborns is

difficult - molecular PCR techniques are not available at CHBAH and ancillary laboratory markers of infection/inflammation may be elevated in neonatal encephalopathy and affected by therapeutic hypothermia[25-29]. To overcome this limitation, we used a higher CRP threshold as a marker of sepsis for probable cases, and therefore are likely to have underestimated the burden of EOS with neonatal encephalopathy.

In conclusion, newborns born with EOS and neonatal encephalopathy are more likely to have moderate or severe encephalopathy and demise. Preventative strategies against EOS to reduce the burden of neonatal encephalopathy is warranted. We also show a high burden of GBS-associated neonatal encephalopathy which further supports the need for maternal vaccination particularly where intrapartum antibiotic prophylaxis is likely to be ineffective.

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Table 1: Clinical and laboratory characteristics of newborns with early-onset sepsis (EOS) and neonatal encephalopathy

	No sepsis n=846 (%)	EOS (confirmed + probable) n=181 (%)	Culture-confirmed sepsis n= 52 (%)	Probable sepsis[1] n=129 (%)	p value[2]	p value[3]	p value[4]
Male sex	408 (48.2)	91 (50.3)	25 (48.1)	66 (51.2)	0.617	0.983	0.707
Median birth weight (IQR)	3082 (2780-3420)	3045 (2770-3465)	3043 (2655-3350)	3045 (2790-3485)	0.738	0.226	0.287
Median gestation[5] (IQR)	39 (38-40)	39 (37-40)	39 (37-40)	39 (38-40)	0.347	0.703	0.280
Normal vaginal delivery	449 (53.1)	109 (60.2)	32 (61.5)	77 (59.7)	0.080	0.235	0.818
HIV-exposed	201 (23.8)	48 (26.5)	11 (21.2)	37 (28.7)	0.432	0.668	0.299
Moderate or severe neonatal encephalopathy[6]	395 (47.9)	109 (61.6)	32 (62.8)	77 (61.1)	0.001	0.040	0.840
Therapeutic hypothermia[6]	298 (36.2)	74 (41.8)	19 (37.3)	55 (43.7)	0.159	0.875	0.435
Seizures in hospital[7]	229 (36.2)	50 (37.3)	18 (48.7)	32 (33.0)	0.814	0.128	0.094
Case fatality rate	89 (10.5)	35 (19.3)	16 (30.8)	19 (14.7)	0.001	<0.001	0.013
I:T ratio ≥0.3[8]	0 (0.0)	9 (5.0)	1 (1.9)	8 (6.3)	<0.001	0.059	0.209
Median CRP[9] (IQR)	3 (1-10)	55 (35-80)	33 (10-76)	57 (41-83)	<0.001	<0.001	0.004

[1]Probable sepsis diagnosed as CRP >32 mg/L or I:T ratio ≥ 0.3; [2] Comparing no Sepsis vs EOS (confirmed + probable); [3]Comparing no Sepsis and Culture-confirmed Sepsis; [4]Comparing Probable sepsis vs Culture-confirmed Sepsis; [5]Data was available for: no Sepsis n=838, EOS (confirmed + probable) n=179, Probable sepsis n=128, Culture-confirmed Sepsis n=51; [6] Data was available for: no Sepsis n= 824, EOS (confirmed + probable) n=177, Probable sepsis n=126, Culture-confirmed Sepsis n=51; [7]Data was available for: no Sepsis n=632, EOS (confirmed + probable) n=134, Probable sepsis n=97, Culture-confirmed Sepsis n= 37; [8] Data was available for: no Sepsis n=826, EOS (confirmed + probable) n=180, Probable sepsis n=128, Culture-confirmed Sepsis n= 52; [9] Data was available for: no Sepsis n= 817, EOS (confirmed + probable) n=181, Probable sepsis n=75, Culture-confirmed Sepsis n= 52.

Table 2: Predictors of mortality in newborns with early-onset sepsis (confirmed + probable) and neonatal encephalopathy

	Overall n=181 (%)	**Demised** n=35 (%)	**Survived** n=146 (%)	p value[1]	aOR (95% CI)[2]	p value[3]
Male sex	91 (50.3)	19 (54.3)	72 (49.3)	0.597	-	-
Median birth weight (IQR)	3045 (2770-3465)	2940 (2575-3280)	3089 (2790-3485)	0.028	1.00 (0.99-1.01)	0.628
Median gestation[4] (IQR)	39 (37-40)	38 (37-40)	39 (38-40)	0.055	0.92 (0.67-1.26)	0.607
Normal vaginal delivery	109 (60.2)	23 (65.7)	86 (58.9)	0.460	-	-
HIV-exposed	48 (26.5)	13 (37.1)	35 (24.0)	0.113	3.72 (1.24-11.16)	0.019
Moderate or severe neonatal encephalopathy[5]	109 (61.6)	33 (94.3)	76 (53.5)	<0.001	6.79 (1.15-40.04)	0.034
Therapeutic hypothermia[5]	74 (41.8)	18 (51.4)	56 (39.4)	0.198	0.62 (0.20-1.95)	0.409
Seizures in hospital[6]	50 (37.3)	16 (69.6)	34 (30.6)	<0.001	3.46 (1.11-10.74)	0.032
Culture-confirmed sepsis[7]	52 (28.7)	16 (45.7)	36 (24.7)	0.013	2.03 (0.65-6.31)	0.220
Median CRP (IQR)	55 (35-80)	65 (28-145)	53 (37-76)	0.461	-	-

[1]Comparing Demised to Survived using Chi-squared or Mann-Whitney U test; [2]Logistic regression reporting adjusted odds ratio adjusting for a p value <0.2 in univariate analysis; [3]p value for adjusted odds ratio; [4]Data was available for: Overall n= 179, Demised n= 34, Survived n=145; [5] Data was available for: Overall n= 177, Demised n= 35, Survived n= 142; [6] Data was available for: Overall n= 134, Demised n= 23, Survived n=111; [7]Data was available for: Overall n= 181, Demised n= 35, Survived n= 146.

Supplementary Table 1: ICD-10 codes from a discharge summary database for physician diagnosed neonatal encephalopathy

ICD-10 code	**Overall n=1027 (%)**	**With EOS (confirmed + probable) n=181 (%)**	**Without EOS n= 846 (%)**
P91.6 Hypoxic Ischemic Encephalopathy	801 (77.9)	138 (76.2)	663 (78.4)
G93.4 Neonatal Encephalopathy	195 (19.0)	37 (20.4)	158 (18.7)
P21.1 Mild and Moderate Birth Asphyxia	11 (1.1)	3 (1.7)	8 (1.0)
P21.0 Severe Birth Asphyxia	20 (2.0)	3 (1.7)	17 (2.0)
P21.9 Birth Asphyxia unspecified	0 (0)	0 (0)	0 (0)
P21 Birth Asphyxia	0 (0)	0 (0)	0 (0)
P20.0 Intrauterine hypoxia first noted before onset of labour	0 (0)	0 (0)	0 (0)
P20.1 Intrauterine hypoxia first noted during labour and delivery	0 (0)	0 (0)	0 (0)
P20.9 Intrauterine hypoxia, unspecified	0 (0)	0 (0)	0 (0)

Supplementary Table 2: Prevalence and incidence estimates of newborns with early-onset sepsis (EOS) and neonatal encephalopathy stratified by organisms cultured

	Prevalence[1] (95% confidence interval) n=1027	Incidence per 1000 live births[2] (95% confidence interval) n=78926
Neonatal Encephalopathy (n=1027)		13.0 (12.2-13.8)
Neonatal Encephalopathy with EOS (confirmed + probable) (n= 181)	17.6 (15.3-20.1)	2.3 (2.0-2.7)
Neonatal Encephalopathy with culture-confirmed sepsis[3] (n=52)	5.1 (3.8-6.6)	0.7 (0.5-0.9)
Neonatal Encephalopathy with probable sepsis[4] (n=129)	12.5 (10.6-14.7)	1.6 (1.4-1.9)
Neonatal Encephalopathy without sepsis (n=846)	82.7 (80.21-84.94)	10.7 (10.00-11.5)
Gram positive bacteria (n=30)	2.92 (1.98-4.14)	0.38 (0.26-0.54)
Gram negative bacteria (n=22)	2.14 (1.35-3.22)	0.28 (0.17-0.42)
Group B Streptococcus (n=17)	1.7 (1.0-2.6)	0.22 (0.13-0.35)
Klebsiella pneumoniae (n=10)	1.0 (0.5-1.8)	0.13 (0.04-0.23)
Escherichia coli (n=5)	0.5 (0.1-1.1)	0.06 (0.02-0.15)
Enterococcus faecalis (n=5)	0.5 (0.1-1.1)	0.06 (0.02-0.15)
Acinetobacter baumannii (n=3)	0.3 (0.06-0.85)	0.04 (0.01-0.11)
Listeria monocytogenes (n=3)	0.3 (0.06-0.85)	0.04 (0.01-0.11)
Enterococcus faecium (n=3)	0.3 (0.06-0.85)	0.04 (0.01-0.11)
Pseudomonas (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Serratia marcescens (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Sphingomonas paucimobilis (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Stenotrophomonas maltophilia (n=1)	0.1 (0.03-0.54)	0.01 (0.00-0.71)
Streptococcus milleri (n=2)	0.2 (0.02-0.70)	0.03 (0.00-0.09)

[1]Prevalence was calculated as a proportion of total neonatal encephalopathy cases (n=1027);
[2]Incidence was calculated as per 1,000 live births (total live births for time period 78926);
[3]Confirmed organism on blood and/or cerebrospinal fluid culture; [4] Probable EOS was a CRP >32mg/L or I:T ratio ≥ 0.3.

Figure 1: Newborns with neonatal encephalopathy at or referred to Chris Hani Baragwanath Academic Hospital from January 2016-June 2018

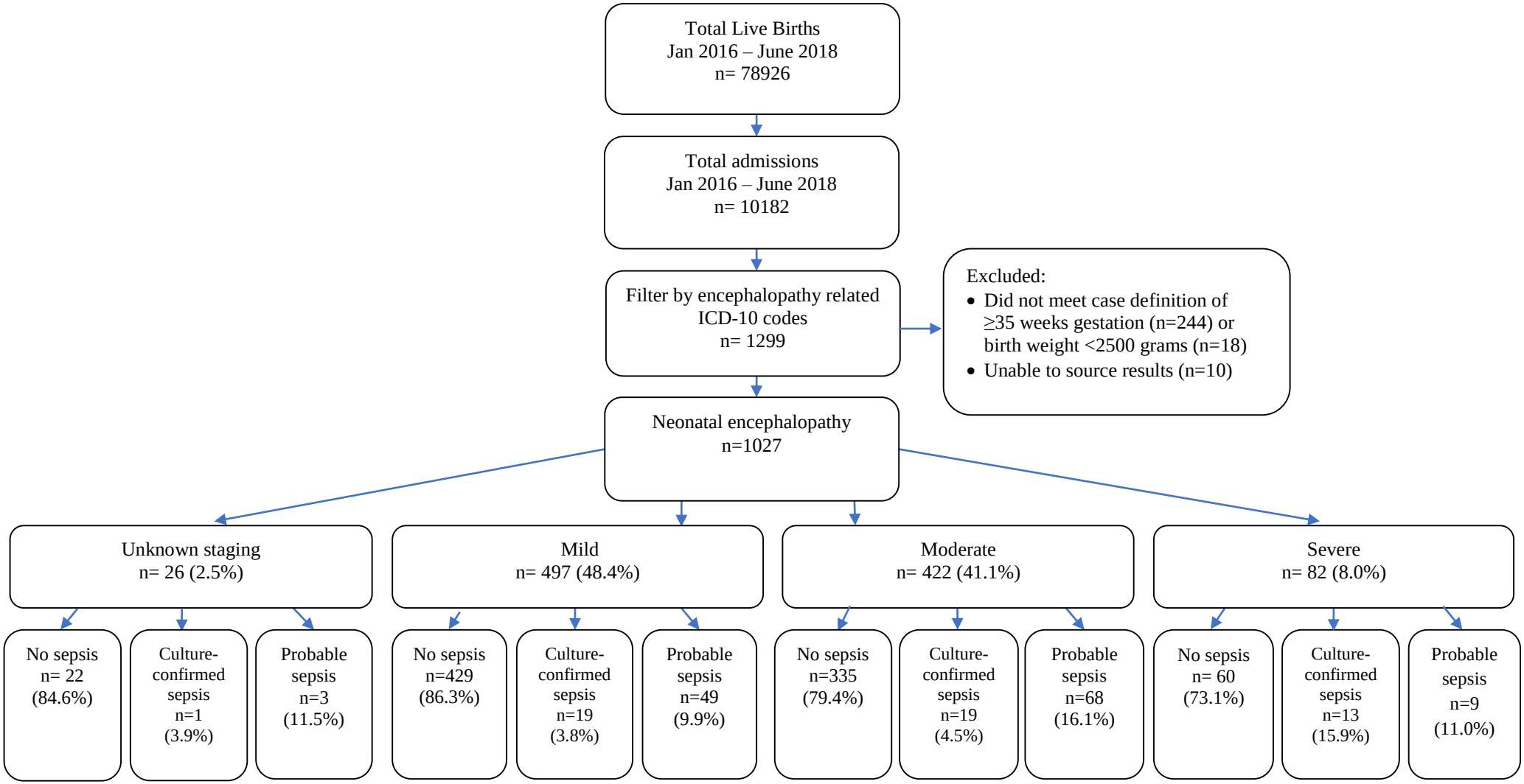
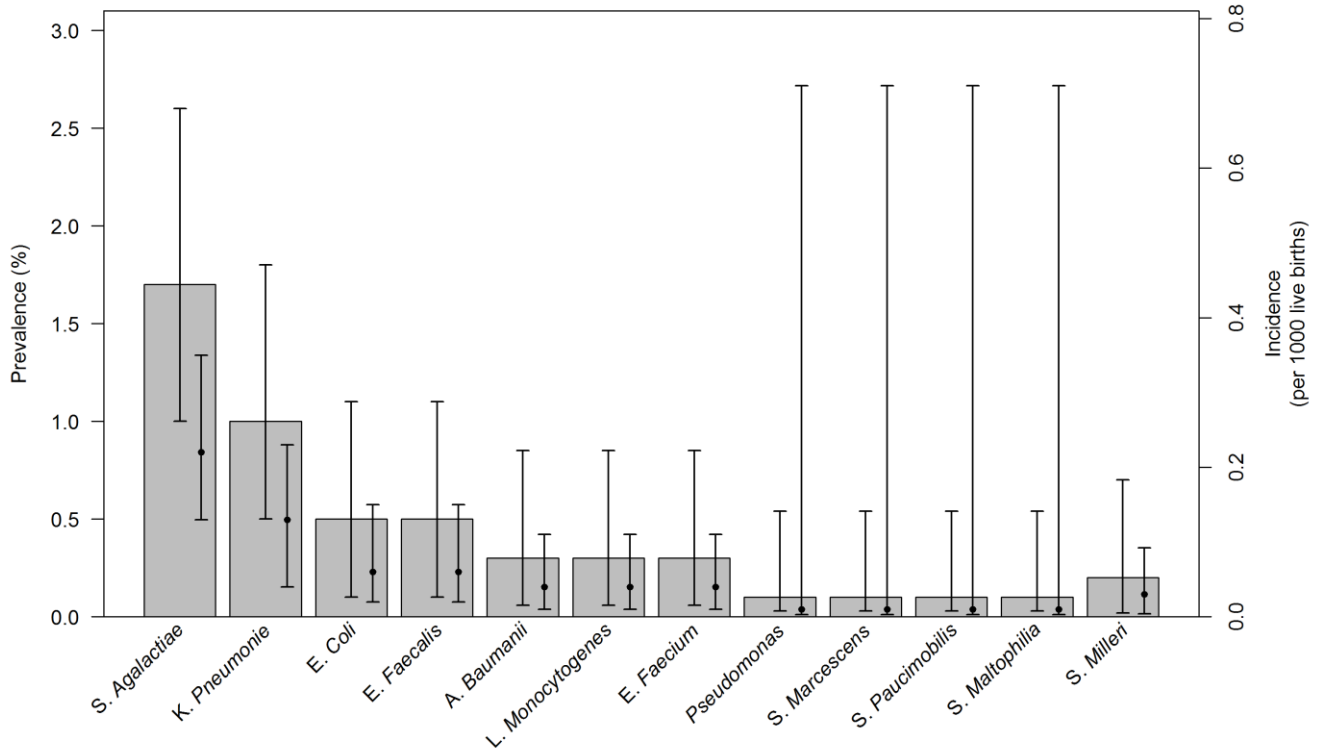


Figure 2: Incidence and organism specific prevalence of newborns with culture-confirmed sepsis and neonatal encephalopathy



MANUSCRIPT 2

NEURODEVELOPMENTAL IMPAIRMENT AT 1-2 YEARS OF AGE IN CHILDREN BORN WITH NEONATAL ENCEPHALOPATHY AND EARLY ONSET SEPSIS

Abstract

Globally, there is a paucity of studies exploring the medium-term neurodevelopmental outcomes in term newborns, born with early onset sepsis and neonatal encephalopathy. In this short report, we describe the neurodevelopmental outcomes (using The Griffith Scales of Mental Development tool) in children at 12 and/or 18-24 months of age that were diagnosed with early onset sepsis and moderate/ severe neonatal encephalopathy at birth. At the 18-24 month visit, NDI was present in 16 (24.6%) children. Visual and auditory impairments were detected in 15 (7.4%) and 3 (1.5%) children respectively. Children with early onset sepsis and moderate or severe neonatal encephalopathy at birth were more likely to have cerebral palsy (36.4% vs 7.3%, p=0.021) compared to those with no sepsis. In conclusion, early onset sepsis in children with moderate or severe encephalopathy is associated with an increased risk of cerebral palsy.

Introduction

Neonatal encephalopathy is estimated to occur in 3.0 per 1000 live births[1], with 99% of all deaths occurring in low middle income countries (LMICs)[2]. Intra-uterine infection increases the risk of neonatal encephalopathy[3], and early onset sepsis (EOS) has been reported in 5-14% of newborns with neonatal encephalopathy in high-income countries[4]. Consequently, 18% of children born with EOS and neonatal encephalopathy had neurodevelopmental impairment (NDI) at 2-3 years of age[4]. Furthermore, the odds of cerebral palsy (CP) is higher (OR: 78.0; 95% CI: 5-406) in children born with EOS and neonatal encephalopathy than in children with only antenatal infection (OR: 7.2; 95% CI: 2.7-20) or intrapartum hypoxia (OR: 2.5; 95% CI: 1-7)[5]. In resource-poor settings with a high burden of EOS, there is limited data on neurodevelopmental outcomes[3,6,7]. We therefore describe the neurodevelopmental outcomes in children at 12 and 18-24 months of age that were born with EOS and neonatal encephalopathy in a large secondary-tertiary care hospital in Johannesburg, South Africa.

Methods

We have previously described the high prevalence (17.6%) of EOS in newborns with neonatal encephalopathy (see manuscript 1). Here, we retrospectively collected neurodevelopmental outcomes data in children at 12 and 18-24 months of age visits that were born with moderate or severe neonatal encephalopathy at or referred to Chris Hani Baragwanath Academic Hospital (CHBAH) between January 2016 and June 2018. Using ICD-10 codes, physician-diagnosed newborns with neonatal encephalopathy were identified from a newborn discharge summary database captured by the Respiratory and Meningeal Pathogens Research Unit. We considered Sarnat stage 1 as mild, stage 2 as moderate and stage 3 as severe neonatal encephalopathy. We defined EOS as a composite of "Culture-confirmed" and "Probable" EOS (see manuscript 1).

Newborns with moderate or severe neonatal encephalopathy were followed up at 12 and/ or 18-24 months of age at a neurodevelopmental clinic run by developmental paediatricians and neonatologists. In this clinic, the Griffith Scales of Mental Development (GMDS) is used to assess development – the GMDS provides a comprehensive developmental profile, highlighting motor, personal-social, cognitive and perceptual developmental skills. The GMDS has been validated for use in South African children and shown to be a culturally fair[8]. At each GMDS assessment, an age equivalent Developmental Quotient (DQ) is calculated. A DQ of 100±15 is considered normal whereas a DQ <85 is considered as NDI. A DQ ≤ 70 is generally accepted as a diagnosis of CP. Physiotherapists, audiologists and occupational therapists are available as the rehabilitation service at the clinic. Children are referred for a hearing test or ophthalmology assessment if clinically indicated.

Using the neurodevelopmental clinic databases (permission was obtained from the gatekeeper), we identified cases of moderate or severe neonatal encephalopathy that completed the 12 month and/or 18-24-month assessment and extracted the following information: Griffith Score, DQ, hearing impairment, visual impairment, cranial ultrasound findings and seizures. We then stratified children into those with EOS (culture-confirmed and probable) and no sepsis. Culture-confirmed sepsis was defined as the identification of pathogenic bacteria (excluding contaminants) on blood and/or CSF within the first 72 hours of life and probable sepsis was defined as a CRP >32 mg/L and/or an immature to total neutrophil ratio ≥0.3 (see manuscript 1).

Categorical variables were reported as proportions and comparisons done using the Chi-squared or Fisher's exact test. Continuous variables were reported as medians and compared using the Mann-Whitney U test. Data was analysed using STATA version 13.1 and a $p<0.05$ was considered statistically significant. The study approved by the University of Witwatersrand Human Research Ethics Committee (HREC number: M181058).

Results

Of 1,027 cases with neonatal encephalopathy at birth, 504 (49.1%) had moderate or severe neonatal encephalopathy. One hundred and fourteen (22.6%) of moderate or severe cases demised in hospital and 187 (47.9%) were lost to follow up (Figure 1). Overall, 203 (52.1%) children attended the NNDC clinic - 184 (90.6%) had moderate and 19 (9.4%) had severe neonatal encephalopathy at birth, and 161 (79.3%) were treated with therapeutic hypothermia (Table 1). Although 121 (66.9%) had seizures in hospital, 69 (34.0%) infants were treated for epilepsy after discharge. Visual and auditory impairments were detected in 15 (7.4%) and 3 (1.5%) children respectively (Table 1).

Of the 203 children, a GMDS was undertaken in 137 (67.5%) children; 72 (52.6%) children were assessed at 12 months, 23 (16.8%) at 18-24 months, and 42 (30.7%) at both visits. At the 12 months and 18-24 months visits respectively, NDI was present in 13 (11.4%) and 16 (24.6%) children. EOS was diagnosed in 45 (22.1%) cases with moderate or severe neonatal encephalopathy at birth (Table 1, Figure 1). Newborns with EOS and neonatal encephalopathy compared to those with no sepsis at birth were not more likely to have cranial ultrasound changes (35.6% vs 30.4%, p=0.780), visual impairment (11.1% vs 6.3%, p=0.552) or hearing impairment (2.2% vs 1.3%, p=0.701). At 18-24 month, 36.4% (n=4) cases with EOS compared to 7.3% (n=4) cases with no sepsis had CP (p=0.021).

Discussion

In this study, we report neurodevelopmental outcomes at 1-2 years of age in term or near-term newborns born with moderate or severe neonatal encephalopathy in a low-middle income setting. We found a high prevalence (24.6%) of NDI at 18-24 months of age in children born with moderate or severe neonatal encephalopathy at birth. Furthermore, albeit our small numbers, children born with EOS and neonatal encephalopathy had a significantly higher prevalence of CP at 18-24 months of age.

Our overall prevalence of CP (12.3%) at 18-24 months was similar to another South African study (11.5%)[9] as well as other high-income countries (10-15%)[7,10]. However, there is a paucity of studies exploring medium-term developmental outcomes in term newborns born with EOS and neonatal encephalopathy[6]. Our prevelence of CP in children with EOS and neonatal encephalopathy is higher than reported in the Netherlands (11.9%)[10], but similar to a systematic review (153 articles, 124 from high income countries) where 36% of newborns with neonatal encephalopathy and sepsis had CP [6].

Limitations of this study are that it was retrospective and many patients were lost to follow up although not dissimilar to another African study that had a follow-up rate of 57% [9]. The low attrition rate of patients at the 12 and 18-24 month visit is because parents are more likely to seek care for children who have developmental concerns. This however would not affect the results comparing newborns with EOS to those without sepsis. Because the diagnosis of EOS is challenging with low blood cultures sensitivity, we included cases with probable sepsis. Furthermore, not all children have GMDS assessment's done at their visits.

In this study, we report a high prevalence of CP in children born with EOS and neonatal encephalopathy. Newborns born with EOS and neonatal encephalopathy should be followed up regularly and screened for NDI to allow for timeous intervention and rehabilitation.

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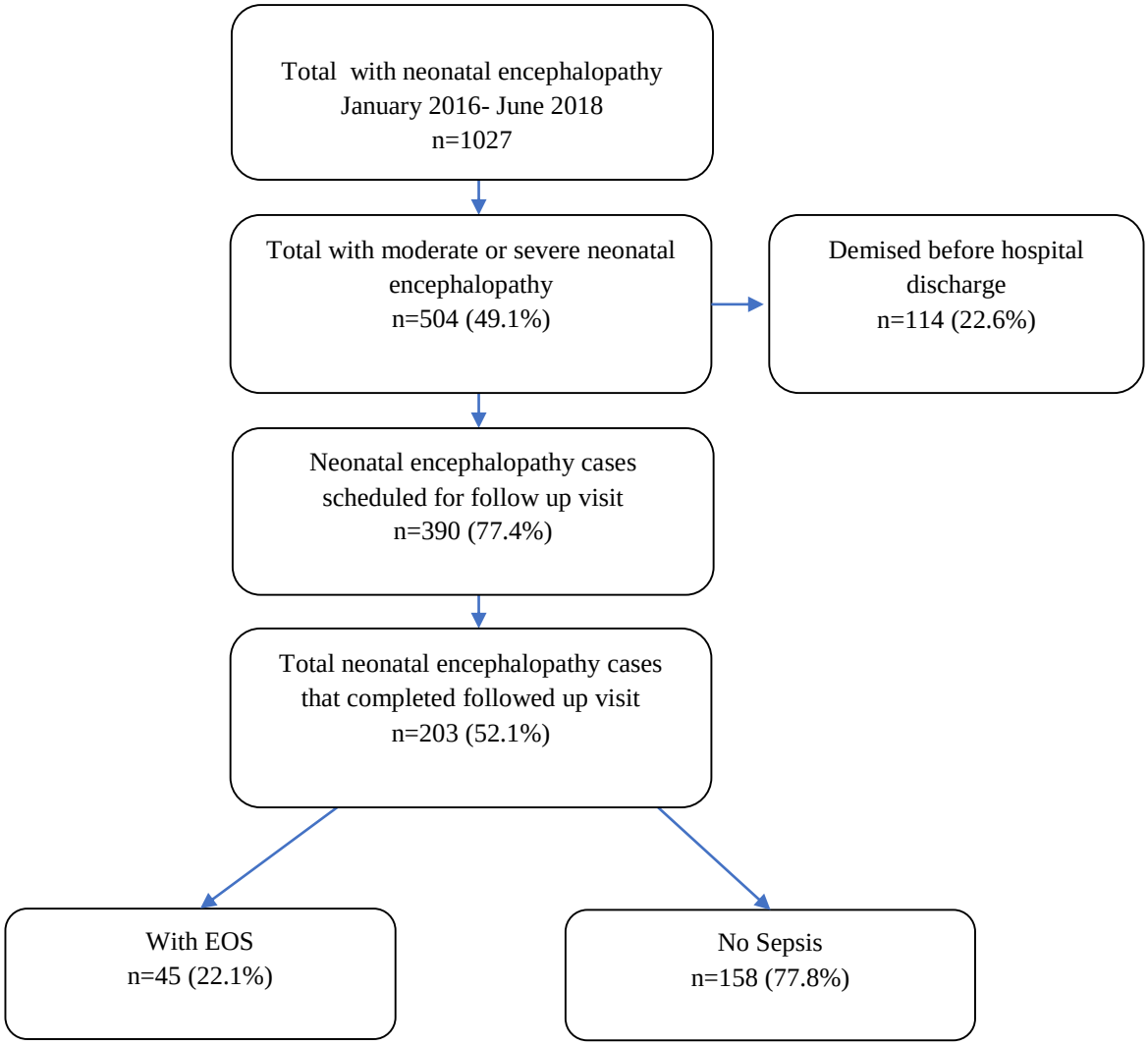
Table 1: Baseline characteristics and neurodevelopmental findings in children born with moderate or severe neonatal encephalopathy

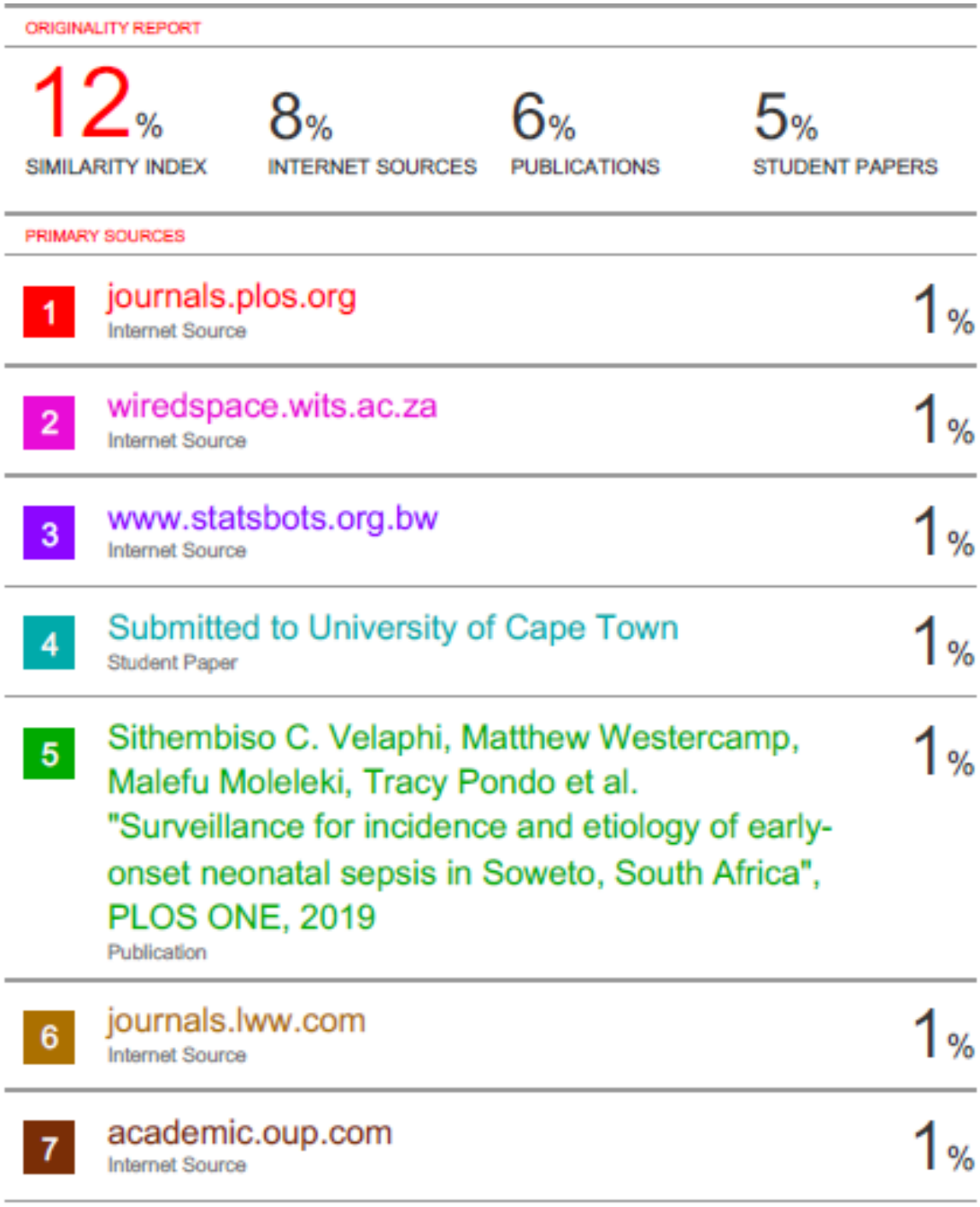
	Overall n=203	**With early onset sepsis** n=45	**No Sepsis** n=158	p value[1]
Median birth weight (IQR)	3200 (2860-3562)	3260 (2835-3500)	3195 (2870-3570)	0.993
Median gestation[2] (IQR)	39 (38-40)	39 (38-40)	39 (38-40)	0.969
Male gender	101 (49.8)	21 (46.7)	80 (50.6)	0.639
Delivery by caesarean	82 (40.4)	19 (42.2)	63 (39.9)	0.777
HIV-exposed	34 (16.8)	7 (15.6)	27 (17.1)	0.808
Seizures in hospital	121 (66.9)	24 (58.5)	97 (69.3)	0.198
Staging[3]				
Moderate	184 (90.6)	39 (86.7)	145 (91.8)	0.300
Severe	19 (9.4)	6 (13.3)	13 (8.2)	
Therapeutic hypothermia				
Yes	161 (79.3)	33 (73.3)	128 (81.0)	0.262
No	42 (20.7)	12 (26.7)	30 (19.0)	
Abnormal cranial sonar				
Yes	64 (31.5)	16 (35.6)	48 (30.4)	0.780
No	98 (48.3)	22 (46.6)	77 (48.7)	
Unknown	41 (20.2)	8 (17.8)	33 (20.9)	
Epilepsy[5]				
Yes	69 (34.0)	15 (33.3)	54 (34.2)	0.683
No	108 (53.2)	26 (57.8)	82 (51.9)	
Unknown	26 (12.8)	4 (8.9)	22 (13.9)	
Visual Impairment[6]				
Yes	15 (7.4)	5 (11.1)	10 (6.3)	0.552
No	153 (75.4)	33 (73.3)	120 (76.0)	
Unknown	35 (17.2)	7 (15.6)	28 (17.7)	
Hearing Impairment[7]				
Yes	3 (1.5)	1 (2.2)	2 (1.3)	0.701
No	163 (80.3)	37 (82.2)	126 (79.8)	
Unknown	37 (18.2)	7 (15.6)	30 (19.0)	
DQ at 12 months	n=114	n=25	n=89	
≤70	8 (7.0)	2 (8.0)	6 (6.7)	0.592
71-84	5 (4.4)	2 (8.0)	3 (3.4)	
85-99	70 (61.4)	16 (64.0)	54 (60.7)	
≥100	31 (27.2)	5 (20.0)	26 (29.2)	
DQ at 18-24 months	n=65	n=11	n=54	
≤70	8 (12.3)	4 (36.4)	4 (7.3)	0.088
71-84	8 (12.3)	1 (9.1)	7 (13.0)	
85-99	36 (55.4)	4 (36.4)	32 (59.3)	
≥100	13 (20.0)	2 (18.1)	11 (20.4)	

[1]Comparing early onset sepsis with no sepsis; [2] Data available for Overall n=201, with early onset sepsis n= 45, no sepsis n=155; [3]Neonatal encephalopathy staging at birth; [4]Cranial ultrasound at six weeks post discharge; [5]On treatment for epilepsy; [6] Vision assessed by ophthalmologist at St Johns Eye Hospital;[7]Hearing assessed by audiologists using the Auditory Brainstem Response Test

Figure 1: Newborns with moderate or severe neonatal encephalopathy and referred to the Neonatal Neurodevelopmental Clinic at Chris Hani Baragwanath Academic Hospital from January 2017 – June 2018



APPENDIX 1: PLAGIARISM DECLARATION AND TURNITIN REPORT



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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

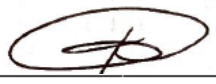
PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Kathleen Car (Student number: 0604562D) am a student registered for the degree of Masters of Medicine (Paediatrics) in the academic year 2017.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 17th September 2020

APPENDIX 2: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr K Car

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M181058

NAME: (Principal Investigator)	Dr K Car
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Neonatal Division Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	The burden of Early Onset Sepsis in neonates with Neonatal Encephalopathy
DATE CONSIDERED:	26/10/2018
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Professor Z Dangor and Dr F Nakwa
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	04/01/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date of the meeting when the study was initially reviewed. In this case, the study was initially reviewed in October and will therefore reports and re-certification will be due early in the month of October each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

KP Car — Principal Investigator Signature

04/1/2019. — Date

APPENDIX 3: MMED RESEARCH PROTOCOL

"The Burden of Early Onset Sepsis in Neonates with Neonatal Encephalopathy"

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Abbreviations

BSID: Bailey Scale of Infant development
BS: Burst Suppression
CHBAH: Chris Hani Baragwaneth Academic Hospital
CFR: Case Fatality Ratio
CLV: Continuous Low Voltage
CP: Cerebral Palsy
CRP: C reactive protein
CSF: Cerebrospinal Fluid
DQ: Developmental Quotient
DNV: Discontinuous Normal Voltage
EOS: Early onset sepsis
FBC: Full blood count
GBS: Group B Streptococcus, *Streptococcus Agalactiae*
GMDS: Griffiths Scales of Mental Development
HIC: High income country
HIE: Hypoxic Ischeamic Encephalopathy
HREC: Human Research Ethics committee
LBW: Low birth weight
LMIC: Low middle income country

NDI: Neurodevelopmental Impairment
NNDC: Neonatal Neurodevelopmental Clinic
NHLS: National Health Laboratory Service
NICU: Neonatal intensive care unit
NICHD: National Institute of Child Health and Human Development
NE: Neonatal Encephalopathy
TICU: Transitional Intensive Care Unit / High care unit
TOBY: Total Body Hypothermia Study
REDCap: Research Electronic Data Capture
RMPRU: Respiratory and Meningeal Pathogens Unit

1.0 Introduction

1.1 Neonatal Encephalopathy

Neonatal Encephalopathy (NE) is defined as a heterogeneous, clinically defined syndrome, characterized by disturbed neurologic function in an infant born at or beyond 35 weeks of gestation. It occurs after birth and is manifested by a reduced level of consciousness or seizures, difficulty with initiating and maintaining respiration, and depression of tone and reflexes (1). NE is estimated to cause 23% of neonatal deaths worldwide, with 99% of these deaths occurring in low middle income countries (LMIC) (2).

Hypoxic Ischemic Encephalopathy (HIE) is defined as cases with NE secondary to an in-utero hypoxia-ischemia event and needs to meet four criteria; the presence of encephalopathy, an umbilical arterial cord pH <7.0 and/or base deficit >12 mmol/l, Apgar scores <5 at 5 minutes, and evidence of multi organ dysfunction (1). HIE is estimated at 1-3 per 1,000 term births in high income countries (3) and 9-13 per 1,000 live births in South Africa (4). Clinical staging systems such as the Sarnat Staging and Thompson Score have been developed to assist clinicians in identifying patients with HIE and NE, and to grade their severity (Appendix 1 & 2) (5,6). The management is largely supportive, however, therapeutic hypothermia is increasingly being recognised as standard-of-care in cases with moderate or severe HIE. Therapeutic hypothermia or ”cooling” is the strategy whereby the core body

temperature is reduced to 33.5-34.5°C for 72 hours, followed by a controlled period of rewarming (3). Therapeutic hypothermia is offered to infants with HIE in accordance with published protocols that were based on large randomized clinical trials such as the TOBY (Appendix 3), ICE and NICHD trials (7,8). Therapeutic hypothermia is able to reduce the combined outcome of death or major neurodevelopmental disability in survivors, with a number needed to treat of 7 (9).

1.2 Early-Onset Neonatal Sepsis

Neonatal sepsis is a clinical syndrome consisting of non-specific signs and symptoms of infection, in the first 28 days of life (10). Early-onset sepsis (EOS) presents within the first 72 hours of life (11), and is primarily the result of vertical acquisition of bacteria from the mother, either transplacentally, ascending infection from the genital tract, or colonization during birth (11,12). In the United States, the incidence of EOS is reported at 0.8-1.0 cases per 1,000 live births, with a case fatality ratio (CFR) of 10.9% (11). Group B *Streptococcus* (GBS) and *Escherichia coli* (*E. Coli*) are the commonest (>70%) cultured organisms for EOS (11,12). The incidence of clinically diagnosed neonatal sepsis in LMICs varies from 4 to 23 per 1,000 live births; with GBS (7-12%), *E. coli* (8% - 19%) and *Klebsiella* species (14% - 25%) commonly cultured (13,14). In South Africa, the incidence of culture-confirmed EOS was reported as 3.4 per 1,000 live births with a CFR of 17% (14). GBS was isolated in 42% of EOS, following with *Streptococcus Viridans* (13%), *Acinetobacter baumannii* (12%) and *E.coli* (9%) (14).

1.3 Diagnosis of EOS

Identification of a pathogen by culture from sterile body fluid (eg: blood or cerebrospinal fluid (CSF)) is considered the gold standard for the diagnosis of EOS. Pathogen detection rates with blood culture samples from infants with sepsis is however low (0.6% to 7.9%) (14). Ancillary investigations may help in the diagnosis of EOS when cultures are negative. Some of these include the full blood count (FBC), C-reactive protein (CRP), Procalcitonin (PCT) and Cytokine profiles (15). From the FBC, the immature-to-total neutrophil ratio (I:T ratio) has the best sensitivity of all the neutrophil indices, but the positive predictive value is only 25%. An I:T ratio (>0.3) is best used to exclude neonatal sepsis, with a negative predictive value of 99% (12). CRP values are best measured at their peak of 24 hours with only 35 - 65% of neonates having a raised CRP (>10mg/L) at the onset of illness (16). Two consecutive CRP values of <10 mg/L, obtained 24 hours apart, have a negative predictive value of 99% (17).

A lumbar puncture (LP) is recommended on neonates with suspected sepsis. Nearly 25% of neonates with blood-culture-positive sepsis have concurrent meningitis (11,12), and up to 38% of neonates with proven meningitis have a negative blood culture (11,12). Diagnosis of neonatal meningitis may be difficult with a considerable overlap between CSF values in neonates, with and without meningitis (17). Adjusting for the number of red blood cells in the CSF is unreliable (11,12) and CSF WBC $\geq$ 21 cells/μL are suggestive of neonatal meningitis (17).

1.4 The association between NE and EOS

There is increasing evidence to show that intra-uterine infection and placental inflammation/ infection are associated with NE in both low and high income settings (18). Inflammation increases the susceptibility of the immature brain to perinatal events that drive the pathogenesis of NE (19). A "dual-hit" hypothesis of infection and hypoxia-ischemia has been described in both high and low income countries (20). It hypothesises that the first insult (maternal infection) renders the perinatal brain more vulnerable to the second (asphyxia) insult (21).

There is a paucity of studies that have described the association between EOS and NE. Studies from HICS report a 5-14% prevalence of EOS among infants with NE (9,22) and an increase in adverse neurodevelopmental outcomes, but not death (22). In the only study undertaken in Africa, maternal and newborn infections were reported as independent risk factors for NE. The strongest associations were seen with foetal inflammation (funisitis) and early neonatal bacteraemia (18). The prevalence of bacteraemia on culture, PCR or both among infants with NE was 3.6%, 6.9% and 8.9% respectively (20). Although early newborn bacteraemia was associated with an eightfold increase odds of NE (20), CFR were not significantly higher for infants with early bacteraemia (44.4%) compared to those without bacteraemia (32.6%) (20).

Maternal intrapartum factors such as chorioamnionitis and prolonged rupture of membranes have been associated with an increased risk (34%) of cerebral palsy (CP) and an increased odds (aOR: 1.5; 95% CI: 1.2-2.0) of early neonatal death (21,23). In high income settings, while antenatal infection (OR: 7.2; 95% CI: 2.7-20) or intrapartum hypoxia (OR: 2.5; 95%

CI: 1-7) individually increase the risk of cerebral palsy, the combination of antenatal infection and hypoxia increase the risk of cerebral palsy many fold greater (OR: 78.0; 95% CI: 5-406) (24).

1.5 Neurodevelopment Impairment Assessment in Children

Neurodevelopment impairment (NDI) occurs in 31% of survivors following intrauterine or neonatal insults, including infections (25). The most commonly reported impairments are learning difficulties and / cognitive and /developmental delay (59%), cerebral palsy (21%), hearing impairment (20%), and visual impairment (18%) (25). In resource-poor settings, there is limited data on NDI following neonatal infections (25).

NDI assessment is complex and multiple childhood development tools exist. Some of the more commonly used tools are the Denver Developmental Scales, Bayley Scale of Infant Development (BSID) and Griffiths Scales of Mental Development (GMDS). The Griffiths Scale provides a comprehensive developmental profile, highlighting motor and personal-social development in addition to the child's cognitive and perceptual skills. In South Africa it has been shown to be a culturally fair and meaningful assessment of the development of children in this setting (26).

1.6 Justification for doing this study

NE contributes to a large burden of deaths and disability worldwide. Studies have shown that EOS is an independent risk factor for NE (20), and when both are present, the risk of cerebral palsy is dramatically increased (24). Despite a high burden of NE in South Africa, the prevalence of EOS in neonates with NE is poorly defined. Furthermore, there is a paucity of data on medium-term outcomes of neonates affected with NE and associated EOS. The aim

of this study, to be conducted in partial fulfilment of a Masters of Medicine in Paediatrics (MMed) degree, is to describe the burden of EOS among neonates born with NE in a large tertiary hospital. Furthermore, this study aims to determine the neurodevelopmental outcomes of neonates with NE and associated EOS.

2.0 Objectives:

1. To determine the prevalence of EOS among neonates with NE.
2. To determine the incidence (per 1,000 live births) and CFR in neonates with NE and associated EOS (culture-confirmed or probable).
3. To determine the proportion of neonates with moderate to severe NE and associated EOS that are managed with therapeutic hypothermia.
4. In the subset of cases that have completed follow-up; to compare the medium-term neurodevelopmental outcomes of neonates with moderate to severe NE and associated EOS versus those with moderate to severe NE without EOS:
    a) The proportion of children with an abnormal Griffiths Score (ie DQ <100) at 12 and 18-24 months
    b) The proportion of children with cerebral palsy at 12 months
    c) The proportion of children with hearing and/or visual impairment at 12 months

3.0 Methods

3.1 Study Design

A retrospective study will be conducted in neonates with NE and associated EOS, born at or referred to CHBAH between January 2016 and June 2018.

3.2 Study Population

There are two public hospitals in Soweto, the CHBAH and district level Bheki Mlangeni District Hospital (BMDH). Approximately 20,000 births occur at CHBAH annually and a further 10,000 births occur at the seven midwife-operated obstetric units (MOU's) within and near Soweto (27). BMDH has limited neonatal services: 20 neonatal beds and 8 Kangaroo mother care beds. Low birth weight (LBW) and presumed ill neonates are assessed and examined by a doctor at the CHBAH. These neonates may be admitted to the neonatal ward, high care/transitional intensive care unit (TICU) or neonatal intensive care unit (NICU). If a neonate is encephalopathic, the neonate will be admitted to TICU or NICU, and investigated for possible causes of the NE. Investigations include an arterial blood gas within the first hour of life, FBC and blood culture. In addition, a Sarnat Staging and Thompson score (Appendix 1 &2) is used to assess the severity of the encephalopathy. Details around the birth, delivery and clinical presentation are discussed with the attending neonatologist to determine whether the neonate would qualify for therapeutic hypothermia using the modified TOBY criteria (Appendix 3). Only those with moderate or severe NE and presumed asphyxia qualify for therapeutic hypothermia (based on available resources) and a lumbar puncture is done on these neonates. In addition, neonates with a positive birth blood culture will get a lumbar puncture irrespective of the diagnosis of NE. A CRP level is also obtained at 24-48 hours.

After discharge, neonates born with moderate or severe NE are followed up at a Neonatal Neurodevelopmental Clinic (NNDC) run by developmental paediatricians and neonatologists, where a paediatric examination and a neurodevelopmental assessment is performed. Neurodevelopmental assessments are undertaken using a Griffiths Mental Developmental Scale. A raw Griffiths Score is calculated to determine the child's developmental quotient

(DQ) for each visit. A DQ score > 100 is considered normal, a DQ < 85 is abnormal and a DQ <75 suggests severe impairment. Physiotherapist, audiologists and other allied rehabilitation services are also available at the NNDC. All patients are referred for a hearing test and ophthalmology examination if clinically indicated.

3.3 Sample Calculation

As this is a retrospective study, no sample size calculation has been done. Based on the available in hospital statistics, the incidence of NE is estimated as 8.8 per 1,000 live births, 60-70% would be moderate or severe NE. In previous studies the prevalence of EOS among infants with NE ranged between 3.6% - 11% in low and high resourced areas (20,22). Therefore, based on a birth rate of 30,000 per annum in this setting, we estimate 660 cases of NE over 2.5 years. Therefore, we estimate 23 to 73 cases of EOS among neonates with NE based on the reported prevalence above. We estimate that at least half of these cases would have completed their 12 months follow up visit.

3.4 Inclusion and Exclusion Criteria

Inclusion criteria	*Exclusion criteria*
All neonates born at or referred to CHBAH	Late onset sepsis (> 72 hours of age)
EOS (< 72hrs) encompassing both: • Culture-confirmed • Probable	Preterm Neonates (<35 weeks)
Encephalopathic neonates (appendix 5)	Contaminants (appendix 5)
Neonates (≥35weeks or ≥2500 grams)	

3.5 Study Method

Objectives 1, 2 and 3:

Using discharge summary information captured by the Respiratory and Meningeal Pathogens Research Unit (RMPRU) (appendix 4) and the CHBAH Neonatal Discharge Summary Research Electronic Data Capture (REDCap)'s existing database (permission will be obtained from the gatekeeper: Prof Madhi and Dr Nakwa respectively), cases of NE from January 2016- June 2018 will be identified.

ICD-10 codes:

- G93.4 Neonatal Encephalopathy
- P21 Birth Asphyxia
- P21.0 Severe Birth Asphyxia
- P21.1 Mild and Moderate Birth Asphyxia
- P21.9 Birth Asphyxia unspecified
- P91.6 Hypoxic ischemic injury of the Newborn
- P20.0 Intrauterine hypoxia first noted before onset of labour;
- P20.1 Intrauterine hypoxia first noted during labour and delivery;
- P20.9 Intrauterine hypoxia, unspecified

Variables that will be extracted from this database are listed in Appendix 4. By cross referencing the RMPRU database, REDCap database, NE register from the CHBAH Neonatal Department and NNDC database, we shall attempt to ensure that cases of NE have not been missed. For each case of NE, laboratory results (FBC, CRP, Blood cultures, CSF cultures, cells and chemistry) will be obtained from the RMPRU database, REDCap database and National Health Laboratory Service Track-Care^{TM} system. Cases of NE will then be stratified into culture-confirmed EOS, probable EOS or no sepsis. EOS, for this study, will be considered the sum of probable and culture-confirmed EOS (Appendix 6).

Culture-confirmed EOS:

- Blood or CSF culture positive

Probable EOS:

- In the absence of culture confirmation, a neonate with any of the following laboratory markers.

  i. CRP > 10mg/L,
  ii. WCC>30x10^9/L or <5x10^9/L,
  iii. Neutropenia with absolute neutrophil count < 1.8 x10^9/L
  iv. Immature to total neutrophil ratio (I:T) >0.3
  v. Elevated CSF WCC > 21
  vi. CSF glucose <1.7mmol

To determine CFR, demised cases will be identified through the neonatal department mortality records, the RMPRU database, REDCap database and the NE registry. Neonates with moderate to severe NE that are managed with therapeutic hypothermia will be identified through the RMPRU database, REDCap database, NNDC and NE database (permission will be sought from the gatekeeper: Dr Nakwa)

Objective 4:

Medium-term neurodevelopmental outcomes of neonates with moderate to severe NE with or without EOS will be obtained from the NNDC and NE database. The assessments of these cases have already been done and captured in the NNDC and NE database. For the subset of neonates that have completed NNDC follow up, the following variables will be analysed at the 12 month visit and 18-24 month visit: age, Griffiths Score, presence of cerebral palsy, hearing impairment, and visual impairment (Appendix 4). At each visit, a raw Griffiths Score is calculated and correlated to age equivalent scores to obtain the Developmental Quotient (DQ). Developmental quotients are interpreted as follows:

- Normal DQ > 100 (115-85 is within normal limits)

- Abnormal DQ < 85
- Severe Impairment (CP): DQ < 75

3.6 Data Analysis

The prevalence of EOS (culture confirmed and probable) will be calculated as a proportion of neonates born with NE. Incidence will be calculated as the number of NE and associated EOS cases per 1,000 live births. The birth rates are captured by the RMPRU from available maternity registries at the CHBAH and MOUs. The CFR will be calculated as the number of deaths divided by the total number of neonates with NE and associated EOS. For objective 3, the proportion of neonates with moderate to severe NE and associated EOS that are managed with therapeutic hypothermia will be calculated as a proportion of all moderate to severe NE cases, and as a proportion of all NE cases.

Neurodevelopmental outcomes (abnormal Griffith's Scores (i.e DQ <100), cerebral palsy, visual or hearing loss) in cases with NE and associated EOS will be compared with cases of NE without sepsis. Data will be imported into the statistical program STATA for data analysis and used to calculate the mentioned measurable outcomes. Categorical variables with be reported as proportions. Comparison between categorical variables will be done using the Chi-squared or Fishers' exact test where appropriate. Continuous variables will be reported as means or medians depending on the distribution of the data. The Students t-test will be used to compare means whereas the Mann-Whitney U test will be used to compare medians. 95% confidence intervals and $p<0.05$ will be considered statistically significant.

4.0 Ethics

As the study is retrospective, there are no risks for participants. Participants in the study will be identified for study purposes with a unique numerical identifier. Participants will incur no

extra costs based on participation in the study. No additional study-specific visits are required.

This protocol will be submitted for approval to the Medical Advisory Committee (MAC) at the CHBAH, the University of the Witwatersrand Human Research Ethics committee (HREC), and registered on the National Health Research Database prior to initiation of the study. Permissions will be sought from Professor Madhi for the RMPRU summary database and from Dr Nakwa for the use of the CHBAH Neonatal Discharge Summary (REDCap) database, NNDC statistics and neonatal death records. A summary of results will be reported to the HREC on completion of the study. Results may be presented at professional clinical meetings and national or international scientific meetings. Results will be submitted for publication in a peer-reviewed journal.

5.0 Anticipated Problems

It is expected that approximately 5% of discharge summaries from the RMPRU database will be missing from an estimated 4000 annual admissions. Discharge summaries are completed by medical doctors ranging from interns to registrars and there may be missing data. Patients may also be lost to follow up and there may be missing data on neurodevelopmental outcomes. A small sample size will also limit data analysis.

6.0 Gant chart

	01/18 – 09/18	09/18-10/18	10/18 – 04/19	04/19– 07/19
Literature review				
Preparing Protocol				
Protocol assessment				
Protocol Revisions				
Ethics Application				
Collecting data				
Data Analysis				
Writing up – thesis				
Writing up Paper				

7.0 Funding

Photocopying & Printing	800
Transport	1000
Total	1800

This will be a self-funded study.

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9.0 Appendices

Appendix 1: Sarnat Stages of Hypoxic Ischaemic Encephalopathy (5)

	Stage I	Stage II	Stage III
Level of consciousness	Hyperalert; irritable	Lethargic or obtunded	Stuporous, comatose
Neuromuscular control Posture Muscle tone Stretch reflexes/ Deep tendon reflexes	 Overeactive Mild distal flexion Normal Overactive	 Diminished Strong distal flexion Mild hypotonia Overactive	 Diminished or absent Intermittent decerebration Flaccid Decreased or absent
Primitive reflexes Suck Moro Grasp	 Normal Weak Strong, low threshold	 Suppressed Weak or absent Weak, incomplete high threshold	 Absent Absent Absent
Autonomic function Pupils Respirations Heart Rate	Sympathetic Mydriasis Spontaneous Tachycardia	Parasympathetic Miosis Spontaneous, occasional apnoea Bradycardia	Both systems depressed Midposition, often unequal; poor light reflex Periodic, apnoea Variable
Seizures	None	Common (onset 6-24 hrs of age)	Uncommon (excluding decerebration)

Appendix 2: Thompsons Score (6)

Sign	0	1	2	3
Tone	Normal	Hypertonic	Hypotonic	Flaccid
Level of Consciousness	Normal	Hyperalert / stare	Lethargic	Comatose
Fits	None	< 3 per day	> 2 per day	
Posture	Normal	Fisting, Cylcing	Strong distal flexion	Decerebrate
Moro	Normal	Partial	Absent	
Grasp	Normal	Poor	Absent	
Suck	Normal	Poor	Absent, bites	
Respiration	Normal	Hyperventilation	Bried Apnea	IPPV (apnea)
Fontenel	Normal	Full not tense	Tense	

- Mild NE : Thompsons score (TS) 6-10,
- Moderate NE : TS 11-14
- Severe NE is a TS > 15

Appendix 3: CHBAH Neonatal Unit Therapeutic Hypothermia Criteria (Modified TOBY Cooling Criteria)

- Born at ≥34 weeks and/or weighing ≥ 2000 grams.
- Apgar score ≤ 5 at 10 minutes or continued need for resuscitation (including endotracheal and bag mask ventilation) for >10 minutes.
- Metabolic acidosis with pH <7.00 or base deficit ≥16 mmol/L based on an arterial blood gas done within 60 minutes of life
- Thompson score ≥ 11
- Encephalopathy present, defined as:
- Lethargy or stupor or coma and at least one of the following:
    - Hypotonia
    - Abnormal reflexes
    - Absent or weak suck
    - Clinical seizures

Category	Moderate Encephalopathy	Severe Encephalopathy
Spontaneous Activity	Decreased activity	No activity
Posture	Distal flexion, complete extension	Decerebrate
Tone	Hypotonia focal or general	Flaccid
Primitive Reflexes	Weak suck or incomplete Moro	Absent suck or Moro
Autonomic Nervous System	Constricted pupils, bradycardia, or periodic/irregular breathing	Deviated/ dilated/ non-reactive pupils, variable HR or apnoea

MUST have at least *ONE* finding in at least *THREE* categories.

- Abnormal amplitude-intergrated electroencephalography (aEEG) for at least 30 minutes. Abnormalities could be any of the following
    - Moderate abnormal background (upper margin of the band above 10μV and lower margin below 5 μV)
    - Severe abnormal background (upper margin of the band below 10μV and lower margin below 5 μV)
    - Normal background with seizure activity
    - Or a Discontinuous normal voltage (DNV) or Burst Suppression (BS), or Continuous Low Voltage (CLV) (moderate to severe tracing (28)

Appendix 4: Variables extracted from RMPRU database

1. Patient related information
    a. Birth Weight
    b. Birth Length
    c. Birth Head Circumference
    d. Gestational Age
    e. Chronological Age
    f. Gender: Male/Female
    g. Date admission
    h. Date of death/discharge/transfer
    i. Route of delivery: NVD/ Caesarean Section
        i. NVD: Foreps/Vacuum/ Breech
        ii. Caesarean Section: Foetal Distress/ Maternal Indication
    j. Apgar: 1 min/5min/10 min
    k. Baby PCR: Done/not done
    l. Baby NVP: yes/no/unknown/Not Applicable
    m. Baby PCR:Positive/ Negative/Unknown
    n. Feeding Type: breast/formula
2. Maternal Details:
    a. Maternal Age
    b. Parity/Gravidity
    c. Antenatal Care: Y/N
    d. Number of babies with this pregnancy: singleton/twins/triplets/other
    e. Maternal TB
    f. Maternal Diabetes
    g. Maternal Hypertension
    h. Antenatal Magnesium Sulphate
    i. Chorioamnionitis
    j. Prolonged Rupture of Membranes
    k. Intrapartum antibiotics
    l. Maternal Steroids
    m. RH: Pos/Neg/unknown
    n. WR: Pos/Neg/Unknown
    o. RVD: Pos/Neg/Unkown
    p. CD4:
    q. ARVS: FC/HAART/PMTCT only/None
    r. Antenatal Steroids: yes/no/N/A
3. Diagnosis / Problem List (listed with ICD-10 codes)
4. Underlying conditions (listed with ICD-10 codes)
5. Admitted in: TICU/NICU/Ward 66
6. Respiratory Support: NCPAP/IPPV/HFOV
7. Immediate Care
    a. Resuscitation at birth: yes/no
    b. Bag mask ventilation without o2/Bag mask ventilation with 02/ intubated/chest compressions/adrenalin/ time to first breath min
    c. ABG: yes/no
    d. Blood gas: ph<1 hr/CO2 < 1 hr/p02<1hr/BE<1hr/lactate/glucose
    e. Lowest temp on admission (Celsius)/ lowest temp within 1st hour of admission (Celsius)

Infections

f. Leucopenia < 5 first 3 days
g. Leucocytosis >25 first 3 days
h. Total white cell count
i. Absolute Neutrophil Count
j. Immature cells Absolute Count
k. Immature cell to Total neutrophil ratio
l. CRP > 10 first 3 days
m. CSF WCC
n. CSF glucose
o. Blood Culture organism
p. CSF Culture organism

8. Major Systemic Findings/examination on D/C
    a. CNS: normal/ hypotonic/ hypertonic
    b. Thompsons Score
    c. Induced Hypothermia: Y/N
    d. Abnormal tests on D/C: Y/N –listed on D/C summary
    e. Hearing test: Y/N/N/A
        i. If yes: findings
9. Outcome: Early Sepsis/IVH/Asphyxia/
10. Central Nervous System
    a. NE
        i. Stage Mild/ Moderate/ Severe
    b. HIE: Y/N
        i. Stage: 1/2/3
    c. IVH:
        i. Grade 1/2/3/4
    d. Seizures: Y/N
        i. Medication: phenobarbitone/phenytoin/lorazepam/dormicum/lignocaine/keppra/topamax/pyridoxine
    e. Hydrocephalus: yes/no
        i. Congenital/post-haemorrhagic/post infective
    f. Induced Hypothermia: Y/N/
    g. Imaging: Sonar/CT Scan/ MRI/Cranial Sonar
    h. Cranial Sonar Findings
    i. Periventricular Leukomalacia: Y/N
    j. Subapneurotic haemorrhage: Y/N
    k. aEEG findings
        i. Not done/normal/continous/discontinuous/burst suppression/low voltage/isoelectric
        ii. Indication: Seizures/cooling/ asphyxia/brain death
        iii. If asphyxia (asphyxia aEEG findings)
            1. Before cooling: normal/continuous/discontinuous/burst suppression/low voltage/isoelectric
            2. At removal of cooling: normal/continuous/discontinuous/burst suppression/low voltage/isoelectric
        iv. Congenital abnormality: yes / no

11. Metabolic/Endocrine
    a. Hypoglycaemia (<2.6mmmol)
    b. Hyperglyceamia (>8.5mmol)

Variables extracted from Neurodevelopmental Clinic database

    c. Age
    d. Griffiths Score
    e. Hearing abnormality
    f. Vision Abnormality
    g. CP: Y/N

Appendix 5: Definitions

1. Neonatal Encephalopathy

Neonatal Encephalopathy (NE) is defined as a heterogeneous, clinically defined syndrome, characterized by disturbed neurologic function in an infant born at or beyond 35 weeks of gestation. It occurs after birth and is manifested by a reduced level of consciousness or seizures, difficulty with initiating and maintaining respiration, and depression of tone and reflexes (1).

2. Hypoxic Ischemic Encephalopathy

Hypoxic Ischemic Encephalopathy (HIE) is defined as cases with NE secondary to an in-utero hypoxia-ischemia event and needs to meet four criteria:

1. The presence of encephalopathy,
2. An umbilical arterial cord pH <7.0 and/or base deficit >12 mmol/l,
3. Apgar scores <5 at 5 minutes,
4. Evidence of multi organ dysfunction (1).

3. Cerebral Palsy

Cerebral palsy (CP) is a disorder of the development of movement and posture, causing activity limitations attributed to nonprogressive disturbances of the fetal or infant brain that may also affect sensation, perception, cognition, communication, and behaviour (29). There are many different modalities to asses children that may have cerebral palsy. For the sake of this study we will consider a child affected with cerebral palsy as scoring a DQ <75

4. I:T ratio

The immature to total neutrophil count is a neutrophil indices used to diagnose early onset sepsis. It is calculated as the absolute number of immature cells divided by the absolute neutrophil count to get a ratio. An I:T ratio >0.3 has a negative predictive value of 99% that there is no sepsis (12).

5. Contaminants

Blood cultures will be considered contaminated if:

- Flagging positive after 48-72 hours
- The following organisms are cultured:

- Coagulase-negative staphylococci (CoNS) - a skin commensal
- Micrococcus species
- Bacillus species
- Corynebacterium species
- Streptococcus viridans

6. Case Fatality Ratio

Case Fatality Ratio (CFR) is defined as the proportion of deaths within a designated population of "cases" (people with a medical condition) over the course of the disease. For this study we shall calculate CFR as the number of Neonates with NNE and associated EOS that have died divided by the total number of affected neonates with NNE and associated EOS.

Appendix 6: Flow Diagram Showing Expected Sample Sizes & Proposed Statistical Analysis from Jan 2016 – June 2018