Necrotising enterocolitis in the Bloemfontein academic complex

By

Tabassum Osman

MBChB
Registrar: Department of Paediatrics and Child Health
Faculty of Health Sciences at the University of the Free State
Bloemfontein, South Africa
Email: osman.tabz@gmail.com

SUPERVISOR

Dr. P Moodley, MBChB, MMed (UFS) FCPaed (SA)
Consultant: Department of Paediatrics and Child Health
Faculty of Health Sciences at the University of the Free State
Bloemfontein, South Africa
Email: parushamoodley@gmail.com

SUBMISSION DATE: June 2020

Submitted in fulfilment of the requirements in respect of the Master's Degree MMed in the Department of Paediatrics and Child Health in the Faculty of Health Sciences at the University of the Free State

DECLARATION

I, Dr Tabassum Osman, declare that the coursework Master's Degree mini-dissertation that I herewith submit in a publishable manuscript format for the Master's Degree qualification MMed at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

DEDICATION

To my family for your unwavering support and encouragement. To my fur children that have sat beside me (and on me) as I completed this manuscript. And to the tiny humans that have inspired me daily to be better.

TABLE OF CONTENTS

List of Tables

ABSTRACT

Background: Necrotising enterocolitis (NEC) is a serious acquired gastrointestinal emergency affecting predominantly premature neonates, especially those with a birthweight of <1500g. Despite advances in neonatal care, the condition still accounts for a high mortality and morbidity. In a resource limited setting, the disease may be more devastating. While other centres in South Africa have reported on the epidemiology, risk factors and outcomes of neonates with NEC, there are no comparable studies to date in the Free State Province. This study aims to lessen this gap.
Objectives: The primary objective was to describe the prevalence and short-term outcomes of infants with NEC in terms of death, discharge or transfer. Secondarily it aimed to describe the presence of known risk factors and the course of illness and to compare all the above parameters between the weight categories - < 1000g, 1001g to 1499g and 1500g to 2000g.
Methodology: This was a retrospective, descriptive cross-sectional study of infants with a birth weight of ≤2000g. There were 184 participants that were included in the study. Medical records and discharge summaries were used to extract relevant data. Descriptive statistics for categorical data and medians and percentiles for numerical data were calculated, per group. The groups were compared by means of Kruskal-Wallis test for numerical data and Chi-square or Fisher's exact test for categorical data. The prevalence was calculated and described by means of 95% confidence interval for the prevalence.
Results: There were 2574 neonatal discharge summaries of babies born with a weight ≤2000g that was screened and after exclusion criteria was applied, 184 neonates with confirmed NEC were identified. The prevalence of NEC was 7.1% [6.2%; 8.2%]. The distribution of cases according to weight were 47 in < 1000g, 101 in 1001 to 1499g and 36 in > 1500g categories. Fifty-six infants (30.4%) died [23 (41.1%) were < 1000g, 28 (50%) were 1001g to 1499g and 5 (8.9%) were >1500g], 106 (57.6%) were discharged and 22 (12%) were transferred to their base hospital. Mortality rates were higher in NEC grade 3A [11 (19.7%), RR 3.22 (95% CL 2.23 ; 4.52), *P*<.001] and NEC grade 3B [28 (50%), RR 3.09 (95% CL 2.07 ; 4.61), *P*<.001]. Significant risk factors per weight category were maternal pre-eclampsia, RDS, mechanical ventilation and blood transfusion.

Conclusion: NEC remains a formidable challenge to clinicians caring for neonates with prevalence and mortality rates comparable to other tertiary neonatal units.

KEYWORDS

Necrotising enterocolitis
Premature infant
Prematurity
Low birth weight
Very low birth weight
Medical NEC
Surgical NEC

Abbreviations

ADEF:	Absent end diastolic flow
BW:	Birth weight
C/S:	Caesarian section
CHDX:	Congenital heart disease
ELBW:	Extremely low birth weight
GA:	Gestational age
GIT:	Gastrointestinal tract
HIV:	Human immunodeficiency virus
LBW:	Low birth weight
NEC:	Necrotising enterocolitis
NICHD:	National institute of child health and human development
NICU:	Neonatal intensive care unit
NVD:	Normal vaginal delivery
PDA:	Patent ductus arteriosus
RDS:	Respiratory distress syndrome
ROM:	Rupture of membranes
TANEC:	Transfusion associated necrotizing enterocolitis
VLBW:	Very low birth weight
RR:	Relative risk
CL:	Confidence limits

LIST OF APPENDICES

1. CHAPTER ONE

1.1. Literature Review

Necrotising enterocolitis in the premature infant

Prevalence

Worldwide, necrotising enterocolitis (NEC) remains a leading cause of neonatal mortality accounting for as much as 20 to 30% of deaths in a neonatal intensive care unit (NICU) [1,2]. Overall the condition has become synonymous with prematurity in which the incidence is inversely proportional to gestational age [3]. Furthermore, as medical advances such as better antenatal care, antenatal steroids and more extensive neonatal interventions allow for higher survival rates of premature infants, so too the population at risk for developing NEC increases [4,5]. It remains evident that the group most at risk are infants with a birth weight below 1500g. It is estimated that approximately 90% of cases of NEC are born prematurely with 12% of those being <1500g at birth [1]. It is estimated that one of ten premature infants with birth weight < 1000g can be diagnosed with NEC [5].

A systematic review in 2018 by Battersby et al. compared the incidence of NEC in 12 high income countries and identified an almost fourfold difference in the rate of NEC in premature infants born below 32 weeks gestation, from 2% in countries such as Japan, Sweden and Switzerland to 7% in the United States of America (USA). It further illustrated that the rate of NEC was higher in infants born with a birth weight of less than 1000g and gestational age less than 28 weeks and that there was an almost fivefold variation in the former category (from 5% to 22%). The study was unfortunately limited as the study designs of articles used from different countries varied in case definitions, inclusion criteria and population and allowed for moderate to high levels of bias. Only 4 of the studies reviewed reported on mortality which ranged from 21.9% to 38%. Its value however was highlighting the lack of consensus on case definition and study designs internationally thereby making it difficult to accurately report on the worldwide variation in incidence of NEC [6].

In a Canadian study by Wendy H Yee et al. using the Canadian Neonatal Network, a cohort of 16669 infants with gestational age <33 weeks over a 5-year period from 25 participating NICUs were analyzed with respect to incidence and timing of onset of NEC. It was found that the overall incidence of NEC was 5.1% (858 infants). There was a significant variation in risk adjusted incidences which considered factors such as gestational age, post-natal steroids, PDA treated by indomethacin and 5minute Apgar score. In addition, the study found that 40% of infants were diagnosed with early onset NEC occurring at a mean age of 7.6 days and had a higher incidence of surgical NEC. The mean age of diagnosis in the late onset group was 32 days and the peak onset of NEC in this cohort was 32 weeks post menstrual age [4]. This data was similar to the findings of the Eunice Kenedy Shriver National Institute of Child health and Human Development (NICHD) Research Network that reported a mean incidence of 3-11% between 1997 to 2000 and 5-15% between 2003 and 2007 in USA [4]. In the USA, NEC is currently estimated to occur in 1-5 cases per 1000 live births. In a further report from the NICHD Neonatal Research Network, NEC had a mean prevalence of 7% in 500g-1500g and up to 15% in <750g category [7]. In a prospective study in England conducted over 2 years between 2012 to 2013, it was found that the incidence (95% CI) per 1000 preterm admissions ranged from 20.0 to 41.1 [8].

Comparably in developing countries, a smaller study done in Pakistan by Shah et al. in which a total of 196 preterm infants (<37 weeks gestation) were included over a 6 month period showed a frequency of NEC of 14.28% with a mean gestational age of patients being 33.61 weeks [9].

In South Africa, incidences of NEC of 2.1 and 3.5 per 1000 live births were reported at Chris Hani Barawagnath Academic Hospital neonatal unit during periods of 1988 and 1994 respectively [3]. In 2014, Velaphi et al. investigated the risk factors for NEC in a setting of high HIV prevalence. During this study over a period of 3 years 110 infants with confirmed NEC were identified with a median birthweight of 1370-1380g and gestational age of 31 weeks [10]. At Groote Schuur Hospital, Joolay et al. compared the incidence of NEC after implementing low cost strategies and change in practices and described a reduction in the number of cases from 52 (2.5% of total admissions) in 2007-2008 to 14 cases (0.6% of total admissions) in 2009 [11]. A study determining the survival of VLBW at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) conducted in 2006/2007 found that NEC accounted for 10% of mortalities in this weight category, being the 4th highest cause of mortality preceded by extreme multi-organ immaturity, hyaline membrane disease and asphyxia [5]. These studies in the major academic centers in South Africa shows the

significant incidence and mortality NEC has on premature infants in our population group and exposes lacking research in this field in the Free State and particularly Bloemfontein Academic Complex.

Furthermore, the financial implications necrotizing enterocolitis has on a neonatal unit is significant, amounting to an estimated 500 million to 1 billion US dollars in the United States annually [2]. Approximately 20% of the cost of running a NICU can be attributed to either the disease or its complications [1] and the length of stay of neonates requiring surgical intervention was estimated to be 60 days longer than that of unaffected infants [2]. If surgery was not performed, infants stayed 20 days longer in hospital [2]. No studies have been done in South Africa to identify these parameters.

Risk Factors:

Numerous risk factors, both antenatally and postnatally has been implicated to the development of NEC. Prematurity as mentioned previously remains the single most independent risk factor with approximately 90% of infants affected by this disease being born premature [3]. Numerous studies have been done to identify and quantify these risk factors. Worldwide very low birth weight continues to be the greatest risk factor for NEC associated death and there is an inverse relationship between the risk of developing NEC with gestational age and birthweight [12].

In the United States a study conducted between 2011 and 2013 identified a racial disparity among NEC associated deaths with black neonates having a higher mortality rate than other racial groups, the reason for which remains unknown [13]. NEC associated death also occurred more commonly in infants born to unmarried mothers, mothers with age <19years and those with no antenatal care [12].

Administration of antenatal steroids to mothers prior to preterm delivery has been found to have a protective effect against these infants developing NEC in the neonatal period and this risk was almost three times higher in infants whose mothers did not receive antenatal steroids [10,13]. In South Africa however, Laher et al. reported in a 2017 publication that there was no significant association between antenatal steroid use and NEC (P=0.81). The antenatal steroid coverage was 44.1% at CMJAH for their study [14].

The role that maternal HIV status plays in the risk of developing NEC has been inconclusive. A study in France described an association between HIV positive mothers and increased risk of NEC, particularly those exposed to Zidovudine [3,10] but similar studies in South Africa did not demonstrate this association. Velaphi et al. in Johannesburg and Karpelowsky et al. in Cape Town found that there was no difference in the risk of NEC, severity of disease and NEC associated mortality between infants exposed and not exposed to HIV infection [10]. More recently though, Riemer et al reported in their 2019 publication that HIV exposed neonates at Groote Schuur hospital had an almost doubled risk of NEC when compared to HIV unexposed neonates. This risk was strongly associated with late onset neonatal sepsis and was increased in the < 1000g weight category. Furthermore, maternal antiretroviral use for > 8 weeks was protective against NEC when compared to HIV exposed infants whose mothers had < 8 weeks or no antiretrovirals [15].

Maternal chorioamnionitis is indirectly associated with NEC as it is a known risk for premature delivery and neonatal sepsis which are both implicated in development of NEC [12]. It has also been found that NEC occurs more frequently in babies of mothers who were treated for chorioamnionitis with Co-Amoxiclav though it is difficult to know whether this association is due to maternal infection itself or the effect of the antibiotic [12].

Severe pre-eclampsia during pregnancy has been implicated in increasing the risk of developing NEC and is thought to be due to decreased fetal umbilical artery blood flow, placental insufficiency and subsequent intrauterine hypo-perfusion and adaptation of the fetus leading to decreased blood flow to the intestines which results in hypoxic ischemic injury to the gut lining [3]. One study demonstrated that infants born to mothers with severe pre-eclampsia and absent or reversed end diastolic blood flow in umbilical vessels were 6 times more likely to develop NEC than infants with normal blood flow [8] while another demonstrated no increased risk [13].

Infant related risk factors are largely related to feeding practices and presence of conditions that directly or indirectly lead to gut mucosal inflammation or ischemia. It has been found that infants who are mechanically ventilated within the first hour of birth had a fourfold lower risk of NEC as they have less hypoxic incidents during the first few days of life and were likely to be initiated on feeds later [12]. Other studies have demonstrated an increased risk of NEC in mechanically ventilated infants due to break in the defensive barriers increasing risk of infection, especially in ELBW infants who are more likely to be intubated [6]. Incorrect placement of endotracheal tube in the esophagus may also predispose to NEC by introducing pathogens into the GIT system [13].

Conditions such as RDS and perinatal asphyxia similarly are associated with a higher risk of developing NEC due to hypoxic injury to gut endothelium caused by an imbalance in vasoactive substances like Endothelin 1 and nitric oxide, that leads to development of inflammation, ischemia and necrosis [3]. Surfactant therapy in hyaline membrane disease is not directly associated with NEC risk [12].

The presence of congenital heart lesions has proven to be contentious in the risk for developing NEC. While some literature describes an increased risk likely due to mesenteric hypo-perfusion and ischemia [16], particularly in infants with patent ductus arteriosus (PDA) where a "steal phenomenon" occurs during diastole in which blood flow to mesenteric arteries from aorta is reduced [8], other studies have demonstrated no increased risk of NEC in cyanotic heart disease [6]. Risk of NEC has also been shown to be increased in infants exposed to indomethacin within the first 48 hours of life, a drug used in preterm infants to close a PDA [8].

Bacterial sepsis in the neonatal period is a leading contributor to NEC associated death after prematurity in most weight categories (VLBW, NBW and Moderately LBW) [6,12]. Common associated organisms that have been isolated include E. coli, Klebsiella, Pseudomonas, Enterobacter and Salmonella [6]. The role of infection in NEC is twofold: infection itself may trigger the inflammatory cascade in the gut which is known in the pathogenesis of NEC and conversely bacterial overgrowth and translocation of gut pathogens into the blood stream may lead to overwhelming sepsis [6]. Furthermore, prolonged use of empiric antibiotics in ELBW in the first 3-5 days postnatally in the presence of negative blood cultures has been associated with an increased risk of NEC and death [17]. This is likely due to broad spectrum antibiotics affecting the natural colonization of the gut with intestinal flora and predisposing to pathogenic bacteria and fungi invasion [16].

Feeding choices is another major contributor to development of NEC. Breastmilk has been shown to be protective against NEC due to substances in it that affect immunity and mucosal barrier, thereby putting formula fed infants at higher risk for developing NEC [13]. Breastmilk assists with colonization of neonatal gut as it contains nonpathogenic bacteria such as bifidobacteria and molecules with antibacterial properties [8]. Immature gut motility and delayed digestion in premature infants directly predisposes to development of NEC. Numerous studies have been conducted to determine the appropriate time of initiation of feeds (early vs late) as well as the progression of feeds in premature infants and its association with NEC. 90% of preterm infants

who develop NEC do so after feeds were initiated [8]. A South African randomized control study conducted at Groote Schuur Hospital evaluated feeding regimens in a resource limited setting. The researchers found that ELBW infants receiving rapid advancement of feeds (36ml/kg/day) and high volume initiation of feeds (24ml/kg/day) were able to tolerate the feeds well and achieved a weight of 1500g and were discharged sooner than the counter group of slow advancement (24ml/kg/day) and low volume (4ml/kg/day) feeds. While limited in its ability to evaluate feed related morbidity, the study did conclude that NEC was not increased in these ELBW infants [18]. The timing of initiation of feeds has also been a contentious issue in evaluating the risk of NEC. While some studies show an increased risk of developing NEC in infants who were initiated on feeds earlier, majority of studies support the conclusion that early enteral feeding does not significantly increase risk of NEC [8,10]. Ultimately a Cochrane met-analyses concluded that the slow advancement and delayed initiation of feeds did not reduce the risk of NEC in preterm, VLBW and growth restricted infants and that slow advancement of feeds lead to increased risk of infection and metabolic complications due to prolonged parental nutrition exposure [18,19].

Transfusion of blood products has been shown to double the risk of developing NEC [20]. One study showed that 27% of infants were diagnosed with NEC within 48 hours of receiving a blood transfusion [16]. There are a number of proposed theories as to why transfusion associated NEC (TANEC) occurs: Severe anemia may lead to impaired gut blood flow, there may be immunological mediators in blood products that cause an immune reaction in the gut and ischemia/ reperfusion injury may occur with transfusion. It has been described that keeping preterm infants nil by mouth during transfusion can decrease the risk of TANEC and this has led to a proposed Cochrane review to further analyze this recommendation [19].

Another contentious factor in developing NEC is the role of probiotic administration. Lactobacillus and Bifidobacterium are amongst the commonly used probiotics and is thought to prevent NEC by promoting gut colonization with beneficial organisms thereby preventing pathogen colonization and improving gut mucosal barrier function and maturity [21,22]. A randomized control trial conducted at Tygerberg Hospital demonstrated a reduced incidence of NEC in the study vs control group that was not specific to HIV status [21]. A limitation of the study was the small sample size. Similarly, a retrospective study in Spain also demonstrated a reduction in NEC, late onset sepsis mortality in infants born < 32 weeks gestation and given routine probiotics [23]. Overall, the

American Paediatric Surgical Association Outcomes and Clinical Trial Committee reviewed the evidence internationally and reported that substantial data supports the routine administration of probiotics to reduce the incidence of severe NEC. The committee however could not make recommendations for ELBW infants and the formulation, timing and duration of supplementation [22].

Clinical Presentation:

NEC presents within one to two weeks of life and can be of sudden or insidious onset [8]. Early onset of disease can occur at a mean age of 7 days while late onset NEC can be delayed to 32 days of age in smaller, more premature infants [4]. Clinical features of NEC can vary depending on severity of disease. Bell et al suggested a staging system in 1978 based on severity and this was later modified by Walsh and Kliegman by adding subcategories in the stages and including signs that differentiate course of disease [8,9] (See below).

Modified Bells Staging (8)

Stage	Systemic signs	Abdominal signs	Radiographic signs	Treatment
IA Suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus	NPO, antibiotics x 3 days
IB Suspected	Same as above	Grossly bloody stool	Same as above	Same as IA
IIA Definite, mildly ill	Same as above	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
IIB Definite, moderately ill	Same as above, plus mild metabolic acidosis and thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus ascites, portal venous gas	NPO, antibiotics x 14 days

IIIA Advanced, severely ill, intact bowel	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as IIA, plus ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
IIIB Advanced, severely ill, perforated bowel	Same as IIIA	Same as IIIA	Same as above, plus pneumoperitoneum	Same as IIA, plus surgery

In the early stages, signs may be nonspecific. In one study abdominal distention was the most common clinical sign, found in 85% of cases, followed by increased pre feed aspirate (70%), lethargy (40%), vomiting (34%) and various other signs like apnea, bradycardia, hypothermia, absent bowel sounds and GIT bleed [13]. Feeding intolerance is common but can also occur in other conditions which affect gut motility including septicemia, gastrointestinal esophageal reflux disease and certain medications such as caffeine and indomethacin. This leads to an over diagnosis of stage 1 disease [7]. As severity progresses other signs may be evident including abdominal wall erythema, tenderness and guarding and presence of an abdominal mass in right lower quadrant. Systemic compromise becomes more marked, necessitating the need for ventilators and/or inotropic support. A South African study at Steve Biko Academic Hospital with 45 neonates with proven NEC demonstrated that a persistent tachycardia is a predictor of progression to perforation [24].

Radiologically, stage 1 disease may present with distended bowel loops while stage 2 consists of the classical radiological signs of a fixed bowel loop, pneumatosis intestinalis and portal venous gas [3]. In the most severe form, pneumoperitoneum is present [3]. This can also occur in spontaneous intestinal perforation, a condition that also affects preterm infants but presents in the first few days after birth and characteristically has minimal gut inflammation and necrosis making it different to NEC [2].

Biochemically, laboratory findings like some of the clinical signs are nonspecific [7]. They include neutropenia, leukocytosis, thrombocytopenia, hyponatremia, metabolic acidosis, elevated C-reactive protein and glucose disturbances [3,9]. Blood cultures may be positive in a third of the cases [9]. Other markers that have been studied include intestinal fatty acid binding protein, urinary D lactate and fecal calprotectin but research and availability of these tests remain limited. More promising research for biomarkers for NEC diagnosis and NEC severity using elevated CCR9+ CD4+ T Cells and CCR9+ IL 7 producing Treg in humans and mice with NEC has been underway [25]. A severe fulminant form of the disease, NEC totalis may present rapidly with widespread gut necrosis and has a poor prognosis [9,26]. Ultimately, a high index of suspicion is needed in higher risk infants particularly preterm and VLBW infants allowing for early detection of onset of NEC.

While the modified Bells staging system has been heavily critiqued owing to the non-specific signs especially in stage 1 of the disease, it remains the most universally accepted diagnostic tool for NEC. The use of plain abdominal x-rays to assess the severity of disease has a high positive predictive value but low sensitivity [27]. This often leads to the overdiagnosis of suspected NEC, cessation of feeds for prolonged periods and overall undernutrition and growth restrictions of these neonates. Other radiological modalities have been investigated especially in predicting the need for surgical intervention in patients without pneumoperitoneum [22]. Ultrasound allows for a more detailed evaluation of bowel wall thickness, perfusion and peristalsis [22]. The use of this modality was investigated during a Chinese retrospective study. They concluded that imaging (radiographic and ultrasound) was the most powerful predictor of surgical NEC. Radiographic portal venous gas had the highest specificity and absent peristalsis on ultrasound had the highest sensitivity [28] . Near infra-red spectroscopy, a modality measuring tissue oxygenation has been investigated. A limitation is that it does not have the ability to assess the entire intestine and while a difference in splanchnic tissue oxygenation has shown in infants who develop NEC, the overall value of using NIRS in preventing NEC is unclear and guarded [22].

Management:

Once diagnosed, NEC can be managed in one of two approaches: medically or surgically and this is determined largely by the severity of disease. In early stages, medical management consisting of bowel decompression, bowel rest (stopping feeds), intravenous broad spectrum antibiotics for 7 to 10 days and total parenteral nutrition is sufficient [1,7]. This said, constant clinical, radiological

and biochemical reevaluation must be done 6 to 8 hourly to detect deterioration and intestinal perforation which requires emergency surgery. Surgery is ultimately required in one third to one half of medically managed cases [3].

Surgical intervention is based on absolute and relative indications in advanced cases of NEC. The only absolute indication is pneumoperitoneum or intestinal perforation [3,7,9]. Relative indications for surgical intervention include: clinical deterioration despite maximal medical therapy requiring increased cardiorespiratory support, presence of abdominal mass, increased abdominal tenderness and distention, persistence of portal venous gas and sudden or worsening acidosis, hyponatremia and thrombocytopenia indicative of ongoing necrosis [3,9]. Van der Schyff and Becker in their Pretoria based study though retrospective and small, challenged the conventional timing of surgical intervention suggesting that often the criteria used for surgery are late signs of advanced disease. They showed that persistent tachycardia in neonates with NEC had a strong association with impending perforation [24].

Two surgical approaches may be used: primary peritoneal drain and laparotomy, each with its advantages and disadvantages. Insertion of peritoneal drains is a bed side procedure that can be lifesaving and assist with resuscitation of the infant by decompressing a tensely distended abdomen and allowing for better ventilation (reduces intra-abdominal pressure) [7]. Release of feculent matter also reduces the risk of contamination [9]. Some paediatric surgeons have reported to use this method as definitive treatment of NEC [3]. Laparotomy allows for surgeon to thoroughly assess and decontaminate the bowel but has the disadvantage of adding stress to the infant body. During laparotomy, several procedures can be performed depending on the extent of necrosis. In limited disease either resection and enterostomy or primary anastomosis can be performed and in multi-segment disease either high jejunostomy (not favored due to high output stoma) or clip and drop method necessitating need for a second surgery within 24-48 hours [3]. The choice of surgical intervention has remained controversial. Moss et al concluded in their study of 117 preterm infants that the type of intervention done did not significantly differ in terms of 90-day post-operative mortality or dependence on TPN as well as length of stay in hospital. The study was however limited by its smaller sample size [29]. In contrast, other studies showed infants treated with peritoneal drains often required a laparotomy anyway and that mortality was increased by more than 50% with peritoneal drains compared to laparotomy [2]. Furthermore, a

multi-centered observational study has suggested that long term neurodevelopment outcome may be better with laparotomy versus peritoneal drainage [21].

Outcomes:

In a South African study of 128 patients at Tygerberg Hospital between 1993 and 1995, 41% of babies had presented with rapid onset, severe disease requiring surgery of which 52% was resectable and of these infants 60% survived till 30 days [26]. Infants with pan-necrosis (NEC totalis) however had the worst prognosis with none of the 19 infants surviving [20]. 20 years later, NEC remains a significant predictor of survival in a South African setting [5]. Arnold et al found that early survival (30 days post operatively) was 69 % overall and 71% in <1500g birth weight group with overwhelming sepsis and pan-necrosis causing most deaths [30]. Late deaths (>30 days post operatively) was largely due to short bowel syndrome or sepsis with intraventricular hemorrhage also contributing [22]. Late complications included late colonic strictures, incisional hernias and adhesive bowel obstruction. Severe neurological deficit and neurodevelopment delay was significant in long term survivors (20% and 49% respectively) [22].

A prospective cohort study by the NICHD Neonatal research network concluded that overall, 49% of ELBW undergoing surgical intervention for NEC or isolated intestinal perforation demised. The median time to death was 8.5 days. The mortality rate in patients with pre-operative diagnosis of NEC was 55,2% [31]. It is well accepted that advanced NEC requiring surgical intervention has a poorer outcome and that mortality for ELBW undergoing surgery is approximately 50% [2,23]. Furthermore, it is found that NEC is associated with a significantly worse neurodevelopment outcome than prematurity itself and the presence of advanced NEC requiring surgical intervention further increases the risk for neurological impairment [32,33]. Growth delay is also significantly worse in ELBW undergoing surgical management for NEC [25].

Overall, a review of the literature available shows that while NEC has been studied in many worldwide centers with respect to prevalence, risk factors, presentation, treatment modalities and outcomes, there is a paucity of local published data to comparatively assess how we fare in the fight against this disease. While deductions from the research thus far can be inferred, a lack of consensus amongst many large research centers makes it difficult to draw definite comparisons to a South African population especially given the gross differences in our demographics. To improve surveillance and management protocols of this devastating condition, local data with respect to current incidence, known risk factors and outcomes is needed. This study aims to

describe necrotizing enterocolitis in neonatal units in the Bloemfontein academic complex between 2016 and 2018.

Research Question: What is the prevalence and outcomes of infants with necrotising enterocolitis in Bloemfontein?

1.2 References

1. Hemanshoo T, Kokila L. UPDATES IN : NECROTISING ENTEROCOLITIS. 1865;10(3):38–43.

2. Neu J, Walker WA. Necrotizing Enterocolitis. N Engl J Med. 2013;364(3):255–64.

3. NECROTISING ENTEROCOLITIS IN PREMATURE INFANTS AT CHRIS HANI BARAGWANATH HOSPITAL . WHAT ARE THE ASSOCIATED FACTORS ? Peter Angura A research report submitted to the Faculty of Health Sciences , University of the Witwatersrand , Johannesburg , in partial . 2013;(January).

4. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK. Incidence and Timing of Presentation of Necrotizing Enterocolitis in Preterm Infants. Pediatrics [Internet]. 2012;129(2):e298–304. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2011-2022

5. Ballot DE, Chirwa T, Ramdin T, Chirwa L, Mare I, Davies VA, et al. Comparison of morbidity and mortality of very low birth weight infants in a Central Hospital in Johannesburg between 2006/2007 and 2013. BMC Pediatr. 2015;15(1):1–12.

6. Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: A systematic review. Arch Dis Child Fetal Neonatal Ed. 2018;103(2):F182–9.

7. Huda S, Chaudhery S, Ibrahim H, Pramanik A. Neonatal necrotizing enterocolitis: Clinical challenges, pathophysiology and management. Pathophysiology [Internet]. 2014;21(1):3–12. Available from: http://dx.doi.org/10.1016/j.pathophys.2013.11.009

8. Battersby CWS, Mandalia S, Fitz-Simon N, Costeloe K, Modi N. G396 A prospective population study of the incidence of severe necrotising enterocolitis in England. Arch Dis Child [Internet]. 2016 Apr 1;101(Suppl 1):A229 LP-A230. Available from:

http://adc.bmj.com/content/101/Suppl_1/A229.3.abstract

9. Shah SS, Saleem M, Mehmood T, Ahmed T. Frequency and Outcome of Necrotizing Enterocolitis in Preterm Neonates. J Ayub Med Coll Abbottabad. 2015;27(1):85–7.

10. Angura P, Velaphi S. Risk factors for necrotising enterocolitis in an HIV-endemic region. Paediatr Int Child Health [Internet]. 2014 Aug 1;34(3):208–15. Available from: https://doi.org/10.1179/2046905514Y.0000000126

11. Joolay Y, Rhoda NR, oke LT, Horn AR, Harrison MC. A reduction of necrotising enterocolitis at Groote Schuur Hospital nursery. South African Med J. 2011;101(11):806.

12. Seeman SM, Mehal JM, Haberling DL, Holman RC, Stoll BJ. Infant and maternal risk factors related to necrotising enterocolitis-associated infant death in the United States. Acta Paediatr Int J Paediatr. 2016;105(6):e240–6.

13. Gane B, Bhat BV, Adhisivam B, Joy R, Prasadkumar P, Femitha P, et al. Risk factors and outcome in neonatal necrotising enterocolitis. Indian J Pediatr. 2014;81(5):425–8.

14. Laher A, Ballot DE, Ramdin T, Chirwa T. A review of antenatal corticosteroid use in premature neonates in a middle-income country. South African Med J. 2017;107(9):768–72.

15. Riemer LJ, Le Roux SM, Harrison MC, Tooke L. Short-term outcomes of HIV-exposed and HIV-unexposed preterm, very low birthweight neonates: a longitudinal, hospital-based study. J Perinatol [Internet]. 2020;40(3):445–55. Available from: http://dx.doi.org/10.1038/s41372-019-0541-4

16. Lu Q, Cheng S, Zhou M, Yu J. Risk Factors for Necrotizing Enterocolitis in Neonates: A Retrospective Case-Control Study. Pediatr Neonatol. 2017;58(2):165–70.

17. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, et al. Prolonged Duration of Initial Empirical Antibiotic Treatment Is Associated With Increased Rates of Necrotizing Enterocolitis and Death for Extremely Low Birth Weight Infants. Pediatrics. 2009;123(1):58–66.

18. Raban S, Santhakumaran S, Keraan Q, Joolay Y, Uthaya S, Horn A, et al. A randomised

controlled trial of high *vs* low volume initiation and rapid *vs* slow advancement of milk feeds in infants with birthweights ≤ 1000 g in a resource-limited setting. Paediatr Int Child Health [Internet]. 2016;36(4):288–95. Available from: https://www.tandfonline.com/doi/full/10.1179/2046905515Y.0000000056

19. Oddie SJ, Young L, Mcguire W. Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database of Systematic Reviews. 2017.

20. Kt Y, Jy K, Sasi A, Tan K, Nm L. Stopping feeds for prevention of transfusion-associated necrotising enterocolitis in preterm infants (Protocol). 2017;(12).

21. Van Niekerk E, Nel DG, Blaauw R, Kirsten GF. Probiotics reduce necrotizing enterocolitis severity in HIV-exposed premature infants. J Trop Pediatr. 2015;61(3):155–64.

22. Eaton S, Rees CM, Hall NJ. Current research in necrotizing enterocolitis. Early Hum Dev [Internet]. 2016;97:33–9. Available from: http://dx.doi.org/10.1016/j.earlhumdev.2016.01.013

23. Uberos J, Aguilera-Rodríguez E, Jerez-Calero A, Molina-Oya M, Molina-Carballo A, Narbona-López E. Probiotics to prevent necrotising enterocolitis and nosocomial infection in very low birth weight preterm infants. Br J Nutr. 2017;117(7):994–1000.

24. Van Der Schyff F, Becker JHR. Predictive Value of a Persistent Tachycardia to Indicate Impending Perforation in Necrotizing Enterocolitis. Am J Perinatol. 2018;35(8):774–8.

25. Nguyen DN, Sangild PT. Pathogenesis and biomarkers for necrotizing enterocolitis: Getting any closer? EBioMedicine [Internet]. 2019;45:13–4. Available from: https://doi.org/10.1016/j.ebiom.2019.06.029

26. Voss M, Moore SW, van I der M, Pieper C. Fulminanting necrotising enterocolitis: outcome and prognostic factors. Pediatr Surg Int. 1998;13(0179–0358):576–80.

27. Soni R, Katana A, Curry JI, Humphries PD, Huertas-Ceballos A. How to use abdominal X-rays in preterm infants suspected of developing necrotising enterocolitis. Arch Dis Child Educ Pract Ed. 2020;105(1):50–7.

28. He Y, Zhong Y, Yu J, Cheng C, Wang Z, Li L. Ultrasonography and radiography findings predicted the need for surgery in patients with necrotising enterocolitis without pneumoperitoneum. Acta Paediatr Int J Paediatr. 2016;105(4):e151–5.

29. Moss RL, Dimmitt RA, Barnhart DC, Sylvester KG, Brown RL, Powell DM, et al. Laparotomy versus Peritoneal Drainage for Necrotizing Enterocolitis and Perforation. N Engl J Med [Internet]. 2006;354(21):2225–34. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa054605

30. Arnold M, Moore SW, Sidler D, Kirsten GF. Long-term outcome of surgically managed necrotizing enterocolitis in a developing country. Pediatr Surg Int. 2010;26(4):355–60.

31. Blakely ML, Lally KP, McDonald S, Brown RL, Barnhart DC, Ricketts RR, et al. Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: A prospective cohort study by the NICHD Neonatal Research Network. Ann Surg. 2005;241(6):984–94.

32. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed. 2007;92(3):193–8.

33. Hintz SR. Neurodevelopmental and Growth Outcomes of Extremely Low Birth Weight Infants After Necrotizing Enterocolitis. Pediatrics [Internet]. 2005;115(3):696–703.

2. CHAPTER TWO

2.1 ABSTRACT

Background: Necrotising enterocolitis (NEC) is a serious acquired gastrointestinal emergency affecting predominantly premature neonates, especially those with a birthweight of <1500g. Despite advances in neonatal care, the condition still accounts for a high mortality and morbidity. In a resource limited setting, the disease may be more devastating. While other centres in South

Africa have reported on the epidemiology, risk factors and outcomes of neonates with NEC, there are no comparable studies to date in the Free State Province. This study aims to lessen this gap.

Objectives: The primary objective was to describe the prevalence and short-term outcomes of infants with NEC in terms of death, discharge or transfer. Secondarily it aimed to describe the presence of known risk factors and the course of illness and to compare all the above parameters between the weight categories - < 1000g, 1001g to 1499g and 1500g to 2000g.

Methodology: This was a retrospective, descriptive cross-sectional study of infants with a birth weight of ≤2000g. There were 184 participants that were included in the study. Medical records and discharge summaries were used to extract relevant data. Descriptive statistics for categorical data and medians and percentiles for numerical data were calculated, per group. The groups were compared by means of Kruskal-Wallis test for numerical data and Chi-square or Fisher's exact test for categorical data. The prevalence was calculated and described by means of 95% confidence interval for the prevalence.

Results: There were 2574 neonatal discharge summaries of babies born with a weight ≤2000g that was screened and after exclusion criteria was applied, 184 neonates with confirmed NEC were identified. The prevalence of NEC was 7.1% [6.2%; 8.2%]. The distribution of cases according to weight were 47 in < 1000g, 101 in 1001 to 1499g and 36 in > 1500g categories. Fifty-six infants (30.4%) died [23 (41.1%) were < 1000g, 28 (50%) were 1001g to 1499g and 5 (8.9%) were >1500g], 106 (57.6%) were discharged and 22 (12%) were transferred to their base hospital. Mortality rates were higher in NEC grade 3A [11 (19.7%), RR 3.22 (95% CL 2.23 ; 4.52), *P* <.001] and NEC grade 3B [28 (50%), RR 3.09 (95% CL 2.07 ; 4.61), *P* <.001]. Significant risk factors per weight category were maternal pre-eclampsia, RDS, mechanical ventilation and blood transfusion.

Conclusion: NEC remains a formidable challenge to clinicians caring for neonates with prevalence and mortality rates comparable to other tertiary neonatal units.

2.2 INTRODUCTION

Necrotising enterocolitis (NEC) is an acquired inflammatory condition predominantly affecting premature and low birth weight infants [1]. Approximately 90% of cases of NEC are born prematurely and it remains evident that the group most at risk are infants with a birth weight <

1500g, accounting for 12% of these cases [1]. It is further estimated that 1 in 10 infants with a birth weight < 1000g can be diagnosed with NEC [2]. Several other risk factors such as perinatal asphyxia, feeding practices, concomitant bacterial sepsis, mechanical ventilation and blood transfusions have been implicated in the disease process [3,4]. While advances in neonatal care have allowed for an increase in survivability of premature infants, the relative unpredictable and devastating nature of the disease still accounts for a high mortality (as much as 20 to 30 %) and morbidity of neonates admitted to a neonatal intensive care unit (NICU) [1,5]. This coupled with the incomplete understanding of the pathogenesis and prevention of NEC warrants further research on this serious condition [5].

The incidence of NEC has been described as inversely proportional to gestational age at birth [6]. Worldwide, the incidence of NEC in high income countries is variable and ranges between 2% and 7% in babies born <32 weeks gestation [7]. In South Africa, an outbreak of NEC was investigated between March to June 2018 in Johannesburg in which 37 infants were diagnosed with NEC over the 4 month period [8]. At Charlotte Maxeke Johannesburg academic hospital, NEC accounted for 10% of mortalities in very low birth weight neonates in 2006/2007 and remained a significant predictor of survival in this weight category in 2013 [9]. It remains a formidable challenge to clinicians caring for neonates, accounting for up to 20% of the cost of running a NICU and a significantly longer stay in hospital compared to unaffected infants [1,10]. Furthermore, long term complications such as neurodevelopmental delay, short bowel syndrome and growth faltering places further burden on the healthcare system long after neonates recover from the primary disease [11]. In a resource limited setting, the disease may prove to be even more devastating. Limited data exists with regards to describing the overall prevalence and outcomes of infants with NEC in South Africa, particularly in the Free State province. This is necessary to determine if our efforts are comparable to other neonatal units and if changes in practice may be warranted. This retrospective study aims to lessen the gap in data on NEC in the province.

2.3 METHODOLOGY

This was a retrospective, descriptive cross-sectional study of infants with a birth weight of ≤2000g admitted with confirmed NEC (Stages 2A to 3B) according to the modified Bells Staging, in the Bloemfontein academic complex during a 3 year period from January 2016 to December 2018.

The in-house neonatal databases and linked meditech system of both Universitas Academic and Pelonomi Regional hospitals were used to identify babies admitted with NEC stages 2A, 2B, 3A and 3B. Surgical NEC cases are generally managed at Universitas Academic Hospital and this is the only referral centre for neonatal surgery for Free State, Northern Cape and Lesotho. Convenience sampling was used. Medical records were retrieved, and relevant data was extracted onto a collection sheet that was later captured on an excel spreadsheet for further analysis. Infants with missing medical records and meditech discharge summaries were excluded. Infants found without a modified Bells staging were retrospectively staged when completing the data sheet using the description of physical signs and radiological features noted in the file. Infants with congenital GIT abnormalities were also excluded.

The primary objective was to describe the prevalence and short-term outcomes of infants with NEC in terms of death, discharge or transfer. Secondarily the author aimed to describe the presence of known risk factors and the course of illness and to compare all the above parameters between the weight categories - < 1000g, 1001g to 1499g and 1500g to 2000g. Demographics, known antenatal risk factors (HIV status, presence of pre-eclampsia, absent end diastolic flow (AEDF), premature pre-labor rupture of membranes (PPROM), chorioamnionitis and antenatal steroid use) and postnatal factors such as mechanical ventilation, bacterial sepsis, blood transfusion, presence of patent ductus arteriosus (PDA) and other congenital heart disease (CHD), birth asphyxia and feeding practices were recorded.

Descriptive statistics namely frequencies and percentages for categorical data and medians and percentiles for numerical data were calculated, per group. The groups were compared by means of Kruskal-Wallis test for numerical data and Chi-square or Fisher's exact test for categorical data. The prevalence of necrotising enterocolitis was calculated and described by means of 95% confidence interval for the prevalence. The analysis was done by the Department of Biostatistics.

2.4 RESULTS

There were 2574 neonatal discharge summaries of babies born with a weight ≤2000g that were screened, of which 438 neonates were found to have NEC as a diagnosis during January 2016

and December 2018. Of this, 223 neonates were diagnosed with NEC grade 1 or suspected NEC and were excluded. Nineteen cases could not be retrospectively graded due to missing files and a further 12 cases had insufficient information on summaries and missing files and was therefore excluded. Ultimately, 184 cases were included in the study. The distribution was as follows: 120 cases (65.2%) occurred at Pelonomi regional hospital and 64 cases (34.8%) occurred at Universitas academic hospital. Twenty-five (13.6%) of the cases were in babies that were out born and subsequently referred to one of the above hospitals. In 30 cases, more than one episode of NEC occurred during the admission period. Three episodes of NEC occurred in 4 cases. The prevalence of one episode was [85.7%; 94.2%], two episodes [5.4%; 13.7%] and three episodes [0.1%; 3.0%]. The overall prevalence of NEC in infants < 2000g was 7.1% [6.2%; 8.2%]. The distribution of cases amongst the weight categories <1000g, 1001 to 1499g and > 1500g were 47, 101 and 36, respectively.

In terms of short-term outcomes, 56 infants (30. 4%) died, 106 (57.6%) were discharged and 22 (12%) were transferred to their base hospital. The median weights at discharge and transfer were 1755g and 1600g, respectively. Of the infants who died, 23 (41.1%) were < 1000g, 28 (50%) were between 1001g and 1499g and 5 (8.9%) were above 1500g in birth weight. The median time from admission to death was 11 days. This was the same for infants < 1000g but 10.5 days for 1001 to 1499g category and 14 days for > 1500g category (P=0.62). The median time from diagnosis of NEC to death was 2 days. Forty-seven deaths occurred during the episode of NEC and was causally related to it while 9 deaths occurred after the resolution of the NEC episode. Eight out of the 9 non-NEC related deaths were because of late onset neonatal sepsis. Of the total deaths there were 12 cases of NEC grade 2A [21.4%, relative risk (RR) 0.19 (95% Confidence limit (CL) 0.11 ; 0.33), P<.001], 8 cases of NEC grade 2B [14.3%, RR 1.23 (95% CL 0.67 ; 2.24), P=0.52], 11 cases of NEC grade 3A [19.7%, RR 3.22 (95% CL 2.23 ; 4.52), P <.001] and 28 cases of NEC grade 3B [50%, RR 3.09 (95% CL 2.07 ; 4.61), P<.001]. The median length of stay was 36 days and the median time to transfer was 52.5 days. The median length of stay for the weight categories were 45 days for < 1000g, 37 days for 1001g to 1499g and 28.5 days for > 1500g (P=0.25).

The maternal and neonatal factors related to NEC (including distribution per weight category) are depicted in tables 1 and 2, respectively.

Table 1: Maternal factors in infants < 2000g with NEC

Variable	n	Frequency/ percentage	Median	Weight category 1[+]	Weight category 2[++]	Weight category 3[+++]	P value
Maternal age	178		27				
Parity	175		2	2*	2*	2*	0.26
Gravidity	175		2	2*	2*	2*	0.96
Multiple pregnancy	184	32 (17.4)		10 (31.3)	13 (40.6)	9 (28.1)	0.18
Twin	32	30 (93.8)		9 (30)	13 (43.3)	8 (26.7)	0.50
Triplet	32	2 (6.2)		1 (50)	0	1 (50)	0.50
Antenatal care	182	163 (89.6)		44 (26.7)	89 (54.6)	30 (18.4)	0.37
HIV Status							
Positive	184	73 (39.7)		16 (21.9)	44 (60.3)	13 (17.8)	0.48
Negative	184	111 (60.3)		31 (27.9)	57 (51.3)	23 (20.7)	0.48
Preeclampsia	183	77 (42.1)		24 (31.1)	45 (58.4)	8 (10.4)	0.02
With AEDF[#]	183	11 (6.0)		6 (54.5)	4 (36.4)	1 (9.1)	0.10
PPROM[$]	183	19 (10.4)		7 (36.8)	10 (52.6)	2 (10.5)	0.39
Chorioamnionitis	183	2 (1.1)		1 (50)	1 (50)	0	0.70
Antenatal Steroids	164	99 (60.4)		31 (31.3)	55 (55.6)	13 (13.1)	0.05
Mode of delivery							
C/S[^]	184	107 (58.2)		30 (28)	59 (55.1)	18 (16.8)	0.45
NVD[^^]	184	77 (41.8)		17 (22.1)	42 (54.5)	18 (23.4)	0.45

+ weight category 1: < 1000g
++ Weight category 2: 1001g – 1499g
+++ Weight category 3 : > 1500g
^ Caesarian section
^^ Normal vaginal delivery
* Median , if unmarked: value indicates frequency and percentage
# Absent end diastolic flow
$ Preterm premature Rupture of membranes

Table 2 Neonatal Factors in infants < 2000g with NEC

Variable	n	Frequency/ percentage	Median	Weight category 1[+]	Weight category 2[++]	Weight category 3[+++]	P value
Sex	184						
Male	184	80 (43.5)					
Female	184	104 (56.5)					
Gestational age	184		30				
24-27weeks	184	29 (15.8)		20 (42.5)	9 (8.9)	0	
28-31weeks	184	109 (59.2)		26 (55.3)	71 (70.3)	12 (33.3)	
32-35 weeks	184	46 (25)		1 (2.1)	21 (20.8)	24 (66.7)	
Birth weight	184		1160	47 (25.5)	101 (54.9)	36 (19.6)	
Apgar Score							
1min	168		6	5.5*	7*	7*	0.01
5min	168		8	7*	8*	8*	0.01
Presence of:							
Asphyxia	182	34 (18.7)		12 (35.3)	18 (52.9)	4 (11.8)	0.26
RDS[#]	181	145 (80.1)		43 (29.7)	79 (54.5)	23 (15.9)	0.01
Mechanical ventilation	181	28 (15.5)		3 (10.7)	22 (78.6)	3 10.7)	0.02
Surfactant therapy	180	38 (21.1)		8 (21.1)	26 (68.4)	4 (10.5)	012
PDA[$]	180	39 (21.7)		12 (30.8)	18 (23.1)	9(23.1)	0.50
Other CHD[^]	180	39 (21.7)		11 (28.2)	20 (51.3)	8 (20.5)	0.90
Bacterial sepsis	182	96 (52.7)		25 (26.1)	58 (60.4)	13 (13.5)	0.10
Blood transfusion	176	111 (63.1)		34 (30.6)	61 (55)	16 (14.4)	0.03
NPO during transfusion	88	53 (60.2)		11 (20.7)	32 (60.4)	10 (18.9)	0.06
Time of diagnosis to transfusion	98		3.5				
Feeds							
Breastmilk	158	119 (75.3)		32 (26.9)	68 (57.1)	19 (16)	0.07
DBM[@]	158	13 (8.2)		5 (38.5)	6 (46.2)	2 (15.4)	0.07
Formula	158	6 (3.8)		0	3 (50)	3 (50)	0.07
Breastmilk/DBM	158	9 (5.7)		1 (11.1)	7 (77.8)	1 (11.1)	0.07
Breastmilk/Formula	158	9 (5.7)		2 (22.2)	2 (22.2)	5 (55.6)	0.07
FM85	155	73 (47.1)		14 (19.2)	45 (61.6)	14 (19.2)	0.17
Time of initiation of feeds	142		1.5	1*	2*	1*	0.01

* median, where not marked indicates frequency and percentage
# Respiratory distress syndrome
$ Patent ductus arterious
^ Congenital heart disease
@ Donor breastmilk

There was a total of 218 episodes of NEC amongst the 184 infants diagnosed during the study period. The distribution of the modified Bells staging grade per episode and weight category is depicted in Table 3. The presentation of the cases during the first episode is illustrated in Table 4.

Table 3. The distribution of the modified Bells staging grade of NEC per episode and weight category

Episode number/ weight	Grade 1A*	Grade 1B*	Grade 2A	Grade 2B	Grade 3A	Grade 3B	P value
Episode 1 (n=184)	7 (3.8)		100 (54.3)	20 (10.9)	11 (6)	46 (25)	0.15
<1000g	3 (6.4)		21 (44.7)	9 (19.1)	2(4.3)	12 (25.5)	
1001- 1499g	2 (2)		60 (59.4)	7 (7)	9 (8.9)	23 (22.8)	
>1500g	2 (5.6)		19 (52.8)	4 (11.1)	0	11 (30.6)	
Episode 2 (n=30)	5 (16.7)	2 (6.7)	18 (60)	2 (6.7)	2 (6.7)	1 (3.3)	0.51
<1000g	2 (15.4)	2 (15.4)	5 (38.5)	2 (15.4)	1 (7.7)	1 (7.7)	
1001- 1499g	3 (21.4)	0	10 (71.4)	0	1 (7.1)	0	
>1500g	0	0	3 (100)	0	0	0	
Episode 3 (n=4)	1 (25)	1 (25)	1 (25)	1 (25)			1.0
<1000g	0	1 (33.3)	1 (33.3)	1 (33.3)			
1001- 1499g	1 (100)	0	0	0			
>1500g	0	0	0	0			

* excluded from study but shown here as part of progression pattern

Table 4: Disease Factors in the first episode of NEC

Variable	n	Frequency/ percentage	Weight category 1^{+}	Weight category 2^{++}	Weight category 3^{+++}	P value
Abdominal distension	183	155 (84.7)	45 (29)	87 (56.1)	23 (14.9)	<0.01
Feed intolerance	182	108 (59.3)	32 (29.6)	61 (56.5)	15 (13.9)	0.09
Bloody stools	183	31 (17)	3 (9.7)	14 (45.1)	14 (45.1)	<0.01
Lethargy	183	57 (31.1)	16 (28.1)	31 (54.4)	10 (17.5)	0.86
Cardiovascular compromise	183	66 (36.1)	21 (31.8)	35 (53)	10 (15.2)	0.29
Pneumatosis	176	146 (83)	33 (22.6)	84 (57.5)	29 (19.9)	0.24
Portal venous gas	179	9 (5.0)	3 (33.3)	3 (33.3)	3 (33.3)	0.29
Pneumoperitoneum	181	39 (21.5)	11 (28.2)	20 (51.3)	8 (20.5)	0.77
Fixed bowel loop	178	10 (5.6)	2 (20)	6 (60)	2 (20)	1.0
Thrombocytopenia	164	69 (42.1)	20 (29)	39 (56.5)	10 (14.5)	0.31
Hyponatremia	165	52 (31.5)	22 (42.3)	25 (48.1)	5 (9.6)	<0.01
Metabolic acidosis	164	65 (39.6)	21 (32.3)	35 (53.8)	9 (13.9)	0.13
Positive blood culture	183	86 (47)	24 (27.9)	47 (54.6)	15 (17.4)	0.75

The median time from admission to diagnosis of the first episode of NEC was 9 days, to second episode was 25.5 days and to third episode was 43.5 days. The time from admission to first episode of NEC did not differ significantly amongst the weight categories. The median age at diagnosis of the first episode of NEC was 9 days in <1000g and 10 days in both 1001g- 1499g and >1500g categories (P=0.91).

As depicted in table 4, abdominal distension was the commonest presenting symptom. Positive blood cultures were found in 47% of cases. In 15 cultures more the one organism was detected. The most frequently cultured organisms were Klebsiella Pneumonia (27), Coagulase negative

staphylococcus (18), Acinetobacter Baumani (15), Enterococcus Faecalis (10), Serratia Marsecens (8) and Enterococcus faecium (6).

In terms of management of infants (Table 5), all infants underwent a degree of medical therapy while surgical management was indicated in 66 cases (35.9%). Majority of the patients had grade 3B disease (69.7%), 10 patients had grade 3A disease (15.2%), 8 patients had grade 2B disease (12.1%) and 2 patients had been classified as NEC grade 2A but needing surgery. Surgical intervention consisted of primary peritoneal drains (n=34, performed at median time from diagnosis of 0 days), laparotomy (n=23 in first episode and n=1 in second episode, median time from diagnosis of 1 day) or a combination thereof. Regarding drain insertion, 28 of the 34 patients (82.3%) had a pneumoperitoneum necessitating the procedure, while the remaining 8 patients did not. Twelve of the patients had a drain as a temporizing measure and then proceeded to laparotomy and 22 patients were managed with drains alone (n=34) while 11 patients had a laparotomy as their primary intervention (n=23). Resection and primary anastomosis (primary repair), resection and ileostomy (15) and laparotomy only (5) were possible procedures (n=23). Three patients underwent a clip and drop procedure at laparotomy of which 1 patient went on to have a second relook laparotomy 2 days later. Twenty-one patients had no surgical intervention done and this was fatal in 90.5% of the cases (2 patients had recovered).

Table 5 : Management of first episode of NEC

Variable	n	Frequency/ percentage	Median	Weight category 1[+]	Weight category 2[++]	Weight category 3[+++]	P value
Medical Management	184	184 (100)		47 (25.5)	101 (54.9)	36 (19.6)	
Bowel rest	184	184 (100)		47 (25.5)	101 (54.9)	36 (19.6)	
Antibiotics	184	182 (98.9)		47 (25.8)	100 (55)	35 (19.2)	0.41
Inotropic support	184	48 (26.1)		15 (31.3)	28 (58.3)	5 (10.4)	0.14
Ventilatory support	184	58 (31.5)		12 (20.7)	35 (60.3)	11 (19)	0.53
Surgical management							
Indicated	184	66 (35.9)		20 (30.3)	35 (53)	11 (16.7)	0.49
Primary peritoneal drains	183	34 (18.6)	0*	8 (23.5)	19 (55.9)	7 (20.6)	0.95
Laparotomy	184	23 (12.5)	1 **	3 (13.1)	11 (47.8)	9 (39.1)	0.03
Primary repair		1		0	1 (100)	0	
Resection and ileostomy		15		2 (13.3)	7 (46.7)	6 (40)	1.0
Laparotomy only		5		1 (20)	2 (40)	2 (20)	1.0

* median time from diagnosis to drain insertion
** median time from diagnosis to laparotomy

Surgical NEC accounted for 46 out of the 56 deaths that occurred (82.1%). Majority of these deaths were in patients who received no surgical intervention (19 out of 21, 33.9% of total deaths) and in those managed with peritoneal drains alone (15 out of 22, 26.8% of total deaths).

Five patients undergoing laparotomy as a primary intervention demised while 6 patients recovered. Seven patients who had both a drain inserted and laparotomy demised.

Possible reasons for laparotomy not being performed despite being indicated included: an assessment of NEC totalis pre operatively (8), ELBW infants not for escalation of care (7), presence of severe IVH and poor prognosis (4), assessed as being too ill for theatre (4), demised prior to transfer to facility where surgery could be performed (5), differing opinion on severity of disease and course of management between neonatal and surgical teams (7) and recovery with pencil drains alone (3 out 22 patients managed with drains alone).

All infants had bowel rest as part of medical therapy. The median time from diagnosis to recommencing feeds was 5 days (n=128). The median time from restarting feeds to achieving full enteral feeds following a diagnosis of NEC was 5 days (n=88). This differed by 1 day in the < 1000g weight category (median 6, P=0.71).

2.5 DISCUSSION

NEC remains a significant challenge in the Bloemfontein academic complex, like many other neonatal units in the country. The uneven distribution of cases amongst both hospitals might be explained by the differing bed capacities of each unit with more infants being admitted to Pelonomi regional hospital monthly. Over a tenth of the cases were born in other hospitals (primary and secondary levels) and this indicates the vastness of the impact of NEC in the Free State.

The prevalence of confirmed cases of NEC in this study of 7.1% is comparable to a report from the NICHD Neonatal research network where the mean prevalence was 7% in infants with a birth weight of < 1500g and went up to 15% in infants < 750g [2]. While grade 1 and suspected cases of NEC were not included in this study, it is important to note that these accounted for 8.6% of the population screened. Arguably, there may be an overdiagnosis of this owing to the lack of disease specific signs and laboratory findings as well as the low sensitivity of plain abdominal radiographs used in the modified Bells staging system [5,12].

Furthermore, the distribution amongst the weight categories < 1000g, 1001g to 1499g and >1500g of 47, 101 and 36 is in keeping with existing data that infants most at risk for NEC are VLBW

babies [1,11]. Interestingly, the study also illustrates that infants can have more than one episode of NEC during their admission, the prevalence of one episode was [85.7%; 94.2%], two episodes [5.4%; 13.7%] and three episodes [0.1%; 3.0%]. Table 3 depicted the distribution of the modified Bells staging grade per episode and weight category. While grade 1 NEC was an exclusion criterion in the study, it was reflected here to illustrate the progression of disease amongst some cases. This adds to the complicated and unpredictable nature of NEC in premature infants and why some infants have multiple episodes of NEC remains poorly understood and under researched [5].

The primary outcomes of NEC investigated in the study were death, discharge and transfer. 30.4% of patients demised during the study period with the worst fatalities occurring in infants between 1001g and 1499g (48.2% of deaths) and with grade 3B disease (50% of deaths). The relative risk of NEC grade 3A and 3B causing death was significant in this study. This was similar to a Chinese study that identified grade 3 disease as a risk factor for mortality in LBW and VLBW infants [13]. In the ELBW category, 23 out of 47 patients demised (48.9%), similar to findings by the NICHD Neonatal Research Network where an overall mortality of ELBW infants of 49% was reported [14]. This indicates that ELBW infants remains a vulnerable group in the neonatal population. A possible reason for the higher fatality rate is the restriction of therapeutic measures and escalation of care in resource limited environments, as was the case in 7 infants not undergoing a laparotomy in this study. The median time from admission to death was 11 days and while this was longer in the >1500g category (14 days), the variations amongst the weight groups were not statistically significant. The study was unfortunately under powered to describe other outcome measures such as growth, duration of TPN use and head circumference due to the limited availability of these parameters retrospectively.

Maternal and neonatal factors associated with NEC were tabulated in tables 1 and 2, respectively. The median gestational age of 30 weeks in this study was similar to a report by Velaphi et al. in their Johannesburg based study where this was found to be 31 weeks, but the median birth weight was substantially lower in our cohort (1160g compared to 1370g in Johannesburg) [15]. The weight category most affected was the 1001g to 1500g group (54.9% of the cases), in keeping with findings that infants < 1500g are most at risk for developing NEC [1].

In this study, 17.4% of the mothers were multiparous, a maternal factor that has been found to be an independent predictor of risk of NEC [16]. Riemer et al. reported that HIV exposed neonates at Groote Schuur hospital had an almost doubled risk of NEC when compared to HIV unexposed neonates [17]. While the role of maternal HIV status in developing NEC has been questionable [15] almost 40% of mothers in this study were found to be HIV positive. Further maternal antenatal infection was not significant in the study. The presence of preeclampsia antenatally was found in 42% of cases which was consistent with previous studies [6] and the distribution amongst the weight categories was a significant finding. The administration of antenatal steroids is known to be protective against NEC [4,15] and the antenatal steroid coverage was 60% in this cohort. It is however important to note that several patients may not have had complete steroid maturity at the time of delivery necessitated by the emergent condition of the mother.

In terms of postnatal factors, the presence of RDS and need for mechanical ventilation within the first hour from birth were significant findings amongst the weight categories. There is conflicting data on the role mechanical ventilation plays with one study reporting a reduced risk of NEC due to less hypoxic incidents [15] while others show an increased risk owing to a break in defensive mechanisms and introduction of pathogens into the gastrointestinal system with incorrect placements of endotracheal tubes [13]. RDS and perinatal asphyxia are associated with higher risk of developing NEC due to hypoxic injury to the gut endothelium [6] and while 80% of cases had a diagnosis of RDS in our setting, only 21% received exogenous surfactant therapy. PDA and presence of other congenital heart disease accounted for almost 22% of cases each and while it is lower than other studies [18], a contributing factor to this was the limited access to emergent echocardiogram facilities at Pelonomi hospital during the study period.

The presence of bacterial sepsis in the neonatal period is a leading contributor to developing NEC [3,4] and in NEC associated death [13,19]. Alarmingly, 52.7% of cases in this study were being treated for neonatal sepsis prior to the diagnosis of NEC and once a diagnosis of NEC was made, 47% of the cases had positive blood cultures. This is higher than in previous reports were blood cultures may be positive in a third of cases [2]. The culture profile is vastly accounted for by gram negative organisms as was found in similar studies [4,13] and the high number of CNS cases puts into question sampling techniques and contaminations. Extended spectrum Beta Lactamase producing

Klebsiella was found to be the commonest organism isolated in patients with neonatal sepsis in a South African tertiary neonatal unit, similar to the findings in our setting [20]. The increasing incidence of multidrug resistant organisms in neonatal units remains concerning and late onset neonatal sepsis still contributed to death in our study even after NEC had resolved.

The transfusion of blood products has been shown to double the risk of NEC and it has been described that keeping preterm infants nil by mouth during the transfusion can reduce this risk [21]. This study demonstrates that 111 patients had received blood products before or during the episode of NEC with a median time from diagnosis being 3.5 days. Under half of these patients were kept NPO but this number was also subject to incomplete record keeping.

Feeding choices is another contributor to development of NEC. Breastmilk has been shown to be protective against NEC due to substances in it that affect immunity and mucosal barrier thereby putting formula fed infants at a higher risk for NEC [4]. Furthermore, numerous studies have been conducted to determine the appropriate timing of initiation and rate of progression of feeds [6,22]. The median time of initiation of feeds was 1.5 days in our study and while majority of infants did receive maternal breastmilk initially, a portion of them also require top up feeding with donor milk or formula.

NEC presents within one to two weeks of life and can be of sudden or insidious onset [15]. The median age of diagnosis was 9 days in our study, but this differed amongst the weight categories. The commonest presenting symptoms remained abdominal distension (84.7%) and feed intolerance (59.3%) which was comparative to other studies [4,18]. Radiological and biochemical findings were not statistically significant amongst the weight categories. The commonest grade of disease found was NEC grade 2A and majority of patients recovered with medical therapy alone.

Evidence is lacking with respect to the timing of initiation of enteral feeding following NEC. Current recommendations suggest withholding feeds for 7 to 10 days in medical NEC and 14 days in surgical NEC but limited data reveals that initiating feeds within 5 days of NEC diagnosis was not associated with adverse outcomes [23]. In our setting the median time from diagnosis to feed initiation was 5 days. It is important to note that some infants did not achieve full feeds owing to

recurrence of NEC or worsening clinical condition. The risk of recurrence is thought to be higher in surgical NEC [23].

One study reports that an estimated 20 to 40% of infants require surgery [11] but this can be as high as 50% in other centers [2]. The need for surgical management is largely determined by the severity of disease with pneumoperitoneum and intestinal perforation being the only absolute indication [2,10,11]. Relative indications include clinical deterioration despite maximal medical therapy requiring increased cardiorespiratory support, presence of abdominal mass, increased abdominal tenderness and distention, persistence of portal venous gas and sudden or worsening acidosis, hyponatremia and thrombocytopenia indicative of ongoing necrosis [2,11]. While 35.9% of the total cases were deemed to require surgical intervention in our setting, only 12.5% of the total cases underwent a laparotomy during the study period. More patients underwent primary peritoneal drainage alone and while research on the superiority of this approach over laparotomy has been conflicting and remains on going, it remains widely practiced as a temporizing measure prior to surgery or in extremely ill and ELBW infants not fit for theatre [24]. In this study, this approach was associated with more fatalities than using a combination of drains and laparotomy or laparotomy alone as interventions. Other contributing factors to surgery not being performed in our setting included patients being too ill for theatre or transfer and being diagnosed with NEC totalis preoperatively. During a Tygerberg study, 41% of cases of NEC presented with rapid onset, severe disease. Interestingly, they concluded that pan necrosis could not be diagnosed preoperatively and if a patient could be stabilized, laparotomy should be performed as at least one half of the patients may have limited necrosis amenable to surgical resection [19]. Surgical NEC accounted for majority of the deaths (82.1%) in our study. While rapid progression of disease and clinical deterioration is inevitable in some cases, the timely diagnosis of surgical NEC, stabilization and transfer of patients is a critical factor in the management that might improve outcomes and therefore warrants further research. A study at Steve Biko evaluated the presence of persistent tachycardia as a predictor of progression to perforation and though this was a small sample size, the usefulness in clinical practice may be valuable [25]. Furthermore research into newer biomarkers for the diagnosis and severity of NEC as well as other radiological modalities such as ultrasonography to determine gut necrosis and predicting the need for surgery is ongoing [24].

Limitation of study:

Unfortunately, the sample size was reduced due to insufficient data for some cases leading to it being excluded from the study. There were 81 medical files missing between both institutions and data had to be extracted from discharge summaries alone. While this was sufficient for most of the cases, discharge summaries are of variable quality and subjective nature and a total of 31 patients were excluded owing to missing information. The study design being retrospective and dependent on case files and summaries, also further limited the availability of pertinent parameters such as dates of feed initiation, blood transfusions and feeding practices related to transfusions.

A strength of this study was its ability to depict the progressive nature of NEC disease and the possibility of recurrence of disease in premature infants. It also illustrated the survival of infants according to the severity of illness and degree of intervention received in our centre.

2.6 CONCLUSION

NEC remains a significant challenge to clinicians caring for neonates in Bloemfontein, Free State. The prevalence and mortality related to NEC described in this study are comparable to local and international tertiary neonatal units. However, the surgical management of severe NEC remains a limited resource in our setting. The high mortality related to NEC warrants further research and the adoption of institutional preventative strategies such as feeding choices and practices, use of probiotics, antibiotic stewardship and practices surrounding blood transfusions to improve these parameters. Furthermore, in order to improve the quality of research in neonatology the adoption of databases such as the Vermont Oxford Network may assist in record keeping and allow for participation in larger, multi-centred studies.

2.7. References

1. Hemanshoo T, Kokila L. UPDATES IN : NECROTISING ENTEROCOLITIS. 1865;10(3):38–43.
2. Huda S, Chaudhery S, Ibrahim H, Pramanik A. Neonatal necrotizing enterocolitis: Clinical challenges, pathophysiology and management. Pathophysiology [Internet]. 2014;21(1):3–12. Available from: http://dx.doi.org/10.1016/j.pathophys.2013.11.009

3. Lu Q, Cheng S, Zhou M, Yu J. Risk Factors for Necrotizing Enterocolitis in Neonates: A Retrospective Case-Control Study. Pediatr Neonatol. 2017;58(2):165–70.

4. Gane B, Bhat BV, Adhisivam B, Joy R, Prasadkumar P, Femitha P, et al. Risk factors and outcome in neonatal necrotising enterocolitis. Indian J Pediatr. 2014;81(5):425–8.

5. Eaton S, Rees CM, Hall NJ. Current research in necrotizing enterocolitis. Early Hum Dev [Internet]. 2016;97:33–9. Available from: http://dx.doi.org/10.1016/j.earlhumdev.2016.01.013

6. NECROTISING ENTEROCOLITIS IN PREMATURE INFANTS AT CHRIS HANI BARAGWANATH HOSPITAL . WHAT ARE THE ASSOCIATED FACTORS ? Peter Angura A research report submitted to the Faculty of Health Sciences , University of the Witwatersrand , Johannesburg , in partial . 2013;(January).

7. Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: A systematic review. Arch Dis Child Fetal Neonatal Ed. 2018;103(2):F182–9.

8. Shonhiwa AM, Shuping L, Feller G, Mabotja MCM, Ho E, Makiwane N, et al. An outbreak of necrotising enterocolitis of unknown aetiology in newborns admitted to a neonatal unit in Gauteng province, March – June 2018. Int J Infect Dis. 2019;79(2019):19.

9. Ballot DE, Chirwa T, Ramdin T, Chirwa L, Mare I, Davies VA, et al. Comparison of morbidity and mortality of very low birth weight infants in a Central Hospital in Johannesburg between 2006/2007 and 2013. BMC Pediatr. 2015;15(1):1–12.

10. Neu J, Walker WA. Necrotizing Enterocolitis. N Engl J Med. 2013;364(3):255–64.

11. Lin PW, Stoll BJ. Necrotising enterocolitis. Lancet. 2006;368(9543):1271–83.

12. Soni R, Katana A, Curry JI, Humphries PD, Huertas-Ceballos A. How to use abdominal X-rays in preterm infants suspected of developing necrotising enterocolitis. Arch Dis Child Educ Pract Ed. 2020;105(1):50–7.

13. Qian T, Zhang R, Zhu L, Shi P, Yang J, Yang C yi, et al. Necrotizing enterocolitis in low birth weight infants in China: Mortality risk factors expressed by birth weight categories. Pediatr Neonatol [Internet]. 2017;58(6):509–15. Available from: https://doi.org/10.1016/j.pedneo.2016.10.004

14. Blakely ML, Lally KP, McDonald S, Brown RL, Barnhart DC, Ricketts RR, et al. Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: A prospective cohort study by the NICHD Neonatal Research Network. Ann Surg. 2005;241(6):984–94.

15. Angura P, Velaphi S. Risk factors for necrotising enterocolitis in an HIV-endemic region. Paediatr Int Child Health [Internet]. 2014 Aug 1;34(3):208–15. Available from: https://doi.org/10.1179/2046905514Y.0000000126

16. Lee J young, Park KH, Kim A, Yang HR, Jung EY, Cho SH. Maternal and Placental Risk

Factors for Developing Necrotizing Enterocolitis in Very Preterm Infants. Pediatr Neonatol [Internet]. 2017;58(1):57–62. Available from: http://dx.doi.org/10.1016/j.pedneo.2016.01.005

17. Riemer LJ, Le Roux SM, Harrison MC, Tooke L. Short-term outcomes of HIV-exposed and HIV-unexposed preterm, very low birthweight neonates: a longitudinal, hospital-based study. J Perinatol [Internet]. 2020;40(3):445–55. Available from: http://dx.doi.org/10.1038/s41372-019-0541-4

18. Wilcock A, Victor S. A survey of necrotising enterocolitis at a tertiary neonatal unit. Arch Dis Child. 2013;98(Suppl 1):A1–117.

19. Voss M, Moore SW, van I der M, Pieper C. Fulminanting necrotising enterocolitis: outcome and prognostic factors. Pediatr Surg Int. 1998;13(0179–0358):576–80.

20. Ballot DE, Bandini R, Nana T, Bosman N, Thomas T, Davies VA, et al. A review of - multidrug-resistant Enterobacteriaceae in a neonatal unit in Johannesburg, South Africa. BMC Pediatr. 2019;19(1):1–9.

21. Kt Y, Jy K, Sasi A, Tan K, Nm L. Stopping feeds for prevention of transfusion-associated necrotising enterocolitis in preterm infants (Protocol). 2017;(12).

22. Raban S, Santhakumaran S, Keraan Q, Joolay Y, Uthaya S, Horn A, et al. A randomised controlled trial of high vs low volume initiation and rapid vs slow advancement of milk feeds in infants with birthweights ≤ 1000 g in a resource-limited setting. Paediatr Int Child Health. 2016;36(4):288–95.

23. Hock AM, Chen Y, Miyake H, Koike Y, Seo S, Pierro A. Initiation of Enteral Feeding after Necrotizing Enterocolitis. Eur J Pediatr Surg. 2018;28(1):44–50.

24. Frost BL, Modi BP, Jaksic T, Caplan MS. New Medical and surgical insights into neonatal necrotizing enterocolitis a review. JAMA Pediatr. 2017;171(1):83–8.

25. Van Der Schyff F, Becker JHR. Predictive Value of a Persistent Tachycardia to Indicate Impending Perforation in Necrotizing Enterocolitis. Am J Perinatol. 2018;35(8):774–8.

3. Appendices

APPENDIX A: Letter of Approval from Research Ethics Committee

UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
HEALTH SCIENCES
GESONDHEIDSWETENSKAPPE

Health Sciences Research Ethics Committee

18-Nov-2019

Dear **Dr Tabassum Osman**

Ethics Number: UFS-HSD2019/0333/2708

Ethics Clearance: **Necrotising enterocolitis in Bloemfontein academic complex**

Principal Investigator: **Dr Tabassum Osman**

Department: **Paediatrics and Child Health Department (Bloemfontein Campus)**

SUBSEQUENT SUBMISSION APPROVED

With reference to your recent submission for ethical clearance from the Health Sciences Research Ethics Committee. I am pleased to inform you on behalf of the HSREC that you have been granted ethical clearance for your request as stipulated below:

Amendment details:
Infants were to be excluded if files were not found. But seeing at this can be extrapolated to majority of patients being excluded based on this criteria, it has been decided to remove this exclusion. If files cannot be found, meditech summary of the infant will be used to complete the data collection sheet. If both file and summary cannot be found, then only will patients be excluded.
Based on the pilot study, the data collection tool was evaluated. Additions have been made as highlighted. In order to track the progress of these infants and count them as one case for the study, the base hospital and transfer hospital was added to the form.
It was found that some infants had more than one episode of NEC during their hospital stay. Episode numbers were therefore added to the sheet.
It was found that sometimes the cause of an infant's demise was related to NEC and occurred during the episode and sometimes it was later in admission, for unrelated reasons. This was added to the data collection tool so that NEC related death can be accurately described

The HSREC functions in compliance with, but not limited to, the following documents and guidelines: The SA National Health Act. No. 61 of 2003; Ethics in Health Research: Principles, Structures and Processes (2015); SA GCP(2006); Declaration of Helsinki; The Belmont Report; The US Office of Human Research Protections 45 CFR 461 (for non-exempt research with human participants conducted or supported by the US Department of Health and Human Services- (HHS), 21 CFR 50, 21 CFR 56; CIOMS; ICH-GCP-E6 Sections 1-4; The International Conference on Harmonization and Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Tripartite), Guidelines of the SA Medicines Control Council as well as Laws and Regulations with regard to the Control of Medicines, Constitution of the HSREC of the Faculty of Health Sciences.

For any questions or concerns, please feel free to contact HSREC Administration: 051-4017794/5 or email EthicsFHS@ufs.ac.za.

Thank you for submitting this request for ethical clearance and we wish you continued success with your research.

Yours Sincerely

Dr. SM Le Grange
Chair : Health Sciences Research Ethics Committee

Health Sciences Research Ethics Committee
Office of the Dean: Health Sciences
T: +27 (0)51 401 7795/7794 | E: ethicsfhs@ufs.ac.za
IRB 0000624; REC 230408-011; IORG0005187; FWA00012784
Block D, Deans Division, Room D104 | P.O. Box/Posbus 339 (Internal Post Box G40) | Bloemfontein 9300 | South Africa
www.ufs.ac.za

APPENDIX B: Permission from Department of Health

health

Department of Health

FREE STATE PROVINCE

29 May 2019

Dr T Osman
Dept. of Paediatrics and Child Health
UFS

Dear Dr T Osman

Subject: Necrotising enterocolitis in Bloemfontein academic complex.

- Please ensure that you read the whole document, Permission is hereby granted for the above – mentioned research on the following conditions:
- Participation in the study must be voluntary.
- A written consent by each participant must be obtained.
- Serious Adverse events to be reported to the Free State department of health and/ or termination of the study
- Ascertain that your data collection exercise neither interferes with the day to day running of the **Universitas and Pelonomi Hospital** nor the performance of duties by the respondents or health care workers.
- Confidentiality of information will be ensured and please do not obtain information regarding the identity of the participants.
- **Research results and a complete report should be made available to the Free State Department of Health on completion of the study (a hard copy plus a soft copy).**
- Progress report must be presented not later than one year after approval of the project to the Ethics Committee of the University of Free State and to Free State Department of Health.
- Any amendments, extension or other modifications to the protocol or investigators must be submitted to the Ethics Committee of the University of Free State and to Free State Department of Health.
- **Conditions stated in your Ethical Approval letter should be adhered to and a final copy of the Ethics Clearance Certificate should be submitted to** [illegible] or [illegible] **before you commence with the study**
- No financial liability will be placed on the Free State Department of Health
- Please discuss your study with the institution manager/CEOs on commencement for logistical arrangements
- Department of Health to be fully indemnified from any harm that participants and staff experiences in the study
- Researchers will be required to enter in to a formal agreement with the Free State department of health regulating and formalizing the research relationship (document will follow)
- You are encouraged to present your study findings/results at the Free State Provincial health research day
- Future research will only be granted permission if correct procedures are followed see [illegible]

Trust you find the above in order.
Kind Regards

Dr D Motau
HEAD: HEALTH
Date: 30/05/19

Head : Health
PO Box 227, Bloemfontein, 9300
4th Floor, Executive Suite, Bophelo House, cnr Maitland and Harvey Road, Bloemfontein
Tel: (051) 408 1646 Fax: (051) 408 1556 e-mail [illegible]

www.fs.gov.za

health
Department of Health
FREE STATE PROVINCE

29 May 2019

Dr T Osman
Dept. of Paediatrics and Child Health
UFS

Dear Dr T Osman

Subject: Necrotising enterocolitis in Bloemfontein academic complex.

Please find below the contact details of CEO's for logistical arrangements.

Universitas Academic Hospital	
Name: Dr M Molokomme **Email**: molokommm@universitas.fs.gov.za **Tel**: 051 405 3557	**PA**: Me M Van Der Berg **Email**: vdbergsa@universitas.fs.gov.za
Pelonomi Hospital	
Name: Mrs. BS Ramodula **Email**: ramodulabs@fshealth.gov.za **Tel**: 051 405 3634	**PA**: Me C Ntlhokoa **Email**: ntlhokc@fshealth.gov.za

Trust you find the above in order.

Kind Regards

APPENDIX C: Permission from Head of Department

UFS·UV [redacted]

The Chair: Health Sciences Research Ethics Committee
Dr SM Le Grange
For Attention: Mrs M Marais
Block D, Room 104,
Francois Retief Building
Po Box 339 (G40)
Nelson Mandela Drive
Faculty of Health Sciences
University of the Free State
Bloemfontein
9300

4 March 2019

Dear Dr SM Le Grange

Dr Tabassum Osman (Student number: **2015357086**)

Necrotising enterocolitis in the Bloemfontein academic complex

I, André Venter, hereby grant Tabassum Osman permission to conduct the above mentioned research project. The research will be completed in accordance with myself as Head of Department of Paediatrics and Child Health and Dr Parusha Moodley as supervisor of this study.

Yours faithfully

Prof A Venter

5/3/2019

Date

Department of Paediatrics and Child Health / Departement Pediatrie en Kindergesondheid

APPENDIX D: Copy of Research protocol approved by the HSREC

Necrotising enterocolitis in the Bloemfontein academic complex

By

Tabassum Osman

Department of Paediatrics and Child Health

Faculty of Health Sciences at the University of the Free State

CANDIDATE

Dr. T Osman
Registrar: Department of Paediatrics and Child Health
Faculty of Health Sciences
University of the Free State

STUDY LEADER

Dr. P Moodley
Consultant: Department of Paediatrics and Child Health
Faculty of Health Sciences
University of the Free State

TABLE OF CONTENTS

List of Tables

List of Figures

Abbreviations

ADEF:	Absent end diastolic flow
BW:	Birth weight
C/S:	Caesarian section
CHDX:	Congenital heart disease
ELBW:	Extremely low birth weight
GA:	Gestational Age
GIT:	Gastrointestinal
HIV:	Human immunodeficiency virus
LBW:	Low birth Weight
NEC:	Necrotizing enterocolitis
NICHD:	National institute of child health and human development
NICU:	Neonatal intensive care unit
NVD:	Normal vaginal delivery
PDA:	Patent ductus arteriosus
RDS:	Respiratory distress syndrome
ROM:	Rupture of membranes
TANEC:	Transfusion associated necrotizing enterocolitis
VLBW:	Very low birth weight

Definitions (43)(44)

Birth weight:	The first weight (recorded in grams) of a live or dead product of conception, taken after complete expulsion or extraction from its mother.
Low birth weight:	Birth weight less than 2500g
Very low birth weight:	Birth weight less than 1500g
Extremely low birth weight:	Birth weight less than 1000g

Gestational Age:	The duration of gestation is measured from the first day of the last normal menstrual period. Gestational age is expressed in completed days or completed weeks
Preterm:	Gestational age of less than 37 completed weeks
Term:	Gestational age of 37 to less than 42 completed weeks
Perinatal asphyxia:	The failure to initiate and sustain breathing at birth

1. Introduction

Necrotising enterocolitis (NEC) is an acquired inflammatory condition of the intestinal tract in which gas forming bacteria causes infection, inflammation and infarction of the gut lining (1). It predominantly occurs in premature and low birth weight infants and attributes to a high morbidity and mortality in this population group (1)(4). As a result this severe gastrointestinal disorder proves to have a high burden of disease in neonatal units, particularly in the low to middle income resource centers (5)(13).

2. Literature Review

Necrotising Enterocolitis in Premature Infant

Prevalence:

Worldwide, Necrotising enterocolitis remains a leading cause of neonatal mortality accounting for as much as 20 to 30% of deaths in a neonatal ICU (1)(2). Overall the condition has become synonymous with prematurity in which the incidence is inversely proportional to gestational age (3). Furthermore, as medical advances such as better antenatal care, antenatal steroids and more extensive neonatal interventions allow for higher survival rates of premature infants, so to the population at risk for developing NEC increases (2)(7). It remains evident that the group most at risk are infants with a birth weight below 1500g. It is estimated that approximately 90% of cases of NEC are born prematurely with 12% of those being <1500g at birth (1). It is estimated that one of ten premature infants with birth weight < 1000g can be diagnosed with NEC (7) .

In a Canadian Study by Wendy H Yee et al. using the Canadian Neonatal Network, a cohort of 16669 infants with gestational age <33 weeks over a 5-year period ending in December 2008 from 25 participating NICUs were analyzed with respect to incidence and timing of onset of NEC. It was found that the overall incidence of NEC was 5.1% amounting to 858 infants diagnosed with NEC stage 2. There was a significant variation in risk adjusted incidences which took into account factors such as gestational age, post-natal steroids, PDA treated by indomethacin and 5min Apgar score. In addition, the study found that 40% of infants were diagnosed with early onset NEC occurring at a mean age of 7.6 days and had a higher incidence of surgical NEC. The mean age of diagnosis in the late onset group was 32 days and the peak onset of NEC in this

cohort was 32 weeks post menstrual age(4). This data was similar to the findings of the Eunice Kenedy Shriver National Institute of Child health and Human Development (NICHD) Research Network that reported a mean incidence of 3-11% between 1997 to 2000 and 5-15% between 2003 and 2007 in the United States of America (USA) (4). In USA NEC is currently estimated to occur in 1-5 cases per 1000 live births. In a further report from the NICHD Neonatal Research Network, NEC had a mean prevalence of 7% in 500g-1500g and up to 15% in <750g category(7). In a prospective study in England conducted over 2 years between 2012 to 2013, it was found that the incidence (95% CI) per 1000 preterm admissions ranged from 20.0 to 41.1 (8).

Comparably in developing countries, a smaller study done in Pakistan by Shah et al in which a total of 196 preterm infants (<37 weeks gestation) were included over a 6month period showed a frequency of NEC of 14.28% with a mean gestational age of patients being 33.61 weeks (9).

In South Africa, incidences of NEC of 2.1 and 3.5 per 1000 live births were reported at Chris Hani Barawagnath Academic Hospital neonatal unit during periods of 1988 and 1994 respectively(3). In 2014, Velaphi et al investigated the risk factors for NEC in a setting of high HIV prevalence. During this study over a period of 3 years 110 infants with confirmed NEC were identified with a median birthweight of 1370-1380g and gestational age of 31 weeks (10). At Groote Schuur Hospital, Joolay et al compared the incidence of NEC after implementing low cost strategies and change in practices and described a reduction in the number of cases from 52 (2.5% of total admissions) in 2007-2008 to 14 cases (0.6% of total admissions) in 2009(11). A study determining the survival of VLBW at Charlotte Maxeke Johannesburg Hospital conducted in 2006/2007 found that NEC accounted for 10% of mortalities in this weight category, being the 4th highest cause of mortality preceded by extreme multi-organ immaturity, hyaline membrane disease and asphyxia (5). These studies in the major academic centers in South Africa shows the significant incidence and mortality NEC has on premature infants in our population group and exposes lacking research in this field in Free State and particularly Bloemfontein Academic Complex.

Furthermore, the financial implications necrotizing enterocolitis has on a neonatal unit is significant, amounting to an estimated 500 million to 1 billion US dollars in the United States annually (2). Approximately 20% of the cost of running a NICU can be attributed to either the disease or its complications (1) and the length of stay of neonates requiring surgical intervention was estimated to be 60 days longer than that of unaffected infants (2). If surgery was not

performed, infants stayed 20 days longer in hospital (2). No studies have been done in South Africa to identify these parameters.

Risk Factors:

Numerous risk factors, both antenatally and postnatally has been implicated to the development of NEC. Prematurity as mentioned previously remains the single most independent risk factor with approximately 90% of infants affected by this disease being born premature (3). Numerous studies have been done to identify and quantify these risk factors. Worldwide very low birth weight continues to be greatest risk factor for NEC associated death (12) and there is an inverse relationship between the risk of developing NEC with gestational age and birthweight(12).

In the United States a study conducted between 2011 and 2013 identified a racial disparity among NEC associated deaths with black neonates having a higher mortality rate than other racial groups, the reason for which remains unknown(13). NEC associated death also occurred more commonly in infants born to unmarried mothers, mothers with age <19years and those with no antenatal care (12).

Administration of antenatal steroids to mothers prior to preterm delivery has been found to have a protective effect against these infants developing NEC in the neonatal period and this risk was almost three times higher in infants whose mothers did not receive antenatal steroids (13)(10).

The role that maternal HIV status plays in the risk of developing NEC has been inconclusive. A study in France described an association between HIV positive mothers and increased risk of NEC, particularly those exposed to Zidovudine (3)(10) but similar studies in South Africa did not demonstrate this association. Velaphi et al in Johannesburg and Karpelowsky et al in Cape Town found that there was no difference in the risk of NEC, severity of disease and NEC associated mortality between infants exposed and not exposed to HIV infection (10).

Maternal chorioamnionitis is indirectly associated with NEC as it is a known risk for premature delivery and neonatal sepsis which are both implicated in development of NEC (12). It has also been found that NEC occurs more frequently in babies of mothers who were treated for chorioamnionitis with Co-Amoxiclav though it is difficult to know whether this association is due to maternal infection itself or the effect of the antibiotic (12).

Severe pre-eclampsia during pregnancy has been implicated in increasing the risk of developing NEC and is thought to be due to decreased fetal umbilical artery blood flow, placental insufficiency and subsequent intrauterine hypo-perfusion and adaptation of the fetus leading to decreased blood flow to the intestines which results in hypoxic ischemic injury to the gut lining (3). One study demonstrated that infants born to mothers with severe pre-eclampsia and absent or reversed end diastolic blood flow in umbilical vessels were 6 times more likely to develop NEC than infants with normal blood flow (8) while another demonstrated no increased risk (13).

Infant related risk factors are largely related to feeding practices and presence of conditions that directly or indirectly lead to gut mucosal inflammation or ischemia. It has been found that infants who are mechanically ventilated within the first hour of birth had a fourfold lower risk of NEC as they have less hypoxic incidents during the first few days of life and were likely to be initiated on feeds later (12). Other studies have demonstrated an increased risk of NEC in mechanically ventilated infants due to break in the defensive barriers increasing risk of infection, especially in ELBW infants who are more likely to be intubated (6). Incorrect placement of endotracheal tube in the oesophagus may also predispose to NEC by introducing pathogens into the GIT system (13).

Conditions such as RDS and perinatal asphyxia similarly are associated with a higher risk of developing NEC due to hypoxic injury to gut endothelium caused by an imbalance in vasoactive substances like Endothelin 1 and nitric oxide, that leads to development of inflammation, ischemia and necrosis (3). Surfactant therapy in hyaline membrane disease is not directly associated with NEC risk (12).

The presence of congenital heart lesions has proven to be contentious in the risk for developing NEC. While some literature describes an increased risk likely due to mesenteric hypo-perfusion and ischemia (16), particularly in infants with patent ductus arteriosus (PDA) where a "steal phenomenon" occurs during diastole in which blood flow to mesenteric arteries from aorta is reduced (8), other studies have demonstrated no increased risk of NEC in cyanotic heart disease (6). Risk of NEC has also been shown to be increased in infants exposed to indomethacin within the first 48 hours of life, a drug used in preterm infants to close a PDA (8).

Bacterial sepsis in the neonatal period is a leading contributor to NEC associated death after prematurity in most weight categories (VLBW, NBW and Moderately LBW) (6)(12). Common

associated organisms that have been isolated include E.Coli, Klebsiella, Pseudomonas, Enterobacter and Salmonella (6). The role of infection in NEC is twofold: infection itself may trigger the inflammatory cascade in the gut which is known in the pathogenesis of NEC and conversely bacterial overgrowth and translocation of gut pathogens into the blood stream may lead to overwhelming sepsis (6). Furthermore, prolonged use of empiric antibiotics in ELBW in the first 3-5 days postnatally in the presence of negative blood cultures has been associated with an increased risk of NEC and death (17). This is likely due to broad spectrum antibiotics affecting the natural colonization of the gut with intestinal flora and predisposing to pathogenic bacteria and fungi invasion (16).

Feeding choices is another major contributor to development of NEC. Breastmilk has been shown to be protective against NEC due to substances in it that affect immunity and mucosal barrier, thereby putting formula fed infants at higher risk for developing NEC (13). Substances lacking in formula milk that is found naturally in breastmilk include Immunoglobulin A, Leucocytes, growth factors, mucin, lysozyme, lactoferrin and cytokines (6). Furthermore, breastmilk assists with colonization of neonatal gut as it contains nonpathogenic bacteria such as bifidobacteria and above mentioned molecules with antibacterial properties (8). Immature gut motility and delayed digestion in premature infants directly predisposes to development of NEC. Numerous studies have been conducted to determine the appropriate time of initiation of feeds (early vs late) as well as the progression of feeds in premature infants and its association with NEC. 90% of preterm infants who develop NEC do so after feeds were initiated (8). A South African randomized control study conducted at Groote Schuur Hospital evaluated feeding regimens in a resource limited setting. The researchers found that ELBW infants receiving rapid advancement of feeds (36ml/kg/day) and high volume initiation of feeds (24ml/kg/day) were able to tolerate the feeds well and achieved a weight of 1500g and discharge sooner than the counter group of slow advancement (24ml/kg/day) and low volume (4ml/kg/day) feeds. While limited in its ability to evaluate feed related morbidity, the study did conclude that NEC was not increased in these ELBW infants(18). The timing of initiation of feeds has also been a contentious issue in evaluating the risk of NEC. While some studies show an increased risk of developing NEC in infants who were initiated on feeds earlier, majority of studies support the conclusion that early enteral feeding does not significantly increase risk of NEC (8)(10) Ultimately a Cochrane met-analyses concluded that the slow advancement and delayed initiation of feeds did not reduce the risk of NEC in preterm, VLBW and growth restricted infants and that slow advancement of feeds lead to

increased risk of infection and metabolic complications due to prolonged parental nutrition exposure (18)(19)

Transfusion of blood products has been shown to double the risk of developing NEC (20). One study showed that 27% of infants were diagnosed with NEC within 48hours of receiving a blood transfusion (16). There are a number of proposed theories as to why transfusion associated NEC (TANEC) occurs: Severe anemia may lead to impaired gut blood flow, there may be immunological mediators in blood products that cause an immune reaction in the gut and ischaemia/ reperfusion injury may occur with transfusion. It has been described that keeping preterm infants nil by mouth during transfusion can decrease the risk of TANEC and this has led to a proposed Cochrane review to further analyze this recommendation (19).

Identifying these known risk factors early can allow for better surveillance and earlier diagnosis and treatment of infants.

Clinical Presentation:

NEC presents within one to two weeks of life and can be of sudden or insidious onset (8). Early onset of disease can occur at a mean age of 7 days while late onset NEC can be delayed to 32 days of age in smaller, more premature infants (4). Clinical features of NEC can vary depending on severity of disease. Bell et al suggested a staging system in 1978 based on severity and this was later modified by Walsh and Kliegman by adding subcategories in the stages and including signs that differentiate course of disease (8)(9) (See below).

Table 1: Modified Bells Staging (8)

Stage	Systemic signs	Abdominal signs	Radiographic signs	Treatment
IA Suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus	NPO, antibiotics x 3 days
IB Suspected	Same as above	Grossly bloody stool	Same as above	Same as IA
IIA Definite, mildly ill	Same as above	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
IIB Definite, moderately ill	Same as above, plus mild metabolic acidosis and thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus ascites	NPO, antibiotics x 14 days
IIIA Advanced, severely ill, intact bowel	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as IIA, plus ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
IIIB Advanced, severely ill, perforated bowel	Same as IIIA	Same as IIIA	Same as above, plus pneumoperitoneum	Same as IIA, plus surgery

In the early stages, signs may be nonspecific and include lethargy, temperature instability, abdominal distention and increased gastric retention. In one study abdominal distention was the most common clinical sign, found in 85% of cases, followed by increased pre feed aspirate (70%), lethargy (40%), vomiting (34%) and various other signs like apnea, bradycardia, hypothermia, absent bowel sounds and GIT bleed (13). Feeding intolerance is common but can also occur in other conditions which affect gut motility including septicemia, gastrointestinal esophageal reflux disease and certain medications such as caffeine and indomethacin. This leads to an over diagnosis of stage 1 disease (7). As severity progresses other signs may be evident including abdominal wall erythema, tenderness and guarding and presence of an abdominal mass in right lower quadrant. Systemic compromise becomes more marked, necessitating the need for ventilators and/or inotropic support.

Radiologically, stage 1 disease may present with distended bowel loops while stage 2 consists of the classical radiological signs of a fixed bowel loop, pneumatosis intestinalis and portal venous gas (3). In the most severe form, pneumoperitoneum is present (3). This can also occur in spontaneous intestinal perforation, a condition that also affects preterm infants but presents in the first few days after birth and characteristically has minimal gut inflammation and necrosis making it different to NEC (2).

Biochemically, laboratory findings like some of the clinical signs are nonspecific(7). They include neutropenia, leukocytosis, thrombocytopenia, hyponatremia, metabolic acidosis, elevated C-reactive protein and glucose disturbances (3)(9). Blood cultures may be positive in a third of the cases (9). Other markers that have been studied include intestinal fatty acid binding protein, urinary D lactate and fecal calprotectin but research and availability of these tests remain limited. A severe fulminant form of the disease, NEC totalis may present rapidly with widespread gut necrosis and has a poor prognosis (9)(26). Ultimately, a high index of suspicion is needed in higher risk infants particularly preterm and VLBW infants allowing for early detection of onset of NEC.

Management:

Once diagnosed, NEC can be managed in one of two approaches: medically or surgically and this is determined largely by the severity of disease. In early stages, medical management consisting of bowel decompression, bowel rest (stopping feeds), intravenous broad spectrum antibiotics for

7 to 10 days and total parental nutrition is sufficient (1)(7). This said, constant clinical, radiological and biochemical reevaluation must be done 6 to 8 hourly to detect deterioration and intestinal perforation which requires emergency surgery. Surgery is ultimately required in one third to one half of medically managed cases (3).

Surgical intervention is based on absolute and relative indications in advanced cases of NEC. The only absolute indication is pneumoperitoneum or intestinal perforation (3)(7)(9). Relative indications for surgical intervention include: clinical deterioration despite maximal medical therapy requiring increased cardiorespiratory support, presence of abdominal mass, increased abdominal tenderness and distention, persistence of portal venous gas and sudden or worsening acidosis, hyponatremia and thrombocytopenia indicative of ongoing necrosis (3)(9). Two surgical approaches may be used: primary peritoneal drain and laparotomy, each with its advantages and disadvantages. Insertion of peritoneal drains is a bed side procedure that can be lifesaving and assist with resuscitation of the infant by decompressing a tensely distended abdomen and allowing for better ventilation (reduces intra-abdominal pressure) (7). Release of feculent matter also reduces the risk of contamination (9). Some paediatric surgeons have reported to use this method as definitive treatment of NEC (3). Laparotomy allows for surgeon to thoroughly assess and decontaminate the bowel but has the disadvantage of adding stress to the infant body. During laparotomy a number of procedures can be performed depending on the extent of necrosis. In limited disease either resection and enterostomy or primary anastomosis can be performed and in multi-segment disease either high jejunostomy (not favored due to high output stoma) or clip and drop method necessitating need for a second surgery within 24-48 hours (3). The choice of surgical intervention has remained controversial. Moss et al concluded in their study of 117 preterm infants that the type of intervention done did not significantly differ in terms of 90-day post-operative mortality or dependence on TPN as well as length of stay in hospital. The study was however limited by its smaller sample size (29). In contrast, other studies showed infants treated with peritoneal drains often required a laparotomy anyway and that mortality was increased by more than 50% with peritoneal drains compared to laparotomy (2). Furthermore, a multi-centered observational study has suggested that long term neurodevelopment outcome may be better with laparotomy versus peritoneal drainage (21).

Outcomes:

In a South African study of 128 patients at Tygerberg Hospital between 1993 and 1995, 41% of babies had presented with rapid onset, severe disease requiring surgery of which 52% was resectable and of these infants 60% survived till 30 days (26). Infants with pan-necrosis (NEC totalis) however had the worst prognosis with none of the 19 infants surviving (20). 20 years later, NEC remains a significant predictor of survival in a South African setting (5). Arnold et al found that early survival (30 days post operatively) was 69 % overall and 71% in <1500g birth weight group with overwhelming sepsis and pan-necrosis causing most deaths (30). Late deaths (>30 days post operatively) was largely due to short bowel syndrome or sepsis wit intraventricular hemorrhage also contributing (22). Late complications included late colonic strictures, incisional hernias and adhesive bowel obstruction. Severe Neurological deficit and neurodevelopment delay was significant in long term survivors (20% and 49& respectively) (22).

In other developing countries such as India, a study described mortality to be 15,3% of cases with stage 2 NEC and 76,9% of cases with Stage 3 disease. Of the infants with stage 3 disease, 4 out of 13 were managed surgically and 3 of these infants demised indicating a high mortality rate (13). A prospective cohort study by the NICHD Neonatal research network concluded that overall 49% of ELBW undergoing surgical intervention for NEC or isolated intestinal perforation demised. The median time to death was 8.5 days. The mortality rate in patients with pre-operative diagnosis of NEC was 55,2% (31). It is well accepted that advanced NEC requiring surgical intervention has a poorer outcome and that mortality for ELBW undergoing surgery is approximately 50% (2)(23) Furthermore, it is found that NEC is associated with a significantly worse neurodevelopment outcome than prematurity itself and the presence of advanced NEC requiring surgical intervention further increases the risk for neurological impairment (32)(33). Growth delay is also significantly worse in ELBW undergoing surgical management for NEC (25).

Overall, a review of literature available shows that while NEC has been studied in many worldwide centers with respect to prevalence, risk factors, treatment modalities and outcomes, there is a paucity of local published data to comparatively assess how we fair in the fight against this disease.

3. Aim

To describe necrotizing enterocolitis in Neonatal Units in the Bloemfontein Academic Complex between 2016 to 2018.

3.1 Primary Objective

To describe the prevalence and short term outcomes (death, discharge or transfer) of infants with a birthweight <2000g admitted with necrotising enterocolitis to neonatal units in the Bloemfontein Academic Complex between 2016 and 2018.

3.2 Secondary Objectives

1. To describe the following known risk factors for Necrotising Enterocolitis in infants with birth weight <2000g:
    a) Antenatal care and antenatal steroid administration
    b) Maternal Peripartum Infection including HIV status
    c) Presence of perinatal asphyxia, RDS and congenital heart lesions
    d) Enteral feeds including type of feeds initiated and rate of feed progression
    e) Transfusion of blood products prior to illness
    f) Bacterial sepsis in the neonatal period

2. To describe the course of illness of low birth weight infants with Necrotising Enterocolitis, namely:
    a) Time taken to achieve full enteral feeds
    b) Intercurrent Infections
    c) Presence of failure to thrive/ poor weight gain
    d) Length of stay in hospital

3. To compare the prevalence, risk factors, course of illness and outcomes between infants with respect to the following weight categories:

a) < 1000g

b) 1001g to 1499g

c) 1500g to 2000g

4. METHODOLOGY

4.1 Study Design

This is a retrospective, descriptive cross-sectional study.

4.2 Sample

4.2.1 Study Population

The Bloemfontein academic complex consists of Universitas Academic Hospital, Pelonomi Regional Hospital, National District Hospital and Free State Psychiatric Hospital. The latter two hospitals are excluded from this study as the Free State Psychiatric Hospital does not care of babies and National District Hospital while providing care for some for stable babies, would otherwise refer ill infants such as those with RDS and NEC to Pelonomi Regional hospital for further management.

The population of the study will therefore be confined to infants born with a birth weight of less than 2000g with confirmed NEC (Stages 2A to 3B) according to the modified Bells Staging (refer to Table 1), admitted in neonatal units in Pelonomi Regional Hospital and Universitas Academic Hospital, during a 3-year period from January 2016 to December 2018. The in-house neonatal databases of both hospitals will be used and it is comprised of an Excel Spreadsheet that is updated on a monthly basis by paediatric registrars rotating through Neonatal ICU. Based on perusal of this, we approximate a population size of about 2000 infants.

4.2.2 Inclusion Criteria

The study will include all infants with a birth weight of <2000g. The modified Bells staging (refer to Table 1) will be used to grade the severity of NEC. Infants will be included if stage 2A, 2B, 3A or 3B disease is present. If grading is not clearly documented in the file, the researcher will retrospectively stage the disease. Infants included must be admitted to either neonatal units at Pelonomi Regional Hospital or Universitas Academic Hospital between January 2016 and December 2018. Infants out of the neonatal period will also be included in the study. If medical records (admission folder) is missing, infants will still be included in the study by using the meditech discharge summary to obtain necessary information.

4.2.3 Exclusion Criteria

Any infant with NEC stage 1 or suspected NEC according to the modified Bells staging will be excluded. Infants with congenital gastrointestinal tract abnormalities will be excluded. Lastly, if medical records (admission folder) and meditech discharge summary is missing, then infants will also be excluded.

4.2.4 Estimated Sample Size

We estimate that over a 3-year period at least 100 cases will be identified between the two hospital's neonatal units.

4.2.5 Method of Selection of Sample Size

Convenience sampling will be used.

4.3 Measurement

Once necessary approvals for the research study is obtained, infants meeting the inclusion criteria will be identified by the researcher using the in-house Neonatal database in both hospitals in

which patient details including name, hospital file number, gestational age, birth weight and diagnosis of all admissions to each unit is recorded. In addition, the Meditech system linked between both hospitals will be used by the researcher to augment identification of cases by screening discharge summaries for Necrotizing enterocolitis as a diagnosis during admission. A list of possible participants will be compiled and patient admission file for the infants identified will be obtained by the researcher from medical records at each hospital. If the patients admission file cannot be found, the meditech discharge summary will be used. The data collection process will be done by the researcher in a private room at the Department of Paediatrics. Each participant will be assigned a unique code by the researcher ensuring that the name and hospital numbers are not used. The relevant information will be extracted from the file and/or discharge summary using a comprehensive data collection sheet (see appendix). If an infant is found to not have a modified Bells staging, the researcher will retrospectively stage the disease (using the standard staging method outlined in Table 1) when completing the data sheet. The researcher will transfer completed data sheets to an excel spreadsheet weekly and once all participants are captured on the spreadsheet this will be sent to the biostatistician for analysis. See below a flow diagram summarizing the above process.

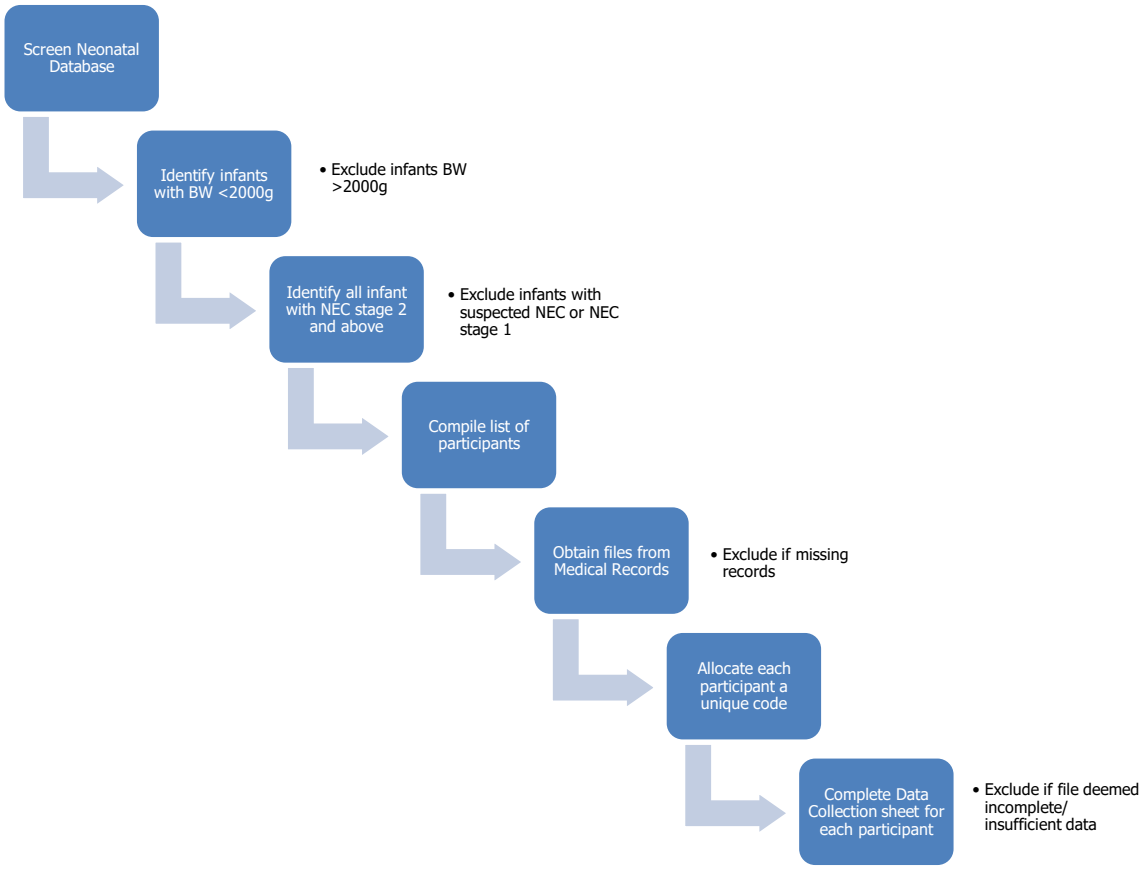

Figure 1: Summary of research study

4.4 Methodological and measurement errors

As a retrospective study the factors needed to be identified is based on chart reviews which may be incomplete or incorrectly recorded. The neonatal databases used to identify cases may also be incomplete. This will be overcome by using the Meditech system in conjunction with in house statistic database to identify all possible cases that can be included.

Data integrity will be maintained by capturing the data sheet on excel spreadsheets twice and then cross checking it. Errors identified in capturing of data will then be corrected by the researcher.

4.5 Pilot Study

A pilot study will be conducted using 6 patients in total that meets the above mentioned inclusion criteria. The first case for each year of the study period (namely 2016 to 2018) will be used from each hospital (therefore 3 cases per hospital). The methodology and data collection sheet will be evaluated. Adjustments to the data sheet will be made if needed. The patients used in the pilot study will later be included in the analysis if no changes were made.

5. Analysis

Descriptive statistics namely frequencies and percentages for categorical data and means and standard deviations or medians and percentiles for numerical data will be calculated, per group. The groups will be compared by means of appropriate statistical tests. The prevalence of necrotising enterocolitis will be calculated and described by means of 95% confidence interval for the prevalence. The analysis will be done by the Department of Biostatistics.

6. Implementation of findings

The research study will firstly be used towards the principal researcher fulfilling her requirements for her MMED. At a later stage, the researcher would like to publish her findings to add to the advancement of knowledge about this condition in a South African setting. Lastly, the researcher would like the findings of the study to guide further treatment protocols and strategies implemented in the neonatal units of both Pelonomi Regional Hospital and Universitas Academic hospital in addressing the impact of necrotising enterocolitis.

7. Time Schedule

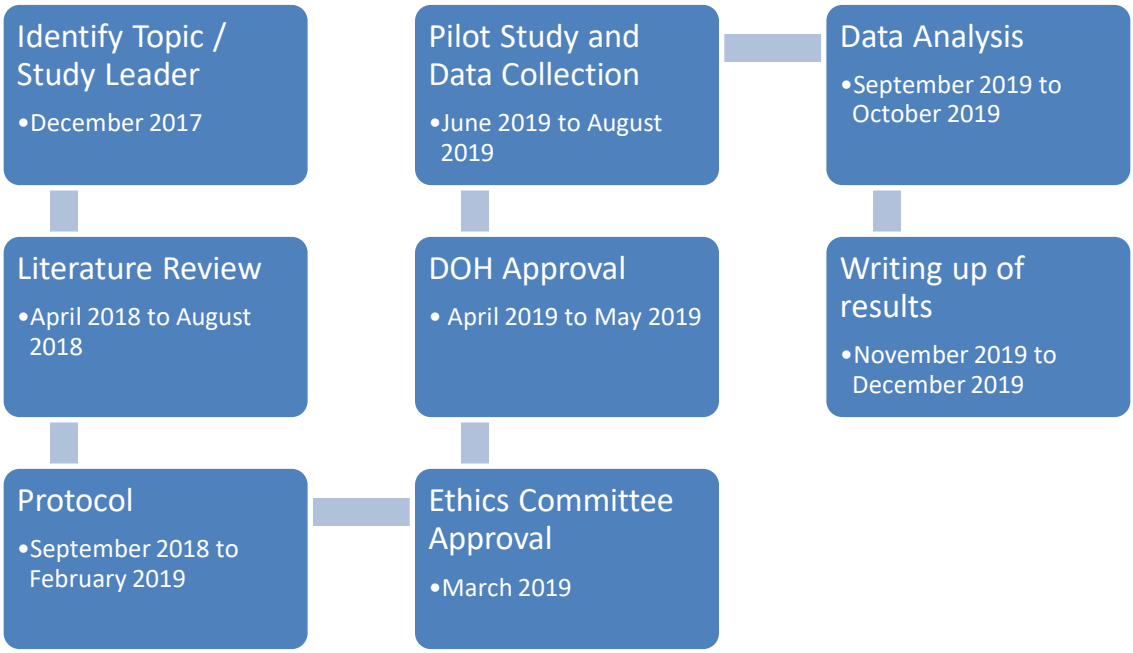

Figure 2: Study time schedule

8. Budget

The costs involved in this study will be for traveling to Universitas and Pelonomi Hospitals for data collection and printing of the data collection sheet. This is estimated to amount to R500 and costs will be covered by the researcher.

Table 2: Budget

ITEM	**COST**
Transport	R200
Printing of data collection sheet	R300
Total	**R500**

9. Ethical Aspects

Patient confidentiality will be maintained by using a coding system where each participant is allocated a unique code that will be used on the data collection sheet. This will be the only identifying detail on the data collection sheet.

A coding form will be used to keep record of which codes are assigned to each patient file and this form will only be privy to the researcher during the data collection phase of the study.

Admission file/records of patients will be used and secured in a private room at the Department of Paediatrics during data collection and once no longer in use will be returned to the medical records Department of each hospital within one week of completion of data collection. Information extracted from the patient files will only be accessible to the research team while the study is underway.

The protocol will be submitted to the Health Sciences Research Ethics Committee (HSREC) of the Faculty of Health Sciences at the University of the Free State and the Free State Department of Health for approval.

Permission from the Head of Department of Paediatrics and Head of Neonatology will be obtained.

There are no conflicts of interest.

10. References

1. Hemanshoo T, Kokila L. UPDATES IN : NECROTISING ENTEROCOLITIS. 1865;10(3):38–43.
2. Neu J, Walker WA. Necrotizing Enterocolitis. N Engl J Med. 2013;364(3):255–64.
3. NECROTISING ENTEROCOLITIS IN PREMATURE INFANTS AT CHRIS HANI BARAGWANATH HOSPITAL . WHAT ARE THE ASSOCIATED FACTORS ? Peter Angura A research report submitted to the Faculty of Health Sciences , University of the Witwatersrand , Johannesburg , in partial . 2013;(January).
4. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK. Incidence and Timing of Presentation of Necrotizing Enterocolitis in Preterm Infants. Pediatrics [Internet]. 2012;129(2):e298–304. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2011-2022
5. Ballot DE, Chirwa T, Ramdin T, Chirwa L, Mare I, Davies VA, et al. Comparison of morbidity and mortality of very low birth weight infants in a Central Hospital in Johannesburg between 2006/2007 and 2013. BMC Pediatr. 2015;15(1):1–12.
6. Battersby C, Santhalingam T, Costeloe K, Modi N. Incidence of neonatal necrotising enterocolitis in high-income countries: A systematic review. Arch Dis Child Fetal Neonatal Ed. 2018;103(2):F182–9.
7. Huda S, Chaudhery S, Ibrahim H, Pramanik A. Neonatal necrotizing enterocolitis: Clinical challenges, pathophysiology and management. Pathophysiology [Internet]. 2014;21(1):3–12. Available from: http://dx.doi.org/10.1016/j.pathophys.2013.11.009
8. Battersby CWS, Mandalia S, Fitz-Simon N, Costeloe K, Modi N. G396 A prospective population study of the incidence of severe necrotising enterocolitis in England. Arch Dis Child [Internet]. 2016 Apr 1;101(Suppl 1):A229 LP-A230. Available from: http://adc.bmj.com/content/101/Suppl_1/A229.3.abstract
9. Shah SS, Saleem M, Mehmood T, Ahmed T. Frequency and Outcome of Necrotizing Enterocolitis in Preterm Neonates. J Ayub Med Coll Abbottabad. 2015;27(1):85–7.
10. Angura P, Velaphi S. Risk factors for necrotising enterocolitis in an HIV-endemic region. Paediatr Int Child Health [Internet]. 2014 Aug 1;34(3):208–15. Available from: https://doi.org/10.1179/2046905514Y.0000000126
11. Joolay Y, Rhoda NR, oke LT, Horn AR, Harrison MC. A reduction of necrotising

enterocolitis at Groote Schuur Hospital nursery. South African Med J. 2011;101(11):806.
12. Seeman SM, Mehal JM, Haberling DL, Holman RC, Stoll BJ. Infant and maternal risk factors related to necrotising enterocolitis-associated infant death in the United States. Acta Paediatr Int J Paediatr. 2016;105(6):e240–6.
13. Gane B, Bhat BV, Adhisivam B, Joy R, Prasadkumar P, Femitha P, et al. Risk factors and outcome in neonatal necrotising enterocolitis. Indian J Pediatr. 2014;81(5):425–8.
14. Laher A, Ballot DE, Ramdin T, Chirwa T. A review of antenatal corticosteroid use in premature neonates in a middle-income country. South African Med J. 2017;107(9):768–72.
15. Riemer LJ, Le Roux SM, Harrison MC, Tooke L. Short-term outcomes of HIV-exposed and HIV-unexposed preterm, very low birthweight neonates: a longitudinal, hospital-based study. J Perinatol [Internet]. 2020;40(3):445–55. Available from: http://dx.doi.org/10.1038/s41372-019-0541-4
16. Lu Q, Cheng S, Zhou M, Yu J. Risk Factors for Necrotizing Enterocolitis in Neonates: A Retrospective Case-Control Study. Pediatr Neonatol. 2017;58(2):165–70.
17. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, et al. Prolonged Duration of Initial Empirical Antibiotic Treatment Is Associated With Increased Rates of Necrotizing Enterocolitis and Death for Extremely Low Birth Weight Infants. Pediatrics. 2009;123(1):58–66.
18. Raban S, Santhakumaran S, Keraan Q, Joolay Y, Uthaya S, Horn A, et al. A randomised controlled trial of high *vs* low volume initiation and rapid *vs* slow advancement of milk feeds in infants with birthweights ≤ 1000 g in a resource-limited setting. Paediatr Int Child Health [Internet]. 2016;36(4):288–95. Available from: https://www.tandfonline.com/doi/full/10.1179/2046905515Y.0000000056
19. Oddie SJ, Young L, Mcguire W. Slow advancement of enteral feed volumes to prevent necrotising enterocolitis in very low birth weight infants. Cochrane Database of Systematic Reviews. 2017.
20. Kt Y, Jy K, Sasi A, Tan K, Nm L. Stopping feeds for prevention of transfusion-associated necrotising enterocolitis in preterm infants (Protocol). 2017;(12).
21. Van Niekerk E, Nel DG, Blaauw R, Kirsten GF. Probiotics reduce necrotizing enterocolitis severity in HIV-exposed premature infants. J Trop Pediatr. 2015;61(3):155–64.
22. Eaton S, Rees CM, Hall NJ. Current research in necrotizing enterocolitis. Early Hum Dev

[Internet]. 2016;97:33–9. Available from: http://dx.doi.org/10.1016/j.earlhumdev.2016.01.013

23. Uberos J, Aguilera-Rodríguez E, Jerez-Calero A, Molina-Oya M, Molina-Carballo A, Narbona-López E. Probiotics to prevent necrotising enterocolitis and nosocomial infection in very low birth weight preterm infants. Br J Nutr. 2017;117(7):994–1000.
24. Van Der Schyff F, Becker JHR. Predictive Value of a Persistent Tachycardia to Indicate Impending Perforation in Necrotizing Enterocolitis. Am J Perinatol. 2018;35(8):774–8.
25. Nguyen DN, Sangild PT. Pathogenesis and biomarkers for necrotizing enterocolitis: Getting any closer? EBioMedicine [Internet]. 2019;45:13–4. Available from: https://doi.org/10.1016/j.ebiom.2019.06.029
26. Voss M, Moore SW, van I der M, Pieper C. Fulminanting necrotising enterocolitis: outcome and prognostic factors. Pediatr Surg Int. 1998;13(0179–0358):576–80.
27. Soni R, Katana A, Curry JI, Humphries PD, Huertas-Ceballos A. How to use abdominal X-rays in preterm infants suspected of developing necrotising enterocolitis. Arch Dis Child Educ Pract Ed. 2020;105(1):50–7.
28. He Y, Zhong Y, Yu J, Cheng C, Wang Z, Li L. Ultrasonography and radiography findings predicted the need for surgery in patients with necrotising enterocolitis without pneumoperitoneum. Acta Paediatr Int J Paediatr. 2016;105(4):e151–5.
29. Moss RL, Dimmitt RA, Barnhart DC, Sylvester KG, Brown RL, Powell DM, et al. Laparotomy versus Peritoneal Drainage for Necrotizing Enterocolitis and Perforation. N Engl J Med [Internet]. 2006;354(21):2225–34. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa054605
30. Arnold M, Moore SW, Sidler D, Kirsten GF. Long-term outcome of surgically managed necrotizing enterocolitis in a developing country. Pediatr Surg Int. 2010;26(4):355–60.
31. Blakely ML, Lally KP, McDonald S, Brown RL, Barnhart DC, Ricketts RR, et al. Postoperative outcomes of extremely low birth-weight infants with necrotizing enterocolitis or isolated intestinal perforation: A prospective cohort study by the NICHD Neonatal Research Network. Ann Surg. 2005;241(6):984–94.
32. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed. 2007;92(3):193–8.
33. Hintz SR. Neurodevelopmental and Growth Outcomes of Extremely Low Birth Weight

Infants After Necrotizing Enterocolitis. Pediatrics [Internet]. 2005;115(3):696–703. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2004-0569

34. Shonhiwa AM, Shuping L, Feller G, Mabotja MCM, Ho E, Makiwane N, et al. An outbreak of necrotising enterocolitis of unknown aetiology in newborns admitted to a neonatal unit in Gauteng province, March – June 2018. Int J Infect Dis. 2019;79(2019):19.
35. Lin PW, Stoll BJ. Necrotising enterocolitis. Lancet. 2006;368(9543):1271–83.
36. Qian T, Zhang R, Zhu L, Shi P, Yang J, Yang C yi, et al. Necrotizing enterocolitis in low birth weight infants in China: Mortality risk factors expressed by birth weight categories. Pediatr Neonatol [Internet]. 2017;58(6):509–15. Available from: https://doi.org/10.1016/j.pedneo.2016.10.004
37. Lee J young, Park KH, Kim A, Yang HR, Jung EY, Cho SH. Maternal and Placental Risk Factors for Developing Necrotizing Enterocolitis in Very Preterm Infants. Pediatr Neonatol [Internet]. 2017;58(1):57–62. Available from: http://dx.doi.org/10.1016/j.pedneo.2016.01.005
38. Wilcock A, Victor S. A survey of necrotising enterocolitis at a tertiary neonatal unit. Arch Dis Child. 2013;98(Suppl 1):A1–117.
39. Ballot DE, Bandini R, Nana T, Bosman N, Thomas T, Davies VA, et al. A review of - multidrug-resistant Enterobacteriaceae in a neonatal unit in Johannesburg, South Africa. BMC Pediatr. 2019;19(1):1–9.
40. Raban S, Santhakumaran S, Keraan Q, Joolay Y, Uthaya S, Horn A, et al. A randomised controlled trial of high vs low volume initiation and rapid vs slow advancement of milk feeds in infants with birthweights ≤ 1000 g in a resource-limited setting. Paediatr Int Child Health. 2016;36(4):288–95.
41. Hock AM, Chen Y, Miyake H, Koike Y, Seo S, Pierro A. Initiation of Enteral Feeding after Necrotizing Enterocolitis. Eur J Pediatr Surg. 2018;28(1):44–50.
42. Frost BL, Modi BP, Jaksic T, Caplan MS. New Medical and surgical insights into neonatal necrotizing enterocolitis a review. JAMA Pediatr. 2017;171(1):83–8.
43. Region SA. South East Asia Regional NEONATAL-PERINATAL DATABASE World Health Organization (South-East Asia Region). Available from: https://www.newbornwhocc.org/pdf/database.pdf
44. Agarwal R, Jain A, Deorari AK, Paul VK. Post-resuscitation management of asphyxiated neonates. Indian J Pediatr. 2008;75(2):175–80.

11. Appendices

APPENDIX A: Modified Bells Staging (8)

Stage	Systemic signs	Abdominal signs	Radiographic signs	Treatment
IA Suspected	Temperature instability, apnea, bradycardia, lethargy	Gastric retention, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus	NPO, antibiotics x 3 days
IB Suspected	Same as above	Grossly bloody stool	Same as above	Same as IA
IIA Definite, mildly ill	Same as above	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
IIB Definite, moderately ill	Same as above, plus mild metabolic acidosis and thrombocytopenia	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA, plus ascites	NPO, antibiotics x 14 days
IIIA Advanced, severely ill, intact bowel	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, DIC, and neutropenia	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as IIA, plus ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
IIIB Advanced, severely ill, perforated bowel	Same as IIIA	Same as IIIA	Same as above, plus pneumoperitoneum	Same as IIA, plus surgery

APPENDIX B: Data Collection Sheet

Enrollment Number:

Demographics :

Date of birth:

Date of admission:

Base Hospital:	Pelonomi		Universitas	Other
Sex:	Male		Female	
Gestational Age:				(In weeks)
	Certain		Uncertain	
Birth weight:				(in grams)

Maternal factors:

Maternal age:
Parity:
Gravidity:

Multiple pregnancy:		Yes	No
		Twin	Triplet
Antenatal Care received:		Yes	No
HIV status:	Positive		Negative Unknown

Presence of the following:

Pre-eclampsia;	Yes	No
Pre-eclampsia with AEDF:	Yes	No
Prolonged ROM:	Yes	No
Chorioamnionitis	Yes	No
	Yes	No
Antenatal Steroids:	Yes	No
Mode of Delivery:	NVD	C/S

Neonatal Factors:

Apgar:	1min		5min
Presence of the following:			
Asphyxia:		Yes	No
RDS		Yes	No
Mechanical Ventilation		Yes	No
Surfactant therapy		Yes	No
PDA		Yes	No
Other CHDx		Yes	No
Bacterial Sepsis		Yes	No
Blood transfusion		Yes	No
	Date		
	NPO	Yes	No

Feeding:

Date of initiation:

Type of feed:

FM85:

Illness:	Episode 1:		Episode 2:		Episode 3	
Date of diagnosis:						
NEC Grade: 2A						
2B						
3A						
3B						
Presence of the following:						
Abdominal distension	Yes	No	Yes	No	Yes	No
Feed intolerance	Yes	No	Yes	No	Yes	No
Blood stool	Yes	No	Yes	No	Yes	No
Lethargy	Yes	No	Yes	No	Yes	No
Cardiovascular compromise	Yes	No	Yes	No	Yes	No
Pneumatosis	Yes	No	Yes	No	Yes	No

Portal Venous gas	Yes	No	Yes	No	Yes	No
Pneumoperitoneum	Yes	No	Yes	No	Yes	No
Fixed bowel loop	Yes	No	Yes	No	Yes	No
Thrombocytopenia	Yes	No	Yes	No	Yes	No
Hyponatremia	Yes	No	Yes	No	Yes	No
Metabolic acidosis	Yes	No	Yes	No	Yes	No
Positive blood culture	Yes	No	Yes	No	Yes	No

Organism

Management:

Medical:	Yes	No
Bowel Rest: Feeds stopped	Yes	No
Antibiotics	Yes	No
Inotropes	Yes	No
Ventilation	Yes	No
Surgery indicated:	Yes	No
Transfer for Management:	Yes	No

Date of transfer:

Hospital to which transferred

Surgical:

Indicated:	Yes	No
Peritoneal pencil drains	Yes	No

Date

Hospital:

Laparotomy	Yes	No

Date

	Hospital:
Intervention at Laparotomy	Resection and ileostomy
	Primary repair
	Laparotomy only
If surgery indicated, reason not performed	

Course of illness:

Date feeds stopped:

Date feeds restarted:
First date full feeds acheived:
Presence of following:

short bowel syndrome	Yes	No
High output ileostomy	Yes	No
Strictures	Yes	No

Final Outcome

Death	Yes	No
	Date	
Cause of Death:	During Episode	After resolution
	NEC Related	Other
Specify:		
Discharge:	Yes	No
	Date	
	Weight	

Transfer: Yes No

Date

Weight

Length of stay

APPENDIX E: Data Collection Sheet

Demographics :						
Date of birth						
Date of admission						
Base Hospital	Pelonomi		Universitas		Other	
Sex	Male		Female			
Gestational Age				(In weeks)		
	Certain		Uncertain			
Birth weight				(in grams)		
Maternal factors:						
Maternal age						
Parity						
Gravidity						
Multiple pregnancy		Yes	No			
		Twin	Triplet			
Antenatal Care received		Yes	No			
HIV status	Positive		Negative		Unknown	
Presence of the following						
Pre-eclampsia		Yes	No			
Pre-eclampsia with AEDF		Yes	No			
Prolonged ROM		Yes	No			
Chorioamnionitis		Yes	No			
Antenatal Steroids		Yes	No			
Mode of Delivery		NVD	C/S			
Neonatal Factors:						
Apgar	1min		5min			
Presence of the following						
Asphyxia		Yes	No			
RDS		Yes	No			
Mechanical Ventilation		Yes	No			
Surfactant therapy		Yes	No			

PDA			Yes	No			
Other CHDx			Yes	No			
Bacterial Sepsis			Yes	No			
Blood transfusion			Yes	No			
		Date					
		NPO	Yes	No			
Feeding:							
Date of initiation							
Type of feed		Breastmilk	DBM	Formula			
FM85		Yes	No				
Illness:		Episode 1		Episode 2		Episode 3	
Date of diagnosis							
NEC Grade	2A						
	2B						
	3A						
	3B						
Presence of the following							
Abdominal distension		Yes	No	Yes	No	Yes	No
Feed intolerance		Yes	No	Yes	No	Yes	No
Blood stool		Yes	No	Yes	No	Yes	No
Lethargy		Yes	No	Yes	No	Yes	No
Cardiovascular compromise		Yes	No	Yes	No	Yes	No
Pneumatosis		Yes	No	Yes	No	Yes	No
Portal Venous gas		Yes	No	Yes	No	Yes	No
Pneumoperitoneum		Yes	No	Yes	No	Yes	No
Fixed bowel loop		Yes	No	Yes	No	Yes	No
Thrombocytopenia		Yes	No	Yes	No	Yes	No
Hyponatremia		Yes	No	Yes	No	Yes	No
Metabolic acidosis		Yes	No	Yes	No	Yes	No
Positive blood culture		Yes	No	Yes	No	Yes	No
Organism							

Management:						
Medical	Yes	No	Yes	No	Yes	No
Bowel Rest: Feeds stopped	Yes	No	Yes	No	Yes	No
Antibiotics	Yes	No	Yes	No	Yes	No
Inotropes	Yes	No	Yes	No	Yes	No
Ventilation	Yes	No	Yes	No	Yes	No
Surgery indicated	Yes	No	Yes	No	Yes	No
Transfer for Management	Yes	No	Yes	No	Yes	No
Date of transfer						
Hospital transferred to						
Surgical:						
Indicated	Yes	No	Yes	No	Yes	No
Peritoneal pencil drains	Yes	No	Yes	No	Yes	No
Date						
Hospital						
Laparotomy	Yes	No	Yes	No	Yes	No
Date						
Hospital						
Laparotomy:						
Intervention at Laparotomy	Resection and ileostomy					
	Primary repair					
	Laparotomy only					
If surgery indicated, reason not performed						
Course of illness:						
Date feeds stopped						
Date feeds restarted						

First date full feeds achieved						
Presence of following						
Short bowel syndrome	Yes	No	Yes	No	Yes	No
High output ileostomy	Yes	No	Yes	No	Yes	No
Strictures	Yes	No	Yes	No	Yes	No
Final Outcome						
Death	Yes		No			
Date						
Cause of Death	During Episode		After resolution			
	NEC Related		Other			
Specify						
Discharge	Yes		No			
Date						
Weight						
Transfer	Yes		No			
Date						
Weight						
Length of stay						

APPENDIX F: Instructions to Authors from SAMJCH

General article format/layout

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

General:

- Manuscripts must be written in UK English (this includes spelling).
- The manuscript must be in Microsoft Word or RTF document format. Text must be 1.5 line spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes). Pages and lines should be numbered consecutively.
- 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)'.
- 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.

SAJCH is a Journal on child health, therefore for articles involving 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 Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Preparation notes by article type

Research

Guideline word limit: 3 000 words (excluding abstract and bibliography)

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. Where appropriate, sample size calculations should be included to demonstrate that the study is not underpowered. 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.

- May include up to 6 illustrations or tables.
- A max of 20 - 25references

Structured abstract

- This should be no more than 250words, 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. It should be able to be intelligible to the reader without referral to the main body of the article.
  - Do not include any references in the abstracts.

Here is an example of a good abstract.

***Scientific letters*/short reports**

These include case reports, side effects of drugs and brief or negative research findings.

Guideline word limit: 1500 words

- Abstract: unstructured, of about 100-150 words
- May include only one illustration or table
- A maximum of 6 references

Editorials

Guideline word limit: 1 000 words

These opinion or comment articles are usually commissioned but we are happy to consider and peer review unsolicited editorials. Editorials should be accessible and interesting to readers without specialist knowledge of the subject under discussion and should have an element of topicality (why is a comment on this issue relevant now?) There should be a clear message to the piece, supported by evidence.

Please make clear the type of evidence that supports each key statement, e.g.:

- expert opinion
- personal clinical experience
- observational studies
- trials
- systematic reviews.

Review articles

Review articlesshould always be discussed with the Editor prior to submission.

Guideline word limit: 4 000 words

These are welcome, but should be either commissioned or discussed with the Editor before submission. A review article should provide a clear, up-to-date account of the topic and be aimed at non-specialist hospital doctors and general practitioners. They should be aligned to practice in South and/or sub-Saharan Africa and not a precis of reviews published in the international literature

Please ensure that your article includes:

- Abstract: unstructured, of about 100-150 words, explaining the review and why it is important
- Methods: Outline the sources and selection methods, including search strategy and keywords used for identifying references from online bibliographic databases. Discuss the quality of evidence.
- When writing: clarify the evidence you used for key statements and the strength of the evidence. Do not present statements or opinions without such evidence, or if you have to, say that there is little or no evidence and that this is opinion. Avoid specialist jargon and abbreviations, and provide advice specific to southern Africa.
- Personal details: Please supply your qualifications, position and affiliations and MP number (used for CPD points); address, telephone number and fax number, and your e-mail address; and a short personal profile (50 words) and a few words about your current fields of interest.

Correspondence (Letters to the Editor)

Guideline word limit: 400 words

Letters to the editor should relate either to a paper or article published by the SAJCH or to a topical issue of particular relevance to the journal's readership

- May include only one illustration or table
- Must include a correspondence address.

Obituaries

Guideline word limit: 400 words

Should be offered within the first year of the practitioner's death, and may be accompanied by a photograph.

Illustrations/photos/scans

- If illustrations submitted have been published elsewhere, the author(s) should provide evidence of 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 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.) consecutively as they are referred to 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. If reference manager software is used, the reference list and citations in text are to be unformatted to plain text before submitting..*

- 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.

  NOTE: no . after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'

APPENDIX G: Turnitin Plagiarism Search Engine Report

Necrotising enterocolitis in the Bloemfontein academic complex

ORIGINALITY REPORT

21%	15%	16%	15%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.medicalcriteria.com Internet Source	2%
2	www.jpss.eu Internet Source	1%
3	Submitted to University of the Free State Student Paper	1%
4	Submitted to University of Witwatersrand Student Paper	1%
5	H.S. Cronjé, E.C. de Coning. "Electrosurgical bipolar vessel sealing during vaginal hysterectomy", International Journal of Gynecology & Obstetrics, 2005 Publication	1%
6	Submitted to University of Cape Town Student Paper	<1%
7	Submitted to University of Liverpool Student Paper	<1%
8	"Invited Lectures", The Journal of Maternal-Fetal & Neonatal Medicine, 2014	<1%

	Publication	
9	hdl.handle.net Internet Source	<1%
10	Submitted to The University of Manchester Student Paper	<1%
11	Submitted to Southern Illinois University Student Paper	<1%
12	samj.org.za Internet Source	<1%
13	eprints.whiterose.ac.uk Internet Source	<1%
14	eprints.nottingham.ac.uk Internet Source	<1%
15	sajhivmed.org.za Internet Source	<1%
16	www.researchsquare.com Internet Source	<1%
17	creativecommons.org Internet Source	<1%
18	www.dovepress.com Internet Source	<1%
19	Submitted to University of Bath Student Paper	<1%

20	relaped.com Internet Source	<1%
21	W. H. Yee, A. S. Soraisham, V. S. Shah, K. Aziz, W. Yoon, S. K. Lee. "Incidence and Timing of Presentation of Necrotizing Enterocolitis in Preterm Infants", PEDIATRICS, 2012 Publication	<1%
22	era.library.ualberta.ca Internet Source	<1%
23	Submitted to University of Southampton Student Paper	<1%
24	www.karger.com Internet Source	<1%
25	www.annualreviews.org Internet Source	<1%
26	thieme-connect.com Internet Source	<1%
27	Grace Hampson, Sarah Louise Elin Roberts, Alan Lucas, David Parkin. "An economic analysis of human milk supplementation for very low birth weight babies in the USA", BMC Pediatrics, 2019 Publication	<1%
28	He, Yu, Ying Zhong, Jialin Yu, Chen Cheng, Zhengli Wang, and Luquan Li. "Ultrasonography	<1%

	and radiography findings predicted the need for surgery in patients with necrotising enterocolitis without pneumoperitoneum", Acta Paediatrica, 2015. Publication	
29	Shehzad Huda, Shabnum Chaudhery, Hassan Ibrahim, Arun Pramanik. "Neonatal necrotizing enterocolitis: Clinical challenges, pathophysiology and management", Pathophysiology, 2014 Publication	<1 %
30	Submitted to Vrije Universiteit Brussel Student Paper	<1 %
31	Peter Angura, Sithembiso Velaphi. "Risk factors for necrotising enterocolitis in an HIV-endemic region", Paediatrics and International Child Health, 2014 Publication	<1 %
32	www.samj.org.za Internet Source	<1 %
33	pediatrics.aappublications.org Internet Source	<1 %
34	"Fundamentals of Pediatric Surgery", Springer Science and Business Media LLC, 2017 Publication	<1 %
35	scholar.ufs.ac.za:8080	

	Internet Source	<1%
36	Submitted to Vrije Universiteit Amsterdam Student Paper	<1%
37	ijn.mums.ac.ir Internet Source	<1%
38	aera.gov.in Internet Source	<1%
39	Submitted to University of Newcastle Student Paper	<1%
40	Submitted to University of Nottingham Student Paper	<1%
41	tel.archives-ouvertes.fr Internet Source	<1%
42	Submitted to University of Arizona Student Paper	<1%
43	Alecia M Thompson. "Necrotizing Enterocolitis in Newborns", Drugs, 2008 Publication	<1%
44	www.firstinfo.nuigalway.ie Internet Source	<1%
45	www.sirirajmedj.com Internet Source	<1%
	www.nature.com	

46	Internet Source	<1%
47	Submitted to Royal Australasian College of Physicians Student Paper	<1%
48	Pierro, A.. "Gastrointestinal surgery in the neonate", Current Paediatrics, 200606 Publication	<1%
49	Submitted to Flinders University Student Paper	<1%
50	Submitted to University of Aberdeen Student Paper	<1%
51	Handbook of Growth and Growth Monitoring in Health and Disease, 2012. Publication	<1%
52	Submitted to Queen Mary and Westfield College Student Paper	<1%
53	epdf.tips Internet Source	<1%
54	Submitted to University of KwaZulu-Natal Student Paper	<1%
55	Duc Ninh Nguyen, Per T. Sangild. "Pathogenesis and biomarkers for necrotizing enterocolitis: Getting any closer?", EBioMedicine, 2019	<1%

	Publication	
56	Molik, K.A.. "Portal venous air: The poor prognosis persists", Journal of Pediatric Surgery, 200108 Publication	<1%
57	Submitted to University Of Tasmania Student Paper	<1%
58	open.uct.ac.za Internet Source	<1%
59	Submitted to National University of Ireland, Galway Student Paper	<1%
60	Submitted to University of College Cork Student Paper	<1%
61	www.cureus.com Internet Source	<1%
62	D. J. Jordaan, A. Prollius, H. S. Cronjé, M. Nel. "Posterior intravaginal slingplasty for vaginal prolapse", International Urogynecology Journal, 2005 Publication	<1%
63	mobile.wiredspace.wits.ac.za Internet Source	<1%
64	E. Van Niekerk, D. G. Nel, R. Blaauw, G. F. Kirsten. "Probiotics Reduce Necrotizing	<1%

	Enterocolitis Severity in HIV-exposed Premature Infants", Journal of Tropical Pediatrics, 2015 Publication	
65	gupea.ub.gu.se Internet Source	<1%
66	Bahubali Gane, B. Vishnu Bhat, B. Adhisivam, Rojo Joy, P. Prasadkumar, P. Femitha, B. Shruti. "Risk Factors and Outcome in Neonatal Necrotising Enterocolitis", The Indian Journal of Pediatrics, 2014 Publication	<1%
67	www.mdpi.com Internet Source	<1%
68	www.science.gov Internet Source	<1%
69	Submitted to University of New South Wales Student Paper	<1%
70	Submitted to University of Warwick Student Paper	<1%
71	www.wits.ac.za Internet Source	<1%
72	"Textbook of Pediatric Gastroenterology, Hepatology and Nutrition", Springer Science and Business Media LLC, 2016 Publication	<1%

73	Jia Liu, Haitao Zhu, Bo Li, Carol Lee, Mashriq Alganabi, Shan Zheng, Agostino Pierro. "Beneficial effects of butyrate in intestinal injury", Journal of Pediatric Surgery, 2020 Publication	<1%
74	Francisca van der Schyff, Jan Becker. "Predictive Value of a Persistent Tachycardia to Indicate Impending Perforation in Necrotizing Enterocolitis", American Journal of Perinatology, 2018 Publication	<1%
75	www.sehop.org Internet Source	<1%
76	eprints.undip.ac.id Internet Source	<1%
77	Rita Prasad Verma, Archana Kota. "Chapter 2 Necrotizing Enterocolitis", IntechOpen, 2019 Publication	<1%
78	adc.bmj.com Internet Source	<1%
79	Submitted to Wageningen University Student Paper	<1%
80	Submitted to The Robert Gordon University Student Paper	<1%
81	Sarah Bombell. "Early trophic feeding for very	

	low birth weight infants", Cochrane Database of Systematic Reviews Reviews, 07/08/2009 Publication	<1 %
82	Submitted to University of Salford Student Paper	<1 %
83	Submitted to University of Texas Health Science Center Student Paper	<1 %
84	M. Arnold. "Long-term outcome of surgically managed necrotizing enterocolitis in a developing country", Pediatric Surgery International, 03/04/2010 Publication	<1 %
85	obgyn.pericles-prod.literatumonline.com Internet Source	<1 %
86	Submitted to University of Melbourne Student Paper	<1 %
87	Submitted to University of Sydney Student Paper	<1 %
88	Submitted to University of Bristol Student Paper	<1 %
89	Submitted to Queen Margaret University College, Edinburgh Student Paper	<1 %
90	Submitted to University of Wisconsin, La Crosse Student Paper	

		<1%
91	Submitted to Jacksonville University Student Paper	<1%
92	Catalina Bazacliu, Josef Neu. "Pathophysiology of Necrotizing Enterocolitis: An Update", Current Pediatric Reviews, 2019 Publication	<1%
93	Giannone, P.J.. "Necrotizing enterocolitis in neonates with congenital heart disease", Life Sciences, 20080213 Publication	<1%
94	Pammi Mohan. "Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in premature infants", Cochrane Database of Systematic Reviews Protocols, 04/23/2008 Publication	<1%
95	Submitted to AUT University Student Paper	<1%
96	Submitted to King's College Student Paper	<1%
97	Submitted to London School of Hygiene and Tropical Medicine Student Paper	<1%