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Is material human immunodeficiency virus positivity a risk factor for the development of necrotizing enterocolitis in premature infants

Ondongo-Ezhet, Claude Christelle Eleonore

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IS MATERNAL HUMAN IMMUNODEFICIENCY VIRUS POSITIVITY A RISK FACTOR FOR THE DEVELOPMENT OF NECROTIZING ENTEROCOLITIS IN PREMATURE INFANTS?

Claude Christelle Eleonore Ondongo-Ezhet

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine in the Department of Paediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand.

Johannesburg, 2015

DECLARATION

I, Claude Christelle Eleonore Ondongo-Ezhet declare that this research report is my own work. It is submitted for the degree of Master of Medicine in the branch of Paediatrics and Child Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

DATE

Dedicated to my parents

Arthur PENE and Leonie PENE

ABSTRACT

Introduction

Little is known about the association between maternal HIV status and the development of necrotizing enterocolitis (NEC) in premature infants. The few studies that have been published give no clear picture. In the light of the high maternal HIV infection in South Africa, it is important to explore the association between maternal HIV infection and all aspects of the paediatric population.

Objectives

The primary objective of this study was to determine if maternal HIV positive status was associated with the development of NEC in preterm infants who were born in one of our academic hospitals.
The second objective was to determine the severity, need for surgery and mortality of preterm infants with NEC according to maternal HIV status.
Finally, the third objective was to determine risk factors associated with NEC.

Method

The study population included preterm newborns less than 1500 grams born at the CMJAH and admitted to the neonatal unit within 24 hours of birth. Data on maternal and infant characteristics were collected from the computerized neonatal database from January 2006 to December 2013.

Results

A total of 2355 infants <1500g constituted the study population. Of these 126 met the inclusion criteria for NEC and 2229 did not. Therefore, large proportions were not entered for a multivariate analysis.

Univariate analysis did not demonstrate an association between maternal HIV positive status and NEC (OR: 1.3, 95% CI: 0.8-1.9, p= 0.2). Therefore it was not entered into the multivariate analysis.

On multivariate analysis antenatal corticosteroids showed a protective association with NEC (OR: 0.2, 95% CI: 0.1-0.4, p< 0.05). Multiple pregnancy and the need for resuscitation at birth was associated with NEC (OR: 1.6, 95% CI: 1-2.5, p= 0.03), (OR: 8, 95% CI: 4.5-15, p< 0.05), respectively.

The analysis also found that severity of NEC, the need for surgery and mortality among infants with NEC did not differ according to maternal HIV status (p= 0.9, p=0.7 and p= 0.4), respectively.

Conclusions

The analysis was not able to demonstrate an association between maternal HIV positivity and the risk of NEC. Risk factors for NEC that were identified were multiple pregnancies and the need for resuscitation at birth. Antenatal corticosteroids were found to have a protective association with NEC.
Finally, severity, need for surgery and mortality did not also differ according to maternal HIV status among the NEC group.

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LIST OF ABBREVIATIONS

- CPAP: Continuous Positive Airway Pressure
- CEO: Chief Executive Officer
- CHBAH: Chris Hani Baragwanath Academic Hospital
- CI: Confidence Interval
- CMJAH: Charlotte Maxeke Johannesburg Academic Hospital
- CMV: Conventional Mechanical Ventilation
- ELBW: Extremely Low Birth Weight
- ET-1: Endothelin-1
- EGF: Epidermal Growth Factor
- HIV: Human Immunodeficiency Virus
- HFOV: High Frequency Oscillation Ventilation
- GM-CSF: Granulocytes-Macrophage Colony Stimulating Factor
- IPPV: Intermittent Positive Pressure Ventilation
- IL: Interleukins
- IVH: Intraventricular Hemorrhage
- NVP: Nevirapine
- NEC: Necrotizing Enterocolitis
- NICU: Neonatal Intensive Care Unit
- NO: Nitric Oxide
- NOS: Nitric Oxide Synthase
- NSW ACT NICU: New South Wales and Australian Capital Territory Neonatal Intensive Care Unit Study group
- OR: Odds Ratio
- PMTCT: Prevention of Mother to Child Transmission Program
- PAF: Platelet Activating Factor
- RDS: Respiratory Distress Syndrome
- RR: Relative Risk
- USA: United States of America
- TNF: Tumor Necrosis Factor
- SD: Standard deviation
- VLBW: Very Low Birth Weight

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1. Introduction

Necrotizing enterocolitis (NEC) is a condition primarily seen in premature infants, where portions of the bowel undergo necrosis as a result of severe inflammation. Any part of the bowel may be involved, but typically the ileum and the proximal colon are affected.[1, 2]

From a neonatal and surgical perspective, it is reported as the most common acquired gastrointestinal disease and contributes to a significant extent to morbidity and mortality in very low birth weight infants (birth weight less than 1500 grams, VLBW infants).

The historical background of the disease goes back to its initial description by Genersich in 1891.[3] The disease occurred in a 45-hour-old premature infant who developed vomiting, cyanosis and abdominal distension. Death occurred rapidly, within 24 hours of being symptomatic. The post mortem examination revealed areas of inflammation and perforation of the ileum. Thereafter, several reports of unexplained perforation of the intestinal tract in the newborn emerged.

Rossier, Sarrut and Delplanque, in 1959, reviewed 15 infants with "malignant enteritis", as termed in 1944 by Willi. The review enabled them to give a thorough clinical and pathological description of the condition. Hence, they described it as ulcerative necrotic enterocolitis of the premature infant.[3] In 1975 Santulli and colleagues described the condition in 64 premature infants. Three factors were reported to be important in the development of the disease: injury to the intestinal mucosa, feeding and bacteria.[4] It was also noted that the disease carried a high morbidity and mortality.

However, improved outcome occurred with the institution of early and aggressive treatment. Since then, numerous descriptions of NEC have provided a better understanding of the disease in terms of its risk factors, pathogenesis, manifestations and mortality. Studies have shown that the incidence of NEC shows a clear and inverse relationship with the birth weight and the gestation age.[1, 5] The incidence and mortality of NEC varies from country to country and from one neonatal unit to the other.

South Africa has experienced a major epidemic with regards to Human Immunodeficiency Virus (HIV) infection since the 1990s. The national department of health in 2011 reported the antenatal HIV prevalence at 29.5% (95% CI: 28.7-30.2).[6]

Recent data from UNICEF in 2014 reported that the estimated number of pregnant women living with HIV delivering in South Africa was 260 000.[7] Little is also known about the association between maternal exposure to HIV and NEC and there are only a few published studies on this in the literature, with conflicting results.

The aim of this study was to test the hypothesis that premature infants born to HIV positive mothers are at greater risk for developing NEC than those unexposed.

2.0 LITERATURE REVIEW

In high income countries such as the United States of America (USA) and Canada the incidence and mortality from NEC is either stable or decreasing as a result of early detection, sepsis management and availability of intensive care facilities.[8] In Canada, population based or multi center epidemiological studies reported that NEC rates stabilized over recent decades for VLBW infants, ranging between 6% and 7%.[9,10,11] In contrast to these findings, a study performed by the New South Wales and Australian Capital Territory Neonatal Intensive Care Unit Study group (NSW ACT NICU), reported a reduction in the incidence of NEC from 12 % in 1986-1987 and 1992-1993 to 6% in 1998-1999 for all infants born at 24 to 28 weeks' gestation.[9] In other developed countries, NEC has a similar incidence and the mortality rate ranges from 12 to 30%.[9,12] The mortality rates are reported to be higher in infants requiring surgery than in those medically treated for NEC with case fatality rates for those requiring surgery as high as 50%.[9]

In South Africa, a recent study conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) neonatal unit reported an incidence of NEC of 5.6% for all VLBW infants admitted in 2006/2007 and 7.3% in 2013.[8] The overall mortality of cases of NEC at CMJAH is high at 43.9% as reported in the same study.[8] Thus the incidence of NEC may be showing an increasing trend. However, it remains similar to the incidence in developed countries such as USA (6 to 7.1%) and Canada (6.6%). [9,10, 11].

As stated previously there are few studies looking at the association of neonates born to HIV positive mothers and NEC.

Desfrere et al, in a retrospective case control study questioned if being born to an HIV-positive mother increased the risk of NEC in premature infants.[13] The study comprised 79 cases of NEC, which were compared with 158 controls. The results showed an increased frequency of HIV positive mothers in infants who subsequently developed NEC (OR, 6.63; 95% CI, 1.26-34.8; p=0.025) suggesting that there is an association between HIV positive mothers and NEC developing in their infants if born prematurely.[13] However, Angura and Velaphi, in a retrospective case control study at the Chris Hani Baragwanath Academic Hospital analyzed risk factors for necrotizing enterocolitis in an HIV- endemic region and found that maternal HIV status was not associated with the development of NEC (OR: 1.14, 95% CI: 0.71-1.82, p=0.58).[14]

Marion et al in a retrospective study found that HIV-exposed infants did not have more severe disease and adverse outcome (stage 3b) than unexposed infants. [25]

Karpelowsky et al assessed the impact of HIV exposure on survival and extent of disease in NEC. They found that neonates with NEC born to HIV positive mothers had a higher mortality compared to those born to HIV negative mothers. However, the study also found that there was no difference in the extent of the disease between the two groups.[15]

2.1 Pathology and Pathophysiology

NEC initially involves the gastrointestinal system, and may then be followed by profound systemic effects. Pathological findings vary and depend on the progression of the disease as well as the presence of underlying factors[16]. Gross examination in most cases reveals distended, hemorrhagic loops of bowel, sub-serosal collections of gas, intra-abdominal fluid collection and intestinal perforation in severe cases. With healing, thickening of the bowel wall, fibrous adhesions and areas of stenosis may develop[16]. Major histological findings in NEC include mucosal edema, hemorrhage, transmural necrosis and acute inflammation secondary to bacterial infiltration[16].

Its pathophysiology is poorly understood. However, immaturity of key functions due to prematurity such as loss of gastrointestinal motility, digestive ability, circulatory regulation, intestinal barrier function and immune defenses are all thought to play a role in its development[17].
Other contributing factors include feeding with formula and colonization by pathological bacteria[17].

2. 2 Prematurity

Prematurity remains the most important and consistent risk factor for NEC. About 90% percent of cases occur in premature infants who have been fed enterally[16]. The majority of patients affected by NEC are VLBW (birth weight less than 1500 grams) infants. The increased susceptibility to the disease is attributed to an immature mucosal barrier response, bowel motility and function, changes in intestinal micro flora, host defense mechanisms and increasing enteral volume.

Full term infants who develop NEC typically have specific risk factors such as congenital heart diseases, sepsis, perinatal asphyxia, polycythemia, and respiratory disease[16]. In fact, any condition that causes reduction in the mesenteric perfusion may allow NEC to develop.

2**. 3 Immature intestinal motility, digestion and function**

Intestinal motility is an important factor in removing antigens presented to the intestinal mucosal barrier from the gut lumen[5]. Fetal studies in both humans and animals proposed that development of gastrointestinal motility begins in the second trimester, but this matures in the third trimester.[14, 15] Study of intestinal motility shows that premature infants have immature motility patterns when compared to full -term infants[5].

Maternal hypertension and fetal disease such as intrauterine growth restriction might induce fetal hypoxia. These can reduce postnatal intestinal motility because of an alteration in the peristaltic mechanism and a poor digestive and absorptive ability.[12] The intrinsic enteric nervous system is affected and predisposes the infants to poor clearance of bacteria, entry of pathogens from the environment, exponential growth of anaerobic bacteria in the small intestine with malabsorption of nutrients.[9] In addition, premature infants do not have the complete ability to fully digest nutrients.[12] Therefore, incompletely digested molecules contribute to intestinal injury and development of NEC.

2.4 Immature intestinal immunity

It is well known that the fetus lacks a functional immune system and has a sterile gastrointestinal tract initially. However, exposure of the newborn infant to bacteria colonized in the mother vaginal canal and the skin as well

as immunological factors in the breast milk result in the gut becoming colonized with bacteria[2].

Immunity of the newborn gut is provided through several mechanisms: enhancement of intestinal salt secretion, water absorption, secretion of IgA, expression of antimicrobial proteins and production of intestinal mucus. However, in the preterm infant, immaturity of those mechanisms results in selective movement of small ions across the intestinal monolayer, inability of secretory IgA to bind to bacteria and to remove harmful pathogens or toxins.[9]

2.5 Abnormal bacterial colonization

Bacteria and viruses have been linked to outbreaks of NEC. Examples of bacteria include: *Clostridium species*, *Klebsiella species* and *Staphylococcus epidermis*.[2] Unfortunately, no single pathogen has been identified as a causative agent. It is important to highlight the ability of the microflora to colonize the epithelium and to ferment unabsorbed nutrients.[2]

Colonization of the gastrointestinal tract in the premature infant is different from the healthy term infant.[11] Premature infants have less species diversity and fewer anaerobic species of *Lactobacillus* and *Bifidobacterium* than term infants.[11] They are also more likely to be populated by pathogenic bacteria, such as *Klebsiella*, *Enterobacter* and *Clostridium* organisms. This imbalance represents a fertile environment for pathological overgrowth of bacteria, which in turn is capable of triggering an inflammatory response.[11]

A case-control study by De la Cochetiere and colleagues analyzing the relationship between the gut colonization and NEC showed evidence of a temporal relationship between abnormal bacterial colonization and the later development of NEC.[18]

2.6 Genetic susceptibility

Although current technology allows for the detection and evaluation of genetic polymorphisms and their influence on disease development, no studies have shown a genetic or familial link with the development of NEC[17].

With regard to the hypothesis of this study, it would be expected that both intestinal immunity and abnormal bacterial colonization in HIV exposed infants may be affected and predispose these infants to NE

2.7 Clinical risk factors

A review article by Bhoomika and Shah summarized the clinical risk factors for NEC.[17]

These included:

- Prematurity (< 28 weeks)
- Enteral feeding (90 % were fed by enteral route)
- Presence of certain bacteria within the gut lumen
- Hypoxic insult
- Intrauterine growth restriction
- Pregnancy induced hypertension
- Placental abruption
- Absent or reversed end diastolic flow velocity
- Umbilical catheterization
- Low Apgar scores
- Blood transfusions

2.8 Protective factors

Several factors have been shown to be associated with a decreased risk of NEC through various mechanisms.

- Human breast milk protects against NEC by inhibiting gut colonization by pathogenic flora, enhancing maturation of the intestinal barrier and controlling the pro-inflammatory response.[19] A study by the National Institute of Child Health and Human Development Neonatal Network has showed that breast milk feeding is associated with a reduced risk of NEC or death after the first two weeks of life among extremely low birth weight infants.[20]

- Probiotics: these are live microorganisms that colonize the gastrointestinal tract. They provide health benefit on the host when administered in adequate amount. The most frequently used probiotics are *Lactobacillus and Bifidobacterium*. They provide protection against the development of NEC by preventing bacterial migration across the intestinal mucosa.[19] In a 2014 Cochrane review twenty four randomized clinical trials and more than 5000 preterm infants were included. The evidence showed that probiotics reduces the risk of severe NEC and all cause mortality in preterm infants.[21]

- Prebiotics are indigestible substances that selectively promote growth and colonization of the probiotic lactobacilli or Bifidobacterium. They have also shown to decrease the incidence of NEC.[17, 19]

Additional protective factors include provision of antenatal corticosteroids, fluid restriction, immunonutrition (*Glutamine and Arginine*) and polyunsaturated fatty acid supplements (Phospholipids), enteral antibiotics, feeding strategies (Trophic feeding and Standardized Feeding Regimens), Synbiotics, *Lactoferrin* supplement, acidification of Gastric contents and Epidermal growth factor (EGF). [17]

2.9 Clinical presentation

The onset of the disease can occur suddenly within a few hours or may be preceded by days of feeding intolerance. Initial symptoms may be subtle and include feeding intolerance, abdominal distension, temperature instability, lethargy, decreased peripheral perfusion, apnea and bloody stools.

Kiegman et al modified the Bell staging criteria for neonatal NEC.[22] It provided a classification according to signs, symptoms, degree of systemic involvement and radiological signs (appendix A).

3.0 METHODOLOGY

3.1 Research question

Does maternal HIV positivity increase the risk of NEC in premature infants?

3.2 Objectives

3.2.1 Primary objective

To determine if maternal HIV positive status is associated with the development of NEC in preterm infants who were born at CMJAH or admitted within 24 hours of birth.

3.2.2 Secondary objective

- To compare the severity, need for surgery and mortality of preterm infants with NEC according to maternal HIV status.

- To determine risk factors for the development of NEC according to maternal and infant characteristics.

3.3 Study design

This was a retrospective descriptive and comparative study

3.4 Source of Data

Data was obtained from the computerized neonatal database between March 2006 and December 2013.

Infants weighing less than 1500g at birth were selected on the basis that most infants with NEC would be found in this group.

3.5 Study Population

A total of 10802 infants were admitted over this period.

8447 patients were excluded for the following reasons:

- Birth weight above 1500 grams
- Infants referred after 24 hours of life to CMJAH.
- Major congenital abnormalities such as gastroschisis, duodenal atresia and others.

Cases were defined as VLBW infants who were diagnosed with NEC stage 2 or 3 based on the Modified Bell's classification (appendix A). Infants with stage 1 NEC were not included as these are regarded only as suspected NEC according to this classification.

Of the 2355 infants who met the inclusion criteria, 126 infants met the inclusion criteria for cases (stage 2 or 3 NEC). The remaining infants 2229 were either not affected by NEC or were suspected cases of NEC, (stage 1) as defined by the Modified Bell's classification and formed the comparison group.

3.6 Sample size

The number of NEC cases meeting the criteria for enrollment predetermined the sample size.

3.7 Characteristics to be analyzed as risk factors

3.7.1 Maternal characteristics

a) Maternal HIV status

b) Multiple pregnancies

c) Maternal steroids

3.7.2 Infant Characteristics

a) Gestational age

b) Birth weight

c) Gender

d) Need for resuscitation

The need for ventilation and the type of feeding were planned to be analyzed as risk factors. However, the database did not differentiate between those ventilated in the early neonatal period and those ventilated as a result of NEC so this could not be analyzed as a risk factor. The data on feeding in the database only specified the type of feeding on discharge and not in the period prior to the development of NEC and this variable also could thus not be analyzed as a risk factor.

3.7.3 Outcome

a) Stage of NEC (severity)
b) Need for surgery
c) Mortality

3. 8 Data processing and analysis

The neonatal data was provided in a Microsoft spreadsheet Excel program.

After identification of cases and non-cases of NEC, the selected data were entered in STATA 12.0 statistical program for analysis.

Summary statistics was used to describe maternal and infant characteristics.

Two sample t-tests with equal variance were used to determine the mean for birth weight and gestational age between the two groups (NEC and non-NEC cases).

Univariate and Multivariate logistic regression analysis were used to determine maternal and infant risk factors associated with NEC.

Odds ratios were reported using a 95% confidence interval and a significant association was reported using a *p*-value of less than 0.05 for both univariate and multivariate analysis.

Finally, the chi square test was used to compare severity, mortality and the need for surgery in the NEC group as well as determine a probable association among variables according to maternal HIV status.

3. 9 Ethics and Authorization

The Protocol Review Committee and the Human Research Ethics Committee of the University of the Witwatersrand approved the study. (Refer to appendix B).

Permission to use the neonatal database was obtained from the Chef Executive Officer of CMJAH (Refer to appendix C).

4.0 RESULTS

4.1 Statistical description of data

Maternal characteristics regarding HIV status, administration of antenatal steroids and multiple pregnancies for infants with and without NEC can be seen in Table 1. The total number of mothers with a positive HIV status in both groups (NEC and non NEC) was 569 (25.5%) and 51 (40.5%), respectively as seen in Table 1. The statistical description also showed that many of the mothers had unknown HIV status and the recording of antenatal steroids in the database was also poor.

Table 1: Statistical description of maternal characteristics

Variables	**NEC (n=126) (%)**	**No NEC (n=2229) (%)**
Maternal HIV status		
Negative	57 (45.2)	840 (37.7)
Positive	51(40.5)	569 (25.5)
Unknown	18 (14.3)	820 (36.8)
Maternal steroids		
Yes	38 (30.4)	767 (34.4)
No	40 (32.0)	275 (12.3)
Unknown	48 (37.6)	1187 (53.3)
Multiple pregnancy		
Yes	28 (22.2)	373 (16.7)
No	92 (73.0)	1737 (78.0)
Unknown	6 (4.8)	119 (5.3)

Neonate's characteristics are seen in Table 2.
The mean birth weight and gestational age for both groups were similar. Others variables such as gender and the need for resuscitation are also seen in Table 2.

Table 2: Statistical description of neonates with NEC as compared to those without NEC

Variables	**NEC (n=126) (±SD) or (%)**	**No NEC (n=2229) (±SD) or (%)**	***p*- value**
Mean birth weight (grams)	1138 (±185)*	1150 (±236)*	0.5
Mean gestational age (weeks)	29 (±2.5)*	28 (±6.3)*	0.1
Gender			
Female	59 (46.8)	1182 (53.0)	0.2
Male	67 (53.2)	1034 (46.4)	
Unknown	0	13(0.6)	
Need for resuscitation			
Yes	92 (73.0)	688 (30.9)	<0.05
NO	20 (15.9)	1541 (69.1)	
Unknown	14 (11.1)	0	

* Standard deviation (SD)

Statistical description of outcomes can be seen in Table 3.
The majority of babies with NEC had stage 3 (60%) and 28.9% required surgery. Overall survival of those with NEC was significantly lower than the group without NEC.

Table 3: Outcomes of neonates with NEC as compared to those without NEC

Outcomes	**NEC n=126 (%)**	**No NEC n=2229 (%)**	***p*- value**
Stage of NEC Stage 2 Stage 3 Unknown	 20 (15.9) 76 (60.3) 30 (23.8)	 N/A	 -
Surgery Yes No Unknown	 36 (28.6) 49(38.9) 41(32.5)	 N/A	 -
Mortality **Yes** **No** **Unknown**	 61(48.4) 65 (51.6) 0	 479 (21.7) 1731(78.3) 19 (0.9)	 <0.05

4.2 Risk factors associated with NEC

It is important to highlight that all variables had missing data. In tables 1, 2 and 3, the missing data in all of the variables used can be seen. However, in order to obtain accurate statistical results unknown data were excluded.
In instances where a high number of missing data was noted, the variable had to be excluded from the statistical analysis.

Statistical description of maternal characteristics, neonates with NEC and those without NEC as well as their outcome, provided an overview of all data available before the analysis.

Table 4 shows the univariate and multivariate analysis on risk factors associated with NEC.

The univariate analysis showed that there was no significant association between maternal HIV and the development of NEC (OR: 1.3, 95% CI: 0.8-1.9 and $p = 0.2$) and it was therefore not entered into the multivariate analysis.

Provision of maternal steroids showed a protective association with NEC on both the univariate and multivariate analysis. Multiple pregnancy and the need for resuscitation at birth also showed an association with NEC as noted in Table 4.

Other variables such as mean birth weight, mean gestational age and gender did not show any association with NEC. Multivariate analysis was not done for those variables.

Table 4: Risk factors associated with NEC on univariate and multivariate analysis.

Characteristics	**Univariate analysis**			**Multivariate analysis**		
	Odds ratio	**95% Confidence interval**	***p*-value**	**Odds ratio**	**95% Confidence interval**	***p*-value**
Maternal HIV status	1.3	0.8-1.9	0.2	-	-	-
Maternal antenatal steroids	0.2	0.1-0.4	**<0.05**	0.2	0.1-0.4	**<0.05**
Multiple pregnancy	1.6	1-2.5	**0.03**	1.8	1-3.3	**0.04**
Mean birth weight	0.9	0.9-1	0.4	-	-	-
Mean gestational age	1	0.8-1	0.5	-	-	-
Gender	0.8	0.5-1.3	0.5	-	-	-
Need for Resuscitation at birth	8	4.5-15	**<0.05**	9	5-16	**<0.05**

4. 3 Severity and Outcome of infant with NEC according to maternal HIV status

The analysis did not demonstrate any difference in severity, need for surgery or mortality among infants with NEC according to maternal HIV status, as can be seen in Tables 5, 6 and 7.

Table 5: Severity of NEC according to maternal HIV status

	Maternal HIV status		
Stage of NEC (severity)	**Positive**	**Negative**	**Total**
Stage 2	20	22	42
Stage 3	31	35	66
Total	51	57	108

Pearson chi2 (1): 0.004, *p*-value: 0.9

Table 6: Need for surgery for NEC according to maternal HIV status

	Maternal HIV Status		
Surgery	**Positive**	**Negative**	**Total**
No	20	24	44
Yes	11	16	27
Total	31	40	71

Pearson chi 2 (1): 0.1, *p* value: 0.7

Table 7: Mortality of NEC infants according to maternal HIV status

	Maternal HIV status		
Mortality	**Positive**	**Negative**	**Total**
Death	27	26	53
Survival	24	31	55
Total	51	57	108

Pearson chi2 (1):0, 57 *p* value: 0.4

5.0 DISCUSSION

As previously stated, data published from this database showed an NEC incidence of 5.6% for VLBW infants at CMJAH in 2006 and an incidence of 7.3% per VLBW in 2013.[8] In the current study, 126 NEC cases meeting the inclusion criteria were found, providing an incidence of 5.6%. Thus, it would seem that most cases of NEC were captured in the routine collection of data for this database when compared with the more detailed data collection that was required for the above studies.

There was a trend towards more mothers in the NEC group testing positive for HIV (40.5%) compared with the control group (25.5%). However, the study was not able to show a significant association between maternal HIV positivity and NEC since this did not reach statistical significance. This finding is consistent with a retrospective case control study by Angura and Velaphi at Chris Hani Baragwanath Academic Hospital.[14]

Administration of antenatal steroids has been shown to improve digestive functions. They also provide an anti-inflammatory effect on the gut. A study by Crowley on the effects of antenatal corticosteroids showed that this drug was associated with a reduction in the incidence of RDS, IVH and NEC.[4] In this analysis, both univariate and multivariate logistic regression showed that the provision of antenatal corticosteroids was a protective factor for NEC. For many infants in the database there was no indication as to whether the mother had received antenatal steroids, but it seemed that a large number of VLBW infants did not have the benefit of this intervention mainly due to late maternal presentation and limited opportunities for the obstetrician to provide antenatal steroids.

Most newborns in both groups (NEC and non NEC) were from singleton pregnancies: 73% and 78%, respectively. Zampieri et al reported that there was an increased incidence of NEC in multiple pregnancies only if the gestational age was less than 28 weeks.[23] Our population was confined to VLBW with a mean gestation age of 29 weeks. Despite a high number of singleton pregnancies compare to multiple pregnancies, the results from the univariate and multivariate analysis in this study revealed that multiple pregnancy was associated with NEC. Therefore, these findings are almost in keeping with the Zampieri study.

Although, most studies have demonstrated that the incidence of NEC shows a clear and inverse relationship with the birth weight and gestational age[1, 5] univariate analysis in this study did not show such an association.

Therefore these were not entered into the multivariate analysis. However, survival of infants with birth weight <1000 grams was low and most died in the early neonatal period. [8] A recent study done by Ballot et al showed a marked improvement in the neonatal survival of neonates weighing between 750 and 900 grams at birth due to provision of surfactant and nCPAP.[8] As the survival of ELBW infants improves future studies in this unit may show the inverse relationship between NEC and birth weight that has been seen in other studies.

Neonatal risk factors for NEC in our population were the need for resuscitation at birth and multiple pregnancy. This was consistent with a Cochrane review by Singh et al on the neonatal outcome of extensive cardiopulmonary resuscitation in the delivery room for infants born at less than 33 weeks gestational age.[24] Since many VLBW infants in our institution have inadequate antenatal and intrapartum care due to poor attendance at antenatal clinic and arrival in the hospital in advanced labour, improvement in these aspects of care may lead to a reduction in the incidence of NEC.

Descriptive analysis of outcomes related to NEC showed that most infants had a severe form of NEC, stage 3 in 60% of participants. However, the degree of severity of the disease among those infants did not differ according to maternal HIV status. These results were consistent with Marion et al who found that HIV exposed newborns with NEC did not have a more severe disease than unexposed newborns.[25]

Mortality among NEC infants with a positive maternal HIV status was not significantly higher compared to HIV negative mothers as seen in table 7. However, Karpelowsky et al found that infants with NEC born to HIV infected mothers had a higher mortality compared to those born to HIV negative mothers possibly explained by confounders such as, sepsis and co morbidity.[15] However, our results did not show a significant increased risk to NEC mortality with regards to maternal HIV status. This was probably because of paucity of data. Therefore, we could not further analyze mortality among stages of NEC. Similarly, a study by Marion et al found that HIV does not worsen outcome in stage 3b NEC and that mortality from NEC did not differ between HIV exposed and non- HIV exposed neonates.[25] Further study is needed to resolve this issue.

The high mortality of NEC cases in this study is similar to that of previous studies in this unit.[8] This may be due to the high incidence of stage 3 NEC.

This in turn may indicate that the early signs of NEC are being missed by the attending staff and should be an area to focus on in the future.

5. 1 Limitations of the study

The retrospective design of the study resulted in many missing data. This study showed no difference in the incidence of NEC on the basis of maternal HIV status. Most of the missing data for this variable occurred in the group who did not develop NEC. There is no reason to believe that, had these data been more complete, the results would have changed, but there is no way to be sure of this. Important variables such as the type of milk feeds provided and ventilation in the early neonatal period could not be analyzed due to inadequate data. However, improvements in data collection in this unit will improve the quality of data for future studies.

6. CONCLUSION

This study did not show an association between maternal HIV positivity and the development of NEC at the CMJAH. Severity, need for surgery and mortality due to NEC also did not differ according to maternal HIV status. Multiple pregnancy and the need for resuscitation at birth were identified as risk factors for NEC whereas antenatal steroids were associated with a reduction in NEC. Improvements in the uptake of antenatal steroids and a reduction in the need for resuscitation at birth may reduce the incidence of NEC at CMJAH, while earlier diagnosis of NEC may improve the survival of those that do develop NEC.

7. References

1. Kathleen M, Dominguez KM, Moss RL. Necrotizing enterocolitis. Clin Perinatol 2012 April; 39 (2): 387-401

2. Kareena L S, van Aerde JE, Thomson BR, et al. Necrotizing enterocolitis: A multifactorial disease with no cure. World J Gastroenterol 2008 April; 14(14): 2142-2161

3. Mizrahi A, Barlow O, Berdon W, et al. Necrotizing enterocolitis in premature infants. J Pediatr 1965; 66(4): 697-706

4. Santulli T.V, Schullinger J.N, Heird W.C, et al. Acute necrotizing enterocolitis in infancy: a review of 64 cases. Pediatrics 1975; 55 (3): 376-387

5. Lianos AR, Moss ME, Pinzon MC, et al. Epidemiology of neonatal necrotizing enterocolitis: a population–based study. Pediatr Perinat Epidemiol 2002; 16 (4): 342-349.

6. Department of Health, 2011. National Antenatal Sentinel HIV and syphilis Prevalence Survey in South Africa.www.health.gov.za

7. UNICEF Statistics HIV/AIDS 2014. www.unicef.org

8. Ballot DE, TE. Chirwa, T.Ramdin et al: Comparison of morbidity and mortality of very low birth weight infants in a central hospital in Johannesburg between 2006/2007 and 2013. BioMedCentral 2015; 13:15-20; doi: 10.1186/s12887-015-0337-4

9. Srinivasan PS, Brandler MD, D'Souza A. Necrotizing Enterocolitis. Clin Perinatol 2008; 35 (1): 251-272.

10. Guthrie SO, Gordon PV, Thomas V, et al: Necrotizing enterocolitis among neonates in the United States. J Perinatology 2003; 23(4): 278-285.

11. Sankaran K, Puckett B, Lee DS, et al: Variation in incidence of necrotizing enterocolitis in Canadian neonatal intensive care units. J Pediatr Gastroenterol Nutr 2004; 39 (4): 366-372.

12. Lin PW, Stoll BJ. Necrotizing enterocolitis. Lancet 2006; 368 (9543): 1271-1283.

13. Desfrere L, de Oliveira l, Goffinet F, el Ayoubi M, Firtion G et al. Increased incidence of necrotizing enterocolitis in premature infants born to HIV-positive mothers. AIDS 2005; 19 (14): 1487-1493

14. Angura P. Necrotizing enterocolitis in premature infants at the Chris Hani Baragwanath Academic Hospital (dissertation). Johannesburg. University of Witwatersrand, 2012.

15. Karpelowsky JS, Van Mil S, Numanoglu A et al. Effect of maternal human immunodeficiency virus status on the outcome of neonates with necrotizing enterocolitis. J. Pediatr. Surg 2010; 45 (2): 315-318

16. Schanler RJ. Pathology and pathogenesis of necrotizing enterocolitis in newborns. www.uptodate.com

17. Bhoomika KP and Shah JS. Necrotizing Enterocolitis in very low birth weight infants: A Systemic Review. Gastroenterology 2012; 2012:562594.

18. De la Cochetiere MF, Piloquet H, des Robert C et al. Early intestinal bacterial colonization and necrotizing enterocolitis in premature infants: Pediatrics 2004; 56 (3): 366-370.

19. Jang Hoon Lee, MD. An update on necrotizing enterocolitis: pathogenesis and preventive strategies. Korean J Pediatr 2011; 54(9): 368-372.

20. Meinzen-Derr J, Poindexter B, Wrage L et al: Role of human milk in extremely low birth weight infant's risk of necrotizing enterocolitis or death. J Perinatology 2009; 29 (1): 57-62

21. Aceti A, Gori D, Barone G et al: Probiotics for the prevention of necrotizing enterocolitis in preterm infants. J Pediatr 2015; 41:89

22. Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: Pathogenesis, classification and spectrum of illness. Curr Probl Pediatr 1987; 17:213-88

23. Zampieri N, Zamboni C, Comoglio FS. Necrotizing Enterocolitis in multiple gestations: comparison with singletons. Minerva pediatr 2012; 64(1): 1-4.
24. Singh A S, Lodha A K, Singhal N et al: Neonatal outcomes following extensive cardiopulmonary resuscitation in the delivery room for infants born at less than 33 weeks gestational age. Resus 2014 Feb; 85(2): 238-243.
25. Marion A, Moore SW: HIV does not worsen outcome in stage 3B necrotizing enterocolitis with current protocols treatment. J Paed surg 2012 Apr; 47(4): 665-672.

APPENDIX A: Modified Bell classification

Table 1. Modified Bell Staging Criteria for NEC

Stage	Classification	Systemic sign	Intestinal sign	Radiological sign
IA	**Suspected NEC**	Temperature instability, apnea, bradicardia, lethargy	Increased pregavage residuals, mild abdominal distension, emesis, guaiac-positive stool	Normal or intestinal dilatation, mild ileus
IB	**Suspected NEC**	Same as above	Bright red blood from rectun	Same as above
IIA	**Proven NEC-mildly ill**	Same as above	Same as above, plus absent bowel sounds, with or without abdominal tenderness	Intestinal dilatation, ileus, pneumatosis intestinalis
IIB	**Proven NEC-moderately ill**	Same as above, pus mild metabolic acidosis, mild thrombocytopenia	Same as above, plus absent bowel sounds, definite abdominal tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same than IIA, plus portal venous gas, with or without ascites
IIIA	**Advance NEC severely ill, bowel intact**	Same as IIB, plus hypotension, bradycardia, severe apnea, combined respiratory and metabolic acidosis, disseminated intravascular coagulation and neutropena	Same as above, plus sign of generalozed peritonitis,marked tenderness and distension of abdomen	Same as IIB, plus definite ascites
IIIB	**Advance NEC severely ill, bowel perforated**	Same as IIIA	Same as IIIA	Same as IIB, plus pneumoperitoneum

Adapted from Lee & Polin, 2003. Treatment and prevention of necrotizing enterocolitis. Semin Neonatol. 2003;8:449–459.

APPENDIX B: Ethic clearance certificate

R14/49 Dr Ondongo-Ezhet

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130742

NAME: (Principal Investigator)	Dr Ondongo-Ezhet
DEPARTMENT:	Paediatrics University of Witwatersrand Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Is Maternal HIV Positivity a Risk Factor for the Development of Necrotising Enterocolitis in Premature Infants in the Neonatal Unit at the Charlotte Maxeke Johannesburg Academic Hospital?
DATE CONSIDERED:	26/07/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Peter Cooper
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/08/2013

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

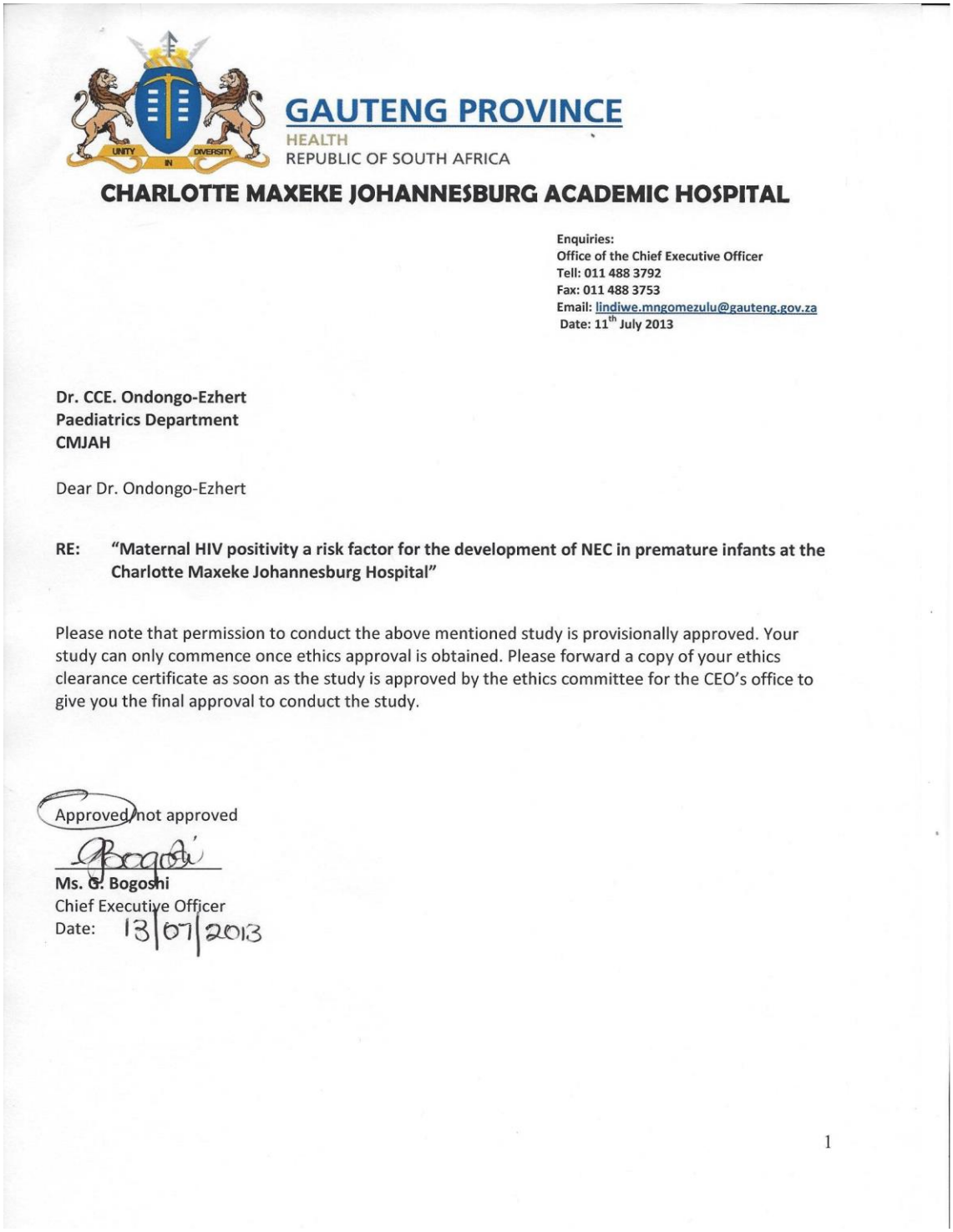
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
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**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX C: Letter of authorization from the hospital CEO to use data for analysis



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries:
Office of the Chief Executive Officer
Tell: 011 488 3792
Fax: 011 488 3753
Email: lindiwe.mngomezulu@gauteng.gov.za
Date: 11th July 2013

Dr. CCE. Ondongo-Ezhert
Paediatrics Department
CMJAH

Dear Dr. Ondongo-Ezhert

RE: "Maternal HIV positivity a risk factor for the development of NEC in premature infants at the Charlotte Maxeke Johannesburg Hospital"

Please note that permission to conduct the above mentioned study is provisionally approved. Your study can only commence once ethics approval is obtained. Please forward a copy of your ethics clearance certificate as soon as the study is approved by the ethics committee for the CEO's office to give you the final approval to conduct the study.

Approved/not approved

Ms. G. Bogoshi
Chief Executive Officer
Date: 13/07/2013

1

APPENDIX D: Data collection sheet for NEC study

Code No (NEC)	
Maternal HIV status	Positive/negative/unknown
Multiple pregnancy	Yes/No/unknown
Maternal steroids	Yes/No/unknown
Gestational age	
Birth weight	
Gender	
Need for resuscitation at birth	Yes/No/unknown
Need for ventilation	Yes/No/unknown
Stage of NEC (Severity)	
Feeding	Breast/Formula/Mixed/unknown
Surgery	Yes/No/unknown
Mortality	Yes/No/unknown