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Maternal, Neonatal and Feeding Type Factors Associated with
Severity of Necrotizing Enterocolitis

Cheryl A. Miner

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

Susan Fullmer, Chair
Dennis L Eggett
Nora Nyland

Department of Nutrition, Dietetics, and Food Science

Brigham Young University

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ABSTRACT

Maternal, Neonatal, and Feeding Type Factors Associated with Severity of Necrotizing Enterocolitis

Cheryl A. Miner
Department of Nutrition, Dietetics and Food Science
Master of Science

Objective: To identify statistical associations with necrotizing enterocolitis (NEC) severity as dichotomized into cases with Bell stage II and III disease.

Study Design: We conducted a retrospective study using eight consecutive years of data from a multihospital healthcare system analyzed NEC severity (Bell stage II vs. III).

Results: We identified 220 neonates with stage \geq II who had 225 separate episodes of NEC (157 stage II and 68 stage III). Those with stage III were born at earlier gestational age ($P < 0.0001$) and lower birth weight ($P < 0.0001$). Diagnosis of NEC occurred on about the same day of life in stage II and stage III cases. Those who developed stage III had significantly higher C-reactive protein ($P < 0.0001$), I/T ratio ($P = 0.0005$), mean platelet volume (MPV) ($P = 0.0001$) and lower pH ($P < 0.0001$) and platelet counts ($P < 0.0001$). Transfusions were more common to those who progressed to stage III ($P < 0.0001$). Regression analysis indicated higher odds of stage III in relationship to the volume of RBC transfusions (OR 2.41, {CI 1.85 to 3.11}, $P < 0.0001$) and pasteurized human milk (PHM) (OR 1.32, {CI 1.07 to 1.62}, $P = 0.0089$). In contrast, feeding early mother's own milk (colostrum) for five days reduced the odds for stage III (OR 0.802, {CI 0.67 to 0.96}, $P = 0.0170$). Those with small bowel resection were less likely to have received mother's own milk before NEC (OR 0.94, {CI 0.89 to 0.99}, $P = 0.019$) and factors predicting death from NEC were a low pH (OR 2.21, {CI 1.27 to 3.85}, $P = 0.0005$) and less colostrum (OR 0.96, {CI 0.94 to 0.99}, $P = 0.003$).

Conclusions: RBC transfusions and PHM increased the odds for stage III NEC, whereas early mother's own milk five days reduced the odds. Mother's own milk with PHM decreased the risk for small bowel resection and early mother's milk decreased the odds for mortality from NEC. Future research and prospective randomized controlled studies are needed to quantify any reduction in NEC severity on the basis of decreasing RBC transfusions and increasing early mother's own milk or colostrum.

Keywords: NEC, surgery, transfusion, feeding, colostrum

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Brigham Young University

SIGNATURE PAGE

of a thesis submitted by

Cheryl A. Miner

This thesis of Cheryl A. Miner is acceptable in its final form including (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory and ready for submission.

Date

Susan Fullmer, Chair

Date

Dennis L. Eggett

Date

Nora Nyland

Date

Susan Fullmer Graduate Coordinator
Nutrition, Dietetics, and Food Science Department

Date

College Dean Rodney Brown

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MANUSCRIPT

Prepared for the Journal of Perinatology

**Maternal, Neonatal and Feeding Type Factors Associated with
Severity of Necrotizing Enterocolitis**

CA Miner¹, RD Christensen², S Fullmer³, and DL Eggett⁴

¹From the Department of Dietetics and the NICU, Utah Valley Regional Medical Center, Intermountain Healthcare Provo, UT, ²the Women and Newborns Program, Intermountain Healthcare, Salt Lake City, UT, ³the Department of Nutrition, Dietetics and Food Science, and ⁴the Department of Statistics, Brigham Young University, Provo, UT, USA

Address correspondence to: Cheryl Miner RD, LD, IBCLC, Utah Valley Regional Medical Center, Department of Dietetics, NICU, 5th Floor, 1034 N, 500 W; Provo, UT, 84604, USA.

SHORT TITLE: NEC Severity

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INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common and devastating inflammatory gastrointestinal emergency in neonates occurring in 1-3 cases per 1000 live births.⁽¹⁻³⁾ Most episodes of NEC are managed successfully using medical intensive care, but certain severe cases require surgery. Reviews and meta-analyses indicate that NEC cases needing surgery have a higher mortality rate, higher costs of care, longer hospital stays among survivors,⁽⁴⁾ and more neurodevelopmental impairment⁽⁵⁻⁷⁾ Thus, if it were possible to somehow prevent NEC cases from progressing to a point where surgery was needed, outcomes would most likely be better and costs lower. However, currently the risk factors associated with NEC severity are not well defined, and it is not clear whether factors associated with severe cases, once identified, could be modified by clinicians in a way that would lower the prevalence of severe cases needing surgery.

Factors associated with developing NEC, of any stage include low gestational age and low birth weight,⁽⁸⁻¹⁰⁾ but little information is available to explain why some neonates with NEC go on to stage III while others do not. Thus, the purpose of our study was to identify demographic, clinical, and laboratory associations with severity of NEC cases. We hypothesized that factors related to NEC differed for the neonate receiving mother's own milk as compared to the formula fed neonate.

METHODS

This study was approved by the Intermountain Healthcare and Brigham Young University Institutional Review Boards as a retrospective de-identified study not requiring individual informed consent. Records of neonates born between January 1, 2002 and December 31, 2009 and admitted to an Intermountain Healthcare Newborn Intensive Care Unit were

reviewed. Intermountain Healthcare is a not-for-profit healthcare company that owns and operates 21 hospitals with labor and delivery services in Utah and Idaho.

Records were included if the diagnosis of Bell stage II or III NEC was identified in the electronic archives. When NEC was identified, radiographic images, radiographic reports, surgical and pathology reports, and physicians and/or nurses notes pertaining to NEC were reviewed. The date of NEC diagnosis was defined as the day of gastric suction tube placement.

To document the occurrence of NEC, we used the criteria originally proposed by Bell and coworkers,⁽¹¹⁾ then subsequently modified by Walsh *et al*,⁽¹²⁾ and later adopted by the Vermont Oxford Network.⁽¹³⁾ This definition required the presence of one or more of the following three clinical signs: 1) bilious gastric aspirate or emesis, 2) abdominal distension, 3) occult or gross blood in stool (no fissure) AND one or more of the following three radiographic findings: 1) pneumatosis intestinalis, 2) hepato-biliary gas, 3) pneumoperitoneum. Stage II NEC diagnosis included hepato-biliary gas or pneumatosis intestinalis radiographic findings along with abdominal distention, bilious gastric aspirate, emesis, occult or gross blood in stool. Stage III NEC diagnosis included all of the above findings plus pneumoperitoneum.

Maternal and neonatal factors along with feeding types at different time periods prior to NEC were considered for statistical models. Maternal factors considered included pregnancy induced hypertension (PIH) and/or HELLP syndrome (a group of symptoms in pregnancy including hemolysis, elevated liver enzymes and low platelet count), substance abuse, smoker during pregnancy, smoker in the home, prolonged rupture of membranes (≥ 18 hours) and race. Neonatal risk factors included: gender, gestational age, birth weight, Apgar scores at 1 minute and 5 minutes, birth number (singleton, twin or triplet/quadruplet), anthropomorphic classification at birth (small, appropriate or large for gestational age), hematocrit ≥ 65 percent

(within the first three days of life), and NEC day of life. Other neonatal factors included the use of histamine receptor antagonist, proton pump inhibitors, vasopressors, or nitric oxide within five days of NEC, positive body fluid cultures within seven days of NEC, and red blood cell (RBC) and platelet transfusions prior to NEC diagnosis. All transfusions were log transformed (natural log) for analysis.

All feeding types (including, mother's own milk, pasteurized human milk (PHM), formula and fortified mother's own milk/formula) were recorded prior to developing NEC and were considered in severity models for NEC. Feedings were grouped into different time periods as follows: 1) the first three days of life (DOL), 2) the first five DOL, 3) the first 14 DOL, 4) the first five days of feedings (any DOL) and 5) feedings prior to NEC diagnosis. The volume of feedings in the different time periods were log transformed for analysis.

All breast feedings were estimated and combined with mother's own milk feedings. For neonates < 38 weeks gestational age at birth, breast feedings were recorded as : day of life (DOL) 1 = 3.5 mL/feeding; DOL 2 - 4 = 5 mL/feeding; DOL 5 = 10 mL/feeding and DOL \geq 6 = 15 mL/feeding. Estimated breast feedings for neonates \geq 38 week gestation included: DOL 1 = 3.5 mL/feeding; DOL 2 = 5 mL/feeding; DOL 3 = 12 mL/feeding; DOL 4 = 25 mL/feeding; DOL 5 = 30 mL/feeding and DOL \geq 6 = 40 mL/feeding.

Stepwise selection regression models (logistic regression for binary variables) were developed using SAS (version 9.2, year 2008, Cary NC) which considered the above maternal factors, neonatal factors, and feeding types in the different time periods. Regression models included: 1) stage II vs. stage III NEC, 2) selected laboratory values within three days of NEC diagnosis and 3) outcomes of bowel resection and death due to NEC. In addition, the lowest pH

(arterial or venous within three days of NEC) was considered in models for the effect on laboratory values, and odds for bowel resection or death from NEC.

Laboratory values that were recorded included the highest C-reactive protein (CRP), highest leukocyte count, lowest neutrophil count, highest ratio of immature neutrophils (metamyelocytes and bands) to total neutrophils (segmented neutrophils plus bands plus metamyelocytes)⁽¹⁴⁾ or I/T neutrophil ratio, lowest pH (arterial or venous), lowest platelet count, and highest mean platelet volume (MPV). These values were collected three days prior to and following NEC diagnosis.

Pathological and surgical reports were reviewed to determine the anatomic area and length of bowel resection. Small bowel resections were grouped as: ≤ 20 cm resected, 20-75 cm resected and >75 cm resected and numerically scored as 1, 2 and 3. Colonic resections were grouped into sections: cecum, ascending, transverse, or descending/sigmoid resections.

Descriptive and clinical characteristics as well as stepwise regression models are reported as number (n), percentages (%), least square means (LSM), standard error of the mean (SEM), means, adjusted odds ratios (OR), 95% confidence intervals(CI) and r^2 values. *P* values were set *a priori* at $\alpha = \leq 0.01$.

RESULTS

A total of 320 neonates were identified with stage II or III NEC born between January 1, 2002 and December 31, 2009 (Figure 1, Appendix C). Ninety six were born at a non-Intermountain Healthcare hospital and transferred for NEC treatment. These ninety six, as well as four others born in an Intermountain Healthcare hospital, lacked pre-NEC data essential for the analysis and therefore were not included in this study leaving 220 neonates with 225 episodes

of NEC (157 stage II and 68 episodes of stage III NEC). One patient had three distinct episodes of stage II NEC, and three patients had one episode of stage II and a subsequent episode of stage III.

Characteristics of those with stage II and stage III NEC are contrasted in Table 1 (Appendix C). Neonates who developed stage III were born at earlier gestational age and lower birth weight and with slightly lower 5 minute Apgar score. The diagnosis of NEC occurred on about the same day of life in stage II vs. stage III cases. No differences were identified in gender, race, singleton vs. multiple birth number, or any of the maternal factors considered.

Laboratory values during the three day period before NEC were more likely to be abnormal among those who developed stage III (Table 2, Appendix C). Specifically, the group that developed stage III had a higher CRP, I/T neutrophil ratio, and mean platelet volume (MPV), and a lower pH and platelet count. However, neutropenia and leukocytosis were not more common in one group than the other.

Neonates with stage III NEC had less total feedings and formula but more PHM given prior to NEC diagnosis. No other differences between feeding types were identified between the two stages (Table 3, Appendix C). RBC and platelet transfusions were more commonly given to those who went on to develop stage III NEC (Table 4, Appendix C). For those who developed stage III, blood and urine cultures were more commonly positive within seven days of NEC. The use of vasopressors and nitric oxide (within five days of NEC) were more common in those who went on to stage III; and death and death ascribed to NEC were also much more common in this group (Table 5, Appendix C).

Stage II vs III NEC Model

Factors identified in regression analysis predicting a greater likelihood of stage III vs. stage II NEC were a greater volume (mL/kg) of RBC transfusions (OR 2.41, {CI 1.85 to 3.11}, $P < 0.0001$) and a greater volume/kg of pasteurized human milk feedings prior to NEC diagnosis (OR 1.32, {CI 1.07 to 1.62}, $P = 0.009$) whereas, factors that predicted a lower likelihood of stage III included early feedings using mother's own milk (first five days of feedings) (OR 0.80, {CI 0.67 to 0.96}, $P = 0.0170$) and an earlier NEC diagnosis (OR 0.94, {CI 0.91 to 0.97}, $P < 0.0001$).

Laboratory Value Models

All regression models for selected laboratory values within three days of NEC did not show any significant findings that were practical or meaningful based on selected maternal, neonatal, or feeding type factors in different time periods studied.

Bowel Resection Models

A regression model for small bowel resection among stage III NEC neonates showed that those with no resection were more likely to have received mother's own milk in combination with pasteurized human milk prior to NEC diagnosis (OR 0.94, {CI 0.89 to 0.99}, $P = 0.0194$). There were no significant maternal, neonatal, or feeding type factors in different time periods associated with colonic bowel resection.

Death from NEC Model

Four factors significantly predicted the likelihood of death from NEC among those that had stage III disease, namely 1) a low pH (OR 2.21, {CI 1.27 to 3.85}, $P = 0.0050$), 2) less percent of early mother's own milk (first 5 days of feedings) any DOL (OR 0.96, {CI 0.94 to

0.99}, $P=0.003$), 3) less total feedings the first 14 DOL (OR 0.62, {CI 0.43 to 0.89}, $P = 0.009$), and 4) less formula feedings the first 14 DOL (OR 0.97, {CI 0.94 to 0.99}, $P = 0.015$).

DISCUSSION

The most significant associations we identified with severe NEC were earlier gestational age, lower birth weight, and more RBC transfusions before developing NEC. Many reports have identified an association of RBC transfusions and the development of NEC,⁽¹⁵⁻¹⁹⁾ but this is the first report demonstrating the association between RBC transfusions and NEC severity. A recent study indicated that 1) 25-30 percent of NEC cases followed a RBC transfusion, generally within 6 to 24 hours, 2) neonates with transfusion-associated NEC were generally born at earlier gestation than those with NEC and no antecedent transfusion, and 3) neonates with transfusion-associated NEC have generally had one or more previous RBC transfusions before the one that precipitates NEC.⁽¹⁵⁻¹⁹⁾

The most significant factor protecting from stage III disease in the present study was early feedings with mother's own milk. This protective effect was not observed for pasteurized human milk. In fact, as the log unit of pasteurized human milk increased before NEC diagnosis, there was a 32% increased likelihood for stage III. Distinct differences exist between pasteurized human milk and fresh maternal milk. Donor milk is pasteurized to avoid potential transmission of infectious agents by heating to 62.5 degrees C for 30 minutes (Holder method), but this process inactivates cellular components of milk, including T and B cells, macrophages, and neutrophils.⁽²⁰⁻²²⁾ Concentration of immunoglobulins A and G are significantly reduced, as

are lactoferrin, ⁽²³⁻²⁵⁾ lysozymes, ^(24,26) and erythropoietin ⁽²⁷⁾ interferon- γ , interleukin – 1 beta, interleukin-10, hepatocyte growth factor, ⁽²⁸⁾ insulin-like factor1 and insulin-like growth factor 2. ⁽²⁹⁾ Some growth factors have been reported to be reduced by pasteurization, including insulin-like growth factor-1 and insulin-like factor-2, ⁽²⁹⁾ However, other factors such as epidermal-like growth factor (EGF) ⁽³⁰⁾ and transforming growth factor-beta, ⁽³⁰⁾ appear capable of withstanding this process. As human milk banking increases, it is critical to understand the effect of pasteurization on immunological components and to improve processing techniques to better preserve these components.

In contrast, early mother's own milk (first 5 days of feedings) was associated with a reduction in odds for severe NEC (stage III). Colostrum as compared to mature maternal milk has many unique features including higher concentrations of secretory IgA, lactoferrin, anti-inflammatory cytokines, oligosaccharides, soluble CD-14, and antioxidants. ⁽³¹⁻³³⁾ Moreover, mothers of the most preterm infants produce colostrum with even higher concentrations of many such factors compared with mothers of term infants. ⁽³⁴⁻³⁶⁾

The observation that colostrum may decrease NEC severity, which generally occurs some three weeks later, is consistent with the recent observations from Gonzalez-Reivera *et al.* ⁽³⁷⁾ that NEC may be a consequence of events occurring soon after birth. It may be possible that feeding colostrum (or lack of) could be such an event.

We found that mother's own milk in combination with PHM had a protective effect on small bowel resection, whereas pasteurized human milk alone did not. The model for large bowel resection showed no maternal, neonatal or feeding type factors that predicted outcome.

We also focused on factors associated with death from NEC and found that early colostrum feedings for five days decreased the odds of mortality. Recently, Meinen-Derr *et*

al.⁽³⁸⁾ also demonstrated an association between human milk feedings given the first two weeks and reduction in death from NEC.

We recognize several limitations of our study, foremost of which are those inherent in retrospective data analyses including susceptibility to error and bias. Other flaws include the many factors unknown to us because they were not recorded such as the reasons or timing of RBC transfusions, differences in feeding practices, differences in the types of fortifiers used, and why colostrum was available for some neonates and not for others.

In conclusion, we sought to find associations with severe NEC using data from a multicentered healthcare database. Indeed we observed that neonates who received more RBC transfusions and less colostrum were more likely to have stage III disease. In addition, those that received less colostrum increased the odds for death from NEC. It is not clear whether successful programs to encourage early colostrum feedings would decrease the prevalence and mortality from stage III NEC in actual practice. Certainly any salutary effect of this practice could have important beneficial effects for neonates, their families, and society. Continued ongoing and future investigative efforts are necessary due to high human and economic costs of stage III NEC disease.

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**APPENDIX A: COMPLETE INTRODUCTION AND NECROTIZING
ENTEROCOLITIS LITERATURE REVIEW**

OVERVIEW

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in neonates. Overall, necrotizing enterocolitis (NEC) rates are between 1-5% for all NICU admissions and occur in approximately 1-3 cases in 1000 live births.⁽¹⁻³⁾ However, among infants with birth weights between 500 and 1500 grams, the rates are approximately 12%.^(1,4) Thus, NEC is a major cause of morbidity and mortality in the very low birth weight premature neonate.(less than or equal to 1500 grams).^(5,6) Due to advances in neonatology, over the past decade, survival of the smaller, more premature infants has increased, resulting in an increase in the number of NEC cases.⁽⁵⁾ Most episodes of NEC are managed successfully using medical intensive care, but certain severe cases require surgery. Several reviews and meta-analyses indicate that NEC cases needing surgery result in a higher mortality rate, higher costs of care, longer hospital stays among survivors,⁽⁷⁾ and more neurodevelopmental impairment.⁽⁸⁻¹⁰⁾ Thus, if it were possible to somehow prevent NEC cases from progressing to a point where surgery was needed, outcomes would most likely be better and cost less. However, currently the variables associated with NEC severity are not well defined, and it is not clear whether such variables could be modified by clinicians that would lower the prevalence of cases needing surgery.

Risk factors associated with an increased likelihood of developing NEC have been identified in the literature such as gestational age and birth weight, but few, if any studies have evaluated the population of NEC (stage II and III) neonates to identify risk factors that increase the likelihood of developing stage III as compared to stage II NEC. The purpose of our study was to identify such factors (maternal, neonatal and feeding types in different time periods) associated with severity of NEC: namely: stage II vs. stage III NEC, severity of laboratory

values and to factors associated with NEC outcomes including postconceptual age (PCA) in weeks to full feedings (140 mL/kg/d) post NEC diagnosis, PCA in weeks to discharge, bowel resection among stage III NEC neonates and death from NEC. We speculated that factors associated with NEC severity and outcomes would be identified between the breast fed vs. formula fed neonate using data existing in a large multihospital healthcare electronic repository which included 21 hospitals with labor and deliveries.

OBJECTIVES AND HYPOTHESES

Objective #1: Identify maternal, neonatal and feeding type factors in different time periods for Bell stage II vs. III NEC

Null hypothesis: There are no differences in factors (maternal, neonatal and feeding types in different time periods) associated between Bell stage II vs. III NEC for the neonate receiving mother's own milk vs. formula.

Alternative hypothesis: The neonate receiving mother's own milk will have a less severe form of NEC as compared to the formula fed as measured by maternal, neonatal and feeding type factors in different time periods.

Objective #2: Identify maternal, neonatal and feeding type factors in different time periods for laboratory values: highest C-reactive protein (CRP), highest leukocyte count, lowest neutrophil count, highest ratio of immature neutrophil (metamylocytes and bands) to total neutrophil count (segmented neutrophils plus bands plus metamylocytes) or I/T ratio, lowest pH, lowest platelet count and highest mean platelet volume in association with stage II and III NEC for the neonate receiving mother's own milk vs. formula.

Null hypothesis: There are no differences in the selected laboratory values (CRP, leukocyte count, neutrophil count, I/T ratio, pH, platelet count and mean platelet volume)

within three days of developing stage II and III NEC between the neonate receiving mother's own milk vs. formula.

Alternative hypothesis: Selected laboratory values (within three days) will be less severe for stage II and III NEC for the neonate receiving mother's own milk vs. formula.

Objective #3: Identify factors (maternal, neonatal and feeding types in different time periods) associated with NEC outcomes for the neonate receiving mother's own milk as compared to the formula fed neonate: post conceptual age (PCA) in weeks to full enteral feedings (140 mL/kg/d), PCA in weeks to discharge, bowel resection (small intestine and colonic resection) and mortality from NEC.

Null hypothesis: There are no differences with factors (maternal, neonatal or feeding types in different feeding periods) for outcomes of post conceptual age (PCA) in weeks to full feedings after NEC diagnosis and PCA in weeks to discharge between neonates receiving mother's own milk vs. formula fed. In addition, there are no differences in maternal, neonatal or feeding type factors in different time periods for bowel resection (small intestine and colonic resection) and mortality between neonates receiving mother's own milk vs. formula.

Alternative hypothesis: Neonates receiving mother's own milk who develop stage II and III NEC have less severe outcomes than formula fed neonates with regards to PCA in weeks to full feedings (140 mL/kg/d) after NEC diagnosis, PCA in weeks to discharge, bowel resection (both small and colonic resection) and mortality from NEC.

LIMITATIONS

We recognize several limitations of our study, foremost of which are those inherent in retrospective data analyses including susceptibility to error and bias. Other flaws include the many factors unknown to us because they were not recorded; such as the reasons for ordering the RBC transfusions and the feeding practices selected, the types of fortifiers used, and why early mother's own milk or colostrum was available for some neonates and not for others.

NECROTIZING ENTEROCOLITIS LITERATURE REVIEW

EPIDEMIOLOGY

Necrotizing enterocolitis rates range from 3 to 7% in all newborn intensive care units (NICU).^(11,12) A database of neonatal hospitalizations found 1.1 cases per 1000 live births⁽¹¹⁾ whereas the Canadian neonatal network had a rate of 1.8 per 1000 live births.⁽¹²⁾ In the NICHD Neonatal Network cohort, rates of NEC were inversely related to birth weight, with NEC affecting 11.5% of infants weighing 401-750 g, 9% of infants 751 to 1000 g, 6% of infants 1001 to 1250 g and 4% infant 1251 to 1500 grams.⁽¹³⁾ Thus, the incidence and mortality rates from NEC are inversely proportional to birth weight and gestational age.^(14,15) NEC only occasionally occurs in the full term infant and is usually associated with predisposing factors such as congenital heart disease, respiratory disease, or asphyxia.⁽¹⁶⁾ There is no consistent association between race, sex, and rates of NEC, however, VLBW black infants are at the greatest risk of death.⁽¹¹⁾

Morbidity rates from NEC are high, ranging from 10-30%^(11,12) which include neurodevelopmental impairment, vision and hearing impairment, failure to thrive due to loss of or dysfunctional bowel, feeding abnormalities, diarrhea, bowel obstruction, or short bowel syndrome. There are also problems related to long-term use of parenteral nutrition including catheter related blood stream infections, cholestasis and liver failure.⁽¹⁵⁾ Many cases of NEC can be managed medically, but roughly 20% to 40% of patients require surgical intervention.^(11,12)

Mortality rates from NEC are also high, ranging from 20-30%, with the highest rates among those requiring surgery.⁽¹⁷⁾ Cases of NEC are usually sporadic, although there have been reports of outbreaks. These outbreaks occur more commonly in crowded nurseries.⁽¹⁸⁾ It has

been suggested that the outbreaks may be due to an infectious etiology, however, a specific organism has never been linked to these episodes. ⁽¹⁹⁾

DIAGNOSIS - Clinical Signs and Symptoms

Necrotizing enterocolitis has an array of presentations including feeding intolerance, abdominal distention, bilious emesis, temperature instability, lethargy, apnea, bradycardia, decreased peripheral perfusion, delayed gastric emptying, bloody stools, and respiratory stress. ^(15,20) These symptoms can occur suddenly within a few hours or may be preceded by several days of feeding intolerance. NEC affects the term infant during the first few days of postnatal life, at the end of the first week of life for neonates greater than 33 weeks gestational age, during the first two-and-a-half-weeks in neonates 28-32 weeks and after more than four weeks in neonates below 28 weeks gestational age. ⁽¹⁵⁾

A systematic description of necrotizing enterocolitis was reported in the literature by Bell *et al* ⁽²¹⁾ in 1978, then, subsequently refined by Walsh *et al*, ⁽²²⁾ and later adopted by the Vermont Oxford Network. ⁽²³⁾ This system assigns necrotizing enterocolitis into three different stages of severity. Stage I criteria includes highly nonspecific findings of feeding intolerance, mild abdominal distention, or both, whereas stage II criteria includes the above conditions as well as radiographic findings of pneumatosis intestinalis or portal venous gas. Stage III criteria includes those in stage I and II and a pneumoperitoneum.

Nonspecific laboratory abnormalities at NEC diagnosis may include neutropenia, thrombocytopenia, hyponatremia, hyperglycemia, metabolic acidosis, as well as bacteria or infectious products isolated from blood, urine, stool or cerebral spinal fluid. ⁽²⁰⁾ Serial C-reactive protein can be useful in the management of NEC since high levels may predict complications

such as strictures, abscess, or need for surgery.⁽²⁴⁾ Early signs of this disease are non specific, thus sepsis may be suspected before NEC is diagnosed.⁽¹⁵⁾

The ileum and proximal colon are the most commonly affected sites in NEC although any segment of the gastrointestinal tract can be involved, including the stomach.⁽²⁵⁾ Severity can range from a small localized mucosal necrosis of a bowel segment to necrosis of the entire small intestine and colon in the most severe cases.

PATHOGENESIS

Etiology

The precise etiology and pathogenesis of NEC is incompletely understood. However, epidemiologic observations strongly suggest a multifactorial cause.⁽²⁶⁾ Predisposing factors, genetic predisposition, intestinal immaturity and an imbalance in microvascular tone accompanied by a strong likelihood of abnormal microbial colonization in the intestine with a highly immuno-reactive intestinal mucosa may be involved in the cascade of events leading to NEC.

Prematurity

Prematurity is the only factor consistently found in epidemiological studies to be an independent determinant of NEC.⁽²⁶⁾ Up to 90% of infants with NEC are of low birth weight (less than or equal to 1500 grams) and the disease is more frequent and severe in those infants with the earliest post-conceptual age.⁽²⁷⁾

Immature Intestinal Motility, Digestion, and Barrier Function

Immature motility, digestion , absorption, immune defenses, barrier function, and circulatory regulation probably predispose the preterm infant to an increased risk of intestinal

injury.⁽²⁸⁾ Intestinal motility is an important factor in clearing antigens presented to the intestinal mucosal barrier from the gut lumen. Studies on fetal animals and humans suggest that development of gastrointestinal motility begins in the second trimester and matures in the third trimester at approximately the eighth month gestation.⁽²⁹⁾ Premature infants can have immature intestinal motility patterns as compared with full term infants and induced fetal hypoxia can reduce postnatal intestinal motility, predisposing the premature infant to NEC.⁽³⁰⁾ Immature motility patterns alter normal peristaltic activity and result in overgrowth of anaerobic bacteria in the small intestine with malabsorption of dietary nutrients.⁽³¹⁾ In addition, premature infants have not yet developed the ability to digest and absorb nutrients, thus contributing to increased carbohydrate load which may serve as substrate for bacteria.⁽³²⁾

Gastric acidity provides a first-line defense against bacterial passage into the proximal intestine. Gastric secretions are limited in the preterm infant, which has been linked to an increased risk of NEC, particularly among infants receiving histamine receptor antagonists.⁽¹³⁾ Lebenthal and Lee⁽³⁵⁾ demonstrated that the function of the exocrine pancreas is limited in infants and may last through the first year of life. Lack of gastric acid and pancreatic secretions and their resulting proteolysis may adversely affect the intestine by allowing a greater bacterial and/or antigenic load to develop. Thus, impaired digestion of nutrients with delayed transit time and bacterial overgrowth could result in intestinal injury with immature host and barrier defenses.

In addition, if structural or biochemical components of the intestinal epithelial barrier are not fully developed, bacteria may gain access to deeper tissues and cause inflammation. These tight junctions between epithelial cells of the intestines create a barrier to fluid and molecules.⁽³⁴⁾ A study has shown that intestinal permeability to macromolecules (including immunoglobulins,

proteins, and carbohydrates) is highest in premature infants, especially in those diagnosed with NEC.⁽³²⁾ Pathogens or toxins may translocate across the leaky intestinal barrier and gain access to deeper tissues and cause inflammation in the preterm infant.

Goblet cells are specialized enterocytes that secrete mucins, forming a thick mucous protective layer over the intestinal mucosa in the small and large intestine. This mucus layer impedes direct microbial-epithelial binding and enhances removal of adherent bacteria.⁽³⁵⁾

Goblet cells in the preterm infant are immature which may lead enhance translocation of bacteria.

Growth factors, growth factor receptors, or their related signal transduction pathways are aberrant in the immature intestine. Epidermal growth factor (EGF) is a major trophic factor for the development of the intestine and the EGF receptor has been identified on the basolateral surface of enterocytes.⁽³⁶⁾ Expression of EGF receptor involved in intestinal maturation is decreased in the preterm infant. Recently, human data suggests a link between EGF production and NEC and using EGF has been suggested to improve epithelial regeneration.⁽³⁷⁾

Trefoil factor peptides (TFF1-3) are part of the protective mechanism operating in the intestinal mucosa and play a fundamental role in epithelial protection and repair.⁽³⁸⁾ A lack of trefoil factor expression in response to NEC in the premature gut may contribute to an insufficient response to reverse mucosal insult observed in NEC.⁽³⁹⁾ This impaired restitution of the mucosa may contribute to the cascade of bowel necrosis.

Immature Intestinal Immunity

The immune defense systems are immature and abnormal in the developing neonate. A possible mechanism for the pathophysiology of NEC is that reduced inflammatory signaling could allow bacterial overgrowth. Premature infants have macrophages that are defective in

producing pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin – 1 β , (IL-1 β), interleukin-6 (IL-6) and interleukin-12 (IL-12).^(40,41) Also, monocytes and T cell production of the anti-inflammatory cytokines interleukin-10 (IL-10) and TGF- β are developmentally delayed in the preterm infant.⁽⁴²⁾ Serum levels of several cytokines and chemokines that recruit inflammatory cells have been reported to be higher in patients with necrotizing enterocolitis than in unaffected preterm infants. Interleukin -8, which is produced by epithelial cells and mediates the migration of neutrophils to the site of inflammation, can cause necrosis and increased production of acute-phase proteins in the gut.^(43,44) Also, newborns have markedly reduced synthesis of secretory IgA and IgG. These immunoglobulins provide local intestinal protection against microorganisms, which may infect the mucosa or enter the body through the gut or respiratory tract.

Hypoxic-Ischemic Injury

It is currently considered unlikely that major perinatal hypoxic-ischemic events contribute to the pathogenesis of necrotizing enterocolitis.⁽⁴⁵⁻⁴⁷⁾ However, hypoxia increases production of vasoconstrictor endothelin-1 and compromises the production of endothelial derived vasodilator nitric oxide (NO) which could play a role in the pathogenic cascade that leads to necrotizing enterocolitis.⁽⁴⁸⁾

Formula Feedings

Enteral feedings have a strong association with NEC. Most NEC cases (90-95%) occur in infants with initiation/reinitiation of feedings or rapid volume advancement of feedings.⁽²⁶⁾ Enteral feedings may disrupt mucosal integrity, blood flow and/or motility and serve as a substrate for bacteria.⁽²⁶⁾ Breast fed infants are 10 times less likely to develop NEC than formula fed infants, suggesting that breast milk contains multiple bioactive factors that influence host

immunity, inflammation and mucosal protection. Breast milk also increases the diversity of gastrointestinal bacterial colonization and contains immunomodulatory factors such as secretory immunoglobulin A, leukocytes, mucin, lysozyme, cytokines, lactoferrin, growth factors (EGF), and oligosaccharides, many of which are not in commercial formulas.

Alternations in Normal GI Microbiological Flora and Infection

The regions of the intestine that are most often associated with NEC are the ileum and proximal colon, which have the highest bacterial loads, and abnormal colonization of the intestinal tract of premature neonates may contribute to NEC. The predominant species of bacteria found in the GI tract of healthy, term, breast fed infants is *Bifidobacteri*.⁽⁴⁹⁾ In contrast, species of *Staphylococcus*, *Enterobacter*, *Enterococcus* and *Clostridia* are the predominant fecal bacterial species in premature neonates undergoing intensive care, with very little colonization of *Bifidobacteria*.^(49,50) *Clostridium perfringens* has been isolated from 40% of infants with NEC⁽⁵³⁾ and species of *Escherichia* and other bacterial, viral and fungal pathogens, including rotavirus and species of *Candida*, have been implicated in the etiology of NEC, however no single pathogen has been identified as a cause of NEC.⁽²⁵⁾ Premature infants are very susceptible to intestinal colonization by pathological bacteria and a high likelihood of exposure to antibiotics on admission to newborn intensive care unit (NICU)⁽⁵²⁾ which could lead to pathogenic bacterial growth.

Abnormal expression of pattern recognition receptors may affect the way in which the intestine in premature infants responds to bacterial colonization. One of the first pro-inflammatory molecules to cross the intestinal barrier is lipopolysaccharide (LPS), which is a component of the outer cell wall of gram-negative bacteria that recognizes and binds to toll like

receptor 4 (TLR-4).⁽⁵³⁾ The circulating LPSs are increased in neonates with NEC which initiate inflammatory signaling cascades within the enterocyte.⁽⁵³⁾

Vasoactive and Inflammatory Mediators

Nitric oxide (NO), a vasodilator in the gastrointestinal tract mediates inhibitory nerve-related relaxation of intestinal smooth muscle and plays a role in regulating gut mucosal blood flow, mucosal permeability, and intestinal motility.⁽⁵⁴⁾ Nitric oxide maintains intestinal microvascular integrity by inhibiting platelet aggregation and leukocyte adhesion. Nitric oxide, in association with peroxynitrite, has anti-microbial properties and play important roles in host defense against pathogens. Researchers have suggested that NO participates in the pathogenesis of NEC by directly damaging the enterocyte monolayer by membrane peroxidation and by disrupting the ability of the mucosa to repair itself.⁽⁵⁴⁾ The degree of nitrotyrosine immunostaining (a marker of NO) correlates with apoptosis in enterocytes of the villi of infants with NEC.⁽⁵⁷⁾

Endothelin -1 is a potent vasoconstrictor agent and is produced in the endothelial cells, submucosal stroma, and circularis muscularis layers of the gut wall. ET-1 production is increased by reduced flow, hypoxia and inflammatory cytokines. When excessive amounts of ET-1 are present, vasoconstriction in the newborn intestine occurs, and recently ET-1 has been shown to be greater in human preterm intestine with NEC.⁽⁵⁵⁾

Platelet Activating Factor (PAF), an inflammatory and vasoconstrictive mediator, is synthesized by neutrophils, macrophages, endothelial cells and enterocytes in response to hypoxia.⁽²⁴⁾ PAF is rapidly degraded by the enzyme, PAF-acetylhydrolase⁽⁵⁶⁾ which is decreased in the human newborn. This imbalance puts the newborn at risk for an elevated PAF response before adequate immune stimuli is developed. Formula does not contain PAF-AH like human

milk, leaving formula fed neonates susceptible to a greater risk for NEC. Neonates with NEC have high levels of PAF and decreased levels of plasma PAF-AH with levels correlating with NEC severity.^(15, 28)

Pro-inflammatory cytokines are produced in response to inflammatory stimuli that communicate to the surrounding tissues at the presence of infection or injury. Several pro-inflammatory cytokines have been implicated in NEC, including tumor necrosis factor- α (TNF- α), Interleukin-1 β , Interleukin-6, Interleukin-8, Interleukin-12 and Interleukin-18.⁽⁴³⁾

Interleukin -8 (IL-8) is triggered in response to various stimuli including LPS, TNF- α , and IL-1 β . Some actions mediated by IL-8 are attraction of neutrophils and basophils to the site of inflammation, neutrophil activation and migration into tissues and production of acute phase proteins.⁽⁴⁴⁾ Plasma IL-8 levels are elevated in infants with NEC and levels correlate with clinical severity.⁽²⁸⁾

Interleukin-12 (IL-12) synthesis and release is early in response to bacteria and viruses. IL-12 exerts its effects by binding to IL-12 receptors present on T cells and NK cells.⁽⁵⁷⁾ IL-12 is potentially an important cytokine in the development of NEC.

Interleukin-18 (IL-18) is a proinflammatory cytokine which induces production of TNF- α and IL-1- β .⁽⁶⁰⁾ IL-18 binds to IL-18 receptors present on macrophages, neutrophils, endothelial cells, smooth muscle cells, and lymphocytes. The risk of NEC has been associated with IL-18 AA genotype.⁽⁵⁹⁾

Interleukin-4 (IL-4) is an immunoregulatory cytokine produced by Th2 cells, mast cells, B cells, and stroma cells. IL-4 promotes Th2 type responses and exerts immunosuppressive effects on macrophages including the suppression of pro-inflammatory cytokine production.⁽⁴³⁾

Studies have shown that very low birth weight infants (less than 1500 grams) with NEC were less likely to possess the IL-4 receptor alpha-chain as compared to infants without NEC.⁽⁵⁹⁾

Interleukin-10 (IL-10) is a regulatory cytokine in the intestine and is synthesized by the Th2 cells, monocytes, and B cells.⁽⁶⁰⁾ Production of anti-inflammatory mediators such as IL-10 is diminished in the newborn as compared to the adult, with preterm infants synthesizing less than term infants. Edelson *et al.*⁽⁶¹⁾ demonstrated increased concentrations of IL-10 with severe NEC.

Genetics

Studies are currently emerging that investigate the potential importance of specific polymorphisms for known NEC-associated inflammatory mediators. This genetic variance may contribute to the individual variances of cytokine responses to inflammatory stimuli.⁽⁶⁰⁾ A family of pathogen recognition receptors has been shown to sense invading bacteria and activate gene transcription pathways that regulate immune and inflammatory responses. The risk of NEC has also been associated with the frequency of the IL-18 AA genotype which is significantly higher in infants with stage III NEC as compared to stages I and II NEC.⁽⁶⁰⁾ Another possible genetic factor is the pro-inflammatory cytokine TNF- α . Pretreatment in animal models with anti-TNF- α reduced the incidence and severity of NEC.^(62,63)

Red Blood Cell Transfusions

An association between the elective transfusion of packed red cells and necrotizing enterocolitis has been reported.⁽⁶⁴⁾ The mechanism by which red cell transfusions are associated with NEC is unclear but may be related to alterations in intestinal blood flow or hypoxia-ischemia events.

MANAGEMENT

Medical

Medical treatment consists of bowel rest and administration of antibacterials to limit bacterial invasion and translocation in stage II and III NEC. Initially, neonates with stage II or III NEC are made NPO (nothing by mouth) and total parenteral nutrition is administered for nutrition support. A full evaluation for systemic infection for bacterial translocation should be performed including blood, urine and possibly cerebrospinal fluid cultures. Parenteral antibacterials should be given for a period of 7-14 days in conjunction with bowel rest.⁽⁴⁶⁾

Clinical monitoring along with cardiopulmonary support is routinely performed. Initial and periodic radiographic evaluations based on clinical status are done to evaluate progression of the disease. Laboratory values including C-reactive protein (CRP), leukocyte counts, neutrophils, platelet counts, electrolytes and acid base status should be monitored based on clinical status and stability of the infant.

Surgical

If an infant does not improve significantly despite medical treatment and if pneumoperitoneum is present, or if clinical, laboratory and/or radiographic findings worsen, surgical management is often required. In fact, 20-40% of neonates with NEC require some type of surgical intervention.^(2,11) For many infants with intestinal perforation, laparotomy is performed with resection of the necrotic portion of the intestine and an ostomy created at the end proximal to the resected bowel.

PREVENTATIVE MEASURES

Numerous approaches have been proposed for the prevention of necrotizing enterocolitis which includes withholding enteral feedings, feeding mother's own expressed breast milk,

administering probiotic agents or prebiotic agents and administering various growth factors. The practice of withholding enteral feedings comes from clinical experience and retrospective reviews suggesting that a rapid increase in feedings increases the likelihood of necrotizing enterocolitis.⁽⁶⁵⁾ However, recent data suggests that withholding feedings may be a dangerous practice because it leads to the prolonged use of parenteral nutrition which results in intestinal atrophy, increased permeability and inflammation. Likewise, a delay in feedings may increase the severity of necrotizing enterocolitis.⁽⁶⁵⁾ The following is a review of preventative measures used with NEC.

Human Milk

Lucas and Cole,⁽⁶⁶⁾ in a prospective multicenter study, confirmed that NEC was 6 to 10 times more common in exclusively formula-fed infants than in those fed human milk alone and 3 times more common than in those who received formula with human milk. Recently, Meinen-Derr *et al.*⁽⁶⁷⁾ have shown a dose related association of human milk feeding with a reduction of risk of NEC or death after the first 2 weeks of life among extremely low birth weight infants (less than or equal to 1000 grams).

The protective effect of breast milk has been correlated with its anti-inflammatory components (IL10), growth factors (EGF), erythropoietin (Epo), lysozyme, and immunoglobulins, as well as pre-and probiotics that modulate intestinal microflora composition to the advantage of the host.⁽⁶⁸⁾ The additional presence of an enzyme, platelet activating factor-acetyl hydrolase (PAF-AH), activity in human milk may also partly explain the protective effect of breast milk.⁽⁶⁹⁾ PAF-AH degrades PAF and is low in neonates under three weeks of age.⁽⁷⁰⁾ However, breast milk alone does not eliminate NEC completely as cases are reported in neonates who have been exclusively breast fed.⁽⁶⁹⁾ A recent study suggested that the exclusive use of

human milk plus a human milk-derived fortifier may result in a lower incidence of necrotizing enterocolitis.⁽⁷¹⁾

Trophic Feeds

Trophic feedings or small volumes of breast milk or formula may overcome gut atrophy and inflammatory responses associated with prolonged bowel rest. Trophic feedings improve the activity of digestive enzymes, enhances the release of digestive hormones and increases intestinal blood flow and motility in premature infants. It has been shown that early trophic feedings do not increase susceptibility to developing NEC, however, best feeding strategies for premature infants are not currently defined.⁽⁷²⁾

Standardized Feeding Regimens and Advancement of Feedings

There is a relationship between the rate of feeding advancement and an increased incidence of NEC.⁽⁷³⁾ A significant decline of 87% in the incidence of NEC and 29% in the risk of developing NEC followed implementation of a standardized feeding regimen in the form of clinical practice guidelines.^(74,75)

Probiotics

Prospective randomized trials during the past decade have evaluated the effects of various probiotics to prevent necrotizing enterocolitis.⁽⁷⁶⁻⁷⁸⁾ The most recently reported multicenter trial suggested that using probiotics decreased the incidence of NEC but did not decrease mortality from NEC. However, there appeared to be a higher incidence of sepsis among infants receiving probiotics, especially in those with a birth weight of less than 750 grams.⁽⁷⁹⁾ Further studies are needed before routine probiotic prophylaxis is recommended in the premature infant.

Prebiotics

Another potential preventative strategy is to administer prebiotics, or nutrients that enhance the growth of potentially beneficial intestinal microbes.⁽⁸⁰⁾ Prebiotic agents include oligosaccharides of inulin, galactose, fructose, lactulose, and other combinations.⁽⁸¹⁾ Although prebiotics appear to alter the consistency and frequency of stools, their efficacy in preventing necrotizing enterocolitis remains unclear. Prebiotics enhance the proliferation of endogenous flora such as *Bifidobacteria*, and require an initial appropriate colonization of the gut, which may be lacking in the very low birth weight (less than or equal to 1500 grams) preterm infant.⁽⁸²⁾

Epidermal Growth Factor (EGF)

EGF is an important constituent of gastrointestinal secretions and has multiple effects upon gut epithelial cells including a stimulatory effect on cell proliferation and migration, induction of mucosal enzyme, trefoil peptide expression, and inhibitory effects on gastric acid secretion.⁽³⁷⁾ It has been shown that preterm neonates with NEC have diminished levels of salivary and serum EGF.⁽⁸³⁾ The use of prophylaxis EGF could be a possible preventative measure since EGF receptors are found in gut epithelial cells of preterm neonates with NEC.⁽⁸⁴⁾ However, researchers warrant the clinical use of EGF since there are a variety of problems and side effects.⁽⁸⁵⁾

Arginine

Intestinal vascular permeability that occurs following an injury allows bacterial translocation across the intestinal mucosa, setting up the inflammation cascade. Endothelial nitric oxide (NO) is an anti-inflammatory agent and vasodilator that is involved in the maintenance of intestinal vascular permeability, mucosal integrity and barrier function.⁽⁸⁶⁾ The plasma levels of the amino acid arginine, a substrate for NO synthase, have been shown to be

low in neonates with NEC.⁽⁸⁷⁾ A study has shown that supplementing neonates with arginine reduces the incidence of all stages of NEC.⁽⁸⁸⁾ However, nitric oxide may not have a significant protective role for the immature and newborn intestines in ischemia-reperfusion as in the mature intestine. Whether the beneficial effects of arginine supplementation in the prevention of NEC are from the synthesis of glutamine and glutamate or to its free radical scavenging action is unknown.^(89,90)

Superoxide Dismutase

Free radicals have been implicated in NEC.⁽⁹¹⁾ Miller *et al.*⁽⁹²⁾ have shown that superoxide dismutase (SOD) prevents damage and attenuates eicosanoid release in a rabbit model of NEC. Recently, a study demonstrated that intestinal ischemia is associated with a shift from nitric oxide (NO) to O₂ production, which is NOS-dependent. Potentially greater injury results from impaired vasodilatation and over-production of reactive oxygen species.⁽⁹³⁾

Acidification of Gastric Contents

Preterm neonates are often hypochlorhydric, and enteric, gram-negative bacteria often colonize their stomachs, especially after gavage feedings.⁽⁹⁴⁾ Carrion and Egan⁽⁹⁵⁾ have documented that acidifying the feedings of preterm neonates to a pH low enough to inhibit gastric bacterial proliferation significantly lowers the risk of NEC.

Polyunsaturated Fatty Acids (PUFA)

Phospholipids are constituents of the mucosal membranes and intestinal surfactant. Their components, arachidonic acid and choline, are also the substrates for intestinal vasodilatory and cytoprotective eicosanoids, and the vasodilatory neurotransmitter, acetylcholine. Long chain PUFAs have been proposed to modulate inflammation and immunity.⁽⁹⁶⁾ Carlson *et al.*⁽⁹⁷⁾ conducted a clinical trial of formula feedings with or without supplementation with PUFA in the

form of egg phospholipids in preterm neonates. It was noted that the incidence of stage II and III NEC was significantly less in the supplemented formula group compared with the control formula group. However, these results need to be interpreted with caution, as the number of neonates in the supplemented formula group (1/34 vs.15/85) was significantly less. More recently, a study has shown that PUFA supplemented formula does not alter the risk of NEC or sepsis.⁽⁹⁸⁾

Enteral Antibiotics

Enteral antibiotics have been used as prophylaxis against NEC in low-birth-weight and preterm infants given the role of bacterial colonization in the pathogenesis of the illness. A systematic review and meta-analysis has reported that the administration of prophylactic enteral antibiotics resulted in a statistically significant reduction in NEC.⁽⁹⁹⁾ However, the possible harmful effects of prophylactic antibiotics, including the development of bacterial resistance, make it difficult to recommend this strategy for prevention of NEC.

Oral Immunoglobulins

Oral immunoglobulins IgA and IgG have been shown to have an immunoprotective effect in the gastrointestinal mucosa. A reduction in the incidence of NEC following feeding an oral IgA-IgG preparation was reported as early as in 1988 by Eibl *et al.*⁽¹⁰⁰⁾ However, a systematic review by Foster and Cole⁽¹⁰¹⁾ identified five trials on oral immunoglobulin for the prevention of NEC, of which three met the inclusion criteria. The oral administration of IgG or an IgG/IgA combination did not result in a significant reduction in the incidence of definite NEC. The researchers concluded that the current evidence does not support the administration of oral immunoglobulin for the prevention of NEC.⁽¹⁰²⁾

Erythropoietin

The presence of erythropoietin (Epo) in human milk and the expression of Epo receptors on intestinal villous enterocytes of neonates suggest that Epo has a role in growth and development of the gastrointestinal tract.⁽¹⁰²⁾ Evidence indicates that the protective effect of rhEPO may be related to inhibition of NO formation.⁽¹⁰³⁾ A recent study demonstrated a role of Epo in the regulation of intestinal epithelial tight junctions and barrier function and suggests the possible use of enteral Epo as a therapeutic agent for gut diseases and NEC.⁽¹⁰⁴⁾

Antenatal Glucocorticoids

Induction of maturation in the developing intestine was first reported by Celano *et al.*,⁽¹⁰⁵⁾ Moog⁽¹⁰⁶⁾ and Neu *et al.*⁽¹⁰⁷⁾ in experimental studies. Other researchers have also shown the beneficial effects of antenatal glucocorticoid therapy on gastrointestinal maturation and function including a reduced uptake of macromolecules from the mucosa, decreased colonization with aerobic bacteria, reduced bacterial translocation to the liver,^(108,109) and increased activity of enzymes like lactase, maltase and sucrase, and Na/K-ATPase.⁽¹¹⁰⁾ A significant reduction in the incidence of NEC following antenatal glucocorticoid therapy was subsequently noted in other studies.^(111,112) However, in contrast to previous studies, it was found recently that exposure to antenatal glucocorticoids was associated with an increased risk for NEC independent of birth weight.⁽¹¹³⁾

Other Experimental Agents

A variety of other experimental agents have been studied in the search for an effective agent for prevention of NEC. These include magnesium,⁽¹¹⁴⁾ nitroglycerin,⁽¹¹⁵⁾ cyclosporin and rapamycin,⁽¹¹⁶⁾ allopurinol,⁽¹¹⁷⁾ somatostatin,⁽¹¹⁸⁾ vitamin A,⁽¹¹⁹⁾ vitamin E,⁽¹²⁰⁾ recombinant human granulocyte colony stimulating factor,⁽¹²¹⁾ hepatocyte growth factor,⁽¹²²⁾ sucralfate,⁽¹²³⁾ L-

carnitine,⁽¹²⁴⁾ interleukin-11,^(125,126) interleukin-10,⁽¹²⁷⁾ pentoxifylline,⁽¹²⁸⁾ pan-caspase inhibitors,⁽¹²⁹⁾ and interferon alpha.⁽¹³⁰⁾

CONCLUSION

Prevention of a multi-factorial illness like NEC is a difficult task because of the poorly understood pathophysiology of the illness. Since mortality and morbidity related to NEC have not changed significantly despite the dramatic advances in neonatal and perinatal care, research in the treatment and prevention of NEC is a priority. Clinical trials of specific “promising” agents need to be designed carefully and include long-term neurodevelopmental outcomes. Given that a single effective agent is unlikely in the near future, an approach utilizing a package of “potentially better practices” (i.e. non aggressive feedings and use of breast milk practices) seems to be the most appropriate strategy to prevent and minimize NEC.

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APPENDIX B: COMPLETE METHODS

The proposal for our study was submitted to Brigham Young University and Intermountain Health Care Institutional Review Boards who required an outline of the study design, hypotheses and objectives. Students were hired through BYU to assist in collection of data pertaining to the study. The students were screened for communicable diseases and issued a temporary badge for identification through Intermountain Health Care. Students also signed confidential forms at Intermountain Health Care. The study was approved by both the Intermountain Health Care and Brigham Young University Institutional Review Boards in June 2009, 2010 and then again in June 2011 as a retrospective de-identified study not requiring individual informed consent. Patients were extracted and identified by the EMPI (electronic medical patient identification) number. Records of neonates were reviewed if born between January 1, 2002 and December 31, 2009, admitted to an Intermountain Health Care Newborn Intensive Care Unit and identified as developing “Bell” stage II or III NEC. Intermountain Health Care is a not-for-profit-healthcare company owning and operating twenty one hospitals with labor and delivery services in Utah and Idaho.

Electronic data was extracted from the Help 2 Intermountain Health Care computer program. Paper records were reviewed at Intermountain Health Care facilities including:

- Iron Mountain in Salt Lake City, Utah
- Primary Children’s Medical Center in Salt Lake City, Utah
- Intermountain Medical Center in Salt Lake City, Utah
- Dixie Regional Medical Center in St George, Utah
- Valley View Medical Center in Cedar City, Utah
- Utah Valley Regional Medical Center in Provo, Utah.

When NEC was identified, radiographic images, radiographic reports, surgical and pathology reports, physicians and nurses notes, and electronic records pertaining to NEC were reviewed. The date of NEC diagnosis was defined as the date the gastric suction tube was placed. A form was designed to collect pertinent demographic, clinical and surgical data (Appendix F).

To document the occurrence of NEC we used the criteria originally proposed by Bell and coworkers,⁽¹¹⁾ then subsequently modified by Walsh *et al.*,⁽¹²⁾ and later adopted by the Vermont Oxford Network.⁽¹³⁾ This definition required the presence of one or more of the following three clinical signs; 1) bilious gastric aspirate or emesis, 2) abdominal distension, 3) occult or gross blood in stool (no fissure) AND one or more of the following three radiographic findings: 1) pneumatosis intestinalis, 2) hepato-biliary gas, 3) pneumoperitoneum. The staging system assigns necrotizing enterocolitis into three stages of severity. Stage I includes criteria that are highly nonspecific such as feeding intolerance, abdominal distention, emesis and/or occult or gross blood in the stool. Stage II NEC criteria are defined as radiographic finding of hepato-biliary gas or pneumatosis intestinalis including criteria from stage 1 NEC, and stage III includes criteria from stage I and II along with pneumoperitoneum.

Maternal, neonatal and feeding type factors at different time periods prior to NEC were considered in all models for NEC. Maternal risk factors were considered in the models of NEC. These included:

- 1) pregnancy induced hypertension (PIH) and/or HELLP syndrome (group of symptoms in pregnancy including hemolysis, elevated liver enzymes and low platelet count)
- 2) substance abuse

- 3) smoking during pregnancy, exposure to a smoker in the home
- 4) rupture of membranes greater or equal to 18 hours
- 5) maternal race

The neonatal risk factors considered in NEC models are listed as follows:

- 1) gender
- 2) gestational age (in weeks)
- 3) birth weight,
- 4) Apgar scores at 1 and 5 minutes
- 5) birth number (singleton, twin or triplet/quadruplet)
- 6) use of histamine receptor antagonist within five days of NEC diagnosis
- 7) use of proton pump inhibitors within five days of NEC diagnosis
- 8) use of vasopressors (dopamine, dobutamine or milrinone) and nitric oxide (NO) within five days of NEC diagnosis
- 9) classification at birth (small, appropriate or large for gestational age)
- 10) hematocrit greater or equal to 65 percent within three days of birth
- 11) positive body fluid cultures within seven days of NEC diagnosis
- 12) NEC day of life (DOL)
- 13) number and volume (mL/kg) red blood cell (RBC) prior to NEC
- 14) number and volume (mL/kg) platelet transfusions prior to NEC.

The total number and volume (mL/kg) of RBC and platelet transfusions were recorded and log transformed (natural log). The lowest pH within three days of NEC was also considered for models of NEC severity for laboratory blood values, bowel resection and death from NEC.

All feeding types (including mother's own milk, fortified mother's own milk, pasteurized human milk (PHM), fortified PHM, regular formula, elemental formula, premature formula and concentrated formula) were recorded to NEC diagnosis and grouped into different time periods. The different feeding time periods included:

- 1) Feedings the first three days of life (DOL)
- 2) Feedings the first five DOL
- 3) Feedings the first 14 DOL
- 4) First five days of feedings (any DOL) and
- 5) Feedings to NEC diagnosis.

All feedings in each time period were log transformed for analysis. Breast feedings were included with mother's own milk and estimated according to gestational age and day of life (DOL). Breast feeding amounts for neonates less than 38 gestational age at birth were estimated as follows:

- DOL 1 = 3.5 mL/feeding
- DOL 2 - 4 = 5 mL/feeding
- DOL 5 = 10 mL/feeding
- DOL ≥ 6 = 15 mL/feeding

Estimated breast feeding amounts for neonates greater or equal to 38 gestational age at birth followed this criteria:

- DOL 1 = 3.5 mL/feeding
- DOL 2 = 5 mL/feeding
- DOL 3 = 12 mL/feeding
- DOL 4 = 25 mL/feeding

- DOL 5 = 30 mL/feeding
- DOL > 6 = 40 mL/feeding.

The rate of the progression of feedings (the first five days of feedings and first five DOL) were recorded. This calculation was done by taking the difference in mL of feedings /kg from one day to the next and averaged the first five DOL and the first five days of feedings.

Feeding types included mother's own milk, pasteurized human milk(PHM), cow milk based formula, soy based formula, premature, hydrolyzed formula, fortified mother's own milk, using human milk fortifier (HMF), fortified pasteurized human milk (PHM) using HMF, fortified mother's own milk using a HMF, fortified PHM using a human milk based fortifier, premature infant formula, premature discharge formula, concentrated cow milk based formula, concentrated soy formula, concentrated hydrolyzed formula, and concentrated premature discharge formula. The feedings in each group were log transformed and percentages were calculated for analysis.

Feedings were combined into different groups which included:

- 1) mother's own milk plus estimated breast feedings plus PHM
- 2) 22-24 cal/oz fortified mother's own milk plus fortified PHM using powdered HMF
- 3) 26-30 cal/oz fortified mother's own milk plus fortified PHM using powdered HMF
- 4) 20 cal/oz formula (cow milk based, soy and hydrolyzed)
- 5) 22-24 cal/oz fortified formulas (cow milk based, soy, and hydrolyzed)
- 7) 26-30 cal/oz fortified formulas (cow milk based, soy, or hydrolyzed)

All feeding groups (first three DOL, first five DOL, first 14 DOL, first five feeding days any DOL, and feedings to diagnosis of NEC) were compared between the two stages of NEC.

Post conceptional age (PCA) in weeks to NEC, PCA to full feedings (140 mL/kg/d) after NEC diagnosis and PCA to discharge was calculated and recorded.

Stepwise selection models (logistic regression for binary variables) were developed using SAS (version 9.2, 2008, Cary, NC) which considered the maternal, neonatal, and feeding type factors in different time periods. Regression models included 1) stage II vs. stage III NEC, 2) selected laboratory values within three days of NEC diagnosis and 3) outcomes of bowel resection and death due to NEC. In addition, the lowest pH (arterial or venous within three days of NEC) was considered in models for the effect on laboratory values, and odds for bowel resection or death from NEC.

Laboratory values recorded included the highest C-reactive protein, highest leukocyte count, lowest neutrophil count, highest immature to total neutrophil ratio,⁽¹⁴⁾ lowest pH, lowest platelet count and highest mean platelet volume. Outcome models for NEC included gestational age to full feedings after NEC diagnosis (140 mL/kg/d), post conceptual age to discharge, small and large bowel resection and mortality from NEC.

Pathology and surgical reports were reviewed to determine the anatomic area and length of bowel resection. Small bowel resections were grouped as follows:

- ≤ 20 cm resected
- 20-75 cm resected
- > 75 cm resected.

Colonic resections were grouped as cecum, ascending, transverse, or descending/sigmoid resections.

Clinical and descriptive characteristics as well as stepwise regression models are reported as number (n), percentages (%), least square means (LSM), standard errors (SE), means, r^2 , adjusted odds ratios (OR), and 95% confidence intervals (95% CI). *P* values were set *a priori* at $\alpha = \leq 0.01$.

APPENDIX C: COMPLETE RESULTS

A total of 320 neonates were identified with stage \geq II NEC and born between January 1, 2002 and December 31, 2009 (Figure 1). Ninety six were born at a non-Intermountain Healthcare hospital and transferred for NEC treatment. These ninety six, as well as four others born within an Intermountain Healthcare hospital, lacked pre-NEC data essential for analysis and therefore, were not included in this study. Finally, a total of 220 neonates were included in the study including 225 NEC episodes (157 stage II and 68 stage III). One patient had three distinct episodes of NEC stage II, and two patients had one episode of stage II and a subsequent episode of stage III. Each episode of stage II or III NEC was recorded separately.

Characteristics of those with stage II and stage III NEC are contrasted in Table 1. Neonates who developed stage III were born at earlier gestational age ($P < 0.0001$) and lower birth weight ($P < 0.0001$) and with slightly lower 5 minute Apgar score ($P = 0.0352$). The diagnosis of NEC occurred on about the same day of life in stage II vs. stage III cases even though stage II neonates were of slightly older post-conceptual age (PCA) at diagnosis. No differences were identified in gender, race, singleton vs. multiple birth number, or any of the maternal factors considered.

Laboratory values during the three day period before NEC were more likely to abnormal among those who developed stage III (Table 2). Specifically, the group that developed stage III had a higher CRP, I/T neutrophil ratio, and MPV, and a lower pH and platelet count. However, neutropenia and leukocytosis were not more common in one group than the other. Several laboratory values were missing during this time period including a total of 31 C-reactive protein values, seven leukocyte counts, seven I/T neutrophil ratios, eleven total neutrophil counts, 82 pHs, eight platelet counts and nineteen mean platelet volumes.

Red blood cell (RBC) and platelet transfusions prior to NEC were much more common among those who had stage III (Tables 3 and 4). The use of vasopressors and/or nitric oxide (NO), within five days of developing NEC, were more common in those who went on to stage III; and death and death ascribed to NEC were also much more common in this group (Table 5).

Model for Stage II vs. Stage III NEC

Factors identified in regression analysis predicting a greater likelihood of stage III vs. stage II NEC were 1) a greater volume (mL/kg) of RBC transfusions (OR 2.413, {CI 1.854 to 3.107} $P < 0.0001$), and 2) a greater volume of pasteurized human milk prior to NEC development (OR 1.316, {CI 1.071 to 1.617}, $P = 0.0089$). Factors that predicted a lower likelihood of stage III included 1) earlier feedings with mother's own milk (colostrum – first five days of feedings) ((OR 0.802, {CI 0.669 to 0.961}, $P = 0.0170$), and 2) an earlier DOL to NEC diagnosis (OR 0.937, {CI 0.906 to 0.968}, $P < 0.0001$)

Models for Laboratory Values

Laboratory values within each stage of NEC included the highest CRP, highest leukocyte count, highest immature neutrophil to total neutrophil ratio, lowest neutrophil count, lowest pH, lowest platelets, and highest mean platelet volume within three days of NEC.

Two factors predicted a greater likelihood of a high CRP within stage II NEC including: 1) a low pH ($P < 0.0001$) and 2) small for gestational age (SGA) ($P = 0.0076$). $r^2 = 0.328$. A low pH ($P = 0.0003$) was identified as the only predictor for a high CRP within stage III NEC. $r^2 = 0.249$.

No significant predictors were identified for a high leukocyte count within stage II NEC. We identified seven factors that predicted a greater likelihood of high leukocytes within stage III

NEC. These included 1) a lower gestational age ($P < 0.0001$); 2) Platelet transfusions ($P = 0.0027$); 3) fortified mother's own milk and PHM (log unit) to DOL 14 ($P < 0.0001$); 4) less mother's own milk and PHM (log unit) to diagnosis of NEC ($P < 0.0001$); 5) PHM (log unit) to DOL five ($P = 0.0007$); percent mother's own milk with PHM (log unit) to NEC diagnosis ($P = 0.0013$); and 6) Less fortified formula (log unit) to NEC diagnosis ($P = 0.0039$). $r^2 = 0.762$.

Two factors predicted a greater likelihood of a high I/T neutrophil ratio within stage II NEC including 1) a low pH ($P < 0.0001$) and 2) a higher percent of PHM the first five DOL ($P = 0.0048$). $r^2 = 0.354$. Four factors that predicted a greater likelihood of a high immature neutrophil to total neutrophil ratio (I/T ratio) within stage III NEC. These included 1) a lower use of proton pump inhibitors ($P < 0.0001$) or histamine receptors antagonists ($P = 0.0021$); 2) fewer red blood cell transfusions ($P = 0.0043$); 3) total feeding (log unit) to NEC diagnosis ($P = 0.0003$); and 4) lower percent of early mother's own milk the first three DOL ($P = 0.0056$). $r^2 = 0.522$.

A single predictor for a low neutrophil count within stage II was a lower birth weight ($P = 0.0004$). $r^2 = 0.235$. For stage III NEC, total feedings (log unit) to NEC diagnosis was the only predictor for a low neutrophil count ($P = 0.0002$). $r^2 = 0.25$.

Four factors predicted a greater likelihood of a low pH within stage II including 1) higher volume (mL/kg) of RBC transfusions ($P = 0.0086$); 2) positive cultures prior to developing NEC ($P = 0.0008$); 3) a lower birth weight ($P = 0.082$) and 4) lower amounts of fortified (22-24 cal/oz) formula (log unit) to NEC diagnosis ($P = 0.0027$). $r^2 = 0.461$. Two factors predicted a greater likelihood of a low pH within stage III NEC included 1) lower amounts of fortified (22-24 cal/oz) formula (log unit) to NEC diagnosis ($P < 0.0001$) and 2) fortified (22-30 cal/oz) formula (log unit) to NEC diagnosis ($P < 0.0001$). $r^2 = 0.312$.

Four factors predicted a greater likelihood of a lower platelet count within stage II NEC. These included 1) a low pH ($P < 0.0001$); 2) lower percent of early mother's own milk the first five DOL ($P = 0.0002$); 3) fewer total feedings (log unit) to NEC diagnosis ($P = 0.0004$); and 4) higher percent mother's own milk combined with PHM the first 14 DOL ($P = 0.0019$). $r^2 = 0.475$. No significant predictors were identified for a low platelet counts within stage III NEC.

Two factors predicted a high mean platelet volume within stage II NEC including: 1) lower percent of early mother's own milk for the first five DOL ($P = 0.0083$) and 2) lower percent of fortified (22-24 cal/oz) mother's own milk combined with fortified (22-24 cal/oz) PHM to the NEC diagnosis ($P = 0.0021$). $r^2 = 0.386$. Four factors predicted a high mean platelet volume within stage III NEC including 1) use of histamine receptor antagonists ($P < 0.0001$); 2) lower maternal substance abuse ($P = 0.0030$); 3) lower total feedings (log unit) to DOL 14 ($P = 0.0052$); and 4) mother's own milk (log unit) to NEC diagnosis ($P = 0.0083$). $r^2 = 0.49$.

We concluded that laboratory values did not show any significant factors that were practically significant or meaningful based on the selected maternal, neonatal, or feeding type factors in different time periods studied.

Models for Post Conceptual Age in Weeks to Full Feedings post NEC

Four factors were identified that predicted a greater likelihood for earlier post conceptual age (PCA) in weeks to full feedings (140 mL/kg/d) within stage II NEC including 1) a lower gestational age ($P < 0.0001$); 2) less total feeding (log unit) to NEC diagnosis ($P = 0.0002$); 3) percent of early mother's own milk the first five DOL ($P = 0.0020$); and 4) lower total feedings (log unit) to DOL 14 ($P = 0.0047$). $r^2 = 0.755$. Four factors predicted a greater likelihood for earlier post PCA to full feedings (140 mL/kg/d) within stage III NEC including: 1) a lower gestational age ($P = 0.0002$); 2) no maternal substance abuse ($P = 0.0023$); 3) less percent PHM

to NEC diagnosis ($P = 0.0006$); and 4) a lower percent of 20 cal/oz formula (cow milk based or soy) the first five feeding days (any DOL) ($P = 0.0056$). $r^2 = 0.683$.

We concluded that PCA to full feedings (140 mL/kg/d) in both stage, did not show any practically significant findings that were meaningful based on the selected maternal, neonatal, or feeding type factors in different time periods studied.

Model for Post Conceptual Age in Weeks to Discharge

Seven factors predicted a lower PCA in weeks to discharge within stage III including: 1) maternal race ($P < 0.0001$) (specifically: hispanics were 45.1 PCA in weeks, whites were 45.6 PCA in weeks and other races were 58.6 PCA in weeks), 2) number of RBC transfusions ($P = 0.0009$), 3) higher gestational age ($P = 0.002$), 4) PHM (log unit) the first five feeding days, any DOL ($P = 0.0003$), 5) less early mother's own milk (log unit) the first three DOL ($P = 0.0013$), 6) the percent early mother's own milk the first five DOL ($P = 0.0049$), and 7) less PHM (log unit) the first three DOL ($P = 0.0073$). $r^2 = 0.697$.

We concluded that regression models for PCA to discharge did not show any practically significant findings that were meaningful based on the selected maternal, neonatal, or feeding type factors in different time periods studied.

Models for Bowel Resection

A regression model for small bowel resection among stage III NEC demonstrated that those with an intact small bowel were more likely to have received mothers own milk combined with PHM to NEC diagnosis (OR 0.936, {CI 0.885 to 0.989}, $P = 0.0194$). A regression model for large bowel resection did not demonstrate any significant maternal, neonatal or feeding type factors influencing colonic resection.

All bowel resection (small or large bowel) comparing mild resection vs. moderate/severe resection plus the expired neonates for stage III NEC showed no predicting factors: maternal, neonatal or feeding types in different time periods were significant.

Model for Death from NEC

Five factors significantly predicted the likelihood of death from NEC among those that had stage III disease included 1) a low pH (OR 2.212, {CI 1.27 to 3.853}, $P = 0.0050$), 2) the absence of PIH or HELLP syndrome (OR 0.018, {CI 0.001 to 0.273}, $P = 0.0038$), 3) less early mother's own milk (log unit) for five days (any DOL) (OR 0.961, {CI 0.937 to 0.986}, $P = 0.0025$), 4) less total feedings (log unit) to DOL 14, (OR 0.618, {CI 0.431 to 0.885}, $P = 0.0087$), and 5) less percent of formula to DOL 14 (OR 0.965, {CI 0.937 to 0.993}, $P = 0.0151$).

A regression analysis demonstrated that gestational age increased by 0.08 weeks for each percent of formula the first 14 DOL ($P < 0.0001$) and gestational age also increased by 0.37 weeks for every log of total feedings to DOL 14 ($P = 0.1228$). Thus, formula and total feedings to DOL 14 were more common among the larger gestational age neonates.

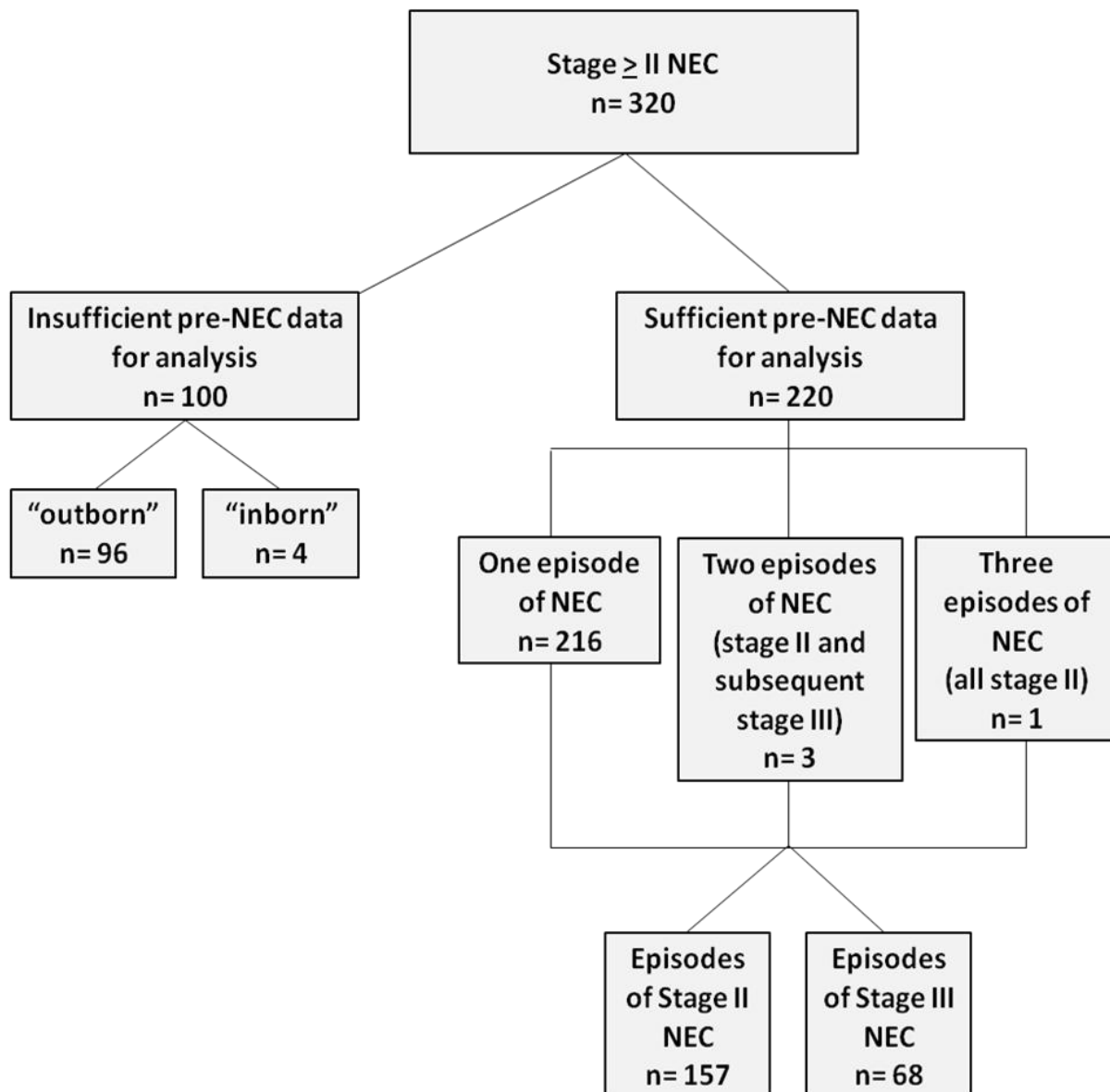


Figure 1 Diagrammatic display of the subjects studied

Table 1 Characteristics of 220 neonates and 225 episodes of stage \geq II NEC

	Stage II n = 157	Stage III n = 68	P-value
Maternal Factors			
	← n (%) →		
PIH and/or HELLP syndrome	29 (18.5)	13 (19.1)	0.909
Smoker	19 (12.1)	7 (10.3)	0.697
Smoker in the home	24 (15.3)	8 (11.8)	0.487
Substance abuse	9 (5.7)	4 (5.9)	0.965
Rupture membranes \geq 18 hrs	32 (20.4)	8 (11.8)	0.260
Unknown	6 (3.8)	4 (5.9)	
Fetal/ Neonatal Factors			
Gender: Male	76 (48.4)	38 (55.9)	0.303
Female	81 (51.6)	30 (44.1)	
Race: White	122 (77.7)	47 (69.1)	0.176
Black	5 (3.2)	1 (1.5)	
Hispanic	21 (13.4)	14 (20.6)	
Other	9 (5.7)	6 (8.8)	
Small for Gestational Age (SGA)	21 (13.4)	10 (14.7)	0.790
Singleton	115 (73.3)	50 (73.5)	0.531
Twin	39 (24.8)	15 (22.1)	
Triplet or Quadruplet	3 (1.9)	3 (4.4)	
Hematocrit \geq 65 percent	4 (2.6)	0 (0.0)	0.184
Proton Pump Inhibitors or Histamine receptor antagonists within 5 days of NEC	99 (63.1)	49 (72.1)	0.191
Least Square Means (95% Confidence Intervals)			
Gestational age (weeks)	32.7 (32.0 - 33.3)	30.0 (29.0 - 30.9)	<0.0001
Birth weight (grams)	1891 (1774-2008)	1371 (1193 – 1549)	<0.0001
Apgar score: 1 minute	5.95 (5.55 – 6.35)	5.34 (4.73 - 5.96)	0.107
Apgar score: 5 minutes	7.83 (7.58 – 8.09)	7.33 (6.93 - 7.72)	0.035
PCA at NEC diagnosis	35.5 (34.9 - 36.1)	32.8 (31.9 - 33.8)	<0.0001
NEC day of life	19.9 (17.1 - 22.7)	20.9 (15.8 - 24.4)	0.946
PCA to full feedings (140 mL/kg/d) after NEC diagnosis	38.55 (37.8 - 39.3)	41.3 (39.8 - 42.7)	0.001
Discharge PCA	40.9 (40.1 - 41.8)	46.5 (44.9 - 48.1)	<0.0001

PIH - pregnancy induced hypertension

HELLP syndrome - a group of symptoms including hemolysis, elevated liver enzymes, and low platelet count

PCA - Post Conceptual Age

Table 2 Laboratory values three days preceding 225 episodes of stage \geq II NEC

	Stage II NEC n= 157		Stage III NEC n = 68		P-value
	Means	s.d. *	Means	s.d.	
Highest C-Reactive Protein (CRP) mg/dL	2.08	4.09	5.26	5.35	<0.0001
Highest Leukocyte count $10^3/\mu\text{L}$	13.34	5.45	16.01	11.98	0.029
Highest I/T neutrophil ratio**	0.26	0.87	0.55	0.29	0.001
Lowest Neutrophil Count $10^3/\mu\text{L}$	4.60	3.29	4.99	6.59	0.570
Lowest pH (arterial or venous)	7.32	0.09	7.15	0.13	<0.0001
Lowest Platelet Count $10^3/\mu\text{L}$	339.3	136.0	175.7	128.7	<0.0001
Highest Mean Platelet Volume (MPV) fL	9.50	1.47	10.65	1.69	<0.0001

* Standard deviation

**Ratio of immature neutrophils (metamyelocytes and bands) to total neutrophils (segmented neutrophils plus bands plus metamyelocytes)

Table 3 Feeding types preceding 225 episodes of stage \geq II NEC

		Stage II n= 157 LSM (95% CI)*	Stage III n = 68 LSM (95% CI)	P-value
Total volume	mL	1252 (953 - 1644)	527 (362 - 828)	0.0012
Mother's own milk (MOM)	mL	164.3 (113.0 - 238.8)	90.1 (51.0 - 159.1)	0.0834
% MOM		31.7 (26.9 - 36.5)	37.6 (30.3 - 45.0)	0.1823
Pasteurized human milk (PHM)	mL	1.5 (1.1 - 1.9)	2.7 (1.9 - 4.1)	0.0072
MOM + PHM	mL	146.8 (98.8 - 218.2)	112.9 (62.1 - 205.3)	0.4705
% MOM and PHM		29.7 (24.9 - 34.5)	39.7 (32.4 - 47.0)	0.0254
22-24 cal/oz MOM using powdered human milk fortifier (HMF)	mL	33.4 (19.5 - 56.9)	19.6 (8.7 - 44.2)	0.2841
22-24 cal/oz PHM using powdered HMF	mL	1.3 (1.0 - 1.7)	2.1 (1.5 - 3.1)	0.0376
22-30 cal/oz MOM + PHM using powdered HMF	mL	37.5 (21.6 - 65.2)	25.95 (11.22 - 60.0)	0.4705
% 22-30 cal/oz MOM + PHM using powdered HMF		26.4 (21.4 - 31.4)	25.4 (17.9 - 32.9)	0.8278
20 cal/oz cow milk based and soy formula	mL	22.8 (14.6 - 35.7)	8.7 (4.4 - 17.2)	0.0210
22-24 cal/oz cow milk based and soy formula	mL	7.2 (4.7 - 11.2)	4.5 (2.3 - 8.9)	0.2540
20 cal/oz Hydrolyzed formula	mL	2.1 (1.6 - 2.9)	1.7 (1.0 - 2.7)	0.4163
22-24 cal/oz Hydrolyzed formula	mL	1.4 (1.1 - 1.7)	1.2 (0.9 - 1.6)	0.4525
20 cal/oz cow milk based, soy and hydrolyzed formula	mL	33.3 (20.8 - 53.1)	10.6 (5.2 - 21.6)	0.0111
% 20 cal/oz cow milk based, soy, and hydrolyzed formula		26.3 (21.0 - 31.5)	19.8 (11.7 - 27.9)	0.1877
22-30 cal/oz cow milk based, soy and hydrolyzed formula	mL	9.7 (6.1 - 15.5)	6.2 (3.0 - 12.7)	0.3045
% 22-30 cal/oz cow milk based, soy, and hydrolyzed formula		11.5 (8.1 - 15.0)	9.3 (4.0 - 14.5)	0.4724

*Least square means (unlogged) and 95% confidence intervals

Table 4 Red blood cell (RBC) and platelet transfusions preceding 225 episodes of stage \geq II NEC

	Stage II NEC n = 157 LSM (95% CI)*	Stage III NEC n = 68 LSM (95% CI)	P-value
RBC Transfusions	1.8 (1.6 – 2.1)	4.0 (3.2 – 4.9)	<0.0001
Platelet Transfusions	1.1 (1.0 – 1.3)	1.7 (1.5 – 2.0)	<0.0001

*Least square means (95% confidence intervals)

Table 5 Morbidity and mortality of 220 neonates with 225 episodes of stage \geq II NEC

	Stage II NEC n = 157	Stage III NEC n= 68	P-value
← n (%) →			
Positive cultures (within 7 days before NEC)			0.0321
Blood	7 (4.5)	7 (10.3)	
Urine	1 (0.6)	3 (4.4)	
None	149 (94.9)	58 (85.3)	
Positive cultures (within 7 days before and after NEC)			0.0001
Blood	27 (17.2)	23 (33.8)	
Urine	6 (3.8)	3 (4.4)	
Multiple	2 (1.3)	7 (10.3)	
None	122 (77.7)	35 (51.5)	
Peritoneal Cultures (within 7 days after NEC)	0 (0)	10 (14.7)	<0.0001
Vasopressors/ nitric oxide (within 5 days before and after NEC)			
Dopamine	7 (4.5)	48 (70.6)	<0.0001
Dobutamine	1 (0.6)	14 (20.6)	<0.0001
Milirone	2 (1.3)	5 (7.4)	0.0159
Nitric Oxide	1 (0.6)	3 (4.4)	0.0491
Small bowel resection			
None	157 (100)	6 (8.5)	<0.0001
<20 cm		27 (39.7)	
20-75 cm		17 (25.0)	
>75 cm		13 (19.1)	
Died before surgery		5 (7.4)	
Colonic resection			
None	157 (100)	22 (32.4)	<0.0001
1 section		7 (10.3)	
2 sections		12 (17.7)	
3 sections		22 (32.4)	
Died before surgery		5 (7.4)	
Total Mortality	5 (3.2)	28 (41.2)	<0.0001
Mortality from NEC	0 (0)	24 (35.3)	<0.0001

Table 6 Feeding types the first 3 days of life preceding 225 episodes of stage \geq II NEC

Variable	Stage II NEC n = 157 LSM (95% CI)*	Stage III NEC n = 68 LSM (95% CI)	P-value
Early mother's own milk (MOM) mL	3.3 (2.6 - 4.20)	2.1 (1.5 - 3.0)	0.0489
% Early MOM	20.5 (15.2 - 25.8)	15.8 (7.8 - 23.8)	0.3329
Pasteurized human milk (PHM) mL	1.3 (1.1 - 1.4)	1.4 (1.2 - 1.7)	0.3480
20 cal/oz cow milk based and soy based formula mL	6.7 (4.8 - 9.3)	3.5 (2.1 - 5.8)	0.0383
% 20 cal/oz cow milk based and soy based formula	38.2 (31.3 - 45.0)	26.3 (15.8 - 36.8)	0.0631

*Least square means and 95% confidence intervals

Table 7 Feeding types the first 5 days of life preceding 225 episodes of stage \geq II NEC

Variable	Stage II NEC n = 157 LSM (95% CI)*	Stage III NEC n=68 LSM (95% CI)	P-value
Early mother's own milk (MOM) mL	12.6 (8.9 - 17.7)	6.1 (3.6 - 10.3)	0.0234
% Early MOM	36.4 (30.0 - 42.9)	35.5 (25.8 - 45.2)	0.8762
Pasteurized human milk (PHM) mL	1.3 (1.1 - 1.5)	1.5 (1.2 - 1.8)	0.4288
22-24 cal/oz early MOM using powdered human milk fortifier (HMF) mL	1.1 (1.0 - 1.2)	1.0 (0.9 - 1.1)	0.3523
20 cal/oz cow milk based and soy based formula mL	11.5 (7.8 - 17.1)	4.5 (2.5 - 8.3)	0.0116
% 20 cal/oz cow milk base and soy based formula	33.8 (27.5 - 40.0)	22.6 (13.1 - 32.2)	0.0564

*Least square means and 95% confidence intervals

Table 8 Feeding types the first 14 days of life preceding 225 episodes of stage \geq II NEC

Variable	Stage II NEC n= 157 LSM (95% CI)*	Stage III NEC n = 68 LSM (95% CI)	P-value
Total volume mL	369 (258 – 529)	136 (79 – 235)	0.0030
Mother's own milk (MOM) mL	78.9 (53.2 – 116.7)	38.5 (20.7 - 67.9)	0.0406
% MOM	44.5 (38.5 - 50.6)	53.0 (43.7 - 62.3)	0.1361
Pasteurized human milk (PHM) mL	1.4 (1.2 – 1.8)	1.8 (1.3 – 2.5)	0.2197
MM + PHM mL	71.4 (47.6 – 107.2)	45.4 (24.4 - 84.1)	0.2195
% MM + PHM	40.0 (33.8 - 45.9)	51.2 (42.1 - 60.4)	0.0410
22-24 cal/oz MOM using powdered human milk fortifier (HMF) mL	11.3 (7.1 – 17.8)	4.5 (2.2 – 9.0)	0.0307
22-24 cal/oz PHM using powdered HMF mL	1.2 (1.0 – 1.4)	1.4 (1.1 – 1.8)	0.3128
22-30 cal/oz MOM + PHM using powdered HMF mL	11.6 (7.3 – 18.6)	5.3 (2.6 – 10.8)	0.0709
% 22-30 cal/oz MOM + PHM using powdered HMF	17.3 (13.3 – 21.3)	13.4 (7.3 – 19.5)	0.2959
20 cal/oz cow milk based or soy based formula mL	12.2 (7.9 – 18.8)	5.3 (2.8 – 10.3)	0.0388
22-24 cal/oz cow milk based or soy based formula mL	3.2 (2.3 - 4.5)	1.8 (1.1 – 3.0)	0.0559
20 cal/oz hydrolyzed formula mL	1.5 (1.2 - 1.8)	1.2 (0.9 – 1.7)	0.3443
22-24 cal/oz hydrolyzed formula mL	1.2 (1.0 - 1.3)	1.0 (0.8 – 1.2)	0.1938
20 cal/oz cow milk based, soy and hydrolyzed formula mL	15.9 (10.3 – 24.7)	6.2 (3.2 – 12.0)	0.0040
% All 20 cal /oz cow milk based, soy, and hydrolyzed mL	23.5 (18.2 – 28.8)	18.2 (10.1 – 26.2)	0.2745
22-30 cal/oz combined fortified cow milk based, soy, and hydrolyzed formula mL	3.6 (2.6 – 5.2)	1.8 (1.0 - 3.0)	0.0277
% combined 22-30 cal/oz cow milk based, soy, and hydrolyzed formula	7.3 (4.41 – 10.1)	4.1 (-0.3 - 8.4)	0.2278

*Least square means and 95% confidence intervals

Table 9 Feeding types the first 5 feeding days (any day of life) preceding 225 episodes of stage \geq II NEC

Variable	Stage II NEC n = 157 LSM (95% CI)*	Stage III NEC n= 68 LSM (95% CI)	P-value
Mother's own milk (MOM) mL	73.8 (53.4- 102.1)	32.5 (19.9 – 53.1)	0.0063
% MOM	60.6 (54.3 – 66.9)	61.9 (52.4 – 71.5)	0.8228
Pasteurized human milk (PHM) mL	1.3 (1.1 – 1.6)	1.7 (1.3 – 2.1)	0.1854
22-24 cal/oz MOM using powdered human milk fortifier (HMF) mL	1.3 (1.1 – 1.5)	1.1 (0.9 – 1.40)	0.4115
20 cal/oz cow milk based and soy formula mL	18.7 (12.3 – 28.5)	6.6 (3.5 – 12.5)	0.0079
% 20 cal/oz cow milk based formula	32.9 (26.8 – 39.1)	25.0 (15.6 - 34.3)	0.1624

*Least square means and 95% confidence intervals

Table 10 Feeding types preceding 225 episodes of stage \geq II NEC

Variable	Stage II NEC n= 157 LSM (95% CI)*	Stage III NEC n = 68 LSM (95% CI)	P-value
Total volume mL	1252 (953 - 1644)	527 (362 - 828)	0.0012
Mother's own milk (MOM) mL	164.3 (113.0 - 238.8)	90.1 (51.0 - 159.1)	0.0834
% MOM	31.7 (26.9 - 36.5)	37.6 (30.3 - 45.0)	0.1823
Pasteurized human milk (PHM) mL	1.5 (1.1 - 1.9)	2.7 (1.9 - 4.1)	0.0072
MOM + PHM mL	146.8 (98.8 - 218.2)	112.9 (62.1 - 205.3)	0.4705
% MOM + PHM	29.7 (24.9 - 34.5)	39.7 (32.4 - 47.0)	0.0254
22-24 cal/oz MOM using powdered human milk fortifier (HMF) mL	33.4 (19.5 - 56.9)	19.6 (8.7 - 44.2)	0.2841
22-24 cal/oz PHM using powdered HMF mL	1.3 (1.0 - 1.7)	2.1 (1.5 - 3.1)	0.0376
22-30 cal/oz MOM+PHM using powdered HMF mL	37.5 (21.6 - 65.2)	25.95 (11.22 - 60.0)	0.4705
% 22-30 cal/oz MOM + PHM using powdered HMF	26.4 (21.4 - 31.4)	25.4 (17.9 - 32.9)	0.8278
20 cal/oz cow milk based and soy formula mL	22.8 (14.6 - 35.7)	8.7 (4.4 - 17.2)	0.0210
22-24 cal/oz cow milk based and soy formula mL	7.2 (4.7 - 11.2)	4.5 (2.3 - 8.9)	0.2540
20 cal/oz hydrolyzed formula mL	2.1 (1.6 - 2.9)	1.7 (1.0 - 2.7)	0.4163
22-24 cal/oz hydrolyzed formula mL	1.4 (1.1 - 1.7)	1.2 (0.9 - 1.6)	0.4525
20 cal/oz cow milk based, soy, and hydrolyzed formula mL	33.3 (20.8 - 53.1)	10.6 (5.2 - 21.6)	0.0111
% 20 cal/oz cow milk based, soy, and hydrolyzed formula mL	26.3 (21.0 - 31.5)	19.8 (11.7 - 27.9)	0.1877
22-30 cal/oz cow milk based, soy and hydrolyzed formula mL	9.7 (6.1 - 15.5)	6.2 (3.0 - 12.7)	0.3045
% 22-30 cal/oz fortified cow milk based, soy, and hydrolyzed formula	11.5 (8.1 - 15.0)	9.3 (4.0 - 14.5)	0.4724

*Least square means and 95% confidence intervals

APPENDIX D: COMPLETE DISCUSSION

The most significant associations with severe NEC we identified were earlier gestational age, lower birth weight and larger volumes of RBC transfusions administered prior to NEC diagnosis. The most significant protective factor from severe NEC we identified was early mother's own milk feedings. However, this protective effect was not observed for pasteurized human milk. However, no cause and effect are claimed with the association of RBC transfusions and severity of NEC.

Many reports have identified an association of RBC transfusions and the development of NEC⁽¹⁻²⁾ but this is the first report demonstrating the association between RBC transfusions and NEC severity. A recent study indicated that 1) 25-30 percent of NEC cases followed a RBC transfusion generally within 6 to 24 hours, 2) neonates with transfusion-associated NEC are generally born at earlier gestational age than those with NEC and no antecedent transfusion, and 3) neonates with transfusion-associated NEC have generally had one or more previous RBC transfusions before the one that precipitates NEC.⁽³⁾ Our study demonstrated an association of RBC transfusions with the severity of NEC.

As the log unit of pasteurized human milk increased prior to NEC diagnosis, there was a 32 percent increased likelihood for stage III. Distinct differences exist between pasteurized human milk and fresh maternal milk. The process of pasteurization (Holder method = 62.5 degrees C for 30 minutes) inactivates cellular components of milk, including T cell and B cells, macrophages, and neutrophils.⁽⁴⁻⁶⁾ Concentration of immunoglobulins A and G are also significantly reduced, as are lactoferrin,⁽⁶⁻⁷⁾ lysozymes,⁽⁸⁾ and erythropoietin.⁽⁹⁻¹⁰⁾ Some growth factors have been reported to be reduced by pasteurization, including insulin-like growth factor-1 and insulin-like factor-2,⁽¹¹⁾ whereas others, like epidermal-like growth factor (EGF) and transforming growth factor-beta appear capable of withstanding heat treatment.⁽¹²⁾ As human

milk banking increases, it is critical to understand the effect of pasteurization on immunological components and to improve processing techniques to better preserve these components.

In contrast, early mother's own milk or colostrum (first five days of feedings) was associated with a reduction in odds for severe NEC (stage III). Colostrum as compared to mature maternal milk has many unique features including higher concentrations of secretory IgA, lactoferrin, anti-inflammatory cytokines, oligosaccharides, soluble CD-14 which binds to whole bacteria and bacterial cell wall components, and antioxidants.⁽¹³⁻¹⁵⁾ Moreover, mothers of the most preterm infants produce colostrum with even higher concentrations of many such factors compared with mothers of term infants.⁽¹⁶⁻¹⁸⁾

The observation that colostrum may decrease NEC severity, which generally occurs some three weeks later, is consistent with the recent observations from Gonzalez-Reivera *et al.*⁽¹⁹⁾ that NEC may be a consequence of events occurring soon after birth. We suggest that feeding colostrum (or lack there of) could be such an event.

Regression models for selected laboratory blood value (highest CRP, lowest leukocytes, highest I/T ratio, lowest neutrophil count, lowest pH, lowest platelet and highest mean platelet volume) demonstrated no meaningful predictors in the severity of NEC. Thus, we concluded that mother's own milk feedings *vs.* formula did not make any significant difference in the severity of NEC as examined by laboratory values.

We found that mother's own milk in combination with PHM had a protective effect on small bowel resection, whereas pasteurized human milk alone did not. However, the model for large bowel resection and severity of small and large bowel resection showed not maternal, neonatal or feeding type factors that predicted outcome.

Post conceptual age to full feedings (140 mL/kg/d) and PCA to discharge showed multiple predictors, but none that were consistent. Thus, we concluded that there were no feeding groups (mother's own milk, PHM or formula) that made a significant difference in achieving an earlier post conceptual age to full feedings post NEC or to discharge.

We also focused on factors associated with death from NEC and found that early colostrum feedings for five days decreased the odds of mortality. Recently, Meizen-Derr *et al.*⁽²⁰⁾ also demonstrated an association between human milk feedings given the first two weeks and reduction in death from NEC. Other factors that decreased the odds of mortality were 1) total feedings to DOL 14 and 2) formula the first 14 DOL. A regression model for gestational age and formula the first 14 DOL indicated that as gestational age increased in weeks, there was an increase in formula use and total feedings given. We suspect that the decreased risk of death by the use of formula and total feedings to DOL 14 was attributed to the larger gestational age neonate who was thus less likely to die from NEC.

We recognize several limitations of our study, foremost of which are those inherent in retrospective data analyses including susceptibility to error and bias. Other flaws include the many factors unknown to us because they were not recorded, namely, the reasons or timing of RBC transfusions, different feeding practices, different types of fortifiers used, and why colostrum was available for some neonates and not for others.

In conclusion, we sought to find associations with severe NEC using data from a multicentered healthcare database. Indeed we observed that neonates who received more RBC transfusions and less colostrum were more likely to have stage III disease. It is not clear whether successful programs to encourage early colostrum feedings would decrease the prevalence of stage III NEC in actual practice. Certainly any salutary effect of such practice changes could

have important beneficial effects for neonates, their families and society. Continued ongoing and future investigative efforts are necessary due to high human and economic costs of stage III NEC disease.

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APPENDIX E: IRB APPROVALS



**Intermountain
Healthcare
Office of Research**

8th Avenue and C Street
Salt Lake City, Utah 84143
801.408.1991
800.321.2107
801.408.6783 Fax
officeofresearch@imail.org

August 24, 2009

Cheryl Miner
Neonatology
Utah Valley Regional Medical Center
1034 N 500 W
Provo, UT 84601

RE: IRB # 1012858 - Feedings and Necrotizing Enterocolitis
Protocol Title: *Types of Feedings affect Necrotizing Enterocolitis (Stage II/III) Presentations and Outcomes: Data from a Multihospital HealthCare system*
On Agenda For: Initial Submission, Expedited Review
Meeting Date: 9/24/2009(to be reported to the committee)
Approved: 8/18/2009
Expires: 8/18/2010

Dear Cheryl Miner:

Your request for **EXPEDITED REVIEW** for the above mentioned research proposal has been reviewed by a member of the Intermountain Healthcare Institutional Review Board and approval has been given for the following:

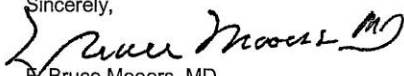
- Protocol Summary Version Dated June 16, 2009
- Waiver of Informed Consent

Approval for this study expires on 8/18/2010. The FDA requires that research projects be reviewed yearly, or more often at the discretion of the IRB Committee. You will receive notification from the IRB Committee when it is time for review of this study. It is your responsibility to respond to this notification or approval for this study will be discontinued. In the meantime, if there are any administrative, procedural or clinical changes please submit them to the IRB for approval prior to making them effective.

It is your responsibility to notify DHHS and/or the FDA, and the Chairperson of the IRB Committee of any occurrence or emergency that seriously increases the risk to or affects the welfare of subjects.

If you have any questions regarding this decision, please contact Elaine Skinner-Ntiri in the IRB Office at (801) 408-6780.

Sincerely,



Bruce Mooers, MD
Chairperson
Intermountain Healthcare
Institutional Review Board





**Intermountain
Healthcare
Office of Research**

8th Avenue and C Street
Salt Lake City, Utah 84143
801.408.1991
800.321.2107
801.408.6783 Fax
officeofresearch@imail.org

July 2, 2010

Cheryl Miner
Neonatology
Utah Valley Regional Medical Center
1034 N 500 W
Provo, UT 84601

RE: IRB # 1012858 - Feedings and Necrotizing Enterocolitis
Protocol Title: *Types of Feedings affect Necrotizing Enterocolitis (Stage II/III) Presentations and Outcomes: Data from a Multihospital HealthCare system*
Meeting Date: 7/22/2010 (to be reported to the Committee)
On Agenda For: Continuing Review of Research – Expedited Review in accordance with 45 CFR 46.110(f)(5)
Approved: 6/16/2010
Expiration Date: 6/16/2011

Dear Cheryl Miner, MD:

The above referenced project has been reviewed by a member of the Intermountain Healthcare Institutional Review Board and continuing approval was recommended.

The FDA requires that research projects be reviewed annually, or more often at the discretion of the IRB. You will be notified when it is time for renewal of this study. It is your responsibility to respond to this notification. If you do not respond, approval of this study will be terminated. In the meantime, if there are any administrative, procedural or clinical changes you will need to submit them to the IRB for approval prior to making them effective.

It is your responsibility to notify DHHS and/or the FDA, and the Chairperson of the IRB Committee of any occurrence or emergency that seriously increases the risk to or affects the welfare of subjects.

If you have questions regarding this decision, please contact Elaine Skinner-Ntiri in the IRB Office at (801) 408-6780.

Approved Items:

- Application for Continuing Review Dated June 15, 2010

Sincerely,

F. Bruce Mooers, MD
Chairperson
Intermountain Healthcare
Institutional Review Board

 **Intermountain
Healthcare
Office of Research**

8th Avenue and C Street
Salt Lake City, Utah 84143
801.408.1991
800.321.2107
801.408.6783 Fax
officeofresearch@imail.org

June 8, 2011

Cheryl Miner
Neonatology
Utah Valley Regional Medical Center
1034 N 500 W
Provo, UT 84601

RE: IRB # 1012858 - Feedings and Necrotizing Enterocolitis
Protocol Title: *Types of Feedings affect Necrotizing Enterocolitis (Stage II/III) Presentations and Outcomes: Data from a Multihospital HealthCare system*
Meeting Date: 6/23/2011 (to be reported to the Committee)
On Agenda For: Continuing Review of Research – Expedited Review in accordance with 45 CFR 46.110(f)(5)
Approved: 6/2/2011
Expiration Date: 6/1/2012

Dear Cheryl Miner:

The above referenced project has been reviewed by a member of the Intermountain Healthcare Institutional Review Board and continuing approval was recommended.

The FDA requires that research projects be reviewed annually, or more often at the discretion of the IRB. You will be notified when it is time for renewal of this study. It is your responsibility to respond to this notification. If you do not respond, approval of this study will be terminated. In the meantime, if there are any administrative, procedural or clinical changes you will need to submit them to the IRB for approval prior to making them effective.

It is your responsibility to notify DHHS and/or the FDA, and the Chairperson of the IRB Committee of any occurrence or emergency that seriously increases the risk to or affects the welfare of subjects.

If you have questions regarding this decision, please contact Elaine Skinner-Ntiri in the IRB Office at (801) 408-6780.

Approved Items:

- Application for Continuing Review Dated May 31, 2011

Sincerely,



F. Bruce Mooers, MD
Chairperson
Intermountain Healthcare
Institutional Review Board

Institutional Review Board
for Human Subjects



Brigham Young University
A-285 ASB Provo, Utah 84602
(801) 422-3841 / Fax: (801) 422-0620

September 25, 2009

Cheryl Miner
1686 E Center
Springville, UT 84663

Re: Presentations and Outcomes of Necrotizing Enterocolitis (Stage II/III) of different feeding types
prior to diagnosis: Data from a Multihospital Health Care System

Dear Cheryl Miner

This is to inform you that Brigham Young University's IRB has approved the above research study.

The approval period is from 9-25-2009 to 9-24-2010. Your study number is X090305. Please be sure to
reference this number in any correspondence with the IRB.

Continued approval is conditional upon your compliance with the following requirements.

All protocol amendments and changes to approved research must be submitted to the IRB and not be
implemented until approved by the IRB.

A few months before this date we will send out a continuing review form. There will only be two
reminders. Please fill this form out in a timely manner to ensure that there is not a lapse in your approval.

If you have any questions, please do not hesitate to call me.

Sincerely,

Lane Fischer, Ph.D., Chair
Santee M.P. Munoz, Administrator
Institutional Review Board for Human Subjects

Institutional Review Board
for Human Subjects



Brigham Young University
A-285 ASB Provo, Utah 84602
(801) 422-3841 / Fax: (801) 422-0620

August 24, 2010

Cheryl Miner
1686 E Center
Springville, UT 84663

Re: X 090305
Presentations and Outcomes of Necrotizing Enterocolitis (Stage II/III) of different
feeding types prior to diagnosis: Data from a Multihospital Health Care System

Dear Cheryl Miner

This is to inform you Brigham Young University's IRB has renewed its approval of the above noted research study.

The approval period is from 8-24-2010 to 9-24-2011. Your study number is X090305. Please be sure to reference either this number and/or the study title in any correspondence with the IRB.

All conditions for continued approval during the prior approval period remain in effect. These include, but are not necessarily limited to the following requirements:

All protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB.

Sincerely,

Lane Fischer, PhD, Chair
Sandee M.P. Munoz, Administrator
Institutional Review Board for Human Subjects

APPENDIX F: MATERIALS

EMPI#	Pt#
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ANTEPARTUM HX: *PIH* *Smoker during pregnancy* *Methamphetamines* *Rupture of membranes:*
HELLP syndrome *Smoker in home* *Cocaine* _____ *hrs*

BIRTH/ TRANSPORT Hx: *Facility* *Discharged* *LOS (d)* *Encounter#*

<i>Birth date</i>	<i>Birthplace</i>			
<i>Admit date</i>				
<i>Admit date</i>				
Hct day 1-3:		PCA at discharge _____ weeks		

DEMOGRAPHICS:

<i>GA</i>	<i>BW</i>	<i>Classification</i>	<i>Birth number</i>	<i>Gender</i>	<i>Apgar Scores</i>		
		<i>SGA AGA LGA</i>		<i>Male</i> <i>Female</i>	<i>1 min</i>	<i>5 min</i>	<i>10 min</i>
Mom's Race: <i>White Hispanic Black Other:</i>							

Gastric Suction Tube Placement **Date** _____ **1st 14 DOL or to NEC _____ mls**

<i>Feedings</i>					
<i>Total amt</i>					
<i>% of total</i>					

Symptoms/ Findings *Abdominal distension* _____ *Pneumatois intestinalis*
Bloody stools _____ *Portal venous gas*
Bilious emesis/ residuals _____ *Pneumoperitoneum*

CULTURES (Blood, urine, CSF) 7 d prior/post gastric suction tube placement From _____ to _____

<i>Date</i>	<i>Site</i>	<i>Results</i>

MEDICATIONS: 5 days prior/ post gastric suction tube placement; From: _____ to _____

Dopamine Nitric Oxide *H2 receptor antagonists* *Proton Pump Inhibitors*
Dobutamine Milrinone *Pepcid (Famotidine)* *Prilosec (Omeprazole)*
Zantac (Ranitidine) *Tagament (Cimetidine)* *Prevacid (Lansoprazole)*

OUTCOMES: *Lived Expired* *NEC Stage II NEC Stage III* *Surgery Date: _____*

All Feedings (ml) prior to GSTP: _____ ml

<i>Feeding Type</i>							
<i>Total Amount (ml)</i>							
<i>% of Total Feedings</i>							

Full Feedings post NEC: _____ **140 cc/kg/d** **PCA: _____ weeks** **Micro** **Labs**

Laboratory values: 3 days prior/post NEC From: _____ to _____

DOL	0	1	2	3	4	5	6	7	8	9	10	11
DATE												
WEIGHT												
Transfusion: RBC												
Transfusion: Platelet												
TYPE of FEEDING												
Total ml												
TYPE of FEEDING												
Total ml												
TYPE of FEEDING												
Total ml												
TYPE of FEEDING												
Total ml												
Emesis												
Enteral fluid (mL/kg/d)												
WBC												
Platelet Count												
Mean Plt Volume												
Platelet Mass (MPV x Plt Count)												
Abs Neutrophil Count												
% Myelocytes												
% Metamyelocytes												
% Bands												
% Segs or Neutrophils/Polys												
I:T <u>Immature cells</u> Total												
CRP												
pH												