RISK FACTORS AND OUTCOMES OF INFANTS WITH NECROTIZING ENTEROCOLITIS: A CASE-CONTROL STUDY

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375457

A Research report submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science.

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DECLARATION

I Noela Holo Bertha Kamanga declare that this Research report is my own, unaided work. It is being submitted for the Degree of Masters of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signature

Date 31/03/3025

DEDICATION

I would like to dedicate this degree to my family:

My husband, Fisher and my children, Chuma and Subira, for their constant support on this journey.

I am grateful to my mother, Elizabeth Mwanukuzi and sister, Catherine Katala for their prayers and encouragement.

Thanks be to God.

PRESENTATIONS AND PUBLICATIONS ARISING FROM THIS RESEARCH

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ABSTRACT

Introduction: Necrotizing enterocolitis (NEC) is a condition associated with high mortality and morbidity. Its pathogenesis is linked to intestinal immaturity, inflammation and enteral feeding. Identifying risk factors for the development of NEC and its mortality can inform targeted preventative strategies.

Aim: The aim of the study was to assess the incidence, characteristics, risk factors and outcomes of infants diagnosed with NEC in a large tertiary neonatal unit in South Africa.

Methods: A prospective case-control study was conducted from May 2022- December 2024 at Chris Hani Baragwanath Academic Hospital. Infants diagnosed with definite NEC (modified Bell's Stage 2 or 3) were included as cases. Each case was matched with 1-2 controls by weight and postnatal age. Demographic characteristics, laboratory findings, management and outcomes of cases and controls were reviewed. Comparisons were performed between cases and controls, and survivors and non-survivors amongst cases using univariate and multivariate logistic regression analyses.

Results: There were 167 cases of NEC enrolled. The incidence of definite NEC was 3.4/1000 live births, 1.4% and 4.0% of all neonatal admissions and very low birth weight infants respectively. The median gestational age, birth weight and postnatal age of cases was 31 weeks, 1455g and 8.5 days respectively. Cases were more likely to have been formula fed (OR 2.00; 95% CI 1.20-3.33); previously exposed to longer duration of antibiotics (OR 1.26; 95% CI 1.14-1.40), having received blood transfusion (OR 27.4; 95% CI 2.09-359) and less likely to have reached full feeds in shorter time (OR-0.88; 95% CI 0.80-0.95). Ninety-one cases (54.5%) had culture-confirmed sepsis. Mortality rate was 49.7%, with ventilation, and hypotension being predictors of mortality

Conclusion:. There was a high incidence of definite NEC, with associated high mortality, mainly in infants who were ventilated or hypotensive post NEC diagnosis. Factors associated with NEC were formula feeding, longer duration of antibiotics and prior blood transfusion.

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ABBREVIATIONS

CHBAH: Chris Hani Baragwanath Academic Hospital

CI: Confidence Interval

HIC: High-Income Country

HIV: Human Immunodeficiency Virus

IQR: Interquartile Range

LMIC: Low and middle-income countries

$MgSO_4$: Magnesium sulphate

NEC: Necrotizing enterocolitis

OR: Odd's Ratio

PDA: Patent ductus arteriosus

PRC: Packed Red Cells

REDCap: Research Electronic Data Capture

SGA: Small for gestational age

VLBW: Very low birth weight

WCC: White Cell Count

SUBMISSIBLE FORMATTED MANUSCRIPT

TITLE PAGE

RISK FACTORS AND OUTCOMES OF INFANTS WITH NECROTIZING ENTEROCOLITIS: A CASE-CONTROL STUDY

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INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating disorder of the gastrointestinal tract in neonates, characterized by inflammation, ischaemia and infection (1). There is resultant necrosis of the bowel and, in severe cases, perforation (2). This condition results in significant morbidity and mortality and is the most common surgical emergency affecting neonates (3). The incidence has been reported as 1.26 per 1000 live births in South African neonatal units, comparable to that of 1-3 per 1000 live births globally (4,5). It has been reported to occur in 2-9% of very low birth (VLBW) weight infants (6). NEC is associated with high mortality rate, ranging from 20-50% (1,4). The diagnosis is made based on clinical features, and confirmed on plain abdominal radiography and, increasingly, abdominal ultrasound scan. NEC is graded according to Bell's modified criteria, stage 1 being suspected NEC, stage 2 is definite NEC and stage 3 is advanced disease (7,8). Stage 2 can further be subdivided into 2a and 2b, where 2a is characterized by pneumatosis intestinalis on X-ray and 2b is characterized by the same X-ray changes, as well as mild metabolic acidosis and thrombocytopaenia. Stage 3 is further divided into 3a, where the same X-ray changes are seen, in conjunction with severe clinical signs such as hypotension and apnoea; and stage 3b has similar clinical features as well as confirmed pneumoperitoneum on X-ray. Treatment includes bowel rest, parenteral nutrition, antibiotics, and severe cases may require surgical intervention, respiratory support and inotropes (1,9) .

Prematurity is a major risk factor for this condition, with approximately 85% of cases occurring in preterm infants (1). NEC is most likely due to poor motility, immature barrier and immune functions of the preterm intestinal tract (3). Most preterm infants have prolonged hospitalization and are often on antibiotics, resulting in dysbiosis (an imbalance between the intestinal flora and pathogenic bacteria) (9,10). Exclusive human milk feeding significantly decreases the risk of developing NEC when compared to formula feeding (11). Human milk contains Bifidobacteria, immunoglobulins, lactoferrin, oligosaccharides and other bioactive components that are protective against NEC (3,12). Although the above risk factors have been described, the pathophysiology is still incompletely understood. Other risk factors that have been implicated in the development of NEC are infection, packed red cell (PRC) transfusion, patent ductus arteriosus (PDA) or any event that exposes the neonate to hypoxia or ischaemia (13). Factors associated with NEC vary from different reports and most of these reports are retrospective studies, which have a high risk of bias (14–17).

The main objectives of this study were to determine the incidence, risk factors and associated mortality in infants diagnosed with definite NEC (modified Bell's stage 2 and 3), hospitalized at the Chris Hani Baragwanath Academic Hospital (CHBAH) neonatal unit, a large public tertiary academic hospital in Johannesburg, South Africa, through conducting a prospective case-control study.

MATERIALS AND METHODS

This was a prospective case-control study which included infants diagnosed with definite NEC matched with controls. All neonates diagnosed as having definite NEC hospitalized and managed between 01 May 2022 and 20 December 2024 were eligible as cases.

Study setting

Cases included any infant admitted in the neonatal unit with definite NEC, diagnosed as stage 2 or 3 NEC according to Bell's modified criteria (7). Both NEC cases diagnosed at CHBAH and NEC referrals from other hospitals were included. For the cases diagnosed at CHBAH, two controls were matched according to birth weight (±100g) and postnatal age (±7 days). The NEC cases referred from other hospitals were not matched with controls. Infants with congenital intestinal abnormalities or severe congenital abnormalities, were excluded for both cases and controls.

The study was conducted at (CHBAH), in Johannesburg, South Africa. The hospital and local clinics conduct approximately 30,000 deliveries with an average of 4,000 admissions to the neonatal unit per year. The neonatal unit has 185 beds, of which 18 are neonatal intensive care beds. Preterm deliveries account for 20% of all deliveries (18). The hospital is a major surgical referral centre for surrounding district and regional hospitals as it is one of the few hospitals offering paediatric surgical services in the Gauteng and North West provinces.

Data collection

The research team was informed by the clinical team at CHBAH of any newly diagnosed NEC cases. Diagnosis of definite NEC was based on clinical diagnosis by the attending paediatrician, which is based on presence of gastrointestinal tract symptoms, abdominal distension and radiologic findings of pneumatosis intestinalis with or without signs of intestinal perforation. Parents were then approached for written informed consent for inclusion into the study, for both cases and controls. Each participant was assigned a case or

control number and data entered onto an anonymized data sheet and then onto the Research Electronic Data Capture (REDCap) database.

Baseline maternal data were collected including demographic characteristics, human immune-deficiency virus (HIV) status, presence of medical conditions, maternal medication including magnesium sulphate (MgSO4), antenatal steroids and antibiotics. Infant data collected included demographic characteristics, delivery mode, Apgar score and need for resuscitation at birth. Data regarding feeding of the infants was collected and included time to first feed, type of feeding, whether human milk (mother's own milk or donor breast milk), formula or mix feeding, administration of FM85® milk fortification and time to full enteral feeds. Other information collected was medical management of controls and cases before NEC diagnosis, these included use of antibiotics, packed red blood cell transfusion (PRC), inotropes, mechanical ventilation and other diagnosis namely PDA. Detailed information regarding all NEC cases was collected including age at diagnosis, stage of disease, laboratory results, presence of infectious organisms, type of surgical management and outcomes. Management such as ventilation, use of inotropes, type of antibiotics was documented.

Statistical analysis

Statistical analysis was performed using TIBCO *Statistica*® 14.0.0.15. For the identification of risk factors for NEC, only the cases diagnosed at CHBAH, and their controls were analysed. Cases diagnosed at other hospitals were not included in this part of the analysis due to differing sepsis rates and protocols between the hospitals. For the descriptive analysis all cases were analysed, including NEC cases referred from other hospitals. Continuous data were presented as means and standard deviations if normally distributed and medians with interquartile ranges (IQR) if not normal distributed. Categorical variables were presented as frequencies and percentages. In comparing the cases and controls or survivors and non-survivors a Chi-square test or Fisher's exact test was performed for categorical variables; and a Student t-test or Mann-Whitney U test for continuous variables, based on whether they were normal distributed or not. Univariate and multivariate logistic regression analysis was performed to determine risk factors for NEC, and these were reported as crude or adjusted odds ratios (OR) with 95% confidence intervals (CI). Variables included in the multivariate analysis were those with p-values <0.10. Odds ratios with 95% CI not including the number one or differences with p-values < 0.05 were considered to be statistically significant. All statistical tests were two-sided.

Ethics

Permission to conduct this study was obtained from the hospital protocol review committee and ethics approval was given by the University of the Witwatersrand Human Research Ethics Committee (Protocol number M220215).

RESULTS

Incidence

There were 256 neonates admitted and diagnosed with NEC between 01 May 2022 and 31 December 2024. Stage I NEC was diagnosed in 70 infants and these were, therefore excluded. These were excluded because the clinical presentation is similar to that of difficult feed intolerance and ileus, making the diagnosis of NEC 1 difficult. After exclusion of stage 1 cases, 186 definite cases remained. Of the 186 definite cases, 160 were diagnosed at CHBAH, and 26 were diagnosed at other facilities (referrals). Nineteen (10.2%) refused consent, thus 167 cases were included in the study **(Figure 1)**. Amongst the enrolled cases, a total of 144 (86.2%) cases were diagnosed at CHBAH and 23 (13.8%) were diagnosed at another facility and referred to for surgical services. During the study period, there were 42,413 live births and 11,108 neonatal admissions, of which 1,886 were very low birth weight (VLBW). The incidence of NEC at CHBAH was 3.8 /1000 live births, 1.4% of all neonatal admissions and 4.5% in VLBW infants. (**Table 1**).

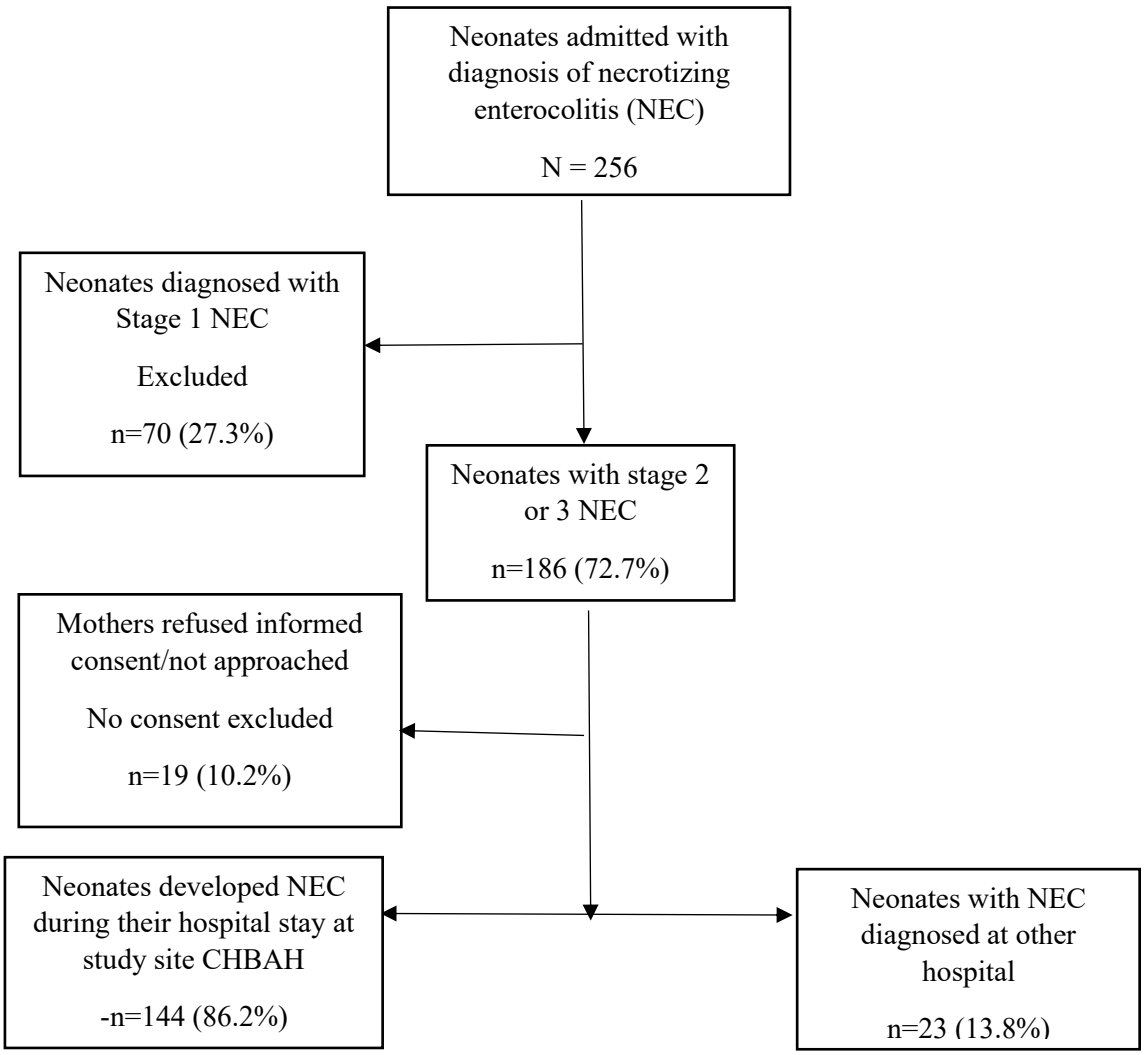

Figure 1: Flow chart of number of patients diagnosed with necrotizing enterocolitis enrolled in the study

Table 1: Incidence of necrotizing enterocolitis (NEC) according to live births, admissions, very low birth weight infants

	Number of live births	All admissions	Admissions of very low birth weight infants (birthweight <1500 grams)
Total numbers	42,413	11,108	1,886
NEC cases	160	160	85
Incidence	3.8/ 1000 live births	1.4%	4.5%

NEC patients (Cases)

Of the 167 cases, 101 (60.5%) were assessed as stage 2 and 66 (39.%) as stage 3 NEC (**Table 2**). The majority (62.3%) were managed medically, with the remaining infants being managed surgically. The median age at diagnosis was 8 days (IQR 5-16). Hypotension requiring inotropes occurred in 58 cases (34.7%). The all-cause mortality rate was 47.9%, with the median age at death being 41 days (IQR 20.5 - 62).

Table 2: Demographic characteristics, severity, management and outcomes of NEC (N=167)

Baseline Characteristics	**Number (%)**
Birth weight, in grams	1310 (1045-1660)*
Gestational age, in weeks	31 (29-33)*
Male sex	95 (56.9)
Apgar scores	9 (8-10)*
Age at diagnosis, in days	8 (5-16)*
Modified Bells Staging	
Stage 2a	54 (32.3)
Stage 2b	47 (28.1)
Stage 3a	15 (9.0)
Stage 3b	51(30.5)
Type of Management	
Non-surgical management	104 (62.3)
Surgical management	63 (37.7)
Type of non-surgical management post diagnosis	
Hypotension requiring inotropes	58 (34.7)
Mechanical ventilation	88 (52.7)
PRC transfusion	62 (37.1))
Type of surgical management	N= 63
Peritoneal drain inserted	20 (31.7)
Subsequent laparotomy	16/20 (80)
No subsequent laparotomy)	4/20 (20)
Laparotomy	43/63 (68.3)
Outcome	
Number died	80 (47.9)
Median age at death, in days	41 (20-62)*

*Median (interquartile range)

Amongst the 63 NEC cases that required surgical intervention, 20/63 (31.7%) were initially managed with peritoneal drain insertion, with 16/20 (80%) of them subsequently being taken for laparotomy. Amongst the cases having initial peritoneal drain and then taken for laparotomy, 3/16 (18.8%) had primary anastomosis, 10/16 (62.3%) had ileostomy/colostomy, and 3 (18.8%) had temporizing surgery (clip and drop) but demised before definitive surgery could be done. Amongst 43/63 (68.3%) cases who were directly taken for laparotomy, 3 (7.0%) had primary anastomosis, 22 (34.9%) had ileostomy/colostomy and 14 (32.6%) had temporizing surgery (clip and drop). Amongst those who had the temporizing surgery, 8 demised before they could have definitive surgery, and the remainder later had definitive surgery. Amongst all those who were taken for laparotomy (59) two patients (2/59; 3.4%)

were assessed to have NEC totalis and two were found to not have surgical disease, therefore no resection was done.

The majority of cases 103 (64.8%) had normal white cell counts (WCC), 50 (31.4%) had leucopaenia the remainer having leucocytosis (**Table 3**). Neutropaenia was observed in 30 cases (20.5%) and 34 cases (21.4%) had thrombocytopaenia. There were some missing data for the WCC parameters, due to intermittent operational challenges at the laboratory. Culture-confirmed sepsis occurred in 54.5% of cases, and the common organisms were *Klebsiella pneumoniae* (n = 41; 42.2%), *Acinetobacter baumannii* (n = 16; 16.5%), *Enterococcus spp.* (n = 14; 14.4%) *and Escherichia coli* (n = 6; 6.1%) .

Table 3: Laboratory findings in infants with necrotizing enterocolitis

Laboratory parameters	Number (%) or Medians (IQR)
Full blood count	
White cell count (WCC) (10^9/L) (N=159)	7.2 (3.9-11.8)*
Leucopaenia (WCC <5.0)	50 (31.4)
Normal (5.0-25.0)	103 (64.8)
Leucocytosis (>25.0)	6 (3.8)
Neutrophils (10^9/L) (N=146)	3.56 (1.6-6.0)*
Neutropaenia (<1500)	30 (20.5)
No neutropaenia) (≥1500)	116 (79.5)
Haemoglobin (g/dL)	13.0 (10.5-15.1)*
Platelets (10^9/L) (N=159)	225 (123-337)*
Thrombocytopaenia (<100)	34 (21.4)
No thrombocytopaenia (≥100)	125 (78.6)
C-reactive protein	N=158
<10 mg/L	66 (41.8)
≥10mg/L	92 (58.2)
Blood cultures	
Culture confirmed sepsis at diagnosis (n, %)	91(54.5)
Organisms cultured (n = 97)	
Klebsiella pneumoniae	41(42.2)
Acinetobacter baumannii	16 (16.5)
Enterococcus spp.	14 (14.4)
Coagulase negative staphylococcus	8 (8.2)
Escherichia coli	6 (6.1)
Candida auris	4 (4.1)
Enterobacter cloacae	2 (2.0)
Serratia marcescens	3 (3.0)
Methicillin Sensitive *Staphylococcus aureus*	1 (1.0)
Candida albicans	1 (1.0)
Pseudomonas aeruginosa	1 (1.0)
Candida parapsilosis	1 (1.0)

*****Median (interquartile range)

Comparing Cases and Controls

There were 144 cases that developed NEC at CHBAH (born and admitted at CHBAH/ local clinics). These were matched with 1-2 controls each with a total of 206 controls being enrolled. There were no significant differences in the maternal characteristics between cases and controls. Maternal age and frequency of comorbidities such as hypertension and diabetes mellitus were similar between the two groups (**Table 4**). Though cases were matched with controls (+/- 7 days); cases had a higher postnatal age than controls (8.5 days [IQR 16-16.5] versus 8 days [IQR 3-15]); p=0.011). Eighty- eight percent of cases were inborn, compared to 91.7% of controls, but this was not statistically significant (p=0.413). There were no statistically significant differences between the gestational age, birth weight, Apgar score or delivery room temperatures between the two groups. The cases had slower weight gain after recruitment compared to controls (7.5g/kg/day [IQR 0-12] versus 13g/kg/day [IQR 10-18]; p< 0.001).

On multivariate analysis, for those neonates that had a longer time (days) to initiate feeds, were less likely to develop NEC by 12% (OR 0.88; 95% CI 0.80-0.95; p=0.003) and those that received any amount of breastmilk versus those that were exclusively formula fed, there was a 50% reduction in developing NEC (OR-0.50;95% CI 0.30-0.83; p=0.008.) The odds of having NEC were higher if infants were fed formula compared to breastmilk feeding (any volume), (OR 2.00; 95% CI 1.20-3.33; p=0.008), on longer duration of previous antibiotics (OR 1.26; 95% CI 1.14-1.40; p<0.001) and received PRC transfusion (OR 27.4; 95% CI 2.09-359; p=0.012) (**Table 5**). Eight (4.8%) of the cases received PRC transfusion within 72 hours of diagnosis compared to one control (0.5%). The odds of having NEC were also 2.3 fold higher in infants who were fed exclusive formula than exclusive breastmilk (OR 2.32; 95% CI 1.37-3.93; p=0.0020, and 2.7-fold higher in those fed breast milk with fortifier than without fortifier (OR 2.69; 95% CI 1.09-6.66;p=0.032).

Table 4: Comparing baseline characteristics of NEC cases and controls

	Cases N=144	Controls N= 206	p-value
Maternal characteristics			
Maternal age, years, median (IQR)	29 (23-34.5)	29.5 (23-35)	0.562
Maternal positive HIV status, n (%)	46/142(32.3)	61/206(29.6)	0.580
Maternal diabetes, n (%)	4/144 (2.8)	6/206 (2.9)	0.940
Maternal hypertensive disorders, n (%)	24/144 (16.6)	31/206(15.0)	0.682
Antenatal antibiotics, n (%)	13/144 (9.0)	14 /206(6.8)	0.444
Antenatal steroids, n (%)	80/143 (55.9)	106/206 (51.4)	0.408
Antenatal $MgSO_4$, n (%)	24/144 (16.7)	48/206 (23.3)	0.127
Delivery by Caesarean section, n (%)	77/143 (53.8)	122/205 (59.5)	0.107
Multiple gestation, n (%)	28/144 (19.4)	46/206 (22.3)	0.513
Infant characteristics			
Birth weight, grams, median (IQR)	1450 (1165-1692)	1450 (1195-1665)	0.940
Gestational age, weeks, median (IQR)	31(29-33)	31(29-33)	0.930
Postnatal age at enrolment, days, median (IQR)	8.5 (6-16.5)	8 (3-15)	0.011
Male sex, n (%)	81(56.2)	102 (49.5)	0.231
Apgar, median (IQR)	9 (8-10)	9 (8-10)	0.402
Temperature of infant recorded in delivery room, ℃, median (IQR)	36.0 (35.0-36.0)	36.0 (35.0-36.0)	0.760
Need for resuscitation at birth #	24 /144(16.6)	23/206 (11.1)	0.140
Weight gain before enrolment, grams/kg/day	0 (-15-1.5)	-4 (-15-2)	0.494
Weight gain after enrolment, grams/kg/day	7.5 (0-12)	13 (10-18)	<0.001

IQR= interquartile range; $MgS0_4$= Magnesium sulphate

Resuscitation at birth includes manual ventilation , with or without chest compressions and administration of adrenaline

Table 5: Comparing feeding, use of antibiotic and in-hospital management between necrotizing enterocolitis (NEC) cases and controls with analyses of risk factors using logistic regression

	Cases N=144* n (%)	Controls N=206 n (%)	Univariate analysis Crude Odds Ratios (95% CI)	P-values	Multivariate analysis Adjusted Odds Ratios (95% CI)	P-values
Feeding patterns						
Age at initiation of feeds, days	2 (2-3)**	3 (2-3)**	0.97 (0.87-1.08)	0.179	N/A	
Days to full enteral feeds	9 (6-12)**	10 (8-12)**	0.94 (0.88-1.00)	0.064	0.88 (0.80-0.95)	0.003
Feeding exclusive formula feeding versus any amount of breastmilk						
Exclusively formula fed (n=109)	56 (40)	53 (26)	1.92 (1.21-3.05)	0.005	2.00 (1.20-3.33)	0.008
Breastmilk or mixed feeding (n=237)	84 (60)	153 (74)	Reference		Reference	
Management of cases (before diagnosis) and controls						
PRC transfusion before diagnosis of NEC	8 (5.6)	1 (0.50)	12.4 (1.53-100)	0.018	27.39 (2.09-359)	0.012
Diagnosed with patent ductus arteriosus	10 (6.9)	7 (3.4)	2.12 (0.79-5.71)	0.137	N/A	
Duration of antibiotic exposure before diagnosis of NEC/ recruitment, days	3.5 (3-5)**	3 (2-4)**	1.18 (1.08-1.29)	<0.001	1.26 (1.14-1.40)	<0.001

*4 patients had not been fed prior to NEC diagnosis

** medians (interquartile ranges)

Abbreviations: CI = confidence interval; N/A = not applicable; NEC = necrotising enterocolitis

Comparing survivors and non-survivors amongst neonates with NEC

On univariate analysis, factors associated with mortality amongst neonates with definite NEC were Modified Bell's stage 3 disease ($p< 0.001$) , the need for ventilation ($p< 0.001$) and culture confirmed sepsis ($p< 0.001$)(**Table 6**). On multivariate logistic regression analysis, after adjusting for birth weight, modified Bell's staging, age at diagnosis, ventilation, hypotension and sepsis, the factors that were associated with mortality were the need for ventilation , with odds of mortality increased by 6 times (OR 6.23 ; 95% CI 2.23- 16.8; $p< 0.001$) and hypotension requiring inotropes, with odds of mortality increased by two times (OR 2.59 ; 95% CI 1.04- 6.47; $p=0.04$).

Table 6: Predictors of mortality in NEC by univariate and multivariate logistic regression model

Variable	Non-survivors N=80 n (%)	Survivors N=87 n (%)	Univariate analysis Crude Odds Ratios (95% CI)	P-values	Multivariate analysis Adjusted Odds Ratios (95% CI)	P- values
Gestational age. weeks	31.0 (29.0-33.0)*	31.3 (30-33)*	0.97 (0.87-1.07)	0.537	N/A	N/A
Birth weight, in grams	1508 (1175-1767)*	1375 (1165- 1695)*	1.00 (0.99-1.00)	0.254	N/A	N/A
Modified Bell's Stage 3	51 (77.3)	15 (22.7)	8.44 (4.11-17.32)	<0.001	2.19 (0.89-5.4)	0.087
Sex, male	50 (62.5)	45 (51.7)	0.64 (0.35-1.19)	0.161	N/A	N/A
Age at diagnosis, days	7 (6-13)	11 (5-19)	0.98 (0.96-1.00)	0.141	N/A	N/A
Ventilation	71 (88.8)	28 (32.2)	16.62 (7.27-37.99)	<0.001	6.17 (2.28-16.70)	<0.001
Hypotension	48 (60)	12 (13.8)	9.38 (4.40-19.96)	<0.001	2.59 (1.04-6.47)	0.041
Sepsis#	57 (71.2)	34 (39.0)	3.86 (2.02-7.39)	<0.001	1.40 (0.61-3.21)	0.426

* median (interquartile range), # Sepsis was defined as culture confirmed sepsis

DISCUSSION

The overall incidence of NEC in this study was 3.8 per 1000 live births and 4.5% amongst VLBW infants. Formula feeding (aOR 2.00), blood transfusion (aOR 27.39) and longer duration of antibiotic exposure(aOR1.26) were identified as risk factors for NEC on multivariate analysis. The rate of culture proven sepsis was high (54.5%), and a substantial proportion of NEC cases had surgical intervention (35.3%). There was a high mortality rate (47.9%), with infants of a lower gestational age more likely to die.

The incidence rates observed in this study are similar to the globally reported incidence of 1-3 cases per 1000 live births, and 2-9% amongst VLBW infants in both high-income countries (HIC) and low-and middle-income countries (LMIC) (1,19,20). A recent study at another public tertiary hospital in Johannesburg reported an incidence of 8.2%, which was higher than that of the present study (21). In that particular study there appears to have been greater survival of ELBW infants, and thus a higher proportion of cases occurring in that category, compared to the present study. Another study from China included all stages of NEC, with a reported similar incidence (4.5%), however when excluding stage 1 NEC, the incidence dropped to 2.5% in VLBW infants. The mortality was 41.7% overall, which was comparable with the present study, but expectedly higher (50.2%) in VLBW infants(15).

The present study showed that prolonged antibiotics exposure was a risk factor for NEC, as previously shown by a retrospective study from Kenya (22). Additionally, a prospective study in a HIC has previously shown that prolonged antibiotic exposure in preterm infants was a risk factor for NEC (23). Several retrospective studies in a narrative review have also found that antibiotic exposure predisposes to NEC It is postulated that antibiotic exposure causes microbial dysbiosis and thus predisposes the infant to NEC (24). However, a previous case-control study from CHBAH did not find any association between antibiotic exposure and NEC, this difference could be explained by the fact that the two studies were conducted approximately 15 years apart and there has since been increased survival of extremely low birth weight infants in this neonatal unit (4) . However, a more recent (data from 2014-2017) prospective matched case-control study conducted in Europe by Berkhout et al. reported that early initiation of antibiotics after delivery in neonates <30 weeks gestation was inversely

associated with NEC(23). The contradictory outcomes of these studies highlight the need for more prospective studies to determine the relationship between prior antibiotic exposure and NEC.

This study found that any breastmilk was protective against NEC and reduced the risk of NEC by approximately 50%. Mother's own breastmilk contains Bifidobacteria and other flora, secretory IgA and microbial proteins which are protective against NEC(24). A prospective matched case-control study conducted in Europe by Berkhout et al. found that after multivariate logistic regression analysis the risk factors for NEC were formula feeding and a longer duration of parenteral feeding (23).

In the present study, NEC was associated with recent PRC transfusion within 72 hours (aOR 27.4). Several mechanisms have been postulated to explain a possible association between PRC transfusion and NEC. The anaemia itself may lead to reduced oxygen delivery to the intestine and, thus, predispose the neonate to NEC (25). Vascular tone is dependent on adequate levels of circulating nitric oxide. Blood transfusion may disrupt expression of nitric oxide synthase, an enzyme important in the production of nitric oxide (26,27). Depletion of nitric oxide results in increased vascular tone and therefore reduced blood supply to the intestinal mucosa, increasing the risk of NEC. Stored red blood cells may have high amounts of free haemoglobin, which may inactivate circulating nitric oxide and activate Toll like receptor-4 (TLR-4), setting off an inflammatory cascade, which may contribute to the development of NEC (25,26). A meta-analysis of observational data by Mohamed et al. (27) found that recent exposure to blood transfusion was associated with NEC, however, other studies have failed to show a causal relationship (23,25). Research regarding the association of PRC transfusion and NEC is ongoing, as this topic is not fully understood.

The reported rate of sepsis amongst NEC cases varies between different tertiary hospitals in South Africa. In the present study, the rate of culture-proven sepsis was 54.5%, which was higher than 44% reported at Tygerberg hospital in Cape Town, South Africa, but lower than 71% at Charlotte Maxeke Johannesburg Academic Hospital in Gauteng, South Africa (16,17). These differences may be due to underlying sepsis rates in the different facilities. The underlying sepsis rates at CHBAH are very high 14.3/ 1000 patient days compared to 3.3/1000 patient days reported at Tygerberg hospital (28,29). The sepsis may also be lower in the study performed in Tygerberg as this study only included neonates requiring admission to

the intensive care unit and presumably did not include the neonates with NEC that remained in the high-care and standard-care units (16).

In the present study, the cases of NEC had a significantly slower weight gain after NEC diagnosis compared to the controls. This has been attributed to functional gastrointestinal insufficiency in a systematic review(30).

In the preset study there was a high mortality rate of 47.9% associated with NEC, and the significant risk factors for mortality using multivariate analysis were ventilation(aOR 6.17) and hypotension(aOR 2.59) . The need for ventilation and the presence of hypotension, requiring inotropic support implies that the infants were sicker than those who did not need ventilation or inotropic support. This mortality rate is similar to that of 43.4% reported for VLBW infants at a similar tertiary public hospital in Johannesburg, but higher than the overall mortality rate of 39.9% (17). A retrospective study in Cape Town showed a higher mortality rate of 52% at 30 days, which may be due to the fact that the infants with NEC were of a lower gestational age and birth weight, 29 weeks and 1185g compared to 31 weeks and 1310g at our institution (16). In that study, significant predictors of mortality were lower gestational age ($p=0.016$), birth weight($p=0.005$) and head circumference ($p<0.001$); as well as shock ($p<0.001$), apnoea ($p=0.027$) and metabolic acidosis($p<0.001$) on admission (16). admission. Similarly to the present study, the study performed in Cape Town also reported the need for invasive ventilation ($p<0.001$) and inotropic support ($p<0.001$) to be associated with mortality(16). A retrospective study by Qian et al found advanced disease, sepsis and small for gestational age (SGA) to be risk factors for mortality (15). Other studies have found extreme low birth weight to be a risk factor for mortality (1). Studies from HIC have reported mortality rates between 21.4% and 28.8%, which is lower than that reported in this present study (31,32). This difference is most likely due to more resources being available for management of these patients in HIC, and the fact that some of the studies reported mortality at 28 days compared to the present study which reported mortality at final outcome or hospital discharge (23,33).

In the present study, the cases had a higher postnatal age than controls. This most likely occurred by chance through controls being enrolled towards the lower extreme of the 7 day limit used to match cases and controls.

STRENGTHS AND LIMITATIONS OF THE STUDY

The main strength of this study was the fact that it was a prospective study, therefore a large amount of information could be collected, including maternal and infant baseline characteristics, information relating to NEC diagnosis, management and outcomes. This was a case control study, which enabled comparison between infants with and without disease. There are few prospective case control studies on NEC from the LMICs, therefore, this research adds valuable information to the body of knowledge on this condition.

The present study was conducted at a single centre; therefore, the findings may not be generalisable to other populations. The diagnosis of definite NEC and interpretation of the radiographs was done by the clinicians managing the infants, with no input from radiologists. This may have led to overestimation or underestimation of the incidence of NEC by the clinicians.

The required number of 288 controls was not achieved, as time availability to consent mothers competed with the researcher's clinical responsibilities, thus missed some controls.

CONCLUSIONS

In conclusion, the results of this study show that NEC remains a relatively common morbidity in neonates, particularly in VLBW infants. This study confirms that formula feeding remains a significant risk factor for development for NEC and therefore exclusive breast feeding should be prioritized as an important preventative strategy. Strategies to improve breastfeeding rates should include establishing a culture of exclusive breast feeding in our communities and setting up donor breastmilk banks. The second major risk factor was blood transfusion. The mechanisms by which blood transfusion is associated with NEC need to be studied further, as it is not clear as to whether this association was through indication for transfusion or the effect of transfusion itself. Prolonged antibiotic exposure is another important risk factor; therefore, antibiotics stewardship should be practised, and unnecessary use of antibiotics should be avoided. NEC had a high mortality rate, therefore preventing NEC should be one of major areas of focus to reduce neonatal deaths.

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STUDY PROTOCOL

TITLE

INCIDENCE, RISK FACTORS AND OUTCOMES OF NECROTIZING ENTEROCOLITIS IN NEONATES ADMITTED AT A TERTIARY PUBLIC HOSPITAL

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BACKGROUND

Introduction

Necrotizing enterocolitis (NEC) is a disorder of the neonatal gastrointestinal tract, characterized by inflammation, ischaemia and infection (1–3). Global incidence is 1-3 cases per 1000 live births, which is similar to that reported in South African studies (4–6). It is common in infants born preterm, with them, accounting for 80-90% of all cases (7).

Risk factors

Common risk factor is being born preterm. In the preterm gut peristalsis is impaired, resulting in stasis and increased bacterial exposure inflammation. Secondly, the intestinal defence mechanisms are immature.

Preterm neonates are often admitted in healthcare facilities for prolonged periods requiring different types of medication, increasing the chances of dysbiosis. Dysbiosis is imbalance between beneficial and harmful intestinal bacteria. This occurs because of gut colonization by hospital organisms, use of H2 blockers and antibiotics, and nosocomial sepsis (7–9).

Formula feeding significantly increases the risk of NEC, related either to total volume, rate of increase of feeds, contamination and lack of protective properties found in breast milk (10,11). Recent blood transfusion has been reported to be another risk factor (1,7,9).

Pathogenesis

The pathophysiology involves inflammation, ischaemia, and feeding. Gut injury results in inflammation, a porous intestinal barrier, allowing bacteria and toxins to penetrate. Ischaemia, focal necrosis, sepsis and perforation develop (7). Intestinal ischaemia may result from asphyxia, umbilical artery catheterization, congenital heart disease or polycythaemia (4) . Approximately 95% of infants with NEC have a history of recent feeding (4). Research is ongoing regarding a possible association between blood transfusion and NEC (1).

Clinical and Pathological Findings

Term infants tend to present in the first few days of life, compared to preterm infants who present later, between one to two weeks of life (5). Clinical presentation of NEC is often difficult to differentiate from that of sepsis. Presenting signs include apnoea, temperature instability, lethargy, irritability, and shock. The abdomen may be distended, tender or

discoloured, with vomiting, delayed gastric emptying, bilious gastric drainage or blood-stained stool (9).

Radiologic and laboratory findings

Abdominal X-rays confirm the diagnosis of NEC. The hallmark feature in diagnosing NEC is pneumatosis intestinalis, which is intramural air produced by gas-forming bacteria. Other features are distension of bowel loops, pneumoperitoneum and portal venous gas. Ultrasonography is a newer diagnostic tool whose use is increasing, as it is more sensitive in diagnosing pneumoperitoneum (12,13). Serological tests such as the C-reactive protein, platelet activating factor, cytokines and metabolic acidosis are non-specific adjuncts.

Assessing Severity of NEC

Disease severity is graded using the modified Bell's criteria. (12,13). It is graded into Stage 1 to 3 based on clinical signs, laboratory and radiologic findings (Table 1).

Stage	**Systemic signs**	**Abdominal signs**	**Radiological Signs**
Ia suspected	Temperature instability Apnoea Bradycardia, Lethargy	Gastric retention Abdominal distension Emesis Haem + stool	Normal or Intestinal dilatation or Ileus
Ib suspected	As above	As above plus Grossly bloody stool	Normal or Intestinal dilatation or Ileus
IIa definite	As above	Gastric retention Abdominal distension Absent bowel sounds +/- abdominal tenderness	Pneumatosis intestinalis Intestinal dilation Ileus
IIb definite	As above plus mild metabolic acidosis thrombocytopenia	A above +Absent bowel sounds +Abdominal tenderness +/- abdominal cellulitis +/- right lower quadrant mass	Pneumatosis intestinalis Intestinal dilation Ileus Ascites
IIIa advanced	Same as IIB plus hypotension bradycardia severe apnoea respiratory and metabolic acidosis DIC neutropenia	+Absent bowel sounds +Marked abdominal tenderness +/- abdominal cellulitis +/- right lower quadrant mass + peritonitis	Pneumatosis intestinalis Intestinal dilation Ileus Ascites
IIIb advanced	As above	+Absent bowel sounds +Marked abdominal tenderness +/- abdominal cellulitis +/- right lower quadrant mass	Pneumatosis intestinalis Intestinal dilation Ileus, Ascites Pneumoperitoneum

Table 1. Modified Bell's staging criteria for NEC

Prevention

Proven preventative strategies are antenatal steroids; human milk feeding and judicious use of antibiotics. Probiotics have been shown to reduce the incidence of NEC, but further research is required to determine the optimal dosing (2).

Management

Medical management entails bowel rest, antibiotics, parenteral nutrition and other supportive treatment such as inotropes and ventilation (4). Stage III NEC is managed surgically.

Outcomes

Mortality from NEC is high, ranging from 20-50% globally and in South Africa (2,5,14). More preterm, extremely low birth weight and small for gestational age infants have increased mortality (2,11). Short term complications are strictures, short-bowel syndrome and disease recurrence. In the long-term intestinal failure, intestinal failure associated liver disease, and neurodevelopmental impairment occur. Severe neurodevelopmental delay occurs in 24-61% of patients, and intestinal failure in 15-35% (15).

JUSTIFICATION FOR THE STUDY

NEC is a devastating disease, with high mortality. Use of human breast milk is associated with reduction in incidence of NEC. A milk bank was recently opened in the neonatal unit at the Chris Hani Baragwanath Academic CHBAH) in October 2020, with increased use of donor breast milk. A lodger facility for mothers was also opened in July 2020, aimed at improving breastfeeding rates. Previous studies from South African institutions reported on incidence of NEC, mortality and surgical outcomes (5,14). However, there have not been any studies on NEC at CHBAH since the opening of the milk bank and lodger facility. This study will assess whether there has been any change in incidence of NEC, risk factors and outcome as a result of these facilities.

PURPOSE OF THE STUDY

Aim

To determine the incidence, characteristics, risk factors, management, outcomes and predictors of mortality of infants with NEC at CHBAH over a one-year period.

Objectives

1.To determine the incidence of NEC in infants admitted to the neonatal unit at CHBAH over a one-year period

2.To describe the demographic and clinical characteristics of infants with NEC

3. To compare the characteristics of infants with NEC and those without disease

4. To determine the mortality rate and duration of hospital stay in infants with NEC

5.To determine predictors of mortality in infants with NEC

METHODOLOGY

Study design

This will be a prospective case-control study conducted in the neonatal unit at CHBAH over a period of two years and eight months.

Study setting

CHBAH is a public, tertiary hospital in Soweto, a township in the south of Johannesburg. There are approximately 20 000 deliveries annually. The neonatal unit has 18 ICU, 40 high care, 80 standard care and 13 Kangaroo Mother Care (KMC) beds. There is a paediatric surgery unit on site. There is a lodging facility, accommodating 42 mothers. Mothers coming from home may visit daily to provide breastmilk. There is a donor breastmilk bank onsite, and due to limited supply, only infants with a birth weight less than 1200g may have access to the milk for a maximum of 14 days. Feeds are started once breastmilk is available. For preterm VLBW infants increases are by 20-30ml/kg/day. Full term infants are started on full feeds from birth unless there is a contraindication. Prior to feeding, the orogastric tube is aspirated to check for gastric residuals. NEC is suspected when there is recurrent vomiting, increased or discoloured gastric aspirates, abdominal distension, or discolouration. According to the unit protocol, when NEC is suspected, feeds are stopped for at least 72 hours; an orogastric tube is inserted for drainage and parenteral nutrition is commenced. Abdominal X-rays, full blood count, CRP, urea, electrolytes, arterial blood gases, blood, urine, and cerebrospinal fluid cultures are done. Antibiotics are commenced. Surgeons are consulted when there is stage IIb or III disease.

Study population

All infants with stage II or III NEC will be included as cases. Each case will be matched with two controls for birth weight (to be within 100 grams) and postnatal age (to be within a week). At CHBAH gestational age is often inaccurate, as many mothers do not attend antenatal clinics. The controls will be infants who do not currently have NEC and have no previous history of NEC.

Inclusion criteria:

Cases:

1. Any infant newly diagnosed with stage 2 or 3 NEC while admitted in the neonatal unit. Inborn and outborn patients will be included
2. Informed consent obtained from the mother

Controls:

1. Any infant admitted in the neonatal unit, who does not currently have NEC, nor previous NEC. Weight within 100g of a case, and born within 1 week of a case
2. Informed consent obtained from caregiver

   If a control subsequently develops NEC, they will no longer be considered a control but will become a case.

Exclusion criteria

Severe congenital malformation, where the infant is not expected to survive (as this will affect mortality/outcomes).

Study procedures

Infants with NEC stages 2 and 3 will be recruited as cases. 2 controls will be recruited for each case. Parents will be approached for informed consent. Cases and controls will be assessed daily for outcome (alive, discharged or died) until 28 days after recruitment. Data shall be collected on a data collection sheet and captured onto Redcap

Study definitions

A booked mother is one who attended antenatal care, unbooked is one who did not.

Born Before Arrival (BBA) refers to an infant who was not born at a health facility.

In this study NEC refers to only modified Bell's stages II and III. See Appendix 1.

Incidence of NEC will be stated as cases per 1000 live births.

28 -day mortality will be defined as any death within 28 days of NEC diagnosis.

A breast milk fed infant is one who receives more than 50% of enteral feeds as breast milk.

Rate of increase of feeds will be the average rate of increase from initiation of feeds until full enteral feeds are attained or until NEC diagnosis if this occurs before full feeds are attained.

Surgical intervention will refer to laparotomy and pencil drain insertion.

Confirmed sepsis will be defined as positive blood, urine or CSF culture.

Suspected sepsis will be defined as clinical signs and laboratory markers such as CRP >10 mg/L, WCC < 5X 10^9/L or > 25 X 10^9/L, Platelet< 100 with negative cultures.

Outcome measures

Primary outcomes

- Incidence of NEC per 1000 live births
- 28-day inpatient mortality rate

Secondary outcomes

- Need for surgical intervention
- Duration of parenteral nutrition
- Time to full enteral nutrition before NEC
- Length of hospital stay
- Weight gain after NEC diagnosis
- Proportion with culture confirmed sepsis at NEC diagnosis

Sample size calculation

A previous study at the same institution reported an overall mortality rate of 19% in NEC cases, the odds of death being 5 times that of the controls. In order to show a significant difference, with 80% power and a p-value of <0.05, an estimated total 489 patients will be required, 163 cases and 326 controls.

Data collection

Maternal and infant information will be collected on a data collection sheet. See Appendix 2.

Data analysis and management

Data will be collected and entered onto Redcap and analysed using Statistica (Stat soft USA, version 13). Categorical variables will be reported as frequencies and percentages, and continuous variables will be reported as means or medians, depending on the distribution of data. The Student's t-test will be used to compare means, and the Mann-Whitney U test will be used to compare medians. Logistic regression analysis will be done to analyse the variables associated with outcomes. A p-value of <0.05 will be considered statistically significant.

ETHICAL CONSIDERATIONS

The confidentiality of the patients will be protected, as no personal details or hospital numbers will be recorded on the database. The data will be stored on Redcap and password protected. A separate database with the identifiers such as names and hospital numbers will

be created on Redcap in order to track the outcomes of the infants. Personal identifiers will not be documented on the data capture sheet nor Redcap. Only the researcher and supervisors will have access to the data. Ethics approval will be requested from the Human Research Ethics Committee at the University of the Witwatersrand, as well as the head of unit, head of department and CEO of Chris Hani Baragwanath Academic Hospital.

TIMELINE

	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
	2021	2022												2023					
Literature review																			
Preparing protocol																			
Protocol assessment																			
Ethics application																			
Data collection																			
Data analysis																			
Write up thesis																			
Write up paper																			

FUNDING

The costs involved in this study will be for stationery, printing, and binding. All costs will be borne by the researcher. No additional funding will be sought.

LIMITATIONS

There may be some missing information from outborn patients.

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APPENDICES

APPENDIX A: DATA COLLECTION

Study number

Case	Control			

Maternal details			**Booking status**					
Age	Gravidity	Parity	Booked			Unbooked		
HIV Positive **CD4** **Viral load**	HIV negative	RPR Positive / Negative	Antenatal antibiotics		Metronidazole		Erythromycin	
			Cephalosporin		Ampicillin	Amoxycillin	Other	
Magnesium sulphate Y N	**Antenatal steroids** **Y N**							
Hypertension	Diabetes	Cardiac	Urinary tract infection		Other		Duration ROM	
Infant details								
Sex			5-minute Apgar score	Weight(g)		Gestational age (wk)		
Male	Female	Ambiguous						
Place of birth			Temperature at birth °C	Perinatal asphyxia Y /N		Cardiac lesion Y/ N		
CHBAH	Local clinic	Other hospital	Born before arrival	H2 receptor blocker/PPI Y/N	Type of feeds and duration			
					Mother's own milk	Donor breast milk	Formula	

Age at first feed (days)		**Age at NEC diagnosis(days)**		**Feed volume at NEC diagnosis(ml/kg/day)**		**Rate of feeds advancement (ml/kg/day)**	
Blood transfusions Y/N		**Transfusion before/ After NEC**		**Timing of blood transfusion before NEC diagnosis**			
Abdominal signs							
Vomiting	Distension	Tenderness	**Type of gastric aspirates**				
Discolouration	Bloody stool	Other	Clear	Milky	Bile stained	"Coffee ground"	Frank blood
Abdominal X-ray findings							
Pneumatosis intestinalis		Sentinel loop		Pneumoperitoneum			
Dilated loops		Portal venous gas		Other			
Infectious organism							
Klebsiella	Acinetobacter Baumanii			Candida Albicans Candida Parapsilosis		CNS	
E. Coli	Candida Auris			MRSA		Enterococcus	
Enterobacter	Pseudomonas			Serratia		Culture negative	Other
Blood results	CRP	WCC		Neutrophils	Hb	Platelet	CRP
Blood glucose	**Arterial blood gas**	pH		HCo3	Base deficit	Lactate	
Sodium	**Potassium**	Chloride		Urea	Creatinine		
Type of antibiotic	Tazocin	Amikacin		Cloxacillin		Fluconazole	
Meropenem	Vancomycin	Amphotericin B		Ceftazidime		Other	

Ventilated Y/N	Hypotensive Y/N	Treatment for hypotension				Normal saline
		Nil	Dobutamine	Dopamine	Adrenaline	Hydrocortisone

Surgical management Y/N	Peritoneal drain	Resection +primary anastomosis	Ileostomy
	Colostomy	Clip and drop	Other

Parenteral nutrition) Y/N	**Duration of parenteral nutrition (days**	Final outcome and age at final outcome	
		Died	Discharged

APPENDIX B: PLAGIARISM DECLARATION

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY

I Noela Holo Bertha Kamanga (Student number:375457) am a student registered for the degree of MSc_(Medicine) in the academic year 2025.

I hereby declare the following:

I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.

I confirm that the work submitted for assessment for the above degree is my own unaided work

except where I have explicitly indicated otherwise.

I have followed the required conventions in referencing the thoughts and ideas of others.

I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.

I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Date 31/03/2025

APPENDIX C: ETHICS

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Noela Kamanga

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M220215

NAME: (Principal Investigator)	Dr Noela Kamanga
DEPARTMENT:	Faculty of Health Sciences Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Incidence, risk factors and outcomes of necrotizing enterocolitis in neonates admitted at a tertiary public hospital
DATE CONSIDERED:	25/02/2022
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the University Registrar's office <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Prof S Velaphi and Dr R Thomas
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	26/04/2022

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions.Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **February** and will therefore be due in the month of **February** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX D: TURNITIN REPORT

Main manuscript 31 March.docx

ORIGINALITY REPORT

7%	5%	4%	0%
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PRIMARY SOURCES

1	www.frontiersin.org Internet Source	3%
2	David J. Hackam. "Necrotizing Enterocolitis - Pathogenesis, Diagnosis, and Treatment", CRC Press, 2021 Publication	2%
3	fortuneonline.org Internet Source	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		

APPENDIX E: AUTHOR GUIDELINES FRONTIERS OF PEDIATRICS JOURNAL

WRITING AND FORMATTING

Title

The title should be concise, omitting terms that are implicit and, where possible, be a statement of the main result or conclusion presented in the manuscript. Abbreviations should be avoided within the title.

Witty and creative titles are welcome, but only if relevant and within measure. Consider if a title meant to be thought-provoking might be misinterpreted as offensive or alarming. In extreme cases, the editorial office may veto a title and propose an alternative.

Authors should avoid:

titles that are a mere question without giving the answer

unambitious titles, for example starting with 'Towards,' 'A description of,' 'A characterization of' or 'Preliminary study on'

vague titles, for example starting with 'Role of', 'Link between', or 'Effect of' that do not specify the role, link, or effect

including terms that are out of place, for example the taxonomic affiliation apart from species name.

Abstract

As a primary goal, the abstract should make the general significance and conceptual advance of the work clearly accessible to a broad readership. The abstract should be no longer than a single paragraph and should be structured, for example, according to the IMRAD format. For the specific structure of the abstract, authors should follow the requirements of the article type or journal to which they're submitting. Minimize the use of abbreviations and do not cite references, figures or tables.

For clinical trial articles, please include the unique identifier and the URL of the publicly accessible website on which the trial is registered.

Manuscript length

We encourage you to closely follow the article word count lengths given in the 'Article types' page of the journals. The manuscript length includes only the main body of the text, footnotes, and all citations within it, and excludes the abstract, section titles, figure and table captions, funding statement, acknowledgments, and references in the bibliography. Please indicate the number of words and the number of figures and tables included in your manuscript on the first page.

Sections

The manuscript is organized by headings and subheadings. The section headings should be those appropriate for your field and the research itself. You may insert up to 5 heading levels into your manuscript (i.e.,: 3.2.2.1.2 Heading Title).

For Original Research articles, it is recommended to organize your manuscript in the following sections or their equivalents for your field.

Introduction
Succinct, with no subheadings.

Materials and methods
This section may be divided by subheadings and should contain sufficient detail so that when read in conjunction with cited references, all procedures can be repeated. For experiments reporting results on animal or human subject research, an ethics approval statement should be included in this section (for further information, see the 'Bioethics' section of our policies and publication ethics.)

Results
This section may be divided by subheadings. Footnotes should not be used and must be transferred to the main text.

Discussion
This section may be divided by subheadings. Discussions should cover the key findings of the study: discuss any prior research related to the subject to place the novelty of the discovery in the appropriate context, discuss the potential shortcomings and limitations on their interpretations, discuss their integration into the current understanding of the problem and how this advances the current views, speculate on the future direction of the research, and freely postulate theories that could be tested in the future.

For further information, please check the descriptions defined in the journal's 'Article types' page, in the 'For authors' menu on every journal page.

Language editing

Frontiers requires manuscripts submitted to meet international English language standards to be considered for publication.

For authors who would like their manuscript to receive language editing or proofreading to improve the clarity of the manuscript and help highlight their research, we recommend the language-editing services provided by the following external partners.

Note that sending your manuscript for language editing does not imply or guarantee that it will be accepted for publication by a Frontiers journal. Editorial decisions on the scientific content of a manuscript are independent of whether it has received language editing or proofreading by these partner services or other services.

Editage
We recommend the language-editing service provided by our external partner Editage. These services may be particularly useful for researchers for whom English is not the primary language. They can help to improve the grammar, syntax, and flow of your manuscript prior

to submission. Frontiers' authors will receive a 10% discount by visiting the following link: editage.com/frontiers.

The Charlesworth Group
We recommend the Charlesworth Group's author services, who has a long-standing track record in language editing and proofreading. This is a third-party service for which Frontiers' authors will receive a 10% discount by visiting the following link: www.cwauthors.com/frontiers..

Language style

The default language style at Frontiers is American English. If you prefer your article to be formatted in British English, please specify this on the first page of your manuscript. For any questions regarding style, we recommend authors to consult the Chicago Manual of Style.

Inclusive language guidelines

Frontiers is an inclusive publisher and we ask that all submissions are in line with our inclusive language policy. When preparing your manuscript for submission, take a mindful approach towards personal biases and a concerted effort to limit their influence. Authors should remove any suggestion or implication of superiority or inferiority of one person over another based on age, gender, race, ethnicity, culture, sexual orientation, disability, religion, or socio-economic class. We ask authors to use inclusive language practices and awareness of diversity, equity, and inclusion into their research and keep it at the forefront during the composition of their findings.

External guidance that may be useful is available at C4DISC's Guidelines on Inclusive Language and Images in Scholarly Communication.

Furthermore, when drafting your work, please take into account the following considerations

In general, seek to avoid

language that could be deemed insulting, profane, or derogatory.

descriptors that identify personal attributes such as age, gender, race, ethnicity, culture, sexual orientation, disability, or health conditions, where they are not critically relevant to the discussion.

any form of language that suggests a particular culture or group as the default or standard.

And where feasible:

proactively ask individuals or groups how they would prefer to be referenced.

adhere to the SAGER guidelines for reference to sex and gender in research.

Remember, the language we use can influence perceptions, evoke emotions, and shape perspectives. Let's work together to nurture an inclusive, respectful, and empowering discourse in science.

Guidelines for artificial intelligence and related technologies

These guidelines cover acceptable uses of generative AI technologies such as Large Language Models (ChatGPT, Jasper) and text-to-image generators (DALL-E 2, Midjourney, Stable Diffusion) in the writing or editing of manuscripts submitted to Frontiers.

Use of AI to create written or visual content

Authors should not list a generative AI technology as a co-author or author of any submitted manuscript. Generative AI technologies cannot be held accountable for all aspects of a manuscript and consequently do not meet the criteria required for authorship.

If the author of a submitted manuscript has used written or visual content produced by or edited using a generative AI technology, this use must follow all Frontiers guidelines and policies. Specifically, the author is responsible for checking the factual accuracy of any content created by the generative AI technology. This includes, but is not limited to, any quotes, citations or references. Figures produced by or edited using a generative AI technology must be checked to ensure they accurately reflect the data presented in the manuscript. Authors must also check that any written or visual content produced by or edited using a generative AI technology is free from plagiarism.

If the author of a submitted manuscript has used written or visual content produced by or edited using a generative AI technology, such use must be acknowledged in the acknowledgements section of the manuscript and the methods section if applicable. This explanation must list the name, version, model, and source of the generative AI technology. We encourage authors to upload all input prompts provided to a generative AI technology and outputs received from a generative AI technology in the supplementary files for the manuscript.

The entire document should be single-spaced and must contain page and line numbers in order to facilitate the review process. The manuscript should be written using either Word or LaTeX. See above for templates.

Abbreviations and nomenclatures

The use of abbreviations should be kept to a minimum. Non-standard abbreviations should be avoided unless they appear at least four times, and must be defined upon first use in the main text. Consider also giving a list of non-standard abbreviations at the end, immediately before the acknowledgments.

Equations should be inserted in editable format from the equation editor.

Italicize gene symbols and use the approved gene nomenclature where it is available. For human genes, please refer to the HUGO Gene Nomenclature Committee (HGNC). New symbols for human genes should be submitted to the HGNC here. Common alternative gene aliases may also be reported, but should not be used alone in place of the HGNC symbol. Nomenclature committees for other species are listed here. Protein products are not italicized.

We encourage the use of Standard International Units in all manuscripts.

Chemical compounds and biomolecules should be referred to using systematic nomenclature, preferably using the recommendations by the International Union of Pure and Applied Chemistry (IUPAC).

Astronomical objects should be referred to using the nomenclature given by the International Astronomical Union (IAU) provided here.

Life Science Identifiers (LSIDs) for ZOOBANK registered names or nomenclatural acts should be listed in the manuscript before the keywords. An LSID is represented as a uniform resource name (URN) with the following format:
urn:lsid:<Authority>:<Namespace>:<ObjectID>[:<Version>]

For more information on LSIDs please see the 'Code' section of our policies and publication ethics.

Enhancing search engine optimization (SEO)

There are a few simple ways to maximize your article's discoverability and search results.

Include a few of your article's keywords in the title of the article

Do not use long article titles

Pick 5-8 keywords using a mix of generic and more specific terms on the article subject(s)

Use the maximum amount of keywords in the first two sentences of the abstract

Use some of the keywords in level 1 headings

References

Preparing and formatting references

Submissions to Frontiers must be grounded in relevant and up to date peer-reviewed, academic research, and this should be reflected in the accompanying reference lists.

Authors are welcome to use online referencing tools in preparation of their manuscript. Some useful resources include RefMe, Zotero, and Mendeley.

The citation of non-academic and non-peer-reviewed sources (e.g. blog posts, website content), as well as anonymous sources or commercial websites should be avoided or kept to a minimum

Authors should avoid citing content that is not directly relevant to the scope of the article and the journal

Reference lists should reflect the current status of knowledge in the field, avoid bias, and not include a high proportion of citations to the same authors or sources, school of thought, etc.

The length of the reference list should be appropriate depending on the article type, covering the relevant literature through sufficient referencing

Authors should ensure that references are accurate, that all links are accessible, and that the citations/references adhere to the reference styles outlined below

Frontiers' journals use one of two reference styles, either Harvard (author-date) or Vancouver (numbered). These formats should be adhered to for the in-text citations and the reference

lists. Please check our help center to find the correct style for the journal to which you're submitting.

All citations of published works in the text, figures, or tables must be in the reference list and vice-versa.

The names of the first six authors followed by et al. and the DOI (when available) should be provided.

Given names of authors should be abbreviated to initials (e.g. Smith, J., Lewis, C.S., etc.).

The reference list should only include articles that are published or accepted.

Unpublished data, submitted manuscripts, or personal communications should be cited within the text only, for article types that allow such inclusions. Where additional details are available, these will be included as footnotes.

For accepted but unpublished works use 'in press' instead of page numbers.

Data sets that have been deposited to an online repository should be included in the reference list. Include the version and unique identifier when available.

Personal communications should be documented by a letter of permission.

Website URLs should be included as footnotes.

Any inclusion of verbatim text must be contained in quotation marks and should clearly reference the original source.

Preprints can be cited provided that a DOI or archive URL is available, and the citation clearly mentions that the contribution is a preprint. If a peer-reviewed journal publication for the same preprint exists, the official journal publication is the preferred source. See the preprints section for each reference style below for more information.

Harvard reference style (author-date)

Reference examples for Frontiers' journals using the Harvard referencing system can be found below. For examples of other sources, and for general questions regarding the Harvard reference style, please refer to the Chicago Manual of Style.

References should include the full last name and first name initials of the first six authors, followed by et al. and the year of publication in brackets.

Alphabetical order is followed for the reference list.

Reference guidelines - Harvard

Vancouver reference style (numbered)

Reference examples for Frontiers' journals using the Vancouver referencing system can be found below. For more examples of citing other documents and general questions regarding the Vancouver reference style, please refer to Citing Medicine.

In-text citations in the Vancouver reference style should be numbered consecutively in order of appearance in the text and identified by Arabic numerals in parenthesis.

Use square brackets for physics and mathematics articles.

The abbreviation ‘Ref’ should not be used, e.g.: [e.g., (1)] should NOT read [e.g. Ref. (1)].

Style for comparing a citation should follow the number format, e.g. [cf. (1)]. The same applies when using ‘see’, e.g. [see (46)].

References should be numbered and listed chronologically, according to the order they appear in the text.

Citation of retracted articles

Authors are responsible for ensuring the integrity of the references they cite, including verifying whether any article they intend to cite has been retracted. Citing retracted articles can undermine the credibility of your manuscript and should only occur where it is essential for the context of your discussion.

Guidelines for citing retracted articles:

Verification: Authors are responsible for checking the status of all cited works before submission. Lists of retracted articles can often be found in databases such as PubMed and the Retraction Watch database. Authors can check the version of the record which is expected to include a visible notice of retraction.

Disclosure: If it is necessary to cite a retracted article, authors must clearly indicate the retraction status in the reference list.

Justification: Authors are encouraged to discuss the reasons for citing the retracted article in the manuscript, explaining its relevance and the implications of its retracted status on the discussion.

Adhering to these guidelines is considered best practice as it helps maintain the scientific integrity of the published literature and ensures that all references within the manuscript contribute validly to the scholarly discourse. Ensuring the accuracy and transparency of citations protects authors from inadvertently perpetuating misinformation and supports the overall trustworthiness of research publications.

Acknowledgements

This is a short text to acknowledge the contributions of specific colleagues, institutions, or agencies that aided the efforts of the authors. Should the content of the manuscript have previously appeared online, such as in a thesis or preprint, this should be mentioned here, in addition to listing the source within the reference list.

Acknowledgements are designed to recognize individuals directly involved in the research. We request that authors avoid personal expressions of gratitude toward figures or entities unrelated or indirectly related to the production of the manuscript. While such acknowledgements are understood to be significant to the authors, they are considered indirectly involved in the research unless they pertain to specific research activities. It

remains the responsibility of the authors to ensure that these guidelines are adhered to in the final publication.

Templates

If working with Word please use our Word templates. If you wish to submit your article as LaTeX, we recommend our LaTeX templates.

For LaTeX files, please ensure all relevant manuscript files are uploaded: .tex file, PDF, and .bib file (if the bibliography is not already included in the .tex file).

During the interactive review, authors are encouraged to upload versions using track changes. Editors and reviewers can only download the PDF file of the submitted manuscript.

Figures, tables, and images

Figures, tables, and images: rights and permissions

All figures, tables, and images will be published under a Creative Commons CC-BY license, and permission must be obtained for use of copyrighted material from other sources (including re-published/adapted/modified/partial figures and images from the internet). It is the responsibility of the authors to acquire the licenses, follow any citation instructions requested by third-party rights holders, and cover any supplementary charges.

For additional information, please see the 'Image manipulation' section of our policies and publication ethics.

Figures and images: style guidelines

We require figures to be submitted individually, in the same order as they are referred to in the manuscript; the figures will then be automatically embedded at the end of the submitted manuscript. Ensure that each figure is mentioned in the text and in numerical order.

For figures with more than one panel, panels should be clearly indicated using labels (A), (B), (C), (D), etc. However, do not embed the part labels over any part of the image. These labels will be replaced during typesetting according to Frontiers' journal style. For graphs, there must be a self-explanatory label (including units) along each axis.

For LaTeX files, figures should be included in the provided PDF. In case of acceptance, our production office might require high-resolution files of the figures included in the manuscript in EPS, JPEG or TIF/TIFF format.

To upload more than one figure at a time, save the figures (labeled in order of appearance in the manuscript) in a zip file and upload them as 'Supplementary material presentation.'

Please note that figures not in accordance with the guidelines will cause substantial delay during the production process.

Captions

Captions should be preceded by the appropriate label, for example 'Figure 1.' Figure captions should be placed at the end of the manuscript. Figure panels are referred to by bold capital letters in brackets: (A), (B), (C), (D), etc.

Image size and resolution requirements

Figures should be prepared with the PDF layout in mind. Individual figures should not be longer than one page and with a width that corresponds to one column (85 mm) or two columns (180 mm).

All images must have a resolution of 300 dpi at final size. Check the resolution of your figure by enlarging it to 150%. If the image appears blurry, jagged, or has a stair-stepped effect, the resolution is too low.

The text should be legible and of high quality. The smallest visible text should be no less than eight points in height when viewed at actual size.

Solid lines should not be broken up. Any lines in the graphic should be no smaller than two points wide.

Please note that saving a figure directly as an image file (JPEG, TIF) can greatly affect the resolution of your image. To avoid this, one option is to export the file as PDF, then convert into TIFF or EPS using a graphics software.

Format and color image mode

The following formats are accepted: TIF/TIFF (.tif/.tiff), JPEG (.jpg), and EPS (.eps) (upon acceptance). Images must be submitted in the color mode RGB.

Images of chemical structures

Chemical structures should be prepared using ChemDraw or a similar program. If working with ChemDraw please use our ChemDraw template. If working with another program please follow the guidelines below.

Drawing settings: chain angle, 120° bond spacing, 18% width; fixed length, 14.4 pt; bold width, 2.0 pt; line width, 0.6 pt; margin width, 1.6 pt; hash spacing, 2.5 pt. Scale 100% Atom Label settings: font, Arial; size, 8 pt

Assign all chemical compounds a bold, Arabic numeral in the order in which the compounds are presented in the manuscript text.

Table requirements and style guidelines

Tables should be inserted at the end of the manuscript in an editable format. If you use a word processor, build your table in Word. If you use a LaTeX processor, build your table in LaTeX. An empty line should be left before and after the table.

Table captions must be placed immediately before the table. Captions should be preceded by the appropriate label, for example 'Table 1.' Please use only a single paragraph for the caption.

Ensure that each table is mentioned in the text and in numerical order.

Large tables covering several pages cannot be included in the final PDF for formatting reasons. These tables will be published as supplementary material.

Tables which are not according to the above guidelines will cause substantial delay during the production process.

During production, tables will be formatted according to Frontiers' house style. Here is an example of a formatted table.

This is a heading	This is a heading
This is a subheading	
This is an example text	This is an example text
This is an example text	This is an example text
This is an example text	This is an example text

Accessibility

We encourage authors to make the figures and visual elements of their articles accessible for the visually impaired. Effective use of color can help people with low visual acuity, or color blindness, understand all the content of an article.

These guidelines are easy to implement and are in accordance with the W3C Web Content Accessibility Guidelines (WCAG 2.1), the standard for web accessibility best practices.

Ensure sufficient contrast between text and its background
People who have low visual acuity or color blindness could find it difficult to read text with low contrast background color. Try using colors that provide maximum contrast.

WC3 recommends the following contrast ratio levels:

Level AA, contrast ratio of at least 4.5:1

Level AAA, contrast ratio of at least 7:1

You can verify the contrast ratio of your palette with these online ratio checkers:

WebAIM

Color Safe

Avoid using red or green indicators
More than 99% of color-blind people have a red-green color vision deficiency.

Avoid using only color to communicate information
Elements with complex information like charts and graphs can be hard to read when only color is used to distinguish the data. Try to use other visual aspects to communicate information, such as shape, labels, and size. Incorporating patterns into the shape fills also make differences clearer; for an example please see below:

Supplementary material

Selecting supplementary material

Data that are not of primary importance to the text, or which cannot be included in the article because they are too large or the current format does not permit it (such as videos, raw data traces, and PowerPoint presentations), can be uploaded as supplementary material during the submission procedure and will be displayed along with the published article. All supplementary files are deposited to figshare for permanent storage and receive a DOI.

Supplementary material is not typeset, so please ensure that all information is clearly presented without tracked changes/highlighted text/line numbers, and the appropriate caption is included in the file. To avoid discrepancies between the published article and the supplementary material, please do not add the title, author list, affiliations or correspondence in the supplementary files.

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presentation (PowerPoint, PDF or Zip files)

image (CDX, EPS, JPEG, PDF, PNG or TIF/TIFF),

table (Word, Excel, CSV or PDF)

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Technical requirements for supplementary images:

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RGB color mode.

For supplementary material templates (LaTeX and Word), see our supplementary material templates.

Submitting information

Choosing where to submit

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We require authors to select the appropriate article type for their manuscript and to comply with the article type descriptions defined in the journal's 'Article types' page, which can be found under the 'About journal' menu in 'For authors' on every Frontiers journal page. Please pay close attention to the word count limits.

Keywords

All article types require a minimum of five and a maximum of eight keywords.

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CrossMark is a multi-publisher initiative to provide a standard way for readers to locate the current version of a piece of content. By applying the CrossMark logo Frontiers is committed to maintaining the content it publishes and to alerting readers to changes if and when they occur.

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'Corrigendum: [Title of original article]'

General Commentaries:
'Commentary: [Title of original article]'
'Response: Commentary: [Title of original article]'

'Editorial: [Title of Research Topic]'

Authors and affiliations

All names are listed together and separated by commas. Provide exact and correct author names as these will be indexed in official archives. Affiliations should be keyed to the author's name with superscript numbers and be listed as follows:

Laboratory, Institute, Department, Organization, City, State abbreviation (only for United States, Canada, and Australia), and Country (without detailed address information such as city zip codes or street names).

Example: Max Maximus1
1 Department of Excellence, International University of Science, New York, NY, United States.

Correspondence

The corresponding author(s) should be marked with an asterisk in the author list. Provide the exact contact email address of the corresponding author(s) in a separate section.

Example: Max Maximus*
maximus@iuscience.edu

If any authors wish to include a change of address, list the present address(es) below the correspondence details using a unique superscript symbol keyed to the author(s) in the author list.

Equal contributions

The authors who have contributed equally should be marked with a symbol (†) in the author list of the doc/latex and pdf files of the manuscript uploaded at submission.

Please use the appropriate standard statement(s) to indicate equal contributions:

Equal contribution: These authors contributed equally to this work

First authorship: These authors share first authorship

Senior authorship: These authors share senior authorship

Last authorship: These authors share last authorship

Equal contribution and first authorship: These authors contributed equally to this work and share first authorship

Equal contribution and senior authorship: These authors contributed equally to this work and share senior authorship

Equal contribution and last authorship: These authors contributed equally to this work and share last authorship

Example: Max Maximus 1†, John Smith2† and Barbara Smith1

†These authors contributed equally to this work and share first authorship

Consortium/group and collaborative authors

Consortium/group authorship should be listed in the manuscript with the other author(s).

In cases where authorship is retained by the consortium/group, the consortium/group should be listed as an author separated by a comma or 'and'. The consortium/group name will appear in the author list, in the citation, and in the copyright. If provided, the consortium/group members will be listed in a separate section at the end of the article.

For the collaborators of the consortium/group to be indexed in PubMed, they do not have to be inserted in the Frontiers submission system individually. However, in the manuscript itself, provide a section with the name of the consortium/group as the heading followed by the list of collaborators, so they can be tagged accordingly and indexed properly.

Example: John Smith, Barbara Smith and The Collaborative Working Group.

In cases where work is presented by the author(s) on behalf of a consortium/group, it should be included in the author list separated with the wording 'for' or 'on behalf of.' The consortium/group will not retain authorship and will only appear in the author list.

Example: John Smith and Barbara Smith on behalf of The Collaborative Working Group.

These guidelines cover acceptable uses of generative AI technologies such as Large Language Models (ChatGPT, Jasper) and text-to-image generators (DALL-E 2, Midjourney, Stable Diffusion) in the writing or editing of manuscripts submitted to Frontiers.

Scope statement

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