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Racial disparities in health outcomes in neonates hospitalized for necrotizing enterocolitis (NEC)

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Racial disparities in health outcomes in neonates hospitalized for necrotizing enterocolitis (NEC)

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## Abstract

Racial disparities in health outcomes in neonates hospitalized for necrotizing enterocolitis (NEC)

By

Lance Kaeo Ching

***Objectives***: Necrotizing enterocolitis (NEC) is an inflammatory bowel disease of newborn infants and a leading cause of significant morbidity and mortality during the neonatal period. The objective of this pilot study is to investigate the relationship between race and two NEC outcomes, case-fatality and length of hospitalization (LOS) among NEC survivors.

***Methods***: A retrospective cohort study was designed. We utilized data collected on all neonatal inpatient admissions to Children's Healthcare of Atlanta (CHOA) between 1 January 2009 and 31 December 2010. Multiple logistic regression analysis was performed to identify independent risk factors for NEC case-fatality. Cox proportional hazard models were used to estimate time-to-hospital-discharge by race.

***Results***: We identified 108 neonates with NEC. Fatality associated with an NEC diagnosis was high at 32.4%, and LOS among NEC survivors averaged 70 $\pm$ 59 days. No significant association was observed between race and survival due to the study's limited statistical power. However, we cannot exclude the possibility of clinically important reductions or increases in either outcome by race. Hispanic infants with NEC had a 3.99 higher odds of death compared to white infants (95% CI: 0.76, 21.00), controlling for gestational age, sex, and payer type. Adjusted estimates for black babies (OR=1.6, 95% CI: 0.57, 4.96) and other race infants (OR=1.7, 95% CI: 0.40, 6.07) were also greater (albeit non-significant) compared to white infants. We did not detect a statistically significant difference in LOS by race in either crude (p=0.3422) or adjusted Cox proportional hazard models (p=0.3860), controlling for gestational age, sex, and payer type. However, the hazard ratio comparing non-Hispanic black neonates to non-Hispanic white infants was 0.85 (95% CI: 0.47, 1.56), indicating slower times to discharge (i.e. longer hospital stays).

***Conclusions***: Our findings are consistent with elevated mortality and LOS for racial minorities. These pilot data provide information on important confounders such as gestational age and birth weight as well as potential modifiable and non-modifiable risk factors. These data will help inform the design of future studies focused on identifying and explaining disparities in both the incidence and outcomes of NEC.

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## Table of Contents

## List of Figures

## List of Tables

## Abbreviations

BPM = beats per minute

CHOA = Children's Healthcare of Atlanta

CI = confidence interval

CORPH = Center for Clinical Outcomes Research and Public Health

ELBW = extremely low birth weight [infants, <1000 g]

g = grams

GBS = Group Beta Streptococcus

HIV = human immunodeficiency virus

ICD-9 = International Classification of Diseases, 9$^{th}$ Revision

IL-4 = interleukin-4

IFN-γ = gamma interferon

LBW = low birth weight [infants, <2500 g]

LOS = Length of Hospital Stay (days)

NDI = neurodevelopmental impairment

NEC = necrotizing enterocolitis

NECSTEPS = Necrotizing Enterocolitis Study Towards Evidence-Based Pediatric Surgery [Trial]

NET = Necrotizing Enterocolitis Trial

NICU = neonatal intensive care unit

NIHCD = National Institute of Child Health and Human Development

NK = natural killer [cell]

OR = odds ratio

PPD = primary peritoneal drainage

RBC = red blood cell

RDS = respiratory distress syndrome

RR = relative risk

SD = standard deviation

SIP = spontaneous intestinal perforation

TANEC = transfusion-associated necrotizing enterocolitis

TNF-α = tumor necrosis factor alpha

VIF = Variance inflation factor

VLBW = very low birth weight [infants, <1500g]

# CHAPTER I

INTRODUCTION

Necrotizing enterocolitis (NEC) is an inflammatory bowel disease of newborn infants and a leading cause of significant morbidity and mortality during the neonatal period. Infants diagnosed with NEC commonly exhibit congested and discolored bowels, feeding intolerances, and bloody stools, among other symptomology(Huda, Chaudhery, Ibrahim, & Pramanik, 2014). In many patients, this condition rapidly progresses to more severe pathology, characterized by gut necrosis (tissue death in the small or large intestines), pneumatosis intestinalis (formation of gas bubbles within the intestinal wall), and pneumoperitoneum (perforation of the intestinal wall) which require surgical intervention. NEC, in fact, is the most common condition for which emergency gastrointestinal surgery is required. Although reports vary considerably by neonatal intensive care unit (NICU), NEC is estimated to affect approximately 2-5% of all NICU admissions or 3-10% of pre-term very low birth weight babies (VLBW, <1500 g) (Kosloske, 1994a; Neu & Walker, 2011; Uauy et al., 1991), with roughly half of cases requiring surgical treatment (Guillet et al., 2006). NEC is also a significant cause of premature mortality. Despite advances in neonatal and surgical care, the overall mortality risk has improved little in the past three decades. Current estimates indicate that death occurs in about 15-30% of VLBW infants with NEC (Caplan & Jilling, 2001; R. H. Clark et al., 2012; Guner et al., 2009; Holman, Stoll, Clarke, & Glass, 1997; Hull et al., 2013). Innovations in NEC prevention and treatment are needed.

The mechanisms involved in initiating inflammation and injury to the gut remain controversial, although NEC is likely the result of several pathophysiological factors imposed on the immature intestine. The modifiable and non-modifiable risk factors are also poorly understood. Several epidemiologic studies reported racial disparities in incidence and mortality of NEC, suggesting that race may be an important predictor of NEC outcomes (Carter & Holditch-Davis, 2008; R. H. Clark et al., 2012; Holman et al., 1997; Llanos et al., 2002). Overall incidence was significantly greater among non-Hispanic black than non-Hispanic white infants and continued to be significant after correction for birth weight (Llanos et al., 2002). A previous study also found that mortality due to NEC is higher for black Americans than for white Americans, even after controlling for birth weight, sex, gestational age, APGAR scores, and geographical region (Holman et al., 1997). Further studies, however, are needed to validate the association between race and NEC outcomes. Moreover, there is a need to investigate other risk factors including maternal factors of black women that may explain racial disparities in NEC outcomes. Given that race is a non-modifiable characteristic, further research to determine its level of contribution to NEC

can equip providers with information needed to alter care plans and account for potential increased sensitivities in the bowels of black preterm infants.

The objective of this study is to investigate racial disparities in diverse NEC outcomes. We utilized data collected on all neonatal inpatient admissions to Children's Healthcare of Atlanta (CHOA) between 2009 and 2010. In 2009, CHOA managed 599,420 patient visits that resulted in 25,346 hospital admissions. The concentration (and diversity) of neonatal patients treated at CHOA provides researchers with an opportunity to conduct a pilot investigation examining the relationship between race and NEC. We describe the population of neonates treated for NEC at CHOA and provide an initial assessment of racial disparities for two NEC outcomes, case-fatality and length of hospital stay. Pilot data will also provide information on potential modifiable and non-modifiable risk factors accounting for racial differences. These data will help inform the design of future studies focused on identifying and explaining disparities in both the incidence and outcomes of NEC.

# CHAPTER II

LITERATURE REVIEW

## A BRIEF HISTORY

Necrotizing enterocolitis (NEC) is not a new disease but one that has been reported since special care units began to house preterm infants. Although the term "necrotizing enterocolitis" was not coined until 1953 through the work of Kurt Schmidt (Schmidt, 1952) and Karl Quaiser (Quaiser, 1952), clinical and post-mortem features of NEC as currently defined can be found in medical reports dating as far back as the early 19th century. Descriptions of NEC disease were seen in foundling hospitals throughout Paris (Billard, 1828) and Vienna (Bednar, 1850), and as the disease occurred in clusters, it was inaccurately regarded as a nosocomial infection in infant hospitals throughout Berlin (Ylppö, 1931) and Zurich (Willi, 1944).

### Early Reports

In 1823, the Paris Athénée de Médecine offered a scientific prize to 'describe, following precise observations, the anatomic characteristics specific for inflammation of the gastrointestinal mucous membrane' (Obladen, 2009). The prize was won by 24-year-old-intern, Charles Billard. Billard completed his medical training at the Hôpital des Enfants Trouvés where more than 5,300 infants were admitted in 1826. Billard gained ample opportunity for clinical and postmortem observations in neonates. By 1828, he published a book and described what is argued as the first case report of NEC. In his publication he describes a neonatal disease which he termed 'gangrenous enterocolitis' to illustrate a small and weak infant with infection, inflammation, and necrosis of the gastrointestinal tract (Billard, 1828):

*"Caroline Jossey, 9 days old ... When opening the body on the next morning ... the terminal ileum is intensely red and swollen, its mucosa friable and the surface covered with blood. When these fluids are removed, the membrane looks rough and bloody; its surface furrowed by numerous wrinkles between which there are deep and black lines with the aspect of being burned by nitric acid. In addition to these blackish furrows, there are a large number of spots or ecchymoses with the same appearance in different regions of the colon."*

Similar reports were made by Alois Bednar in 1850 in a series on 25 infants in a Vienna hospital (Bednar, 1850). Bednar, the hospital's director, described these patients as displaying non-specific clinical

signs that rapidly progressed and resulted in death and who had "gangrenous enterocolitis" upon post-mortem examination:

*"… the mucosa of small and large intestines swollen, injected, in the colon often a large number of millet-sized dirty-dark red spots ... In addition, the mucosa including the submucous tissue frequently corroded ... in many areas of the small intestine yellow grey infiltrates with a tendency towards gangrene."*

**20th Century to Present**

Despite evidence of NEC disease in anecdotal and small case series reports from the 19th century (Obladen, 2009), NEC remained relatively rare and was not formally recognized until special care units facilitated the survival of premature infants. Before the advent of modern neonatal intensive care, prognosis of very immature infants was poor and most did not survive beyond the initial critical days after birth for NEC to manifest. In fact, Alexander Schaffer, a well-known Baltimore pediatrician who coined the term *neonatology*, did not mention NEC in his 1965 issue of 'Diseases of the Newborn' (Schaffer, 1965), considered one of the most highly recognized handbooks on newborn diseases at the time.

The clinical description of NEC began to emerge in the first part of the 20th century, with the opening of special care nurseries throughout Europe. In 1938, Thomas Botsford and Cecil Krakower anatomically described a defining feature of NEC disease, pneumatosis intestinalis, in 6 infants (Botsford, 1938). Then in 1951, Ann Arbor radiologist Arthur Stiennon observed pneumatosis by X-ray (Stiennon, 1951). The modern era of NEC was ushered in by W.E. Berdon from New York Babies Hospital, who in 1964 characterized the whole spectrum of radiographic features of NEC in 21 infants (Berdon et al., 1964). In 1978, Martin Bell combined historical, clinical, and radiographic data to classify NEC into three stages based on the severity of the clinical presentation and recommended treatment strategies (Bell et al., 1978). Bell's staging criteria for NEC was further modified by Walsh and Kleigman and is now a widely accepted classification (Walsh & Kliegman, 1986). The introduction of exogenous surfactant in 1990 to treat preterm infants with respiratory problems changed the face of NEC around the globe (see section, 'Pre- and Post-Surfactant Era'). These treatments combined with other advances in clinical care meant that more neonates were surviving the traditional diseases of prematurity and were now at risk of acquiring NEC (Holman et al., 1997; Horbar, Wright, & Onstad, 1993). By the turn of the century, NEC

became the most widely recognized gastrointestinal emergency among neonates in the world, affecting 3-10% of very-low-birth-weight infants in Canada and the United States and killing approximately 500 infants per year in the United States (Holman et al., 1997).

Despite incremental advances in our understanding of the clinical presentation and pathophysiology of this disease, NEC pathogenesis remains unclear and its distinction from related disorders ambiguous. Universal prevention of this serious and often fatal disease continues to elude us in the 21st century.

## NEC CLASSIFICATION

The clinical manifestations associated with NEC vary considerably among infants and exist on a spectrum, ranging from a very slow, indolent course to a rapidly progressive illness resulting in death in just a few hours. This variation presents a challenge to clinicians aiming to diagnose the disease at the earliest and least severe stage of pathogenesis. Diagnosis generally relies on a combination of clinical symptoms, radiographic signs, and/or data attained by abdominal ultrasound. Assigning disease severity for NEC is also important in the diagnosis and management of the disease.

### Bell Modified Staging Criteria

Bell Staging has traditionally been the standard since 1978 in assigning severity of disease to NEC cases. Three stages were outlined to enhance the recognition and diagnosis of NEC and provide guidance on effective treatments (Bell et al., 1978). As our understanding of NEC evolved, the proposed staging was modified to incorporate further specificity into each disease stage (Table 2.1) (Walsh & Kliegman, 1986). Newly included in the staging criteria are lab values of acidosis (increased acidity in the blood or tissues), thrombocytopenia (decreased platelets), neutropenia (low white blood cells), and hyponatremia (electrolyte imbalance), in addition to intestinal signs of absent bowel sounds and abdominal tenderness. Ascites (accumulation of fluid in the abdominal cavity) as demonstrated by ultrasound is also incorporated into the modified staging criteria by Walsh and Kleigman. Even with subsequent changes, however, it has recently been suggested that the Bell Staging criteria are outdated. Nonetheless, Bell Staging remains the standard of practice for diagnosing and staging NEC in the NICU.

**Table 2.1: Modified Bell Staging Criteria for NEC**

| STAGE | SYSTEMIC SIGNS | INTESTINAL SIGNS | RADIOLOGICAL SIGNS | TREATMENT |
|---|---|---|---|---|
| **Ia** Suspect NEC | Temperature instability, apnea, bradycardia, lethargy | Unremarkable, poor feeding, vomiting, some abdominal distension | Unremarkable | Bowel rest, IV fluids, gastric decompression tube, laboratory tests and close monitoring, educate and support parents |
| **Ib** Suspect NEC | Same as Ia | Stage Ia + bright blood stools | Same as Ia | Same as Ia |
| **IIa** Proven, mildly ill | Mild metabolic acidosis, mild thrombocytopenia | Decreased or absent bowel sounds, mild abdominal tenderness | Pneumatosis intestinalis, bowel loops, sentinel loops | Serial radiographs, continue vigilant monitoring of patient's clinical status, gastric decompression, antibiotics, surgical consult |
| **IIb** Proven, moderately ill | Metabolic acidosis, thrombocytopenia, hyponatremia | Absent bowel sounds, marked abdominal tenderness, abdominal wall discoloration | Stage IIa + portal venous gas, possible ascites | Stage IIa + supportive treatment |
| **IIIa** Advanced, intact bowel | Stage II + hypotension, respiratory acidosis, neutropenia | Stage II + marked distension of abdomen | Stage II + ascites | Stage II + surveillance for gram negative bacteria |
| **IIIb** Advanced, bowel perforated | Same as IIIa | Same as IIIa | Stage IIIa + pneumoperitoneum | Stage IIIa + emergency surgery, postoperative care |

- **Stage I**, includes patients who present the mildest symptoms. Clinical manifestations include temperature instability, lethargy, apnea (breathing suspension), and bradycardia (low heart rate under 60 beats per minute, bpm). The infant may vomit, present with a mildly distended abdomen, exhibit bright red blood in the stool, and/or have feeding intolerances. Stage I diagnoses are considered suspect NEC cases since many stage I presentations are commonly associated with other conditions. Over-diagnosis of stage I NEC, thus, is common.

- **Stage II**, includes suspect NEC cases whose disease has progressed to include pneumatosis intestinalis, a hallmark radiological sign of NEC disease. Stage II patients generally present signs that are more indicative of NEC than stage I and thus, are considered proven NEC cases. Abdominal distention and tenderness may be marked, and blood tests may show thrombocytopenia or

hyponatremia. Bowel loops that create functional or mechanical obstructions in the intestines may also present upon radiographic evaluation.

- **Stage III**, includes advanced NEC cases. These infants show a deterioration of vital signs (e.g. low blood pressure) and may have marked gastrointestinal bleeding, requiring surgical intervention. In addition to the symptoms present in stages I and II, stage III is characterized by portal venous gas (accumulation of gas in the portal vein) and pneumoperitoneum (perforation of the intestinal wall) with gut necrosis. In a subset of patients, the disease takes on a rapid and fulminating course wherein the entire bowel is irreversibly damaged. This severe form of NEC, termed 'NEC totalis' or 'fulminant NEC', has a mortality of more than 90% (Henry & Lawrence Moss, 2005; Lambert et al., 2012).

**Alternate Classification System**

Since Bell's 1978 work, new data have emerged prompting a debate about further revisions to the Bell Stage classifications. Some researchers such as *Gordon* and colleagues proposed abandoning the Bell system altogether in favor of one that differentiates between "medical NEC" versus "surgical NEC" cases (P. V. Gordon, Swanson, Attridge, & Clark, 2007). It is widely inferred that infants with NEC receiving an operation (surgical NEC) harbor a more severe form of the disease than those treated without surgery (medical NEC) (see section "Disease Management") (Guthrie et al., 2003). Although there are no large-scale cohort studies directly evaluating the mortality of surgical versus medical NEC by birth weight categories, a classification system based on such data could potentially afford a more accurate determination of prognosis and may be used as a basis for future quality improvement efforts. Gordon's Classification also focuses on improving the specificity of the intestinal disease diagnosis by excluding cases of spontaneous intestinal perforation, or SIP (P. V. Gordon et al., 2007). SIP is a separate clinical entity from NEC and requires different management considerations. SIP commonly presents as pneumoperitoneum without pneumatosis or portal venous gas but is readily misclassified as Bell's stage IIIb. A diagnosis of SIP is difficult to make without surgical resection. Thus, some epidemiological studies may overestimate the prevalence of NEC (Huda et al., 2014). Gordon's Classification focuses on the different etiologies of the two diseases in an attempt to provide a clearer distinction between NEC and SIP cases.

### NEC Reductionism

More recently, Gordon's system was coupled with a concept called 'NEC reductionism', which categorizes confirmed NEC cases into 5 reproducible clinical subsets based on disease etiology: (i) ischemic NEC, (ii) NEC associated with packed red blood cell (RBC) transfusions, (iii) NEC associated with cow milk intolerance, (iv) NEC associated with prematurity and delayed feeding, and (v) NEC associated with allergies or contagions (P. Gordon, Christensen, Weitkamp, & Maheshwari, 2012). By classifying NEC according to proposed risk factors, NEC reductionism improves one's ability to predict the types (and timing) of triggers that precipitate NEC. The new classification groups put forth by *Gordon* and colleagues have neither been widely cited nor incorporated into practice, to date. However, they provide an analysis of current neonatal intestinal disease diagnoses that warrants further discussion.

## EPIDEMIOLOGY

### Incidence, Prevalence, & Mortality

NEC is one of the most common and devastating gastrointestinal emergencies in newborn infants. It is also considered one of the most difficult to eliminate, and thus, has become a priority of scientific study. The latter half of the 20$^{th}$ century witnessed an increase in NEC cases as medical technology improved the survival of small infants. NEC is estimated to affect 1 to 3 in 1,000 live births and 2% to 5% of infants who are admitted to NICUs in the United States, or roughly 2,500 to 5,000 incident cases annually (Stoll, 1994). The mean prevalence is about 7% among infants with a birth weight between 500 and 1500 g (Guillet et al., 2006; Holman et al., 2006; Horbar et al., 2002).

NEC also results in significant morbidity and mortality. 10% to 30% experience morbidities ranging from neurodevelopmental complications to vision and hearing impairments, failure to thrive, feeding abnormalities, diarrhea, bowel obstruction, and short bowel syndrome (Lee & Polin, 2003; P. W. Lin & Stoll, 2006). Although many NEC patients are managed medically, roughly 20% to 40% require surgery given inadequate treatments and no effective preventative strategies (Guillet et al., 2006; P. W. Lin & Stoll, 2006). Some of these cases are due to progression of disease during medical treatment, but many infants also present acutely with severe disease and require immediate operation. The estimated death risk associated with NEC is between 15% to 30% (Fitzgibbons et al., 2009; Guillet et al., 2006; Guthrie et al., 2003; Holman et al., 2006; Llanos et al., 2002; Luig & Lui, 2005). The highest risks occur

among infants requiring surgery. The case fatality among medical NEC cases ranges between 5% and 10% (P. W. Lin & Stoll, 2006). In comparison, the case fatality associated with surgical intervention is between 23% and 36%, although some studies show fatality as high as 50% (P. W. Lin & Stoll, 2006).

**Pre- & Post-Surfactant Eras**

In 1990, exogenous surfactant was introduced to treat infants with respiratory distress syndrome (RDS). Widespread use of surfactant was linked to significant improvements in infant survival, particularly among preterm babies (Horbar et al., 1993). *Holman* and colleagues examined annual U.S. death certificate data for 1979 through 1992 to compare NEC infant mortality in the pre- and post-surfactant eras (Holman, Stehr-Green, & Zelasky, 1989; Holman et al., 1997). From 1979 to 1985 (pre-surfactant era), NEC mortality decreased significantly from14.5 deaths per 100,000 live births to 10.2 deaths per 100,000 live births. This decrease was attributed primarily to improvements in neonatal care. From 1990 through 1992 (post-surfactant era), however, NEC mortality risk increased to 12.3 deaths per 100,000 live births at a time when *overall* neonatal and post-neonatal mortality risk declined in the United States (Guyer, Strobino, Ventura, & Singh, 1995). These data suggest that improvements in survival of smaller, more immature infants – those at greatest risk for NEC – may be accompanied by increases in NEC-associated infant mortality. As more preterm infants survive early causes of prematurity, the pool of infants at risk for acquiring (and dying of) NEC is likely to grow. In the past 30 years since the introduction of surfactant, NEC mortality has improved little (Caplan & Jilling, 2001). Identifying risk factors for NEC might improve disease diagnosis and lead to interventions that decrease the risk of NEC in preterm infants.

**Economic Costs**

The financial burden of NEC to the U.S. health care system is substantial. Hospitalization costs alone are estimated at $500 million to $1 billion per year (Neu & Walker, 2011). To understand some of these costs, several groups examined the impact of NEC on length of hospitalization stay (Bisquera, Cooper, & Berseth, 2002; Ganapathy, Hay, & Kim, 2012). *Bisquera* and colleagues determined the impact of NEC on length of stay for two NICUs at Baylor College of Medicine. During a 2-year period from 1992-1994, medical NEC survivors were hospitalized ($\pm$SD) on average 95 $\pm$ 42 days, or roughly 22 days more than other preterm infants (73 $\pm$ 27 days) ($p<0.001$). Surgical NEC survivors had

hospitalization stays of 142 ± 65 days that exceeded those of other preterm infants (82 ± 31 days) by 60 days ($p<0.001$) (Bisquera et al., 2002). Based on length of stay, estimated hospital charges for infants with medical NEC averaged $73,700 in excess of controls, and $186,200 more for infants with surgical NEC. In total, the additional yearly hospital charges for NEC in this single neonatal community were $6.5 million, or $216,666 per survivor. Similar findings were captured in a later study conducted for the State of California in 2007 (Ganapathy et al., 2012). Medical NEC cases in California averaged 11.7 days (95% CI: 6.9 to 16.5 days) longer in the hospital than infants without NEC and had hospitalization charges in excess of $74,004 (95% CI: $47,051 to $100,957). Surgical NEC cases in California averaged 43.1 days (95% CI: 36.3 to 50 days) longer in the hospital than infants without NEC and had hospitalization charges in excess of $198,040 (95% CI: $159,261 to $236,819). Both studies demonstrate that a diagnosis of NEC often leads to long hospitalization periods which are very costly for the individual patient as well as the neonatal community.

Hospital expenditures alone, however, provide only very conservative cost estimates of NEC, since there are many adverse consequences beyond the initial hospitalization period (see section "NEC Morbidities"). Infants who survive advanced NEC are at increased risk of having poor long-term physiological and neurodevelopmental growth. For example, bowel resection among survivors is associated with complications such as short gut syndrome, a condition that causes malabsorption of essential vitamins and minerals and requires prolonged administration of parenteral nutrition (Blakely, Gupta, & Lally, 2008; Cikrit, West, Schreiner, & Grosfeld, 1986; Ladd et al., 1998). There is also growing evidence of a possible link between surgical NEC and neurodevelopmental impairment, leading to poor health outcomes in the long-term (Bedrick, 2004; Cikrit et al., 1986; Dilli, Eras, Ozkan Ulu, Dilmen, & Durgut Sakrucu, 2012; Hintz et al., 2005; Rees, Pierro, & Eaton, 2007; Schulzke, Deshpande, & Patole, 2007; Simon, 1994; Sonntag et al., 2000; Ta et al., 2011; Tobiansky, Lui, Roberts, & Veddovi, 1995; Wadhawan et al., 2014). In 2013, *Ganapathy* and colleagues published one of the first studies examining long-term health care costs with NEC (Ganapathy, Hay, Kim, Lee, & Rechtman, 2013). They compared the health care costs incurred after hospitalization among survivors of medical and surgical NEC cases at 6 months up to 3 years of age. Two-hundred fifty NEC survivors (177 medical, 73 surgical) and 2,909 matched controls were available for follow-up. Medical NEC infants incurred significantly higher healthcare costs than matched controls between 6-12 months of age (mean incremental cost = U.S.

$5,112 per infant), however, no significant difference was seen after 12 months. All-inclusive healthcare costs among surgical NEC survivors, however, continued to be higher than matched controls beyond 6 months. Surgical NEC survivors incurred an additional $60,000 more in healthcare costs than matched controls over the period from 6 months to 3 years of age. These findings demonstrate that the healthcare costs of children who survive NEC continue to accumulate during the early child development period after hospitalization.

The long-term economic burden of NEC confirms the need for more research towards improved risk-reduction strategies. Approximately 1.5% of live births in the United States, or 64,500 infants, were VLBW in 2007. If we estimate the incidence of NEC among VLBW babies to be 7-10%, a 55% reduction in the risk of NEC and surgeries for NEC would avert 2,483-3,548 NEC cases annually. This reduction would lead to an estimated savings of U.S. $212 million in hospitalization costs alone (Ganapathy et al., 2012). Apart from these direct savings, prevention of NEC also entails indirect savings associated with prevention of NEC mortality. Studies on the value of statistical life have placed a value of $5 million for the life of an infant for even small reductions in mortality risk (Murphy & Topel, 1999). *Ganapathy et al.* estimated that a 55% reduction in NEC-associated mortality would avert 323-461 deaths annually, leading to savings of $1.6-2.3 billion (Ganapathy et al., 2012). Understanding the long-term economic burden of NEC will aid policy makers, healthcare providers, and patients to make informed decisions in providing care for infants at high risk for NEC. The factors driving long-term NEC costs (e.g. treatment for post-surgical complications, treatment of infections, and costs associated with prolonged parenteral nutrition), however, are poorly understood. Moreover, little is known regarding the cost-effectiveness of novel therapeutic strategies to mitigate the risk of developing NEC or reduce its long-term impact on infant growth and development. Further investigation of these topics is needed.

## NEC RISK FACTORS

NEC is a complex, multi-factorial disease whose etiology and pathogenesis remain elusive. The leading hypothesis is that NEC is preceded by an ischemic or toxic event that causes damage to the immature gastrointestinal mucosa and loss of mucosal integrity (Neu & Walker, 2011). The consequent breakdown of the mucosal barrier coupled with an impaired ability of the mucosa to heal leads to a self-perpetuating vicious cycle that results in severe NEC, shock, sepsis, and sometimes death (Hsueh et al.,

2003; Patole, 2005). Despite extensive clinical and basic research on NEC in the last few decades, the nature of the insult is incompletely defined and may vary between infants. Ultimately, NEC is likely the end result of a confluence of many predisposing factors. In this section, we provide a comprehensive review of several well-described NEC risk factors.

**Prenatal Risk Factors**

In premature infants, NEC usually presents several weeks following birth. Because NEC has not been described in a fetus, several investigators have suggested that prenatal factors are unlikely to contribute to its etiology (Huda et al., 2014). Recent evidence, however, suggests that maternal conditions during pregnancy could potentially trigger events that damage the vasculature and intestinal mucosa predisposing the infant to NEC (Gephart, McGrath, Effken, & Halpern, 2012). Possible risk factors present in the prenatal course include maternal drug use (Czyrko, Del Pin, O'Neill, Peckham, & Ross, 1991; Hand, Noble, McVeigh, Kim, & Yoon, 2001; Lopez, Taeusch, Findlay, & Walther, 1995; Stout et al., 2008), maternal hypertensive disease including pregnancy-induced hypertension (Bashiri et al., 2003; Manogura et al., 2008), maternal infections (Chokoe, Wright, Bezuidenhout, Moore, & Smith, 2012; Desfrere et al., 2005; Schmitz, Weizsaecker, Feiterna-Sperling, Eilers, & Obladen, 2006), and problems related to placental blood flow that may result in a growth-restricted newborn (Bashiri et al., 2003; Manogura et al., 2008).

*Maternal Hypertension*: Vascular disorders related to maternal hypertension may play an important role in the pathophysiology of NEC. Maternal hypertensive disorders alter utero-placental circulation, which effectively reduce the supply of oxygen and nutrition to the fetus. Pre-eclampsia, for example, is a hypertensive disorder characterized by reduced utero-placental blood flow, which results in progressive deterioration and sclerosis of arteries in the placenta (Shah, 2001; Sheppard & Bonnar, 1999). Chronic hypertension is also associated with pathological changes in the placenta (Regnault, Galan, Parker, & Anthony, 2002). Placental and fetal compensatory mechanisms are induced to alleviate the debilitating effects of prolonged oxygen deprivation (Shah, 2001; Sheppard & Bonnar, 1999). These include increased red cell production, restriction of fetal growth and activity, increased oxygen extraction from the blood, and a redistribution of blood flow (often away from the bowel) to vital organs. The outcome of

such changes is ischemic insult (restriction of oxygen-rich blood from tissues) and an increase in the risk of NEC.

Several clinical and laboratory studies support the theory of hypertension-induced asphyxia with redistribution of blood away from the fetal intestines leading to ischemia as the pathophysiological basis of NEC. In a cross-sectional study (N=211), *Bashiri* and colleagues found that mild pre-eclampsia (11.8% vs. 2.6%, p=0.04), severe pre-eclampsia (35.5% vs. 12.9%, p=0.01), and chronic hypertension (29.4% vs. 5.7%, p<0.001) were significantly higher among mothers of neonates with NEC compared to neonates without NEC (Bashiri et al., 2003). All three conditions are characterized by poor placental blood flow and hypoxia *in utero*, with resultant intestinal ischemia. Multiple logistic regression analysis revealed that maternal hypertension was an independent risk factor for the development of NEC, controlling for pregestational diabetes, birth weight, and gestational age (OR=5.21, 95% CI: 1.64 to 16.58). In a separate study, *Malcolm* and colleagues reported a significant association between NEC and abnormal umbilical blood flow velocity, as measured by Doppler ultrasound (Malcolm, Ellwood, Devonald, Beilby, & Henderson-Smart, 1991). Infants with high-resistance flow patterns in the placenta exhibited greater fetal hypoxemia and were at increased risk of NEC. These findings were confirmed by three separate independent studies (Baschat et al., 2000; Craigo, Beach, Harvey-Wilkes, & D'Alton, 1996; Murdoch, Sinha, Shanmugalingam, Smith, & Kempley, 2006). Future efforts are focused on determining whether common treatments for maternal hypertension can be employed to reduce the risk of NEC in infants. Thus far, treatments such as magnesium sulfate which is administered prior to delivery do not appear to confer a significant protective effect against the neonatal occurrence of NEC (Ghidini, Espada, & Spong, 2001).

*Illicit Drug Use*: Prenatal substance abuse is an ongoing concern across socioeconomic lines due to the characteristic physical and mental developmental problems that result from drug abuse during pregnancy. Among pregnant women aged 15 to 44 years who participated in the 2006 National Survey on Drug Use and Health, 4.0% reported having used illicit drugs within one month of the survey (Koren, Hutson, & Gareri, 2008). An increasingly popular illicit drug among pregnant women which is also linked to NEC risk is cocaine. A nationwide survey carried out at 36 U.S. urban teaching hospitals indicated that 11% of the women cared for at those hospitals tested positive for cocaine during pregnancy (Chasnoff, Bussey, Savich, & Stack, 1986). These findings are consistent with estimations by *Birchfield* and colleagues who

concluded that the frequency of infants exposed prenatally to cocaine range between 2.6% and 11% of all live births (Birchfield, Scully, & Handler, 1995).

Various neonatal health and developmental problems are directly related to fetal exposure to drugs, alcohol, and other chemical agents (Addis, Moretti, Ahmed Syed, Einarson, & Koren, 2001; MacGregor et al., 1987; C. Moore, Negrusz, & Lewis, 1998). Cocaine, for example, is associated with younger gestational age at delivery, lower birth weight, and increased incidence of preterm labor and delivery (MacGregor et al., 1987). Cocaine increases maternal blood pressure and reduces uterine blood flow, impairing the transfer of oxygen and nutrients to the fetus (T. R. Moore, Sorg, Miller, Key, & Resnik, 1986; Woods, Plessinger, & Clark, 1987). Given the potent vascoconstrictive properties of cocaine, it has been hypothesized that *in utero* cocaine exposure could retard blood flow to the intestines, increasing risk of NEC.

The association between cocaine use and NEC is supported by studies in both animal models (Buyukunal, Kilic, Dervisoglu, & Altug, 1994; Kilic, Buyukunal, Dervisoglu, Erdil, & Altiok, 2000) and human neonates (Hand et al., 2001; Lopez et al., 1995). In one case-control study (matched on race, sex, and birth weight), infants born to mothers with a positive history and/or positive toxicology screen for cocaine were compared to infants not exposed to cocaine. The matched odds ratio for developing NEC stage II or III was 2.5 (95% CI: 1.17 to 5.32), where 11 of 51 cases (21.6%) and 23 of 204 controls (11.3%) were exposed to cocaine ($p=0.02$) (Czyrko et al., 1991). In a separate study, *Lopez* and colleagues determined that infants developing NEC were not only more likely to have been born to a mother with a positive cocaine test result but the age of onset of NEC was also likely to be earlier (Lopez et al., 1995). Overall, 12% (28 of 231) of cocaine-exposed infants developed NEC stage II or III versus 3% (34 of 1053) in the non-exposed group ($p<0.05$). Among cases, 8% of cocaine-exposed and 2% of non-exposed survivors had NEC early by day 7 ($p<0.05$), versus 20% and 5% by day 28 after birth ($p<0.05$). Antenatal cocaine exposure, thus, may promote NEC with earlier onset. Further studies examining the relative effects of timing and dosage of cocaine (as well as other illicit substances) on the developing fetus need to be explored.

*Human Immunodeficiency Virus (HIV)*: Numerous studies have examined the relationship between maternal infections and various pregnancy-related outcomes. One well-described association is the relationship between HIV-positive maternal status and risk of NEC in preterm infants (Chokoe et al.,

2012; Desfrere et al., 2005; Schmitz et al., 2006). The human immunodeficiency virus, or HIV, is an infection of the immune system. The predominant and most visible targets of HIV are $CD4^+$ T-lymphocytes, which play an essential role in the body's adaptive immune response to infection (Mehandru et al., 2004; Veazey et al., 1998; Veazey & Lackner, 1998). HIV is also polytropic, meaning that it can infect (or at least bind to) many cell types in the body (Mörner et al., 1999; Willey et al., 2003; Y. Zhang et al., 2000). Among the cells now reported to be infectable, at least *in vitro,* include monocytes, macrophages, dendritic cells, B-cells, natural killer (NK) cells, eosinophils, mast or basophils, thymic epithelial cells, megakaryocytes, NKT-cells, γδ T-lymphocytes, and T-cells. These cells are integral to formation of an effective cellular and intestinal mucosal barrier against penetrating bacteria, toxins, and other antigens (Mannick & Udall, 1996). Their demise signifies the onset of immune deficiency. Immunologic deterioration in HIV-positive mothers leads to higher rates of genital colonization (Mussi-Pinhata et al., 2007), chorioamnionitis of the fetus (inflammation of fetal membranes due to infection) (Mussi-Pinhata et al., 2007), and lower transfer of protective antibodies across the placenta (de Moraes-Pinto et al., 1998). HIV-uninfected infants of HIV-positive mothers also exhibit immunological deficiencies, including aberrant cytokine profiles that may make them more prone to infections (Clerici et al., 2000; Nielsen et al., 2001). NEC represents the end result of a complex interplay between the intestinal mucosal barrier and pathogenic invasion (Caplan & Jilling, 2001). Abnormalities in maternal immune function due to HIV infection combined with the immaturity of the mucosal barrier in infants (Mannick & Udall, 1996) increase the risk of infection and injury to the fetal gut, which may promote pathogenesis of NEC.

In a matched case-controlled study in a single NICU spanning 8.5 years of admissions (79 NEC, 158 controls), *Desfrere* and colleagues demonstrated a significant relationship between maternal HIV status and NEC incidence (Desfrere et al., 2005). HIV-positive status was more frequent in the NEC group compared to controls; 7 of 79 NEC (8.9%) cases and 2 of 158 (1.3%) controls were born to HIV-positive mothers (p=0.01). The adjusted odds ratio comparing neonates of HIV-positive mothers to controls was 6.63 (95% CI: 1.26 to 34.8, p=0.025). *Karpelowsky et al.* also showed that neonates with NEC born to HIV-positive mothers have a higher fatality than NEC infants born to HIV-negative mothers (OR=4.8, 95% CI: 1.7 to 14.2), due possibly to increased severity or extent of disease (Karpelowsky, van Mil, Numanoglu, Leva, & Millar, 2010). Overall, 13 of 22 (59%) NEC cases born to HIV-positive

mothers died compared with 10 of 48 (21%) NEC infants born to HIV-negative mothers (p=0.003). Although these studies do not determine a causal relationship, the authors recommend judicious monitoring of NEC in premature infants born to HIV-positive mothers.

Another (yet poorly described) possibility is that HIV is acting as a confounder, and the actual increased risk of NEC may be secondary to antiretroviral drug exposure (Desfrere et al., 2005; Schmitz et al., 2006). The standard care of HIV-positive women and newborn infants to prevent vertical transmission is chemoprophylaxis with oral zidovudine, which is started between 14 and 34 weeks of pregnancy and continued until the onset of labor for the mother and after delivery for the newborn (Connor et al., 1994). Zidovudine can be used alone or in combination with other antiretroviral drugs. This drug may increase the risk of NEC through its recognized adverse effects, including anemia, granulocytopenia, and grastro-intestinal toxicity (Desfrere et al., 2005; Schmitz et al., 2006). Further investigation, however, is needed to validate these findings. Little is known about the potential effects of other antiretroviral drugs, including nucleoside reverse transcriptase inhibitors (e.g. didanosine or lamivudine) and protease inhibitors (e.g. indinavir, ritonavir or nelfinavir) used during pregnancy.

**Infant-Specific Characteristics**

In this section we discuss infant-specific characteristics, particularly the importance of gestational age, birth weight, race, and genetics in the development of NEC.

*Gestational Age & Birth Weight*: The single most important risk for NEC is prematurity, where low birth weight and early gestational age are associated with increased mortality and morbidity of the preterm infant. NEC is almost exclusively a disorder of preterm babies, wherein over 90% of affected infants are born before 34 weeks gestation. Full-term and near-term infants constitute the remaining 10% of cases.

The frequency and severity of NEC are inversely proportional to birth weight and gestational age. One large, case-control study conducted by the National Institute of Child Health and Human Development (NICHD) Neonatal Research Network established birth weight-based prevalence benchmarks for NEC. In total, 11,072 infants born between 1 September 1998 and 31 December 2001 were examined, of which, 787 (7.1%) developed NEC. When stratified by birth weight, NEC prevalence increased with decreasing birth weight: NEC affected 11.5% of infants weighing 401 to 750 g, 9.1% of infants 751 to 1,000 g, 6.0% 1,001 to 1,250 g, and 3.9% 1,251 to 1,500 g. NEC mortality is also inversely

proportional to birth weight (Fitzgibbons et al., 2009). The mortality risk for infants classified as being *extremely* low birth weight (ELBW, <1000 g) with NEC is 35% to 50%, whereas infants classified as being *very* low birth weight (VLBW, <1500 g) with NEC is 10% to 30%.

The etiology of NEC in preterm groups differs from the etiology in full-term populations (Gephart et al., 2012). Several hypotheses have been proposed to explain the different patterns of neonatal susceptibility observed between NEC cases born early and those born at term. One hypothesis is that babies in the two groups are simply exposed to different sets of risk factors which put them at differential risk for NEC. Premature babies are more likely to be treated with H2 blockers (acid reducers), indomethacin (non-steroidal anti-inflammatory drug), glucocorticoids, or antibiotics which can alter the premature gut biome. They are also more likely to experience birth asphyxia and congenital defects (specifically cardiac or gastrointestinal anomalies) that can negatively impact the supply of blood to the small intestines, leading to gut injury and inflammation. Full-term and late preterm babies are more likely to develop NEC from other risk factors, including intrauterine growth retardation, polycythemia (elevated red blood cell count), hypoglycemia (low blood sugar), gestational diabetes, and being born to a mother with chorioamnionitis. Several risk factors are also common to both infant populations. Maternal drug exposure, HIV positivity, and early onset sepsis are all associated with NEC, regardless of gestational age. Further studies are needed to identify common mechanisms of gut insult that may lead to NEC.

*Race/Ethnicity*: The study of race and its impact on NEC development is not well understood. Most epidemiologic studies to date report racial disparities, with non-Hispanic blacks showing higher overall incidence and mortality compared to other races (Carter & Holditch-Davis, 2008; R. H. Clark et al., 2012; Hogue, Buehler, Strauss, & Smith, 1987; Holman et al., 1997; Llanos et al., 2002; Sappenfield et al., 1987). Early studies attributed this racial difference to the increased incidence of prematurity among black infants (Holman et al., 1989; Kleinman & Kessel, 1987). *Holman* and colleagues, however, later reported that mortality due to NEC is higher for black American infants than for white American infants (RR=1.6, 95% CI: 1.3 to 1.8) even after controlling for birth weight and other characteristics (sex, gestational age, APGAR scores, and geographical region) (Holman et al., 1997). A partial explanation may be due to differences in the timing of death. Low-birth weight black neonates are significantly less likely to die in the first week of life than white neonates from all-causes, suggesting that more black infants survive the early causes of prematurity to make up the cohort at risk for necrotizing enterocolitis

(Holman et al., 1989). Another explanation may be related to increased rates of gut colonization by Group Beta Streptococcus (GBS) among black women because of decreased access to prenatal care (Carter & Holditch-Davis, 2008). GBS-positive colonization could contribute to decreased oxygenation to the bowel or bacterial proliferation, initiating the NEC process (for more on this topic, see section "Altered Gut Microbiome"). Black women who experience such infections are also at increased risk for preterm birth (Holditch-Davis, Scher, Schwartz, & Hudson-Barr, 2004). These findings emphasize the need to examine maternal factors of black women that might contribute to NEC development.

Not all studies, however, are consistent with these findings. In a separate cohort study conducted for the years 1999-2004, *Guner et al.* determined that black and white neonates in the State of California had similar survival risks, while Hispanic neonates had a 1.44 greater odds of mortality due to NEC compared to white, black, and Asian neonates (95% CI: 1.05 to 1.95) (Guner et al., 2009). Overall, 18.1% of Hispanic neonates died of NEC versus 11.9% of white, black, and Asian neonates (p=0.022). Socioeconomic factors, including insurance status (State funded vs. HMO/private RR = 1.10, 95% CI: 0.83 to 1.48, p=0.50) and parental median household income (<$30,000 vs. >$30,000 RR = 1.45, 95% CI: 0.95 to 2.22, p=0.078) were not predictors of mortality. Possibly, the diverse and robust population dynamics of California are different than other regions across the U.S. This finding is alarming when we consider current and future predictions about the demographic makeup of the U.S. By 2050, the Hispanic share of the U.S. population could be as high as 29%, up from 16.9% in 2012 (Passel & D'Vera, 2008). The black proportion of the population is also projected to rise slightly to 13%. In contrast, non-Hispanic whites which constitute 63% of the current population, will decrease to half or slightly less than half (47%) of the population by 2050, becoming a minority. As the face of America changes, the importance of understanding racial disparities in NEC health outcomes grows.

*Genetics*: Although a genetic basis for NEC has not been defined, emerging technologies now permit investigators to examine the potential implications of genetic polymorphisms and their influence on NEC disease development. Investigation of factors that might cause a genetic predisposition for NEC might allow specific treatments or preventative strategies for infants at most risk for this disease.

One area of intense interest is the potential importance of specific polymorphisms for known NEC-associated inflammatory mediators, particularly cytokines, which may predispose the bowel to inflammation and injury (Treszl, Tulassay, & Vasarhelyi, 2006). Cytokines are small molecules or

proteins that are secreted by cells such as T-lymphocytes, and they play an essential role in the immune response to invading pathogens. They are functionally divided into two broad groups – those that are pro-inflammatory (Th1) and those that are anti-inflammatory (Th2). Th1-type cytokines such as gamma interferon (IFN-γ) produce pro-inflammatory responses that kill intracellular parasites and perpetuate autoimmune responses. Excessive inflammation, however, can lead to uncontrolled tissue damage initiating the NEC process. Thus, Th2-type cytokines such as interleukin-4 (IL-4) are essential for balancing Th1 cytokines.

The ability of individuals to synthesize (and respond to) different cytokines varies greatly and may account, at least in part, for inter-individual differences in NEC susceptibility. Preliminary studies in VLBW infants suggest that neonates with NEC are less likely to be carriers of a specific mutant allele that affects IL-4 receptors on cells (Treszl et al., 2006). This variant affects the IL-4 receptor α gene and is associated with enhanced transduction of IL-4 signals which promotes a more pronounced Th2 response. Researchers speculate that the elevated anti-inflammatory response of this genetic polymorphism is a protective factor against NEC. The risk of NEC has also been associated with the IL-18 AA genotype. The frequency of the AA genotype is significantly higher in infants with stage III NEC compared to stages I and II (Treszl et al., 2006). The AA genotype may contribute to intestinal inflammation and adversely affect the outcome of NEC through altered IL-18 levels, a cytokine that induces IFN-γ and amplifies Th1 cytokine production. Another cytokine of interest is the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α). In animal models, pretreatment with anti-TNF-α reduced the incidence and severity of NEC (Halpern et al., 2006; Seitz et al., 2005). Investigators have yet to report a genetic link between TNF-α gene variants and NEC disease (Treszl et al., 2001). Future studies are focused on identifying other genetic polymorphisms affecting the quantity and quality of the cytokine response as well as understanding the genetic basis of other conditions such as prematurity and low gestational age that may put neonates at higher risk for NEC.

**Clinical Course Factors**

The events of the clinical course include risk factors experienced during the first days to weeks of life. Factors attributed to greater NEC risk include enteral feedings or medications that alter gut flora and

enhance inflammatory responses, delivery room resuscitation and mechanical ventilation, and injuries resulting from blood transfusions.

*Enteral Feeding*: The role that enteral feeding plays in the pathogenesis of NEC is an important area of investigation. Enteral feeding refers to the delivery of nutritionally complete food directly into the neonatal intestinal lumen, usually through a feeding tube. 90% to 95% of infants who develop NEC have been fed enterally or have had a recent history of feeding volume advancement (Lee & Polin, 2003). Although the mechanism is not well understood, enteral feeding is reported to disrupt mucosal integrity, blood flow, and motility (Lee & Polin, 2003; Pietz, Achanti, Lilien, Stepka, & Mehta, 2007). Raising milk intake increases metabolic demands, which may be taxing for the infant who must expand mesenteric blood flow to meet those demands (Horton, 2005). In turn, intestinal hypoxemia – a condition where intestinal blood is abnormally low in oxygen – may result. Unabsorbed nutrients in the small and large intestine also provide a bacterial substrate that can lead to enteric bacterial proliferation and invasion of the bowel wall (see section, "Altered Gut Microbiome") (Kliegman, 2003). The enteric bacteria produce intraluminal gas leading to distention, pneumatosis intestinalis, increased intraluminal pressure, and the resultant decreased blood flow (D. A. Clark & Munshi, 2014; Eckburg et al., 2005; Kliegman, 2003).

Altering feeding regimens and nutritional strategies are powerful measures clinicians can take to reduce the risk of NEC. However, striking a balance between supporting neonatal growth and curbing a baby's risk of developing NEC is challenging. Attempts to refine feeding strategies have focused on the time of initial enteral feeding, initial volume, rate of advancement, and type of feedings over the neonatal course, as well as understanding the characteristics of microorganisms present in the gastrointestinal tract (Pietz et al., 2007). Research in these areas, however, is conflicting and a consensus has not been reached on most basic feeding parameters.

Several randomized controlled trials (RCTs) were conducted to determine the optimal rate of feeding advancement. An early trial comparing rapid (20 mL/kg per day) versus slow (10 mL/kg per day) advancement of feeding was halted prematurely due to an increase in the incidence of NEC in the rapidly advancing group (10% vs. 1.4%) (Berseth, Bisquera, & Paje, 2003). Conversely, others have evaluated fast feeding advancement with vigilant exclusion of infants on the basis of NEC risk (e.g. birth weight < 1000 g, history of mechanical ventilation, presence of gastrointestinal abnormalities, respiratory distress,

history of vasopressors, low APGAR scores) and found a protective effect against NEC in patients who had enteral feedings advanced more aggressively (Book, Herbst, & Jung, 1976; Caple et al., 2004; Rayyis, Ambalavanan, Wright, & Carlo, 1999). Neonatologists continue to debate this issue. On the one hand, fast fed infants require fewer days to full feedings and regain birth weight more rapidly, which means fewer central catheter days, lower risk for acquired bloodstream infections, and quicker gut development. A cautionary approach, however, involving minimal enteral nutrition with small increases for extremely premature infants helps to prime the gut epithelium. This approach also permits adaptability which may help in decreasing feeding intolerance. Further study in this area is needed for subgroups of premature infants displaying varying degrees of illness.

Another area of debate focuses on the type of enteral nutrition that neonates should receive. The American Academy of Pediatrics endorses the use of human milk (Terrin et al., 2009). Breast fed infants are 6 to 10 times less likely to develop NEC than infants fed formula exclusively and 3 times less likely than infants receiving a mixture of breast milk and formula (Lucas & Cole, 1990). Breast milk also reduces the severity of NEC disease (Patole, 2005). This protective effect is afforded through both nutritional and immunologic mechanisms. Breast milk contains a host of bioactive and immunomodulatory factors, anti-inflammatory components, and immunoglobulins that influence host immunity, inflammation, and strengthen mucosal protection (Caplan, Amer, & Jilling, 2002; Donovan, 2006; Hanson, 1999). These factors are not provided in commercially available neonatal formula preparations. Breast milk also contains pre- and probiotics that modulate intestinal microflora composition and diversity, which prevents overgrowth of pathogenic strains. Despite the advantages of human milk, however, it is important to appreciate that breastfeeding alone is unlikely to eliminate NEC, as cases are reported in neonates who have been fed exclusively with human breast milk (Patole, 2005).

*Altered Gut Microbiome*: Prior to birth, the newborn gastrointestinal tract is sterile, but bacterial colonization occurs within hours (D. A. Clark & Munshi, 2014; Claud & Walker, 2001). Contact with the mother's vaginal flora begins this process, which is further developed by oral or enteral feedings and exposure to the environment. Under optimal conditions, the neonate develops a rich and diverse intestinal microbiota that is involved in maintenance and homeostasis and protection of the host against hospital-acquired pathogens (Collier-Hyams & Neish, 2005). In certain instances, the gut mucosa is weakened and breached by pathogenic bacterial (or viral) strains. Aberrant colonization and bacterial overgrowth leads

to the production of food-induced toxic by-products (Boccia, Stolfi, Lana, & Moro, 2001), excessive gas due to fermentation of carbohydrates (D. A. Clark & Munshi, 2014; Eckburg et al., 2005; Kliegman, 2003), and presentation of unique microbial molecular patterns that are capable of altering the epithelial barrier and triggering an inflammatory cascade (Claud & Walker, 2001, 2008; C. R. Martin & Walker, 2006). These pathogens also compete with beneficial commensal bacteria involved in mucosal immunity. The excessive inflammatory response combined with reductions in the normal gut flora support the role of bacterial colonization in the pathophysiology of NEC.

This hypothesis is supported by several lines of evidence: First, NEC is absent *in utero* when the fetal gut is sterile, and the timing of onset of NEC occurs 8 to 10 days post-partum when anaerobic bacteria have colonized the gut. Second, infants with NEC frequently have concomitant bacteremia and endotoxemia in regions of the intestine (ileum and proximal colon) that are most often associated with NEC (Schwiertz et al., 2003). Pathogenic proteobacteria also constitute as much as 70% of the organisms found in the stools of children with NEC, within three days preceding the clinical diagnosis (Mai et al., 2011). Third, experimental NEC does not occur in germ-free animals (Morowitz, Poroyko, Caplan, Alverdy, & Liu, 2010). Fourth, supplementation with pre- and probiotics facilitates colonization of a balanced, non-pathogenic flora in the gut and is associated with decreased NEC risk (Alfaleh & Anabrees, 2014; H. C. Lin et al., 2005). Finally, prolonged use of antibiotics (Alexander, Northrup, & Bizzarro, 2011; Cotten et al., 2009; Faix, 2012; Weintraub et al., 2012) and/or H2-blockers (Bilali, Galanis, Bartsocas, Sparos, & Velonakis, 2013; Dalton & Schumacher, 2012; Guillet et al., 2006; H. C. Lin, Su, & Chen, 2006; Patole, 2006) soon after birth is associated with increased risk of NEC in ELBW infants, presumably because these drugs disrupt normal flora diversity in the neonatal intestinal tract. Antibiotics destroy both pathogenic as well as beneficial bacterial strains and H2 antagonists reduce the acidity of the stomach which potentiates the growth of bacteria.

Concerted effort has focused on identifying a specific pathogen responsible for causing the intestinal inflammation of NEC, much like how *Helicobacter* species cause gastric ulcers. Several bacterial and viral species have been cultured in outbreaks of NEC (Eckburg et al., 2005). These include viruses (e.g. coronavirus, rotavirus, enterovirus, and parvovirus B19) (Genzel-Boroviczeny, Jager, & Schatzl, 2012; Rousset et al., 1984; Sharma, Garrison, et al., 2004), as well as pathogenic bacterial strains (e.g. *E. coli*, *Klebsiella* species, *Enterobacter* species, and *Staphylococcus* species) (Carlisle, Poroyko,

Caplan, Alverdy, & Liu, 2011; Powell et al., 1980; Smith et al., 2011; C. Zhang et al., 2012). Recently, abnormal colonization of stools with *Clostridium perfringens* has been correlated with development of NEC (de la Cochetiere et al., 2004). *C. perfringens* was isolated from 40% of infants with NEC, compared with 13% of controls (p=0.005). Despite efforts, however, no single pathogen has been consistently identified as causative. Possibly, the ability to colonize the epithelium, ferment unabsorbed nutrients, and induce exaggerated pro-inflammatory responses is a more important consideration than the strain itself.

*Mechanical Ventilation*: In the past three decades, use of a ventilator to assist or replace natural breathing has been a primary means for decreasing mortality among preterm ELBW infants (Lau, Ambalavanan, Chakraborty, Wingate, & Carlo, 2013). Approximately 80% of ELBW infants demonstrate signs of respiratory decompensation at birth and require respiratory support (Lemons et al., 2001). Although the use of ventilators has increased infant survival, prolonged dependence on one may also lead to adverse outcomes including increased risk for NEC. The act of intubation and the indwelling endotracheal tube may destroy the integrity of the infant's esophageal mucosal barrier, increasing the risk for infection (Flidel-Rimon et al., 2004). Invading pathogens of the esophagus could lead to the introduction of pathogens to the bowel, initiating the NEC process. Once NEC disease has set in, the need for mechanical ventilation escalates because the intestinal inflammation caused by NEC increases metabolic demands leading to further respiratory decompensation. The result is a vicious, self-perpetuating cycle.

*Gregory* and colleagues examined whether mechanical ventilation was a predictor for NEC in premature neonates in a single center (N=247), using a case-control design. Infants who required mechanical ventilation during the early neonatal period had a 12.6 higher odds of developing NEC stage II or III than infants who did not have this physiologic need ($p<0.001$) (Gregory, 2008). Additional predictors of significance between NEC and non-NEC groups were noted for other stabilization or resuscitation interventions that occurred at the time of birth, including bag-mask ventilation ($p<0.002$), endotracheal tube intubation in the delivery room ($p<0.001$), and hemodynamic support ($p <0.0001$). In a separate analysis using the Kids Inpatient Data, *Carter and Holditch-Davis* identified length of time on a ventilator and the number of infections in the neonatal period as predictors of NEC (Carter & Holditch-Davis, 2008). Mechanical ventilation had a positive relationship with NEC (Pearson $r = 0.25$, $p=0.002$), such that the risk of developing NEC increased as the number of days on respiratory support increased.

This association may be explained by the very strong positive relationship observed between mechanical ventilation and the number of infections experienced by a preterm infant (Pearson $r = 0.73$, $p<0.0001$). The log odds ratio indicated that each additional infection increased the odds of developing NEC by 2.6 times. Fifty percent of the 24 infants with NEC in this study had 1 or more infections before developing NEC, whereas only 31% of the remaining 110 infants without NEC had infections during their hospitalization. These findings underscore the significant relationship between hypoxic events requiring increased respiratory support and the role of infection in the development of NEC. These studies also support the need for early extubation and crucial infection control practices in the healthcare setting.

*Transfusion-Associated NEC (TANEC)*: Transfusion-associated necrotizing enterocolitis, or TANEC, is described as necrotizing enterocolitis that arises within 48 hours of a packed RBC transfusion. TANEC is concerning to clinicians because neonates are among the most heavily-transfused patients, with more than half of all VLBW infants receiving one or more transfusions during their hospital stay (Strauss, 1997). Case reports and retrospective studies reveal that as many as 20% to 40% of VLWB infants with NEC receive one or more RBC transfusions in the 2-48 hours prior to onset of NEC (Bednarek et al., 1998; Blau et al., 2011; Christensen et al., 2010; Couselo, Aguar, Ibanez, Mangas, & Garcia-Sala, 2011; El-Dib, Narang, Lee, Massaro, & Aly, 2011; Ghirardello, Lonati, Dusi, Pugni, & Mosca, 2011; Josephson et al., 2010; Mally et al., 2006; McGrady et al., 1987; Paul et al., 2011; Singh et al., 2011; Stritzke, Smyth, Synnes, Lee, & Shah, 2013; Wan-Huen, Bateman, Shapiro, & Parravicini, 2013). Compared with infants who develop NEC unrelated to transfusion, TANEC neonates tend to be of lower birth weight and of earlier gestation at birth, and they have a delayed onset at 3-5 weeks of post-natal age (Amin, Remon, Subbarao, & Maheshwari, 2012). TANEC cases also tend to be more severe, require prolonged hospitalization as well as surgical intervention, and have high mortality. These differences suggest that transfusion-associated NEC is a distinct pathogenic entity. To date, there is no proven pathogenic mechanism, although several plausible explanations have been proposed. Suggested theories include anemia leading to impaired gut blood flow, exposure to biologically active mediators such as free hemoglobin, cytokines, or broken red cell fragments in the transfused blood which can trigger immunologic reactions in the gut mucosa, and ischemia/reperfusion injury associated with transfusion (Mohamed & Shah, 2012).

The association between blood transfusions and NEC was first described by *McGrady et al.* in 1987. During their investigation of an outbreak of 33 cases of NEC they reported increased odds of NEC disease among infants receiving RBC transfusions (OR=15.1, 95% CI: 2.59 to 92.51) (McGrady et al., 1987). Recently, *Mohamed and Shah* published a meta-analysis of 9 studies on transfusion-associated NEC and confirmed the increased odds of NEC within a 48-hour period following an RBC transfusion (Mohamed & Shah, 2012). Meta-analysis on five studies that reported *unadjusted* estimates of exposure showed increased odds of NEC after recent transfusion (pooled OR=3.91, 95% CI: 2.97 to 5.14, $I^2$=58%). Also, meta-analysis of four studies reporting *adjusted* estimates revealed a similar but lower risk of NEC (pooled OR=2.01, 95% CI: 1.61 to 2.50, $I^2$=91%). Exploration of statistical heterogeneity in the adjusted estimates revealed that one study involving a review of 2,123 VLBW infants reported divergent results. In contrast to the other three studies, *Harsono et al.* reported a protective effect associated with transfusion (Harsono, Talati, Dhanireddy, & Elabiad, 2011). Twenty-six of 43 infants who developed NEC (60%) presented disease within 48 hours of receiving a blood transfusion. After controlling for birth weight, gender, and a history of umbilical artery catheter insertion, infants who received a transfusion were less likely to develop NEC than those who did not receive a transfusion (OR=0.30, 95% CI: 0.15 to 0.60). *Harsono et al.* hypothesized that blood transfusions may protect against NEC by reducing the effects of chronic anemia and consequent tissue hypoxia. Exclusion of *Harsono et al.* from meta-analysis led to disappearance of statistical heterogeneity (pooled OR=2.48, 95% CI: 1.97 to 3.12, $I^2$=0%) (Mohamed & Shah, 2012).

Management of transfusion-associated NEC will require adoption and strict adherence to transfusion guidelines. However, differences exist in the criteria for transfusion both within units and across providers. This variability in transfusion practices was exemplified by a study by *Bednarek et al*. In 1998, they compared transfusion practices in VLBW infants across six NICUs and found tremendous variability among units in the quantity of blood transfused over an infant's stay (Bednarek et al., 1998). High ($\geq$2 transfusions, average $\geq$56 ml/kg blood volume, range 56-203) versus low (1 transfusion, average $\geq$38 ml/kg blood volume, range 0-96) transfusing units can vary by as much as 70 ml/kg over an infant's NICU stay. NICUs with fewer transfusions had a lower incidence of NEC (OR=0.3, 95% CI: 0.1 to 0.8). Dangers from multiple and large-volume transfusions include exposure to multiple donors, viruses, preservatives used in blood products, and iron and volume overload. Efforts to standardize

transfusion practices will require further research, including prospective, multi-center trials evaluating the scientific merits of limiting transfusion volumes. Strategies to decrease RBC transfusions with replacement recombinant erythropoietin or with its synthetic, longer-acting homologues such as darbepoietin may also be of interest (Amin et al., 2012). Erythropoietin and darbepoietin are hormones that can be used therapeutically to stimulate red blood cell production naturally, thereby minimizing the need for transfusion.

## DISEASE MANAGEMENT

### Medical Management

Most patients affected by NEC can be managed medically. When clinical, laboratory, and radiographic findings are suspicious for NEC, initial management generally includes bowel rest, abdominal decompression with a gastric tube, hydration, and correction for underlying conditions such as hypotension, metabolic acidosis, and hyponatremia (Gregory, Deforge, Natale, Phillips, & Van Marter, 2011; Patole, 2007). Since infants who develop NEC can rapidly deteriorate, close monitoring is recommended. Serial abdominal examinations and radiographs should be performed, including laboratory assessments of blood, urine, and sputum to determine whether the illness has progressed (Dominguez & Moss, 2012). In certain instances, administration of broad spectrum antibiotics is warranted for possible sepsis and an anti-fungal if bowel perforation is suspected or confirmed (Dominguez & Moss, 2012; Gregory et al., 2011; Patole, 2007). Although the duration of antibiotic treatment is not clear, typical practices are 7 to 14 days. Additional supportive care, including increased ventilator support, blood transfusions, and management of blood pressure with pressors may be necessary (Dominguez & Moss, 2012). Medical management of NEC cases is considered appropriate as long as the patient's condition is stable or improving.

### Surgical Management

Roughly 20% to 40% of NEC cases require surgical intervention (Guthrie et al., 2003; Luig & Lui, 2005; Sharma et al., 2006; Sharma, Tepas, Mollitt, Pieper, & Wludyka, 2004). In most cases, surgical consultation is recommended for patients who are unresponsive to medical management with a deteriorating clinical status (Hall & Pierro, 2012; Kosloske, 1994b). The only absolute indications for surgery are bowel perforation, as indicated by pneumoperitoneum, and/or the presence of stool or bile in

the abdominal cavity, both of which require emergency action (Blakely et al., 2008; Henry & Lawrence Moss, 2005; Kosloske, 1994a). The presence of portal venous gas is also readily used as an indication for operative intervention (Henry & Lawrence Moss, 2005). Other relative indications for surgery include erythema of the abdominal wall, the presence of fixed loops by abdominal radiograph, and a palpable abdominal swelling. These indications must be taken in context with the overall clinical condition of the patient, since they are neither specific nor sensitive.

Two commonly used methods for treating advanced NEC with intestinal perforation are laparotomy and primary peritoneal drainage (PPD) without laparotomy. Laparotomy is an invasive surgical procedure involving a large incision through the abdominal wall to gain access into the abdominal cavity. Laparotomy was the traditional form of surgery that physicians relied upon for decompressing the gut and/or removing necrotic bowels. In 1977, placement of a peritoneal drain to relieve abdominal pressure was described by *Ein* and colleagues (Ein, Marshall, & Girvan, 1977). In a subsequent report, they demonstrated a 46% survival for patients treated using PPD (Janik & Ein, 1980). They also noted that 40% of this group improved to the degree that they never required operation. PPD was initially proposed for patients considered too unstable for laparotomy and was intended only as a temporizing measure until definitive treatment could be performed. Over time, PPD has become an accepted alternative to laparotomy and is used as a definitive form of treatment rather than just a temporizing measure. However, the relative benefits of each method continue to be debated.

Over the 25-year-period from 1980 to 2005, more than 20 studies involving over 1,300 infants have been published in an attempt to determine whether laparotomy or PPD is a better treatment method for children with NEC. Most of these studies, however, comprised case series data from a single institution where patients were assigned to a treatment based on surgeon preference or institutional practice pattern. In 2006, *Blakely* and colleagues conducted one of the first large-scale prospective cohort studies involving 16 centers through the National Institute of Child Health and Human Development (NIHCD) Neonatal Network (Blakely et al., 2006). A total of 156 ELBW infants were treated by either PPD or laparotomy. Initial findings suggested that laparotomy had a decreased risk of adverse outcomes (death or neurodevelopmental impairment) relative to the PPD group (OR=0.39, 95% CI: 0.18 to 0.86). However, the two treatment groups were not similar in their initial comparisons. The PPD group had a younger gestational age, younger age at operation, smaller birth weight, greater use of vasopressors, and

lower mean blood pressure, among other factors. After adjusting for these group differences, the initial outcomes favoring laparotomy were no longer evident (OR=0.44, 95% CI: 0.16 to 1.2), suggesting that both treatment options are equally effective at preventing negative outcomes.

These studies were followed by two large multicenter, randomized clinical trials comparing PPD with laparotomy in NEC:

- *Necrotizing Enterocolitis Study Towards Evidence-Based Pediatric Surgery (NECSTEPS) Trial*: The NECSTEPS trial was conducted at North American tertiary care centers (Moss et al., 2006). The primary outcome was survival at 90 days. Secondary outcomes were length of hospitalization and dependence on parenteral nutrition. In total, 117 infants with perforated NEC were randomized to either PPD or laparotomy (55 PPD, 62 laparotomy). Mortality at 90 days in the two groups was similar at 34.5% (19 of 55) of the PPD group and 35.5% (22 of 62) of the laparotomy group (p=0.92). Secondary outcomes were also similar. The mean length of stay of hospitalization ($\pm$SD) for the PPD and laparotomy groups were 126 $\pm$ 58 days and 116 $\pm$ 56 days, respectively (p=0.43). The risk of parenteral nutrition dependence was 47.2% of the PPD group and 40.0% of the laparotomy group (p=0.53). These findings suggest that the type of surgical intervention is not a significant determinant of outcome.

- *Necrotizing Enterocolitis Trial (NET)*: The NET trial was an international multicenter randomized clinical trial covering patients across 13 countries (Rees et al., 2008). In total, 69 ELBW infants with perforated NEC were randomized to either PPD or laparotomy (35 PPD, 33 laparotomy, 1 withdrew). The primary outcome was survival at 6 months. Secondary outcomes included length of hospitalization, need for ventilator support, and dependence on parenteral nutrition. Mortality at 6 months in the two groups was similar at 51.4% (18 of 35) of the PPD group and 63.6% (21 of 33) of the laparotomy group (p=0.3). Secondary outcomes were also similar. The median length of hospitalization for the PPD and laparotomy groups were 74 days (range 18-227) and 85 days (31-193), respectively (p=0.95). At 1 month, 67% of the PPD group and 54% of the laparotomy group were ventilator dependent (p=0.5). Finally, the risk of parenteral nutrition dependence at 1 month was 57% of the PPD group and 59% of the laparotomy group (p=1). Thus, similar to the NECSTEPS trial, there were no significant differences in any of the primary or secondary outcome measures between

the treatment groups. This finding is supported by a meta-analysis of both the *Moss et al.* and *Rees et al*. trials, which showed no survival advantage to PPD over laparotomy (RR of mortality at 90 days = 1.05, 95% CI: 0.71 to 1.55) (Rees et al., 2008). The mortality risk was similar in both trials despite differences in geographical coverage, suggesting the findings are widely generalizable.

Evidence from these studies suggests that once NEC has progressed to the point of perforation, the type of surgical intervention does not influence survival. Further focus will need to be directed toward the examination of morbidities, including long-term outcomes such as neurodevelopmental impairments, in the assessment of treatment options. Finally, these studies indicate that once surgery is required, prognosis is likely to be poor, a finding that underscores the need for newer treatments and/or effective prevention strategies.

## NEC MORBIDITIES

Infants who survive NEC commonly experience short- and long-term sequelae, which differ depending on the clinical presentation and requirement of medical or surgical management. These include gastrointestinal morbidities such as short bowel syndrome and intestinal strictures as well as neurodevelopmental delays. Early detection and treatment may reduce the risk of morbidity and mortality.

### Short-Bowel Syndrome

Short-bowel syndrome is a condition in which nutrients are not properly absorbed because a large part of the intestinal tract is missing, dysfunctional, or has been surgically removed. Approximately 25% of patients with NEC experience some degree of short-bowel syndrome (Henry & Moss, 2008). When a portion of the bowel is lost, the remaining bowel is exposed to an increased volume of gastric and small-bowel secretions. To compensate, there is an up-regulation of nutrient absorption by the remaining bowel, made possible by an increase in the production rate and height of crypt cells in the gut. These changes increase the functional surface area available for absorption. Since some regions of the bowel respond more prominently to this process than others (e.g. the ileum), the location (and length) of the residual intestine after surgery is an important determinant in gastrointestinal dysfunction (Collins, Georgeson, Vicente, Kelly, & Figueroa, 1995). Other factors that determine the severity of short-bowel syndrome include the length of the colon present (since the colon also has absorptive function), the age at resection,

and the time allowed for adaptation (Henry & Moss, 2008). Also, most cases of short-bowel syndrome require long-term parenteral nutrition, which increases the risk of catheter-related bloodstream infections and liver complications (Goulet & Sauvat, 2006). Evidence-based strategies to minimize the risk of short-bowel syndrome are lacking, although a variety of treatment strategies for coping with the condition are under investigation. These efforts may be facilitated by large-scale prospective registries of patients undergoing resection for NEC.

**Intestinal Strictures**

An intestinal stricture, also known as a stenosis, is a narrowing of the gastrointestinal tract leading to obstruction of the bowel. Although the long-term impact of strictures is unclear, there is evidence of diverse complications ranging from abdominal pain to cramping, bloating, nausea, constipation, and vomiting. 10% to 35% of NEC patients suffer from intestinal strictures caused by damaged intestinal mucosa, regardless of management strategy (Butter, Flageole, & Laberge, 2002; Lemelle, Schmitt, de Miscault, Vert, & Hascoet, 1994; Schimpl, Hollwarth, Fotter, & Becker, 1994). Management of strictures generally requires surgery. One surgical approach is complete removal of the stricture. However, resection may have an impact on gastrointestinal functioning of these patients, as this group is already at high risk for short-bowel syndrome. A surgery called ‘strictureplasty’ was recently introduced and involves enlarging the width of the strictured intestine. The impact of this and other approaches, however, remain unclear.

**Neurodevelopmental & Growth Outcomes**

An association exists between NEC and poor neurodevelopmental outcomes in children who survive NEC. This association has been observed since the early 1980s when *Stevenson* and colleagues published a groundbreaking retrospective cohort study which showed that less than half of children surviving NEC are neurologically normal at 3 years of follow-up (Stevenson, Kerner, Malachowski, & Sunshine, 1980). The most common neurodevelopmental impairments (NDI) include cerebral palsy, visual impairment, hearing impairment, cognitive impairment, and low psychomotor index (Rees et al., 2007; Schulzke et al., 2007). NEC is also more likely to be associated with growth delays in height, weight, and head circumference (Hintz et al., 2005). The biological events following NEC leading to neurodevelopmental impairment are unknown. However, one hypothesis is that cerebral injury is caused

by a surge of pro-inflammatory cytokines experienced during surgery, and again potentially with recurrent sepsis and suboptimal nutrition (Schulzke et al., 2007).

Several studies have elucidated the important issue of adverse neurodevelopmental outcomes in children with NEC. *Schulzke* and colleagues performed a meta-analysis of 8 studies (N=4,239) and reported an almost 2-fold higher odds of long-term neurodevelopmental impairment in VLBW children with NEC (combined odds ratio = 1.82, 95% CI: 1.46 to 2.27) (Schulzke et al., 2007). Additional analysis of 4 studies (N=347) showed that patients requiring surgical intervention are at increased odds for adverse neurodevelopmental and growth outcomes compared with medical NEC cases (combined OR = 1.99, 95% CI: 1.26 to 3.14). Similarly, a meta-analysis of 10 studies by *Rees* and colleagues revealed a combined odds ratio of neurodevelopmental impairment of 1.58 for those with NEC compared to those without NEC (95% CI: 1.25 to 1.99), and a 2-fold increased odds for NDIs when comparing surgically managed NEC versus medically managed NEC (combined OR = 2.34, 95% CI: 1.51 to 3.60) (Rees, Pierro, & Eaton, 2007). Recent evidence suggests that differences in neurodevelopmental outcomes may also exist between infants treated by laparotomy versus those treated by peritoneal drainage, however, no conclusive difference has been established (Blakely et al., 2006). These findings underlie the necessity of further studies to evaluate the effect of treatment modality on long-term neurodevelopmental outcome.

## SUMMARY

Despite advances in neonatal intensive care and significant gains in premature infant survival, NEC remains one of the most significant complications of premature birth. The onset of the disease is often insidious, yet progression can be rapid. Infants who develop NEC are at increased risk of death, infection, and long-term health consequences, including intestinal sequelae and aberrant growth and neurodevelopment. Most of the morbidity and mortality from the disease is observed in patients who require surgical intervention. Therefore, preventative strategies are likely to have the greatest impact in positively influencing outcomes. However, its pathogenesis and, more importantly, its prevention remain unresolved. The only consistently shown risk factor is prematurity, and clinical parameters do not seem to accurately predict NEC risk. The limitation of small sample sizes and assignment bias are evident in most studies, re-emphasizing the need for rigorously designed clinical studies on which we can model our practice.

# CHAPTER III

METHODOLOGY

## STUDY DESIGN

### Study Type & Data Source

This study is a retrospective cohort analysis utilizing data collected on neonatal inpatient admissions to Children's Hospital of Atlanta (CHOA). CHOA is one of the largest pediatric hospitals in the country serving patients from locations throughout the State of Georgia in its hospitals, emergency rooms, and Level III NICU facilities. CHOA collects and stores medical record data collected by local staff using uniform definitions and input into the EPIC medical record system. Records are subjected to automated checks for quality and completeness and returned if corrections are needed. Each child that visits a CHOA medical facility is given a medical record number which allows for tracking of the same individual through time.

As part of an ongoing collaboration with CHOA, the Birth and Neonatal Outcomes interest group within the Center for Clinical Outcomes Research and Public Health (CORPH) developed a secondary database of all neonatal inpatient admissions to CHOA facilities between 1 January 2009 and 31 December 2010. Examples of variables extracted from CHOA's EPIC system include: (i) admission and discharge diagnoses (International Classification of Disease 9$^{th}$ Revision, or ICD-9), (ii) population sources (e.g. delivery hospital transfer, outpatient), (iii) length of hospital stay (days), (iv) birth characteristics (e.g. gestational age, birth weight, method of delivery, APGAR score), (v) sociodemographic information (e.g. race, payer status), and (vi) medications received. Neonatal visits were defined as visits among infants aged 28 days or less, where age was calculated by subtracting the birth date from the date of admission.

### Study Population

The study population includes all neonatal CHOA admissions with a documented diagnosis of NEC, by modified Bell stage I and above, from 1 January 2009 to 31 December 2010.

*NEC Definition*: NEC was diagnosed either by direct observation of intestine at operation or pathologic examination or by using a set of strict clinical criteria. A clinical diagnosis of NEC was made based on at least 1 physical finding (bilious gastric aspirate or emesis, abdominal distention, blood in stool without evidence of a rectal fissure) and at least 1 radiographic finding (pneumoperitoneum, pneumatosis intestinalis, or hepatobiliary gas). Patients with a confirmed NEC diagnosis were identified by ICD-9

codes 777.5 or 777.50. ICD-9 coding was also used to determine disease severity by Bell's stage criteria (777.51 to 777.53, stages I to III).

*Inclusion & Exclusion Criteria*: Our analysis includes neonates (≤28 days of age) of all birth weights and gestational ages, as well as singleton and multiple gestation infants. The data set did not permit us to differentiate the study population between cases receiving an operation ("surgical NEC") and those treated without surgery ("medical NEC"). However, because the study locations are surgical centers, the overwhelming majority of cases included in this analysis are surgical. Analysis was limited to infants with an NEC diagnosis.

**NEC Outcomes**

The primary outcomes are **NEC-associated case-fatality** and **length of hospital stay (LOS)**. Case-fatality was examined for all NEC infants from the time of diagnosis during the initial hospitalization until discharge. Infants who were initially discharged or transferred and subsequently died were not followed. LOS is defined as the number of days between admission to CHOA and discharge.

*Variables*: The primary exposure variable, **race/ethnicity**, was categorized into four levels - non-Hispanic white, non-Hispanic black, Hispanic, and other/unknown. Asian/Pacific Islander and Native populations (Native American/Eskimo/Aleut/Native Hawaiian) were grouped together in the other/unknown category since the sample size was less than 1% of the overall NEC cohort.

Our analysis examines several potential predictors of NEC outcomes.

- Infant and Birth Characteristics: Prematurity and low birth weight were examined since they are most commonly recognized as a contributor to increased mortality and morbidity (Holman et al., 1997). APGAR scores, which provide a general summary of the health of newborn children, were also evaluated given the availability of this data. **Birth weight** was categorized into three levels (<1000 g, 1000-2500 g, and 2500 g or more) of roughly similar proportions of subjects. **Gestational age** (≤27 weeks, 27-33 weeks, ≥33 weeks) and **APGAR scores at 1 minute** (<5, 5-10) and **5 minutes** (<7, 7-10) were analyzed using previously published categories (Bashiri et al., 2003; Holman et al., 1997). Finally, **birth number** (singleton, multiple gestation) and the **sex** (male, female) of the neonate were

considered and examined as dichotomous variables. In sensitivity analyses, birth weight and gestational age were also analyzed as continuous, linear terms and by using finer categories.

- Hospital risk factors of NEC are poorly understood. We examined two hospital characteristics - **delivery method** is categorical (Cesarean section, vaginal birth) while **ventilation time** (days) is continuous.
- To identify economic and institutional health care disparities among NEC patients of different races, we analyzed NEC outcomes according to **insurance status** (state-based funding, HMO/private coverage, or paid-out-of-pocket/other coverage). We did not investigate whether **parental income** was related to case fatality or length of hospital stay, as this data was not available.

## ANALYSIS PLAN

**Descriptive and Bivariate Analyses**: To describe the characteristics of the population under study, we report how diverse exposures and potential confounders are distributed across NEC cases (by race) and indicate whether NEC infants who died versus those who survived differ with respect to these characteristics. We also report how these characteristics are distributed and vary among NEC patients with longer hospital stays compared to those with shorter hospital stays. Continuous variables are described by mean and standard deviation; categorical variables were defined by frequencies of each category.

**Logistic Regression Analysis**: Logistic regression analysis permits an examination of the relationship between several independent variables and NEC case-fatality which is a dichotomous dependent variable. Logistic regression is well suited for epidemiologic studies of dichotomous outcomes because it permits modeling of multiple variables (including continuous variables) and provides overall tests of significance. Moreover, in logistic modeling, all independent variables can be treated as exposures and potential confounders simultaneously. We report the crude odds ratios with 95% confidence intervals as well as adjusted odds ratios using the final adjusted model determined through the model selection procedure. Wald p-values representing the combined significance across all levels of a variable are provided.

**Model Selection**

We describe the process used to select an accurate yet parsimonious linear or logistic regression model. Four models are specified: (i) The first is the **maximum model**, which may include the main exposure variable, covariates, and possible cross-product terms representing the interaction between each covariate with the main exposure. All subsequent models, including the **fully adjusted model**, the **most parsimonious model**, and the **final model** are derived from the maximum model, using a sequential procedure known as *backward elimination*. Backward elimination starts with the maximum model and eliminates variables one by one. At each step, the parameter with the least significant p-value is removed. (ii) Backward elimination of the cross-product terms is considered first to derive the **fully adjusted model**. The fully adjusted model is considered the "gold standard" against which all measures of association (e.g. odds ratios) are compared to assess for confounding. It includes all of the main effect terms under consideration, along with any significant cross-product terms. A strict stay level of 0.01 was specified (slstay) (iii) Next, backward elimination of the main effect terms is considered to derive the **most parsimonious model**. The most parsimonious model includes the minimal set of variables that validly describe the association between the exposure and the outcome. Meaning, it shows no evidence of confounding against the fully adjusted model. It includes the exposure variable, any significant cross-product terms, main effect terms that are part of the cross-product terms, and confounders of the exposure. A very low slstay level of 0.0001 was specified to give every main effects parameter a chance to be dropped and tested for confounding. Adjusted estimates falling 10% above or below the "gold standard" are considered potential confounders and are retained as main effects in the model. (iv)The **final model** is usually a variation of the most parsimonious model. Parameters may be added (or omitted) based on findings in the literature, tests of significance, efforts to improve precision, or reasons related to publication. To assess each model's "goodness of fit," we report the **Hosmer-Lemeshow chi-square statistic**. This test assesses whether or not the observed event rates match expected event rates in subgroups of the model population.

**Test for Multicollinearity**: Multicollinearity occurs when two or more predictor variables in a multivariable model are highly correlated. Although multicollinearity does not reduce the predictive power or reliability of the model as a whole, it can complicate model selection and lead to erroneous statements regarding the usefulness (or importance) of various variables for predicting the response. Collinearity was assessed between each variable $X_i$ and the other predictors in the final regression

equation by observing the variance inflation factor. A VIF of around 5 is indicative of moderate multicollinearity, and a VIF of 10 or more indicates severe multicollinearity. A VIF of 1 indicates an absence of multicollinearity.

**Tests for Effect Modification & Confounding**: Effect modification was assessed using Breslow-Day tests for heterogeneity in stratified analysis and by assessing the significance of cross-product terms in logistic regression and Cox proportional hazards regression. Specifically, we examined whether the association between NEC case-fatality or length of hospital stay and race, adjusted for other variables, is modified across levels of the other covariates. In the absence of interaction, crude and adjusted odds ratios (without interaction terms) were compared to determine the presence of confounding. Adjusted values falling more than 10% above or below the crude estimate are considered potential confounders.

**Survival Analysis**: The Cox proportional hazard estimator was used for estimating time-to-event end points. The main assumption is proportionality. Meaning, the ratio of hazards is constant and does not depend on time. The proportional hazards assumption was assessed by inclusion of a time-dependent covariate in the Cox model; specifically, a cross-product term for the interaction between our main exposure (race) and LOS time. If the time dependent covariate is significant then that predictor is not proportional. A p-value of less than 0.05 was considered statistically significant. Comparisons across groups were conducted by log-rank test. Survival time is length of stay in the hospital, or time to discharge.

**Data Analysis**: Statistical analyses were conducted using SAS version 9.3 software.

# CHAPTER IV

RESULTS

**DESCRIPTIVE & BIVARIATE ANALYSES**

This study is an examination of neonates with an NEC diagnosis. To describe the characteristics of this population, we report how diverse exposures and potential confounders are distributed across subjects (Tables 3.1 and 3.2). Complete data on race, sex, ventilator time, length of hospital stay, survival outcome, and total hospital costs are available on all NEC patients. All but two cases also have available gestational age and birth weight information, and all but a single patient has information on payment type. Information regarding Apgar scores (1-minute and 5-minute), birth number, and delivery method are available for the majority of cases but may be unknown or missing for several patients.

Between 1 January 2009 and 31 December 2010, there were 599,520 patient visits to Children's Healthcare of Atlanta that resulted in 25,346 hospital admissions. Overall, we identified 108 neonates with a diagnosis of NEC. Some of the NEC cases in the study were rated by clinicians according to severity using Bell's Staging criteria. Four cases (5.6%) were categorized as stage I, 35 (49.3%) as stage II, and 32 (45.1%) as stage III. Thirty-seven patients have no available information for staging. Given the already limited sample size, all patients regardless of staging category were included in the final study population, including stage I suspect NEC cases. More than three-quarters of infants are preterm (n=59, 55.7%) or extremely preterm babies (n=21, 19.8%) born at 33 weeks of gestation or less, and most are very low (n=54, 51.0%) or extremely low birth weight (n=35, 33.0%) infants weighing 2,500 g or less. Twenty-six (24.5%) infants were born near or at full term (>33 weeks of gestation), and 17 (16.0%) were of normal birth weight at delivery (>2,500 g). Apgar summaries based on an evaluation of each newborn's respiration, heart rate, muscle tone, reflexes, and skin color were performed at 1-minute (n=83) and 5-minutes (n=84) following birth. The 1-minute score determines how well the baby tolerated the birthing process, and the 5-minute score provides an indication of how well the baby is doing outside the mother's womb. Sixty-two (74.7%) patients had a normal Apgar rating above a score of 5 at 1-minute and 73 (86.9%) patients had normal Apgar ratings above a score of 7 at 5-minutes.

We also report the frequency of NEC cases by diverse demographic and hospital risk factors. More than half of cases are male (n=61, 56.5%). By race, 35 (32.4%) are non-Hispanic white, 48 (44.4%) non-Hispanic black, 9 (8.3%) Hispanic, and 16 (14.8%) are Alaska Native American, Native Hawaiian, Asian, or multi-racial. Sixty-four (83.1%) cases were known singleton births versus 13 multiple gestation births (16.9%), and 58 (64.4%) were born by Cesarean section versus 32 (35.6%) vaginally.

**Table 3.1: Characteristics of study population, by NEC and survival status (CHOA, 2009-2010)**

| *Characteristics* | *NEC* N (col %)* | *Death* N (row %)^ | *No Death* N (row%) | $X^2$ (d.f.)† | *p-value* | *Fisher's p-value*†† |
|---|---|---|---|---|---|---|
| **TOTAL** | **108** | **35 (32.4%)** | **73 (67.6%)** | | | |
| *NEC Stage* | | | | | | |
| I | 4 (5.6%) | 1 (25.0%) | 3 (75.0%) | | | |
| II | 35 (49.3%) | 12 (34.3%) | 23 (65.7%) | | | |
| III | 32 (45.1%) | 11 (34.4%) | 21 (65.6%) | 0.1468(2) | *0.9292* | *1.0000* |
| *Unknown/Missing* | *37* | *11* | *26* | | | |
| *Gestational Age, wk* | | | | | | |
| Extremely Premature (<27) | 21 (19.8%) | 9 (42.9%) | 12 (57.1%) | | | |
| Preterm (27-33) | 59 (55.7%) | 19 (32.2%) | 40 (67.8%) | | | |
| Near & Full Term (>33) | 26 (24.5%) | 6 (23.1%) | 20 (76.9%) | 2.0872(2) | *0.3522* | -- |
| *Unknown/Missing* | *2* | *1* | *1* | | | |
| *Birth Weight, grams* | | | | | | |
| <1000, ELBW | 35 (33.0%) | 14 (40.0%) | 21 (60.0%) | | | |
| 1000-2500, LBW | 54 (51.0%) | 15 (27.8%) | 39 (72.2%) | | | |
| >2500 | 17 (16.0%) | 5 (29.4%) | 12 (70.6%) | | | -- |
| *Unknown/Missing* | *2* | *1* | *1* | 1.5220(2) | *0.4672* | |
| *1-min Apgar Score* | | | | | | |
| <5 | 21 (25.3%) | 7 (33.3%) | 14 (66.7%) | | | |
| 5-10, normal | 62 (74.7%) | 16 (25.8%) | 46 (74.2%) | 0.4436(1) | *0.5054* | -- |
| *Unknown/Missing* | *25* | *12* | *13* | | | |
| *5-min Apgar Score* | | | | | | |
| <7 | 11 (13.1%) | 7 (63.6%) | 4 (36.4%) | | | |
| 7-10, normal | 73 (86.9%) | 17 (23.3%) | 56 (76.7%) | 7.6259(1) | *0.0058* | *0.0106* |
| *Unknown/Missing* | *24* | *11* | *13* | | | |
| *Sex* | | | | | | |
| Female | 47 (43.5%) | 14 (29.8%) | 33 (70.2%) | | | |
| Male | 61 (56.5%) | 21 (34.4%) | 40 (65.6%) | 0.2608(1) | *0.6096* | -- |
| *Race* | | | | | | |
| White | 35 (32.4%) | 8 (22.9%) | 27 (77.1%) | | | |
| Black | 48 (44.4%) | 17 (35.4%) | 31 (64.6%) | | | |
| Hispanic | 9 (8.3%) | 5 (55.6%) | 4 (44.4%) | | | |
| Asian/Multi-Racial/Other | 16 (14.8%) | 5 (31.3%) | 11 (68.8%) | 3.8671(3) | *0.2762* | -- |
| *Multibirth* | | | | | | |
| No | 64 (83.1%) | 22 (34.4%) | 42 (65.6%) | | | |
| Yes | 13 (16.9%) | 2 (15.4%) | 11 (84.6%) | 1.8163(1) | *0.1778* | *0.3239* |
| *Unknown/Missing* | *31* | *11* | *20* | | | |
| *Delivery Method* | | | | | | |
| Cesarean Section | 58 (64.4%) | 17 (29.3%) | 41 (70.7%) | | | |
| Vaginal | 32 (35.6%) | 9 (28.1%) | 23 (71.9%) | 0.0141(1) | *0.9055* | -- |
| *Unknown/Missing* | *18* | *9* | *9* | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Payer Type* | | | | | | |
| Public | 29 (27.1%) | 12 (41.4%) | 17 (58.6%) | | | |
| Private | 59 (55.1%) | 18 (30.5%) | 41 (69.5%) | | | |
| Other | 19 (17.8%) | 4 (21.1%) | 15 (78.9%) | 2.2852(2) | *0.3190* | -- |
| *Unknown/Missing* | *1* | *1* | *0* | | | |

*Column percentages presented, excludes unknown/missing values. ^Row percentages presented.
†Chi-square test, d.f. = degrees of freedom. Excludes unknown/missing data.
††Fisher's Exact test computed for variables with expected cell sizes of <5 counts.

**Table 3.2: Characteristics of CHOA population with NEC (CHOA, 2009-2010)**

| *Characteristic* | *N* | *Min* | *Q1* | *Median* | *Q3* | *Max* | *Mean* | *Std Dev* |
|---|---|---|---|---|---|---|---|---|
| *Ventilator Time (days)* | 101 | 1.0 | 3.0 | 12.0 | 26.0 | 117.0 | 17.6 | 19.5 |
| *Total Hospital Costs* | 108 | $0.00 | $33,229 | $248,971 | $569,545 | $11,780,883 | $460,970 | $1,154,211 |

As most cases in this cohort are surgical, the majority received ventilator support to assist or replace natural breathing (n=101) (Table 3.2). The median ventilator time was 12 days, but ranged from a single day to 117 days. The median cost of being hospitalized at a CHOA facility for NEC during this period was $248,971 (range, $0.00 to $11,780,883). More than half of patients (n=59, 55.1%) covered these costs through private insurance (Table 3.1). Twenty-nine patients (27.1%) utilized public forms of payment such as Medicaid. Nineteen patients (17.8%) either paid out-of-pocket or utilized other forms of coverage such as Tricare or Champus for uniformed service members and their beneficiaries, or Peach State Health Insurance which is a managed care program for Medicaid members, including uninsured, eligible children living in Georgia.

**NEC SURVIVAL**

NEC is a leading cause of significant morbidity and mortality during the neonatal period. Thirty-five deaths, representing 32.4% of all NEC cases, were documented (Table 3.1). Overall, no significant difference was observed in the distribution of any variable by survival status ($p>0.05$), as determined by Mantel-Haenszel Chi Square Statistic or by Fisher's Exact Test. Although NEC survivors and non-survivors do not differ statistically with respect to these diverse characteristics, our findings provide a suggestion of higher levels of death among minority populations and by various characteristics. A larger proportion of extremely preterm (9 of 21, 42.9%) and preterm NEC infants (19 of 59, 32.2%) died compared with full-term infants (6 of 26, 23.1%). Also, a higher proportion of extremely low birth weight infants with NEC died (14 of 35, 40.0%) compared with low birth weight (15 of 54, 27.8%) or normal birth weight infants (5 of 17, 29.4%). More infants with abnormal Apgar ratings at 1-minute (7 of 21,

33.3%) and 5-minutes (7 of 11, 63.6%) died compared to infants assigned normal Apgar scores at birth at 1-minute (16 of 62, 25.8%) and 5-minutes (17 of 73, 23.3%).

We also examined the distribution of diverse demographic and hospital risk factors by survival status. Among 61 male infants with NEC, 21 (34.4%) died compared with 14 of 47 (29.8%) female infants. Also, among 64 singleton babies with NEC, 22 (34.4%) died compared with 2 of 13 (15.4%) multiple gestation babies. Similar proportions of NEC babies born by Cesarean section (17 of 58, 29.3%) and babies born vaginally (9 of 32, 28.1%) resulted in death. By race, 5 of 9 (55.6%) Hispanic, 17 of 48 (35.4%) non-Hispanic black, and 5 of 16 (31.3%) other race infants with an NEC diagnosis resulted in death. In contrast, only 8 of 35 (22.9%) non-Hispanic white infants with NEC died. Moreover, a larger proportion of individuals utilizing public forms of coverage (12 of 29, 41.4%) died compared with persons utilizing private coverage (18 of 59, 30.5%) or who paid-out-of-pocket (4 of 19, 21.1%).

*Logistic Regression Analysis*

Logistic regression was used to examine the associations between diverse categorical and dichotomous exposures with NEC case-fatality. A more thorough examination of the race-survival relationship is also explored. Odds ratio estimates (with 95% confidence intervals) representing crude and adjusted models are reported, as well as Wald p-values representing the combined significance of all levels of each variable.

**Table 3.3: Crude and adjusted associations of risk factors and NEC case-fatality (CHOA, 2009-2010)**

| *Characteristic* | *Unadjusted* | | | | *Birth Weight-Adjusted* | | | |
|---|---|---|---|---|---|---|---|---|
| | *N* | *OR* | *95% CI*[†] | *p-value*[††] | *N* | *OR* | *95% CI* | *p-value* |
| *NEC Stage* | | | | | | | | |
| I (*ref*) | 71 | 1 | -- | *0.9303* | 71 | 1 | -- | *0.9066* |
| II | | 1.57 | (0.15, 16.72) | | | 1.70 | (0.16, 18.56) | |
| III | | 1.57 | (0.15, 16.94) | | | 1.54 | (0.14, 17.22) | |
| *Gestational Age, wk* | | | | | | | | |
| Extremely Premature (<27) | | 2.50 | (0.71, 8.78) | | | 4.86 | (0.43, 55.38) | |
| Preterm (27-33) | | 1.58 | (0.55, 4.59) | | | 3.53 | (0.40, 31.04) | |
| Near & Full Term (>33) (*ref*) | 106 | 1 | -- | *0.3596* | 106 | 1 | -- | *0.4445* |
| *Birth Weight, grams* | | | | | | | | |
| <1000, ELBW | | 1.60 | (0.46, 5.55) | | | -- | -- | |
| 1000-2500, LBW | | 0.92 | (0.28, 3.07) | | | -- | -- | |
| >2500 (*ref*) | 106 | 1 | -- | *0.4704* | | -- | -- | -- |
| *1-min Apgar Score* | | | | | | | | |
| <5 | | 1.44 | (0.49, 4.20) | | | 0.61 | (0.30, 1.21) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5-10, normal (*ref*) | 83 | 1 | -- | *0.5065* | 83 | 1 | -- | *0.1549* |
| *5-min Apgar Score* | | | | | | | | |
| <7 | | 5.77 | (1.51, 22.08) | | | 0.59 | (0.26, 1.36) | |
| 7-10 (*ref*) | 84 | 1 | -- | *0.0106* | 84 | 1 | -- | *0.9561* |
| *Sex* | | | | | | | | |
| Female (*ref*) | 108 | 1 | -- | *0.6098* | 106 | 1 | -- | *0.7247* |
| Male | | 1.24 | (0.55, 2.81) | | | 1.16 | (0.50, 2.68) | |
| *Race* | | | | | | | | |
| White (*ref*) | 108 | 1 | -- | *0.2995* | 106 | 1 | -- | *0.3459* |
| Black | | 1.85 | (0.69, 4.96) | | | 1.68 | (0.61, 4.61) | |
| Hispanic | | 4.22 | (0.91, 19.55) | | | 4.13 | (0.88, 19.46) | |
| Asian/Multi-Racial/Other | | 1.53 | (0.41, 5.74) | | | 1.55 | (0.41, 5.92) | |
| *Multibirth* | | | | | | | | |
| No (*ref*) | 77 | 1 | -- | *0.1928* | 76 | 1 | -- | *0.1466* |
| Yes | | 0.35 | (0.07, 1.71) | | | 0.29 | (0.06, 1.54) | |
| *Delivery Method* | | | | | | | | |
| Cesarean Section (*ref*) | 90 | 1 | -- | *0.9055* | 90 | 1 | -- | *0.9695* |
| Vaginal | | 0.94 | (0.36, 2.45) | | | 0.98 | (0.37, 2.58) | |
| *Payer Type* | | | | | | | | |
| Public (*ref*) | 107 | 1 | -- | *0.3273* | 105 | 1 | -- | *0.4051* |
| Private | | 0.62 | (0.25, 1.57) | | | 0.59 | (0.23, 1.50) | |
| Other | | 0.38 | (0.10, 1.43) | | | 0.45 | (0.12, 1.74) | |

†C.I. = Confidence Interval. ††Wald Chi-square statistics for the combined significance of each predictor.

Indicators of Infant Health: We report odds ratio estimates representing the associations between diverse indicators of disease severity (NEC Stage) and infant health (gestational age, birth weight, Apgar scores) with survival outcome among neonates with NEC (Table 3.3). Overall statistical significance was not observed for most predictors, however, important trends are observed. As predicted, patients with stage II (OR=1.57, 95% CI: 0.15, 16.72) and advanced stage III NEC (OR=1.57, 95% CI: 0.15, 16.94) exhibit higher overall odds of death compared to stage I cases, even after controlling for birth weight. Our data also indicate a trend toward higher odds of death with decreasing levels of gestational age, birth weight, and Apgar scores. The crude odds of death due to NEC among extremely premature infants (<27 weeks) is 2.50 the odds in babies born to full-term (95% CI: 0.71, 8.78), and the odds of death among preterm infants (27-33 weeks) is 1.58 the odds of full-term babies (95% CI: 0.55, 4.59). Similar estimates were reached after birth weight adjustment, however, neither crude (p=0.3596) nor birth weight-adjusted analyses (p=0.4445) reached statistical significance. The odds ratio among extremely low birth weight

infants (<1000 g) is 1.60 (95% CI: 0.46, 5.55), and the odds ratio among preterm infants (27-33 weeks) approached the null value (OR=0.92, 95% CI: 0.28, 3.07). Although our analyses do not support an association between survival and Apgar scores at 1-minute (p=0.5065), a significant association was observed for Apgar scores obtained 5-minutes post-partum (p=0.0106). At 5-minutes, infants with abnormal ratings below a score of 7 exhibited 5.77 higher odds of death compared to infants with normal Apgar ratings (95% CI: 1.51, 22.08). The significance of this association, however, was lost after controlling for birth weight (p=0.9561). Moreover, a switchover in the odds ratio was observed for both Apgar 1-minute and Apgar 5-minute ratings, suggesting that birth weight is a confounder of the Apgar and survival relationship.

Demographic and Hospital Risk Factors: We report odds ratio estimates representing the associations between demographic and hospital risk factors (sex, multi-birth, delivery method, payer type) and infant survival among NEC neonates (Table 3.3). Overall, statistical significance was not reached for any predictor in the crude or adjusted logistic analyses. The birth weight-adjusted OR for males with NEC compared to females is 1.16 (95% CI: 0.50, 2.68), suggesting a weak albeit non-significant association (p=0.7247). The birth weight-adjusted estimate for vaginal births compared to births by Cesarean section also approached the null (OR=0.98, 95% CI: 0.37, 2.58, p=0.9695), suggesting the absence of a strong, significant association. Multiple gestation babies with NEC exhibit decreased odds of death compared to singleton babies with NEC (adjusted OR=0.29, 95% CI: 0.06, 1.54), however, this association was non-significant (p=0.1466). Finally, NEC families utilizing private (adjusted OR=0.59, 95% CI: 0.23, 1.50) or other, non-public forms of insurance (adjusted OR=0.45, 95% CI: 0.12, 1.74) to cover hospital costs exhibited lower odds of death compared to families utilizing public forms of payment (p=0.4051).

Race and NEC Survival

We investigate racial disparities in NEC survival using logistic regression analysis. Although our crude analyses revealed that race, overall, is not a significant predictor of survival outcome (p=0.2995), infants of non-Hispanic black (OR=1.85, 95% CI: 0.69, 4.96) and Hispanic mothers (OR=4.22, 95% CI: 0.91, 19.55) have almost 2-fold upward to 4-fold higher odds of death compared to non-Hispanic whites (Table 3.3). Multi-racial, Asian, Native Hawaiian, and Alaska Native neonates with NEC also exhibit a

1.53 (95% CI: 0.41, 5.74) higher odds of death compared to non-Hispanic whites. These results provide a suggestion of racial disparities in NEC case-fatality.

We also examined the race-survival association by controlling for diverse risk factors (Table 3.4). Tests for effect modification were performed by the addition of a cross-product term to each logistic model, and homogeneity p-values for the combined significance of the cross-product terms are reported. In the absence of interaction, crude estimates were compared to adjusted estimates as a test of confounding. Adjustments were considered only for variables with minimal data missingness in order to maintain similar crude and adjusted population subsets for comparison. Overall, race remained a statistically non-significant predictor of survival after controlling individually for NEC stage, gestational age, birth weight, sex, or payer type. No evidence of interaction was detected with any covariate ($p>0.05$). All predictors, however, with the exception of sex confounded the race-survival association, as evidenced by differences in the adjusted ORs 10% above or below the crude estimates.

**Table 3.4: Association of race with NEC fatality, adjusted for other characteristics (CHOA, 2009-2010)**

| *Adjusted For*[*] | *N* | *Race* | *OR* | *95% CI*[†] | *p-value*[††] | *Homogeneity p-value of interaction term*[^] |
|---|---|---|---|---|---|---|
| *Unadjusted (crude)* | 108 | White (*ref*) | 1 | -- | *0.2995* | -- |
| | | Black | 1.85 | (0.69, 4.96) | -- | -- |
| | | Hispanic | 4.22 | (0.91, 19.55) | -- | -- |
| | | Other | 1.53 | (0.41, 5.74) | -- | -- |
| *Gestational Age, wk* | 106 | White (*ref*) | 1 | -- | *0.3185* | 0.9881 |
| | | Black | 1.57 | (0.57, 4.38) | -- | -- |
| | | Hispanic | 4.39 | (0.93, 20.70) | -- | -- |
| | | Other | 1.45 | (0.38, 5.50) | -- | -- |
| *Birth Weight, grams* | 106 | White (*ref*) | 1 | -- | *0.3459* | 0.9996 |
| | | Black | 1.68 | (0.61, 4.61) | -- | -- |
| | | Hispanic | 4.13 | (0.88, 19.46) | -- | -- |
| | | Other | 1.55 | (0.41, 5.92) | -- | -- |
| *Sex* | 108 | White (*ref*) | 1 | -- | *0.2917* | 0.8632 |
| | | Black | 1.93 | (0.71, 5.22) | -- | -- |
| | | Hispanic | 4.20 | (0.90, 19.50) | -- | -- |
| | | Other | 1.54 | (0.41, 5.76) | -- | -- |
| *Payer Type* | 107 | White (*ref*) | 1 | -- | *0.3690* | 0.6599 |
| | | Black | 1.98 | (0.71, 5.51) | -- | -- |
| | | Hispanic | 3.98 | (0.76, 20.90) | -- | -- |
| | | Other | 1.73 | (0.45, 6.65) | -- | -- |

[*]For categories of adjustment variables
[†]C.I. = Confidence Interval. [††]Wald Chi-square statistics for the combined significance of each predictor.
^Test for interaction. Homogeneity p-value for the significance of the combined cross-product terms.

**Table 3.5: Race & NEC fatality, different categorizations of gestational age and birth weight (CHOA, 2009-2010)**

| *Adjusted For:* | *Race* | *N* | *OR* | *95% CI*[†] | *p-value*[††] |
|---|---|---|---|---|---|
| *Unadjusted (crude)* | White (*ref*) | 108 | 1 | -- | *0.2995* |
| | Black | | 1.85 | (0.69, 4.96) | -- |
| | Hispanic | | 4.22 | (0.91, 19.55) | -- |
| | Other | | 1.53 | (0.41, 5.74) | -- |
| *Gestational Age, wk* (2- levels) | White (*ref*) | 106 | 1 | -- | *0.3074* |
| <33, ≥33 | Black | | 1.70 | (0.62, 4.63) | -- |
| | Hispanic | | 4.37 | (0.93, 20.62) | -- |
| | Other | | 1.44 | (0.38, 5.43) | -- |
| *Gestational Age, wk* (3- levels) | White (*ref*) | 106 | 1 | -- | *0.3185* |
| <27, 27-33, >33 | Black | | 1.57 | (0.57, 4.38) | -- |
| | Hispanic | | 4.39 | (0.93, 20.70) | -- |
| | Other | | 1.45 | (0.38, 5.50) | -- |
| *Gestational Age, wk* (7-levels) | White (*ref*) | 106 | 1 | -- | *0.3225* |
| <24, 25-26, 27-28, 29-30, 31-32, 33-34, >34 | Black | | 1.55 | (0.53, 4.55) | -- |
| | Hispanic | | 4.44 | (0.93, 21.28) | -- |
| | Other | | 1.60 | (0.40, 6.39) | -- |
| *Gestational Age, continuous* | White (*ref*) | 106 | 1 | -- | *0.3322* |
| | Black | | 1.51 | (0.90, 20.34) | -- |
| | Hispanic | | 4.29 | (0.34, 5.01) | -- |
| | Other | | 1.31 | (0.83, 1.02) | -- |
| *Birth Weight, grams* (2-levels) | White (*ref*) | 106 | 1 | -- | *0.3026* |
| <2500, ≥2500 | Black | | 1.76 | (0.65, 4.80) | -- |
| | Hispanic | | 4.32 | (0.92, 20.20) | -- |
| | Other | | 1.49 | (0.39, 5.63) | -- |
| *Birth Weight, grams* (3-levels) | White (*ref*) | 106 | 1 | -- | *0.3459* |
| <1000, 1000-2500, >2500 | Black | | 1.68 | (0.61, 4.61) | -- |
| | Hispanic | | 4.13 | (0.88, 19.46) | -- |
| | Other | | 1.55 | (0.41, 5.92) | -- |
| *Birth Weight, grams* (10-levels) | White (*ref*) | 106 | 1 | -- | *0.3629* |
| <500, 500-749, 750-999, 1000-1249, | Black | | 1.59 | (0.56, 4.55) | -- |
| 1250-1499, 1500-1749, 1750-1999, | Hispanic | | 4.29 | (0.86, 21.42) | -- |
| 2000-2249, 2250-2499, >2500 | Other | | 1.37 | (0.34, 5.58) | -- |
| *Birth Weight, continuous* | White (*ref*) | 106 | 1 | -- | *0.3383* |
| | Black | | 1.55 | (0.56, 4.30) | -- |
| | Hispanic | | 4.26 | (0.90, 20.20) | -- |
| | Other | | 1.38 | (0.36, 5.22) | -- |
| *Gestational Age + Birth Weight, continuous* | White (*ref*) | 106 | 1 | -- | *0.3299* |
| | Black | | 1.51 | (0.54, 4.23) | -- |
| | Hispanic | | 4.29 | (0.91, 20.33) | -- |
| | Other | | 1.30 | (0.34, 4.99) | -- |

[†]C.I. = Confidence Interval. [††]Wald Chi-square statistics for the combined significance of each predictor.

Adjustments for gestational age and birth weight were also analyzed using broad to finer categories of each predictor (Table 3.5). Adjustments using different parameterizations of gestational age and birth weight produced estimates falling more than 10% away from crude values, further supporting our finding that these predictors confound the race-survival relationship. Of particular note is the stepwise reduction in the adjusted OR for non-Hispanic black infants compared to white babies as finer categories of gestational age are utilized in the adjustment: 2-level (OR=1.70), 3-level (OR=1.57), 7-level (OR=1.55), continuous (OR=1.51). A similar trend was observed when using different parameterizations for birth weight: 2-level (OR=1.76), 3-level (OR=1.68), 7-level (OR=1.59), continuous (OR=1.55). Although adjustments with finer categories of gestational age or birth weight led odds ratios closer to the null, even with tight control for gestational age or birth weight, point estimates for non-Hispanic black infants showed a 50% higher odds of death compared to non-Hispanic whites ($p>0.05$). Overall, our analyses suggest that several variables, including gestational age and birth weight, are important confounders of the race-case fatality relationship.

Final Adjusted Model - Race and Survival

Model building was utilized to derive a multiple logistic regression model that examines diverse risk factors and considers potential effect modifiers and confounders of the race-survival association. Table 3.6 outlines the procedure used to derive the *fully adjusted logistic model*, the *most parsimonious logistic model*, and the *final logistic model* from the maximum model using backward elimination. The initial *maximum model* includes the main exposure variable (race), 4 covariates (gestational age, birth weight, sex, payer type), and several cross-product terms representing the interaction between each covariate and the main exposure. Only predictors with minimal data missingness were considered to maximize the utility of this limited data set.

The *fully adjusted model* was derived by applying backward elimination to the interaction terms first. Each cross-product term did not contribute significantly ($p>0.01$) to the model and was eliminated. Thus, the fully adjusted model contains only main effect terms: race, gestational age, birth weight, sex, and payer type. Next, backward elimination of the main effect terms was considered to derive the *most parsimonious model*. All main effects were initially removed because each parameter did not contribute significantly to the model ($p>0.01$). However, elimination of payer type changed the main exposure estimates by more than 10% relative to the fully adjusted model. Given the presence of confounding,

payer type was returned to the model. No evidence of confounding was detected after dropping the main effect terms for gestational age, birth weight, or sex. In turn, the most parsimonious model contains the following main effects: race and payer type. The *final model* contains the main effects for race and payer type, as well as gestational age and sex. Although gestational age is not a statistically significant predictor of NEC survival and confounding was not indicated in our model building exercise, our analyses using different parameterizations of gestational age (Table 3.5) coupled with evidence from the literature suggests that it is an important confounder of the race-survival relationship. Gestational age but not birth weight was returned to the model since our analyses revealed collinearity between these predictors (data not shown), and gestational age (p=0.6144) had a slightly lower overall p-value compared to birth weight (p=0.6457). Sex was returned to the model since it is a predictor of outcomes in the literature.

**Table 3.6: Model Building – Backward Elimination of Cross-product and Main Effect Terms**

Initial Model: **Logit ( P(D=1 | RACE, GEST, WEIGHT, SEX, PAYER) )**
**$= b_0 + [b_1\text{-}b_3][RACE_{1\text{-}3}] + [b_4\text{-}b_5][GEST_{1\text{-}2}] + [b_6\text{-}b_7][WEIGHT_{1\text{-}2}] + b_8[SEX] + [b_9\text{-}b_{10}][PAYER_{1\text{-}2}] + [b_{11}\text{-}b_{15}][RACE_{1\text{-}3}]*[GEST_{1\text{-}2}] + [b_{16}\text{-}b_{20}][RACE_{1\text{-}3}]*[WEIGHT_{1\text{-}2}] + [b_{21}\text{-}b_{23}][RACE_{1\text{-}3}]*[SEX] + [b_{24}\text{-}b_{28}][RACE_{1\text{-}3}]*[PAYER_{1\text{-}2}]$**

HL GOF Test^: **p-value=0.8600**

Backwards Elimination of Cross-Product Terms

| | Cross-Product Term | Removed or retained p-value |
|---|---|---|
| **Step 1:** | race*Gestational Age | 0.9959 (removed) |
| **Step 2:** | race*Birth Weight | 0.9409 (removed) |
| **Step 3:** | race*Payer Type | 0.8410 (removed) |
| **Step 4:** | race*Sex | 0.4443 (removed) |

Fully Adjusted Model: **Logit ( P(D=1 | RACE, GEST, WEIGHT, SEX, PAYER) )**
**$= b_0 + [b_1\text{-}b_3][RACE_{1\text{-}3}] + [b_4\text{-}b_5][GEST_{1\text{-}2}] + [b_6\text{-}b_7][WEIGHT_{1\text{-}2}] + b_8[SEX] + [b_9\text{-}b_{10}][PAYER_{1\text{-}2}]$**

HL GOF Test**: p-value=0.7843**

Backwards Elimination of Main Effects Terms

| | Fully Adjusted Model | Odds Ratio |
|---|---|---|
| | White (*ref*) | 1 |
| | Black | 1.73 (95% CI: 0.58, 5.17) |
| | Hispanic | 3.65 (95% CI: 0.69, 19.32) |
| | Asian/Multi-Racial/Other | 1.70 (95% CI: 0.43, 6.76) |
| | **Main Effects Term** | **Removed or retained p-value** |
| **Step 1:** | **Sex** | **0.9365 (removed)** |
| | White (*ref*) | 1 |
| | Black | 1.75 → no confounding |
| | Hispanic | 3.65 → no confounding |
| | Asian/Multi-Racial/Other | 1.69 → no confounding |

| | | |
|---|---|---|
| **Step 2:** | **Weight** | **0.6457 (removed)** |
| | White (*ref*) | 1 |
| | Black | 1.67 → no confounding |
| | Hispanic | 3.99 → no confounding |
| **keep** | Asian/Multi-Racial/Other | 1.56 → no confounding |
| **Step 3:** | **Payer Type** | **0.6233 (removed but returned to model)** |
| | White (*ref*) | 1 |
| | Black | 1.52 → confounding |
| | Hispanic | 4.37 → confounding |
| | Asian/Multi-Racial/Other | 1.45 → confounding |
| **Step 4:** | **Gestational Age** | **0.6144 (removed)** |
| | White (*ref*) | 1 |
| | Black | 1.85 → no confounding |
| | Hispanic | 3.81 → no confounding |
| | Asian/Multi-Racial/Other | 1.68 → no confounding |
| Most Parsimonious Model: **Logit ( P(D=1 \| RACE, PAYER) )** $= b_0 + [b_1\text{-}b_3][RACE_{1\text{-}3}] + [b_4\text{-}b_5][PAYER_{1\text{-}2}]$ | | |
| HL GOF Test: **p-value=0.9907** | | |
| Final Adjusted Model: **Logit ( P(D=1 \| RACE, GEST, SEX, PAYER) )** $= b_0 + [b_1\text{-}b_3][RACE_{1\text{-}3}] + [b_4\text{-}b_5][GEST_{1\text{-}2}] + b_6[SEX] + [b_7\text{-}b_8][PAYER_{1\text{-}2}]$ | | |
| HL GOF Test: **p-value=0.7906** | | |

^Hosmer-Lemeshow goodness of fit test (p-value).

A summary of each model is provided in Table 3.7. Hosmer-Lemeshow p-values for the fully adjusted, most parsimonious, and final logistic models are 0.7843, 0.9907, and 0.7906, respectively, indicating that all models are well-calibrated. Overall, race was not a significant predictor of NEC survival after controlling for gestational age, sex, and payer type (p=0.4261). However, point estimates indicated a higher odds of death for non-Hispanic black, Hispanic, and other race neonates compared to non-Hispanic white infants. Hispanic babies with NEC have a 4.0 higher odds of death compared to non-Hispanic white infants (95% CI: 0.76, 21.00), holding all other variables constant. Non-Hispanic black babies (95% CI: 0.57, 4.96) and babies of other race (95% CI: 0.40, 6.07) each share a 1.6-1.7 higher odds of death compared to non-Hispanic white babies, holding all other variables constant. Despite the limited size of our pilot study population, these findings suggest that survival outcome among NEC babies differs by race. Gestational age (p=0.6266), sex (p=0.9393), and payer type (p=0.6223) remained non-significant predictors in the final adjusted logistic regression model, which may have been similarly affected by power.

**Table 3.7: Summary: fully adjusted, most parsimonious, final models. Race and Survival (CHOA, 2009-2010)**

| *Characteristic* | *Fully Adjusted Model* | | | *Parsimonious Model* | | | *Final Model* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *OR* | *95% CI*[†] | *p-value*[††] | *OR* | *95% CI* | *p-value* | *OR* | *95% CI* | *p-value* |
| *Race* | | | | | | | | | |
| White (*ref*) | 1 | -- | 0.4675 | 1 | -- | 0.3690 | 1 | -- | 0.4261 |
| Black | 1.73 | (0.58, 5.17) | | 1.98 | (0.71, 5.51) | | 1.68 | (0.57, 4.96) | |
| Hispanic | 3.65 | (0.69, 19.32) | | 3.98 | (0.76, 20.90) | | 3.99 | (0.76, 21.00) | |
| Other | 1.70 | (0.43, 6.76) | | 1.73 | (0.45, 6.65) | | 1.56 | (0.40, 6.07) | |
| *Gestational Age, wk* | | | | | | | | | |
| <27 | 3.95 | (0.31, 50.89) | | | | | 1.94 | (0.48, 7.80) | |
| 27-33 | 3.34 | (0.36, 30.94) | | | | | 1.55 | (0.51, 4.72) | |
| >33 (*ref*) | 1 | -- | 0.5494 | | | | 1 | -- | 0.6266 |
| *Birth Weight, grams* | | | | | | | | | |
| <1000, ELBW | 0.36 | (0.02, 5.37) | | | | | | | |
| 1000-2500, LBW | 0.32 | (0.03, 3.67) | | | | | | | |
| >2500 (*ref*) | 1 | -- | 0.6443 | | | | | | |
| *Sex* | | | | | | | | | |
| Female (*ref*) | 1 | -- | 0.9365 | | | | 1 | -- | 0.9393 |
| Male | 0.96 | (0.38, 2.42) | | | | | 1.04 | (0.42, 2.53) | |
| *Payer Type* | | | | | | | | | |
| Public (*ref*) | 1 | -- | 0.6215 | 1 | -- | 0.4055 | 1 | -- | 0.6223 |
| Private | 0.82 | (0.29, 2.31) | | 0.83 | (0.30, 2.28) | | 0.82 | (0.29, 2.28) | |
| Other | 0.49 | (0.12, 2.06) | | 0.40 | (0.10, 1.58) | | 0.49 | (0.12, 2.05) | |
| **HL GOF Test:**[^] | (N=105) p-value=0.7843 | | | (N=107) p-value=0.9907 | | | (N=105) p-value=0.7906 | | |

[†]C.I. = Confidence Interval. [††]Wald Chi-square statistics for the combined significance of each predictor.
[^]Hosmer-Lemeshow goodness of fit test (p-value).

## LENGTH OF HOSPITAL STAY (LOS)

We assess overall length of stay in the hospital among neonates treated for NEC at CHOA (Table 3.8). Since death can be a reason that an infant has a short hospital stay, analysis was limited to NEC neonates who survived to discharge (n=73). We report both median and average length of hospitalization times, as well as p-values to assess whether mean LOS differs between groups. Overall, survivors of NEC stayed at Level III CHOA facilities for a median of 55 (range, 1-229) days, or an average ($\pm$SD) of 68.9 $\pm$ 59.4 days. Although average LOS did not differ significantly between levels of any variable ($p>0.05$), a general trend toward increasing LOS was observed with increasing categories of disease severity and by decreasing categories of gestational age, birth weight, and Apgar scores. The average LOS among female survivors was 77.5 $\pm$ 65.2 days, compared with males who stayed an average of 61.9 $\pm$ 53.8 days, although this difference was not significant ($p=0.2681$). By race, non-Hispanic blacks held the longest

times, averaging 82.3 ± 65.0 days in the hospital compared with non-Hispanic white neonates at an average of 62.7 ± 58.8 days, Hispanics at 54.3 ± 52.0 days, and other race neonates at 52.0 ± 42.7 days (p=0.3965). Multiple gestational babies (101.6 ± 71.3 days) and babies born by Cesarean section (75.1 ± 60.2 days) had higher mean LOS times, compared with singleton infants (80.3 ± 56.7) and babies delivered vaginally (63.7 ± 58.8 days). Persons utilizing public forms of payment had the shortest hospitalization times with a mean of 57.7 ± 54.5 days, compared with persons using private insurance (69.7 ± 60.2) or other forms of payment (79.5 + 64.0 days) (p=0.5852).

**Table 3.8: Length of Hospital Stay (days), by different characteristics (CHOA, 2009-2010)**

| *By Characteristic* | *N* | *Median (Range)* | | *Mean ± SD* | *p-value** |
|---|---|---|---|---|---|
| ***Overall LOS of survivors, days*** | **73** | **55.0** | **(1.0 – 229.0)** | **68.9 ± 59.4** | **--** |
| *NEC Stage* | | | | | |
| I | 3 | 108.0 | (90.0 – 151.0) | 116.3 ± 31.3 | 0.3472 |
| II | 23 | 57.0 | (1.0 – 229.0) | 79.0 ± 71.6 | |
| III | 21 | 72.0 | (1.0 – 168.0) | 64.2 ± 48.0 | |
| *Unknown/Missing* | *26* | *42.0* | *(1.0 – 198.0)* | *58.3 ± 56.6* | |
| *Gestational Age, wk* | | | | | |
| Extremely Premature (<27) | 12 | 79.0 | (1.0 – 168.0) | 70.7 ± 58.6 | 0.9533 |
| Preterm (27-33) | 40 | 67.0 | (1.0 – 229.0) | 70.8 ± 65.5 | |
| Near & Full Term (>33) | 20 | 51.5 | (8.0 – 198.0) | 65.9 ± 49.8 | |
| *Unknown/Missing* | *1* | *35.0* | *(35.0 – 35.0)* | *35.0 ± 0.0* | |
| *Birth weight, grams* | | | | | |
| <26.5 | 7 | 90.0 | (31.0 – 168.0) | 99.6 ± 49.9 | 0.5287 |
| 26.5-35 | 14 | 18.5 | (1.0 – 178.0) | 54.3 ± 62.8 | |
| 36-70.5 | 31 | 71.0 | (1.0 – 229.0) | 72.5 ± 65.2 | |
| >70.5 | 20 | 47.5 | (8.0 – 198.0) | 64.6 ± 50.4 | |
| *Unknown/Missing* | *1* | *35.0* | *(35.0 – 35.0)* | *35.0 ± 0.0* | |
| *1-minute Apgar Score* | | | | | |
| <5 | 14 | 94.0 | (2.0 – 229.0) | 93.2 ± 70.6 | 0.1677 |
| 5-10, normal | 46 | 54.0 | (1.0 – 198.0) | 67.8 ± 56.1 | |
| *Unknown/Missing* | *13* | *27.0* | *(1.0 – 152.0)* | *46.8 ± 52.2* | |
| *5-minute Apgar Score* | | | | | |
| <7 | 4 | 94.0 | (80.0 – 151.0) | 104.7 ± 31.7 | 0.2890 |
| 7-10, normal | 56 | 54.0 | (1.0 – 229.0) | 71.5 ± 61.2 | |
| *Unknown/Missing* | *13* | *27.0* | *(1.0 – 152.0)* | *46.8 ± 52.2* | |
| *Sex* | | | | | |
| Female | 33 | 57.0 | (1.0 – 217.0) | 77.5 ± 65.2 | 0.2681 |
| Male | 40 | 48.5 | (1.0 – 229.0) | 61.9 ± 53.8 | |
| *Race* | | | | | |
| White | 27 | 43.0 | (1.0 – 198.0) | 62.7 ± 58.8 | 0.3965 |

| | | | | | |
|---|---|---|---|---|---|
| Black | 31 | 63.0 | (1.0 – 229.0) | 82.3 ± 65.0 | |
| Hispanic | 4 | 51.0 | (2.0 – 113.0) | 54.3 ± 52.0 | |
| Asian/Multi-Racial/Other | 11 | 55.0 | (1.0 – 108.0) | 52.0 ± 42.7 | |
| *Multibirth* | | | | | |
| No | 42 | 71.5 | (2.0 – 229.0) | 80.3 ± 56.7 | 0.2974 |
| Yes | 11 | 98.0 | (3.0 – 217.0) | 101.6 ± 71.3 | |
| *Unknown/Missing* | *20* | *19.0* | *(1.0 – 109.0)* | *27.1 ± 31.3* | |
| *Delivery Method* | | | | | |
| Cesarean Section | 41 | 63.0 | (1.0 – 217.0) | 75.1 ± 60.2 | 0.4688 |
| Vaginal | 23 | 55.0 | (1.0 – 229.0) | 63.7 ± 58.8 | |
| *Unknown/Missing* | *9* | *35.0* | *(1.0 – 152.0)* | *54.2 ± 59.8* | |
| *Payer Type* | | | | | |
| Public | 17 | 43.0 | (1.0 – 178.0) | 57.7 ± 54.5 | 0.5852 |
| Private | 41 | 57.0 | (1.0 – 217.0) | 69.7 ± 60.2 | |
| Other | 15 | 63.0 | (6.0 – 229.0) | 79.5 ± 64.0 | |

*Analysis of variance (ANOVA) to assess whether group means differ.

Hazard Analysis

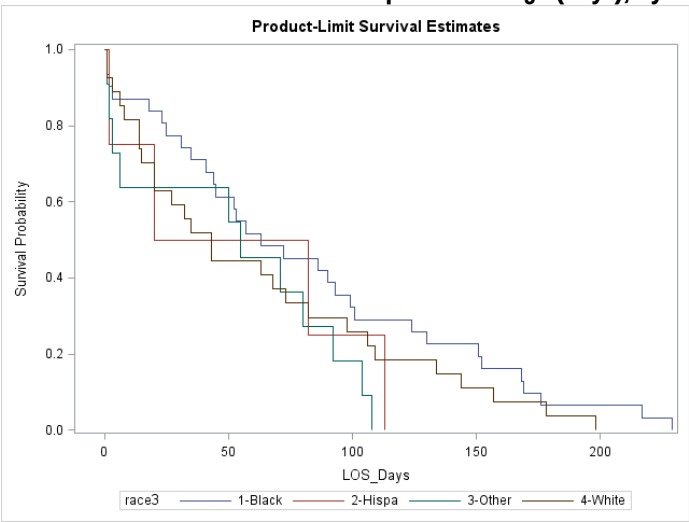
Cox proportional hazard models were used to analyze time to hospital discharge by race (Figure 3.1, Table 3.9). Hazard ratios greater than 1 indicate the better outcome of shorter hospital lengths (i.e. faster discharge), while hazard ratios falling below 1 indicate longer periods of hospital stay and possibly more serious disease (i.e. slower time to discharge). Compared to non-Hispanic whites, the crude rate of being discharged from a CHOA facility is 0.74 (95% CI: 0.44, 1.25) times slower for black neonates but 1.21 (95% CI: 0.42, 3.49) times faster for Hispanics and 1.35 (95% CI: 0.66, 2.77) times faster for other race infants. We also report adjusted Cox proportional hazard estimates, controlling individually for gestational age, birth weight, sex, or payer type, or controlling for gestational age, sex, and payer type simultaneously. In the fully adjusted hazard model where gestational age, sex, and payer type are controlled for, the hazard ratio comparing non-Hispanic black neonates to non-Hispanic white infants is 0.85 (95% CI: 0.47, 1.56). Compared to white babies, the hazard ratio for hospital discharge is 1.55 (95% CI: 0.48, 4.98) times greater for Hispanics and 1.62 (95% CI: 0.73, 3.55) times greater for other race infants. Overall, we do not find a statistically significant difference in LOS by race in either the crude ($p=0.3422$) or final adjusted models ($p=0.3860$). However, the potential for a subject with NEC to stay in the hospital for longer periods is greatest for non-Hispanic blacks, followed by non-Hispanic whites, Hispanics, and other race infants. No significant interaction between race and LOS time was observed for any hazard model ($p>0.05$), indicating our analyses fulfill the proportional hazards assumption.

**Table 3.9: Cox proportional hazard ratios for race and hospital stay length (CHOA, 2009-2010)**

| *Adjusted For:* | *By Race* | *N* | *Hazard Ratio* | *95% CI*[†] | *P-value*[††] | *LOS*Race interaction p-value*[^] |
|---|---|---|---|---|---|---|
| *Unadjusted (Crude)* | White (*ref*) | 73 | 1 | -- | *0.3422* | *0.9031* |
| | Black | | 0.74 | (0.44, 1.25) | | |
| | Hispanic | | 1.21 | (0.42, 3.49) | | |
| | Other | | 1.35 | (0.66, 2.77) | | |
| *Gestational Age* | White (*ref*) | 72 | 1 | -- | *0.2781* | *0.7938* |
| | Black | | 0.72 | (0.41, 1.24) | | |
| | Hispanic | | 1.30 | (0.44, 3.84) | | |
| | Other | | 1.41 | (0.69, 2.92) | | |
| | | | | | | *0.6505* |
| *Birth Weight* | White (*ref*) | 72 | 1 | -- | *0.3140* | |
| | Black | | 0.79 | (0.45, 1.39) | | |
| | Hispanic | | 1.28 | (0.44, 3.73) | | |
| | Other | | 1.55 | (0.71, 3.37) | | |
| *Sex* | White (*ref*) | 73 | 1 | -- | *0.4069* | *0.9094* |
| | Black | | 0.76 | (0.45, 1.30) | | |
| | Hispanic | | 1.25 | (0.43, 3.62) | | |
| | Other | | 1.35 | 0.66, 2.77) | | |
| *Payer Type* | White (*ref*) | 73 | 1 | -- | *0.4036* | *0.8548* |
| | Black | | 0.80 | (0.46, 1.39) | | |
| | Hispanic | | 1.28 | (0.41, 3.97) | | |
| | Other | | 1.48 | (0.68, 3.23) | | |
| *Gestational Age, Sex, Payer Type* | White (*ref*) | 72 | 1 | -- | *0.3860* | *0.7169* |
| | Black | | 0.85 | (0.47, 1.56) | | |
| | Hispanic | | 1.55 | (0.48, 4.98) | | |
| | Other | | 1.62 | (0.73, 3.55) | | |

[†]CI = Confidence Interval. [††]Wald Chi-Square Type-3 test for overall significance. ^Test of proportional hazards assumption, p-value for interaction term of race*LOS_Days.

**Figure 3.1: Survival Plot – Time to Hospital Discharge (days), By Race**



# CHAPTER V

CONCLUSIONS

## SUMMARY & OVERVIEW

Efforts to elucidate racial disparities to better prevent and reduce mortality and other negative outcomes associated with necrotizing enterocolitis have, thus far, been met with limited success. In this pilot study, we examine the relationship between race (as well as other sociodemographic and hospital risk factors) and NEC case-fatality. Our investigation also provides one of the first and few examinations of length of hospitalization by race.

In a large data set of infants derived from NICUs and hospital facilities across the state of Georgia, we identified 108 NEC cases during a two-year study period. Fatality associated with a diagnosis of NEC was high at 32.4%, which is close to expected national estimates of 15-30% (Caplan & Jilling, 2001; R. H. Clark et al., 2012; Holman, Stoll, Clarke, & Glass, 1997; Hull et al., 2013). LOS among NEC survivors averaged 70 $\pm$ 59 days which is slightly lower than reported LOS estimates at other U.S. medical facilities. *Bisquera et al*., for example, reported an overall LOS of 95 $\pm$ 42 days for medical NEC survivors and 142 $\pm$ 65 days for surgical NEC survivors admitted to NICUs at Texas Children's Hospital and Ben Taub General Hospital (Bisquera et al., 2002). Possibly, our study underestimated LOS due to a large number of patients (n=25) who were discharged to facilities outside of CHOA where follow-up on hospitalization time was not possible. The small sample size may be explained by several factors. First, CHOA is primarily a surgical center. Surgical intervention is required in as many as 20% to 40% of cases (Guillet et al., 2006; P. W. Lin & Stoll, 2006). A low study N, thus, may be explained by exclusion of a large number of statewide medically-treated NEC patients. A large proportion of surgical NEC may also explain the high case-fatality. Fatality among surgical NEC ranges between 23% and 36% versus 5% and 10% among medical cases (P. W. Lin & Stoll, 2006). Second, NEC is a complex diagnosis relying on a combination of clinical symptoms, radiographic signs, and/or data attained by abdominal ultrasound. Clinical uncertainty in the determination of disease may lead to an underestimation of true NEC counts. Misclassification of disease or miscoding in CHOA's EPIC medical record system is a third although unlikely possibility. Confirmation of patient diagnoses by detailed review of all patient medical records was not feasible. Finally, differences may exist in applied exclusion or inclusion criteria or how NEC was defined in this study compared to previous investigations. Further discussion of the study's limitations is reviewed below.

Overall, with a study population of 108, study power was limited to detect a significant association between race and survival or length of hospital stay. However, we cannot exclude the possibility of clinically important reductions or increases in either outcome by race. In terms of NEC survival, the frequency with which Hispanic neonates died due to NEC was higher than that of other races. These results are in agreement with findings by *Guner et al.* who reported a 1.44 greater odds of death among Hispanic neonates compared to white, black, or Asian neonates (95% CI: 1.05 to 1.95, p=0.022) (Guner et al., 2009). In addition to these findings, our study revealed increased odds of fatality (albeit non-significant) among black and other race infants with NEC, even after controlling for gestational age, sex, and payer type. Several studies to date have reported similar findings, particularly for black neonates (Carter & Holditch-Davis, 2008; Holman et al., 1997; Llanos et al., 2002). Thus, our survival data compare well with previous population-based investigations.

In terms of LOS, the potential to stay in the hospital for longer periods following an NEC diagnosis was greatest for non-Hispanic blacks, followed by non-Hispanic whites, Hispanics, and other race infants. It is curious that Hispanic and other race survivors have hazard ratios that are greater than white infants (even after controlling for gestational age, sex, and payer type), indicating quicker times to discharge from the hospital; although it must be noted that the confidence intervals are wide for these groups due to the majority of the study population being non-Hispanic black or white. Given the higher case-fatality, one would hypothesize greater overall illness among Hispanic and other race infants and thus, longer periods in the hospital. Possibly, the longer hospitalization periods among black and white infants may be explained by unaccounted comorbidities or medical conditions that disproportionately affect these racial groups. To date, there are no studies that have specifically looked at these questions in regards to race and LOS among NEC survivors to compare or contrast with our findings.

Patient comorbidities can exacerbate NEC disease severity and worsen outcomes. One of the most important patient-specific factors that influences neonatal outcomes is birth weight. This is clearly demonstrated in our study where low birth weight (as well as low gestational age and Apgar scores) correlated with increased fatality risk and length of hospital stay. Although our study's statistical power was limited, the point estimates for Hispanic and black neonates versus white neonates were still suggestively elevated even with fine scale control of gestational age and birth weight. The confounding assessment, therefore, is suggestive of independent impacts of birth weight/gestational age and race on the

outcomes. Possibly, other underlying factors including socioeconomic factors may also explain the racial trends in NEC survival and LOS. Together, these findings underscore the need for a national agenda to decrease the incidence of premature births. In this study, the highest odds of death were observed in the lowest gestational age and birth weight groups, but the highest proportion of deaths was accounted for by infants with gestational ages at birth between 27-33 weeks. These results are comparable to a study by *Llanos et al.* (Llanos et al., 2002). Strategies to decrease the incidence of prematurity in this group, therefore, may have the greatest impact on reducing NEC mortality.

Gender, multiple birth, and delivery method were not recognized as significant risk factors of NEC case-fatality or LOS in this study. However, this finding is likely due to limited power and these factors should continue to be evaluated. Of the 35 infants who died, 21 were male and 14 were female, suggesting that female infants with NEC experience less death. Also, 17 deaths were among infants born by Cesarean section compared with 9 infants born vaginally. Multiple birth infants and babies born by Cesarean section also had higher overall LOS. These findings are consistent with reports that multiple gestation babies and babies born by Cesarean section are at increased risk for prematurity (J. A. Martin & Taffel, 1995).

Socioeconomic factors may contribute to racial disparities in NEC fatality and length of hospital stay. To account for the potential contribution of parental socioeconomic status to NEC fatality and LOS, we examined patients' insurance status. We did not find insurance type to be a significant case-fatality or LOS predictor in this cohort. However, it is interesting that patients utilizing private or other forms of payment had lower overall odds of death compared to patients utilizing public forms of payment. Hispanic neonates with the highest odds of death utilized a greater proportion of public funds than any other race, while non-Hispanic white neonates with the lowest case-fatality risk utilized a greater proportion of private funding (Supplemental Table 1). Non-Hispanic black and other race infants utilized all three forms of payment type more equally and exhibited moderate fatality risks that fell somewhere in between that of white and Hispanic infants. Further research is needed in this area. Recently, *Joseph et al.* demonstrated that lower income is associated with increased risk of gestational diabetes and preterm birth (Joseph, Liston, Dodds, Dahlgren, & Allen, 2007). Based on this finding, one would postulate that if the greatest risk factor for NEC is low birth weight, income and NEC mortality ought to correlate as well.

Therefore, one approach to test whether socioeconomic factors contribute to racial disparities in NEC outcomes may be to link patients (by race) to individual income data.

## STUDY LIMITATIONS

Several limitations of the current analysis deserve discussion.

*Data Set Limitations*: First, this study is a retrospective cohort analysis of clinical associations. Inherent in any retrospective study design using existing medical record data are unknown errors based on medical record documentation. Although the EPIC system is rich with patient information and clinical case findings, flawed results are to be expected when the medical record is the sole source of study data. Also, as a secondary data analysis, the original data were not collected with the intention of investigating the effects of multiple variables on NEC in preterm infants. Therefore, the data is burdened by moderate levels of missingness for a number of important potential confounders which can be a source of significant bias. Several variables were excluded from the final logistic regression model in order to maximize the usefulness of our already limited study population. Data missingness also hampered our ability to select an ideal study population during the design stage. For example, stage I NEC is regarded as suspect, and most epidemiological studies will exclude these cases from analysis. Exclusions are also usually recommended for multiple gestation babies since these infants are at increased risk for prematurity. However, more than a third of patients had unreliable or missing information regarding NEC disease severity, and a third of patients had missing data on birth number. Another limitation of retrospective studies is that they are susceptible to confounding by unmeasured variables.

We also had limited access to identifying information for many other potential confounders and risk factors, including biological, medical history, and social environment data. Information is not available to describe the relationship between the type, timing, and rate of advancement of enteral feeds and NEC outcomes. Medication- and surgical-treatment codes are absent, preventing a firm distinction between medical NEC and surgical NEC cases. We also could not determine whether NEC was the primary admission diagnosis for the subjects in this study, as the database does not allow one to make temporal relationships between various diagnoses. The implication is that it is not possible to determine the impact NEC had over the clinical course of each neonate. Finally, our study does not allow for reporting of long-term outcomes such as neurodevelopmental effects. Longer follow-up will be necessary to assess whether there are neurodevelopmental differences between racial groups.

*Diagnosis and Outcomes*: NEC is a complex disease with multiple predisposing factors. Diagnosis relies heavily on thorough medical and laboratory investigation. Few protocols for standardized diagnosis of NEC have been published, and there is wide variability in both the content and frequency with which existing protocols are implemented across state lines, counties, and even between physicians in a single facility. Disease confirmation is particularly difficult without pathological or gross examination which can lead to misdiagnosis. For this study population, a distinction between SIP and stage III surgical NEC cases with intestinal perforation could not be delineated for all patients. Similarly, data on deaths and LOS may be unreliable. As many as 25 patients were discharged to facilities outside of CHOA where information on their health outcomes could not be followed. Thus, information on death number and length of time spent in the hospital may be inaccurate. To determine whether this discrepancy is a meaningful source of bias, follow-up is needed to establish whether any misreporting of exposures or outcomes differs by race; meaning, if the misclassification is differential. Without consensus on a scheme for certifying NEC and outcome data, the reporting of trends for these categories will remain suspect.

*External Validity*: A significant study limitation could be that our findings may not be generalizable to all NEC neonates. Differences in the socioeconomic and demographic characteristics of our study population may lead to poor external validity. Although Children's Healthcare of Atlanta serves patients throughout the state of Georgia in its hospitals, emergency rooms, and Level III NICU facilities, the study's findings were derived from a sample of children with a higher proportion of non-Hispanic black children and few Hispanic, multi-racial, Asian, Native Hawaiian, or Alaska Native people. Moreover, since CHOA is primarily a surgical center we are limited to the most severe disease. Our findings may only be generalizable to the most severe NEC patients who require surgical intervention, since medical NEC patients are less likely to pass through a CHOA facility.

*Study Power*: The study findings for NEC survival and length of hospital stay are limited by the number of participants included in the comparison groups for each outcome. Despite a large starting inpatient population, we only identified 108 cases of NEC over the two years of study. Lack of statistical power is evidenced by the absence of statistical significance across most NEC predictors in our multivariate analyses, including those established in the literature. However, the analysis was a pilot study to assess whether there was any provocative evidence in our population of a relationship between race and NEC

outcomes. Although not statistically significant, our results are consistent with elevated fatality and hospital length of stay for racial minorities which should be further explored in larger studies.

Finally, a brief comment must be made in regards to utilization of the odds ratio as opposed to the relative risk in our multivariate analysis of the race-survival association. Although both are appropriate measures, the odds ratio will generally overstate the risk ratio because the outcome (death) is common. This point is demonstrated in Supplemental Table 2. Although the adjusted odds ratio does overestimate the relative risk, the difference is not considerable. The largest overestimation is observed for Hispanic neonates (compared to white neonates) with an adjusted OR of 4.0 compared to an adjusted RR of 2.5.

**FUTURE DIRECTIONS**

In light of our study's limitations, several recommendations are suggested. First, researchers seeking to use information documented in the medical record as study data should consider a prospective cohort design. This design will allow researchers to review the medical record for completeness and accuracy as the patient is being cared for by the clinical team. A prospective design also permits inclusion of risk factors and potential confounders that are absent in our current data set. Since NEC case-fatality and LOS are fairly short-term outcomes requiring less than a year of follow-up for most patients, a cohort design is feasible. To improve the study's statistical power, a multisite study or substantially longer study period may be considered. This approach this will provide access to a greater number of patient records, and in turn, a larger sample of data. Replication of this study across multiple NICU sites would increase not only the sample size of the comparison groups but also the representative nature of the findings.

The results of this study emphasize the need to have complete data on potential confounders such as gestational age, birth weight, and payer type. Researchers should also strive to get complete information on Apgar scores, birth number, delivery method, and NEC stage, as these are potential confounders of the race and NEC fatality or LOS relationship. Potentially, some of this information may be obtained by linking back to individual birth and death records. Data on NEC stage and birth number are also important for making appropriate patient exclusions such as stage I suspect NEC and multiple gestation babies. Appropriate exclusions should also be considered for infants with other major birth defects such as anencephaly or cardiac defects.

Finally, this work further emphasizes the need to determine additional risk factors accounting for racial disparities in NEC survival and LOS. Racial disparities in primary health care use, including prenatal care, may offer one explanation for the observed disparities in NEC fatality and LOS by race. In a recent study evaluating racial disparities in the advice received from primary care providers, *Kogan et al.* found that black women were more likely to report not receiving advice about breast feeding (Kogan, Kotelchuck, Alexander, & Johnson, 1994). Low prevalence of in-hospital use of maternal breast milk among black infants has also been cited by *Lawrence* (Lawrence R & RM, 1999). As breast milk may have a protective effect against NEC, decreasing its use may increase the risk of this disease. Thus, the possibility that health care inequalities are involved in the high prevalence of NEC observed in minorities deserves further study. Complete data regarding access to breast milk, and utilization of hospital resources such as enteral feedings, antibiotics, RBC transfusions, and respiratory support are needed.

Efforts must also extend to maternal factors to understand their level of contribution. For example, Group Beta Streptococcus (GBS) colonization was found to occur more often in black women because of decreased access to prenatal care. Factors such as GBS-positive colonization could contribute to decreased oxygenation to the bowel or proliferation of bacteria, which can contribute to NEC and promote negative outcomes in the preterm infant. In addition to risk factors experienced by the infant post-partum, risk factors experienced during the prenatal course should also be examined, such as maternal drug use, maternal hypertension, and maternal infections such as HIV positivity. Finally, unidentified biological or genetic differences between races may be partly responsible. For example, there is increasing interest to identify genetic polymorphisms for known NEC-associated inflammatory mediators, such as cytokines, which may predispose the bowel to inflammation and injury (Treszl et al., 2006). Preliminary studies in VLBW infants showed that neonates with NEC are less likely to be carriers of a specific mutant allele that affects IL-4 receptors on cells, which is important for promoting an anti-inflammatory Th2 response (Treszl et al., 2006). Further study is needed to determine whether differences exist in the prevalence of specific mutations that affect the quality and/or quantity of the cytokine response among different races. We may also consider an examination distinguishing between medical vs. surgical NEC cases or preterm vs. full-term infants since recent evidence suggests that NEC risk factors may differ between these groups (Gephart et al., 2012).

## SUPPLEMENTAL TABLES

**Supplemental Table 1: Characteristics of study population, by Race (CHOA, 2009-2010)**

| *Characteristics* | *Overall* | *White* | *Black* | *Hispanic* | *Other* | *$X^2$ (d.f.)*[†] | *p-value* | *Fisher's p-value*[††] |
|---|---|---|---|---|---|---|---|---|
| **TOTAL** | **108** | **35** | **48** | **9** | ***16*** | | | |
| *NEC Stage* | | | | | | | | |
| I | 4 (5.6%) | 0 | 2 (6.7%) | 1 (20.0%) | 1 (6.7%) | | | |
| II | 35 (49.3%) | 12 (57.1%) | 12 (40.0%) | 2 (40.0%) | 9 (60.0%) | | | |
| III | 32 (45.1%) | 9 (42.9%) | 16 (53.3%) | 2 (40.0%) | 5 (33.3%) | *5.2888(6)* | *0.5073* | *0.4436* |
| *Unknown/Missing*[*] | *37* | *14* | *18* | *4* | *1* | | | |
| *Gestational Age, wk* | | | | | | | | |
| <27 | 21 (19.8%) | 4 (11.4%) | 14 (30.4%) | 1 (11.1%) | 2 (12.5%) | | | |
| 27-33 | 59 (55.7%) | 20 (57.2%) | 23 (50.0%) | 5 (55.6%) | 11 (68.7%) | | | |
| >33 | 26 (24.5%) | 11 (31.4%) | 9 (19.6%) | 3 (33.3%) | 3 (18.8%) | *7.0523(6)* | *0.3160* | *0.3627* |
| *Unknown/Missing* | *2* | *0* | *2* | *0* | *0* | | | |
| *Birth Weight, grams* | | | | | | | | |
| <1000, ELBW | 35 (33.0%) | 9 (25.7%) | 19 (41.3%) | 3 (33.3%) | 4 (25.0%) | | | |
| 1000-2500, LBW | 54 (51.0%) | 18 (51.4%) | 22 (47.8%) | 3 (33.3%) | 11 (68.8%) | | | |
| >2500 | 17 (16.0%) | 8 (22.9%) | 5 (10.9%) | 3 (33.3%) | 1 (6.2%) | *7.8823(6)* | *0.2469* | *0.2637* |
| *Unknown/Missing* | *2* | *0* | *2* | *0* | *0* | | | |
| *1-min Apgar Score* | | | | | | | | |
| <5 | 21 (25.3%) | 6 (20.0%) | 12 (33.3%) | 1 (16.7%) | 2 (18.2%) | | | |
| 5-10, normal | 62 (74.7%) | 24 (80.0%) | 24 (66.7%) | 5 (83.3%) | 9 (81.8%) | *2.2066(3)* | *0.5306* | *0.6355* |
| *Unknown/Missing* | *25* | *5* | *12* | *3* | *5* | | | |
| *5-min Apgar Score* | | | | | | | | |
| <7 | 11 (13.1%) | 2 (6.7%) | 8 (21.6%) | 0 | 1 (9.1%) | | | |
| 7-10, normal | 73 (86.9%) | 28 (93.3%) | 29 (78.4%) | 6 (100.0%) | 10 (90.9%) | *4.5121(3)* | *0.2112* | *0.2914* |
| *Unknown/Missing* | *24* | *5* | *11* | *3* | *5* | | | |
| *Sex* | | | | | | | | |
| Female | 47 (43.5%) | 13 (37.1%) | 25 (52.1%) | 3 (33.3%) | 6 (37.5%) | | | |
| Male | 61 (56.5%) | 22 (62.9%) | 23 (47.9%) | 6 (66.7%) | 10 (62.5%) | *2.6269(3)* | *0.4528* | -- |
| *Multibirth* | | | | | | | | |
| No | 64 (83.1%) | 22 (81.5%) | 27 (81.8%) | 8 (100.0%) | 7 (77.8%) | | | |
| Yes | 13 (16.9%) | 5 (18.5%) | 6 (18.2%) | 0 | 2 (22.2%) | *1.8989(3)* | *0.5936* | *0.6973* |
| *Unknown/Missing* | *31* | *8* | *15* | *1* | *7* | | | |
| *Delivery Method* | | | | | | | | |
| Cesarean Section | 58 (64.4%) | 23 (67.7%) | 24 (68.6%) | 7 (77.8%) | 4 (33.3%) | | | |
| Vaginal | 32 (35.6%) | 11 (32.3%) | 11 (31.4%) | 2 (22.2%) | 8 (66.7%) | *6.1796(3)* | *0.1032* | *0.1238* |
| *Unknown/Missing* | *18* | *1* | *13* | *0* | *4* | | | |
| *Payer Type* | | | | | | | | |
| Public | 29 (27.1%) | 7 (20.0%) | 11 (23.4%) | 7 (77.8%) | 4 (25.0%) | | | |
| Private | 59 (55.1%) | 26 (74.3%) | 24 (51.1%) | 1 (11.1%) | 8 (50.0%) | | | |
| Other | 19 (17.8%) | 2 (5.7%) | 12 (25.5%) | 1 (11.1%) | 4 (25.0%) | *20.3064(6)* | *0.0024* | *0.0030* |
| *Unknown/Missing* | *1* | *0* | *1* | *0* | *0* | | | |

[*] Column percentages exclude unknown/missing values.
[†] Chi-square test, d.f. = degrees of freedom. Excludes unknown/missing data.
[††] Fisher's Exact test computed for variables with expected cell sizes of <5 counts.

**Supplemental Table 2: OR vs. RR - Final Model, Race and Survival (CHOA, 2009-2010)**

| *Characteristic* | *Odds Ratio* | *95% CI*[†] | *p-value*[††] | *Relative Risk* | *95% CI* | *p-value* |
|---|---|---|---|---|---|---|
| *Race* | | | | | | |
| White (*ref*) | 1 | -- | 0.4261 | 1 | -- | 0.3412 |
| Black | 1.68 | (0.57, 4.96) | | 1.44 | (0.65, 3.18) | |
| Hispanic | 3.99 | (0.76, 21.00) | | 2.45 | (1.00, 6.01) | |
| Other | 1.56 | (0.40, 6.07) | | 1.39 | (0.53, 3.64) | |
| *Gestational Age, wk* | | | | | | |
| <27 | 1.94 | (0.48, 7.80) | | 1.52 | (0.60, 3.86) | |
| 27-33 | 1.55 | (0.51, 4.72) | | 1.30 | (0.61, 2.77) | |
| >33 (*ref*) | 1 | -- | 0.6266 | 1 | -- | 0.6476 |
| *Sex* | | | | | | |
| Female (*ref*) | 1 | -- | 0.9393 | 1 | -- | 0.8293 |
| Male | 1.04 | (0.42, 2.53) | | 1.07 | (0.59, 1.92) | |
| *Payer Type* | | | | | | |
| Public (*ref*) | 1 | -- | 0.6223 | 1 | -- | 0.4670 |
| Private | 0.82 | (0.29, 2.28) | | 0.84 | (0.46, 1.54) | |
| Other | 0.49 | (0.12, 2.05) | | 0.57 | (0.21, 1.51) | |
| | | (N=105) | | | (N=105) | |

[†]C.I. = Confidence Interval. [††]Chi-square statistic for the combined significance of each predictor.

**SAS CODING**

```
libname a "T:\EpiProjs\NACH\Lance thesis\Data";
proc contents data=a.nec;
run;

*Create NEC2 dataset with added changes;
data nec2;
set a.nec;

If race2="Multi-Racial" then race2="Other/Declined";
If race2="Asian" then race2="Other/Declined";

*Input values for patients with missing gestational age;
If patient_MRN=1277877 then ped_gest_age2=27;
If patient_MRN=2008862 then ped_gest_age2=29;
If patient_MRN=2010868 then ped_gest_age2=33;
If patient_MRN=3016382 then ped_gest_age2=27;
If patient_MRN=3017758 then ped_gest_age2=29;
If patient_MRN=3028215 then ped_gest_age2=27;
If patient_MRN=3042855 then ped_gest_age2=24;
If patient_MRN=3047971 then ped_gest_age2=29;
If patient_MRN=6240234 then ped_gest_age2=25;
If patient_MRN=6245953 then ped_gest_age2=27;
If patient_MRN=6256739 then ped_gest_age2=25;

*Input values for patients with missing birth weight;
If patient_MRN=2008862 then PED_BIRTH_WT=39.7;
If patient_MRN=2010868 then PED_BIRTH_WT=66.1;
If patient_MRN=3016382 then PED_BIRTH_WT=30.8;
If patient_MRN=3017758 then PED_BIRTH_WT=57.3;
If patient_MRN=3028215 then PED_BIRTH_WT=17.6;
If patient_MRN=3042855 then PED_BIRTH_WT=30.8;
If patient_MRN=6240234 then PED_BIRTH_WT=39.7;
If patient_MRN=6245953 then PED_BIRTH_WT=30.8;
If patient_MRN=6256739 then PED_BIRTH_WT=39.7;

*Input death data;
If patient_MRN=2005899 then death=1;
If patient_MRN=3010408 then death=1;
If patient_MRN=3118051 then death=1;
If patient_MRN=3122101 then death=1;
If patient_MRN=2140955 then death=1;
If patient_MRN=2133301 then death=1;
If patient_MRN=3094114 then death=1;
If patient_MRN=2090122 then death=1;
If patient_MRN=2100381 then death=1;
If patient_MRN=3090231 then death=1;
If patient_MRN=3086399 then death=1;
If patient_MRN=2093253 then death=1;
If patient_MRN=3047971 then death=1;
If patient_MRN=3068029 then death=1;
If patient_MRN=2073885 then death=1;
If patient_MRN=1285541 then death=1;
If patient_MRN=2052460 then death=1;
If patient_MRN=2068802 then death=1;
If patient_MRN=3059036 then death=1;
If patient_MRN=2061421 then death=1;
If patient_MRN=2053632 then death=1;
If patient_MRN=3042855 then death=1;
If patient_MRN=2047927 then death=1;
If patient_MRN=3017758 then death=1;
If patient_MRN=2038598 then death=1;
```

```
If patient_MRN=3026759 then death=1;
If patient_MRN=3028095 then death=1;
If patient_MRN=2031507 then death=1;
If patient_MRN=3028215 then death=1;
If patient_MRN=1294162 then death=1;
If patient_MRN=3026454 then death=1;
If patient_MRN=2029094 then death=1;
If patient_MRN=3016382 then death=1;
If patient_MRN=2019566 then death=1;
If patient_MRN=2008862 then death=1;
If death=" " then death=2;

*Identifying patients with gestational age ICD data;
array d (43) icd1 icd2 icd3 icd4 icd5 icd6 icd7 icd8 icd9 icd10 icd11
icd12 icd13 icd14 icd15 icd16 icd17 icd18 icd19 icd20 icd21 icd22 icd23
icd24 icd25 icd26 icd27 icd28 icd29 icd30 icd31 icd32 icd33 icd34 icd35
icd36 icd37 icd38 icd39 icd40 icd41 icd42 icd43 ;
do i = 1 to 43;
if substr(d(i),1,6)= "777.51"  then stage="3-Stage 1";
if substr(d(i),1,6)= "777.52"  then stage="1-Stage 2";
if substr(d(i),1,6)= "777.53"  then stage="2-Stage 3";
end;

If multi_birth="Unknown" then multi_birth="";

*Categorize gestational age - 3 levels;
if ped_gest_age2 <27 then gest="1-Extremely Preterm";
if 27 <= ped_gest_age2 <= 33 then gest="2-Preterm";
if ped_gest_age2 > 33 then gest="3-Full Term";
if ped_gest_age2 = "" then gest="";

*Categorize gestational age - 7 levels;
if ped_gest_age2 <=24 then gestb=1;
if 24 < ped_gest_age2 <= 26 then gestb=2;
if 26 < ped_gest_age2 <= 28 then gestb=3;
if 28 < ped_gest_age2 <= 30 then gestb=4;
if 30 < ped_gest_age2 <= 32 then gestb=5;
if 32 < ped_gest_age2 <= 34 then gestb=6;
if 34 < ped_gest_age2 then gestb=7;
if ped_gest_age2 = "" then gestb="";

*Categorize gestational age - 2 levels;
if ped_gest_age2 <=33 then gestc="1";
if ped_gest_age2 > 33 then gestc="2";
if ped_gest_age2 = "" then gestc="";

*Convert ounces to grams;
grams = PED_BIRTH_WT / 0.035274;

*Categorize birth weight - 3 levels;
if grams <1000 then weight=1;
if 1000 <= grams <= 2500 then weight=2;
if grams > 2500 then weight=3;
if grams = "" then weight="";

*Categorize birth weight - 2 levels;
if grams <2500 then weightb=1;
if grams >=2500 then weightb=2;
if grams = "" then weightb="";

*Categorize birth weight - 10 levels;
if grams <500 then weightc=1;
if 500 <= grams < 750 then weightc=2;
```

```
if 750 <= grams < 1000 then weightc=3;
if 1000 <= grams < 1250 then weightc=4;
if 1250 <= grams < 1500 then weightc=5;
if 1500 <= grams < 1750 then weightc=6;
if 1750 <= grams < 2000 then weightc=7;
if 2000 <= grams < 2250 then weightc=8;
if 2250 <= grams < 2500 then weightc=9;
if grams >= 2500 then weightc=10;
if grams = "" then weightc="";

*Categorize Apgar scores;
if APGAR_1_minute <=3 then one=1;
if 4 <= APGAR_1_minute <= 7 then one=2;
if 8 <= APGAR_1_minute <= 10 then one=3;
if APGAR_1_minute = "" then one="";

if APGAR_1_minute <5 then oneb=1;
if 5 <= APGAR_1_minute <= 10 then oneb=2;
if APGAR_1_minute = "" then oneb="";

if APGAR_5_minute <=3 then five=1;
if 4 <= APGAR_5_minute <= 7 then five=2;
if 8 <= APGAR_5_minute <= 10 then five=3;
if APGAR_5_minute = "" then five="";

if APGAR_5_minute <7 then fiveb=1;
if 7 <= APGAR_5_minute <= 10 then fiveb=2;
if APGAR_5_minute = "" then fiveb="";

if sex='M' then sex2='1-M';
if sex='F' then sex2='2-F';
if sex='' then sex2='';

if race2='White,Non-Hispanic' then race3='4';
if race2='Black/African-Amer' then race3='1';
if race2='Hispanic' then race3='2';
if race2='Other/Declined' then race3='3';
if race2='' then race3='';

if multi_birth="Yes" then multi=1;
if multi_birth="No" then multi=2;
if multi_birth="" then multi="";

if delivr_meth2="Vaginal" then delivery=1;
if delivr_meth2="Cesarean Section" then delivery=2;
if delivr_meth2="" then delivery="";

if payor_name3="PUBLIC" then payer='3-Public';
if payor_name3="PRIVATE" then payer='1-Private';
if payor_name3="OTHER" then payer='2-Other';
if payor_name3="" then payer='';

if los_days > 0 then status = 0;
if los_days = 0 then status = 1;
run;
proc contents data=nec2;
run;


**TABLES 3.1 and 3.2 - Characteristics of NEC Population at CHOA;
proc freq data=nec2;
tables stage gest weight oneb fiveb sex2 race3 multi delivery payer death;
run;
```

```
proc means data=nec2 n min q1 median q3 max mean std;
var  vent_days los_days total_cost;
run;
*Characteristics of NEC Population by Death;
proc freq data=nec2;
tables (stage sex2 race3 delivery payer multi)*death /nocol nopercent chisq fisher;
run;
proc freq data=nec2;
tables (stage sex2 race3 delivery payer multi)*death /nocol nopercent missing;
run;
proc freq data=nec2;
tables (gest weight oneb fiveb)*death /nocol nopercent chisq fisher;
run;
proc freq data=nec2;
tables (gest weight oneb fiveb)*death /nocol nopercent missing;
run;


**Supplemental Table 1 - Characteristics of population by Race;
proc freq data=nec2;
tables (stage gest weight oneb fiveb sex2 multi delivery payer)*race3/norow nopercent
chisq fisher;
run;
proc freq data=nec2;
tables (stage gest weight oneb fiveb sex2 multi delivery payer)*race3/norow nopercent
missing;
run;


**TABLE 3.3 - Crude and Adjusted Estimates - Survival;
*NEC Stage;
proc logistic data=nec2;
class stage (PARAM=ref REF="3-Stage 1");
model death (event="1") = stage;
run;
proc logistic data=nec2;
class stage (PARAM=ref REF="3-Stage 1")
weight (PARAM=ref REF="3");
model death (event="1") = stage weight;
run;
*Gestational Age;
proc logistic data=nec2;
class gest (PARAM=ref REF="3-Full Term");
model death (event="1") = gest;
run;
proc logistic data=nec2;
class gest (PARAM=ref REF="3-Full Term")
weight (PARAM=ref REF="3");
model death (event="1") = gest weight;
run;
*Birth weight;
proc logistic data=nec2;
class weight (PARAM=ref REF="3");
model death (event="1") = weight;
run;
*Apgar 1 minute;
proc logistic data=nec2;
class oneb (PARAM=ref REF="2");
model death (event="1") = oneb;
run;
proc logistic data=nec2;
class oneb (PARAM=ref REF="2")
weight (PARAM=ref REF="3");
```

```
model death (event="1") = one weight;
run;
*Apgar 5 minute;
proc logistic data=nec2;
class fiveb (PARAM=ref REF="2");
model death (event="1") = fiveb;
run;
proc logistic data=nec2;
class fiveb (PARAM=ref REF="2")
weight (PARAM=ref REF="3");
model death (event="1") = five weight;
run;
*Sex;
proc logistic data=nec2;
class sex2 (PARAM=ref REF="2-F");
model death (event="1") = sex2;
run;
proc logistic data=nec2;
class sex2 (PARAM=ref REF="2-F")
weight (PARAM=ref REF="3");
model death (event="1") = sex2 weight;
run;
*Race;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4");
model death (event="1") = race3;
run;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
weight (PARAM=ref REF="3");
model death (event="1") = race3 weight;
run;
*Multibirth;
proc logistic data=nec2;
class multi (PARAM=ref REF="2");
model death (event="1") = multi;
run;
proc logistic data=nec2;
class multi (PARAM=ref REF="2")
weight (PARAM=ref REF="3");
model death (event="1") = multi weight;
run;
*Delivery Method;
proc logistic data=nec2;
class delivery (PARAM=ref REF="2");
model death (event="1") = delivery;
run;
proc logistic data=nec2;
class delivery (PARAM=ref REF="2")
weight (PARAM=ref REF="3");
model death (event="1") = delivery weight;
run;
*Payer Type;
proc logistic data=nec2;
class payer (PARAM=ref REF="3-Public");
model death (event="1") = payer;
run;
proc logistic data=nec2;
class payer (PARAM=ref REF="3-Public")
weight (PARAM=ref REF="3");
model death (event="1") = payer weight;
run;
```

```
**TABLE 3.4 - Race and survival adjusted for other characteristics;
*Gestational Age Adjusted;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
gest (PARAM=ref REF="3-Full Term");
model death (event="1") = race3 gest;
run;
*Birth Weight Adjusted;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
weight (PARAM=ref REF="3");
model death (event="1") = race3 weight;
run;
*Sex Adjusted;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
sex2 (PARAM=ref REF="2-F");
model death (event="1") = race3 sex2;
run;
*Payer Type Adjusted;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
payer (PARAM=ref REF="3-Public");
model death (event="1") = race3 payer;
run;


**Table 3.5: Race and NEC Survival - different parameterizations of gestational age
and birth weight;
*Gestational Age adjusted - 2 levels;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
gestc (PARAM=ref REF="2");
model death (event="1") = race3 gestc;
run;
*Gestational Age Adjusted - 7 levels;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
gestb (PARAM=ref REF="7");
model death (event="1") = race3 gestb;
run;
*Gestational Age Adjusted - continuous;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4");
model death (event="1") = race3 ped_gest_age2;
run;
*Birth Weight Adjusted - 2 levels;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
weightb (PARAM=ref REF="2");
model death (event="1") = race3 weightb;
run;
*Birth Weight Adjusted - 10 levels;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
weightc (PARAM=ref REF="10");
model death (event="1") = race3 weightc;
run;
*Birth Weight Adjusted - continuous;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4");
model death (event="1") = race3 grams;
```

```
run;
*Gest and BW Adjusted - continuous;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4");
model death (event="1") = race3 ped_gest_age2 grams;
run;


**Tables 3.6 and 3.7: Model Building Procedure;
*Initial Maximum Model - with interaction terms;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
gest (PARAM=ref REF="3-Full Term")
weight (PARAM=ref REF="3")
sex2 (PARAM=ref REF="2-F")
payer (PARAM=ref REF="3-Public");
model death (event="1") = race3 gest weight sex2 payer
race3*gest race3*weight race3*sex2 race3*payer/
hierarchy=single
selection=backward
slstay=0.01 include=5 details
lackfit;
run;
*Fully Adjusted Model - main effect terms;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
gest (PARAM=ref REF="3-Full Term")
weight (PARAM=ref REF="3")
sex2 (PARAM=ref REF="2-F")
payer (PARAM=ref REF="3-Public");
model death (event="1") = race3 gest weight sex2 payer/
hierarchy=single
selection=backward
slstay=0.01 include=1 details
lackfit;
run;
*Final Model;
proc logistic data=nec2;
class race3 (PARAM=ref REF="4")
gest (PARAM=ref REF="3-Full Term")
sex2 (PARAM=ref REF="2-F")
payer (PARAM=ref REF="3-Public");
model death (event="1") = race3 gest sex2  payer/lackfit;
run;


**Supplemental Table 2 - RR - Fully Adjusted Model;
proc genmod data=nec2;
class race3 (PARAM=ref REF="4")
gest (PARAM=ref REF="3-Full Term")
sex2 (PARAM=ref REF="2-F")
payer (PARAM=ref REF="3-Public");
model death = race3 gest sex2 payer/dist=bin link=log type3;
estimate 'Black' race3 1 0 0;
estimate 'Hispanic' race3 0 1 0;
estimate 'Other' race3 0 0 1;
estimate 'Extremely Preterm' gest 1 0;
estimate 'Preterm' gest 0 1;
estimate 'Male' sex2 1;
estimate 'Private' payer 1 0;
estimate 'Other' payer 0 1;
run;
```

```
**TABLE 3.8 - LOS;
*Create new data set including only survivors;
data nec3;
set nec2;
where death=2;
run;
*Overall LOS;
proc means data=nec3 n median min max mean std;
var los_days;
run;
*LOS by NEC Stage;
proc sort data=nec3;
by stage;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by stage;
run;
proc glm data=nec3;
class stage;
model los_days = stage;
means stage;
run;
*LOS by gestational age;
proc sort data=nec3;
by gest;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by gest;
run;
proc glm data=nec3;
class gest;
model los_days = gest;
means gest;
run;
*LOS by birth weight;
proc sort data=nec3;
by weight;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by weight;
run;
proc glm data=nec3;
class weight;
model los_days = weight;
means weight;
run;
*LOS by Apgar 1-minute;
proc sort data=nec3;
by oneb;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by oneb;
run;
proc glm data=nec3;
class oneb;
model los_days = oneb;
means oneb;
run;
```

```
*LOS by Apgar 5-minute;
proc sort data=nec3;
by fiveb;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by fiveb;
run;
proc glm data=nec3;
class fiveb;
model los_days = fiveb;
means fiveb;
run;
*LOS by sex;
proc sort data=nec3;
by sex2;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by sex2;
run;
proc glm data=nec3;
class sex2;
model los_days = sex2;
means sex2;
run;
*LOS by race;
proc sort data=nec3;
by race3;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by race3;
run;
proc glm data=nec3;
class race3;
model los_days = race3;
means race3;
run;
*LOS by birth number;
proc sort data=nec3;
by multi;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by multi;
run;
proc glm data=nec3;
class multi;
model los_days = multi;
means multi;
run;
*LOS by delivery method;
proc sort data=nec3;
by delivery;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by delivery;
run;
proc glm data=nec3;
class delivery;
model los_days = delivery;
```

```
means delivery;
run;
*LOS by payer type;
proc sort data=nec3;
by payer;
run;
proc means data=nec3 n median min max mean std;
var los_days;
by payer;
run;
proc glm data=nec3;
class payer;
model los_days = payer;
means payer;
run;


**Table 3.9: Cox Proportional Hazard Analysis;
*Unadjusted Model;
proc phreg data=nec3;
class race3;
model los_days*status(1) = race3;
contrast 'black' race3 1 0 0/estimate=exp;
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;
*Controlling for gest;
proc phreg data=nec3;
class race3 gest;
model los_days*status(1) = race3 gest;
contrast 'black' race3 1 0 0/estimate=exp;
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;
*Controlling for weight;
proc phreg data=nec3;
class race3 weight;
model los_days*status(1) = race3 weight;
contrast 'black' race3 1 0 0/estimate=exp;
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;
*Controlling for sex;
proc phreg data=nec3;
class race3 sex2;
model los_days*status(1) = race3 sex2;
contrast 'black' race3 1 0 0/estimate=exp;
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;
*Controlling for payer;
proc phreg data=nec3;
class race3 payer;
model los_days*status(1) = race3 payer;
contrast 'black' race3 1 0 0/estimate=exp;
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;
*Controlling for gestational age, sex, payer type;
proc phreg data=nec3;
class race3 gest sex2 payer;
model los_days*status(1) = race3 gest sex2 payer;
contrast 'black' race3 1 0 0/estimate=exp;
```

```
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;
*Test proportional hazards assumption - interaction between race3 and LOS_Days;
proc phreg data=nec3;
class race3;
model los_days*status(1) = race3 x;
x = los_days * race3;
contrast 'black' race3 1 0 0/estimate=exp;
contrast 'hispanic' race3 0 1 0/estimate=exp;
contrast 'other' race3 0 0 1/estimate=exp;
run;


**Figure 1 - Survival Curves and Log-Rank Tests;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata race3;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata stage;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata gest;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata weight;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata oneb;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata fiveb;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata sex;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata multi;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata delivery;
run;
proc lifetest data=nec3 plots=S;
time los_days*status(1);
strata payer;
run;
```

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