Master of Medicine (MMed) : Paediatrics

A TWO-YEAR REVIEW OF NECROTISING ENTEROCOLITIS IN VERY LOW BIRTH WEIGHT INFANTS (<1500g) IN A SOUTH AFRICAN TERTIARY HOSPITAL

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DECLARATION

I, **Mbalenhle Purity Gumede**, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 11 /11 / 2019

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ACKNOWLEDGEMENTS

God, it is done! Amen

Myself, for getting to the point of completion.

Dr Lloyd Tooke, the most patient supervisor. Thank you for your time, flexibility and the impeccable attitude with which you met all the MMed challenges

Dr Adelaide Masu, for assistance with some of the statistical analysis

Dr Heloise Buys, my registrar supervisor, for your additional guidance and support

Family and friends for the encouragement, particularly my youngest sister Londeka Gumede in the final phases

ABSTRACT

Background: There is paucity of local data on the profile of preterm very low birth weight (VLBW) infants who develop moderate to severe necrotising enterocolitis (NEC) and their outcomes.

Methods: A retrospective folder review of VLBWs who developed Modified Bell's stage II NEC or higher at Groote Schuur Hospital (GSH) nursery between January 2012 and December 2013 was performed. Outcomes were defined as requirement for surgery and mortality.

Results: Forty seven infants were included (5% incidence). Gestational ages ranged from 25 to 36 weeks, 53% were <1 000 g. The trend was for bigger infants to manifest disease earlier. The HIV exposure rate was 40% which was significantly higher than the background institutional exposure rate, but HIV exposure did not increase their mortality risk (p =0.82). Common findings at diagnosis included an elevated CRP >10 mg/L (60%) and subserosal gas radiologically (84%). Half the patients received mechanical ventilation, 38% required inotropes. The mortality rate was 64%. Three of the five infants that received surgery survived.

Conclusion: Despite a similar incidence to global counterparts, our VLBW infants have severe NEC disease often requiring advanced life support, with a high mortality rate. HIV exposure may increase the risk of NEC development.

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CHAPTER ONE

Introduction

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INTRODUCTION

Necrotising Enterocolitis (NEC) remains a common and serious gastrointestinal medical emergency occurring almost predominantly amongst the premature very low birth weight (VLBW) infants. The aetiology is multi-factorial and our understanding of the pathophysiology continues to evolve. It is described as there being an interplay between various factors such as immaturity of the gut, immaturity of the immune system and microbial dysbiosis affecting the premature gut which all invariably leads to mucosal injury

The incidence worldwide is 2-7 %, but also varies within institutions (1, 2). NEC associated morbidity in survivors includes short-term surgical complications and long-term neurodevelopmental problems, with more severe neurodevelopmental impairment seen in those with disease that requires surgical intervention (3, 4). The mortality rate is 20-50 % in VLBWs, and even higher in the more premature and surgically treated infants but also varies between institutions (5, 6).

The timing of development of NEC is inversely proportional to gestational age, usually occurring in the second or third week of life in premature VLBWs and earlier in term infants. It classically affects the terminal ileum and the ascending colon (7). The severity varies as classified in the Modified Bell's staging criteria (Appendix 1), with the more severe forms requiring intensive care support and surgical intervention.

There has been a worldwide increase of in preterm deliveries and births over the past two decades with 11.1 % of infants now being born prematurely (<37 completed weeks of gestation) (8). A corresponding increase in survival rates of premature infants has also been witnessed with advances in neonatology and improvement in obstetric care (5). Locally a similar pattern has been exhibited at a central hospital in Johannesburg (9). However, there are still global disparities with

higher premature infant survival rates seen in high income countries compared to those in developing countries. This overall increase in premature infant survival rates has led to more infants living long enough to develop NEC and this plays a role in contributing to the neonatal and under 5 mortality rates.

Deaths occurring during the neonatal period account for almost half the causes of mortality in the under 5 years (U5) populations with prematurity and its complications responsible for 35% of these, particularly in low income countries (10, 11).

This makes NEC in premature infants a significant disease to study, particularly in the context of developing countries if we are to contribute in achieving the targets of the third United Nations Sustainable Development Goal.

LITERATURE REVIEW

Although NEC is not a completely preventable disorder, it has well documented protective and risk factors.

PROTECTIVE FACTORS

- ANTENATAL STEROIDS

The use of antenatal corticosteroids in expected preterm deliveries matures the intestinal system amongst other benefits and has been associated with a decrease incidence in NEC (12). An analysis of the 2011 WHO Multicountry Survey on Maternal and Newborn Health (WHOMCS) data obtained from 29 countries located in all the continents revealed that this intervention is underutilized in low and middle-income countries (13)

- FEEDING

A mother's own, fortified breast milk is the ideal milk for growth and prevention of NEC amongst other benefits to a preterm infant (14). Human milk has several protective components including: *Secretory IgA,* which binds to the intestinal lumen and prevents bacteria translocation through the wall; *Oligofructose,* which enhances colonisation of the gut by bifidobacteria and also prevents colony by pathogenic bacteria. It also *contains Platelet Activating Factor Acetylhydrolase (PAF-A)*, which metabolizes one of the proinflammatory markers platelet activating factor (PAF) noted in the pathogenesis of NEC (15). PAF-A activity is up to five times higher in the milk of mothers of preterm babies compared to those of term babies (16).

A mother's own unpasteurized milk provides more biologically active molecules compared to pasteurized human milk and the quantity of raw maternal milk consumed compared to pasteurized human milk inversely correlates with NEC development (14, 17).

It is acceptable practice to use pasteurized human donor milk as an alternative to augment the mother's own, this has more favourable outcomes compared to formula. A 2014 Cochrane review by Quigley et al. of over 1000 infants compared the effect of formula vs. donated human milk on preterm or low birth weight infants. It showed that although formula was associated with more in-hospital growth, the risk of developing NEC is up to three times higher compared to those who receive donated human milk whether nutrient-fortified or not (18, 19)

No decrease in the risk of NEC has not been demonstrated when comparing slower (15-20ml/kg/day) versus faster (30-35ml/kg/day) rates of feeds advancement in VLBWs. Neither has a risk reduction been proven when feed introduction is delayed compared to commencing feeds early (20, 21)

- PROBIOTICS

Microbial dysbiosis is one of the currently recognized pathogenesis of NEC in premature infants. Probiotics, which also occur naturally in the breastmilk, can be administered as enteral supplementation to help promote good, symbiotic gut bacteria. The oral probiotics were shown to decrease the incidence, severity and mortality associated with NEC in a 2014 Cochrane review. A study conducted in South Africa by Van Niekerk et al. further demonstrated a decrease in NEC severity in HIV exposed preterm VLBW infants (22-24).

However, further studies looking at the dosing and appropriate probiotic strains for routine administration are still required.

RISK FACTORS

- MATERNAL PROFILE

Maternal conditions that may lead to placental insufficiency and compromised blood flow to the intestines increase the risk of NEC development. Maternal methamphetamine use and hypertensive disorders in pregnancy are probably of greater interest in our South African population. Methamphetamine exposure, though only documented for term infants, increases the risk of developing NEC (25-27). In Western Cape, methamphetamine use is particularly endemic in the community (28).

Pre-eclampsia and eclampsia are a common indication for premature delivery of infants globally and has also been shown to be a risk factor for NEC (29, 30).

- PREMATURITY

Prematurity alone is the most consistent independent risk factor for developing NEC largely due to an immature gut and immune system (31, 32). The immature gut has loose “tight junctions” which makes it easier for bacteria to invade it’s mucosa, has less secretory IgA and is colonized by fewer protective intestinal microflora, Bifidobacterium, amongst other factors (32, 33).

- ANTIBIOTICS

The use of prolonged broad-spectrum empiric antibiotics alters intestinal commensal flora thus increasing the risk of NEC (34-36). Prevention of acquired infections and judicious use of antibiotics is highly recommended. A decrease in incidence of NEC was seen at Groote Schuur Hospital with changes in these practices, development of standard operation protocols and hand hygiene vigilance (37)

- HIV EXPOSURE

It is inconclusive whether HIV exposure contributes to increasing the risk of developing NEC, its severity or its outcomes.

In 2005 Desfrere et al. from Italy suggested that there might be an increased association of NEC in HIV exposed premature infants (38). Locally, in 2010 Karpelowsky et al at Red Cross War Memorial Children's Hospital, Cape Town, showed that premature HIV exposed infants requiring NEC surgery had a higher mortality rate compared to those who were HIV unexposed (39). However in 2012 Arnold's group from Tygerberg Children's Hospital, Stellenbosch, found that HIV exposure did not worsen the severity of disease or outcome of their infants with severe NEC and Angura et al in Johannesburg found that HIV exposure did not increase the risk of developing NEC (40, 41).

There are also differences in the breast milk composition of HIV-Infected and HIV-Uninfected mothers of premature VLBW infants that have also been noted to exist and contributory in the development of NEC. One study showed the lower concentrations of human milk oligosaccharide disialyllacto-N-tetraose (DSLNT) in the breast milk of HIV infected mothers to be associated with an increased incidence of necrotising enterocolitis (42).

Furthermore, a case study in Germany suggested a possible link between NEC and intravenous Zidovudine (AZT) exposure. This was observed in a term baby who developed NEC post AZT administration in the absence other identifiable risk factors, however this has not been described yet in premature infants (43).

- RACE

Black ethnicity increases the risk of NEC in some populations (44).

- BLOOD TRANSFUSION

The phenomena of red blood cell "Transfusion Associated Necrotising Enterocolitis" (TANEC) is defined as NEC occurring within 48hrs of transfusion. A recent review of a multicentre observational cohort study in Georgia over a four year period between 2010 -2014 revealed that it is the severity of anaemia rather than the blood transfusion itself that is associated with increased NEC, transfusion merely was a surrogate marker (45). Preventative methods of decreasing anaemia requiring transfusion like delayed cord clamping should be encouraged (46)

DIAGNOSIS

The modified Bell staging for NEC is a combined clinical and radiological classification of NEC widely used to classify severity (Appendix 1). The commonest clinical presentation is an ileus with gastric retention, abdominal distension, vomiting and perforation; contrary bloody stools are the least common (47). Systemic presentation includes apnoeas and hypotension.

There are no specific diagnostic biochemical or septic markers, however several markers support the diagnosis.

Hällström et al in a Finnish study described a laboratory parameter pattern that should increase a clinician's index of suspicion for developing NEC and/or resultant intestinal perforation in a premature infant. In their prospective study of 140 premature infants, 19% developed NEC and they found that a metabolic acidosis that persists, declining platelets, persistent hyponatremia <130 mEq/l and rising blood glucose levels on several successive days might indicate a developing NEC. Furthermore a leukocyte count of >30 x 10(9)/L, a pH <7.25, and a blood glucose increase by 1.5 mmol/L or more within 24 hours predict NEC with intestinal perforation (48).

Kenton et al. further supports that severe thrombocytopenia (<100 000/mm3) in early disease stages suggests significant bowel necrosis and worsening illness, it is also likely associated with an increase in mortality (49). In another group of neonates early neutropenia seen in small for

gestational age infants compared to appropriately grown ones is associated with poor prognosis in NEC (50).

An elevated C-reactive protein (CRP) is usually present in moderate and severe NEC. A persistence in elevation of serial CRPs can be associated with complications of NEC (51).

Primary invasion of the intestine by pathogenic bacteria has also been reported, blood cultures are positive in 20-30% of cases of NEC. Common isolates include Enterobacteria (Escherichia coli, Klebsiella pneumoniae), Clostridium spp. enteric pathogens (salmonellae, Coxsackie B2 virus, coronavirus, rotavirus), and occasional pathogens Bacteroides fragilis (52, 53)

Abdominal radiography is the choice of investigation. Classical radiological findings range from abnormal gas pattern as seen in ileus in early NEC, progressing to the pathognomonic pneumatosis intestinalis, portal venous gas (PVG) and in severe cases pneumoperitoneum. Sentinel loops (a fixed loop of intestines) suggests necrosis and/or perforation. Traditionally PVG was considered a poor prognosticator and indication for surgery, however a review of 194 neonates diagnosed with NEC done by Sharma et al at a unit in Florida between 1991-2003 did not support this, as there was no difference in the rate of survival in those with and without PVG (54). Radiological presentation appears to vary with gestational age - intramural gas and PVG are seen more commonly in those with older term gestational ages compared to those of younger premature gestational age (47)

MANAGEMENT

The management depends on the severity of disease manifestation - this includes bowel rest with parenteral nutrition, empiric treatment of possible underlying infection and surgery where indicated. Intensive care support and longer nursery admission is often required which has cost implications in our resource limited settings.

The absolute indication for surgical intervention is disease leading to bowel perforation. Appropriate timing of surgery requires input from paediatric surgery taking into consideration the clinical stability and biochemical changes of the patient (55). Viable bowel preservation is the goal; laparotomy and percutaneous drainage may be of similar efficacy in initial management of perforations in extremely low birth weight infants (56, 57). Late post-surgery complications include formation of stricture, short bowel syndrome and much rarer enteroceles, intra-abdominal abscesses.

There is a paucity of data on the profile of infants who develop NEC and their outcome in SA, this study will look at this at a tertiary institute in Western Cape.

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CHAPTER TWO

Journal Manuscript

Journal Guidelines

Appendices

A TWO-YEAR REVIEW OF NECROTISING ENTEROCOLITIS IN VERY LOW BIRTH WEIGHT INFANTS (<1500G) IN A SOUTH AFRICAN TERTIARY HOSPITAL

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ABSTRACT

Background: There is paucity of local data on the profile of preterm very low birth weight (VLBW) infants who develop moderate to severe necrotising enterocolitis (NEC) and their outcomes.

Methods: A retrospective folder review of VLBWs who developed Modified Bell's stage II NEC or higher at Groote Schuur Hospital (GSH) nursery between January 2012 and December 2013 was performed. Outcomes were defined as requirement for surgery and mortality.

Results: Forty seven infants were included (5% incidence). Gestational ages ranged from 25 to 36 weeks, 53% were <1 000 g. The trend was for bigger infants to manifest disease earlier. The HIV exposure rate was 40% which was significantly higher than the background institutional exposure rate, but HIV exposure did not increase their mortality risk (p =0.82). Common findings at diagnosis included an elevated CRP >10 mg/L (60%) and subserosal gas radiologically (84%). Half the patients received mechanical ventilation, 38% required inotropes. The mortality rate was 64%. Three of the five infants that received surgery survived.

Conclusion: Despite a similar incidence to global counterparts, our VLBW infants have severe NEC disease often requiring advanced life support, with a high mortality rate. HIV exposure may increase the risk of NEC development.

INTRODUCTION

NEC remains a common and serious gastrointestinal medical emergency occurring predominantly amongst preterm VLBW infants. It remains a noteworthy condition in the era of increased preterm deliveries and significant advances in neonatology and obstetric care leading to increased survival rates of preterm infants(8). It further contributes to the under 5 (U5) year mortality rates, where half the deaths occur in the neonatal period with complications of prematurity accounting for 35% of these, particularly in low income countries like South Africa(SA)(10).

The incidence is reported to be 2-7% globally with institutional variation in incidence and stage of NEC studied(1, 2). The timing of development of NEC is inversely proportional to gestational age, typically occurring in the second or third week of life in preterm VLBW neonates and classically affecting the terminal ileum and the ascending colon. The aetiology is multi-factorial and our understanding of the pathophysiology continues to evolve. It is described as there being an interplay between various factors such as immaturity of the gut, immaturity of the immune system and microbial dysbiosis affecting the premature gut which leads to mucosal injury. Other associated risk factors include inadequate antenatal steroid use in preterm deliveries, feeding practices particularly infant formula feeding, race, severe anemia and HIV exposure.

Pathogenic bacteria may lead to NEC by primary invasion of the intestines (53).
Morbidity associated with survival of NEC includes short-term surgical complications e.g. short bowel syndrome and long-term neurodevelopmental impairment more profound than that secondary to prematurity alone(3). Furthermore, more severe neurodevelopmental impairment is seen in those VLBW infants with NEC who require surgical intervention(4).The mortality rates in different institutions vary between 20-50%, however the consistent trend is a higher mortality in the very preterm and in surgically treated infants(5, 6). There is a paucity of data on the profile of

VLBW infants who develop this significant condition and their outcome in SA and other middle- or low-income countries. The objective of this study was therefore to describe the epidemiology, clinical features, relevant special investigations and outcomes of a cohort of VLBW infants in Cape Town, South Africa.

METHODS

The study was conducted at Groote Schuur Hospital (GSH), a tertiary neonatal unit in Cape Town, South Africa. At the time of the study, GSH neonatal unit admitted approximately 3 200 of 40 000 newborns from the West Metro region of Cape Town yearly, with over 500 of these being VLBW.

A retrospective folder review of infants who developed NEC at GSH from January 2012 up until December 2013 was performed. These infants were identified using the Vermont Oxford Network (VON) database of VLBW infants, a global database consisting of over 1000 neonatal intensive care units, of which GSH is one of the largest contributors. All VLBW infants who are admitted at GSH are entered into the VON database.

Inclusion criteria were all neonates weighing ≤1 500 g who developed NEC Bell stage II or higher at GSH. **(Table 1)**. Infants excluded were those referred to the unit already with a diagnosis of NEC, infants with primary gastrointestinal abnormalities and infants whose records could not be located.

The data obtained from individual patient folders were entered onto a Microsoft Excel spreadsheet. Patient demographics included gestational age, birth weight, race, day of NEC occurrence, risk factors - such as type of infant feed and HIV exposure amongst others, diagnostic investigations and management. All abdominal radiographs were reviewed by the principal investigator and a neonatologist.

All data were analysed descriptively which included means and standard deviations for normally distributed data and medians and inter-quartile ranges for non-normally distributed continuous data. Relative risks with 95% confidence intervals and p values were used to compare outcomes between deaths and survivors. Bivariate analysis was used to compare differences in the death and survivor groups; Chi-squared tests for categorical data, T-tests for normally distributed numeric

data and the Mann Whitney U test for non-parametric numeric data. The Shapiro Wilk testing was used to assess if data was normally distributed. Statistical significance was set at $p<0.05$

Approval by the Human Research Ethics Committee of the University of Cape Town Faculty of Health Sciences was obtained (HREC Ref: 776/2016)

RESULTS

Sixty-three of 1 032 (6%) VLBW infants admitted over the two-year period were identified as having a diagnosis of NEC from the VON database. 16 were excluded from the study: 5 had developed NEC elsewhere, 1 patient had a duodenal atresia repair prior to developing NEC and 10 folders could not be located. The study sample was 47 infants (5% incidence).

Maternal profile

Five mothers did not receive any antenatal care and one of them delivered an infant with congenital syphilis. 36 mothers (77%) received at least one dose of antenatal steroids (ANS). This is higher than the background rate of 58% steroid exposure of all VLBWs over the same time period (p=0.01). Amongst those that developed NEC, the use of ANS did not protect against the need for surgery (p=0.69).

Of the 21 (45%) mothers who had a hypertensive disorder, 20 had early onset pre-eclamptic toxemia (EOPET) and one had pre-existing chronic hypertension. The incidence of pre-eclamptic toxemia (PET) was similar to the background rate of 47% for all VLBW births at GSH and was the commonest indication for early delivery.

19 mothers (40%) were HIV-positive. 11 of these mothers were on at least 3 months of antiretroviral therapy (ART) as per the 2010 national Prevention of Mother to Child Transmission (PMTCT) guidelines applicable at the time. The other 8 mothers had either less than 3 months or no ART.

Table 1. Summary of NEC Modified Bell Staging

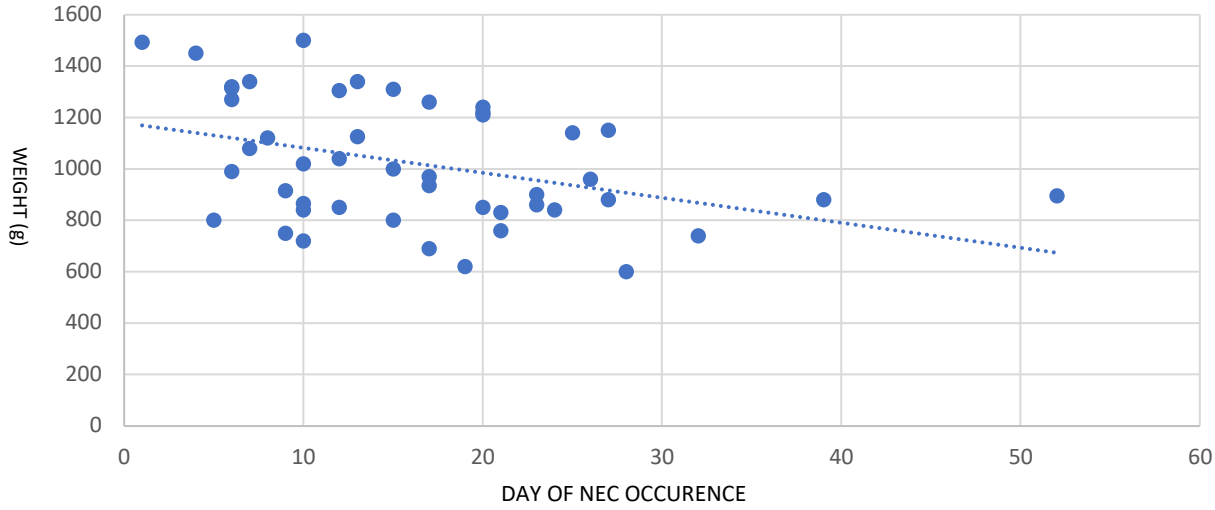
STAGE	CLASSIFICATION OF NEC	SYSTEMIC SIGNS	ABDOMINAL SIGNS	RADIOGRAPHIC SIGNS
1A	Suspected NEC	Clinically ill	Absent bowel sounds, abdominal distension, Increased nasogastric aspirates, vomiting	Normal/mild intestinal dilatation, mild ileus
1B	Suspected NEC		Grossly bloody stools	
2A	Definite, mildly ill	Mild-moderate illness	Absent bowel sounds	Intestinal dilatation, ileus, pneumatosis intestinalis, portal venous gas
2B	Definite, moderately ill			As above + Ascites
3A	Advanced, severely ill, intact bowel	Severely ill (apnoea, bradycardia, hypotension. Mixed metabolic/respiratory acidosis, DIC	Marked tenderness, marked distension, peritonitis	
3B	Advanced, severely ill, perforated bowel			All above + pneumoperitoneum

Infants

The infants' demographics showed that 57% (n= 27) were black Africans and 43% (n= 20) were of mixed ancestry. Their gestational ages ranged from 25 weeks to 36 weeks. 25 (53%) were below 1000 g

Most (77%) of these infants developed NEC in the first 3 weeks of life, with 17 (36%) occurring in the first 10 days **(Figure 1).** The trend was for the larger infants to develop NEC earlier than the smaller ones

Figure 1. Graph demonstrating day of NEC occurence according to birth weight



Feeding:

Sixty percent of the infants received exclusive breastmilk (either mother's own or pasteurized donated milk). **(Figure 2.)** All mothers who were HIV-positive pasteurized their milk. 5 of the 19 (26%) HIV exposed infants also received some formula feed(s).

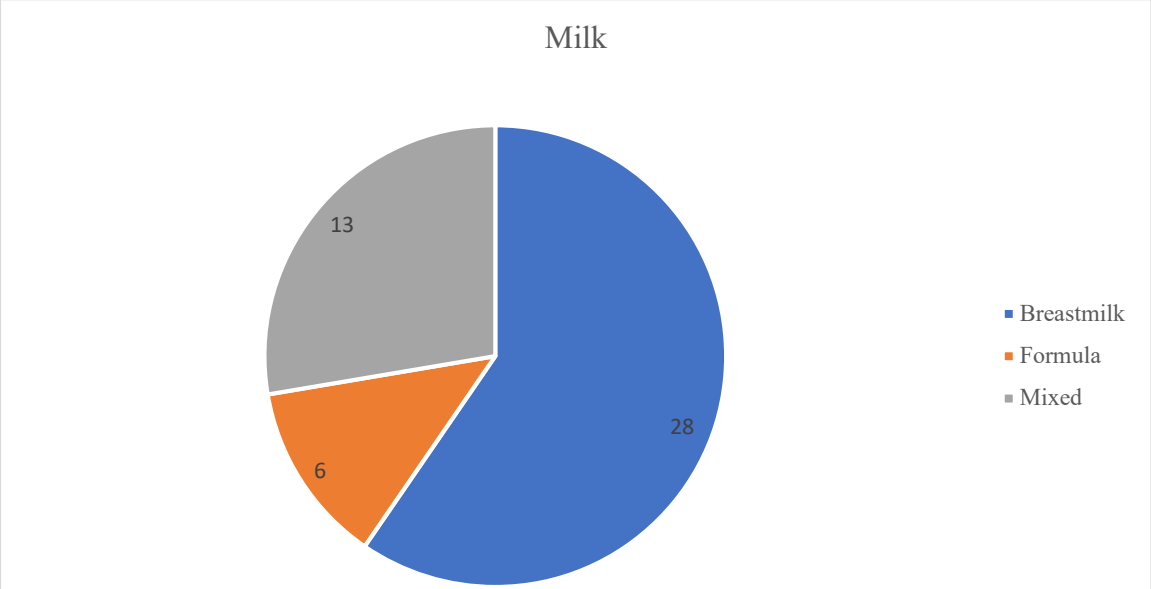


Figure 2. Graph showing milk feeds distribution in the

Four babies (8%) developed NEC within 48 hours of receiving a red blood cell transfusion i.e. transfusion associated NEC (TANEC). Their haemoglobin ranged between 6.8- 9.9 g/dL on the day of transfusion.

Laboratory and radiological Diagnosis:

At diagnosis and initial work-up, an elevated CRP >10 mg/dL was the most commonly found laboratory marker in this group (60% of infants). A quarter of the infants were thrombocytopenic **(Table 2)** There were 14 (30%) infants with a positive blood culture growth: Gram-negative organisms especially ESBL *Klebsiella* (n= 4) and E.Coli (n= 3) were the most commonly found.

Table 2. Laboratory markers at the time of diagnosis

	Range	Mean	Comments
CRP (mg/L)	1 - 146	30	60% had a CRP <10
WCC (x10^9/L)	0.95 - 27	10	17% neutropenic
Platelets (x10^9/L)	19 - 743	360	25% thrombocytopenic

Radiologically, the commonest finding at diagnosis was the 'soap bubble' appearance which may indicate subserosal gas (84% of all abdominal radiographs). Linear pneumatosis intestinalis (submucosal gas) was seen in 45% of the images, 29% showed portal venous gas and 2% had pneumoperitoneum.

Management

Fifty-three percent (n= 25) of the babies required mechanical ventilation and 38% (n=13) additional inotropic support. TPN was commenced in 23 babies, of these who received TPN 60% received more than 7 days of TPN. Blood products including platelets, fresh frozen plasma were given in 38%.

Outcome

There was a high mortality rate of 64% (n= 30) (**Figure 3).** Most deaths (60%) occurred in the infants born ≤ 1 000 g. There was a trend towards statistical significant in the difference in mortality between this weight category and the 1 001-1 500 g infants (p= 0.05). Twenty (42%)

infants died within 48hrs of presentation. Babies with HIV exposure did not have a significantly increased risk of death (p=0.82)

Laparotomies were performed on five babies at Red Cross War Memorial Childrens' Hospital.

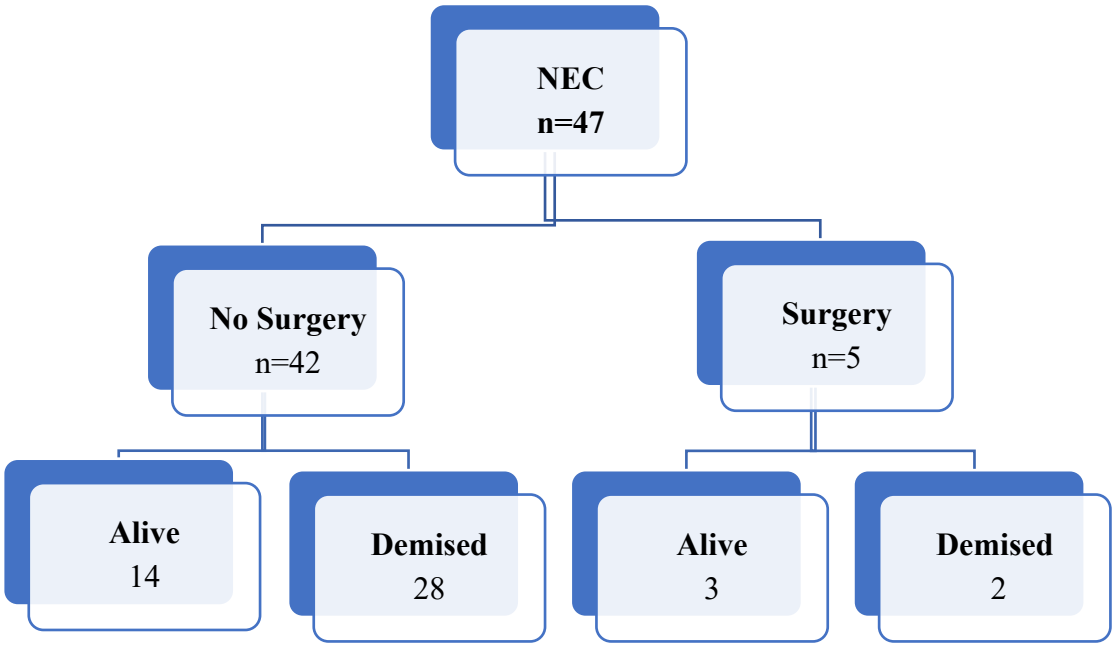


Figure 3. Illustration showing NEC patient outcomes

DISCUSSION

Although our NEC incidence during this study fell within the 2-7% expected for VLBW infants, we had a higher (64%) mortality rate than is usually quoted, with most of the deaths, 20 of 30 (60%), occurring in the <1000g infants. Despite intensive care, two thirds of those infants that died did so within 48 hours with many of them being too unstable to transfer for surgery. Only 5 infants underwent a laparotomy.

In our cohort there were 19 (40%) HIV-exposed infants, a significantly higher rate than that reported overall for HIV-exposure rate of 20% in VLBWs during that time period (p=0.001)(58). This may support a hypothesis that HIV exposure is associated with an increased risk of developing NEC.

The literature is divided about whether HIV-exposure contributes to the risk of developing NEC, its severity or its outcomes. In 2005 Desfrere et al., in Italy suggested that there might be an increased association of NEC in HIV exposed preterm infants(38). A local study in 2010, from Red Cross War Memorial Children's Hospital showed that preterm HIV-exposed infants requiring NEC surgery had a higher mortality rate compared to those who were HIV-unexposed (39).

Lower concentrations of human milk oligosaccharide disialyllacto-N-tetraose (DSLNT) in the breast milk of HIV infected mothers has also been described to be associated with an increased incidence of necrotising enterocolitis(42). A German case study suggested a possible association of intravenous Zidovudine used as prophylaxis in PMTCT with NEC development, this was seen in a term infant who developed NEC shortly after exposure with no other risk factors (43), this however has not yet been described in preterm infants. Two local studies from Cape Town and Johannesburg in 2012 and 2014 respectively, reported that HIV-exposure did not increase the risk of developing NEC, and neither did it worsen the severity or outcome of those with stage III NEC (40, 41).

The maternal condition of PET is known globally as the commonest indication for preterm delivery, and is also postulated as a risk factor for NEC(30). At GSH however, almost half the mothers of VLBW infants are delivered due to EOPET by emergency caesarean section and there is no difference in the incidence of PET in the mothers of the babies who develop NEC.

A 2017 Cochrane review stated that the use of antenatal steroids (ANS) in anticipated preterm births has been shown to reduce the risk of NEC (RR 0.50, 95% CI 0.32-0.78) (12). In this study, 77% of mothers received antenatal steroids which is close to the 80% seen in high income countries(HIC) (13). This was significantly higher than the background rate of maternal steroid administration (58%) in our unit. This could possibly be explained by the hypothesis that infants

who were not steroid exposed would have a higher chance of dying in the first few days of life due to respiratory distress syndrome and therefore never develop NEC

Infant factors

NEC timing

The timing of development of NEC is inversely proportional to gestational age. Our study showed similar findings and the trend was for the larger infants to develop NEC earlier than the smaller ones. Over one third of the infants developed NEC within the first 10 days of life, this was in keeping with global patterns(7)

Feeds

A 2014 Cochrane review by Quigley et al., of over 1 000 infants compared the effect of formula versus donated human milk on preterm or low birth weight infants. It showed that although formula was associated with more in-hospital growth, the risk of developing NEC is up to three times higher compared to those who receive donated human milk (18). Approximately 40% of the infants received some formula feed(s) at some point during their hospitalization due to either milk unavailability from their mother or their ineligibility for donor breastmilk as per our unit's qualifying criterion (i.e. >1 200 g at the time). Of note, a mother's own unpasteurized milk provides more biologically active molecules compared to pasteurized human milk and the quantity of raw maternal milk consumed compared to pasteurized human milk inversely correlates with NEC development (16).

TANEC

Four infants who required a standard blood transfusion developed NEC within 48hrs post transfusion. These had been transfused according to the unit's restrictive blood transfusion policy which allows a lower hemoglobin threshold for transfusing preterm infants (37).

This phenomenon of red blood cell TANEC has been defined as NEC occurring within 48 hours of transfusion. This association has been described as early as 1987(59), the main hypothesis was possibly low velocity flow of the mesenteric blood in the post-transfusion state predisposing to intestinal injury.

These theories of TANEC have been dispelled in more recent studies. Of note is the multicenter observational cohort study in Atlanta, Georgia conducted between 2010 -2014 which revealed that it is the severity of anemia rather than the blood transfusion itself that is associated with increased NEC. Transfusion was deemed merely a surrogate marker of severity(45)

Diagnosis

The diagnosis is made using the Modified Bell's staging criteria for NEC (clinical and radiological), there are no specific diagnostic biochemical or septic markers, however several markers support the diagnosis such as increased inflammatory markers and abnormal white cell and platelets blood counts. A Finnish prospective study of 140 preterm neonates where 19% developed NEC found that a leukocyte count of >30 x 10(9)/L, a pH <7.25, and a blood glucose increase by 1.5 mmol/L or more within 24 hours predict NEC with intestinal perforation (23) Most of the patients in our study had a white cell and platelet count in the normal ranges at diagnosis with only 25% classified as thrombocytopenic. However, thrombocytopenia may only develop later in the course of the disease.

An elevated C-reactive protein (CRP) is usually present in moderate and severe NEC. A persistence in elevation of serial CRPs can be associated with complications of NEC (51). A raised CRP>10 mg/L was present in 60% of our infants at diagnosis. Due to peaking of CRP levels at 36-

48hrs after the start of inflammation it is likely that some of the infants may have only had a raised CRP the day after diagnosis.

Primary invasion of the intestine by pathogenic bacteria has also been reported and blood cultures are positive in 20-30% of cases of NEC. Common isolates include *Enterobacteriaceae (Escherichia coli, Klebsiella pneumoniae*), Clostridium spp. and enteric pathogens (salmonellae, coxsackie B2 virus, coronavirus, rotavirus)(53). Similar rate of positive blood cultures was observed in our study (30%), however resistant strains of Gram-negative organisms were the most cultured organisms - especially ESBL *Klebsiella* (n= 4) and E.Coli (n= 3)

Management and outcomes

The management depends on the severity of disease manifestation, it includes bowel rest with parenteral nutrition, empiric treatment of possible underlying infection, ventilatory and circulatory support, and surgery where indicated. The absolute indication for surgical intervention is disease leading to bowel perforation.

Upon establishing a diagnosis of NEC, all the infants in this study were transferred to the NICU, they were initiated on empiric second line antibiotics as per the unit's microbiological profiling at the time. Only half the patients received TPN, mostly because of the many deaths soon after diagnosis. TPN was required for 5-14 days. Fifty-three percent required invasive ventilation within 24hours of diagnosis. 13 infants required inotropic support. The magnitude of ventilatory and inotropic support required, illustrates the severity of illness which also has both resource and cost implications.

The mortality rate is 20-50% in different institutions, whilst our rate in this study was particularly high at 64%, with the majority being amongst the <1000g infants. Only 5 infants received laparotomies of which three survived to discharge. As surgery takes place off-site at Red Cross War Memorial Children's hospital many babies were too unstable to transfer. There was incomplete data regarding short-term neurodevelopmental follow-up.

The major limitations to this study were the retrospective nature and the small sample size. The 10 missing folders not analysed further undermined the power of the study. The setting was a tertiary unit with a higher than usual maternal cohort having a diagnosis of gestational hypertension, therefore we cannot generalize findings to other less specialized hospitals.

Future study considerations should include a bigger size sample, the role that introduction of probiotics has played and exploring further the role of HIV/ ARV exposure in the development of NEC in the context of the current PMTCT Option B+ regimen which uses more ART compared to the previous regimen.

CONCLUSIONS

In this study of 47 VLBW infants with NEC, there was a high mortality (64%) with death within 48hrs of diagnosis being common, particularly in those < 1 000 g (20 of 30 infants). A raised CRP >10 mg/L was the most reliable biochemical marker at presentation on the day of NEC occurrence and the most commonly seen radiological sign was sub-serosal gas. One third of the VLBW infants developed NEC within the first 10 days of life. Whilst HIV-exposure did not increase the risk of mortality, there is possibly an associated increased risk of NEC development with HIV exposure and this finding warrants further investigation.

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JOURNAL GUIDELINES

South African Journal of Child Health (SAJCH)

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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.

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 250 words, with the following recommended headings:

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

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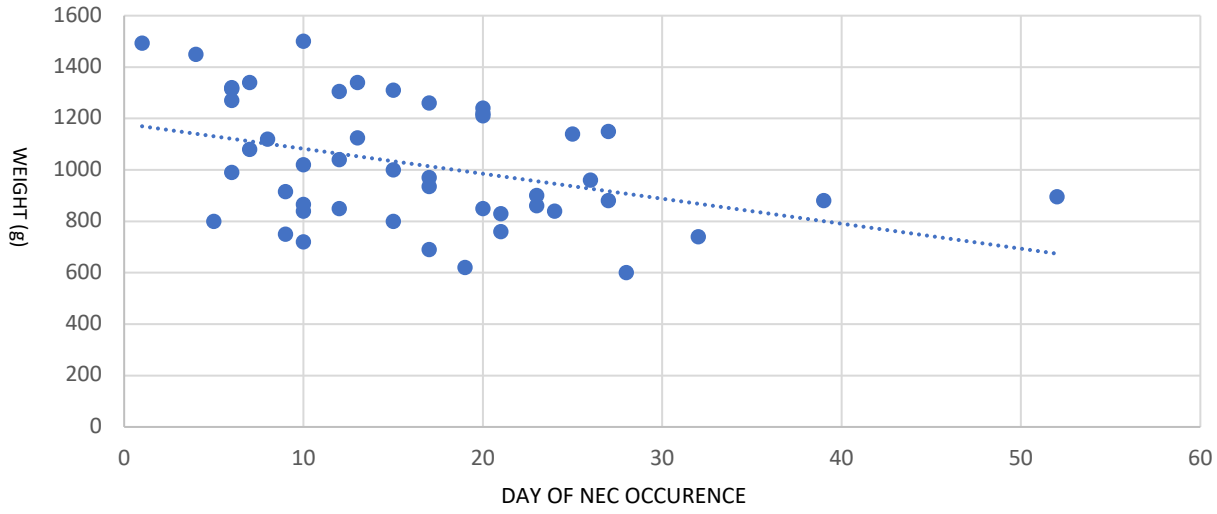

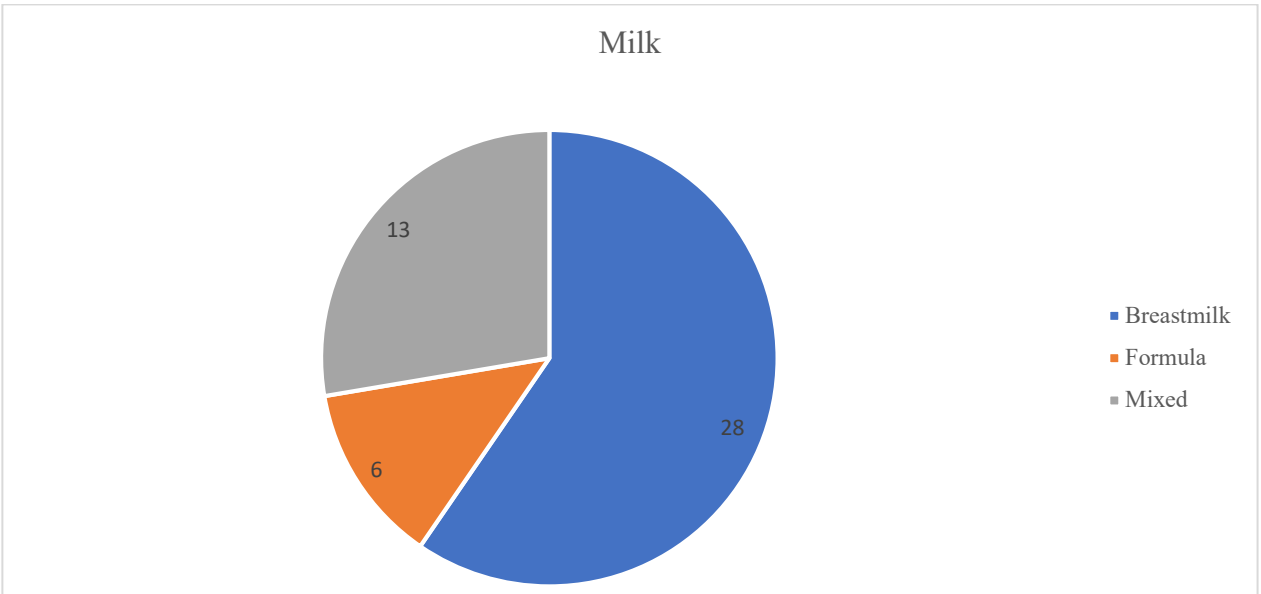

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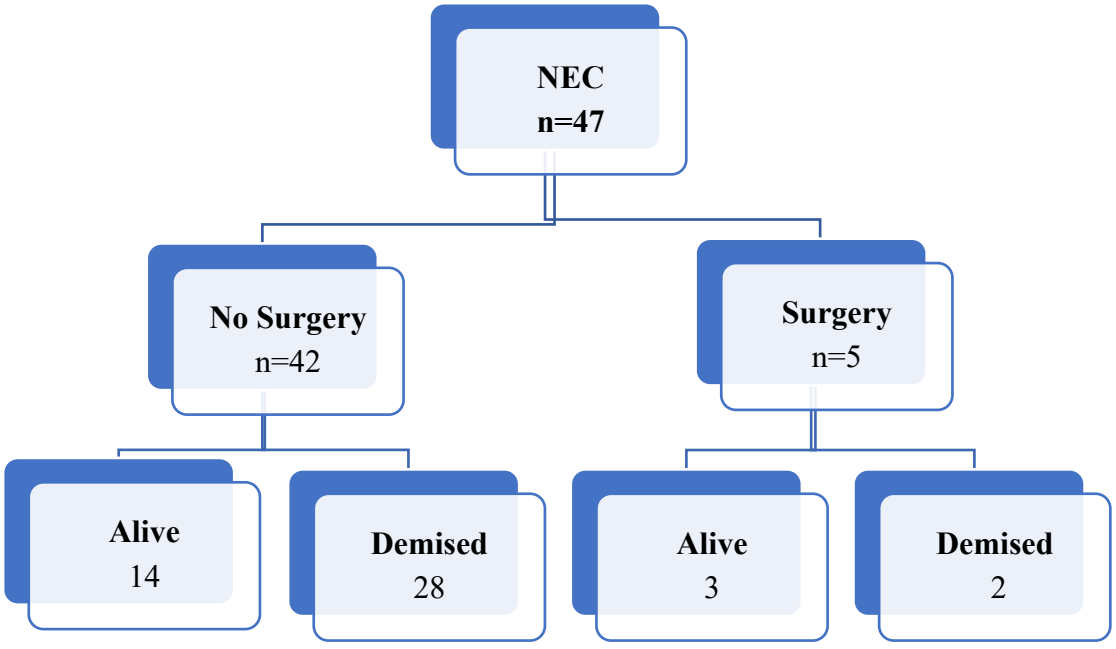

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