REVIEW OF RISK FACTORS AND SURVIVAL FOR LATE ONSET SEPSIS IN VERY-LOW-BIRTH-WEIGHT INFANTS

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Declaration

I, Koki Octovia Mashile, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Paediatrics at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature of candidate

14th day of April 2022 in Johannesburg

Acknowledgements

I would like to give a heartfelt thanks to my Supervisors Prof Ballot and Dr Saggers for their guidance and unwavering support. Many thanks are also extended to the staff at Charlotte Maxeke Johannesburg Academic Hospital involved in the data collection for the neonatal unit.

Dedication

I dedicate this work to my mother, who worked tirelessly to give me a better life.

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Abbreviations

ANC:	Antenatal care
B/C:	Blood culture
BMV:	Bag Mask Ventilation
CLD:	Chronic Lung Disease
CMJAH:	Charlotte Maxeke Johannesburg Academic Hospital
CMV:	Conventional Mechanical Ventilation
CoNS:	Coagulase Negative Staphylococcus
C/S:	Caesarean Section
EOS:	Early Onset Sepsis
HIV:	Human Immunodeficiency Virus
ICD:	Intercostal Drainage
IV:	Intravenous
IVH:	Intraventricular Haemorrhage
KMC:	Kangaroo Mother Care
LMICS:	Low-and-middle-income countries
LOS:	Late Onset Sepsis
NCPAP:	Nasal Continuous Positive Airway Pressure
NEC:	Necrotizing enterocolitis
NNJ:	Neonatal Jaundice
NNS:	Neonatal Sepsis
NVD:	Normal Vaginal Delivery
PDA:	Patent Ductus Arteriosus
PTT:	Phototherapy
RDS:	Respiratory Distress Syndrome
ROP:	Retinopathy of Prematurity
SA:	South Africa
TPN:	Total Parenteral Nutrition
VLBW:	Very Low Birth Weight

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Submissible paper

Review of risk factors and survival for late onset sepsis in very-low-birth-weight infants

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Abstract

Background: Despite advances in neonatal care that have improved survival of very-low-birth-weight (VLBW) infants, neonatal sepsis (NNS) remains a common cause of morbidity and mortality.

Objectives: To review risk factors and short-term outcomes of late onset sepsis (LOS) in VLBW infants at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), South Africa.

Methods: This was a secondary analysis of an existing database of VLBW infants admitted at CMJAH from 1st January 2014 to 31st December 2018. Characteristics of infants with LOS were compared with those infants without LOS to elicit factors associated with LOS.

Results: A total of 1974 VLBW infants were enrolled for the study and 698/1974 (35.4%) had LOS. Factors associated with LOS after multivariate logistic regression were patent ductus arteriosus (PDA) ($p = 0.038$), blood transfusion ($p = <0.001$), necrotising enterocolitis (NEC) ($p = 0.025$), surgery for NEC ($p = 0.013$) and surgery for any other reason ($p = 0.043$). The mortality rate was increased in infants with LOS compared to those without LOS, 31.23% versus 16.38% ($p < 0.001$). There were 901 episodes of sepsis in 698 infants with LOS. The majority of the sepsis episodes were caused by Gram-positive organisms 450/901 (49.9%). Gram-negative and fungal organisms were responsible for 353/901 (39.2%) and 98/901 (10.9%) of the LOS respectively.

Conclusion: A third of VLBW infants in the study developed LOS. Factors associated with LOS in VLBW infants are NEC, surgery, PDA and blood transfusion. Mortality was increased in those VLBW infants with LOS. Sepsis caused by Gram negative organisms is associated with increased mortality.

KEYWORDS: Neonatal sepsis, very low birth weight, risk factors, late onset sepsis.

Introduction

Neonatal sepsis (NNS) is a common cause of morbidity and mortality worldwide, especially in low-and-middle-income countries (LMICs).[1] In South Africa, (SA), NNS accounts for 11.6% of all neonatal deaths making it the third leading cause of neonatal deaths.[2] In studies done in Ethiopia, Cameroon and Central Vietnam, NNS accounted for 31.0%, 32.0% and 37.8% of neonatal deaths respectively.[3, 4, 5]

NNS is classified as early-onset sepsis (EOS) or late onset sepsis.[6, 7, 8] LOS – which is more common than early onset sepsis (EOS) – presents after 72 hours of life.[6, 7] In a South African study done at a tertiary hospital in 2012, the incidence of NNS was 10.3 per 100 admissions, of which LOS accounted for 83.7% of cases of NNS.[9] LOS is from organisms acquired in the hospital or the community (for infants delivered at home or discharged home).[1, 8] LOS often occurs while the infants are in the neonatal unit and the infection may be transmitted by direct contact with hospital personnel, parents, visitors – most commonly through hand contamination or contaminated equipment.[1]

The group mostly affected by LOS are VLBW infants.[1,8] A number of factors predispose VLBW infants to infection, such as: immunological immaturity, multiple invasive procedures, prolonged intravenous (IV) access, mechanical ventilation and hospital stay, use of total parenteral nutrition or other invasive procedures.[1,8] Organisms implicated in LOS are fungi and bacteria – the latter being more common. In a study done at CMJAH retrospectively for 2012, bacterial infection was the cause of LOS in 89.8% of cases and fungi were responsible for 10.1% of cases of LOS.[9] Organisms causing NNS change over time, dependent on the environmental pathogens.[9] This is evident in two studies done at CMJAH at different periods of time, ten years apart.[9, 10] Motara *et al*. reported Gram positive organisms being the predominant cause of LOS and years later Lebea *at al*. reported Gram negative organisms as the commonest cause.[9,10] LOS is associated with increased mortality and neurodevelopment delay.[1]

LOS and its outcomes increase hospital costs which becomes a serious burden in LMICS. Risk factors for NNS are clearly documented in the literature but there are few studies done, especially in Africa, that look into risk factors of LOS in VLBW infants. It is important to know which factors play a major role in causing LOS so that infection prevention and control

measures can be implemented or revised to reduce the incidence of these factors. Therefore, the aim of this research is to describe the risk factors associated with LOS in VLBW infants in Johannesburg, SA.

Methods

This was a secondary analysis of an existing database performed at CMJAH neonatal unit. CMJAH is a central referral hospital in Johannesburg, SA. The study population included all VLBW infants admitted within 48 hours of life from the 1st January 2014 to 31st December 2018. Infants with birth weight less than 500g and those who died within the first 72 hours of life were excluded.

LOS was defined as positive blood culture of organisms deemed to be significant after 72 hours of life. Organisms cultured but considered to be contaminants were *Bacillus Spp*, *Corynebacterium Spp* and *Streptococcus viridans*. Patients with the above-mentioned organisms cultured were included in the group without LOS. Although there is lack of consensus on the role of Coagulase Negative Staphylococci (CoNS) in NNS, these were considered significant pathogens in this study.[11,12]

The following definitions were used in the study:

- Necrotising enterocolitis was defined as NEC II or III according to the Bell's staging criteria.[13]
- Resuscitation at birth was defined as the need for ventilation with bag-valve-mask or T-piece resuscitator or chest compressions.
- Chronic lung disease (CLD) was defined as persistent oxygen requirements up to or beyond 28 days of chronological age.
- Retinopathy of prematurity (ROP) was defined as ROP stage 3 or 4 according to the International Committee for the Classification of ROP.[14]
- Invasive respiratory support included conventional mechanical ventilation and high frequency oscillator ventilation.
- Non-invasive respiratory support included modes of ventilation that do not require the patient to be intubated, such as nasal continuous positive airway pressure (NCPAP) and high flow nasal cannula oxygen.

- A workup to exclude sepsis was done in infants who had clinical symptoms and signs of infection and post-surgery. Empiric antibiotics were initiated when sepsis was suspected and antibiotics were targeted when culture results were available.
- Other surgery included all surgery excluding NEC surgery.

Ethics

The research protocol was approved by the Human Research Ethics Committee of the University of Witwatersrand. Ethics clearance certificate number M190862.

Database

The neonatal records were retrieved from the REDCap (Research Electronic Data Capture) database, hosted by the University of Witwatersrand.[15] The information was captured at discharge for all infants admitted to the neonatal unit and for the purpose of quality control was verified at several stages of collection. Permission was granted by the host of the REDCap database at the neonatal unit of CMJAH to access the data.

Data Analysis

Data was entered into an MS Excel (version 2013) spreadsheet for data cleaning and imported to IBM SPSS (version 25) for analysis. Continuous variables were described using mean and standard deviation for the variables with a normal distribution. Median and interquartile ranges were used for variables with non-normally distributed data. Categorical variables were described using frequencies and percentages. The study population was divided into two groups, one with LOS and the other with no LOS; associated factors and outcomes were compared between the two groups. Continuous variables were compared using the Student's t-test or Mann-Whitney U analysis. Categorical variables were compared using the Chi-squared analysis. Only valid cases were analysed for each variable (i.e., missing values were excluded). A *p*-value of less than 0.05 was considered significant. A binary logistic regression was done considering LOS as the outcome variable, to determine adjusted odd ratios for factors associated with LOS.

Results

A total of 2435 VLBW (<1500g) infants were admitted during the study period, 1 974 infants fulfilled the inclusion criteria (see Figure 1); 35.36% (698/1974) developed late onset sepsis.

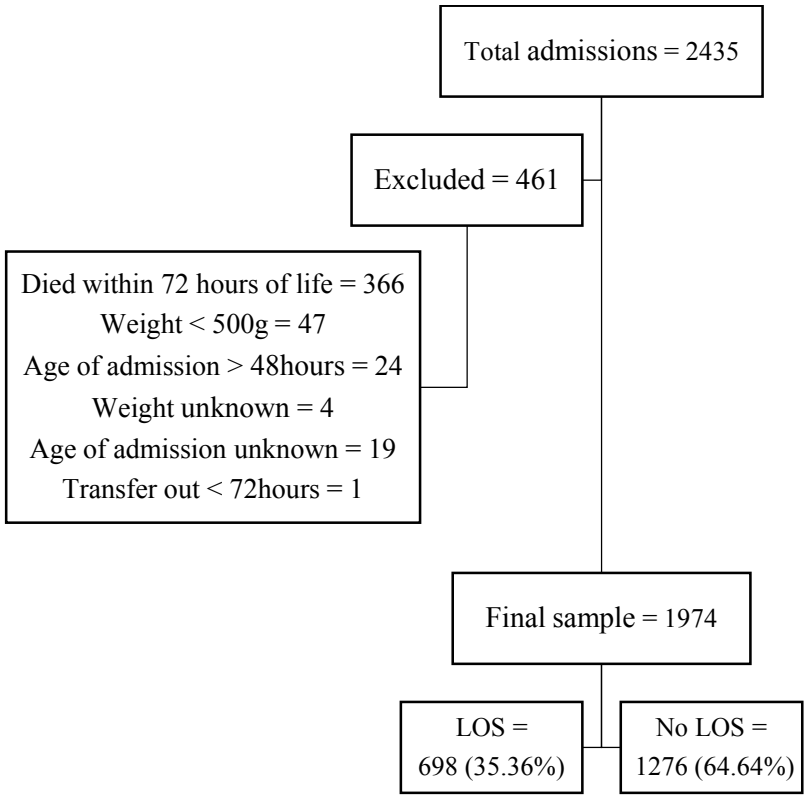

Figure 1: Study participants included in the review of risk factors and survival for late onset sepsis in very low birth weight infants admitted to Charlotte Maxeke Johannesburg Academic Hospital, 1st January 2014 – 31st December 2018

Patients' characteristics are summarized in Table 1 (below). The majority of patients were inborn (n = 1664/1973, 84.33%) and delivered via caesarean section (n = 1129/1955, 57.75%). Most patients were female (n = 1067/1967, 54.25%). The median maternal age was 28.0 years (IQR 24.0 – 33.0 years). The median gravidity was 2 (IQR 2 – 3). There was an antenatal care attendance rate of 78.72% (n = 1554/1974). Antenatal steroids were received in 53.24 % of cases (n = 1051/1974). 30.50 % (602/1974) of mothers tested positive for HIV.

Table 1: Characteristics of very-low-birth-weight infants included in the study admitted to Charlotte Maxeke Johannesburg Academic Hospital, 1st January 2014 – 31st December 2018

Clinical characteristics	VLBWI included (n=1 974) (Median, IQR)
Gestational age, weeks	29 (28-31)
Birth weight, grams	1150 (960-1320)
Duration of non-invasive ventilation, days	2 (1-5)
Duration of invasive ventilation, days	4 (2-8)
Length of hospital stay, days	31 (19 -49)

VLBWI = very-low-birth-weight infants, IQR = inter-quartile range

Resuscitation in the delivery room was required in 863/1974 (43.72%) of the patients. Respiratory distress syndrome (RDS) was diagnosed in 1748/1974 (88.55%) of the patients, 1392/1974 (70.52%) required NCPAP at some time during their admission. There were 619/1974 (31.36%) infants with CLD, of which 394/619 (63.65%) of the patients received postnatal steroids. NEC occurred in 175/1974 (8.87%) and PDA was diagnosed in 213/1974 (10.79%) of the infants. Of the infants screened for ROP, 108/670 (16.12%) developed ROP. The overall survival of the VLBW infants was 78.42 % (1548/1974).

The total sample was divided into two groups, those with LOS and those who had no LOS. The variables were compared between the groups. The significant variables relating to the development of LOS are summarized in Table 2 (below).

Table 2: Factors significantly associated with outcome (LOS vs. No LOS) in very low birth weight infants admitted to the neonatal unit at Charlotte Maxeke Johannesburg Academic Hospital, 1st January 2014 – 31st December 2018

Variable	LOS	NO LOS	P-value	OR	95% CI
Patient characteristics					
Birth weight, grams, (median, IQR)	1060 (900- 1220)	1207 (1020 – 1360)	<0.001	0.99	0.99-1.00
Gestational age, weeks, (median, IQR)	27 (27- 30)	28 (28-31)	<0.001	0.86	0.82-0.89
Mode of delivery, n/N (%): NVD C/S	319/692 (46.10) 373/692 (53.90)	510/1263 (40.38) 753/1263 (59.62)	0.014	0.78	0.65-0.95
5-minute Apgar score, (median, IQR)	8 (7- 9)	9 (7 -9)	<0.001	0.91	0.87-0.96
Resuscitation at birth, n/N (%)	345/698 (49.43)	518/1276 (40.60)	<0.001	1.43	1.18-1.72
Respiratory conditions, interventions and complications					
RDS, n/N (%)	653/698 (93.55)	1095/1276 (85.82)	<0.001	2.39	1.7-3.37
Surfactant administered, n/N(%)	569/698 (81.52)	830/1276 (65.05)	<0.001	2.37	1.89-2.96
Nasal septal necrosis, n/N(%)	28 /698(4.01)	11 /1276(0.86)	<0.001	4.80	2.38-9.71
Pneumothorax, n/N (%)	12/698 (1.72)	9/1276 (0.71)	0.036	2.46	1.03-5.87
NCPAP, n/N(%)	593/698 (84.96)	799 /1276(62.62)	<0.001	3.37	2.66-4.27
CMV, n/N (%)	278/698 (39.83)	173/1276(13.56)	<0.001	3.37	2.66-4.27
Duration of non-invasive respiratory support, days (median, IQR)	4 (1- 8)	2 (1 -3)	<0.001	1.07	1.05 -1.10
Duration of invasive respiratory support, days (median, IQR)	6 (3- 12)	4 (2- 7)	<0.001	1.06	1.03 -1.09
Oxygen on Day 28, n/N (%)	358/ 698(51.30)	261/1276 (20.45)	<0.001	4.00	3.34-5.0
Steroids for CLD, n/N (%)	213/698 (30.52)	181/1276(14.18)	<0.001	2.65	2.1-3.32
Hospital course					
Spontaneous intestinal Perforation, n/N (%)	10/698 (1,43)	6/1276 (0,47)	0.023	3.07	1.11-8.5
NEC 2 – 3, n/N (%)	118 /698(16.91)	57/1276 (4.47)	<0.001	4.35	3.1-6.0
NEC surgery, n/N (%)	30/698(4.30)	14/1276 (1.10)	<0.001	4.35	3.1-6.0
Other surgery, n/N (%)	32/698 (4.58)	7/1276 (0.59)	<0.01	8.71	3.82-19.8
NNJ requiring PTT, n/N (%)	490/698 (70.20)	785/1276(61.52)	<0.001	1.47	1.20-1.79
PDA, n/N(%)	145/698 (20.77)	68/1276 (5.33)	<0.001	4.6	3.43-6.32
Blood transfusion, n/N (%)	526/698 (75.36)	370 /1276(28.99)	<0.001	7.48	6.06-9.24
KMC, n/N (%)	219/698(31.38)	603/1276(47.26)	<0.001	0.51	0.42-0.62
Length of stay, days, (median, IQR)	46 (26 -65)	27 (17- 40)	<0.001	1.03	1.02-1.04

IQR = inter-quartile range; CI = confidence interval; OR = odd ratio, RDS = respiratory distress syndrome, NCPAP = nasal continuous positive airway pressure, CMV = conventional mechanical ventilation, LOS = late-onset sepsis, CLD = Chronic lung disease, NEC = necrotizing enterocolitis, PDA = patent ductus arteriosus, NNJ = neonatal jaundice, PTT = phototherapy, KMC = kangaroo mother care

There were no significant differences between the groups with regards to maternal age, parity or gravity, place of birth, use and duration of high flow oxygen, undergoing exchange transfusion and major birth defect. No significant association was observed between LOS and ROP.

After an adjustment by multivariate logistic regression, the variables that remained significantly associated with LOS are summarized in table 3 below, while the rest of the variables as previously indicated in Table 2 were found to be not associated with increased risk of LOS.

Table 3: Factors significantly associated with outcome (LOS vs. No LOS) in very low birth weight infants after multivariate logistic regression

Variable	OR	95% CI	P-value
NEC 2 – 3	0.34	0.13- 0.87	0.025
NEC surgery	6.26	1.46- 26.82	0.013
Other surgery	0.22	0.05- 0.98	0.043
PDA	0.48	0.24- 0.96	0.038
Blood transfusion	7.48	6.06-9.24	<0.001

CI = confidence interval; OR = odd ratio, LOS = late-onset sepsis, NEC = necrotizing enterocolitis, PDA = patent ductus arteriosus.

There were 90l episodes of sepsis in 698 infants who had LOS. The majority of the sepsis episodes were caused by Gram-positive organisms including CoNS 450/901(49.9%). Gram-negative and fungal organisms were responsible for 353/901 (39.2%) and 98/901 (10.9%) of LOS respectively. Some patients 203/698 (29.08%) had more than one organism (from different classes) as cause of LOS.

There was an increased mortality in the group with LOS as compared to the group without LOS (31.23% vs. 16.38%, $p < 0.001$). An increased mortality was observed in patients who had LOS caused by Gram-negative organisms as compared to Gram-positive organisms (49.20% vs. 19.40%, $p < 0.001$). There were no significant differences in survival between those with LOS caused by bacterial or fungal organisms.

Discussion

This study showed that there are multiple risk factors other than immunological immaturity that predispose VLBW infants to LOS, the majority being the complications of prematurity and its interventions. The rate of mortality was higher in the LOS group compared to the group without LOS, similar to other studies in VLBW infants.[8, 16] LOS caused by Gram-negative organisms caused higher mortality. A study done by Ballot *et al.* in the same unit 4-years prior to our study found the same result, however that study included term infants and EOS.[17]

In our study, the incidence of LOS in VLBW was 35.4%, which is higher than the 25.0% reported in the multicentre study by Stoll *et al*.[16] Even with the centre-to-centre variability reported by Stoll *et al*. of 11.5 – 32.4%, our study showed a higher incidence. The reason for these results might be because the current study was conducted 11 years later when there have been advances in the care for VLBW infants, where more survive to later develop LOS. Both studies included CoNS as a significant cause of LOS: the study by Stoll *et al.* had higher numbers of CoNS sepsis (55%) compared to our study (44%). Other studies have found a lower incidence of LOS than our study: Hornik *et al*. found an incidence of 12.2%, Escalante *et al*. found an incidence of 22.2%.[8, 18] Hornik *et al.* performed their study eight years prior to our study and only looked at patients admitted in NICU where space and care is optimal. Escalante *et al.* performed theirs in 2001 to 2013 and included centres in the private sector in South America.

The majority of LOS was caused by Gram-positive organisms, followed by Gram-negative organisms. Some patients had more than one episode of LOS, caused by organisms from different classes. In studies done at the same institution eight years apart but in all infants

including VLBW infants there was a shift in predominance from Gram-positive organisms in 2002-2003 to Gram-negative organisms in 2012.[9, 10] Our study's findings concur with studies done by Stoll *et al.* and Hornik *et al.* which stated that Gram-positive organisms were responsible for the majority of LOS in VLBW infants.[8, 16]

There appears to be an increasing trend of fungal septicaemia. In the studies done in the same institution, the incidence of fungal LOS was 7.8% in 2002-2003 and 10.1% in 2012.[9, 10] In our study the incidence is yet higher at 10.9%. This could be because this study was only focusing on LOS in VLBW infants (unlike the other studies which looked at all infants) or it could represent an increased prevalence of fungal infection. Our study still revealed an increased incidence of fungal LOS as compared to studies done in VLBW infants in other countries which again supports an increase in the prevalence of fungal sepsis over the years.[8, 16] The overuse of broad spectrum antibiotics has increased antimicrobial resistance and rate of fungal sepsis.[1, 19]

After adjustment with multivariable logistic regression, our study showed that LOS was not directly proportional to smaller birth weight or gestational age at birth. The complications of prematurity which are directly proportional to lower birth weight and GA could have been the cause of the association prior the adjustment as the median for birth weight and gestational age was lower in the LOS group. Previous studies have found an association between lower GA and LOS.[16,18] One would have expected lower gestational age and birth weight to be directly proportional to LOS as these patient tend to stay longer in hospital and may be predisposed to sepsis while awaiting weight gain and resolution of complications of prematurity.

Our study revealed that there was no association between place of birth, antenatal care (ANC), antenatal steroids (ANS), sex and multiple gestation, which agrees with the study by Stoll *et al*.[16] However, Escalante *et al.* reported antenatal care and steroids to be protective against LOS.[18] This makes sense in that perinatal steroids decrease the incidence of neonatal complications (RDS, NEC, etc.) which would predispose patients to sepsis. Perhaps, our study showed no protection against LOS by ANC and ANS because the ANC attendance rate is poor (78.7%) as well as ANS coverage rate (46.8%).

Mode of delivery did not show significant association with sepsis in our study, a finding in contrast with that found by Escalante *et al*.[18], while Stoll *et al*. found no association between NVD and LOS.[16] Antenatal antibiotics were associated with LOS in other studies.[8, 18] Although we did not look at maternal antibiotic use, maternal chorioamnionitis (in which most of the mothers received antenatal antibiotics) was found to have no association, which concurs with study by Stoll *et al.* which reported that there was no association between LOS and antenatal antibiotics.

Some complications of prematurity are associated with LOS and further prolonged hospital stay in preterm babies. There was a significant association of LOS with NEC and PDA, findings were also supported by studies done in VLBW infants.[8,16,18] PDA results in increased pulmonary blood flow and interstitial oedema which predispose to LOS. Meanwhile, sepsis is implicated in the pathogenesis of NEC.

Alterations in the skin and/or mucous membrane barriers predispose to sepsis, as was evident in our study, where increased number of patients who had undergone surgery had LOS. Improvements can be made to wound care in these patients. With regards to the type of NEC surgery, laparotomy with ileo/colostomy was associated with increased risk of LOS as compared to laparotomy with primary closure in our study.

An interesting observation in this study which was not found in the literature was the association of blood transfusion with LOS. This association could be as a result of LOS causing anaemia in addition to other causes of anaemia which include prematurity that requires transfusion. This observation also puts into question the sterility followed during blood transfusion. There was no significant association in patients that had exchange transfusion for severe NNJ.

Surprisingly, kangaroo mother care (KMC) does not seem to be protective against LOS in our study. This is in contrast with a meta-analysis review by Boundy *et al*. that reported KMC to

have decreased the risk of neonatal sepsis.[20] The type of KMC done most commonly at CMJAH is continuous KMC where the mother stays with the baby in the KMC cubicle. Typically, this separates them from other sick infants and the staff caring for them. KMC also favours rapid weight gain and consequently babies get discharged early before developing episodes of sepsis. One would have expected KMC, particularly the type done at CMJAH, to decrease the risk of LOS.

After adjusting for complications of prematurity, LOS was not associated with prolonged hospital stay in our study. LOS is commonly associated with prolonged hospital stay in other studies.[8, 16, 18, 21] This is a challenge that hospitals are faced with as complications of prematurity contribute to long hospital stay. Preventing complications of prematurity will reduce the duration of hospital stay and costs. Patients should be discharged as soon as they are well and discharge weights need to be revised.

Study Limitations

This was a retrospective review of an existing database. As such, some important factors stated to be risk factors of LOS in the literature such as central lines, total parenteral nutrition and their duration could not be analysed. Furthermore, we did not follow up the infants post discharge to assess the long-term complications of LOS such as neurological impairment.

Conclusion

A third of VLBW infants in the study developed LOS during their stay in neonatal unit while awaiting weight gain or resolution and treatment of complications of prematurity. Certain factors have been noted to be associated with LOS, such as PDA, NEC, blood transfusion and surgery. The majority of LOS were caused by Gram-positive organisms, however Gram-negative organisms were associated with increased mortality. The prevalence of fungal sepsis is increasing. LOS increases neonatal morbidity and mortality. Interventions required to manage the sepsis and other conditions associated with prematurity, pose a financial constraint to the health sector.

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Approved research protocol

REVIEW OF RISK FACTORS AND SURVIVAL FOR LATE ONSET SEPSIS IN VERY LOW BIRTH WEIGHT INFANTS

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1.0 GLOSSARY

NNS:	Neonatal sepsis
EOS:	Early onset sepsis
LOS:	Late onset sepsis
CMJAH:	Charlotte Maxeke Johannesburg Academic Hospital
VLBW:	Very low birth weight
IV:	Intravenous
TPN:	Total parenteral nutrition
RDS:	Respiratory distress syndrome
BPD:	Broncho-pulmonary dysplasia
ROP:	Retinopathy of Prematurity
IVH:	Intraventricular haemorrhage
PVL:	Periventricular leukomalacia
PDA:	Patent ductus arteriosus
NEC:	Necrotizing enterocolitis
HIV:	Human Immuno-deficiency Virus
PCR:	Polymerase chain reaction
NNJ:	Neonatal Jaundice
B/C:	Blood culture
CoNS:	Coagulase negative staphylococcus
CFU/ml:	Colony-forming units per millilitres
REDCap:	Research Electronic Data Capture

2.0 LITERATURE REVIEW

Neonatal Sepsis is one of the common causes of neonatal morbidity and mortality worldwide.(1) In South Africa (S.A.), neonatal sepsis (NNS) accounts for 11.6% of all neonatal deaths (2) making it the third leading cause of neonatal deaths. In studies done in Ethiopia, Central Vietnam and Cameroon neonatal sepsis accounted for 31.0, 32.0, and 37,8% of neonatal deaths respectively. (3, 4, 5) In the United States, bacterial sepsis of newborn babies was ranked as the ninth leading cause of neonatal deaths in 2015. (6) Despite advances in neonatal care that have improved survival of very low birth weight (VLBW) infants, neonatal sepsis still remains an important cause of morbidity and mortality,(1) especially in underdeveloped and developing countries.

Neonatal sepsis is classified as early or late onset sepsis according to the infant's age at the onset of symptoms and signs.(7,8,9) Early onset sepsis (EOS) presents within the first 72 hours of life while late onset sepsis (which is more common than EOS) presents after 72 hours of life.(7,8) In a South African study done at a tertiary hospital(10), the incidence of neonatal sepsis was 10,3 per 100 admissions, of which late onset sepsis (LOS) accounted for 83,7% of cases of NNS. In the United Kingdom and Switzerland, LOS accounted for the majority of neonatal sepsis.(11,12)

Generally, EOS is caused by organisms acquired from the mother before or during delivery, whereas LOS is from organisms acquired in the hospital or the community (for infants delivered at home or discharged home).(1,9) LOS often occurs while the infants are in the neonatal unit and the infection may be transmitted by direct contact with hospital personnel,

parents, visitors (most common source from this groups is hand contamination) or contaminated equipment.(1)

Infants that are mostly affected by LOS are the VLBW infants.(1,9) A number of factors predispose VLBW infants to infection, such as: immunological immaturity, multiple invasive procedures, prolonged hospital stay, prolonged intravenous (IV) access, use of total parenteral nutrition or other invasive procedures.(1,9)

Organisms implicated in LOS are fungi and bacteria – the latter being more common. In a study done at Charlotte Maxeke Johannesburg Academic hospital (CMJAH) , bacterial infection was the cause of LOS in 89,1% of cases and fungi were responsible for 10.1% cases of LOS.(10) Fungal sepsis is usually acquired during prolonged hospital stay in preterm babies.(1) Organisms causing neonatal sepsis change over time, dependent on the environmental pathogens.(10) This is evident in two studies done at CMJAH at different periods of time, 10 years apart.(10,13) The study done by Motara *et al*. showed predominance of Gram-positive organisms as the cause of LOS which accounted for 57,5% of LOS and 33,2%% of cases were caused by Gram-negative pathogens.(13) In the follow up study done by Lebea *at al*. Gram-negative organism were the leading cause of LOS by 48,7% and Gram-positive organism at 41.1%.(10) The above studies also show that over the past years there has been a re-emergence of Gram-negative organisms causing LOS.

There is a lack of an accepted consensus on the definition for neonatal sepsis.(14) Historically, the presence of a positive blood culture constituted the gold standard for the presence of neonatal sepsis.(14) To some extent, this has fallen out of favour as some infants with bacterial infections may have a negative blood culture but with clinical signs of sepsis and this is referred

to as clinical sepsis.(14) Coetzee *et al.* defined neonatal sepsis as a clinical syndrome consisting of non-specific symptoms and signs of infection accompanied by bacteraemia in the first 28 days of life.(7)

Therefore, making a diagnosis of LOS is challenging as the clinical signs are nonspecific and the initial manifestation may involve only one symptom or system or it can occur concurrently with a variety of non-infectious conditions.(1) The clinical manifestations of neonatal sepsis may include temperature instability, lethargy, hypotension, poor perfusion with pallor, mottled skin, tachy- or bradycardia, apnoea, respiratory distress, irritability, seizures, feeding intolerance, abdominal distension, jaundice, bleeding and glucose instability.(1,7) Clinical sepsis is confirmed to be neonatal sepsis by a positive culture from a sterile site, including blood, cerebrospinal fluid or urine.(8) This however also poses difficulties as culture results are not immediately available.

Blood culture (B/C) results can be false negative in true sepsis when inadequate volume of blood is collected for culture. This is a common problem in infants as there are usually technical difficulties with collecting blood and the fear of causing anaemia. In a study done in Texas, it was found that 60% of B/C results will be falsely negative if only 0.5mls of blood is sampled in low colony count (less than 4 CFU/ml) sepsis. (15) A great number of infants are at risk of sepsis even with a low colony count of 4 or less. (1, 15) The recommended volume of blood for culture is 1-2millilitres of blood. (15) A blood culture may be positive in infants without sepsis, when the specimen is contaminated. In a study done in Vojvodina, the percentage of contaminated blood cultures was 16.4%, and after a strict introduction of sterile procedures and a checklist, the rate of contaminated B/C reduced to 7,6%. (16) Staphylococcus epidermis (a coagulase negative staphylococcus) is one of the commonest contaminants (16) and this causes

difficulties when making a decision whether to continue or stop antibiotics. Positive blood culture with coagulase negative staphylococcus (CoNS) is most likely a true sepsis if 2 blood cultures are positive, blood culture flagged positive within 48 hours and clinical condition of the infant is suggestive of sepsis or sepsis biomarkers are raised.(7)

The current practice with treatment of LOS is to start antibiotics empirically as soon as sepsis is suspected while awaiting results. Thereafter, treatment can be stopped early (i.e. after 24-48 hours) if cultures are negative, biomarkers of sepsis are normal and in the absence of clinical signs of sepsis.(7) If cultures are positive, antibiotics should be changed to target the organism cultured. The antibiotics used as empiric treatment should cover both Gram-positive and Gram-negative organisms. (1,7) Empiric antibiotics should cover organisms known to be the common causes of LOS in that area,(7) therefore ongoing surveillance is required in all the institutions. Empiric antibiotics differ according to site of the patient and change over time because there is re-emergence of new organisms with time. Antimicrobial stewardship programmes are important to guide clinicians to use antibiotics appropriately.

There are a wide range of factors associated with LOS. Other than mortality, LOS is associated with prolonged hospital stay, prolonged mechanical ventilation, need for total parenteral nutrition (TPN) and neurodevelopment delay.(1) A report from the National Institute of Child Health in 1994(6) stated that VLBW infants with LOS had significant longer duration of mechanical ventilation and also prolonged hospital stay. The number of days in hospital were increased by 25 days even after adjustment of other complications of prematurity.(6) All these factors or conditions further predispose them to infection, some of VLBW infants develop more than one episode of sepsis before discharge. LOS and its outcomes increase hospital costs and that becomes a serious burden in developing and underdeveloped countries.

Risk factors of neonatal sepsis are clearly documented in the literature but there are few studies done especially in Africa that look into risk factors of LOS in VLBW infants. It is paramount to know which factors play a major role in causing LOS so that infective control measures can be implemented or revised to reduce the incidence of these factors. The aim of this research is therefore to describe risk factors of LOS in VLBW infants that apply in our setting.

3.0 AIMS AND OBJECTIVES

Aim: To determine the risk factors and short-term outcomes of late onset sepsis in VLBW infants.

Objectives:

- To determine the prevalence of LOS among VLBW infants admitted to CMJAH.
- To identify risk factors of LOS in VLBW infants.
- To assess survival to discharge of VLBW infants diagnosed with LOS.
- To determine the percentage of Gram-positive, Gram-negative and fungal organisms.

4.0 DEFINITIONS

i. Infant – a child presenting within the first 28 days of life. A preterm infant will be classified as an infant according to their chronological age corrected for gestational age (e.g., a 5-week-old preterm infant born at 28 weeks will be corrected to 31 weeks and hence is an infant.)

ii. Very low birth weight – birth weight < 1500g.

iii. Preterm – infant born before 37 weeks gestational age

iv. Confirmed sepsis – positive culture of organism deemed to be significant.

v. Neonatal sepsis – sepsis that occurs in an infant during the neonatal period.

vi. Early onset sepsis – onset of sepsis, confirmed by culture, before 72 hours of life.

vii. Late onset sepsis – onset of sepsis, confirmed by culture after 72 hours of life in infants

5.0 METHODS

5.1 Study Design:

This is a retrospective cross-sectional, case-control study of an existing database of all VLBW infants admitted to the Neonatal unit from 1st January 2014 to 31st December 2018.

Case group: all infants with culture positive sepsis.

Control group: all infants without sepsis or with clinical signs of sepsis but negative cultures.

5.2 Setting:

The study will be done at CMJAH Neonatal Unit, a tertiary hospital in Johannesburg, South Africa.

5.3 Sample Population:

The sample population includes all VLBW infants admitted to CMJAH Neonatal unit

5.3.1 Inclusion Criteria:

All VLBW infants admitted to CMJAH Neonatal Unit within 48 hours of life from 1st January 2014 to 31st December 2018.

5.3.2 Exclusion Criteria:

- Birth weight less than 500g.
- All VLBW who died within the first 72 hours of life.
- All VLBW infants whose information is missing or incomplete.

5.4 Data Collection

Data will be extracted from the existing database of VLBW infants admitted at CMJAH Neonatal unit which is completed at discharge. Discharge information is captured for all infants admitted to the CMJAH Neonatal unit on a computerized database. Data is managed using Research Electronic Data Capture (REDCap) which is hosted by the University of the Witwatersrand.(18) REDCap is a secure, web-based programme that has been designed to aid data capture for the purpose of clinical audit and quality improvement. The information is verified at several different stages of collection. The data will be collected and entered onto a prepared data sheet (Appendix A), which will capture the following variables: demographic information (e.g., Maternal details, antenatal care, antenatal steroids, place of birth, mode of delivery, gestational age, birth weight, maternal syphilis, chorioamnionitis, tuberculosis, and HIV status), hospital stay (e.g. Apgars, resuscitation at birth respiratory diagnosis, surfactant administration, respiratory support initially and at 36 weeks, duration of stay), laboratory information (e.g. Bacterial or fungal pathogens and site of sepsis), other neonatal complications (e.g. NNJ, ROP, PDA, NEC, BPD, birth defect, other surgery) and outcome – died or survived.

5.5 Statistical Analysis

Data will be described using standard statistical methods. Continuous variables will be described using mean and standard deviation for those variables with a normal distribution and

median and range for variables with non-normally distributed data. Categorical variables will be described using frequencies and percentages. The study population will be divided into two groups, one with positive cultures and the other with negative cultures; associated factors and outcome will be compared between the two groups. Continuous variables will be compared using the Student's t-test or Mann Whitney U analysis, as appropriate. Categorical variables will be compared using the Chi-Square analysis. A P-value of less than 0.05 will be considered significant. Adjusted odd ratios for factors associated with sepsis will be determined using binary logistic regression.

Sample Size

There are approximately 450 VLBW admissions per annum to the CMJAH neonatal unit. It is estimated that 20% will be excluded, thus the final estimated sample size is 1800 for the five-year period. The prevalence of sepsis is about 30% - so there will be 600 cases and 1200 controls.

6.0 ETHICAL CONSIDERATIONS

The protocol will be submitted to the Human Research Ethics Committee of the University of Witwatersrand and the investigation will commence only once clearance from the committee has been obtained. As this study is retrospective and information will be collected from the existing hospital records database, no consent will be required from participants. Names, hospital number of participants or any details that could lead to identification of the participants will be de-identified. All the information obtained during the course of the study will be confidential.

7.0 LIMITATIONS

As this will be a retrospective study, variables not documented on the existing database (for example: TPN, central lines, use of proton pump inhibitors, etc) will not be included as part of the study, and not all records are complete. The other limitation is that patients will not be followed up after discharge to assess outcome after LOS. The time of onset of sepsis in relation to various risk factors cannot be determined.

8.0 TIMELINE

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Literature Review												
Preparing Protocol												
Protocol assessment												
Ethics application												
CMJAH application												
Data collection												
Data Analysis												
Writing up dissertation												

9.0 COST/ FUNDING

The researcher will be solely responsible for all funds required for this project, which will primarily cover administration costs such as stationery and printing, estimated at R5000.

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11.0 APPENDIX A

Data Collection Sheet

Study number:

Maternal details:

Age:

Parity:

Gravidity:

Antenatal care: ` Yes/No

HIV exposed: Yes/No

RPR positive: Yes/No

Antenatal steroids given: Yes/No

Maternal chorioamnionitis: Yes/No

Maternal TB: Yes/No

Delivery:

Place of birth: Inborn/Outborn

Mode of delivery: NVD C/S

Infant details:

Birth weight: g

Gestational age: weeks

Head circumference at birth:

Gender:

Multiple gestation:

Apgar score at 5 mins: Birth

HIV PCR : Not applicable/Not done/Negative/Positive

Initial resuscitation in the delivery Room Yes / No

Temperature within 1 hour of admission:

Early onset neonatal sepsis Yes/No

Hospital stay:

Age on admission in hours:

Respiratory diagnosis: RDS/Congenital pneumonia/Pulmonary haemorrhage

Surfactant therapy: Yes/No

Respiratory support after initial resus: Nil/Oxygen/NCPAP/CMV/HFOV

Respiratory support at 36 weeks: Nil/Oxygen/NCPAP/CMV/HFOV

Steroids for BPD: Yes/No

Age at outcome:

Outcome: Died/Discharged/Transferred

Cause of Death:

Other neonatal complications:

Stage 3 or 4 Retinopathy of Prematurity Yes/No

Grade 3 or 4 IVH Yes/No

PDA: Yes/ No.

NEC: Yes/No

Surgery for NEC: Yes/No

Other surgery: Yes/No

Pneumothorax: Yes/No

Blood transfusion: Yes/No

NNJ requiring phototherapy: Yes/No

Congenital anomalies: Yes/ No

KMC: Yes/No

Breastfed on discharge Yes/No

Laboratory results:

Sepsis after day 3: Yes/No

Sepsis site: Blood/Urine//CSF/Arthritis

Bacterial pathogen: Yes/No

Organism: Gram-positive/Gram-negative

Fungal pathogen: Yes/No

SAMJ Author Guidelines

Manuscript preparation for South African Medical Journal

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, *full* affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g., 'intravenous (IV)' or 'Department of Health (DoH)'.
- Include sections on Acknowledgements, Conflict of Interest, Author Contributions and Funding sources. If none is applicable, please state 'none'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g., µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counsellors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - Background: why the study is being done and how it relates to other published work.
  - Objectives: what the study intends to find out
  - Methods: must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - Results: first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - Conclusion: must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.

- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide consent to republication obtained from the copyright holder.
- Figures must be numbered in Arabic numerals and referred to in the text e.g. '(Fig. 1)'.
- Each figure must have a caption/legend: Fig. 1. Description (any abbreviations in full).
- All images must be of high enough resolution/quality for print.
- All illustrations (graphs, diagrams, charts, etc.) must be in PDF or jpeg form.
- Ensure all graph axes are labelled appropriately, with a heading/description and units (as necessary) indicated. Do not include decimal places if not necessary e.g. 0; 1.0; 2.0; 3.0; 4.0 etc.

- Scans/photos showing a specific feature e.g., *Intermediate magnification micrograph of a low malignant potential (LMP) mucinous ovarian tumour. (H&E stain).* –include an arrow to show the tumour.
- Each image must be attached individually as a 'supplementary file' upon submission (not solely embedded in the accompanying manuscript) and named Fig. 1, Fig. 2, etc.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:
Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:
Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:
Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must <u>not</u> be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]

- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 not 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
  - On the CrossRef homepage, paste the article title into the 'Metadata search' box.
  - Look for the correct, matching article in the list of results.
  - Click Actions > Cite
  - Alongside 'url =' copy the URL between { }.
  - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references

  - Government Gazettes:

  National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

  In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

  - Provincial Gazettes:

  Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

  - Acts:

  South Africa. National Health Act No. 61 of 2003.

  - Regulations to an Act:

  South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

  - Bills:

  South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

  - Green/white papers:

  South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

  - Case law:

  Rex v Jopp and Another 1949 (4) SA 11 (N)

  Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

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- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
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CLEARANCE CERTIFICATE NO. M190862 MED19-07-125

NAME: (Principal Investigator)	Dr Koki Octovia Mashilo
DEPARTMENT:	Paediatrics Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Review of risk factors and survival for late onset sepsis in very low birth weight infants
DATE CONSIDERED:	30/08/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Daynia Ballot and Robin Saggers
APPROVED BY:	L B Penny Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/09/2019

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 in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized 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 the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed August and will therefore be due in the month of August each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

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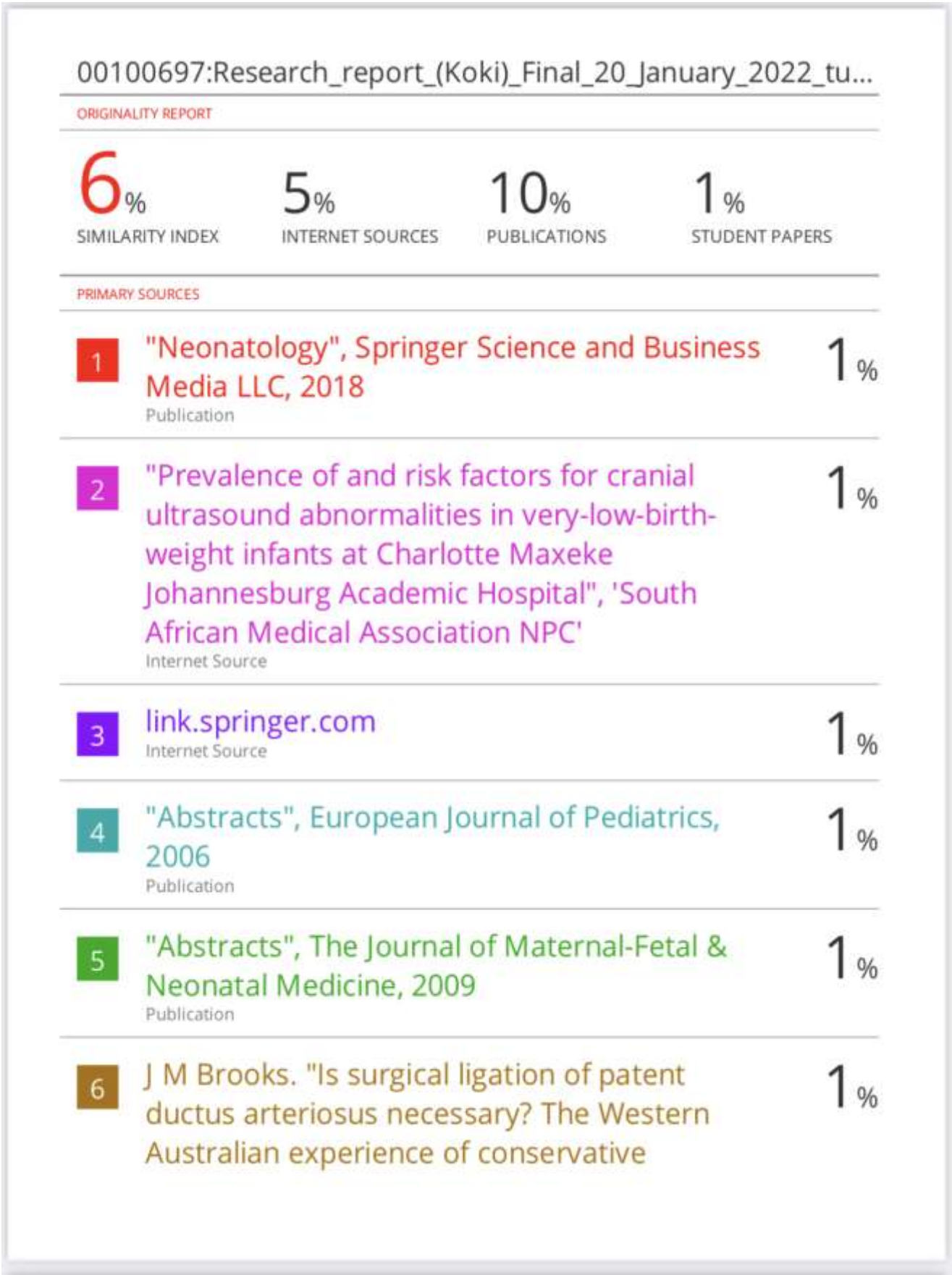

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