UNIVERSITY OF CALIFORNIA,
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Impact of Concurrent Bloodstream Infections in Infants with Necrotizing Enterocolitis and Intestinal Failure

THESIS

submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in Biomedical and Translational Science

by

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DEDICATION

To

Babies born too soon

May your health be restored.
May your lives be long and happy.
May your parents be blessed with the joy of seeing you grow and thrive.
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ABSTRACT OF THE THESIS

Impact of Concurrent Bloodstream Infections in Infants with Necrotizing Enterocolitis and Intestinal Failure

By

Siobhan Poling Smith, MD

Master of Science in Biomedical and Translational Science

University of California, Irvine, 2015

Professor Sherrie Kaplan, Chair

Objective To examine the microbiology and outcomes related to bloodstream infections (BSIs) in neonates with intestinal failure (IF) due to necrotizing enterocolitis (NEC).

Study Design I performed a retrospective review of very low birth weight (VLBW) neonates with late-onset BSI at a single center over 8.5 years. Recurrence and frequencies of BSI pathogens were compared between neonates with and without IF. Pathogen frequencies and mortality were compared for NEC-associated BSI (≤72 hours from NEC diagnosis) and post-NEC BSI (>72 hours after NEC diagnosis) in neonates with and without IF.

Results 177 neonates with 234 BSIs were studied. Compared to other VLBW neonates with BSI, IF patients (n=45) suffered more often from recurrent BSI (44% vs. 20%, p=0.01), and frequently had gut-bacteria BSI (51% vs. 28%, p=0.01). NEC-associated BSIs in IF patients had similar pathogen profiles to BSIs in other VLBW neonates, while post-NEC BSIs in IF patients were more commonly due to gut bacteria (48% vs. 23%, p<0.00). Mortality for IF patients with post-NEC BSI was the same as for the cohort as a whole (17%). IF patients with NEC-associated BSI had higher odds of death (OR 3.9) than other neonates (35% vs. 15%, p=0.02).
**Conclusions** IF patients commonly have recurrent BSI and gut-bacteria BSI. The microbiologic etiology of post-NEC BSI in neonates with IF is different from BSI in other VLBW neonates, and is more commonly of gut origin. IF patients with NEC-associated BSI have a significantly higher odds of death than other VLBW neonates with BSI.
CHAPTER 1. INTRODUCTION

Study Overview

Late-onset neonatal sepsis, particularly when associated with bloodstream infection (BSI), is a serious complication of premature birth. Late-onset sepsis is associated with increased morbidity and mortality, increased length of stay in the neonatal intensive care unit (NICU), increased antibiotic utilization, poor neurological outcome among survivors, and increased cost of hospitalization.\(^1\) Much progress has been made in prevention of late-onset sepsis through programs aimed at prevention of central-line associated blood stream infections. Unfortunately, ventilator associated pneumonia and necrotizing enterocolitis (NEC), an intestinal complication of prematurity, remain important foci for late-onset sepsis. There is a substantial body of data that suggests greater severity of late-onset sepsis when it coincides with NEC.\(^2-4\)

This is a retrospective study of a cohort of 177 very low birth weight (VLBW) infants with BSI hospitalized in the NICU at the Children’s Hospital of Orange County (CHOC) between July of 2005 and December of 2013. In this descriptive study, I aim to characterize the types of BSIs suffered by this cohort of infants. I am particularly interested in the types of BSI suffered by patients with and without NEC. I propose that infants with the most severe cases of NEC (those who require surgery and develop intestinal failure [IF]) suffer different types of BSI from those with more benign NEC, and from premature babies without NEC. Characterization of BSIs in infants suffering from NEC associated IF may add to our understanding of the pathophysiology of this most severe form of this devastating complication of prematurity.
Advances in Neonatal Care Since the 1980s

Since the 1980s, great advances have been made in the care of premature infants. The past three decades have seen numerous technological advances, such as the administration of antenatal corticosteroids, use of pulmonary surfactant and high frequency oscillatory ventilation, as well as improvements in the availability and organization of neonatal intensive care. Infants who several decades ago would have died due to prematurity are surviving from very early gestational ages. For infants receiving intensive care at the threshold of viability (24 weeks gestational age), survival in the 2000s in the United States was around 55% compared to survival of around 15% in the 1980s.6-7

Most importantly, many premature infants are surviving the neonatal period without previously prevalent morbidities. Premature infants are at risk for major lung, brain, eye and intestinal diseases as well as infections. Immature lungs may suffer from respiratory distress syndrome requiring mechanical ventilation. This therapy may lead to bronchopulmonary dysplasia (abnormal lung development). Administration of antenatal corticosteroids and surfactant replacement therapy have reduced the severity of respiratory distress syndrome;8 added to oscillatory mechanical ventilation these therapeutic modalities have reduced the frequency of bronchopulmonary dysplasia.9 Brain complications of prematurity include bleeds (intraventricular hemorrhage) and tissue necrosis (periventricular leukomalacia), leading to poor cognitive and motor development as well as seizure disorders. Antenatal corticosteroids have reduced the incidence of intraventricular hemorrhage.10 Eye complications can arise due to damage of the visual cortex or from direct damage of the retina (retinopathy of prematurity), and
may lead to blindness. Better understanding of the contribution of oxygen therapy to
retinopathy of prematurity has helped reduce the incidence of this disease.\textsuperscript{11} In the
modern era of neonatal intensive care, compared to 30 years ago, premature infants have
better pulmonary, neurologic and ophthalmologic outcomes, but the same is not true of
intestinal outcomes. Increased survival of these subjects has resulted in a larger
population at risk for infections and intestinal complications of prematurity including
NEC.

**Advances Are Still Needed: Neonatal Necrotizing Enterocolitis**

Despite advances in prevention and treatment of many complications of
prematurity, little progress has been made towards reducing incidence and complications
of NEC.\textsuperscript{12} VLBW infants ($\leq 1500$g) suffer from NEC at a rate of up to 10\% with mortality
rates up to 30\%.\textsuperscript{13} Incidence is even higher among those $<1000$ grams. Many patients
who survive NEC go on to suffer life-long intestinal as well as neurologic disability.
Infants with NEC often require surgical resection of necrotic bowel leading to
dependence on intravenous (aka parenteral) nutrition. Patients diagnosed with NEC who
undergo surgical resection, and are subsequently dependent on parenteral nutrition (PN)
for at least 6 weeks, are classified as having NEC associated IF. Up to 43\% of patients
with IF requiring liver and intestinal transplant developed this condition as a sequela of
NEC.\textsuperscript{14}

Although NEC is primarily a disease of the intestinal tract, multiple studies have
found an association between NEC and poor neurodevelopmental outcomes.\textsuperscript{15} One such
case-control study from the year 2000 found that 55\% of infants suffering from NEC but
only 22.5% of matched controls were severely mentally retarded at 20 months of corrected gestational age.\textsuperscript{16} Infants requiring surgery for NEC have even poorer neurodevelopmental outcomes.\textsuperscript{15}

Thanks to advances in care, more premature infants are surviving the neonatal period only to be faced with NEC and its associated morbidity and mortality. To realize the full benefit of the past three decades of advances in neonatal care, we must make similar advances in the prevention and treatment of NEC.

In addition to the mortality and morbidity suffered by individual patients and their families, this disease poses a significant financial burden to our medical system. The total estimated yearly cost of caring for infants with acute NEC in the United States is between $500 million and $1 billion.\textsuperscript{13} Children who survive NEC with short bowel syndrome, the most severe intestinal sequela of NEC, have an estimated mean cost of $1.5 million for five years of care for a single child.\textsuperscript{13} Because NEC affects an extremely fragile population of patients for whom intensive care is costly, this disease poses a large financial burden despite its rarity.

**Blood Infections and NEC: Working Towards a Better Understanding of this Disease Process**

Advances in prevention and treatment are needed to reduce the disease burden of NEC. To improve our approach to the NEC patient we must develop a better understanding of this disease. Since NEC was first recognized as a distinct disorder in the 1950s the exact pathophysiology of this condition has remained unclear.\textsuperscript{17} NEC is a
complex disease with multiple proposed mechanisms and is now believed to have a multifactorial etiology, including an infectious component.

Bacterial translocation through a compromised intestinal mucosal barrier is an important feature of NEC. Translocation may contribute to the relatively high prevalence of BSI in infants with NEC. In a large study published in 2014, 39% of infants with NEC suffered from at least one BSI. NEC patients suffering from BSI are likely to be sicker than those who do not have blood infection during their hospital stay and BSI concurrent with the onset of NEC has been shown to be a risk factor for death. My study explores the microbial profile and timing of BSIs associated with NEC. I believe that infants with the most severe cases of NEC (those going on to suffer from IF) suffer BSIs with different microbial profiles and different timing than infants with less severe cases of NEC. Understanding the microbiology of BSIs in infants with NEC associated IF will shed light on the origin of these infections.

Objectives

This descriptive study aims to profile BSI pathogens, BSI recurrence, timing of BSIs in relation to NEC diagnosis and patient outcomes in patients with NEC associated IF compared to other VLBW neonates. Additionally, I tested the following specific hypotheses:
Hypothesis One

- Hypothesis 1a. Compared to BSI in other VLBW neonates, BSIs occurring during the onset of NEC in infants who go on to develop IF are more often due to gut bacteria, and/or more often polymicrobial (due to more than one pathogen).
- Hypothesis 1b. Compared to other VLBW neonates, infants who suffer from BSI during the onset of NEC and go on to develop IF have higher mortality.

Background for Hypothesis One

Infants with Post-NEC IF have been previously shown to be susceptible to recurrent BSI and likely to have BSI within a week of diagnosis with NEC. Researchers have also demonstrated that BSIs occurring within three days of NEC diagnosis (NEC-associated BSI) are commonly due to gut bacteria. I believe the infants contracting these NEC-associated infections are the same group of infants who will go on to develop IF, thus infants who develop IF should have NEC-associated BSIs predominantly with gut bacteria.

Hypothesis Two

- Hypothesis 2a. Compared to BSI in other VLBW neonates, post-NEC BSIs in patients suffering from IF are more often caused by gut bacteria, and/or more often polymicrobial.
- Hypothesis 2b. Compared to other VLBW neonates, infants with IF who suffer from post-NEC BSI have higher mortality.

Background for Hypothesis Two

Prior studies have shown that BSIs occurring more than 3 days after onset of NEC (post-NEC BSI) revert to the pathogen profile of general NICU infections. The
prevailing theory is that post-NEC infections are due to iatrogenic risks shared by all NICU infants (such as indwelling venous catheters). However, I believe that in the NEC patients with the most severe disease (IF), the disruption of the gut integrity persists beyond resolution of clinical signs and symptoms of NEC. The result of this ongoing intestinal compromise is recurrent BSIs with bacteria of gut origin.

Differences in BSI pathogens and BSI timing in patients with Post-NEC IF could point towards a different course of disease in these infants. Infants with this more severe form of NEC may suffer a chronic intestinal disease process not seen in patients with milder NEC.

Better understanding of the differences between BSIs in patients with differing severities of NEC may help clinicians develop better targeted treatment and preventive strategies for these neonates. Understanding of NEC associated IF as a chronic rather than acute disease process may also shape future research into the pathophysiologic mechanisms of this poorly understood condition.
CHAPTER 2. BACKGROUND

Necrotizing Enterocolitis (NEC)

NEC is a devastating and unpredictable multifactorial intestinal disease of prematurity affecting up to 5% of neonatal intensive care patients and up to 10% of infants weighing less than 1500g at birth.\textsuperscript{13} Despite current treatment approaches, NEC related morbidity and mortality remain high. Up to 30% of infants who suffer from NEC will not survive to hospital discharge.\textsuperscript{13} Many of those who do survive are left with long-term intestinal and neurological sequelae. To develop more effective and targeted treatment and preventive strategies for NEC, the medical and scientific community must first develop a more clear understanding of inciting factors, disease process and diagnostic strategy.

Clinical Course

Infants with NEC suffer intestinal inflammation and bowel wall necrosis in varying degrees of severity leading to a broad spectrum of symptoms. Affected infants frequently display feeding intolerance with emesis, abdominal distention and tenderness, abdominal wall discoloration and bloody stools. Infants with NEC also commonly have signs and symptoms of systemic illness, such as apnea with oxygen desaturation, bradycardia, lethargy and temperature instability. More severe cases of NEC lead to extensive bowel necrosis, intestinal perforation and sepsis.\textsuperscript{14} Intestinal perforation requires surgical intervention to either drain peritoneal fluid or resect necrotic bowel. Approximately half of affected infants require surgical intervention.\textsuperscript{20}
Infants with extensive bowel resection can go on to develop short bowel syndrome wherein intestinal absorptive surface area has been so severely reduced that patients become reliant on intravenous (parenteral) nutrition. PN carries with it risk of infection and liver disease. PN must be administered through an intravenous catheter threaded deeply into a large central vein to allow medication to be delivered safely. These central lines (CLs) pose greater risk of BSI, namely central line associated BSI (CLABSI), than a standard intravenous catheter placed in a small peripheral vein in the hand or arm. Reliance on parenteral nutrition may also lead to cholestatic liver disease, requiring liver transplant in severe cases. Infants reliant on parenteral nutrition for an extended period of time are said to be suffering from IF.

In addition to intestinal morbidity, infants surviving NEC also suffer poor neurodevelopmental outcomes. A case-control study from the year 2000 found that 55% of infants suffering from NEC but only 22.5% of matched controls were severely mentally retarded at 20 months of corrected gestational age. Infants requiring surgery for NEC have even poorer neurodevelopmental outcomes. NEC leads to an excessive systemic inflammatory response, which is thought to explain the link between NEC and neurodevelopmental delay.

**At Risk Population**

The population at-risk for NEC has been well characterized. NEC is a disease of prematurity and low birth weight; the lower the birth weight, the greater the incidence of NEC. The vast majority of cases occur in infants born before 36 weeks gestation (90-95% of cases). The remaining 5-10% of cases likely represent separate disease entities with a different pathophysiologic (likely ischemic) etiologies. Conditions that have been called
“NEC” in full term infants are usually associated with underlying disorders that may lead to bowel wall ischemia such as perinatal asphyxia, polycythemia, respiratory distress syndrome, congenital heart disease and other congenital anomalies.\textsuperscript{24}

A slightly higher incidence of NEC has been demonstrated among infants of male sex and black race. Higher rates of NEC among black infants may be due to higher rates of prematurity in this group.\textsuperscript{25} NEC commonly presents around two weeks of life but presentation can be delayed to approximately one month of age in smaller and more premature infants.\textsuperscript{26} The more premature the infant, the later the presentation of NEC.

**Incidence**

NEC affects 1-5\% of all newborns admitted to NICUs in the United States: between 0.5 and 5 infants per 1000 live births.\textsuperscript{14, 27} Since its recognition in the 1950s, the incidence of NEC has remained essentially unchanged despite improvements in neonatal care, likely due to improved survival rates in the smallest of premature infants.\textsuperscript{13, 28} NEC has a 5\% incidence in premature babies <33 weeks of gestational age\textsuperscript{26} and up to a 10\% incidence in VLBW infants (401 to 1500g birth weight).\textsuperscript{14} The at-risk population is increasing due to advances in care and survival of extremely preterm infants.

**Cause**

The onset of NEC in at-risk infants has been clearly linked to enteral feeding, particularly with infant formula. Over 90\% of infants who develop NEC have been fed enterally prior to developing the disease.\textsuperscript{29} A risk reduction has been shown for infants fed breast milk rather than formula.\textsuperscript{30} Other than the link to enteral feeding no other clear inciting factor has been identified; rather, it seems that a combination of many risk factors leads to development of this disease, including but not limited to genetic
predisposition, immaturity of gut motility and mucosal barrier, immune system immaturity and imbalance of gut microbial flora.

**Pathogenesis**

NEC develops as a consequence of the immature gut’s maladaptive reaction to enteral feeding in the setting of abnormal microbial colonization.

**Immature GI Tract**

The immature GI tract is at risk for NEC due to poor motility, poor mucosal barrier function, and altered immune defense. Preterm infants have impaired gut motility. Poor motility leads to longer transit times through the intestinal lumen, which may lead to bacterial overgrowth. The immature intestinal mucosal barrier in premature infants may allow bacterial invasion due to permeability between adjacent epithelial cells (poor integrity of intracellular tight junctions) and lack of important antibodies in mucosal secretions (reduced secretory immunoglobulin A). The immature intestine is predisposed to triggering an excessive inflammatory response to microbial presence. Compared to adult intestinal cells, intestinal cells in premature infants have more receptors that recognize bacterial presence (toll-like receptor 4) and a deficiency in an important immune inhibitory molecule (inhibitory factor κB). These differences in immune regulation of the immature gut predispose the premature infant to an exaggerated immune response to gut microbes. In NEC poor motility increases the bacterial load in the premature gut, poor mucosal barrier allows these bacteria to more easily penetrate the bowel wall, and an exaggerated immune response to invading bacteria leads to immune-mediated intestinal necrosis.
Abnormal Bacterial Colonization

Outbreaks of NEC have been documented and have led to theories of an infectious basis for development of this disease.\textsuperscript{34} Although gram-negative blood infections are common in NEC patients,\textsuperscript{2} varied pathogens cause these infections and no particular pathogen has been clearly linked to development of NEC. Premature infants undergoing intensive care frequently receive broad-spectrum antibiotics that alter their intestinal microbial profile. Infants in the NICU are predominantly colonized with species of \textit{Staphylococcus}, \textit{Enterobacter}, \textit{Enterococcus} and \textit{Clostridium}, while term breast-fed infants are predominantly colonized with \textit{Bifidobacteria} \textit{spp}. Enteric colonization with nosocomial microbes has been postulated to lead to an exaggerated immune response not seen with normal commensal bacteria.\textsuperscript{35}

Blood Infections and NEC

Blood stream infections (BSI) are common among NEC patients, with as many as 43\% of infants with NEC suffering from at least one BSI.\textsuperscript{2} Infants with NEC may suffer from BSI at the time of diagnosis of NEC and/or later during the course of hospitalization. BSIs at the time of NEC diagnosis are thought to be related to bacterial translocation through the intestinal wall.\textsuperscript{18} In fact, one of the hallmark radiographic signs of NEC, “pneumatosis intestinalsis”, or air in the bowel wall, is thought to be a result of bacterial fermentation within the wall as it moves from intestinal lumen to systemic circulation.\textsuperscript{36} It has been widely speculated that BSIs occurring after the acute phase of NEC has passed are due to iatrogenic risk factors such as the presence of CLs required for administration of PN.\textsuperscript{13, 25}
Mortality

In 2002, Stoll et al. reported that 18% of 1313 VLBW neonates with BSI died during NICU stay. In 2006, Holman et al. found an overall in-hospital fatality rate for NEC of 15% for 4463 neonates. In 2014, Bizzarro et al. reported that 46% of 69 infants suffering from BSI within 3 days of NEC diagnosis died. Late-onset sepsis and NEC both cause significant mortality in premature neonates, and the combination of NEC and sepsis appears to lead to a significantly higher risk of death.

Diagnosis

NEC is a challenging illness to diagnose. Because the initial symptoms overlap with many other conditions of prematurity, clinicians may not recognize NEC until it has progressed to a more advanced stage. On the other hand if clinicians are overly cautious, they run the risk of treating many unaffected infants. Adding to the diagnostic challenge, the definition of NEC has evolved, and some groups of infants who historically would have been diagnosed with NEC are now classified as suffering from other intestinal diseases. The lack of validated and objective diagnostic criteria make it difficult to establish a diagnosis of NEC.

The most widely used diagnostic criteria were developed in an era before surfactant therapy. In the 1970s, the majority of NEC cases occurred in term and near-term infants because without surfactant therapy a large proportion of premature infants did not survive long enough to develop NEC. In the 1970s, the lack of uniformly accepted diagnostic criteria for NEC led Bell and colleagues to develop a schema for diagnosing and staging NEC. The Bell Criteria for diagnosis of NEC was the first such attempt to create a systematic method of diagnosis and to stratify different levels of NEC.
severity. Bell’s 1978 paper chronicled the use of his classification system to provide graded intervention based on NEC severity. This staging system included clinical and radiographic signs and was based on expert opinion. Bell analyzed outcomes of treatment for 48 patients based on this staging. In Bell’s study none of the patients with Stage 1 NEC died or progressed to more advanced stages of NEC, 4 of 28 died in the Stage 2 group and 7 of 10 died in the Stage 3 group. The lack of mortality or disease progression in the Stage 1 group led Bell to question the certainty of NEC diagnosis in this group and Stage 1 of NEC was named “suspect”.

Bell Criteria for NEC Diagnosis

Stage I (Suspect NEC – Recommended care: supportive and diagnostics to rule out other causes)
Any one or more of the following:
- Derangement of vital signs
- Poor feeding, emesis, distension, may have blood in stool
- Radiographs showing mild ileus

Stage II (Definite NEC – Medically treated)
Any one or more of the following:
- Above signs or symptoms plus persistent or occult gastrointestinal (GI) bleeding
- Radiographs may show: Distension with ileus/ Edema in bowel wall/ Unchanging dilated bowel loops/ Pneumatosis intestinalis/ Portal venous gas

Stage III (Advanced NEC – Surgically treated)
Any one or more of the following:
- Above signs or symptoms plus deterioration of vital signs, septic shock, GI bleed
- Radiographs may show: Pneumoperitoneum

The clinical signs included in Bell’s criteria are entirely non-specific and may be seen with any number of systemic illnesses. The radiographic findings of pneumatosis intestinalis (bubbly lucency in the bowel wall) and portal venous gas have more recently been shown to have 100% specificity for NEC in infants with clinically suspected disease. Unfortunately these findings are not present in the majority of infants diagnosed
with NEC; the sensitivity for pneumatosis intestinalis and portal venous gas are 44% and 13% respectively.\(^{38}\)

In the 1980s, researchers began to recognize that use of the Bell Criteria allowed for many infants with other acquired neonatal intestinal diseases to be misclassified as NEC patients. Thus began the revision of the NEC diagnostic algorithm. The first proposed change was the removal of Bell Stage 1 to reduce inclusion of non-specific ileus.\(^{28}\) Bell himself had suggested that Stage 1 includes many infants without true NEC. The first major revision came in 1987 when Kleigman and colleagues proposed the Modified Bell Criteria that provide for grading of severity within each of the three stages of NEC.

**Modified Bell Criteria\(^ {39}\)**

Stage I - Suspected NEC
- **IA** Same as Bell I except blood in stool must be occult
- **IB** Stage IA signs and symptoms with bright red blood per rectum

Stage II - Definite NEC
- **IIA** – “Mildly ill”
  - Same as Stage I but with diminished bowel sounds and radiographs may show: Distension with ileus/Pneumatosis intestinalis
- **IIB** – “Moderately ill”
  - Same as IIA but with definite abdominal tenderness, mild metabolic acidosis, mild thrombocytopenia, ascites, and radiographs may show portal venous gas

Stage III – Advanced NEC
- **IIIA** – “Severely ill, bowel intact”
  - Same as Bell III but without radiographic pneumoperitoneum
- **IIIB** – “Severely ill, bowel perforated”
  - Above signs and symptoms plus radiographic pneumoperitoneum
  - Requiring surgical intervention

The Modified Bell Criteria is the most commonly used diagnostic system for NEC research. The National Institute of Child Health and Human Development’s Neonatal Research Network Registry compiles data on NEC occurrence according to the Modified Bell Criteria. Most studies classify patients as suffering from NEC only if they are
Modified Bell stage IIB or greater. Patients with Modified Bell stage IIB have mild laboratory abnormalities (metabolic acidosis, thrombocytopenia), diminished bowel sounds and abdominal tenderness and may or may not have radiographic findings of ileus, unchanging dilated bowel loops, pneumatosis intestinalis or portal venous gas. Because radiographic signs are the only aspect of this diagnostic algorithm truly specific to NEC, and they are not required for diagnosis, this staging system relies heavily on the clinician’s suspicion of NEC. More recent studies have shown that these clinical signs and symptoms are not sufficient to predict which patients will experience a mild versus severe disease course.40

Many patients with spontaneous intestinal perforation (SIP) have historically been included in datasets as Modified Bell stage IIIB NEC patients due to the presence of free peritoneal air on abdominal radiographs. SIP involves focal perforation of the small bowel without extensive necrosis. SIP usually has a very early onset, occurring during the first week of life. No relationship has been found between enteral feeding and the development of SIP and this disease can frequently develop before initiation of any enteral feeds. SIP has been linked to the use of postnatal anti-inflammatory drugs (indomethacin and ibuprofen) and steroids.41-42 SIP has a distinct histopathology involving destruction of the muscularis interna intestinal layer but no disruption in the integrity of the intestinal mucosa.43 In contrast to SIP, NEC produces extensive coagulative necrosis of the intestinal mucosa. Newer classification systems have focused on reducing the inclusion of patients with SIP by requiring free peritoneal air to be accompanied by signs more specific for NEC such as the specific radiographic findings
of pneumatosis and portal venous gas. The exclusion of patients with SIP from NEC diagnosis is a concern for researchers and clinicians alike.

**Prevention and Treatment**

Because many factors contribute to manifestation of this disease, it warrants multiple treatment approaches including modification of feeding regimens, use of PN, antibiotics and sometimes surgery. Prebiotics, probiotics and treatments targeting immune system dysfunction are also being explored.

Since NEC only occurs once enteral feedings have been initiated, prevention techniques have historically focused on withholding feeds. More recent research has shown that small volume enteral feeding with human milk and human milk fortified formulas provides some degree of protection against NEC.

Probiotics have also been shown to reduce the severity of NEC but, due to lack of standardized probiotic formulations, many NICUs have not implemented probiotic use into common practice. Since many clinicians are hesitant to administer live bacteria to preterm patients, supplementation with prebiotics, substrates known to encourage growth of beneficial bacteria (e.g. fructooligosaccharide), has been proposed as a preventative strategy for NEC.

Several studies have explored novel therapies for NEC that target the exaggerated immune response of the immature gut. Supplementation with oral immunoglobulins G and M have not been proven to provide any benefit. Epidermal growth factor supplementation has shown only minor improvement in rates of NEC. In two small studies supplementation with oral arginine decreased incidence of NEC. There is not
yet enough evidence to support routine use of immune-modulating supplements for the
treatment of NEC.

Typically once definitive diagnosis is made (Modified Bell stage greater than IIa),
broad-spectrum antibiotics are administered and feeds are withheld for 7 to 14 days. PN
is initiated if infants are restricted from enteral feeding for periods of 7 days or more.
Frequent abdominal x-rays are obtained to monitor progression of disease and assess for
intestinal perforation.¹⁴

Surgery is indicated in cases with intestinal perforation, and approximately half of
infants suffering from NEC require surgery.⁵⁰ Surgeries range from the less invasive
placement of a peritoneal drain, to the more invasive exploratory laparotomy with bowel
resection and creation of a stoma. Two large studies have addressed differences between
NEC outcomes for patients receiving peritoneal drains compared to exploratory
laparotomy with bowel resection. Both concluded that there is not a difference in
survival.⁵¹-⁵² But one of these studies suggests that infants do not clinically improve with
peritoneal drainage and often go on to require laparotomy.⁵² A systematic review of
several studies concluded a 50% higher mortality for NEC patients undergoing peritoneal
drainage and a reduced risk of death and neurodevelopmental impairment in those
undergoing laparotomy.¹³ Both of these surgical techniques are still in common practice
and the debate over which technique is superior is far from settled. However, it is clear
that once surgery is required outcomes may be poor, thus the need for methods of early
diagnosis and prediction of which neonates are at greatest risk of developing NEC.
Aim of This Study

This study will answer the unanswered question of whether infants with IF as the result of NEC suffer from a prolonged period of gut inflammation leading to recurrent gut bacteria BSI and a greater risk of death.
CHAPTER 3. METHODS

Study Site

Children’s Hospital of Orange County is a tertiary and quaternary care referral center in the city of Orange, serving the metropolitan area between Los Angeles, Riverside and San Diego, California. CHOC runs a 54 bed, level 4 NICU.

Cohort

The study population was comprised of 177 VLBW neonates with late-onset BSI (LO-BSI) who were treated in the CHOC NICU between July 1, 2005, and December 31, 2013.

Data Collected

Since July 1, 2005, the infectious disease research division at CHOC has maintained a database of all positive blood cultures obtained in the institution. The CHOC internal review board (IRB) has approved the compilation of this de-identified database. Informed consent was waived as this study met the conditions of minimal risk to study subjects. This database includes date and time of culture, site (peripheral or central line) from which blood specimen was obtained, and species type for all laboratory confirmed positive blood cultures deemed non-contaminant by an infectious disease specialist. Cultures positive for *Acinetobacter lwoffii*, *Micrococcus spp* and diptheroids were considered contaminants. Cultures positive for coagulase negative *Staphylococci* were determined to be non-contaminants if at least 2 positive cultures were obtained for
the same pathogen, or if one positive culture was obtained but the patient had signs and symptoms of sepsis (as determined by the clinician) warranting antibiotic treatment for at least 72 hours.

The 177 patients included in this study represent a subset of patients in the BSI database. CHOC IRB approval was also obtained for completion of this study. Additional information about patients was obtained from the medical record. Data collected from the medical record included gestational age, birth weight, gender, length of NICU stay, use of medical equipment such as ventilators and CLs, duration of treatment with PN, treatments given for patent ductus arteriosus, types of surgical interventions, discharge diagnoses and mortality.

**Definitions**

**Necrotizing Enterocolitis**

Diagnosis of NEC was determined based on chart review according to the following criteria:

NEC was suspected by neonatal physician and patient had either:

1. Surgical evidence of necrotic bowel.

   Or

2. Specific signs of NEC on abdominal x-ray as evidenced by one of the following:
   a. Pneumatosis intestinalis
   b. Portal venous gas
   c. Free intraperitoneal air
Or

3. Radiographic findings on abdominal x-ray associated with NEC but not meeting the above criteria, and treatments initiated for NEC by withholding feeds, starting PN and administering broad-spectrum antibiotics for at least 72 hours.

NEC-associated radiographic findings:

   a. Dilated loops of bowel unchanged in appearance between consecutive films more than 6 hours apart (“fixed dilated loops”)
   b. Ileus pattern
   c. Absence of bowel gas

Date of onset of NEC was defined as the date of surgical intervention with evidence of necrotic bowel (see 1 above) or the date specific radiographic signs of NEC were found (see 2 above) or the date treatment for NEC was initiated (see 3 above).

Infants with SIP have bowel perforation, which may result in free intraperitoneal air visible on abdominal radiograph. I excluded these infants (n = 13) from my NEC cohort by reviewing surgical reports for pathologic evidence of SIP and requiring NEC to be the treating neonatologist’s leading clinical diagnosis.

**Post-Necrotizing Enterocolitis Intestinal Failure (IF)**

Post-NEC IF was defined as follows, in accordance with the definition described by Cole and colleagues in 2011.\(^9\) Infants were classified as having Post-NEC IF if they suffered from NEC with abdominal surgical intervention and were subsequently reliant on PN for \(\geq 6\) weeks. Surgical interventions included laparoscopy, laparotomy, bowel
ostomy creation, bowel resection and intraperitoneal drain placement. Patients with NEC and abdominal surgery who were reliant on PN, but died before 6 weeks of PN could be completed, were also classified as having IF. The IF classification of patients who died within 6 weeks of PN initiation is unique from the definition used by Cole and colleagues.

**Bloodstream Infection (BSI)**

BSI episodes were also defined in accordance with Cole and colleagues. For each patient, positive blood cultures were grouped according to date of collection. A BSI episode consisted of all positive blood cultures obtained from 0 to 4 days apart. A positive blood culture obtained more than 4 days after the last positive culture of a BSI episode was considered to be part of a separate BSI episode. Blood cultures were assessed in the clinical laboratories at CHOC and the neighboring St. Joseph Hospital using a fluorescent detection system for the presence of CO₂ production (Bact/alert; bioMerieux, Basingstoke, Hants, UK, or Bactec; Becton Dickinson, Sandy, Utah) and species were identified using standard procedures. Only late onset BSIs (LO-BSI: onset >72 hours after birth) were included in this analysis.

BSI episodes were classified as polymicrobial or monomicrobial. *Polymicrobial BSI* episodes were those episodes in which multiple pathogens were cultured either from a single blood draw or from separate blood draws considered to be part of the same episode. *Monomicrobial BSI* episodes were those in which only a single pathogen was identified for all positive cultures in that episode. Occasionally the specific species of *Staphylococcus* was not identified for cultures positive for coagulase negative
Staphylococci (CoNS). In these cases if CoNS was identified more than once in the same BSI episode, the antibiotic resistance profiles were compared to determine if these represented a single or multiple species. BSI episodes with multiple species of CoNS were considered polymicrobial.

BSI episodes were classified as gut bacteria BSI if the species cultured included gram-negative organisms, Enterococcus spp or Lactobacillus spp and were monomicrobial or polymicrobial consisting of only these pathogens.

BSI episodes in patients with NEC were classified according to their timing with regards to date of NEC diagnosis, in accordance with the definition used by Bizzarro and colleagues in 2014.² BSIs were considered NEC-associated BSI if they included a positive culture obtained within 72 hours of NEC diagnosis. BSI episodes occurring more than 72 hours before NEC diagnosis, or more than 72 hours after NEC diagnosis, were considered pre-NEC and post-NEC BSIs respectively.

In patients who died, deaths were classified according to their timing with respect to BSI episodes and with respect to NEC diagnosis. Deaths were considered BSI-attributable or NEC-attributable if occurring 0 to 7 days after BSI episode or NEC diagnosis respectively, and BSI-associated or NEC-associated if occurring 0 to 28 days after BSI episode or NEC diagnosis.

Length of Hospital Stay

Length of stay was defined as the time from birth through discharge from CHOC. Birth was used as the beginning of stay because all infants were admitted to a hospital at birth, most were initially admitted to CHOC, but some were transferred from a referring
hospital. Upon discharge some patients were transferred to an outside hospital, but their longer length of stay could not be captured in this study because information about discharge from outside hospitals was not available in the CHOC medical record.

**Very-Low-Birth-Weight**

Because NEC is a disease of premature infants, I decided to study only the low birth weight infants in the BSI database. I selected infants based on birth weight because weight is more accurately determined than gestational age. This analysis included only VLBW infants, defined as those with birth weights 401 to 1500g. Infants were not selected according to gestational age.

**Gastrointestinal (GI) Pathology**

GI conditions listed as discharge diagnoses were recorded for all patients. All GI conditions were recorded including but not limited to common conditions such as feeding intolerance, hernias, gastro-esophageal reflux, cholestasis, and NEC, and less common conditions such as meconium ileus, esophageal atresia and imperforate anus. Surgical GI conditions such as bowel resection, ostomies and percutaneous endoscopic gastrostomy tubes were also recorded. For the purposes of analysis, infants with NEC were only considered to have comorbid GI pathology if they had GI conditions unrelated to NEC. The diagnoses of feeding intolerance, short bowel syndrome, cholestasis due to prolonged PN administration, and surgical interventions for NEC were considered to be part of the NEC episode and not labeled as comorbidities.
Statistical Analyses

The IBM SPSS Statistics v. 21.0 software package (Armonk, New York) was utilized for data analyses. Descriptive data for the entire patient cohort was expressed as mean and standard deviation for continuous data, and percentage and number for dichotomous data. Characteristics of patients with and without IF were compared in the entire cohort. For continuous data, comparisons were made via independent samples t-test; for dichotomous data, comparisons were made via \( \chi^2 \) analysis.

Univariable analyses of distribution of specific pathogens causing BSI in patients with and without IF were conducted. Pathogen types for BSI in infants with and without IF were compared in the entire patient cohort. These comparisons were made for gram-negative BSI, gram-positive BSI, polymicrobial BSI and gut pathogen BSI (see definitions above) via logistic regression analysis, and presented as odds ratios (OR) with 95% confidence intervals (CI).

Occurrence of NEC-Associated BSI and Post-NEC BSI (see definitions above) were analyzed in the cohort of patients suffering from NEC. Comparison was made between patients with and without IF via \( \chi^2 \) analysis and presented as P values. Recurrence of BSI was analyzed among those patients surviving to NICU discharge. Comparison among these surviving patients was made between patients with and without IF via \( \chi^2 \) analysis and presented as a P value.

Patient characteristics were compared for those who died vs. survived in the entire cohort. For continuous data, comparisons were made via independent samples t-test; for dichotomous data, comparisons were made via \( \chi^2 \) analysis. All comparisons were presented as P values.
All-cause mortality was compared between patients with IF vs. without IF among all patients, as well as among the subset of patients with NEC. All-cause mortality was also compared between patients with IF and NEC-associated BSI vs. all others; and between patients with IF and post-NEC BSI vs. all others. Comparisons were made using bivariable and multivariable logistic regression analysis and presented as ORs and adjusted ORs with 95% CIs. Consistent with previous studies of mortality in VLBW neonates with NEC and BSI, adjusted models accounted for gestational age, birth weight and gender.²

Pathogen types were compared between NEC-associated BSI events in IF patients vs. BSI events in patients without IF, and post-NEC BSI events in IF patients vs. BSI events in patients without IF. These comparisons were made for gram-negative BSI, gram-positive BSI, polymicrobial BSI and gut pathogen BSI using bivariable logistic regression analysis and presented as ORs with 95% CIs.
CHAPTER 4. RESULTS

a. Study population

In the VLBW population of 177 neonates, all of whom suffered from BSI, 74 (42%) were diagnosed with NEC (see Figure 1).

Fifty NEC patients had surgical interventions for their NEC (68% of those with NEC). Forty-five NEC patients developed IF (61% of those with NEC or 90% of those with surgical NEC). Two hundred and thirty-four BSI episodes were recorded for these 177 subjects. Infants with IF (n=45) suffered more BSIs per infant than any other group (1.6 vs. 1.2 BSI episodes per patient, p = 0.02 calculated using independent samples t-test). Infants without NEC had an average of 1.2 BSIs per subject. Infants with medically managed NEC had an average of 1.3 BSIs per subject. Those with surgically managed NEC, not complicated by IF, had only one BSI each.

Figure 1. Study Population
b. Characteristics of neonates with and without IF

Comparisons were made between patients with IF (n=45) and all other patients in the cohort (see Figure 2).

The non-IF comparison group included NEC patients treated medically (n=24), NEC patients treated surgically who did not go on to develop IF (n=5) and patients not suffering from NEC (n=103).
The mean gestational age of the total VLBW cohort (with and without IF) was 26
weeks (ranging from 23 – 35 weeks) (see Table 1).

| Table 1. Characteristics of Patients with and without IF Among 177 VLBW NICU Patients with LO-BSI, July 2005-December 2013 |
|-----------------------------------------------|---------------|-----------------|---------|
| GA (weeks)* | 26 ± 3 | 26 ± 3 | 26 ± 2 | 1.00 |
| BW (g)*  | 860 ± 262 | 874 ± 261 | 856 ± 263 | 0.69 |
| Male* | 61% (108) | 58% (26) | 62% (82) | 0.60 |
| Length of NICU Stay* | 106 ± 49 | 127 ± 51 | 98 ± 46 | < 0.00 |
| DOL Onset of First BSI* | 39 ± 36 | 53 ± 42 | 34 ± 32 | 0.01 |
| Diagnosed With NEC* | 42% (74) | all | 22% (29) | - |
| DOL Nec Onset* | 22 ± 16 | 25 ± 18 | 18 ± 12 | 0.05 |
| Days on PN** | 52 ± 36 | 82 ± 40 | 41 ± 28 | -** |
| Ventilator* | 51% (90) | 61% (27) | 47% (62) | 0.12 |
| Central Line* | 82% (145) | 71% (32) | 86% (113) | 0.04 |
| NSAID Use for PDA* | 52% (92) | 53% (24) | 52% (68) | 0.86 |
| SIP* | 8% (14) | 2% (1*) | 10% (13) | 0.12 |
| Non-NEC GI Pathology* | 51% (90) | 36% (16) | 56% (74) | 0.02 |

*Mean ± SD. P was calculated using the independent samples t-test.
* % (no. of patients). p was calculated using chi^2 analysis.
*At time of diagnosis of first BSI
**This comparison was not made because a minimum of 42 days on PN were required for inclusion in the IF group.
NEC - Neonatal Necrotizing Enterocolitis, IF - NEC associated intestinal failure
GA - gestational age, BW - birth weight, DOL - day of life, PN - Parenteral Nutrition, SIP - Spontaneous Intestinal Perforation

The mean birth weight was 860 g (ranging from 450 – 1491 g). Sixty-one percent of
subjects studied were male (108/177). The mean length of stay in the NICU was 106 days
(ranging from 4 – 263 days). The age of onset for first BSI varied greatly within this
cohort, with an average onset of 39 days of life (ranging from 4 – 228 days of life). Forty-
two percent of patients in this cohort were diagnosed with NEC (74/177). For the 74
patients with NEC, on average, diagnosis was made on day 22 of life (ranging from 4 –
77 days of life). Average duration of PN administration was 52 days with a wide range
including one patient who was treated with PN for only one day and another who
received PN for 172 days. Notably, all patients in this cohort were treated with PN at
some point during their hospitalization. Half of patients in this cohort were on mechanical
ventilation at the time of first BSI (90/177). Most (82%) had at least one central venous
line in place at the time of first BSI (145/177). Half of all patients received non-steroidal
anti-inflammatory drug (NSAID) therapy for closure of a patent ductus arteriosus (92/177). SIP was rare (<8%) in this cohort (14/177). Roughly half of the patients in this cohort had GI pathology other than NEC listed on their discharge diagnoses (90/177).

There were no significant differences between infants with and without IF in gestational age, birth weight, gender, use of mechanical ventilation, NSAID use for patent ductus arteriosus closure or diagnosis of SIP. Statistical comparison of days on PN and diagnosis with NEC are not presented since NEC was a prerequisite to be classified as having IF, and ≥42 days on PN was a requirement for inclusion in the IF group.

Infants with IF stayed in the NICU on average one month longer than those without IF (127 days vs. 98 days; P < 0.00). Infants with IF experienced their first BSIs on average 19 days later than infants without IF (53 days of life vs. 34 days of life, P = 0.01).

Neonates going on to develop IF were diagnosed with NEC on average one week later than those with less complicated NEC (25 days of life vs. 18 days of life, P = 0.05).

Patients with IF were significantly less likely to have comorbid GI pathology (36% vs. 56%; P = 0.02). Patients with IF were less likely to have a CL at time of first BSI (71% vs. 86%, P = 0.04).
c. BSI characteristics: Pathogen types for neonates with and without IF

I characterized distribution of microbes cultured from 234 LO-BSIs in this population of 177 VLBW neonates. Polymicrobial BSIs were uncommon yet slightly more frequent among patients suffering from IF (18% vs. 13%) (see Table 2).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>All BSIs (n=234)</th>
<th>BSIs in IF Patients (n=71)</th>
<th>BSIs in Patients without IF (n=163)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymicrobial</td>
<td>15% (35)</td>
<td>18% (13)</td>
<td>13% (22)</td>
</tr>
<tr>
<td>Monomicrobial (n=199)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>23% (46)</td>
<td>31% (18)</td>
<td>20% (28)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>19</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>5</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Gram-Positive</td>
<td>69% (138)</td>
<td>59% (34)</td>
<td>74% (104)</td>
</tr>
<tr>
<td>CoNS</td>
<td>86</td>
<td>21</td>
<td>65</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>14</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>31</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Fungi</td>
<td>8% (15)</td>
<td>10% (6)</td>
<td>6% (9)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>7</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Yeast Not Further Identified</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

% (no. of BSIs)

Gram Positive Other - Micrococcus, Streptococcus intermedius, Kocuria kristiane, Lactobacillus
Klebsiella - Species oxytoca and pneumoniae
Staphylococcus aureus - includes MRSA
CoNS - epidermidis, capitis, Not aureus, haemolyticus, warneri, sciuri, lugdunensis, hominis, auricularis
Other Fungi - Malassezia furfur
The majority of BSI episodes were monomicrobial (85%, 82% and 87% for all, IF and non-IF patients respectively). For these monomicrobial infections, specific pathogen type and distribution were recorded.

Gram-negative BSIs made up approximately one-quarter to one-third of infections, with a slightly higher frequency among patients with IF (23%, 31% and 20% for all, IF and non-IF patients respectively). Among monomicrobial gram-negative BSIs, *Klebsiella spp* were the most frequently cultured pathogen for all patient groups (41%, 50% and 36% of gram-negative BSIs for all, IF and non-IF patients respectively). *E. coli* was another commonly cultured gram-negative organism (30%, 33% and 29% of gram-negative BSIs for all, IF and non-IF patients respectively). Other gram negatives were present in cultures much less frequently.

Gram-positive BSIs were more common among non-IF patients compared to those with IF (74% vs. 59% of BSIs). Coagulase negative *staphylococci* (CoNS) were the most common pathogens, gram-positive or otherwise in all groups, making up 43% of all monomicrobial infections and 62% of gram positive BSIs. Approximately two-thirds of all gram-positive infections were due to CoNS (62%, 62% and 63% of gram-positive for all, IF and non-IF patients respectively). *Staphylococcus aureus* was the next most common gram-positive pathogen making up only 16% of monomicrobial infections and 22% of gram-positive infections. *S. aureus* was much more common among patients without IF (27% vs. 9% of gram-positive BSIs). *Enterococcus spp* represented 10% of all gram-positive BSIs. *Enterococcus* BSIs were much more common among infants with IF (21% vs. 7% of gram-positive BSIs). Other gram-positive pathogens were rare in this
cohort. Fungi were cultured infrequently, representing fewer than 10% of all monomicrobial BSIs.

Statistical comparison of pathogen types for patients with and without IF are seen in Table 3 below. Nearly one-third (31%) of patients suffered from at least one gram-negative BSI (54/177).

<table>
<thead>
<tr>
<th>BSI Type</th>
<th>All Patients (n=177)</th>
<th>IF Patients (n=45)</th>
<th>Patients without IF (n=132)</th>
<th>IF vs. No IF OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-Negative</td>
<td>31% (54)</td>
<td>44% (20)</td>
<td>26% (34)</td>
<td>2.3 (1.1, 4.7)* p = 0.02</td>
</tr>
<tr>
<td>Gram-Positive</td>
<td>75% (133)</td>
<td>73% (33)</td>
<td>76% (100)</td>
<td>0.9 (0.4, 1.9) p = 0.75</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>18% (32)</td>
<td>29% (13)</td>
<td>14% (19)</td>
<td>2.4 (1.1, 5.4)* p = 0.03</td>
</tr>
<tr>
<td>Gut Pathogens</td>
<td>34% (60)</td>
<td>51% (23)</td>
<td>28% (37)</td>
<td>2.7 (1.3, 5.4)* p = 0.01</td>
</tr>
</tbody>
</table>

% (no. of patients)

*Associations with p ≤ 0.05

Odds ratios calculated using logistic regression analysis.

Gram Negative - Patients with gram negative BSI during one or more monomicrobial or polymicrobial BSI events
Gram Positive - Patients with gram positive BSI during one or more monomicrobial or polymicrobial BSI events
Polymicrobial - Patients with one or more polymicrobial BSI events
Gut Pathogens - Patients with one or more gut pathogen BSI events (Gram Negatives, Enterococcus and Lactobacillus): Monomicrobial or polymicrobial consisting of only gut pathogens.

Gram-positive infections were common, occurring in at least one BSI episode for three-quarters of all patients (133/177). Polymicrobial infections were uncommon, occurring in less than one-quarter of patients (32/177). One-third of patients (60/177) experienced a BSI episode consisting of only gut pathogens (gram-negatives, *Enterococcus spp* and/or *Lactobacillus spp*).
Infants with and without IF had similar frequency of gram-positive BSI (73% vs. 76%). Infants who went on to develop IF were more likely to be infected with gram-negative bacteria (44% vs. 26%; OR 2.3, 95% CI 1.1 - 4.7), gut pathogens (51% vs. 28%; OR 2.7, 95% CI 1.3 - 5.4) and to suffer from polymicrobial BSIs (29% vs. 14%; OR 2.4, 95% CI 1.1 - 5.4).

d. BSI onset in relation to NEC diagnosis for neonates with and without IF

BSI episodes were classified according to their onset with relation to the diagnosis of NEC. Thirty-one percent of NEC patients (23/74) experienced a BSI within three days of NEC diagnosis (NEC-associated BSI) (see Table 4).

<table>
<thead>
<tr>
<th></th>
<th>All NEC Patients</th>
<th>IF Patients</th>
<th>NEC without IF</th>
<th>IF vs. No IF (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC Associated BSI*</td>
<td>31% (23/74)</td>
<td>38% (17/45)</td>
<td>21% (6/29)</td>
<td>0.13</td>
</tr>
<tr>
<td>Post-NEC BSI**</td>
<td>76% (47/62N)</td>
<td>74% (25/34)</td>
<td>79% (22/28)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

% (proportion of patients)
P was calculated using χ2 analysis.
*BSI within 72 hours of NEC diagnosis
**BSI >72 hours after NEC diagnosis
NAnalysis done among those surviving to NICU discharge
NEC-associated and post-NEC BSI are neither required nor mutually exclusive: 7 NEC patients had both NEC associated and post-NEC BSIs, 6 NEC patients had neither.

Infants who went on to develop IF experienced NEC-associated BSIs more often than infants without IF, however this result was not statistically significant (38% vs. 21%; P = 0.13). BSIs occurring more than three days after NEC diagnosis (post-NEC BSI) were analyzed among those patients who survived to NICU discharge (62 NEC patients).
Analysis was restricted to this population because death cut short the possibility of contracting post-NEC BSIs. Post-NEC BSIs were common among NEC patients regardless of whether or not they went on to develop IF. Seventy-six percent of surviving NEC patients (47/62) experienced post-NEC BSI. Seventy-four percent (25/34) of those developing IF and 79% (22/28) of those without IF experienced post-NEC BSI.

e. Recurrence of BSI

Nearly half of infants with IF suffered recurrent BSI (42%: 19/45) (see Figure 3).

![Figure 3. Distribution of Recurrent BSI](image)

Infants with IF suffered up to five BSI episodes in a single patient but most IF patients with recurrent BSI experienced only 2 BSIs each (79%: 15/19). Surgical NEC patients not developing IF suffered no recurrent BSIs. Nearly one-third of medically managed
NEC patients experienced recurrent BSI (29%: 7/24). Medically managed NEC patients experienced up to four BSIs per patient, with the majority of medical NEC patients with recurrent BSI only experiencing two BSIs each (71%: 5/7). Relatively few infants without NEC suffered recurrent BSI (19%: 20/103). The maximum number of BSIs in a single patient without NEC was three but the vast majority of non-NEC patients with recurrent BSI suffered only two BSIs each (95%: 19/20).

f. Recurrence of BSI for neonates with and without IF

Analysis of recurrence of BSI was performed in the subset of patients who survived to NICU discharge (147 patients) to avoid comparing groups with unequal likelihoods of BSI recurrence (those who died and those who survived) (see Table 5).

| Table 5. Recurrence of LO-BSI Among Survivors with and without IF Among 147 VLBW NICU Patients Surviving to Hospital Discharge |
| --- | --- | --- | --- |
| | All Patients Surviving to Discharge (n=147) | Surviving IF Patients (n=34) | Surviving Patients without IF (n=113) | IF vs. No IF (p) |
| >1 Episode of BSI* | 26% (38) | 44% (15) | 20% (23) | 0.01 |

P was calculated using $\chi^2$ analysis.
* During NICU stay

Twenty-six percent of the cohort experienced recurrent BSI (38/147). Surviving patients with IF were significantly more likely to experience recurrent BSI (44% vs. 20%, $P = 0.01$).
g. Mortality outcomes

Gestational age, birth weight, gender, timing of onset for first BSI, frequency of NEC diagnosis, presence of CL during first BSI, use of NSAIDs, frequency of SIP diagnosis and frequency of diagnosis with GI pathology other than NEC were not significantly different between infants who did and did not survive (see Table 6).

Table 6. Characteristics of Patients who Did and Did Not Survive to Hospital Discharge Among 177 VLBW NICU Patients with LO-BSI

<table>
<thead>
<tr>
<th></th>
<th>Died (n=30)</th>
<th>Survived (n=147)</th>
<th>Died vs. Survived (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA (weeks)*</td>
<td>27 ± 3</td>
<td>26 ± 3</td>
<td>0.59</td>
</tr>
<tr>
<td>BW (g)*</td>
<td>824 ± 282</td>
<td>868 ± 258</td>
<td>0.41</td>
</tr>
<tr>
<td>Male</td>
<td>63% (19)</td>
<td>61% (89)</td>
<td>0.84</td>
</tr>
<tr>
<td>Length of NICU Stay*</td>
<td>75 ± 72</td>
<td>112 ± 41</td>
<td>0.01</td>
</tr>
<tr>
<td>DOL Onset of First BSI*</td>
<td>47 ± 59</td>
<td>37 ± 29</td>
<td>0.39</td>
</tr>
<tr>
<td>Diagnosed With NEC*</td>
<td>40% (12)</td>
<td>42% (62)</td>
<td>1.00</td>
</tr>
<tr>
<td>Days on PN*</td>
<td>37 ± 34</td>
<td>55 ± 36</td>
<td>0.01</td>
</tr>
<tr>
<td>Ventilator*</td>
<td>73% (22)</td>
<td>46% (67)</td>
<td>0.01</td>
</tr>
<tr>
<td>Central Line*</td>
<td>80% (24)</td>
<td>82% (121)</td>
<td>0.80</td>
</tr>
<tr>
<td>NSAID Use for PDA*</td>
<td>37% (11)</td>
<td>55% (81)</td>
<td>0.07</td>
</tr>
<tr>
<td>SIP</td>
<td>13% (4)</td>
<td>7% (10)</td>
<td>0.26</td>
</tr>
<tr>
<td>Non-NEC GI Pathology*</td>
<td>40% (12)</td>
<td>53% (78)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*Mean ± SD. P was calculated using the independent samples t-test.
* % (no. of patients). p was calculated using χ2 analysis.
* At time of diagnosis of first BSI
* During NICU stay
NEC - Neonatal Necrotizing Enterocolitis
IF - Post-NEC intestinal failure
GA - gestational age, BW - birth weight, DOL - day of life
PN - Parenteral Nutrition, SIP - Spontaneous Intestinal Perforation

Those who survived had an average length of stay more than one month longer than those who died (112 days vs. 75 days, P=0.01). Those who survived were on PN for an average of more than 2 weeks longer than those who died (55 days vs. 37 days, P=0.01). Patients who died had a higher frequency of mechanical ventilation at the time of first BSI (73% vs. 46%, P=0.01).
h. Timing of mortality in relation to BSI

Sixteen percent of infants suffering from NEC (12/74) and 17% (18/103) of infants without NEC died (see Figure 4).

Only one infant with medically managed NEC died (4%). Among infants with surgically managed NEC all deaths occurred in infants with IF; none of the infants with surgical NEC without IF died (0/5). There was 22% mortality in infants suffering from surgical NEC (11/50) and 24% mortality among those with IF (11/45).

Among NEC patients fewer than half of deaths (5/12; 42%) were BSI-attributable (death within 7 days of BSI); all BSI-attributable deaths were suffered by IF patients. Three quarters of the deaths (9/12) were BSI-associated (death within 28 days of BSI). The one death among patients with medically managed NEC was a BSI-associated death (died 24 days after BSI episode).
In infants not suffering from NEC nearly two-thirds of deaths (11/18; 61%) were attributable to BSI and more than three-quarters (14/18; 78%) of deaths were associated with BSI.

**i. Timing of Mortality in Relation to NEC**

Infants with NEC suffered 40% of the deaths in this cohort (12/30) (see Figure 5).

![Figure 5. Temporal Association of Mortality with NEC](image)

**Figure 5. Temporal Association of Mortality with NEC**

NEC Attributable Death: Death occurring 0-7 days after diagnosis of NEC
NEC Associated Death: Death occurring 0-28 days after diagnosis of NEC

Only one patient had a NEC-attributable death (death within 7 days of NEC diagnosis), this NEC-attributable death occurred in a patient with IF. Two-thirds of those who died with NEC (8/12) had NEC-associated deaths (died within a month of NEC diagnosis). Among those NEC patients who died, mean survival after NEC diagnosis was 42 days.
j. Mortality for all neonates with and without IF

Thirty neonates in this study died during NICU stay (17%) (see Table 7).

<table>
<thead>
<tr>
<th>All-Cause Mortality for Patients with and without IF</th>
<th>IF Patients</th>
<th>Patients without IF</th>
<th>IF vs. No IF</th>
<th>Adjusted Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients (n=177)</td>
<td>IF Patients (n=45)</td>
<td>Patients without IF (n=132)</td>
<td>IF vs. No IF OR (95%CI)</td>
<td>Adjusted Comparison IF vs. No IF aOR (95%CI)*</td>
</tr>
<tr>
<td>mortality</td>
<td>17% (30)</td>
<td>24% (11)</td>
<td>14% (19)</td>
<td>1.9 (0.8, 4.4) p = 0.13</td>
</tr>
</tbody>
</table>

% (no.of patients)

OR/aOR calculated by bivariable/multivariable logistic regression analysis

*Associations with p ≤ 0.05

*Died during NICU stay

*Adjusted model accounts for: gestational age, birth weight and gender

Patients with IF had higher mortality than patients without IF (24% vs. 14%). However, this difference was not statistically significant. Differences in mortality between patients with and without IF remained insignificant when adjusted for gestational age, birth weight and gender.

k. Mortality for NEC patients with and without IF

Among patients with NEC, IF patients had a significantly higher odds of death (24% vs. 3% mortality; OR 9.1, 95% CI 1.1, 74.5) (see Table 8).

<table>
<thead>
<tr>
<th>All-Cause Mortality for Patients with and without IF</th>
<th>IF Patients</th>
<th>NEC Patients without IF</th>
<th>IF vs. No IF OR (95%CI)</th>
<th>Adjusted Comparison IF vs. No IF aOR (95%CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All NEC Patients (N=74)</td>
<td>IF Patients (n=45)</td>
<td>NEC Patients without IF (n=29)</td>
<td>IF vs. No IF OR (95%CI)</td>
<td>Adjusted Comparison IF vs. No IF aOR (95%CI)*</td>
</tr>
<tr>
<td>mortality*</td>
<td>16% (12)</td>
<td>24% (11)</td>
<td>3% (1)</td>
<td>9.1 (1.1, 74.5)* p = 0.04</td>
</tr>
</tbody>
</table>

% (no. of patients)

OR/aOR calculated by bivariable/multivariable logistic regression analysis

*Associations with p ≤ 0.05

*Died during NICU stay

*Adjusted model accounts for: gestational age, birth weight and gender

This difference remained significant when adjusting for gestational age, birth weight and gender.
1. Results for hypothesis one: NEC-associated BSI in IF patients

- **Hypothesis 1a.** Compared to BSI in other VLBW neonates, BSIs occurring during the onset of NEC in infants who go on to develop IF are more often due to gut bacteria, and/or more often polymicrobial.

Using an event-wise dataset, wherein each BSI event (n=234) represents a separate data point, I analyzed the pathogen types isolated from NEC-associated BSIs in IF patients “IF/NEC-associated BSI” (n=18) compared to those BSIs occurring in VLBW NICU patients without IF (n=163) (see Figure 6).
Three BSIs that were both NEC-associated and post-NEC were included as NEC-associated for this analysis. This analysis excluded 53 BSIs occurring in IF patients that were not NEC-associated (pre-NEC and post-NEC BSIs).

There were no significant differences in frequencies of pathogen types for IF/NEC-associated BSIs vs. BSIs in non-IF patients (see Table 9).

| Table 9. Frequency of Pathogen Types NEC-Associated BSIs* Among IF Patients vs. BSIs Among Other VLBW Patients 181 LO-BSIs in 149 VLBW NICU Patients |
|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| BSI Type                                   | NEC-Associated BSIs in IF Patients (n=18)   | BSIs in Other VLBW Patients (n=163)         |
|                                            |                                            |                                              |
| Gram-Negative                              | 28% (5)                                    | 21% (35)                                    |
|                                            | 1.4 (0.5, 4.2)                             | p = 0.54                                    |
| Gram-Positive                              | 72% (13)                                   | 77% (125)                                   |
|                                            | 0.8 (0.3, 2.4)                             | p = 0.67                                    |
| Polymicrobial                               | 28% (5)                                    | 13% (22)                                    |
|                                            | 2.5 (0.8, 7.6)                             | p = 0.12                                    |
| Gut Pathogens                              | 39% (7)                                    | 23% (38)                                    |
|                                            | 2.1 (0.8, 5.8)                             | p = 0.15                                    |

*Associations with p ≤ 0.05
Odds ratios calculated using logistic regression analysis.
Analysis done among NEC-associated BSIs for IF patients and all BSIs for non-IF patients: excludes 53 pre and post-NEC BSIs occurring in 28 IF patients.
Three BSIs that were both NEC-associated and post-NEC were included as NEC-associated infections for this analysis.
Gram Negative - Gram negative bacteria cultured during a monomicrobial or polymicrobial BSI event.
Gram Positive - Gram positive bacteria cultured during a monomicrobial or polymicrobial BSI event.
Polymicrobial - BSI events with multiple pathogens cultured.
Gut Pathogens - One or more gut pathogens (Gram Negatives, Enterococcus and Lactobacillus) cultured during a BSI event that was monomicrobial or polymicrobial consisting of only gut pathogens.

Gram-negative pathogens were more common in IF/NEC-associated BSIs vs. BSIs in non-IF patients (28% vs. 21%). Gram-positive infections were slightly less common in
IF/NEC-associated BSIs (72% vs. 77%). Polymicrobial infections were more common in IF/NEC associated BSIs (28% vs. 13%). Infections with gut pathogens were also more common in IF/NEC associated BSI (39% vs. 23%).

- **Hypothesis 1b.** *Compared to other VLBW neonates, infants who suffer from BSI during the onset of NEC and go on to develop IF have higher mortality.*

Patients with IF/NEC-associated BSI had significantly higher mortality compared to other patients (35% vs. 15%; OR 3.1, 95% CI 1.0 – 9.2) (see Table 10).

<table>
<thead>
<tr>
<th>Table 10. All-Cause Mortality IF Patients with NEC-Associated BSI vs. Other VLBW Patients in 177 VLBW NICU Patients with LO-BSI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Patients</strong></td>
</tr>
<tr>
<td>(N=177)</td>
</tr>
<tr>
<td>Mortality*</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

% (no. of patients)
OR/aOR calculated by bivariable/multivariable logistic regression analysis
*Associations with p ≤ 0.05
*Died during NICU stay
*Adjusted model accounts for: gestational age, birth weight and gender

When adjusted for gestational age, birth weight and gender, odds of death for patients with IF/NEC-associated BSI remained significantly higher than for other VLBW NICU patients (OR 3.9, 95% CI 1.2 – 11.9).
m. Results for hypothesis two: post-NEC BSI in IF patients

- **Hypothesis 2a.** Compared to BSI in other VLBW neonates, post-NEC BSIs in patients suffering from IF are more often caused by gut bacteria, and/or more often polymicrobial.

Using an event-wise dataset, wherein each BSI event (n=234) represented a separate data point, I analyzed the pathogen types isolated from post-NEC BSIs in IF patients “IF/post-NEC BSI” (n=50) compared to those BSIs occurring in VLBW NICU patients without IF (n=163) (see Figure 7).

![Figure 7. BSIs Compared for Post-NEC in IF vs. BSIs in Non-IF Patients](image)

Pre-Nec: BSI > 72 hours prior to NEC diagnosis
NEC-associated: BSI within 72 hours of NEC diagnosis
Post-NEC: BSI >72 hours after NEC diagnosis

Three BSIs that were both NEC-associated and post-NEC were included as post-NEC for this analysis.
Analysis excludes 21 pre-NEC and NEC-associated BSIs occurring in IF patients.
Three BSIs that were both NEC-associated and post-NEC were included as post-NEC for this analysis. This analysis excluded 21 BSIs occurring in IF patients that were not post-NEC (pre-NEC and NEC-associated BSIs).

Gram-negative pathogens were twice as common in IF/post-NEC BSIs vs. BSIs in non-IF patients (40% vs. 21%; OR 2.4, 95% CI 1.2 - 4.8) (see Table 11).

### Table 11. Frequency of Pathogen Types

<table>
<thead>
<tr>
<th>BSI Type</th>
<th>Post-NEC BSIs in IF Patients (n=50)</th>
<th>BSIs in Other VLBW Patients (n=163)</th>
<th>Post-NEC BSI in IF Patients vs. BSIs in Other VLBW Patients OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-Negative</td>
<td>40% (20)</td>
<td>21% (35)</td>
<td>2.4 (1.2, 4.8)* p = 0.01</td>
</tr>
<tr>
<td>Gram-Positive</td>
<td>58% (29)</td>
<td>77% (125)</td>
<td>0.4 (0.2, 0.8)* p = 0.01</td>
</tr>
<tr>
<td>Polymicrobial</td>
<td>15% (7)</td>
<td>13% (22)</td>
<td>1.0 (0.4, 2.6) p = 0.93</td>
</tr>
<tr>
<td>Gut Pathogens</td>
<td>48% (24)</td>
<td>23% (38)</td>
<td>3.0 (1.6, 5.9)* p &lt; 0.00</td>
</tr>
</tbody>
</table>

% (no. of BSI events)

*BSI >72 hours after NEC diagnosis

*Odds ratios calculated using logistic regression analysis.

Analysis done among post-NEC BSIs for IF patients and all BSIs for non-IF patients: excludes 21 pre-NEC and NEC-associated BSIs occurring in 15 IF patients.

Three BSIs that were both NEC-associated and post-NEC were included as post-NEC infections for this analysis.

Gram Negative - Gram negative bacteria cultured during a monomicrobial or polymicrobial BSI event.

Gram Positive - Gram positive bacteria cultured during a monomicrobial or polymicrobial BSI event.

Polymicrobial - BSI events with multiple pathogens cultured.

Gut Pathogens - One or more gut pathogens (Gram Negatives, Enterococcus and Lactobacillus) cultured during a BSI event that was monomicrobial or polymicrobial consisting of only gut pathogens.

Gram-positive infections were less common among IF/post-NEC BSIs (58% vs. 77%; OR 0.4, 95% CI 0.2 - 0.8). Polymicrobial infections were similarly uncommon in IF/post-
NEC BSIs and BSIs in non-IF patients (15% vs. 13%; OR 1.0, 95% CI 0.4 – 2.6).

Infections with gut pathogens were twice as common in IF/post-NEC BSIs (48% vs. 23%; OR 3.0, 95% CI 1.6 – 5.9).

- **Hypothesis 2b.** Compared to other VLBW neonates, infants with IF who suffer from post-NEC BSI have higher mortality.

Patients with IF/post-NEC BSI had similar mortality to other patients (17% for both groups) (see Table 12).

<table>
<thead>
<tr>
<th>Table 12.</th>
<th>All-Cause Mortality</th>
<th>IF Patients with Post-NEC BSI vs. Other VLBW Patients in 177 VLBW NICU Patients with LO-BSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Patients</td>
<td>Patients with IF and Post-NEC BSI</td>
</tr>
<tr>
<td></td>
<td>(N=177)</td>
<td>(n=30)</td>
</tr>
<tr>
<td>Mortality*</td>
<td>17% (30)</td>
<td>17% (5)</td>
</tr>
</tbody>
</table>

% (no. of patients)
OR/aOR calculated by bivariable/multivariable logistic regression analysis
*Associations with p ≤ 0.05
*Died during NICU stay
*Adjusted model accounts for: gestational age, birth weight and gender

Adjusting for gestational age, birth weight and gender did not change the odds of death for patients with IF/post-NEC BSI vs. other patients (OR 1.0, 95% CI 0.3 – 2.8).
CHAPTER 5. DISCUSSION

Advances in prevention and treatment are needed to reduce the disease burden of NEC. This disease threatens significant morbidity and mortality for individual patients, and is a significant financial burden to the healthcare system. To improve our clinical approach to the neonate with NEC we must develop a better understanding of this disease. My study explored the microbial profile and timing of BSIs associated with IF in an attempt to better understand the natural history of IF. Few prior studies have stratified NEC according to IF.\textsuperscript{2, 53} Data from this study suggest that the IF population suffers from a prolonged state of intestinal inflammation leading to repeated blood infections that differ from infections in other NICU babies. The microbiology of the infections suffered by IF patients also suggests that NEC may be a chronic condition requiring treatment for weeks and months beyond the initial acute onset. Additionally, recognizing IF as a unique subpopulation of NEC may help clinicians to choose more appropriate empiric antimicrobial therapy for these patients.

This study found that IF was very common among NEC patients treated surgically (90%), and that IF patients were at high risk for recurrent BSI (44% vs. 20%, \( p = 0.01 \)). IF patients frequently had BSI with gut pathogens, both at the time of NEC diagnosis (39%), and after NEC diagnosis (48%). IF patients appeared to suffer a high mortality rate (24%), and an even higher mortality rate (35%) when acute NEC was accompanied by BSI. I found significantly higher odds of death (OR 9.1) for patients with IF when I restricted my analysis to infants suffering from NEC (24% vs. 3% mortality, \( p = 0.04 \)). Restricting this analysis to the NEC group for the mortality analysis allowed for the assessment of deaths that were more likely to be related to NEC. The analysis within the
entire VLBW cohort included a greater proportion of fatalities related to other conditions such as cardiovascular and pulmonary dysfunction.

Consistent with previous studies, these findings illustrate that suffering from acute NEC concurrently with BSI puts neonates at great risk of death.\(^2\) By analyzing IF patients as a separate population I have revealed a post-NEC BSI pathogen profile that prior studies have failed to capture.\(^19\) My results suggest that the IF population suffers from ongoing intestinal permeability long after the acute onset of NEC, leading to more frequent BSI and BSIs that are microbiologically different from BSIs in other VLBW infants.

This study revealed the interesting finding that patients with NEC who went on to develop IF had a later onset of disease. IF patients were diagnosed with NEC at an older age than other NEC patients (25 days of life vs. 18 days of life, \(P = 0.05\)), and IF patients had their first BSI at an older age (53 days of life vs. 34 days of life, \(P = 0.01\)). Additionally IF patients were less likely to have a CL at the time that the first BSI was diagnosed (71% vs. 86%, \(P = 0.04\)), possibly indicating that clinicians did not conclude that these infants were sick enough to require a CL at that time. These findings may signify either a later onset or a later recognition of disease. These findings may even suggest a different pathophysiology of NEC; a more prolonged subclinical gut inflammatory process may be taking place in IF patients. If patients suffer from unrecognized subclinical NEC for days prior to formal diagnosis, late initiation of treatment could account for their more severe clinical course.

Recognition of a phenomenon of subclinical NEC may lead to implementation of monitoring for gut inflammation. Better diagnostic techniques, beyond the modified Bell
criteria, will be necessary for early recognition of subclinical NEC; a sensitive and specific biomarker is needed to provide a definitive early diagnosis for this devastating disease before radiologic changes can be recognized. Stool calprotectin is currently being evaluated as a maker of NEC and in the future may prove useful in monitoring for intestinal inflammation in asymptomatic VLBW neonates.54

- **Hypothesis 1a.** Compared to BSI in other VLBW neonates, BSIs occurring during the onset of NEC in infants who go on to develop IF are more often due to gut bacteria, and/or more often polymicrobial.

The results of this study do not support the hypothesis that NEC-associated BSIs in IF patients have a different pathogen profile from BSIs in other NICU patients. I expected to find a higher frequency of gram-negative, gut bacteria, and polymicrobial BSIs in NEC-associated infections among IF patients. This hypothesis was based on previous research demonstrating a high frequency of gram-negative BSI in NEC-associated infection.2 I presume that at the time of acute NEC onset (within 72 hours of diagnosis) bacteria can more easily translocate across the compromised intestinal barrier and invade the blood. Since IF patients suffer the most devastating intestinal sequelae of NEC, they most likely to also suffer from severe intestinal compromise at the time of NEC onset, and thus contract gut bacteria BSIs more frequently. However, I found no significant differences in the frequencies of gram-positive, gram-negative, polymicrobial or gut pathogen BSIs between these groups. Surprisingly, NEC-associated infections were most frequently due to gram-positive pathogens (72% of 18 BSIs). Because many gram-positive species are colonizers of the skin, I would have expected more gram-positive BSIs in those babies.
developing infections due to the use of invasive medical equipment (intravenous lines/endotracheal tubes: risk factors shared by most NICU patients) and more gut pathogen BSIs in those babies with severe intestinal pathology.

In support of my hypothesis, gut pathogens were common among NEC-associated BSIs for IF patients and there was a trend towards a higher frequency of gut pathogens in NEC-associated BSIs for IF patients (39% vs. 23% of infections in other VLBW patients). This difference was not statistically significant, possibly related to the small sample size for NEC-associated infections. Some of these infections were also included in the gram-positive analysis because the Enterococcus spp are both gram-positive and gut bacteria. Although I believe the trend towards a larger number of gut pathogen BSIs would have proven significant in a larger study, I am not able to say with certainty that there is a difference between these infections and infections in other VLBW NICU patients.

• **Hypothesis 1b.** *Compared to other VLBW neonates, infants who suffer from BSI during the onset of NEC and go on to develop IF have higher mortality.*

In my study many IF patients with NEC-associated BSI died (35% of 17 patients), and the odds of dying were approximately 3 times greater among these infants compared to other VLBW babies. Adjusting for potential confounding factors (gestational age, birth weight and gender) did not significantly change the odds of death in this patient sample. Infants who suffered the double insult of severe NEC (IF) and concurrent BSI may have had high mortality due to the NEC/BSI combination itself, or may have been generally sicker and more prone to infection, thus more likely to die from any number of
comorbidities. The relationship between NEC-associated infection and death warrants the attention of clinicians caring for these babies. Recognizing the fragility of patients suffering from concurrent NEC and BSI is the first step towards developing strategies to reduce this high mortality rate.

- **Hypothesis 2a.** Compared to BSI in other VLBW neonates, post-NEC BSIs in patients suffering from IF are more often caused by gut bacteria, and/or more often polymicrobial.

This study showed significant differences in pathogens causing post-NEC BSI in IF patients compared to BSI in other VLBW NICU patients. Post-NEC BSI in IF patients appeared to be more commonly due to gut pathogens and gram-negative bacteria than BSI in other VLBW NICU patients. This result suggests that BSIs affecting IF patients after the acute phase of NEC has subsided (more than 3 days after NEC onset) are not simply nosocomial infections due to risk factors associated with hospitalization (intravenous lines/endotracheal tubes), but are fundamentally different and related to intestinal pathology. These results support the conjecture that NEC in these patients is an ongoing inflammatory disease that compromises intestinal integrity for an extended period of time, and may continue to put infants at risk of blood infection for weeks or months after the acute NEC episode.

Due to the diversity of intestinal flora, I hypothesized that BSIs arising from bacterial translocation across a compromised intestinal barrier would commonly be polymicrobial. My findings do not support this hypothesis; polymicrobial BSIs were uncommon in both groups analyzed (15% and 13%). Although BSIs in IF patients are not more frequently
polymicrobial, the higher odds of infection with gut and gram negative bacteria clearly point towards an intestinal source for post-NEC BSI in IF patients.

- **Hypothesis 2b.** *Compared to other VLBW neonates, infants with IF who suffer from post-NEC BSI have higher mortality.*

  I did not find mortality differences in IF patients with post-NEC BSI. Because I expected these patients to suffer from more virulent blood infections (gut pathogen infections), I also expected that these patients would die more frequently than other VLBW NICU patients. I found that VLBW NICU patients with BSI suffer a high mortality burden (17%) regardless of severity of intestinal pathology and regardless of BSI type. Although IF patients may suffer repeated BSIs in the NICU with pathogens of gut origin, the risk of death in these patients is similar to the risk of death for all VLBW NICU patients with BSI.

  This study was designed as a follow-up to a 2012 study by Cole *et al.* titled *Bloodstream Infections in Very Low Birth weight Infants with Intestinal Failure* and a 2014 study by Bizzarro *et al.* titled *Concurrent Bloodstream Infections in Infants with Necrotizing Enterocolitis.* Cole explored the pathogens and characteristics of LO-BSI in infants with IF. Bizzarro explored the pathogens and characteristics of NEC-associated BSIs. My study combined both of these concepts and explored NEC-associated and post-NEC BSIs in neonates with and without IF.

  Cole found in a 3-year retrospective multicenter study that BSIs in IF patients were frequently due to gram-positive pathogens; of 104 BSIs in patients with IF, 28% were
gram-negative and 55% were gram positive.\textsuperscript{19} My study revealed similar frequencies of monomicrobial gram-negative and gram-positive BSIs in IF patients (31\% and 59\% of BSIs respectively). The high frequency of gram-positive BSI in IF patients may have been due to the risk posed by invasive medical equipment (CLs and endotracheal tubes), or potentially these gram-positive infections were of intestinal origin. Coagulase negative \textit{staphylococci} (CoNS), a very common group of gram-positive pathogens, have been shown to colonize the intestines of hospitalized neonates as young as 3 days old,\textsuperscript{55} thus CoNS BSIs may be due to intestinal bacterial translocation in infants with NEC. Unfortunately, clinical laboratories cannot distinguish between CoNS of skin vs. intestinal origin, so therefore it is uncertain whether observed CoNS BSIs were due to skin bacteria.

Cole also reported that NEC-associated BSIs were common among patients with IF; 45\% of 78 IF patients suffered from BSI within 1 week of NEC diagnosis. I found a slightly lower frequency of BSI at the time of NEC diagnosis (38\% of 45 IF patients). Cole also found that post-NEC BSIs were common in IF; 52\% of IF patients suffered post-NEC BSI. Analyses of data from my study revealed a higher frequency of post-NEC BSI in this population (74\%). Differences in our findings may be due to differences in the definition of NEC-associated BSI; my analysis only included those BSIs within 3 days of onset of NEC while Cole considered BSI within 7 days of NEC diagnosis to be NEC-associated. My study reveals that IF patients frequently suffer from post-NEC BSI.

Additionally, Cole found that IF patients rarely suffer recurrent post-NEC BSI; 18\% of 78 IF patients and only 5\% of 402 non-surgical NEC patients. I found many more recurrent BSIs in patients with and without IF and a significant difference in BSI
recurrence in IF vs. non-IF cases (44% vs. 20% respectively, p = 0.01). The discrepancy between Cole and my findings may be partially attributable to my analysis including all cases of \( \geq 2 \) BSIs while Cole includes only cases with \( \geq 2 \) post-NEC BSIs.

My findings are largely consistent with Cole’s; both studies demonstrate that IF patients are a fragile patient population frequently suffering from NEC-associated BSI, and post-NEC BSI. Additionally, both studies show that gram-positive BSIs are the most common BSI type affecting IF patients. Unfortunately we are unable to say what portion of these gram-positive BSIs are in fact due to intestinal translocation vs. iatrogenic risks from CLs and endotracheal tubes.

In a 20-year retrospective single center study published in 2014, Bizzarro found that, of 57 monomicrobial NEC-associated BSIs, 67% were due to gram-negative and 30% were due to gram-positive pathogens. They theorized that the high prevalence of gram-negative infection at NEC onset was due to translocation of gut bacteria across the intestinal wall and dissemination into the blood during this time of acute intestinal compromise. Compared to Bizzarro, my study found far fewer gram-negatives among NEC-associated infections; only 25% of 24 NEC-associated BSIs, (28% of 18 NEC-associated BSIs in IF patients). This discrepancy may be due to differences in intestinal bacterial colonization between neonates at CHOC vs. Yale-New Haven Children’s Hospital in Connecticut, where Bizzarro’s cohort of patients were hospitalized. Early exposure to broad-spectrum antibiotics, use of human milk and human milk fortified formulas for enteric feeds, and differing NICU bacterial colonization patterns are external factors that may have differed between these two centers and may have altered the
intestinal microbiome of NICU infants. Furthermore, Bizzarro’s study spans 20 years, while my study examined a more recent population of premature infants. Our populations may have been significantly different due to changes in survival of premature neonates between the two study periods. Because acute NEC alters intestinal permeability, I expect NEC-associated BSIs to frequently be of gut bacteria origin. Bizzarro’s findings suggest that the majority of NEC-associated BSIs may originate in the gut. The NEC-associated BSIs in my study may also have originated in the gut, if the two cohorts of infants in these studies had different intestinal microbial profiles.

*Enterococcus spp* are important gram-positive gut pathogens that may also cause BSI in the setting of a compromised intestinal barrier. Classification of BSIs as gut pathogen infections rather than simply gram-negative infections allowed me to include *Enterococcus spp* in my analysis. My study cohort had a higher proportion of gut-pathogen BSI than gram-negative BSI. Gut pathogen BSIs made up 38% of 24 NEC-associated BSIs, (39% of 18 NEC-associated BSIs in IF patients). The difference in frequency of gram-negative BSI and gut pathogen BSI for these NEC-associated infections illustrates the importance of analyzing gut pathogens as a separate category. Bizzarro’s study did not analyze gut microbes as a separate category of NEC-associated infection. It is possible that such an analysis may have found an even higher prevalence of BSI of intestinal origin.

Bizzarro found that, of 69 monomicrobial post-NEC BSIs, 32% were gram-negative, 58% were gram-positive, and similar frequencies of gram-negative and gram-positive BSI were observed among VLBW NICU patients not suffering from NEC.
Those researchers argued that post-NEC BSIs are no different from BSIs suffered by VLBW NICU patients without NEC, and are generally due to the use of CLs and endotracheal tubes. I found that, for 50 post-NEC BSIs in IF patients, there were significant differences in pathogens compared to other VLBW patients: 40% of IF/post-NEC BSIs were gram-negative vs. 21% of other BSIs (p = 0.01), and 58% of IF/post-NEC BSIs were gram-positive vs. 77% of other BSIs (p = 0.01). My analysis differed from that of Bizzarro in two important ways: First, I included both monomicrobial and polymicrobial BSI. Second, I performed my analysis on the subset of NEC patients suffering from IF. I chose to analyze post-NEC BSI in IF patients because I hypothesized that these patients suffered from a prolonged chronic course of gut inflammation leaving them vulnerable to repeated bouts of BSI due to translocation of bacteria across a compromised intestinal wall. My results provide evidence in favor of a different pathogen profile in post-NEC infections among IF patients and suggest a chronic intestinal permeability in those NEC patients suffering from IF.

Prior studies have suggested that post-NEC infections are similar to infections suffered by all infants with CLs; thus when infants present with signs of post-NEC BSI many clinicians expect to find a CLABSI. The treatment for CLABSI is removal and replacement of the infected CL. IF patients are reliant on CLs for nutrition, and many of these lines must be placed surgically. Removing a CL in an IF patient exposes these delicate infants to the risks associated with surgery and subjects them to a period of time without needed PN. My study revealed a pathogen profile for post-NEC BSI in IF patients not revealed in prior studies suggesting that post-NEC infections in IF patients
frequently originate from the gut. Clinicians should take into account this possible source for BSI, and avoid removing CLs without clear evidence that a CL is the source of infection.

NEC has previously been thought of as an acute and rapidly progressing illness, doing most of its damage during the first week of illness. My results suggest that in a subset of patients (IF patients), NEC may cause chronic intestinal inflammation. I hope the understanding of NEC as an intestinal chronic inflammatory condition will inform future research into the pathogenesis of this disease.

Multiple studies have explored genetic markers to predict predisposition to NEC as well as biochemical markers to make early diagnosis of infants suffering from NEC. These studies have so far been unsuccessful in finding reliable genetic or biochemical markers. I have shown that the subgroup of NEC patients with IF suffer a prolonged inflammatory process with unique long-term intestinal consequences. It is also plausible that IF patients may have a unique genetic predisposition to NEC with IF, or have a biomarker that would allow clinicians to predict a risk of IF and target preventive strategies and early treatments appropriately. Future research in NEC genetics and biomarkers should include analyses of infants going on to suffer IF.

**Limitations**

My definition of IF was adapted from the definition used by Cole in their 2011 study of BSIs in VLBW infants with IF. My IF definition differed from Cole’s in my inclusion of infants who had surgery for NEC and died on PN prior to completing six weeks of PN therapy. These patients had surgery, were started on PN and, if given the
opportunity, they may have continued on PN for the requisite six weeks. I believe that the exclusion of these neonates from the IF group led Cole to miss an important portion of IF patients. In my study, of 11 deaths in the IF group, six deaths were among infants who had not yet completed six weeks of PN therapy. The length of PN therapy completed by these infants ranged from 9 days to 40 days with a mean of 22 days. The decision to classify these infants as suffering from IF may have skewed my findings towards greater mortality in the IF group. This decision was supported clinically and has helped to reveal important differences between infants with and without IF.

This work was subject to the inherent limitations of a retrospective study. The database from which I drew my sample included only patients with BSI; I could not make comparisons to patients without BSI. The physicians caring for these patients did not use a consistent diagnostic algorithm for diagnosing NEC (such as the Bell criteria). To determine date of NEC onset, I relied on clinician diagnosis expressed in written notes, treatments initiated, radiographic evidence and pathological findings. My method of determining date of NEC onset may be less precise than if the clinician had provided a diagnosis at the time of onset using standardized criteria. I did not have access to follow-up data beyond the period of initial hospitalization. Upon discharge from CHOC some patients were transferred to other NICUs and thus had longer NICU stays than I was able to capture. These transferred patients may have experienced BSIs or deaths that I was unable to capture in this study. The medical record from which I collected data did not clearly record the timing and type of enteric feeds, so I was unable to assess the impact of human milk feeding on BSI, IF or mortality. I did not collect data on length of intestinal
resection, which could be useful as a potential explanation for progression from surgical NEC to IF.

This was a single center study and not necessarily generalizable to other sites that may have different NICU bacterial colonization patterns, and may serve patients with different risk of IF due to differing socioeconomic and genetic backgrounds. Because NEC is a rare disease and this data was collected at a single center, my sample size was small. The small sample size particularly impacted my ability to explore the pathogen profile of NEC-associated BSI. Future studies of these infections should draw from large multicenter databases to achieve the power required for exploration of these rare infections.

Finally, because blood cultures were analyzed in a clinical rather than research laboratory, they did not include analysis of molecular markers that would allow determination of which *staphylococcal* BSIs are of gut origin.\(^{57}\) Future works should include analyses of bacterial molecular markers in blood and stool to determine which BSIs are truly of gut origin.

**Conclusion**

This study indicated that neonates with IF are at risk for multiple post-NEC BSIs of gut origin. This finding suggests that NEC in these infants is a chronic intestinal inflammatory condition. Although many of these infants are also at risk for CLABSI, due to reliance on PN delivered through a CL, clinicians should have a high index of suspicion for BSI of gut origin in these infants. Understanding the pathogen profile of BSIs in these patients will help clinicians choose appropriate empiric antimicrobial
therapy, and avoid exposing these infants to the risks associated with replacing CLs in the absence of definitive evidence of CLABSI.

Additionally, I have shown that IF patients with NEC-associated BSI are at increased risk of death (35%) above and beyond an already elevated risk of death for VLBW NICU patients with BSI (17%). The double insult of severe intestinal pathology (NEC/IF) and concurrent BSI potentially contributes to this high mortality burden. Such a high mortality rate warrants the attention of scientists and clinicians alike. Genetic and biochemical markers are needed to help clinicians identify these cases prior to the onset of signs and symptoms of NEC. Future studies may have greater success elucidating such biomarkers by exploring genetic and biochemical markers among IF patients, who suffer the most severe and chronic consequences of this devastating disease of prematurity.
REFERENCES


### APPENDIX A.

#### Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BSI</td>
<td>Bloodstream infection</td>
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<tr>
<td>CHOC</td>
<td>Children’s Hospital of Orange County</td>
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<tr>
<td>CL</td>
<td>Central line (intravenous)</td>
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<tr>
<td>CoNS</td>
<td>Coagulase negative Staphylococci</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>IF</td>
<td>Intestinal failure</td>
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<tr>
<td>IRB</td>
<td>Internal Review Board</td>
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<tr>
<td>LO-BSI</td>
<td>Late-onset bloodstream infection</td>
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<tr>
<td>NEC</td>
<td>Necrotizing enterocolitis</td>
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<tr>
<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
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<tr>
<td>PN</td>
<td>Parenteral nutrition</td>
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<tr>
<td>SIP</td>
<td>Spontaneous intestinal perforation</td>
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<tr>
<td>VLBW</td>
<td>Very low birth weight</td>
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