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Terri Marin Date

Mesenteric Perfusion Pattern Changes as the
Result of Packed
Red Blood Cell Transfusions
in Preterm Infants

By

Terri Marin
Doctor of Philosophy

Nursing

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Mesenteric Perfusion Pattern Changes as the Result of Packed Red Blood Cell Transfusion in Preterm Infants

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An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in

Nursing

2012

Abstract

Mesenteric Perfusion Pattern Changes as the Result of

Packed Red Blood Cell Transfusion in Preterm Infants

By Terri Marin

Necrotizing enterocolitis is the most serious gastrointestinal emergency encountered by very low birth weight (VLBW) infants. Approximately half of the 4500 preterm infants affected annually require surgical intervention, with associated mortality rates of 30%-50%. Extensive research has determined that NEC pathogenesis is most likely multifactorial; however, prematurity is the only definitive predictor. Clear predictive and prevention strategies for this disease remain unknown and its incidence unchanged.

Recent evidence demonstrates a temporal relationship between packed red blood cell (PRBC) administration and NEC development. Although the underlying pathophysiology of this occurrence is unknown, leading theories suggest gastrointestinal immaturity and the age of blood infused may substantially increase the risk for transfusion-related NEC. Therefore, perfusion alterations as a result of changing blood flow subsequent to transfusion and the age of blood administered may increase the risk for ischemic insult.

This observational, prospective study endeavored to identify changes in mesenteric tissue perfusion by monitoring differential tissue oxygenation using near-infrared spectroscopy in preterm infants receiving blood transfusions. In addition, the relationship between the age of blood infused and perfusion pattern alteration was observed.

Thirty-three transfusion events were observed. It was concluded that the most immature infants demonstrated lower mesenteric perfusion following PRBC administration. The administration of PRBCs greater than six days old was also associated with decreased mesenteric perfusion. Four infants developed NEC temporally associated with PRBC transfusions, occurring within 48 hours of blood infusion. Infants who developed transfusion-related NEC were gestationally younger, more likely to have received enteral feedings during the transfusion, received larger volumes of feedings and received greater volumes of blood than infants who did not develop transfusion-related NEC.

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Table of Contents

List of Tables

List of Figures

Chapter I

Introduction

NEC is a devastating disease of prematurity characterized by bowel inflammation, ischemia and necrosis (Petrosyan, et al., 2009). Of the 4500 VLBW infants affected annually, approximately 48% require surgery, and 30-50% will not survive (Holman, et al., 2006; Stoll, et al., 2010). NEC survivors face long-term morbidities often associated with debilitation, neurologic and sensory impairment, and feeding difficulties requiring subsequent surgical intervention (Hintz, et al., 2005; Martin, et al., 2010; Pike, et al., 2012; Sharma et al., 2006; Stoll, et al., 2004; Vohr, et al., 2000). Hospital costs associated with this disease may exceed $5 billion annually in the United States (Bisquera, et al., 2002). Without sufficient prediction and prevention strategies to reduce the incidence of NEC, the associated economic burden will undoubtedly continue to rise.

Statement of the Problem

Recent studies have established an association between packed red blood cell (PRBC transfusions and NEC onset (Christensen et al, 2010; Josephson et al, 2010; Mohamed et al., 2012). Scientific evidence demonstrates that transfusion-related NEC is an authentic pathogenic entity among the preterm population with a greater incidence in VLBW infants < 1500 grams and unknown causality (Blau, et al., 2011; Christensen, Wiedmeier, et al., 2010; El-Dib, et al., 2011; Ghirardello, et al., 2011; Josephson, et al., 2010; Mally, et al., 2006; McGrady, et al., 1987; Paul, et al., 2011; Singh, et al., 2011). The impact of enteral feedings during and following PRBC administration on mesenteric perfusion patterns and tissue oxygenation is unknown. Research has suggested that PRBC storage length may increase the risk for microcirculation impairment due to cellular degradation, loss of deformability, intracellular constituent loss and nitric oxide depletion (Gladwin, Crawford, & Patel, 2004; Gladwin & Kim-Shapiro, 2009; Kim-Shapiro, et

al., 2011). It is not clear if red blood cell transfusions themselves or the age of the red cells being infused may alter mesenteric perfusion in the preterm infant. Currently, there are no established guidelines for transfusion procedures for VLBW infants and practice remains physician and/or institution specific (Kasat, Hendricks-Muoz, & Mally, 2011; Strauss, 2010). Further examination of the effect on mesenteric perfusion patterns and tissue bed oxygenation before, during and subsequent to PRBC transfusions and enteral feedings will address these specific gaps in scientific knowledge and further our understanding related to the pathophysiology and risk factors inherent to this disease process.

Purpose of the Study

The purpose of this study was to examine mesenteric tissue oxygenation patterns using near infrared spectroscopy (NIRS) in preterm infants < 37 weeks gestational age receiving packed red blood cell transfusions, with and without concurrent enteral feedings. NIRS measures differential oxygen uptake in tissue beds, reflecting perfusion status and preferential blood shunting during periods of hemodynamic instability (Marin & Moore, 2011; Reber, Nankervis, & Nowicki, 2002). Continuous NIRS measurements of cerebral, mesenteric and renal beds before, during and subsequent to PRBC transfusions allowed direct evaluation of differential organ perfusion. Additionally, this study endeavored to identify other risk factors for transfusion-related NEC, including the temporal relationship between enteral feeding and transfusion event, medication administration, and influence of the age and quality of blood transfused. The specific aims and research questions undertaken in this observational, prospective investigation were:

Specific Aim 1: Evaluate cerebral and mesenteric tissue oxygenation patterns using near infrared spectroscopy (NIRS) rSO_2 measurements before, during and subsequent to packed red blood cell (PRBC) transfusions with and without feedings to identify variations in tissue oxygenation delivery in preterm infants < 37 weeks gestational age.

H1: Mesenteric tissue oxygenation patterns in preterm infants < 37 weeks gestational age will be negatively affected during and following PRBC transfusions.

H2: Preterm infants < 37 weeks gestation age who receive enteral feedings during a PRBC transfusion event will exhibit greater negative impact on mesenteric tissue oxygenation patterns than those not fed during a PRBC transfusion event.

RQ 1.1: What are the differences in cerebral and mesenteric tissue oxygenation patterns during and subsequent to PRBC administration in preterm infants who receive an enteral feeding during a transfusion event compared to those who do not receive a feeding during a transfusion event?

RQ 1.2: What temporal relationship exists following transfusions between altered tissue perfusion patterns and feedings?

Specific Aim 2: Examine the association between the age of blood transfused and changes in mesenteric tissue oxygenation patterns using NIRS rSO_2 measurements in preterm infants < 37 weeks gestational age receiving a PRBC transfusion.

RQ 2.1: What changes occur in mesenteric tissue oxygenation patterns (rSO_2) when preterm infants are transfused with PRBCs that have been stored for > 6 days compared to those that received PRBCs stored for ≤ 6 days, controlling for feeding status?

Specific Aim 3: Determine if mesenteric tissue oxygenation patterns differ in preterm infants who do and do not develop NEC following PRBC transfusions, with and without feedings and the temporal relationship between NEC onset, feedings and PRBC transfusion.

RQ 3.1: What mesenteric tissue oxygenation patterns are noted in preterm infants who develop NEC compared to those who do not following a PRBC blood transfusion when no feedings are received during the transfusion event?

RQ 3.2: What mesenteric tissue oxygenation patterns are noted in preterm infants who develop NEC compared to those who do not following a PRBC blood transfusion when feedings are given during the transfusion event?

RQ 3.3: For preterm infants who develop NEC following a transfusion, what is the temporal relationship between transfusion event and NEC onset?

Chapter II

Background and Significance

Introduction

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency encountered by premature very low birth weight (VLBW) infants in the United States, affecting approximately 4500 premature infants < 37 weeks corrected gestational age each year, with approximately half requiring surgery (Holman et al., 2006). NEC is characterized by bowel inflammation, ischemia and necrosis. Mortality rates are 30-50% among those requiring surgery, and those who survive are at significant risk for complicated life-long morbidities (Holman, et al., 2006). NEC typically occurs within the first six weeks of life with a higher incidence in VLBW infants ≤ 1500 grams (Neu, Mshvildadze, & Mai, 2008; Stoll et al., 2010). Costs associated with this disease are an estimated $5 billion annually attributed to prolonged hospitalizations, surgery and prolonged required therapeutics (Bisquera, Cooper, & Berseth, 2002). The pathogenesis of NEC is most likely multifactorial, but the specific processes involved still remain imprecise. Despite four decades of extensive research to determine specific associations and causal factors, prevention strategies remain elusive and its incidence unchanged, underscoring an enormous need for further research (Abdullah, 2008; Petrosyan, Guner, Williams, Grishin, & Ford, 2009).

The innate immune system in preterm infants may be inefficient to mount an appropriate response to invading pathogens (Strunk, Currie, Richmond, Simmer, & Burgner, 2011); therefore, any condition that increases the risk for enteric bacterial introduction or intestinal injury may increase the risk for NEC. Although multiple risk factors predisposing preterm infants to NEC have been identified, prematurity is the only uniform and clear predictor with risk inversely related to gestational age (Guthrie et al., 2003; Lin & Stoll, 2006). Known risk factors for NEC

include enteral feedings, packed red blood cell transfusions, congenital heart disease, bacterial sepsis and respiratory distress syndrome (Blau et al., 2011; Petrosyan, et al., 2009; Thompson et al., 2011). Morbidities associated with NEC survivors include poor neurodevelopment outcome, short bowel syndrome, feeding difficulties relating to bowel strictures, failure to thrive, sepsis, total parenteral nutrition cholestasis, and vision and/or hearing impairment often requiring long-term therapies (Hintz et al., 2005; Martin et al., 2010; Pike et al., 2012; Sharma et al., 2005; Stoll et al., 2004; Vohr et al., 2000).

Clinical Presentation of NEC

Clinical manifestations of NEC vary from subtle symptoms to overt fulminant sepsis. Therefore, a classification system, known as Bell's Staging Criteria for NEC (Bell et al., 1978; Walsh & Kliegman, 1986), is commonly used to diagnose an infant presenting with NEC symptoms (see figure 1). Some infants may present with non-specific symptoms such as increased apneic events, bradycardia, desaturation, feeding intolerance, abdominal distention and bloody stools without radiographic changes and may gradually demonstrate disease progression. These preterm infants are often difficult to diagnose and intestinal injury may occur insidiously prior to intervention. Others demonstrate sudden deterioration characterized by severe sepsis, radiographic evidence of pneumatosis and/or gastrointestinal perforation, ascites, cardiopulmonary decompensation and profound metabolic acidosis (Thompson & Bizzarro, 2008). Unfortunately, research has not established specific pathogenic causes relating to severity of NEC onset illustrating the need for further research.

Stage	Symptoms	Radiographic findings
IA Suspected	Gastric residuals, abdominal distention, emesis, heme-positive stool	Normal or intestinal dilation, mild ileus

IB Suspected	Same as above plus grossly bloody stool	Same as IA
IIA Definite, Mildly Ill	Same as above, plus absent bowel sounds with or without abdominal tenderness	Intestinal dilation, ileus, pneumatosis intestinalis
IIB Definite, Moderately Ill	Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass	Same as IIA plus ascites
IIIA Advanced, severely Ill, bowel intact	Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention	Same as IIA, plus ascites
IIIB Advanced, severely Ill, bowel perforation	Same as IIIA	Same as IIIA plus pneumoperitoneum

Figure 1-1. Bell's Staging Criteria for Necrotizing Enterocolitis. Adapted from Walsh& Kliegman, 1986. Reproduced with permission from ElsevierLimited, Kidlington, Oxford, United Kingdom.

Disease Management

Approximately 52% of preterm infants with NEC can be managed medically; however, 48% will require surgical intervention (Stoll, et al., 2010). Medical management consists of bowel rest, withholding feedings, gastric decompression and antimicrobial therapy. Some infants develop hemodynamic instability and may require intravenous vasopressor support. Surgical intervention may be required in those with severe onset or disease progression (Thompson & Bizzarro, 2008). Exploratory laparotomy, bowel resection for necrosis and ostomy formation are general surgical procedures required for Bell's stage III and greater. Peritoneal drain placement may precede surgical laparotomy, but the benefits of this approach remain controversial, as

studies have shown increased mortality rates in preterm infants subjected to delayed laparotomy (Blakely et al., 2006; Sola, Tepas Iii, & Koniaris, 2010). Therefore, the decision to perform surgery remains case-specific influenced by disease severity and clinical presentation.

NEC Pathogenesis

Necrotizing enterocolitis in prematurity involves intestinal bacterial colonization, immune response, gut barrier disruption and ischemia and the degree of gastrointestinal immaturity heavily impacts these factors. Whether ischemia is the initial insult or result of these components is poorly understood.

Inflammation. Research has consistently shown that intestinal inflammation is central to the development of NEC (McElroy et al., 2011; Nanthakumar, Fusunyan, Sanderson, & Walker, 2000; Viscardi, Lyon, Sun, Hebel, & Hasday, 1997). In the presence of bacterial colonization, an immune response is activated stimulating the cytokine cascade which consists of proinflammatory and counterinflammatory mediators. The role of cytokines and mediators is to incite, maintain and moderate the inflammatory response. The gastrointestinal system is the major source of cytokine production in healthy individuals (Edelson, Bagwell, & Rozycki, 1999.) However, the immature intestine may lack the capability to regulate this response, disproportionally releasing greater amounts of potent vasoconstrictors potentially increasing the risk for ischemia (Nankervis, Giannone, & Reber, 2008). Additionally, the preterm gut epithelial mucosal surfaces are vulnerable to injury due to the inability to form tight junctions (TJs) and prevent permeability in the presence of inflammatory cytokines (Clark et al., 2006; Han, Fink, & Delude, 2003). The final pathway for mucosal injury may be activated by reactive oxygen species production in response to leukocyte migration and adhesion to the site of injury (Hsueh et al., 2003). In sum, deficient epithelial barrier function leads to mucosal disruption, bacterial

invasion and subsequent inflammation and seems to favor ischemia as a secondary event triggered by these processes (Young, Kingma, & Neu, 2011).

The tenuous balance of inflammatory mediators released in response to bacterial invasion may substantially contribute to NEC development in the immature gut. Histologic findings from human NEC intestinal tissue reveal that Endothelin-1 levels and P-selectin expression are elevated (Nowicki et al., 2005; Stefanutti et al., 2005). P-selectin is an adhesion molecule which stimulates neutrophil epithelial adherence during inflammation (Stefanutti, et al., 2005). Endothelin-1 is a potent vasoconstrictor stimulated in response to the cytokine cascade (Nowicki, et al., 2005). The culmination of these processes suggests heightened leukocyte influx to injured mucosal surfaces with concomitant blood vessel vasoconstriction. Proinflammatory mediators capable of potent vasoconstriction, such as tumor necrosis factor-α (TNF-α), platelet activating factor (PAF) and interleukins (IL-1β and IL-6), are elevated in preterm infants with NEC (Chan, Wong, & Luk, 2009; Harris et al., 1994; Hsueh, et al., 2003; Hsueh, Caplan, Tan, MacKendrick, & Gonzalez-Crussi, 1998; McElroy, et al., 2011; Soliman et al., 2010). However, recent evidence suggests vasodilatory anti-inflammatory mediators (IL-8 and IL-10) are also present in infants with NEC, and may be reflective of an attempt to regulate the effect of proinflammatory cytokines following the initial inflammatory stimulus (Edelson, et al., 1999). Studies indicate that the extent of the anti-inflammatory response may be insufficient favoring greater vasoconstriction with proinflammatory cytokine predominance (Edelson, et al., 1999; Frost, Jilling, & Caplan, 2008).

Perfusion alteration. In non-disease states, endogenous nitric oxide (NO, produced from arginine by nitric oxide synthase, NOS) maintains normal vessel homeostasis through its vasodilatory effects (Petrosyan, et al., 2009). Studies have shown that endothelial nitric oxide synthase (eNOS) is diminished in NEC infants reducing NO formation, possibly related to

gastrointestinal epithelial barrier injury. This imbalance results in ongoing cytokine-induced vasoconstriction in the presence of inflammation (Nowicki et al., 2007). Experimental animal studies have shown that during later stages of NEC, there is altered NOS activity which results in NO reduction and formation of reactive oxygen species capable of intense vasoconstriction and subsequent ischemic injury (Whitehouse et al., 2010). Nitric oxide also inhibits platelet aggregation; therefore, in the presence of NO depletion, the risk for microclot formation may increase and be further augmented in the presence of PAF activated by the immune response (Ewer et al., 2004; Hsueh, et al., 1998; Jones, Barrett, Moraes, Gibbins, & Jackson, 2012). Interrelation of all these processes may explain the histologic findings of gross coagulation congestion and ischemic changes commonly observed in NEC tissues (Ewer, et al., 2004).

Variations in intestinal blood flow induced by inflammatory mediators and circulatory dysfunction of the immature gut may support the role of ischemic-reperfusion injury in NEC development. In non-disease states, the preterm intestine is characterized by low vascular resistance controlled by a delicate balance between regulators that favor vasodilation, mainly nitric oxide (NO) (Nankervis, Dunaway, & Nowicki, 2001; Nowicki, 2005). Studies have shown that blood flow alteration directly affects NO production; there is an increased production of NO to flow stimulus producing dilation (Nowicki, 1998; Reber, Mager, Miller, & Nowicki, 2001). Low perfusion followed by high perfusion may significantly alter vasculature homeostasis in the preterm intestine, and prolonged low perfusion states of greater than 50% below baseline may alter NO production to a significant degree triggering an ischemic event (Nowicki, 1998). Therefore, conditions that increase the risk for perfusion alteration and blood flow velocity changes may contribute substantially to the development of ischemia-reperfusion episodes and subsequent intestinal injury.

Enteral Feedings. The introduction of enteric bacteria via enteral feedings may play a role in NEC pathogenesis due to the potential for bacteria colonization prompting an immune response (Bjornvad et al., 2008; Cilieborg, Boye, Molbak, Thymann, & Sangild, 2011; Jiang et al., 2011; Nanthakumar, et al., 2000). No single specific pathogen has been associated with the development of NEC (Cilieborg, et al., 2011). Preterm infants are at risk for gut dysfunction due to decreased mass, function and immune response (Sangild, 2006). Additionally, gastric motility is delayed in the preterm intestine until approximately 34 weeks gestation, increasing the possibility for noxious substrate accumulation, intestinal distention and consequent mucosal surface damage (Lin, Nasr, & Stoll, 2008; Sase et al., 2005). However, delayed feedings and slow advancement of feeds have not been shown to reduce the incidence of NEC (McGuire & Bombell, 2008; Rayyis, Ambalavanan, Wright, & Carlo, 1999; Salhotra & Ramji, 2004). Breast milk remains highly preferable to formula feedings, likely due to its protective immunologic factors, and studies have further established that preterm infants exclusively fed breast milk have lower NEC incidence (Bjornvad, et al., 2008; Chauhan, Henderson, & McGuire, 2008; Kliegman, Walker, & Yolken, 1993; McKeown et al., 1992; Mihatsch et al., 2001; Neu, et al., 2008; Sangild, 2006). Using piglet models, Bjornvad et al (2008) examined gut response following delayed enteral feedings and total parenteral nutrition (TPN) in the first 3 days of life. NEC developed only after enteral feedings were introduced, and more often following an initial TPN-only period. Gut maturation and NEC resistance was higher in pigs fed colostrum compared to those fed formula, subsequent to TPN and delayed initial feeding (Bjornvad, et al., 2008). This investigation supports the premise that enteral feedings are a prerequisite for NEC development, but early feedings, especially with breast milk may stimulate gut maturation and may lower the risk for NEC. Optimal feeding regimens including type, duration and rate of advancement have been extensively studied in relation to risk for NEC development; however, no uniform consensus

exists regarding these issues (Gregory & Connolly, 2012; McGuire & Bombell, 2008; Schurr & Perkins, 2008).

Genetics and NEC

Recent evidence suggests that genetic variants in immunomodulating genes may be involved in NEC susceptibility. Scientific inquiry in this arena is limited yet evolving, and further research is needed to establish definitive evidence. The bulk of scientific investigations regarding genetics have focused on the susceptibility of the preterm infant's host defense system to invading pathogens when genetic mutations are present within cytoplasmic pattern recognition receptors (PRRs). It has been postulated that if abnormal gene expression is present within the innate immune response system, the ability to fight infection is diminished increasing the risk for NEC development (Henderson et al., 2009; Szebeni et al., 2006; Treszl et al., 2003; Treszl et al., 2001; Zouali et al., 2005).

Investigations examining genetic mutations of cytokines involved in the inflammatory process have failed to produce significance. Zouali at al (2005) found that a mutation in the CARD15/NO2 gene, which modulates the immune system response to gut flora, was not associated with NEC development (Zouali, et al., 2005). Others have found that polymorphisms in interleukins were not associated with NEC development (Henderson, et al., 2009; Szebeni, et al., 2006; Treszl, et al., 2003). Genetic mutations in cytokines are commonly single nucleotide polymorphisms (SNPs), and can be inherited leading to an increased predisposition to disease susceptibility, but not all SNPs are abnormal or disease producing (Duff, 2006). Therefore, these studies were conducted on the premise that inherited cytokine genetic variation may predispose preterm infants to NEC development due to their inability to appropriately respond to enteric

pathogenic bacteria invasion. Unfortunately, no specific cytokine genetic mutations have been identified associated with NEC development to support these theories.

Banyasz et al (2006) conducted a study examining the relationship between genetic polymorphisms of vascular endothelial growth factor (VEGF) and perinatal complications. This study found a significant relationship between preterm infants who developed NEC and carriers of the VEGF-2578A mutant allele, adjusting for gestational age, sepsis, patent ductus arteriosis and cardiac failure (Banyasz et al., 2006). VEGF genes are key modulators for nitric oxide synthesis and also regulate vascular permeability and angiogenesis. Therefore, it seems logical that decreased production of VEGF due to genetic mutation may predispose preterm infants to mesenteric vasoconstriction and subsequent ischemic events if nitric oxide production is impaired or vascular permeability is altered. Larger studies are needed to further explore this potential contributor to NEC development, and may prove beneficial if the presence of parental genotype for the mutant allele is associated with neonatal genotype expression.

The complexity of NEC pathophysiology underscores the need for further research to identify specific etiologies that will lead to effective prediction and preventive strategies. Research suggests a multifactorial pathogenesis is most probable; however, specific combinations of these factors to predict NEC onset remain unknown. Prematurity is the primary predictor and is directly correlated with gastrointestinal immaturity (Lin, et al., 2008). Enteral feedings have long been identified as a major risk factor for NEC, and further evidence implicates immune response and cytokine cascade activation as key contributory components. Recent evidence has linked packed red blood cell (PRBC) administration to NEC onset in a temporal fashion, and this phenomenon deserves further inquiry (Blau, et al., 2011; Christensen, 2011; Christensen, Lambert, et al., 2010; El-Dib, Narang, Lee, Massaro, & Aly, 2011; Josephson et al., 2010; Mally et al., 2006; McGrady et al., 1987; Paul et al., 2011; Singh et al., 2011).

Transfusion-Related NEC

Anemia is a common plication of due to iatrogenic blood loss and deficient erythropoiesis. Thus, the majority of VLBW premature infants are exposed to packed red blood cell (PRBC) transfusions during their NICU hospitalization (Aher, Malwatkar, & Kadam, 2008; Luban, 2008). The timing of PRBC administration in relation to NEC development may have important implications, as most preterm infants will receive their first transfusion in the first 4 weeks of life (Maier et al., 2000) which is within the same timeframe for NEC development. Several retrospective studies have associated PRBC transfusions with NEC onset, and have further identified a temporal relationship which occurs immediately and up to 48 hours post transfusion (Blau, et al., 2011; Carter, Holditch-Davis, Tanaka, & Schwartz, 2012; Christensen, Lambert, et al., 2010; Josephson, et al., 2010; Mohamed, A. & Shah, P.S., 2012; Singh, et al., 2011). Specific pathophysiologic mechanisms related to this occurrence are unknown. The following have been suggested: prolonged storage of blood (Gladwin & Kim-Shapiro, 2009; Kim-Shapiro, Lee, & Gladwin, 2011), concomitant enteral feedings (El-Dib, et al., 2011; Krimmel, Baker, & Yanowitz, 2009), perfusion-reperfusion injury (Agwu & Narchi, 2005; Cortez et al., 2011), or blood hyperviscosity (Cheromcha & Hyman, 1988). Although each of these may contribute to the development of NEC, it is more likely that a combination of factors may exist increasing the risk for transfusion-related NEC. As some of these factors may be controllable to reduce NEC risk, determination of interrelated mechanisms and associated pathophysiology necessitates further investigation.

Recent studies examining transfusion-related NEC. High mortality and morbidity rates are associated with PRBC transfusions in the adult population (Koch et al., 2008; Marik & Sibbald, 1993; Shander, Javidroozi, Ozawa, & Hare, 2011). The effects of PRBC transfusions

and the specific association with NEC in the preterm population have only recently gained attention among neonatal researchers. In 1987, McGrady et al reported an odds ratio of 15.1 for NEC development subsequent to PRBC transfusion for preterm infants < 1500 grams in their NICU (McGrady, et al., 1987). For unexplained reasons, this report did not alarm neonatal clinicians because no further studies were conducted regarding this phenomenon for several years. In 1998, Bednerak et al, as part of a larger study to evaluate a Score for Neonatal Acute Physiology (SNAP II), discovered that institutions with lower transfusing rates had fewer cases of NEC (Bednarek et al., 1998). Eight years later, in 2006, Mally et al found a 35% incidence for transfusion-related NEC (Bell's stage IIB or greater) in preterm infants < 1500 grams occurring 22 ± 5 hours subsequent to the transfusion (Mally, et al., 2006). Preterm infants with transfusion-related NEC had lower gestational ages and birth weights, greater postconceptual ages and lower pre-transfusion hematocrits than those who did not develop transfusion-related NEC. Although not statistically significant, preterm infants with transfusion-related NEC received older blood (12±10 versus 6±4 storage days). Also of interest, all preterm infants who developed transfusion-related NEC in this study were receiving full enteral feedings at the time of transfusion (Mally, et al., 2006). This report generated much concern among the neonatal research community prompting further retrospective investigations examining the relationship between NEC and PRBC transfusions.

Since 2010, several studies have retrospectively analyzed the prevalence, demographics and associated characteristics of preterm infants who develop transfusion-related NEC compared to those who do not. Christensen et al (2010) reported 30% of surgical NEC cases were related to PRBC administration within a 48 hour window, and these preterm infants were chronologically older with lower birth weights and gestational ages (Christensen, Lambert, et al., 2010). Josephson et al (2010) found that preterm infants with late onset NEC (> 4 weeks of age) had

received more PRBC transfusions than those with early-onset NEC (< 4 weeks of age), had lower birth weights and greater intensive care needs. In addition, preterm infants who receive multiple transfusions from multiple donors had an increased risk for late onset transfusion-related NEC (Josephson, et al., 2010). Blau et al (2011) found that transfusion-related NEC (defined as onset within 48 hours post transfusion) occurred in 25% of all NEC cases at greater postconceptual age (mean 31 weeks), lower birth weights and pre-transfusion hematocrits (Blau, et al., 2011). Similar studies report NEC develops 24-48 hours following PRBC transfusions (Paul, et al., 2011) and is associated with lower hematocrits concluding that anemia severity may increase NEC development in preterm infants (Singh, et al., 2011). A recent meta-analysis including 12 retrospective studies examined all variables related to transfusion related-NEC (Mohamed & Shah, 2012). This study reported that transfusion-related NEC was associated with younger gestational age, of lower birth weights and were more likely to have patent ductus arteriosis and requiring mechanical ventilator support than those who acquired NEC not related to transfusions (Mohamed & Shah, 2012). This meta-analysis included many of the studies previously described, but additionally found one study that reported a protective effect for NEC development when transfusions were given (Mohamed & Shah, 2012). This contradiction in findings is important, illustrating that not all institutions are experiencing the phenomenon of transfusion-related NEC. Therefore, the need for further research related to specific risk factors associated with this occurrence is crucial. In summary, transfusion-related NEC seems to be an authentic entity with higher prevalence in VLBW infants. The incidence has been reported between 25-35% occurring at older postconceptual ages when compared to preterm infants who develop NEC unrelated to PRBC transfusions. (Ghirardello, Lonati, Dusi, Pugni, & Mosca, 2011; Josephson, et al., 2010).

Prolonged storage of blood. Prolonged storage of red cells has been suggested as one mechanism increasing the risk for transfusion-related NEC. To minimize multiple donor

exposure and reduce infection risks, preterm infants may receive multiple transfusions from one unit of stored blood (Luban, 2008). Banked red blood cells (RBCs) stored over a period of time undergo biochemical, metabolic, and molecular changes, a phenomenon known as "storage lesion" (Agwu & Narchi, 2005; Almac & Ince, 2007; Bennett-Guerrero et al., 2007; Chin-Yee, Arya, & d'Almeida, 1997; Ho, Sibbald, & Chin-Yee, 2003; Tinmouth & Chin-Yee, 2001). Although the life span of in vivo RBCs is 90 to 120 days, the life span of stored blood is shortened by half, with most red cells only surviving 45 days, even when stored in a preservative solution (Antonelou, Kriebardis, & Papassideri, 2010). Accelerated aging results in cellular degradation and loss of constituents required for energy, mainly 2, 3 diphosphoglycerate (2,3 DPG) and adenosine triphosphate (ATP). The breakdown of the RBC membrane forms microvesicles which may potentially release harmful products capable of oxidation and further cellular structural deterioration (Kriebardis et al., 2008). The net effect is increased cellular rigidity and decreased energy production increasing the risk for hemolysis, release of intracellular hemoglobin and cellular death. Intravascular nitric oxide is rapidly depleted in the presence of plasma free hemoglobin leading to altered vessel homeostasis, platelet aggregation and potential microsludging in capillary beds (Kim-Shapiro, et al., 2011; Roback, Neuman, Quyyumi, & Sutliff, 2011). Administration of stored blood may be involved in stimulation of an inflammatory response, as cytokines and other proinflammatory mediators accumulate during storage. The degradation of RBCs as they age may induce leukocyte influx causing endothelial damage, tissue destruction and possible organ failure (Stack, Baril, Napychank, & Snyder, 1995). The immature intestinal environment may be extremely vulnerable to these effects. Adult studies have demonstrated significant adverse outcomes directly related to the administration of older blood, especially > 14 days old (Koch, et al., 2008; Marik & Sibbald, 1993; Purdy, Tweeddale, & Merrick, 1997). However, studies among the neonatal population are limited and this issue remains unresolved. Mally et al (2006) found a trend towards transfusion-related NEC when

older RBCs were given (Mally, et al., 2006), but others found no association related to the age of blood (Christensen, Lambert, et al., 2010; Josephson, et al., 2010; Paul, et al., 2011). Theoretical concern related to storage lesion is plausible; and given these conflicting reports, further research is needed before this potential risk factor can be dismissed.

Enteral feedings during transfusions. The effect of enteral feeding during and following PRBC transfusions has been less emphasized in prior studies, but reported findings suggest a potential association and deserve further consideration (Krimmel, 2009; El-Dib, 2011). Doppler studies reveal superior mesenteric artery blood flow is reduced post transfusion when enteral feedings are given (Krimmel, et al., 2009) and El-Dib et al (2011) found that withholding feedings during transfusions significantly reduced the incidence of transfusion-related NEC (El-Dib, et al., 2011). Christensen (2010) found that preterm infants who developed transfusion-related NEC were more often receiving larger feeding volumes and cow's milk formula (Christensen, Lambert, et al., 2010). The impact of enteral feedings given during a transfusion on mesenteric perfusion alteration to incite NEC development remains unknown. Further, it remains questionable if NEC onset was imminent irrespective of receiving a PRBC transfusion (Morini & Bagolan, 2011). Prospective research analyzing mesenteric perfusion patterns pre-, during and post-transfusion and associated events would address these gaps in evidence.

Perfusion-reperfusion injury. Prospective studies examining the effect of PRBC transfusion on mesenteric blood flow and perfusion are extremely limited. Kemply et al (1992) conducted a study to evaluate superior mesenteric artery (SMA) blood flow in preterm infants with and without NEC unrelated to PRBC transfusions. Their data indicate increased SMA blood flow velocity in those with confirmed NEC when compared to suspected NEC and non-NEC cases. Prior to NEC development, however, SMA blood flow velocity was very low in one subject with subsequent increases at the time of NEC onset (Kempley & Gamsu, 1992). The

authors concluded that NEC onset may be preceded by low blood flow with subsequent rise in blood flow as the result of postischemic hyperemia (Kempley & Gamsu, 1992). A pivotal study by Krimmel et al (2009) used Doppler technology to evaluate SMA blood flow in preterm infants pre- and post-transfusion. Their results showed SMA blood flow diminished post transfusion in preterm infants < 1250 grams and was further diminished in the postprandial state (Krimmel, et al., 2009). The explanation for these findings is conjectural, but may relate to low-perfusion status prior to PRBC administration and the inability of the immature mesenteric circulatory system to adapt to increased blood volume post-transfusion. The effect of enteral feedings on impaired blood flow may be related to increased metabolic demands required for digestion further impairing circulatory regulation. The overall implications of these findings suggest an increased susceptibility to altered perfusion subsequent to PRBC transfusions in the presence of enteral feedings (Krimmel, et al., 2009).

Blood hyperviscosity. Older studies suggest blood hyperviscosity causing microvessel occlusion may contribute to NEC development (Dunn et al., 1985; LeBlanc, D'Cruz, & Pate, 1984; Wilson, del Portillo, Schmidt, Feldman, & Kanto Jr, 1983). The possibility for this occurrence seems plausible, especially in the presence of altered RBC structure due to storage lesion and the related effects of nitric oxide depletion, vasoconstriction and platelet aggregation. Further, if large volumes of PRBCs are administered in a relatively short time frame rapidly increasing hematocrit levels, blood hyperviscosity following PRBC transfusions may indeed increase the risk for microvasculature occlusion and ischemic bowel insult. However, the level to which hematocrit must be raised to produce this response is poorly understood. Incorporating this risk factor in future research evaluating the effects of transfusion-related NEC would address this contention.

Near Infrared Spectroscopy

Scientific evidence evaluating the effect of PRBC transfusion in the presence of enteral feedings on mesenteric tissue oxygenation is unknown. Near infrared spectroscopy (NIRS) technology allows direct observation of oxygen extraction at the tissue level reflecting perfusion status, and is an optimal method for evaluating changes in oxygen consumption (Kaufman, Almodovar, Zuk, & Friesen, 2008; Tina et al., 2009; Wolf & Greisen, 2009; Wolfberg & du Plessis, 2006; Wong et al., 2008). Near-infrared spectroscopy measures the difference between oxyhemoglobin and deoxyhemoglobin in the tissue bed through infrared spectral absorption and is reported as the regional oxygen saturation (rSO_2) (Marin & Moore, 2011). The use of NIRS technology in the neonatal population is extensively discussed in Chapter 3, Article 1 of this dissertation. Although normal values for preterm infants do not exist, reference ranges have been suggested for stable preterm infants in the first three weeks of life revealing that cerebral oxygenation remains fairly stable (60-80%), with greater variability in mesenteric (40-60% for infants 32-33 weeks; 28-58% for infants 29-30 weeks) and renal (65-95%) beds (McNeill, Gatenby, McElroy, & Engelhardt, 2011). Physiologically, this is reflective of fluctuating metabolic demands and preferential blood shunting. Cortez et al (2011) evaluated mesenteric perfusion patterns in preterm infants in the first 14 days of life. This study found statistically significant decreased mesenteric perfusion patterns in the presence of feeding intolerance when compared to preterm infants tolerating feedings. Feeding intolerance was defined as withholding feedings for 24 hours or greater without evidence of medical or surgical NEC. Two 24 week gestation infants (twins) in this study developed early onset NEC (day of life 11) not related to PRBC transfusions. The mesenteric rSO_2 patterns demonstrated by these preterm infants were strikingly different, as the rSO_2 values plummeted to the lowest values read by NIRS devices (15%) and exhibited frequent “signal drop out”(Cortez, et al., 2011). Both studies demonstrated decreased gut perfusion patterns during the first 9 days of life, with subsequent increases around

days 10-14. Researchers attributed these findings to transitional gastrointestinal vascular resistance changes from fetal to extrauterine life (Cortez, et al., 2011; McNeill, et al., 2011).

Cerebro-splanchnic oxygenation ratio. Near infrared spectroscopy rSO_2 values may also be evaluated in a ratio format, known as cerebro-splanchnic oxygenation ratio (CSOR). This value is derived by dividing mesenteric rSO_2 by cerebral rSO_2. Fortune et al (2001) were the first to suggest that CSOR values < 0.75 may be indicative of potential intestinal injury (Fortune, Wagstaff, & Petros, 2001). However, to appropriately incorporate CSOR values when analyzing perfusion patterns, absolute values must be known. In the presence of cerebral autoregulation and stable perfusion patterns, a decrease in CSOR would directly reflect decreased mesenteric oxygenation. If cerebral patterns are impaired, an increase in CSOR could reflect low cerebral perfusion, not improved mesenteric perfusion. Cerebral autoregulation is often lost in critically ill preterm neonates (Soul et al., 2007) and in these circumstances, CSOR values may be misleading if used to evaluate mesenteric perfusion only.

Bailey et al (2012) postulate that CSOR values may be useful to identify preterm infants who may benefit from PRBC transfusions In this study, investigators found that preterm infants with CSOR values < 0.73 prior to PRBC administration demonstrated greater clinical improvement than those with CSOR values > 0.73 (Bailey, Hendricks-Muñoz, & Mally, 2012). Clinical improvement was defined as > 50% decrease in apnea, bradycardia and desaturation episodes, > 10% reduction in mean heart rate, decrease in fraction of inspired oxygen (FiO_2) and increase in feeding volume within 24 hours post transfusion. It should be noted that the preterm infants enrolled in this study were stable and not receiving antimicrobial therapy for suspected or confirmed sepsis, limiting the generalizability of these findings. Furthermore, the implications of this study must be interpreted with caution. CSOR values analyzed independently of absolute cerebral and mesenteric rSO_2 values may be misleading, and therefore should only be clinically

applied after ascertaining cerebral perfusion is not impaired. This approach ensures that changes in CSOR values accurately reflect changes in mesenteric perfusion thus allowing direct evaluation of improvement or impairment.

Transfusions and NIRS monitoring. Another study conducted by Bailey et al (2011) using near infrared spectroscopy technology examined cerebral and mesenteric tissue oxygenation changes before, during and twelve hours following PRBC infusion in preterm infants (Bailey, Hendricks-Munoz, Wells, & Mally, 2010). Their findings suggest that mesenteric oxygenation improves midway through a transfusion, but at twelve hours post transfusion, levels begin to decline. Cerebral oxygenation, however, immediately rose with PRBC administration and remained steady throughout the study period. The effect of enteral feedings during and following transfusions was not evaluated, and no infants developed NEC during this study. These investigators also found that pre-transfusion hemoglobin levels did not correlate with cerebral or mesenteric oxygenation patterns suggesting that severity of anemia cannot predict altered tissue perfusion (Bailey, et al., 2010).

Enteral Feedings and NIRS monitoring. Dave and colleagues (2009) evaluated cerebral and mesenteric oxygenation patterns using NIRS technology in stable growing preterm infants receiving full volume enteral feedings. This study found that cerebral patterns remained unchanged postprandially, while mesenteric perfusion significantly increased (Dave et al., 2009). Therefore, in the non-anemic, non-transfused state, mesenteric tissue oxygenation improves when feedings are given; however, according to Krimmel's study (2009) mesenteric blood flow diminishes subsequent to transfusions when enteral feedings are given. Hence, there seems to be a paradox regarding gut response when enteral feedings are given during and subsequent to transfusions. Based on these studies, and the unresolved issues surrounding NEC antecedent PRBC transfusion, it seems pragmatic to examine organ tissue bed oxygenation fluctuations in

the presence of PRBC transfusions and enteral feedings to better understand whether these events alone or in combination increase the risk for perfusion alteration that may lead to ischemia and possibly NEC development.

Conclusion

Necrotizing enterocolitis is a serious disease of prematurity and is a leading cause of morbidity and mortality in the preterm population. Prevention and prediction strategies remain ineffectual due to the obscurity of NEC etiology. Although many risk factors have been identified, individual and combined pathogenic causes continue to elude clinicians. Recent evidence suggests packed red blood cell transfusions are associated with NEC development, and enteral feedings may further enhance this risk. However, not all preterm infants who receive PRBC transfusions develop NEC, and for those that do, identified risk factors are somewhat variable. Therefore, this problem deserves further investigation to identify underlying pathophysiologic mechanisms and associated risk factors in order to establish safe and effective transfusion guidelines that will decrease transfusion-related NEC incidence in the preterm population.

Chapter III

Methodology

Design

Using Near-infrared spectroscopy technology, INVOS 5100C Cerebral/Somatic Oximeter (Covidien, Boulder, CO), an FDA approved device, this observational, prospective study was designed to address the following Specific Aims:

Specific Aim 1: Evaluate cerebral and mesenteric tissue oxygenation patterns using near infrared spectroscopy (NIRS) rSO_2 measurements before, during and subsequent to packed red blood cell (PRBC) transfusions with and without feedings to identify variations in tissue oxygenation delivery in preterm infants < 37 weeks gestational age.

Specific Aim 2: Examine the association between the age of blood transfused and changes in mesenteric tissue oxygenation patterns using NIRS rSO_2 measurements in preterm infants < 37 weeks gestational age receiving a PRBC transfusion.

Specific Aim 3: Determine if NIRS rSO_2 tissue oxygenation patterns differ in preterm infants who do and do not develop NEC following PRBC transfusions, with and without feedings.

The outcome of interest for Specific Aims 1 and 2 was altered perfusion patterns, a precursor to ischemia that may ultimately result in NEC. The outcome of interest in Specific Aim 3 is the clinical diagnosis of NEC according to Bell's staging criteria (Bell et al., 1978), with or without surgical intervention. To gain better understanding of the quality of the stored blood, the age of blood transfused was recorded and blood samples from all donor units was collected prior to transfusions and measured for 2,3-DPG and plasma free hemoglobin. These values were

recorded for secondary analysis. Volume and duration of blood transfused, days of storage and irradiation was recorded. Mesenteric perfusion was analyzed using Near Infrared Spectroscopy (NIRS) before, during and 48 hours following each transfusion event. Feedings were recorded according to volume, type, route, duration, caloric density, and time given in relationship to transfusion.

Study Variables

The conceptual and operational definitions of all variables in this study are presented in Table 3-1. Variables are categorized by demographic data, transfusion factors, feeding factors, gastrointestinal immaturity factors and perfusion factors.

Recruitment and Setting

This study was conducted at Emory University Hospital, Midtown Level IIIB Neonatal Intensive Care Unit, and subjects were recruited from November 30, 2010 to December 31, 2011. Prior to subject recruitment, nursing and medical directors, neonatal nurse practitioners and nursing staff in the neonatal intensive care unit (NICU) were informed of study purpose, procedures and recruitment plan. Copies of study protocol, Emory institutional review board approval notice and informed consent were stored in study binder in the Nursing Director's office. Patients were recruited by the study primary investigator (PI) and research team members. Attending neonatologists in the study NICUs contacted the PI if an infant was to receive a PRBC blood transfusion and parental consent was obtained by a research team member. Parents were given study procedure forms, copy of informed consent including contact names and phone numbers of research PI. Parents were informed that study enrollment included NIRS monitoring for all subsequent PRBC transfusions during entire NICU hospitalization, and that they could choose to withdraw their infant from this study at any time without negative consequences. Only

one infant was withdrawn from study prior to completion of data collection in the 48 hour monitoring timeframe.

Table 3-1.

Conceptual and Operational Definitions of Study Variables.

	Conceptual Definition	Operational Definition
Demographic Variables		
Gestational age	Time elapsed between the first day of the last normal menstrual period and the day of delivery, or number of completed weeks (AAP, 2004).	Gestational age at birth measured in weeks. Additional measurement confirmed by Ballard Gestational Exam, dates of confinement, ultrasound, or any combination of these methods. Information collected from the medical record.
Corrected gestational age	Represents the age of the child from the expected date of delivery (AAP, 2004).	Calculated by adding number of chronological days since birth adjusted to weeks (3 days /7) to gestational age at birth. If multiple transfusion events occurred separated by 7 or more days for one subject, corrected gestational age for each transfusion event was calculated.
Postnatal Age	Time elapsed after birth	Measured by days of life since birth

		at time of transfusion event.
Birth weight	Actual weight of infant at time of birth	Initial infant's weight measured in grams immediately following birth and documented on Labor and Delivery Record.
Current weight	Actual weight of infant at time of study enrollment or subsequent transfusion.	Initial infant's weight expressed in grams measured day of transfusion event and documented in patient record. If multiple transfusion events occurred separated by 7 or more days for one subject, current weight for each transfusion event was recorded.
Gender	Male or female sex	Male or female sex. If ambiguous genitalia present and without associated anomalies, sex will be confirmed with chromosome analysis or stated as ambiguous in the absence of confirmatory chromosomal analysis.
Race/Ethnicity	Heritable phenotypic characteristics divided by group: African-American, Caucasian, Asian, Pacific Islander or American Indian/Alaskan. Ethnicity defined	Subject race/ethnicity based on parental race/ethnicity. If race is mixed, explanation of specific parental combination recorded.

	as ethnic group either Hispanic or non-Hispanic.	
Transfusion Factors		
Age of Blood	Number of days blood was stored prior to transfusion to subject	Number of days from date of blood unit donation to transfusion event
Components of Blood	Constituents altered during accelerated aging of stored blood known to be associated with cellular degradation	Adenine triphosphate (ATP); 2,3-diphosphoglycerate (2,3 DPG); plasma free hemoglobin levels
Storage lesion	Cellular degradation due to microvesicular formation as a result of prolonged storage	Measurement of certain constituents known to be associated with cellular degradation: Adenine triphosphate (ATP); 2,3-diphosphoglycerate (2,3 DPG); plasma free hemoglobin levels
Type of blood	Blood group and Rh factor	Information collected from each donor blood unit for blood group and Rh factor.
Duration of transfusion	Transfusion event length	Number of hours from beginning to end of transfusion event
Time between transfusions	Amount of time (if any) between two transfusions given in less than 7 day period.	Length of time between end of first transfusion event and beginning of second transfusion event reported in hours.

Feeding Factors		
Type	Type of enteral feeding infant received at each feeding: breast milk, type of formula or combination of both	Breast milk (BM), Premature formula (PEF), Transitional premature formula (Enfacare), not receiving feedings (NPO).
Caloric density	Caloric density of breast milk via addition of human milk fortifier; preparation of premature formula to provide specific caloric density to promote growth and weight gain	Measured in kcal/oz for each enteral feeding event
Volume	Volume of enteral feeding administered at each feeding	Volume received over 24 hour period, measured in ml/kg/day. Full feedings = 150ml/kg/day or more.
Duration	Length of time for enteral feeding administration	Duration of time in minutes for each enteral feeding event
Route	Route enteral feeding was administered	Method of enteral feeding administration for each event: bottle (PO), orogastric tube (OG), or combination (PO/OG)
Frequency	Time interval in which enteral feedings were administered	Interval between feeding events measured in hours (Q3=every three hours)
Tolerance/Intolerance	Ability of subject to adequately	Amount, color and consistency of

	digest enteral feeding	gastric residuals obtained prior to each orogastric feeding. Amount measured in milliliters. Intolerance noted as feedings held for abdominal distention, large gastric residuals, or emesis.
Feeding Status during Transfusion event	Subject received or did not receive concurrent enteral feeding event during transfusion event	If feedings were held or administered during transfusion event, and if feeding was held; time until feedings restarted (measured in hours).
GI Immaturity Factors		
Impaired Circulatory Regulation	Imbalance between vasoconstriction and vasodilation predisposing immature intestine to ischemia	Directly related to degree of prematurity as determined by corrected gestational age. Degree of impairment increases with lower corrected gestational age.
Impaired Barrier Function	Decreased structural (epithelial) and biochemical (enterocytes) barriers in the preterm infant	Directly related to degree of prematurity as determined by corrected gestational age. Degree of impairment increases with lower corrected gestational age.
Immature Immune Response	Inefficient inflammatory response to intestinal injury in the immature gut	Directly related to degree of prematurity as determined by

	secondary to immature immune defense system.	corrected gestational age. Degree of impairment increases with lower corrected gestational age.
Impaired Motility	Lack of effective gastrointestinal motility due to premature birth prior to third trimester. Increased vulnerability for accumulation of noxious substances potentially causing gastrointestinal epithelial damage, impaired nutrient absorption and inadequate digestion.	Directly related to degree of prematurity as determined by corrected gestational age. Degree of impairment increases with lower corrected gestational age.
Perfusion Factors		
Intestinal Perfusion	Blood flow through the intestine that provides adequate oxygenation to tissues.	Near infrared spectroscopy measurement of mesenteric, cerebral and renal tissue oxygenation reported as regional oxygenation (rSO_2).
Normal Perfusion	Adequate blood flow through the intestine that provides optimal tissue oxygen delivery	rSO2 measurement within reference ranges established for stable premature infants in the first 3 weeks of life (McNeill et al., 2011).
Altered perfusion	Inadequate intestinal blood flow that provides suboptimal tissue oxygen	rSO_2 measurement outside reference ranges established for stable

	delivery	premature infants in the first 3 weeks of life (McNeill et al., 2011). Changes greater than 25% reduction from baseline rSO_2 values were analyzed over time for duration of reduction and total amount of percentage change.
CSOR	Cerebro-splanchnic oxygenation ratio measuring the relationship between regional tissue oxygenation differences.	Mesenteric rSO_2 value divided by cerebral rSO_2 value. Measurements < 0.75 indicative of altered splanchnic perfusion if cerebral perfusion within reference range.
Ischemia	Tissue damage related to inadequate oxygenation of tissue bed	-
Bacterial colonization	Intestinal bacteria colonization in sufficient quantity to stimulate an immune response with subsequent release of inflammatory mediators capable of vasoconstriction	-
Mucosal Disruption	Presence of intestinal epithelial damage	-
NEC	Bowel inflammation, ischemia and necrosis which affects portions or entire bowel	Confirmed diagnosis of NEC based on Bell's staging criteria (Bell et al., 1978).

Sample and Sample Size. The sample included premature infants of all races and gender who were admitted to the study NICU, and met Inclusion/Exclusion criteria. Informed consent from parent/parental guardian was obtained prior to placing near-infrared spectroscopy sensors.

Inclusion criteria:

1) < 37 weeks gestational age
2) To receive a PRBC transfusion
3) Hemodynamically stable as defined by not requiring intravenous vasopressor support

Exclusion criteria:

1) ≥37 weeks gestational age
2) Receiving intravenous vasopressor support
3) Current or previous diagnosis of necrotizing enterocolitis
4) Intraventricular hemorrhage Grade III or greater
5) Congenital anomalies

Rationale for inclusion/exclusion criteria were any infant with a condition that renders instability due to hemodynamics may confound the measured perfusion status and alter the results being analyzed. In addition, the purpose of this study was to identify associations between altered mesenteric perfusion patterns in stable preterm neonates possibly increasing the risk for NEC when receiving transfusions with and without enteral feedings.

Instrumentation

Near infrared spectroscopy (NIRS) monitoring was used to measure cerebral, mesenteric and renal tissue oxygenation values, as defined by regional oxygenation saturation levels (rSO_2). Three site monitoring was used to evaluate differential tissue bed oxygenation. Adhesive sensor probes were applied to the forehead for cerebral monitoring, periumbilical area for mesenteric monitoring and flank area for renal monitoring and left in place for entire study period (48 hours following completion of transfusion). Sensor probes were replaced every 4 hours or as needed for dislodgement, poor signal quality or skin irritation. There were no incidences of skin abrasions due to sensor probe placement during this investigation. The time on the NIRS monitor was recorded on the Subject data form when sensors were initially placed on each subject. Additionally, the time on the wall clock in the NICU was recorded. Discrepancies between these recorded times were adjusted during data transcription and prior to data analysis.

Data Collection

Vital signs (heart rate, respiratory rate and blood pressure) were obtained using Hewlett-Packard cardiovascular monitoring devices and recorded on nursing flowsheets per standard NICU protocol. Heart and respiratory rates were recorded 1 hour prior to each PRBC transfusion, every 15 minutes for the first hour of the transfusion, every 30 minutes for the duration of the transfusion, and 1 hour subsequent to the transfusion. Heart rate is an accepted measure of adequate cardiac output in infants and was measured as a reflection of circulatory regulation. Pulse oximetry monitoring using Sensormetics devices producing arterial saturation levels were recorded at the same time intervals. Feedings were recorded to include volume, type, duration, time initiated, route, and caloric density. All NIRS data was downloaded to a research computer by the PI within 24 hours following the study period.

Procedure

Study protocols were written and approved by Emory Institutional Review Board (Appendix A). Parental/guardian informed consents were obtained prior to application of NIRS sensor probes and device (Appendix A). Patient history data was documented on subject data forms by research team members. Patient physiologic data was collected by nursing staff as per institution guidelines on transfusion forms and patient flowsheets, and this data was transferred to study protocol forms by research team members. All data records were locked securely in cabinets located in Children's Healthcare of Atlanta, Egelston Hospital research office. Research team members responsible for applying NIRS device completed Subject Data Form and Flowsheet Data Forms (See Appendix B) for entire study period. Flowsheets were completed for:

1) Vital Signs (heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, arterial saturation per pulse oximetry (SaO_2).
2) Feedings: Time started, duration, type, amount, frequency, route, gastric residual volume obtained prior initiation of feeding and any additional information related to feeding intolerance.
3) Medications: Time started or given, duration, type, dosage, route.
4) Laboratory data: All laboratory data collected during study period, including hemoglobin and hematocrit levels prior to transfusion.
5) Procedures: Documentation including type, time start and completion of any procedures performed on patient during study period (in addition to red blood cell transfusions).

Blood bank director was notified of subject enrollment via electronic mail and phone. Blood samples were then collected from the parent donor units by blood bank personnel prior to

administration. Two vials of red blood cells from parent donor unit were collected and transported per PI to Roback lab within 24 hours of collection. Protocols (Appendix B) for laboratory specimen collection from parent donor units were stored on shelf located in the blood bank within a research binder labeled "NIRS Study". Blood bank personnel completed subject data collection sheet for each blood sample collected (Appendix B). These samples were analyzed per Roback laboratory procedures for 2,3-DPG and plasma free hemoglobin levels and were recorded on data collection sheets (Appendix B) and placed in research binder in Roback Laboratory. The PI collaborated with blood bank directors, personnel and Roback laboratory personnel throughout study period to ensure proper sample collection and documentation of all data were being performed. The PI collected all documents from blood bank and Roback laboratory on a monthly basis.

Routine patient care was not interrupted for study procedures. The NIRS sensor probes and device were placed immediately following parental consent to obtain baseline data prior to the beginning of the transfusion. However, no transfusion was delayed for study purposes. Following the removal of the NIRS device and data download, all infants were followed for NEC development until NICU discharge, death or transfer to another institution.

Statistical analysis

All data were checked for completeness and any outliers. Using SAS version 9.2 statistical software, mean rSO_2 measurements of cerebral, mesenteric and renal tissue beds were calculated in 30 minute intervals from raw 30-second data time points. Cerebro-splanchnic ratios (CSOR) were also computed for each time point using Microsoft Excel software by dividing mesenteric rSO_2 value by cerebral rSO_2 value. Using SAS version 9.2 statistical software, these values were also averaged over 30-minute intervals for each case. These means were then examined over time using multi-level modeling and linear regression.

Using SPSS statistical software, correlations and T-tests were used to test for significant associations among demographic, transfusion and feeding variables. To reduce the large number of temporal measurements, thirty minute mesenteric means were examined for every hour during the transfusion. Time was referenced relative to the end of each transfusion event. So, negative time points were for time before and during the transfusion, the end of the transfusion was denoted at time=0, and positive time points were after the transfusion. Some infants did received two transfusions. For these infants, the "time" points were reset relative to the end of the second transfusion (the clock restarted for each transfusion). Overall there were 33 transfusions for 24 cases. Due to the nested nature of the data, with numerous gut measurements over time within a given transfusion and transfusions nested within subjects, 3-level multi-level linear models (MLM) were used to difference between subjects, evaluate changes over time and time-by-covariate interaction effects. The 3 levels of the MLM models included time nested within transfusion (level-1), transfusions nested within subject (level-2) and differences between subjects (level-3). Intercepts were treated as random; variance components methods were employed; and maximum likelihood estimation was used. While there were over several hundred time points, there were only 24 subjects and sometimes less due to missing data on one or more covariate, so the significance level was set to alpha=0.10. All p-values are reported, but significance was noted for p-values < 0.10.

Chapter IV

Results and Publications

Introduction

This chapter will describe subject enrollment, demographic data, transfusion event characteristics, enteral feeding event characteristics and study results related to each Specific Aim, hypothesis and research question. The final section contains publications submitted to peer-reviewed journals related to this dissertation.

Subject Enrollment

Enrollment characteristics are listed in Table 4-1. Nineteen subjects were enrolled who received a total of 33 transfusions. When subjects were enrolled, they remained in the study until discharge, transfer, death or development of exclusion criteria. If multiple transfusion events were separated by greater than 7 days, these separate transfusions were treated as independent events and demographic data were re-entered and assigned a different study identification number. This approach ensured accurate interpretation of perfusion pattern changes related to gastrointestinal maturity reflective of increased gestational age, postnatal age and changing therapeutics for newly developed diagnoses. Additionally, nine subjects received "split" volume transfusions (full volume divided into two aliquots separated by 12 hours) or two full volume transfusions in less than a 7 day period. Because corrected gestational age and postnatal age did not change for these events, demographics were not re-entered. Each transfusion event was independently monitored (n=33); however, subject demographics totaled 24 to account for those who received multiple transfusions separated by 7 or more days. Analysis of MP (MP) specifically focused on each transfusion event relative to corrected gestational age: transfusion

event one (single full volume events and first "split" event), or transfusion event two (either split volume or full volume received in less than 7 day period).

Table 4-1

Subject enrollment characteristics

Subject	Single transfusion event	Transfusion events during study separated by < 7 days	Transfusion events during study separated by > 7 days	Length of time in study (days)	Termination Reason
1		2*		24	DC
2		2*		30	DC
3		2*		2	TR-NEC
4		2*		38	DC
5		2*	2†	66	DC
6			2†	68	DC
7	1			41	DC
8			2†	80	DC
9			2†	79	DC
10		2**		6	TR-NEC
11		2**		2	TR-NEC
12		2**		3	TR-NEC
13	1			82	DC
14	1			24	DC
15	1			50	DC
16	1			39	DC
17	1			1	WD
18	1			9	DC
19		2*		27	DC
		Totals			
19	7	18	8		
Total # transfusion events		33			
# associated with single demographic data	7	9	8		
Total # of events with demographic data		24			

Note: *Denotes split full volume transfusion events divided into two aliquots separated by 12 hours. **Denotes two full volume transfusion events administered in less than 7 day period. †Denotes transfusion events administered in greater than 7 day period with subsequent demographic data re-entry. DC, discharge to home; TR-NEC, developed necrotizing enterocolitis and therefore met exclusion data; WD, withdrawn at parental request.

Table 4-2 lists subject recruitment and enrollment characteristics over the 13-month study period. There were 11 parental refusals generally stating they did not want their infant to participate in research. One parent withdrew their infant at 23 hours subsequent to a single full-volume transfusion event, due to dissatisfaction with excessive equipment related to the NIRS device.

Table 4-2

Subject recruitment and enrollment characteristics over 13-month study period

Month	Year	Number enrolled	Multiple transfusions with data re-entry	Number of refusals	Number of withdrawals
November	2010	1	0	0	0
December	2010	3	0	0	0
January	2011	2	0	0	0
February	2011	3	2	2	0
March	2011	0	3	1	0
April	2011	3	0	0	0
May	2011	1	0	2	0
June	2011	2	0	0	0
July	2011	0	0	2	0
August	2011	0	0	1	0
September	2011	2	0	1	1
October	2011	1	0	1	0
November	2011	0	0	0	0
December	2011	1	0	1	0
Total		19	5	11	1

Note: Nineteen subjects enrolled, and further delineates subjects who received multiple transfusions and demographic re-entry (n=24). There were 11 refusals over the 13-month

enrollment period and one withdrawal prior to the conclusion of the monitoring period (at 23 hours post-transfusion event).

Sample Characteristics

Demographic data for each subject at time of enrollment are listed in Table 4-3. Of the total 19 participants, 7 were female and 12 were male. The majority of the sample were African-American (84%) and three (16%) were Caucasian. Demographic data for each event (including re-entered data of multiple transfusions) are listed in Table 4-4. The mean gestational age at birth for all events was 27.57(± 2), and 31.6 (± 2.8) for corrected gestational age. There was a wide range for postnatal age (1-56 days), mean 28.4 (± 17). All subjects were categorized by physical exam as appropriate for gestational age (AGA).

Table 4-3

Demographic data for each subject at time of enrollment

Subject	GA (weeks)	PNA (days)	cGA (weeks)	Birth Weight (g)	Current Weight (g)	Gender	Race
1	24.7	50	31.8	630	1505	M	B
2	29	31	33.4	1210	1655	F	B
3	29	31	33.4	1080	1635	F	B
4	30	23	33.3	1505	1885	M	B
5	28	1	28.1	1060	1070	M	B
6	25.8	28	29.8	920	1163	F	B
7	25	35	30	770	1057	F	B
8	26.3	23	29.6	814	870	F	B
9	26.3	24	29.7	932	1020	M	B
10	24	11	25.6	705	710	F	C
11	27	22	30.1	1000	1196	M	B
12	26	8	27.1	803	797	M	B
13	27.6	8	28.7	980	880	M	C
14	30	24	33.4	1160	1417	M	B
15	28.8	17	31.2	1265	1316	M	B
16	32	3	32.4	1650	1615	M	B
17	30	55	37.9	1170	2050	M	C
18	29	40	34.7	1315	2570	M	B
19	29	26	32.7	1180	1633	F	B

Mean/SD	27.8 ± 2.15	24.2 ± 14.6	31.1 ± 3	1060.5 ± 266.7	1370.7 ± 477.8

Note: GA, gestational age at birth; cGA, corrected gestational age; PNA, postnatal age; M, male; F, female; B, black (African-American); C, Caucasian.

Table 4-4

Demographic data associated with transfusion event

Event	Birth GA (weeks)	cGA (weeks)	PNA (days of life)	Birth Weight (grams)	Current Weight (grams)
1	24.7	31.8	50	630	1505
2	29	33.4	31	1210	1655
3	29	33.4	31	1080	1635
4	30	33.3	23	1505	1885
5	28	28.1	1	1060	1070
6	25.8	29.8	28	920	1163
7	28	30	14	1060	1000
8	25	30	35	770	1057
9	26.3	29.6	23	814	870
10	26.3	29.7	24	932	1020
11	25.8	32.4	46	920	1445
12	26	33.6	53	932	1474
13	26	33.6	53	814	1570
14	28	36	56	1060	1976
15	24	25.6	11	705	710
16	27	30.1	22	1000	1196
17	26	27.1	8	803	797
18	27.6	28.7	8	980	880
19	30	33.4	24	1160	1417
20	28.8	31.2	17	1265	1316
21	32	32.4	3	1650	1615
22	30	37.9	55	1170	2050
23	29	34.7	40	1315	2570
24	29	32.7	26	1180	1633
Mean/SD	27.57 ± 2	31.6 ± 2.8	28.4 ± 17	1039 ± 243.7	1396 ± 449.8

Note: GA, gestational age at birth; cGA, corrected gestational age; PNA, postnatal age; M, male; F, female; B, black (African-American); C, Caucasian.

Table 4-5 presents therapeutic data related to all transfusion events. Seventeen transfusion events were associated with concurrent caffeine citrate administration and 7 with antimicrobial therapy (2 for confirmed gram negative sepsis, 5 for clinical sepsis). Five subjects from the sample were not requiring oxygen supplementation and were on room air (FiO2 = 0.21), 13 were receiving oxygen supplementation via nasal cannula (FiO2 range 0.21-1.0), three were receiving nasal cannula positive airway pressure (NCPAP)(FiO2 range 0.26-0.35), one was on nasal cannula positive airway pressure with delivered respiratory rate and intermittent sigh (SiPAP) (FiO2 0.3) and two subjects were on mechanical ventilation (FiO2 range 0.22-0.36).

Table 4-5

Therapeutic data for each transfusion event

Event	Caffeine citrate	Antibiotics prior to transfusion	Reason for Antibiotics	FiO_2 (%)	Mode of delivery	TR-NEC?	Time to TR-NEC onset (hours)
1	Y	Y	Clinical sepsis	30	NC	N	-
2	N	N		100	NC	N	-
3	N	N		21	RA	Y	0.5
4	Y	N		26	NC	N	-
5	Y	Y	Clinical sepsis	22	VENT	N	-
6	Y	Y	Gram negative	25	NC	N	-
7	Y	N		30	NCPAP	N	-
8	Y	Y	Clinical sepsis	21	NC	N	-
9	Y	N		26	NCPAP	N	-
10	Y	N		30	NC	N	-
11	Y	Y	Clinical sepsis	21	NC	N	-
12	Y	N		21	NC	N	-
13	Y	N		30	NC	N	-
14	N	N		21	RA	N	-
15	Y	Y	Gram negative	36	VENT	Y	38.5
16	Y	N		21	RA	Y	11.5
17	Y	Y	Clinical sepsis	35	NCPAP	Y	<1
18	Y	N		30	SIPAP	N	-
19	N	N		21	RA	N	-
20	Y	N		25	NC	N	-
21	N	N		30	NC	N	-
22	N	N		30	NC	N	-

23	N	N	21	RA	N	-
24	Y	N	21	NC	N	-

Note: Y, yes; N, no; Clinical sepsis, subject presented with symptoms and laboratory data consistent with sepsis but negative blood cultures; RA, room air with no respiratory assistance; NC, nasal cannula; NCPAP, nasal cannula positive airway pressure; SIPAP, continuous positive airway pressure with intermittent sigh and delivered respiratory rate; VENT, mechanical ventilation; FiO2, fraction of inspired oxygen; TR-NEC, necrotizing enterocolitis developed following second split transfusion event within 48 hours subsequent to event.

Transfusion Event Characteristics

Data specific to transfusion events are listed in Tables 4-6 and 4-7. All transfusions were administered based on a diagnosis of anemia of prematurity, except for event five (5). Six transfusion events were "split" transfusions: one full volume transfusion (15-20cc/kg) divided into two aliquots given 12 hours apart. Three events were two full volume transfusions administered in < 72 hour window (detailed description of the nature of these events are outlined in Table 4-1). Fifteen transfusion events were single full volume transfusion of 10-20cc/kg. Four subjects were the result of placental abruption births; however, three of these were greater than 22 days old at the time of the studied transfusion event (events 1, 2 and 17) and all of these events were split transfusions. The fourth subject entered the study at day of life one (event 5), and received 2 subsequent transfusions on separate occasions > 7 days apart (events 7 and 14). On these subsequent events, this subject was 14 and 56 days of life, respectively. Therefore, the association with anemia related to the placental abruption at birth (not to anemia of prematurity) for this particular subject was most likely relevant during event 5, but not for the subsequent transfusion events. The hematocrit level was determined prior to each transfusion event according to routine NICU policy. The mean hematocrit level was 26.24 (± 3.9).

Table 4-6

Transfusion specific data for all transfusion events

Event	Split transfusion?	Hct mg/dl	Number of transfusions received in study	Number of transfusions received since birth	Time between transfusion (hours)
1	Y	23.4	1	3	12
2	Y	21.3	1	1	12
3	Y	19.5	1	1	12
4	Y	27.6	1	1	12
5	N	37.6	1	2	-
6	N	29.2	1	8	-
7	N	27.9	2	2	-
8	N	27	1	3	-
9	N	26.7	1	7	-
10	N	28.8	1	3	-
11	N	27.3	2	9	-
12	N	24.6	2	5	-
13	N	30.6	2	9	-
14	Y	21.6	3	4	12
15	Y	28	1	8	67
16	Y	21.9	1	2	21
17	Y	28.8	1	1	24
18	N	28.8	1	2	-
19	N	23.1	1	1	-
20	N	28.4	1	1	-
21	N	26.8	1	1	-
22	N	22.5	1	1	-
23	N	24	1	2	-
24	Y	24.3	1	1	12

Note: This table indicates transfusion events that were split (events in which full volume transfusions were divided in half separated by 12 hours), or two full volume events separated by more than 12 hours, but less than 72 hours. Hct, hematocrit level immediately prior to transfusion event; number of transfusions in study, the total number of each transfusion in this study for each event—these refer to single full volume or split transfusions; number of transfusions since birth, several subjects received multiple transfusions prior to study enrollment.

Table 4-7 provides information related to the age of donor blood administered and days of irradiated for each unit per transfusion event. Transfusion one and two refer to split transfusion events. The mean age of blood for the first transfusion event was 6.88 ± 2.4 days and for the second transfusion event was 8.3 (± 2.8). Mean days of irradiation for the first transfusion event were 3.13 (± 2.2) and for the second event were 4.3 (± 2.8). There was a direct linear correlation between the age of blood and days of irradiation which was statistically significant ($p<.0001$). The average volume of blood transfused for the first event was greater than for the second transfusion event; however, there were only 9 second transfusion events.

Table 4-7

Transfusion event characteristics related to blood age, length of irradiation and volume

Event	Age of blood 1st tx (days)	Irradiation days 1st tx	Age of blood 2nd tx (days)	Irradiation days 2nd tx	Volume of 1st tx (cc/kg)	Volume of 2nd tx (cc/kg)
1	6	1	6	1	7.5	7.5
2	13	8	13	8	7.5	7.5
3	7	4	7	4	7.5	7.5
4	10	6	10	6	7.5	7.5
5	7	3	-	-	10	-
6	7	3	-	-	15.5	-
7	6	2	-	-	16	-
8	6	2	-	-	15	-
9	4	1	-	-	15	-
10	5	2	-	-	15	-
11	6	3	-	-	15	-
12	7	3	-	-	15	-
13	7	3	-	-	15	-
14	6	2	6	2	10	10
15	7	4	10	7	20	15
16	7	3	7	3	15	15
17	7	3	8	4	15	16
18	14	10	-	-	15	-
19	5	2	-	-	15	-
20	5	2	-	-	15	-
21	6	2	-	-	15	-
22	4	0	-	-	15	-

23	7	4	-	-	20	-
24	6	2	6	2	10	10
Mean±SD	6.88 ± 2.4	3.13± 2.2	8.3 ± 2.8	4.3 ±2.8	13.6 ±3.6	9.7 ± 4.9

Note: The mean age of blood and irradiation lengths were greater for second split transfusion event. The mean volume of the first split transfusion event was greater than the second split transfusion event.

Transfused Packed Red Blood Cell (PRBC) Characteristics

Appendix C presents an overview of all PRBC characteristics related to transfusion event, age, irradiation length, volume, type and events that received donor blood from the same donor unit. This table also specifies events associated with transfusion-related NEC (TR-NEC). Events 17 and 18 received blood from the same parent donor unit, and both developed TR-NEC subsequent to the second split full volume transfusion event. Other events that received blood from the same donor unit that were not associated with TR-NEC development were: subjects 1 and 2; 7 and 8; 9, 10 and 11; and 12 and 13. Additionally, mean pre-transfusion hematocrit levels were slightly lower for the TR-NEC group compared to the non-NEC group (24.55 ± 4.56 v. 26.58 ± 3.76). The major difference between the TR-NEC and non-NEC group was the volume of the second transfusion event (13.38 ± 3.94 cc/kg compared to 6.7 ± 3.37 cc/kg). The mean total volume of blood received for those who developed TR-NEC (27.75 ± 8.77) compared to those who did not develop TR-NEC (15.6 ± 2.23) was statistically significant ($p < .0001$). No subjects received direct donor blood and all subjects received blood type/Rh factor O positive except one subject who received O negative (subject 19).

Feeding Characteristics

Table 4-8 lists enteral feeding characteristics for each transfusion event which specifies volume, type, caloric density, route and if feedings were held during each transfusion event. If feedings were held, the number of hours until feedings resumed is also listed for each event. Feedings were held for 57% of the transfusion events, and this decision was made by the attending neonatologist independent of this study. For events in which feedings held, six were < 30 weeks corrected gestational age (average volume of feeds = 55.3cc/kg), and seven were ≥ 31 weeks corrected gestational age (average volume of feeds = 122.9cc/kg). The majority of transfusion events were associated with orogastric-only feedings (71%), and 21% received a combination of orogastric and bottle feedings. Only one event was associated with all feedings per bottle and/or breastfeeding. One event was NPO (nothing by mouth) for the entire study period. The duration of feedings ranged from bolus (typically given over 5-10 minutes) to two hours. Formula feedings were given to 57% events associated with feedings, and 43% were associated with breast milk enteral feedings. Events associated with breast milk feedings had lower cGA ($p = .061$) and postnatal age ($p=.016$), were given lower volumes of feedings ($p=.066$), and lower current weights ($p=.034$) than events associated with formula feedings.

Table 4-8

Enteral feeding characteristics related to transfusion event

Event	On feedings prior to transfusion?	Type	Route	Volume (cc/kg/day)	Caloric density (kcal/oz)	Fed during transfusion	Time feedings resumed post-transfusion (hours)
1	Y	PF	OG/PO	150	24	Y	-
2	Y	PF	OG/PO	145	24	Y	-
3	Y	PF	OG	181	24	Y	-
4	Y	PF	OG	132	24	Y	-
5	Y	BM	OG	15	20	N	23
6	Y	BM	OG	89	20	Y	-
7	Y	BM	OG	136	22	N	1

8	N	-	-	NPO	-	-	-
9	Y	BM	OG	92	20	N	1
10	Y	BM	OG	15	20	N	15
11	Y	PF	OG	33	20	Y	-
12	Y	PF	OG	92	20	N	1
13	Y	PF	OG	143	22	N	1.5
14	Y	PF	OG/PO	145	24	N	4
15	Y	BM	OG	56	20	N	4
16	Y	PF	OG	147	24	Y	-
17	Y	PF	OG	100	20	Y	-
18	Y	BM	OG	18	20	N	20
19	Y	PF	OG/PO	175	24	N	4.5
20	Y	BM	OG	145	24	N	7
21	Y	BM	OG	20	20	N	21
22	Y	BM	PO	214	22	Y	-
23	Y	PF	OG/PO	140	24	N	4
24	Y	PF	OG	142	24	Y	-
Mean±SD				112.86 ± 55.04	22.09 ± 1.9		

Note: Y, yes; N, no; BM, breast milk; PF, premature formula; OG, orogastric feedings; PO, bottle feedings (per os); NPO, nothing by mouth; cc/kg/day, volume of feedings given every 3 hours totaled for 24 hour period and divided by subject's current weight. Full volume feedings were defined as ≥ 150cc/kg/day; kcal/oz, kilocalories per ounce of premature formula or human milk fortifier added to breast milk.

TR-NEC compared to non-NEC events

Table 4-9 presents demographic differences between events associated with TR-NEC development (n=4) and events in which no TR-NEC occurred (n=20). TR-NEC events had lower gestational age at birth and lower postnatal age (PNA) than non-NEC events. Hematocrit levels for TR-NEC events were slightly lower than non-NEC events. Using independent unpaired t-test to evaluate the means of the total amount of blood given (including split and two volume events), the mean volume for TR-NEC events was greater than non-NEC events ($p < .001$). There were no other major differences between these groups.

Table 4-9

Comparison of TR-NEC and non-NEC event characteristics

	TR-NEC (n=4)	Non-NEC (n=20)
	Mean ± SD	Mean ± SD
Gestational age at birth (weeks)	26.5 ± 2.1	27.78 ± 1.97
Postconceptual age (weeks)	29.07 ± 3.47	32.14 ± 2.49
Postnatal Age (days)	18 ± 10.55	30.5 ± 17.5
Birth weight (g)	897 ± 173.01	1067.35 ± 249.16
Current weight (g)	1084.5 ± 423.63	1458.55 ± 438.21
Volume of feedings (cc/kg/day)	113.25 ± 44.51	112.78 ± 58.25
Hematocrit (%)	24.55 ± 4.56	26.58 ± 3.76
Volume of 1st transfusion (cc/kg)	14.38 ± 5.15	13.45 ± 3.42
Age of Blood 1st transfusion (days)	7.0 ± 0	6.85 ± 2.62
Irradiation 1st transfusion (days)	3.5 ± .577	3.05 ± 2.4
Volume of 2nd transfusion (cc/kg)	13.38 ±3.94	6.7 ± 3.37
Age of Blood 2nd transfusion (days)	8.5 ± 2.12	8.2 ± 3.19
Irradiation 2nd transfusion (days)	5.5 ± 2.12	3.8 ± 3.03

Note: Comparison of means and standard deviation for major variable between events associated with TR-NEC and non-NEC. TR-NEC events were of lower weight and gestational age. Hematocrit values were slightly lower for TR-NEC events. Total volume of blood given to TR-NEC events was greater than non-NEC ($p<.001$).

Study Findings Addressing Specific Aims

Results related to Specific Aim 1

Specific Aim 1. Evaluate cerebral, mesenteric and renal tissue oxygenation patterns using near infrared spectroscopy (NIRS) rSO_2 measurements before, during and subsequent to packed red blood cell (PRBC) transfusions with and without feedings to identify variations in tissue oxygenation delivery in preterm subjects < 37 weeks gestational age.

Hypothesis 1: Mesenteric tissue oxygenation patterns in preterm subjects < 37 weeks gestational age will be negatively affected during and following PRBC transfusions**.**

MP Changes related to Transfusion Event. MP (MP) overall was not negatively affected during packed red blood cell (PRBC) transfusion event, although fluctuations were highly variable following the transfusion event and seemed to be associated with feedings (See H2; RQ 1.1 and 1.2). MP during and subsequent to PRBC transfusions varied related to corrected gestational age. To fully evaluate these responses, the sample was striated by corrected gestational age (cGA) to capture gastrointestinal maturational changes that may have impacted the response to each transfusion event (See Figure 4-1). These graphs were separated by transfusion event (first or second) and by TR-NEC and non-NEC cases. Gestational categories were as follows: < 30 weeks (n= 9), 30-< 33 weeks (n= 11) and > 33 weeks (n= 13), where n = number of transfusion events. There were no second transfusion events for those in the < 30 week category.

MP patterns were analyzed over time related to corrected gestational age (cGA), split transfusion event (1 or 2), non-NEC and TR-NEC cases. Non-NEC events with lower gestational ages (< 30 weeks) had the lowest MP throughout and following the first transfusion event, and as

gestational age increases, MP increased over time. All groups showed transient improvement in MP immediately following the transfusion event (except for one infant in the TR-NEC group), with a trend for MP to further decrease over time subsequent to the transfusion. However, for the non-NEC events, this trend did not continue for second transfusion events, exhibiting a decreased trend over time. Multilevel Linear Models (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of cGA as well as interaction effects between cGA and time. Statistically, for every 1 week increase in cGA, there was an increase in MP by 2.81 points ($p = .032$).

Figure 4-2 displays MP means with 95% Confidence Intervals for all subjects over specific time points to demonstrate a global response to transfusion event: during transfusion, 12, 24, 36 and 48 hours post transfusion. There is an obvious difference related to increased age during the first transfusion event in non-NEC cases; older subjects have higher overall means. However, this trend does not continue for those who received a second transfusion event. Further, subjects $\geq$ 33 weeks cGA did not show any improvement over time during the second transfusion event, and subjects 30-32 weeks demonstrated only slight improvement immediately following the transfusion event which then stabilized and remained stable with no increases throughout the study period. From this data, first transfusion events ending means were slightly higher than beginning baseline means, but lower for second transfusion events.

TR-NEC subjects exhibited extremely different patterns for both transfusion events. All subjects developed TR-NEC either during or following the second transfusion event (see table 4-5 for onset time). No increases in MP were seen for any TR-NEC cases during the first transfusion event; however, there were substantial fluctuations following the second transfusion event. These dramatic increases in MP patterns may have been related to initial therapeutics

related TR-NEC onset and further disease progression. A discussion regarding this data is extensively discussed in Publication 3 of this dissertation.

Cerebro-splanchnic ratio (CSOR) patterns related to Transfusion Event. Cerebro-splanchnic ratio (CSOR) patterns are shown in Figure 4-3. Using a reference value of 0.75, events with lower gestational ages had lower CSOR values. However, there were less pronounced increases in CSOR values immediately following the transfusion event. TR-NEC events demonstrated greater improvement in CSOR values following the second transfusion event. For all events, Multilevel Linear Models (MLM) were used to evaluate expected CSOR temporal patterns nested within transfusion events and to test for overall impact of cGA as well as interaction effects between cGA and time: for every 1 week increase in cGA, there was an expected increase in CSOR value by 0.051 ($p<.0001$).

In summary, MP in non-NEC cases was lower overall for lower cGA, and demonstrated greater reduction following the transfusion event. During the second transfusion event, there was less improvement in mesenteric values during and following the transfusion event, and an overall negative impact for those ≥ 33 weeks. However, overall MP did not fall below beginning values for non-NEC cases during the first transfusion event. CSOR data was similar, in that increased cGA was significantly associated with increased MP.

Hypothesis 2. Preterm subjects < 37 weeks gestation age who receive enteral feedings during a PRBC transfusion event will exhibit greater negative impact on mesenteric tissue oxygenation patterns than those not fed during a PRBC transfusion event.

Research Question 1.1. What are the differences in mesenteric tissue oxygenation patterns during and subsequent to PRBC administration in preterm subjects who receive an

enteral feeding during a transfusion event compared to those who do not receive a feeding during a transfusion event?

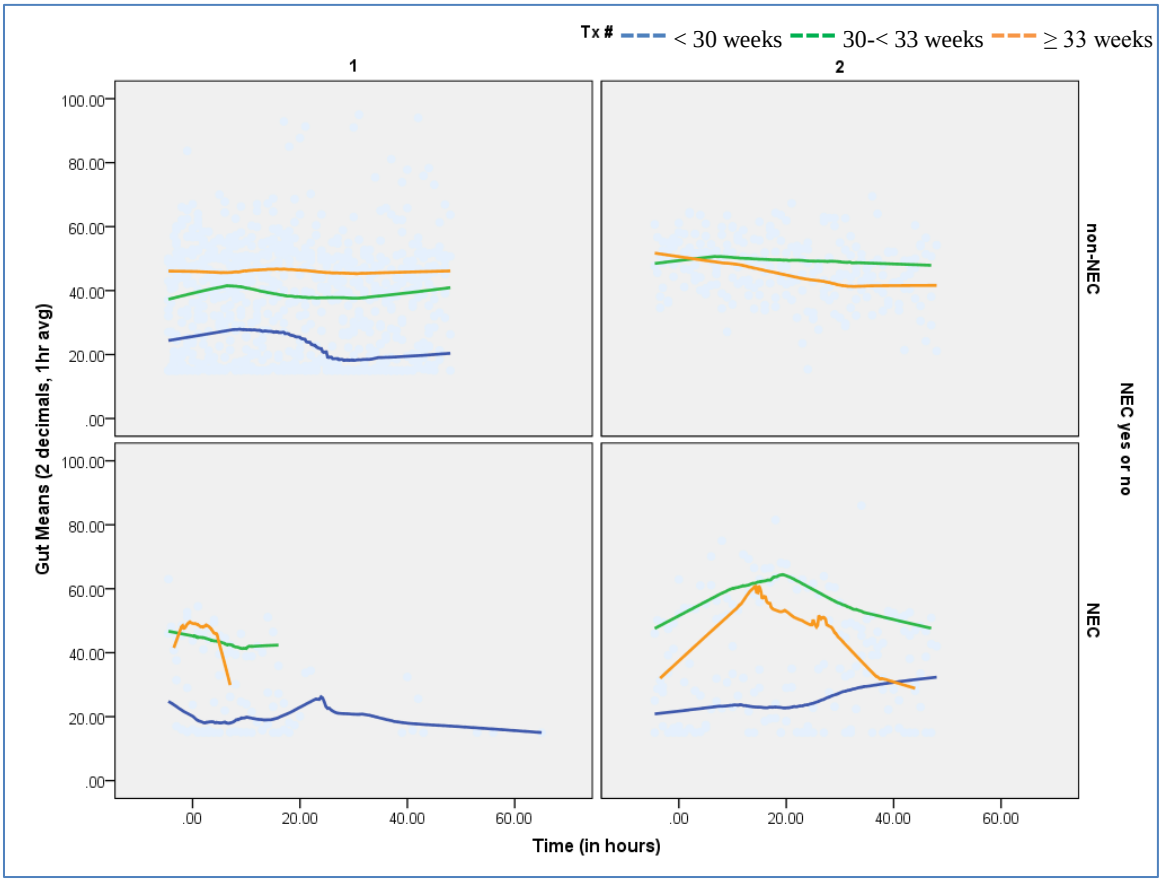

Figure 4-1. MP patterns over time categorized by corrected gestational age, transfusion event and TR-NEC v. non-NEC cases. Multilevel Linear Models (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of cGA as well as interaction effects between cGA and time. MP patterns lowest associated with < 30 weeks. As age increases, MP increases. For every one week increase in cGA, MP increased by 2.81 points (p=.032). The lines in each graph are Loess smoothing fit lines using weighted least squares.

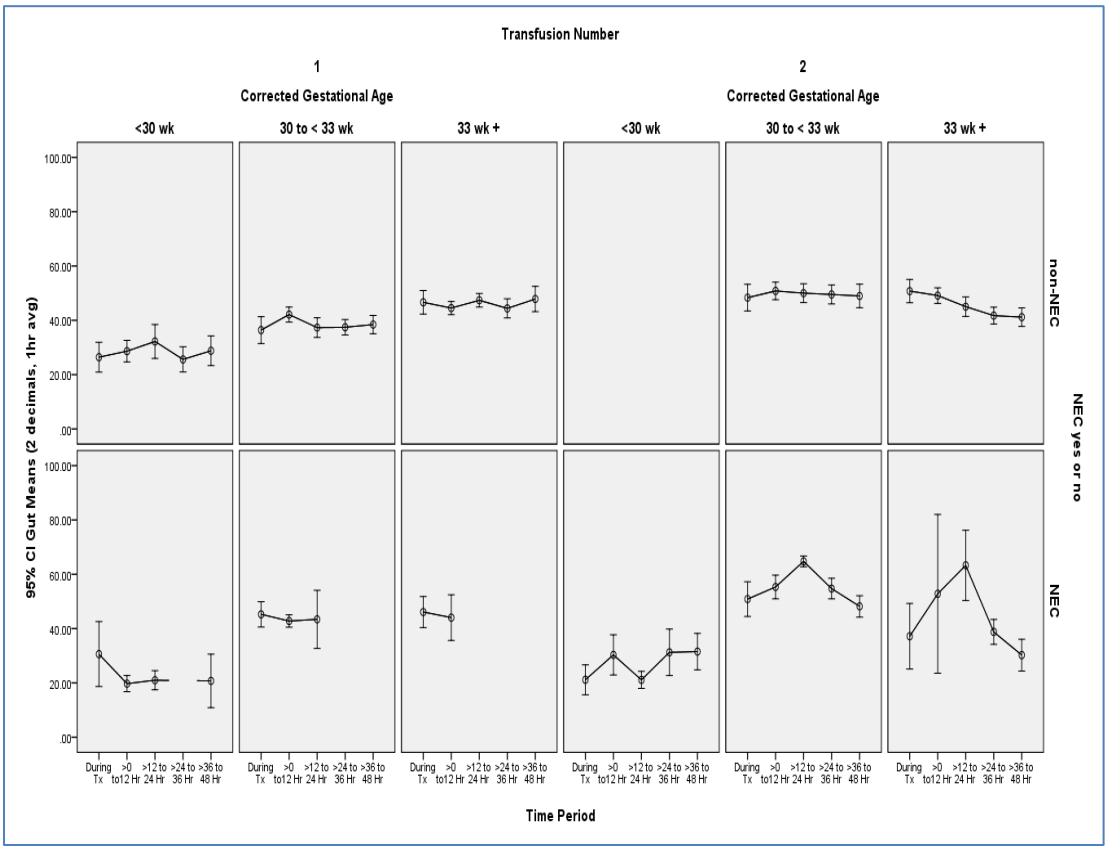

Figure 4-2. MP means plotted over specific time points related to transfusion event. MP means categorized by cGA, transfusion event and non-NEC and TR-NEC cases. Time points for MP means: during transfusion, end of transfusion event to 12 hours post-transfusion, > 12-24 hours, >24-36 hours and >36 to 48 hours post-transfusion event. Ending means were greater for all categories for the first transfusion event in the non-NEC cases, lower for the TR-NEC cases. In the second transfusion category, ending MP means were similar for the 30 to < 33 week category, but lower for the > 33 week category in the non-NEC cases. There was great variability during the second transfusion event for TR-NEC cases.

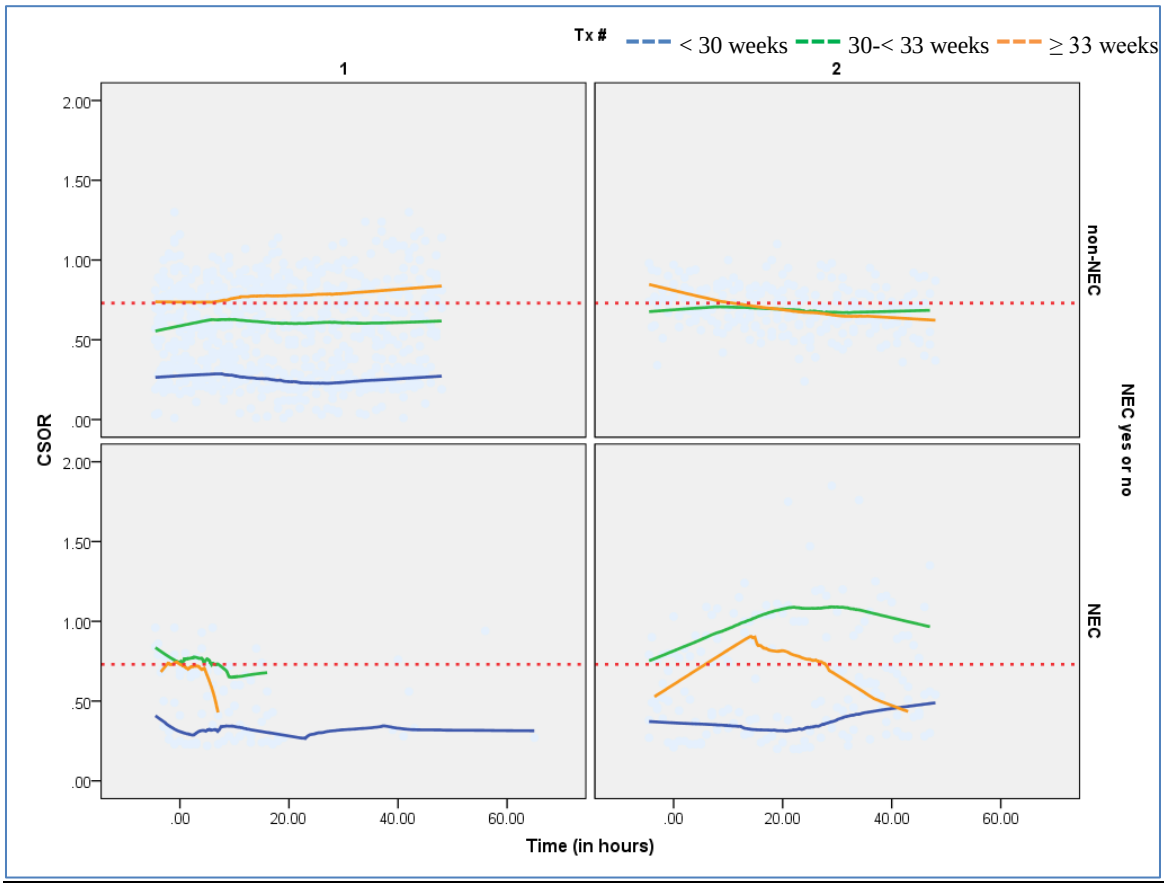

*Figure 4-3.*Cerebro-splanchnic (CSOR) means over time categorized by corrected gestational age, transfusion event and TR-NEC v. non-NEC cases. Multilevel Linear Models (MLM) were used to evaluate expected CSOR temporal patterns nested within transfusion events and to test for overall impact of cGA as well as interaction effects between cGA and time. As age increases, CSOR increases. For every one week increase in cGA, CSOR increased by 0.51 points ($p<.0001$). The lines in each graph are Loess smoothing fit lines using weighted least squares.

MP related to enteral feeding and transfusion event. To address this hypothesis and research question, events were divided into two groups: fed during transfusion and not fed during transfusion. MP and CSOR values were then analyzed over time. For transfusion event one: events associated with concurrent enteral feedings during transfusion event one exhibited higher overall MP than those not fed in both TR-NEC and Non-NEC cases. In contrast, for non-NEC

cases who received a second transfusion, events associated with concurrent enteral feeding exhibited lower overall MP (Figure 4-4).

Referring to Figure 4-4, time point .00 indicates the end of the transfusion event. Events associated with concurrent enteral feedings during the first transfusion had MP 10.23 points higher than events not associated with concurrent enteral feedings during the transfusion event (95% CI 1.7-18.78; $p = 0.02$). However, transfusions associated with concurrent enteral feedings was associated with negative slopes over time subsequent to transfusion event (time slope = -0.16; $p<.0001$). In contrast, transfusions associated with no concurrent enteral feeding event demonstrated positive slopes over time following the transfusion (time slope = .056, $p=0.019$). In addition, no events in the < 30 weeks cGA category received two transfusion events, and only one case was associated with enteral feedings during the transfusion event.

Cerebro-splanchnic patterns related to enteral feeding and transfusion event. Cerebro-splanchnic ratios showed similar patterns between subjects fed and not fed during transfusion events (See Figure 4-5). CSOR values were lower in non-NEC cases not associated with enteral feedings during the first transfusion event, and higher for those not fed during the second

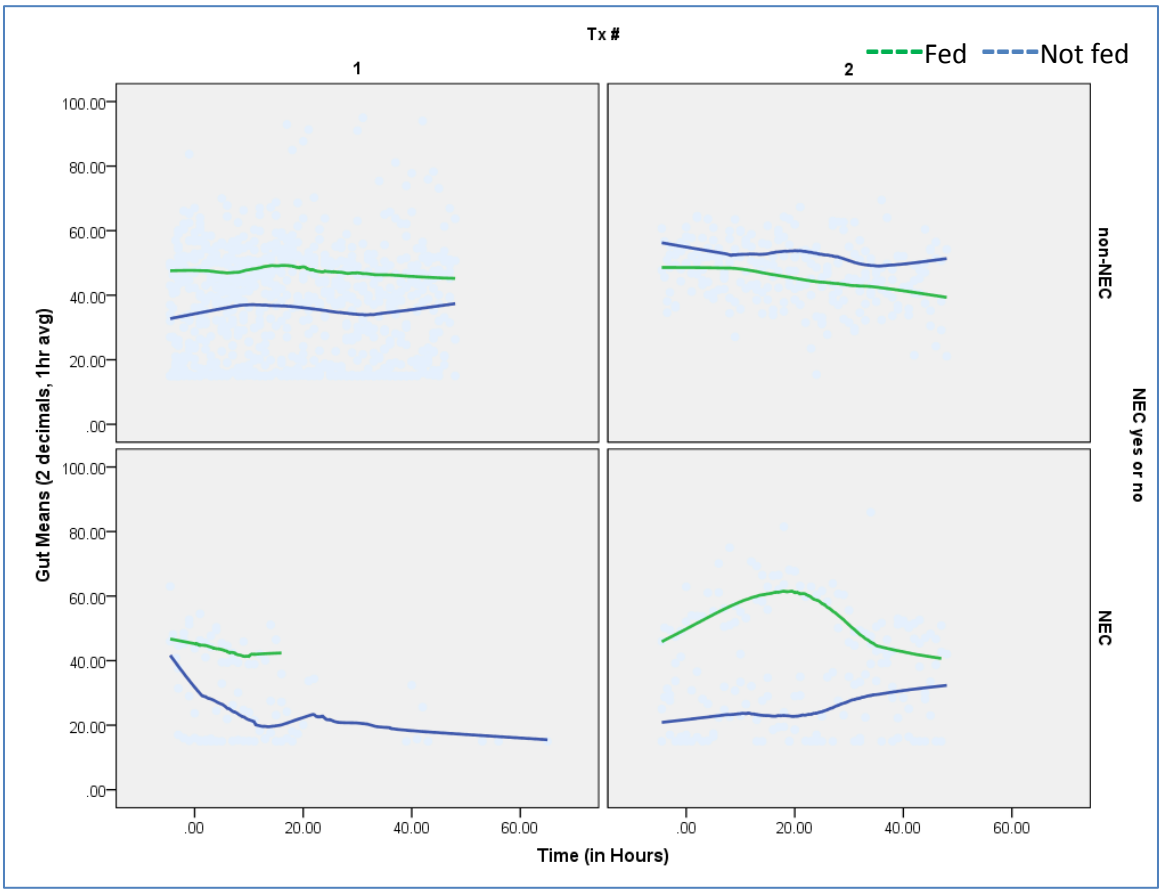

*Figure 4-4.*Comparison of MP for events associated with feedings or no feedings. Graphs separated by TR-NEC and non-NEC cases, and transfusion event. Multilevel Linear Models (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of feedings as well as interaction effects between feedings and time.. First transfusion events associated with concurrent enteral feeding events had higher MP than events associated with no enteral feeding events (p=.02). Non-NEC cases had lower MP during the second transfusion event. Concurrent feedings during transfusion event was associated with decreased MP over time (p<.0001), and no concurrent feedings during transfusion event was associated with increased MP over time (p=.019). The lines in each graph are Loess smoothing fit lines using weighted least squares.

transfusion event. For TR-NEC cases, CSOR values were generally lower for the one event not associated with feeds during both transfusion events, and higher for events associated with feedings. However, the change in slope for CSOR values was not statistically significant. For no feedings during transfusions, CSOR values slightly increased over time (time slope = .00071, p=0.084) and for feedings during transfusion, their slope was basically flat (time slope = .000142, p=.45).

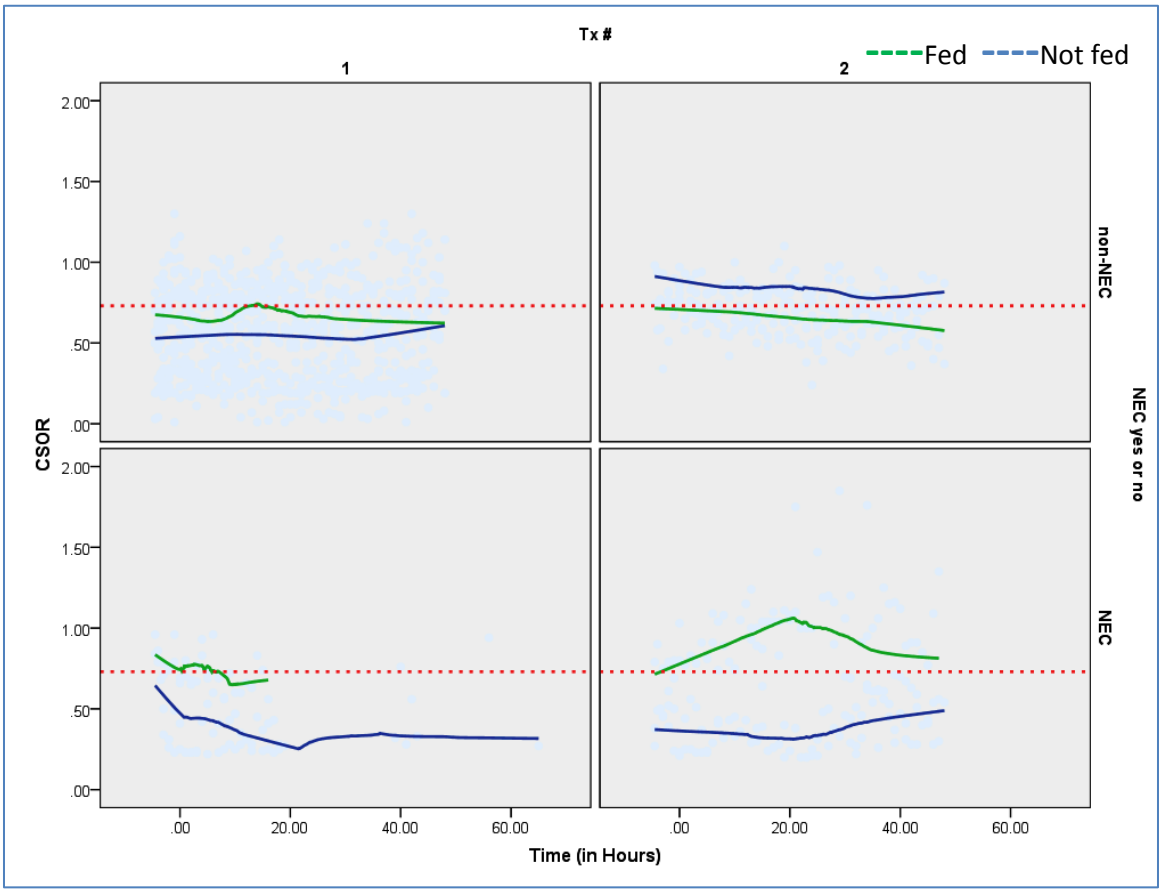

*Figure 4-5.*Comparison of CSOR values for events associated with feedings or no feedings.

(MLM) were used to evaluate expected CSOR temporal patterns nested within transfusion events and to test for overall impact of feedings as well as interaction effects between feedings and time.. Graphs separated by TR-NEC and non-NEC cases and transfusion event. Events associated with

no enteral feedings during transfusions, CSOR values slightly increased over (p=0.084) and for those associated with feedings during transfusion, there was no statistical difference (p=.45). The lines in each graph are Loess smoothing fit lines using weighted least squares.

In summary, events associated with enteral feedings during transfusion had higher means overall, but this effect was confounded because most of these subjects were older (> 30 weeks gestation) and the majority of subjects < 30 weeks corrected gestation had enteral feedings held during transfusion event. However, this effect was opposite during the second transfusion event in which feeding events were associated with lower overall MP than those not associated with enteral feedings. Furthermore, no subjects < 30 weeks gestation in the non-NEC group received a second transfusion; therefore, this group did not confound these results.

Research Question 1.2. What temporal relationship exists following transfusions between altered tissue perfusion patterns and feedings?

MP related to feeding volumes. To understand temporal changes that occurred during and following transfusion events, it is necessary to explain the relationships between overall MP pattern changes related to volume and type of feeding. An interaction effect occurred between MP and feeding volumes: greater volumes of feedings were significantly associated with higher MP (p=.007). However, older subjects (higher cGA and PNA) received larger volumes of enteral feedings (p=.003). So, there seemed to be a logical interaction in that older subjects had higher MP and were fed larger volumes of milk. Plots are shown in Figure 4-6 representing MP changes over time related to feeding volumes; divided into events receiving ≤ 140cc/kg/day and > 140cc/kg/day. (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of feeding volume as well as interaction effects between feeding volume and time. For every 1 point increase in feeding volume (increase of 1cc/kg/day), MP was expected to increase by 0.12 points (p=0.007). CSOR values (Figure 4-7)

showed similar patterns and this relationship was also statistically significant (p=.001). For every 1cc/kg increase, CSOR values were expected to increase by .0002 points. The relationship between feeding type (formula v. breast milk) and MP patterns differed. There was no significant relationship between events associated with breast milk feeding and time slope (p=0.719) or formula and time slope (p=0.976). Therefore, the volume of feeding, but not type of feeding, significantly influenced MP changes over time following transfusion events.

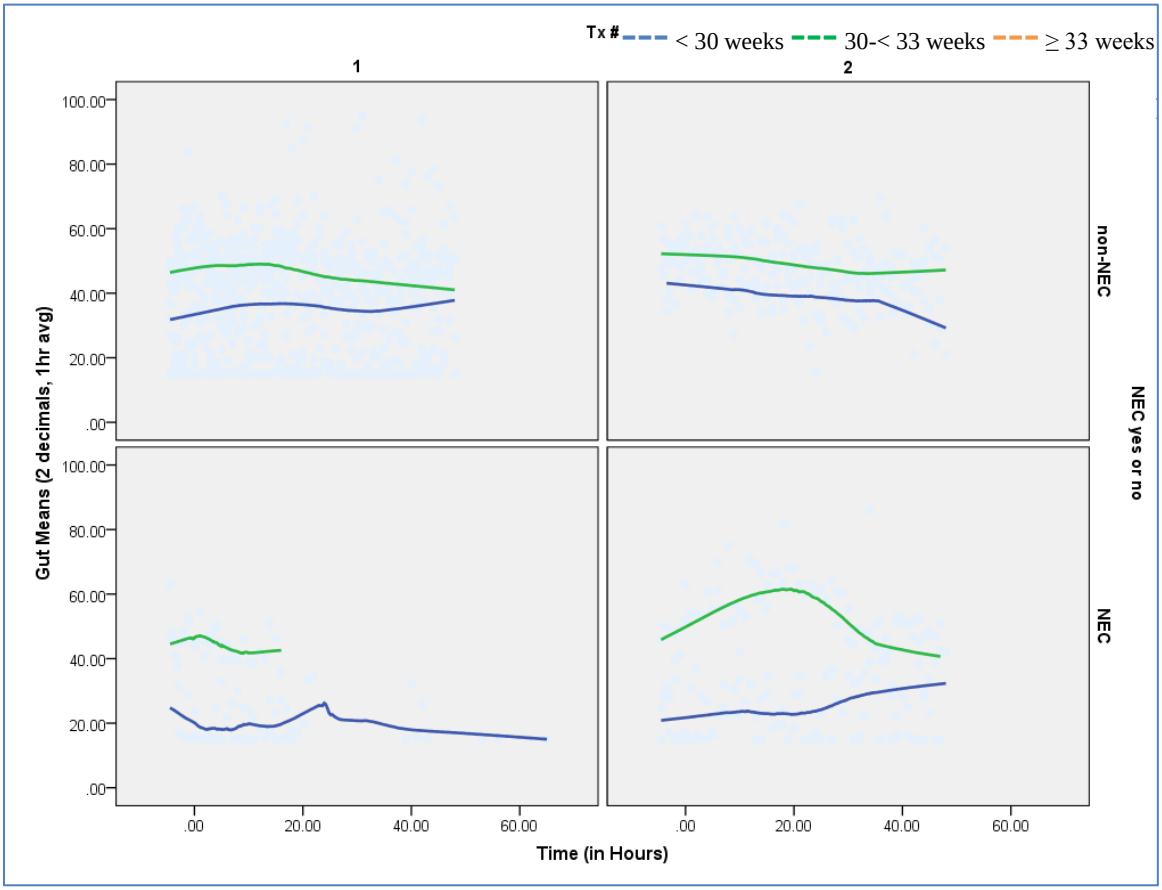

Figure 4-6. MP changes over time related to feeding volumes per transfusion event (volumes divided into ≤ 140cc/kg/day and > 140cc/kg/day); graphs separated by TR-NEC and non-NEC cases. (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of feeding volume as well as interaction effects between feeding volume and time: For every 1 point increase in feeding volume (increase of 1cc/kg/day),

MP was expected to increase by 0.12 points (p=0.007). The lines in each graph are Loess smoothing fit lines using weighted least squares.

Temporal changes in MP related to enteral feedings and transfusion event. In addition to the information previously described, there were positive and negative fluctuations in MP patterns surrounding feeding events throughout the 48 hour post-transfusion period for all non-NEC cases. Changes in TR-NEC perfusion patterns will be described in Specific Aim 3 section. Variability of MP between subjects was highly significant (p< .0001); therefore, each subject displayed unique patterns related to their gestational age, postnatal age, feeding status during transfusion event and feeding volumes. A common pattern emerged for most non-NEC cases. Although overall means slightly improved for first transfusion events, there were wide fluctuations with enteral feeding events. For most feeding events, MP was higher than during or at the end of the feeding event. Just prior to the subsequent feeding event (all subjects were fed every 3 hours), MP elevated. Therefore a pattern developed in which reductions in MP was commonly seen subsequent to feeding events in the post-transfusion state. These fluctuations continued for many non-NEC cases throughout the study period. . Exceptions to this pattern were subjects 9 and 10 (twins) whose MP were very low (at or near 15) for most of the study period.

In summary, feedings given during transfusions did not negatively affect MP patterns in non-NEC cases; but seemed to affect pattern fluctuations post-transfusion throughout the entire study period for most subjects. MP was higher for events associated with greater volumes (> 140cc/kg/day), but was not associated with type of feeding administered (breast milk or formula).

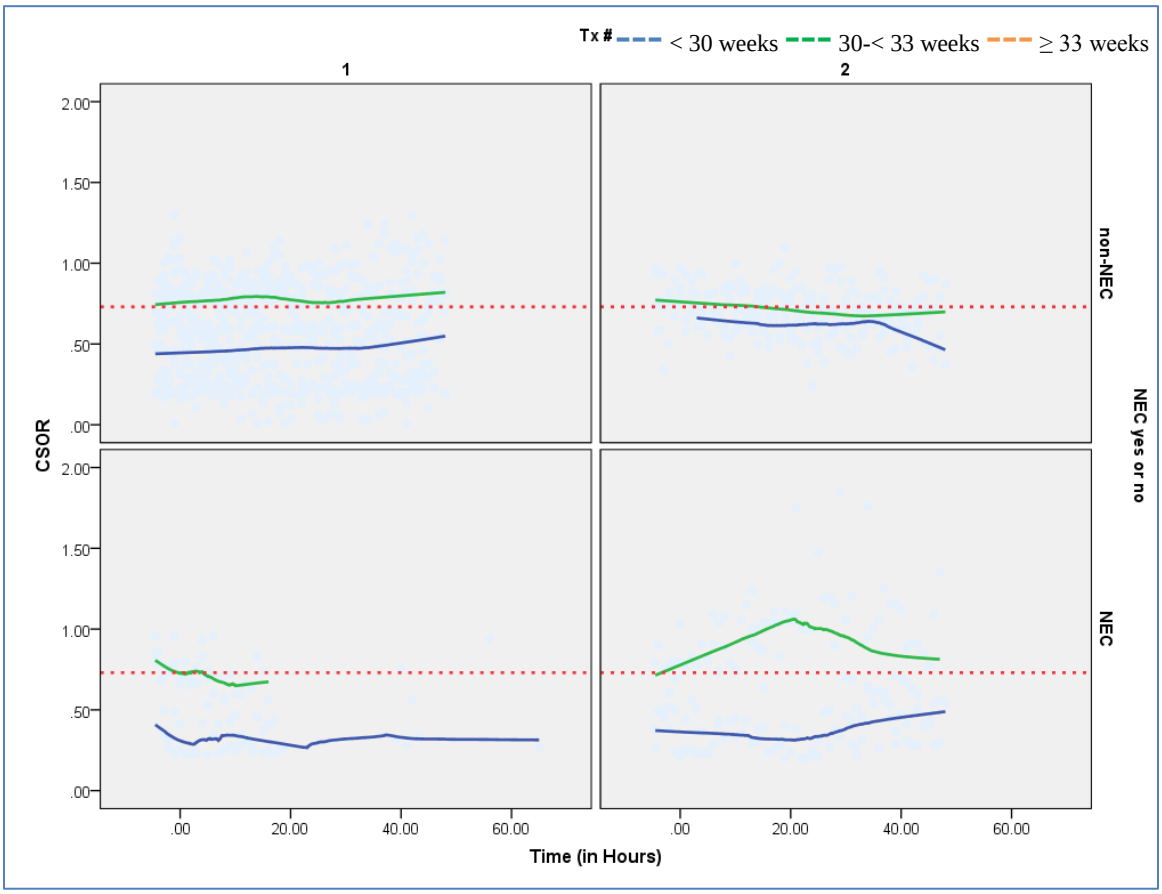

Figure 4-7. CSOR changes over time related to feeding volumes. Graphs separated by TR-NEC and non-NEC cases, and transfusion event. (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of feeding volume as well as interaction effects between feeding volume and time.. Volumes divided into ≤ 140cc/kg/day and > 140cc/kg/day. For every 1 point increase in feeding volume (increase of 1cc/kg/day), CSOR was expected to increase by 0.002 points ($p<0.001$). The lines in each graph are Loess smoothing fit lines using weighted least squares.

Results related to Specific Aim 2

Specific Aim 2. Examine the association between the age of blood transfused and changes in mesenteric tissue oxygenation patterns using NIRS rSO_2 measurements in preterm subjects < 37 weeks gestational age receiving a PRBC transfusion.

Research Question 2.1. What changes occur in mesenteric and cerebral tissue oxygenation patterns (rSO_2) when preterm subjects are transfused with PRBCs that have been stored for > 6 days compared to those who receive PRBCs stored for ≤ 6 days, controlling for feeding status?

MP and the age of blood infused. The range of the age of blood for events in this study was 4-14 days, with irradiation length 0-10 days. Overall, events associated with older blood had lower MP (See Figure 4-8). From this graph, it is apparent that cases receiving blood 10, 13 and 14 days old had negative MP over time following the transfusion, than those who received younger blood. (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of the age of blood as well as interaction effects between age of blood and time. MP was evaluated over time related to the age of blood for each event into ≤ 6 days old (Figure 4-9) and > 6 days old (Figure 4-10) and categorized by corrected gestational age. There was a significant interaction between the age of blood and time ($p=0.007$); events associated with blood > 6 days old had negative slopes over time. Twelve transfusion events were associated administration of blood ≤ 6 days old, and 12 events were associated with administration of blood > 6 days old. All TR-NEC subjects received 7 day old blood, and their MP patterns are seen in the lower graphs in Figure 4-8. As previously found, younger subjects < 30 weeks gestational age had lower overall MP means; however, all subjects who received blood > 6 days old demonstrated negative slopes for longer periods of time following transfusion events and 4 of these subjects developed TR-NEC. Because there was a direct linear correlation

between the age of blood and irradiation length (r=.968, p< 0.001), there was also a statistically significant difference between these variables (p=.001). CSOR values followed the same trending patterns; as the age of blood increased, there was a decrease in CSOR means over time (p=0.063).

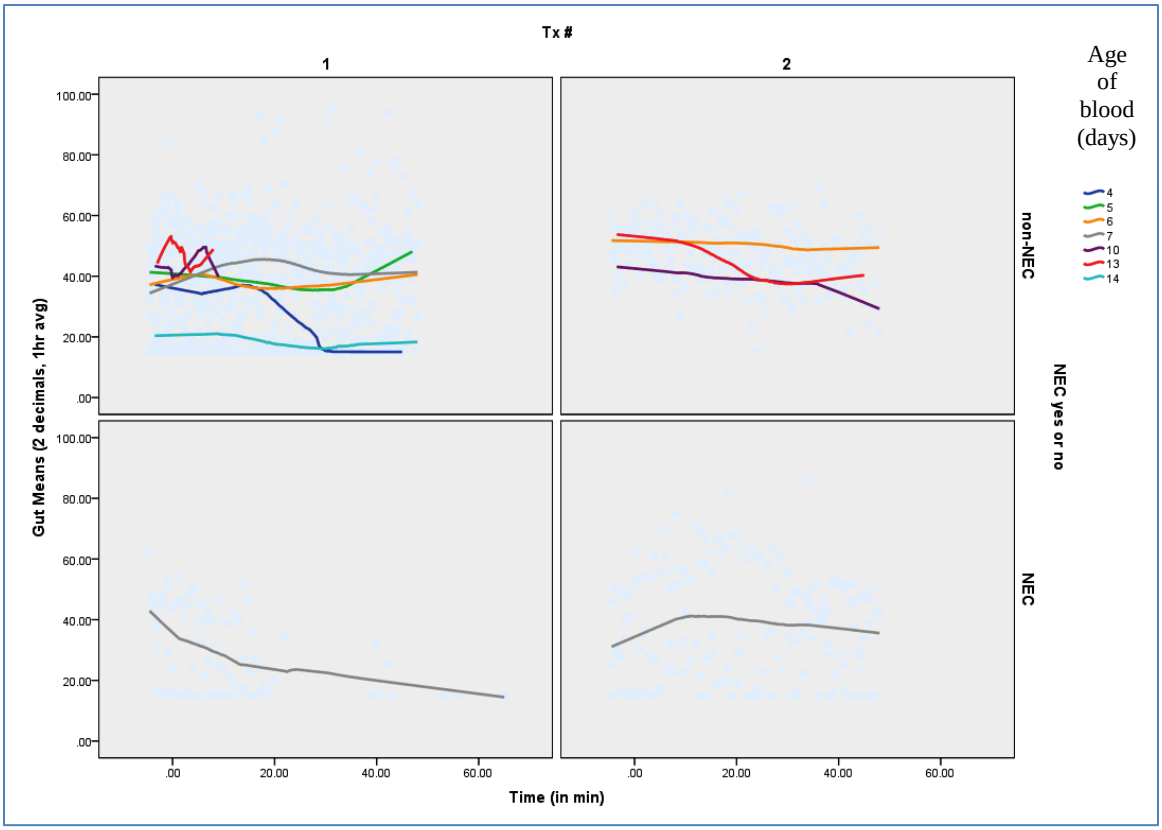

Figure 4-8. MP over time related to age of blood. Plots of MP patterns associated with the age of blood infused over time for each transfusion event. Graphs separated by TR-NEC and non-NEC cases. The range of blood for all events was 4-14 days old. The lines in each graph are Loess smoothing fit lines using weighted least squares.

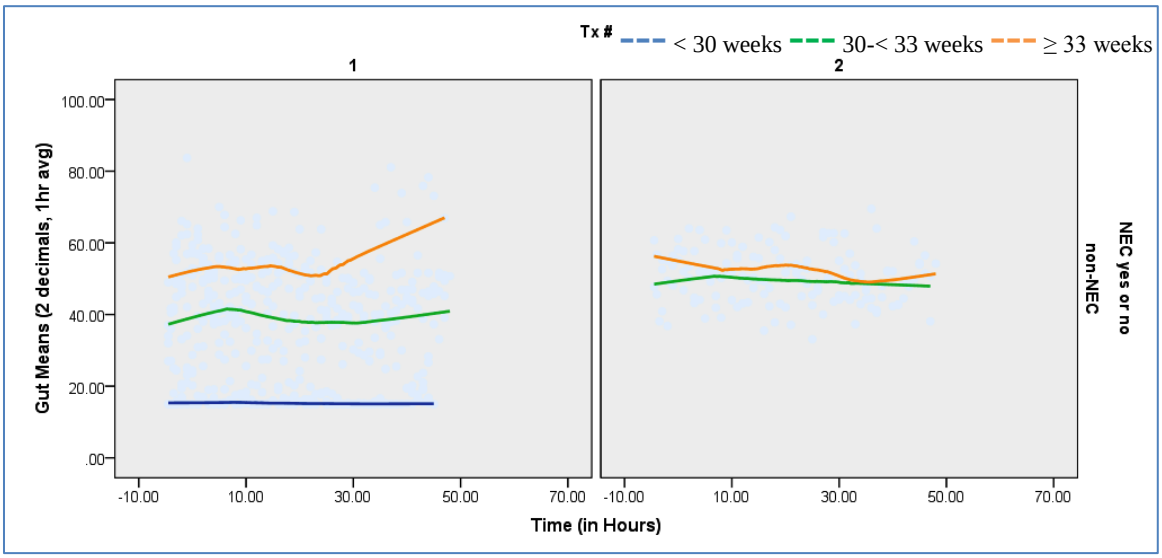

Figure 4-9. MP patterns associated with transfusion events receiving < 6 day old blood. No TR-NEC events received blood ≤ 6 days old. MP patterns remained unchanged or increased over time subsequent to the first transfusion event. There was a decreased trend over time following the second transfusion event for those ≥ 30 weeks cGA. The lines in each graph are Loess smoothing fit lines using weighted least squares.

Results related to Specific Aim 3

Specific Aim 3. Determine if NIRS rSO_2 tissue oxygenation patterns differ in preterm subjects who do and do not develop TR-NEC following PRBC transfusions with and without feedings.

Research Question 3.1. What mesenteric tissue oxygenation patterns are noted in preterm subjects who develop TR-NEC compared to those who do not following a PRBC blood transfusion when no feedings were received during the transfusion event?

Research Question 3.2. What mesenteric tissue oxygenation patterns are noted in preterm subjects who develop TR-NEC compared to those who do not following a PRBC blood transfusion when feedings are given during the transfusion event?

Research Question 3.3. For preterm subjects who develop TR-NEC following a transfusion, what is the temporal relationship between transfusion event and TR-NEC onset?

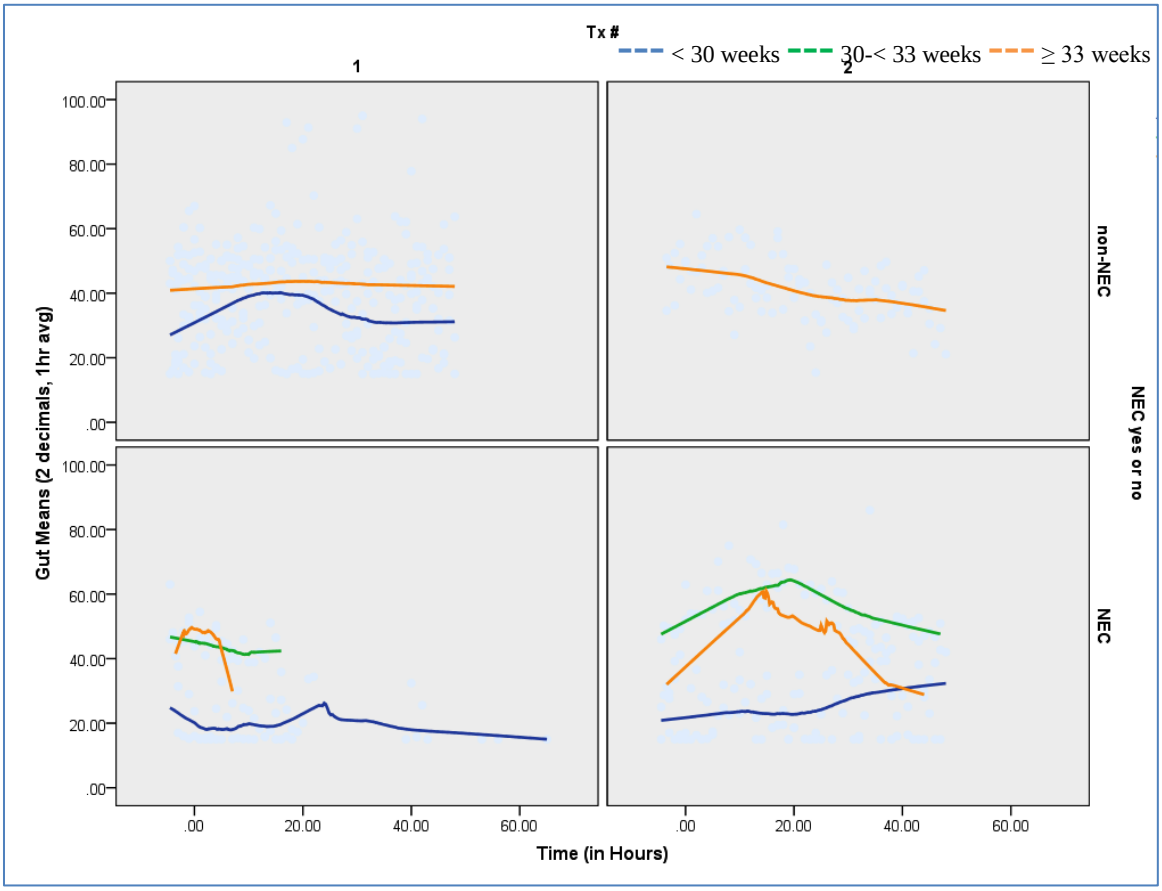

Figure 4-10. MP over time associated with transfusion events receiving > 6 day old blood. Graphs separated by TR-NEC and non-NEC cases and transfusion event. All events associated with TR-NEC received > 6 day old blood. (MLM) were used to evaluate expected MP temporal patterns nested within transfusion events and to test for overall impact of age of blood as well as interaction effects between age of blood and time. Events associated with administration of blood > 6 day old demonstrated decreased MP patterns over time subsequent to transfusion event (p=.063). The lines in each graph are Loess smoothing fit lines using weighted least squares.

MP in TR-NEC cases. Publication 3 of this dissertation addresses in detail the MP pattern changes in preterm subjects who developed TR-NEC. Although not statistically

significant (p=0.175), mean PNA for TR-NEC cases was less than non-NEC cases. To accurately explain the changes in MP patterns observed, the case-series publication compared TR-NEC cases to four preterm subjects of similar corrected gestational ages so that gastrointestinal maturational changes would be similar among the comparison groups. We concluded that MP patterns were different in cases that developed medical TR-NEC compared to those that developed surgical TR-NEC, and that percent changes from baseline were very different between these cases. We also found that MP patterns between TR-NEC and non-NEC cases were descriptively different. However, MP were lower for all subjects < 30 weeks corrected gestational age (cGA), and the TR-NEC cases had a mean cGA of 26.5 (± 2.1); with only one infant > 30 weeks cGA (Subject 3; 33.5 weeks cGA). Additionally, 3 of the 4 TR-NEC cases were associated with concurrent enteral feedings during the transfusion event, and all were receiving preterm formula. The mean volume of feedings for the 3 TR-NEC events associated with enteral feedings was 142.67 cc/kg/day (± 40.67) which was higher than the mean of all non-NEC events (n=7) associated with enteral feedings 129.29 (± 56.15); however, using unpaired independent-test, this difference was not statistically significant (p=0.72). However, three of the four TR-NEC events were associated with greater volumes of blood infused in < 72 hours (2 full volume transfusions of ≥ 15cc/kg) and compared to the total volume of blood given for all non-NEC events, this was statistically significant (p<.0001). No other events in the entire sample were associated with this volume of blood within the same timeframe.

Near-infrared spectroscopy directly revealed impaired MP during the onset of TR-NEC, but not cerebral or renal perfusion. In addition, conventional monitoring systems did not detect physiologic alterations until after clinical symptoms of TR-NEC presented. In all cases of TR-NEC, pulse oximetry values did not decrease simultaneously with diminished mesenteric rSO_2 values. Heart rate, respiratory rate and blood pressure monitoring remained stable until clinical symptoms of TR-NEC presented (abdominal distention, feeding intolerance and radiographic

changes). Subject 15, who eventually developed gastrointestinal perforation, maintained adequate pulse oximetry readings (low 90s) throughout the period of extensively decreased mesenteric rSO_2 readings (from baseline of 63 to 15) for an extended period of time from the first transfusion event until pneumoperitoneum was observed on abdominal radiograph.

This study found that TR-NEC observed in this study was related to younger gestational age subjects who were more likely to be fed during the transfusion event, received formula feedings in larger volumes than subjects who did not develop TR-NEC. All TR-NEC cases developed subsequent to the second transfusion event and occurred < 48 hours subsequent to the transfusion. We further found that conventional physiologic monitoring systems were unable to detect MP reductions until clinical symptoms were apparent.

Conclusions

Preterm subjects with lower gestational age have overall lower mesenteric MP prior to, during and subsequent to PRBC transfusions. As corrected gestational age increases, MP improves. Receiving a second transfusion event (split) does not improve MP, but is associated with MP declines over time. Receiving enteral feedings during a first or single volume transfusion event does not negatively impact MP; however, if a second transfusion is received (split volume) MP reduces with concurrent enteral feeding administration. TR-NEC events are associated with declining MP during first transfusion events; however, during second transfusion events, MP is highly variable with initial increases subsequent to TR-NEC onset followed by dramatic declines following therapeutic interventions and disease progression. Only one infant showed dramatic improvement in MP subsequent to TR-NEC onset, but this was following peritoneal drain placement for pneumoperitoneum.

The administration of older blood is associated with lower MP values and is not related to gestational age. Subjects who received blood > 6 days old demonstrated more negative slopes

over longer periods of time than subjects receiving blood ≤ 6 days old. In addition, four subjects who received blood > 6 days old developed TR-NEC within 48 hours of receiving a PRBC transfusion.

Subjects who developed TR-NEC have lower gestational age, younger postconceptual age and lower birth weights and current weights than subjects who did not develop TR-NEC. These subjects were also more likely to receive feedings during the transfusion event, and fed a bovine formula. Additionally, TR-NEC cases received greater volumes of transfused blood infused in a shorter timeframe relative to subjects who did not develop TR-NEC.

Introduction of Publications

Publication 1:

Title: **Understanding Near Infrared Spectroscopy**

Authors: Terri Marin, MSN, NNP-BC

James Moore, MD, PhD

Journal: Advances in Neonatal Care

Citation: Marin, T., & Moore, J. (2011). Understanding near-infrared spectroscopy. *Advances in*

neonatal care, 11(6), 382-388.

Purpose of publication and relation to this dissertation: This publication explains the technology of near-infrared spectroscopy (NIRS) in terms of what it measures, how it works and current application in the preterm population. A comprehensive review of previous studies using NIRS to evaluate perfusion patterns in pretem infants was presented. The content of this publication is directly related to the use of NIRS for the current disseration study, and supportive references relative to the reliability and validity of this measurement tool were described.

Publication 2:

Title: **Transfusion-Related Necrotizing Enterocolitis: A Conceptual Framework**

Authors: Terri Marin, MSN, NNP-BC

Ora Strickland, PhD, RN

Journal: The Journal of Perinatal and Neonatal Nursing

Status: This publication has been accepted for publication and is currently under final editorial review following requested revisions.

Purpose of publication and relation to this dissertation: Current pathogenic theories related to gastrointestinal immaturity in prematurity, packed red blood cell transfusions and enteral feedings were extensively discussed as they interrelate to form the conceptual framework for transfusion-related NEC. This framework was developed from these theories to guide the current dissertation study specifically focusing on gastrointestinal perfusion as the central measurement. Previous studies supporting the development of this conceptual framework were presented, and how the current study was designed incorporating these elements.

Publication 3:

Title: **Red Blood Cell Transfusion-Associated NEC in VLBW Infants: A Near-Infrared Spectroscopy Investigation (NIRS)**

Authors: Terri Marin, MSN, NNP-BC

James Moore, MD, PhD

John Roback, MD, PhD

Niki Kosmetatos, MD

Paul Weiss, MPH

Melinda Higgins, PhD

Linda McCauley, PhD, RN

Ora Strickland, PhD, RN

Cassandra Josephson, MD

Journal: Transfusion Medicine

Status: Submitted for Review

Purpose of Publication and relation to this dissertation: This case-series describes a portion of the results from the current dissertation study, specifically focusing on the infants that developed transfusion-related necrotizing enterocolitis. A comparison between these infants and four similar infants from this study was presented. This article addresses Specific Aim 3 of this dissertation concerning the patient characteristics, transfusion characteristics, perfusion patterns and temporal association of NEC development subsequent to packed red blood cell transfusions and the differences exhibited between infants that developed NEC and those that did not.

Publication 1:

Understanding Near Infrared Spectroscopy

Terri Marin, MSN, NNP-BC

James Moore, MD. PhD

Understanding Near-Infrared Spectroscopy

Research has demonstrated that the use of Near-Infrared Spectroscopy (NIRS) to monitor tissue perfusion at the bedside is a feasible, non-invasive and beneficial technology. Its use in cardiac, neurologic and gastrointestinal disease shows significant potential to further understanding of tissue perfusion pathology not reflected in current routine bedside monitoring modalities.[1-8] Current methods employed to evaluate oxygen delivery and tissue consumption are frequently non-specific. For neonates in the intensive care unit, these methods include urine output, lactate measurements, capillary refill time, blood pressure, oxygen saturation and others. Alterations in tissue perfusion are associated with pathologic conditions of prematurity that result in morbidity or mortality. Due to the immaturity of the vasculature tree, coupled with a narrow range of pressure autoregulation; even slight fluctuations in tissue perfusion can potentially result in ischemia leading to end organ damage.[9,10] NIRS, a new technology to the neonatal intensive care unit (NICU), allows for continuous measurement of tissue oxygenation which reflects perfusion status, and enables clinicians to directly monitor fluctuations in real-time. To potentially intervene and decrease harmful hypoperfusion episodes, clinicians must have a comprehensive understanding of what, when, and how perfusion is measured using NIRS.

What is NIRS?

NIRS was introduced in the 1970s as a technology which was capable of noninvasive monitoring of oxygenation in living tissue.[11] NIRS utilizes light

wavelengths (700-1000nm) similar to pulse oximetry for measuring tissue oxygenation. Pulse oximetry, however, depends on pulsatile blood flow and measures only the oxyhemoglobin in arterial blood as it leaves the heart. NIRS measures the difference between oxyhemoglobin and deoxyhemoglobin, which reflects oxygen uptake in the tissue bed. This measurement is reported as the *regional* oxygen saturation (rSO_2). NIRS measures the balance of oxygen that is delivered minus what is extracted at the tissue level. Because of the unique design of this technology, clinicians can directly monitor fluctuations in tissue oxygenation as they occur. During hemodynamic stability, tissue oxygenation uptake differs between regions of the body. Cerebral uptake is generally higher due to higher metabolic demands, while renal and splanchnic uptake is slightly lower indicating less metabolic activity. In NIRS measurement, this translates to normal cerebral readings being generally lower than somatic measurements, (60-80), and splanchnic/renal measurements being generally higher than cerebral (65-90).[12-14]To explain this concept further, lower NIRS readings can indicate two possibilities: (1) increased oxygen extraction at the tissue level, or (2) decreased blood flow altering oxygen delivery to tissues in the region being measured. Therefore, it is helpful to evaluate NIRS measurements in proportion for appropriate pathophysiologic interpretation. During episodes of hemodynamic instability, the neonatal intestine may be vulnerable to hemodynamic instability,[15] while other tissue beds may not. Using NIRS, this phenomenon can readily be examined because it provides real-time tissue oxygenation status that can guide interventions to treat underlying pathology and can further demonstrate the immediate physiologic response to intervention strategies.

Baseline trends must be obtained in all infants monitored with NIRS (see Figure 1) and due to inherent variability of these measurements, it is important to monitor baseline data over an adequate amount of time (several hours).[14] In addition, baseline values tend to change with increasing postnatal age, and may be affected by factors such as anemia, hypotension and acidosis.[13,14] Data suggest that cerebral rSO_2 values in stable preterm infants remains fairly constant, while larger fluctuations in renal and mesenteric measurements are common.[13,14] Therefore, the objective in NIRS measurement is to observe persistent and/or frequent changes from baseline that are greater than 15%.[14] Others have suggested that interpretation of NIRS measurement in ratio format (cerebral to somatic) provides a relative comparison between differential regional perfusion. Known as a cerebro-splanchnic oxygenation ratio (CSOR), values < 0.75 have been correlated with an increased risk for mesenteric ischemia. [5]

While NIRS technology is very similar to pulse oximetry, the difference is *what* is being measured. Studies have shown that pulse oximetry alone is insufficient in detecting tissue level hypoxia because only arterial oxygen saturation is being measured; it does not reveal if enough blood flow or oxygen delivery to a particular tissue bed actually occurs. One study found that arterial desaturation (SpO_2) in infants coincides with diminished mesenteric perfusion; however, when arterial saturations improved, mesenteric perfusion was delayed.[16] It has also been shown that in infants and children experiencing apnea, NIRS can detect cerebral oxygenation decreases one minute before pulse oximetry reflects the same decrease.[17] NIRS is highly correlated with superior vena cava saturations and is a better indicator of systemic perfusion than pulse oximetry in monitoring infants with single ventricle heart defects.[1] Data using NIRS has also

suggested that when arterial saturations fall to 70-80%, cerebral perfusion may not be compromised, but renal tissue perfusion is likely impaired, increasing the potential for ischemic organ injury.[2]

How does NIRS work?

NIRS monitors regional tissue oxygenation (rSO_2), by placing probes on different areas of the body such as the forehead (cerebral), abdomen (mesentery), and lower back (renal) (see Figure 2). Each probe consists of a light source and 2 photo-detectors to measure tissue oxygen levels at different tissue depths (see Figure 3). One path length measures surface level tissue oxygenation, and the other path length measures deep tissue oxygenation. The photons emitted from the light source scatter in the tissue bed and those that are not absorbed, are returned to the skin photodetector. By measuring the amount of light returned to the skin, NIRS values represent the amount of spectral absorption that is occurring in the tissue bed. This measurement represents the average of arterial, venous, and capillary oxygenation at the tissue level.[18] This rSO_2value represents a 'weighted average' of tissue oxygenation, with approximately 75–80% of the signal originating from venous measurement.[19]

The accuracy of NIRS has been correlated with invasive measurements such as SvO_2[3,20]jugular venous saturation[21]and gastric tonometry. [22]Studies have found strong correlations between tissue oxygenation index (TOI) measured by NIRS (the amount of oxygen extracted at the tissue level) and invasive measurement of cerebral blood flow and venous saturations.[3,20,21] NIRS has also been extensively utilized to monitor cerebral perfusion during cardiothoracic surgery.[3,4, 8, 23-27] Data have shown that rSO2 measurements were good correlates of cerebral perfusion to accurately detect cardiac

shunting pre- and post-operatively. In one study, data showed that prolonged low cerebral rSO2 values in postoperative infants were significantly associated with ischemia and subsequent periventricular leukomalacia. [23]Invasive gastric tonometry has also been shown to accurately correlate with mesenteric rSO_2 values.[22] Gastric tonometry calculates intramucosal pH (pHi) based on intraluminal pCO_2 and low gastric pH is an early warning of compromised mesenteric perfusion.[22] Kaufman found that anterior wall rSO2 measurements significantly correlated with pHi ($p < .0001$), SVO2 ($p < .001$) and a negatively correlated with serum lactate ($p < .0001$).[22] These studies support the use of NIRS to measure tissue oxygenation non-invasively and provide indices that can monitor trending pattern changes in real-time due to perfusion variability.[13,14]

The importance of NIRS technology is its ability to detect differences in tissue oxygen uptake that may not routinely be recognized using current routine modalities. The inability of the neonatal gut to maintain adequate blood flow during periods of hemodynamic instability may predispose the intestine to hypoperfusion and subsequent ischemia.[15] Prompt therapeutic interventions to minimize injury during these times could be possible with the use of NIRS monitoring.

How is NIRS being used in Neonatology?

Cerebral perfusion

NIRS has gained attention in Neonatology because of the ability to simultaneously detect differences in regional tissue oxygen uptake in different organ tissue beds. For infants undergoing cardiothoracic surgery, perioperative cerebral and mesenteric oxygen saturation monitoring with NIRS has proven beneficial in detecting

changes from baseline indicating possible hypoperfusion of tissue beds. During repair of complex cardiac conditions, continuous monitoring of cerebral perfusion is valuable as infants undergo cardiopulmonary bypass and hypothermia. Studies have shown that NIRS offers greater reliability than transcranial Doppler,[28] and aids in preventing potential intraoperative neurologic insult.[29] Preoperatively, NIRS can aid in monitoring tissue oxygenation of cerebral and somatic vascular beds in those awaiting corrective surgery, especially those receiving mechanical ventilation. In hypoplastic left heart syndrome (HLHS) infants awaiting palliation, NIRS can provide improved oxygenation monitoring of cerebral and somatic tissues.[4] Since these patients typically undergo pulmonary and systemic pressure changes, pulse oximetry monitoring of arterial saturations alone may not provide sufficient and accurate information regarding tissue oxygenation status. In a recent report from Children's Hospital of Wisconsin, investigators found that infants with HLHS continuously monitored with NIRS demonstrated fewer ventilation days than those not monitored with NIRS.[4] In this study and others, NIRS technology aids in overall management therapies of infants with complex cardiac disease.

NIRS has been used to monitor cerebral perfusion during and following patent ductus arteriosis (PDA) ligations; however, study findings varied.[30] One study found increased oxygen extraction (decreased NIRS readings) during this procedure, while others[26,31] found no significant changes. However, in these particular studies, post ligation monitoring times differed. Zaremella et al found decreased cerebral readings at 14 and 27 minutes following the procedure,[30] while Huning et al found no changes up to 10 minutes post procedure.[26] A third study followed infants for 1 hour post ligation

finding an immediate and acute increase in cerebral oxygenation, but values returned to normal and remained at pre-procedure baseline for 1 hour post PDA clip.[31]These findings suggest that variability in cerebral perfusion exists among neonates undergoing PDA ligation, and using NIRS may increase detection of changes in tissue oxygenation that could prompt potential intervention, especially for those on mechanical ventilation or receiving supplemental oxygen for respiratory compromise.

It has been recommended that NIRS is a suitable screening mechanism to monitor perfusion status in infants receiving indomethacin for PDA closure.[32,33] The impact of blood flow changes during pharmacologic induced PDA closure can easily and feasibly be monitored with NIRS, serving as a less expensive alternate to multiple Echocardiograms. It has been demonstrated that regional perfusion changes may be used as a predictor of the need for an Echocardiogram, and regional (cerebral and somatic) perfusion changes occur in extremely low birth weight (ELBW) infants treated with indomethacin.[32] Underwood and colleagues found that cerebral rSO_2decreases and somatic rSO_2increases following indomethacin administration, suggesting that indomethacin decreases cerebral blood flow and does not significantly increase following ductal closure; but the effect of PDA closure on renal blood flow may be negated by increased post-ductal blood flow.[32] However, other investigators found that cerebral rSO_2 was diminished in infants with PDA compared to those without, and a subsequent rise in cerebral rSO_2 readings occurred 36 hours following indomethacin administration.[33] Since impaired neurodevelopmental outcomes are potentially associated with hemodynamically significant PDA in preterm infants,[34]and variance exists in study findings, NIRS offers a

reliable method to easily monitor these vulnerable infants in an attempt to intervene sooner to improve cerebral oxygenation and blood flow.

Somatic perfusion

Studies examining mesenteric (splanchnic) perfusion using NIRS are limited. Interpretation of the rSO_2 of the mesenteric bed is challenging due to the hollow structure of the intestine, peristalsis and large surface area. Furthermore, studies have shown that mesenteric tissue oxygenation is highly variable.[13,14] Because NIRS can simultaneously monitor perfusion at different sites, calculating a ratio of these differences may provide more accurate information of differential tissue oxygenation to certain organs. By placing a probe just below the umbilicus, it is possible to monitor mesenteric perfusion, and then analyze this saturation compared to cerebral readings. As previously discussed, this is known as the CSOR measurement. Fortune[5] was the first to demonstrate the potential significance of this value. During hypotensive states, splanchnic tissue perfusion may be diminished and blood may be preferentially diverted to vital organs.[15] By simultaneously measuring cerebral and splanchnic regions with NIRS, Fortune found that infants with confirmed acute ischemic bowel had CSOR < 0.75 (splanchnic NIRS value divided by cerebral NIRS value), and those with non-ischemic bowel had an average CSOR 0.96 (0.83-1.02). In this study, 40 neonates were examined; 10 with acute abdominal disease, 29 with normal abdomens, and 1 with hypoxic-ischemic encephalopathy. Of the 10 neonates with acute abdomens, 5 had confirmed NEC. Two site NIRS (cerebral and splanchnic) were applied within 24 hours of NICU admission and measurements were obtained daily for a total of 5 minutes at each reading. The results demonstrated that all 5 infants with NEC demonstrated a CSOR < 0.75.[5] Given that NEC

affects approximately 4500 infants each year, and of those requiring surgical intervention mortality rates are 30-50% ,[35] a technique to monitor for its occurrence would be beneficial. Further, the pathogenesis of NEC remains elusive complicating treatment and prevention strategies.[36,37] NIRS provides an avenue to increase awareness of splanchnic tissue ischemia, even when arterial oxygenation is normal. By using a CSOR cutoff value of 0.75, it may be possible to identify infants at risk for developing this devastating disease.

Other studies have also examined splanchnic perfusion patterns to identify alterations associated with common disease processes. One case report found significant mesenteric desaturation using NIRS in an infant during the early stages of NEC who also had congenital heart disease.[6] It is not known whether ischemia is the initiating or resultant factor contributing to NEC development; however, in this particular case study, reduced gut tissue oxygenation existed during early phases supporting the premise that ischemia may play a role in NEC pathophysiology.[6] In another case report by Meier et al,[7] mesenteric saturation greatly improved following PDA ligation. Prior to surgery, NIRS splanchnic readings averaged only 16% and increased to a mean of 34.1 +/- 12.6% immediately after the surgical ligation of a large PDA. This report demonstrates the potential for mesenteric hypoxia in patients with a hemodynamic significant PDA and the potential predisposition for gut ischemia prior to treatment. The reason for using a cerebral tissue oxygenation in ratio format is that cerebral oxygenation may be maintained through autoregulatory processes in the stable preterm infant,[2,14] while mesenteric circulation may be compromised.[15]However, VLBW infants are at risk for loss of cerebral autoregulation, especially in the first days of life and during

hemodynamic instability.[9,10,38] NIRS monitoring of both vascular beds offers a feasible system to non-invasively monitor for compromised tissue oxygenation that otherwise may not be recognized for a much longer time increasing the risk for organ injury.

NIRS has also been used to evaluate splanchnic perfusion patterns in premature infants tolerating full feeds. It has been shown that splanchnic tissue oxygen extraction actually increases postprandially in preterm infants receiving full orogastric bolus feedings, while cerebral oxygenation remained unchanged.[39] Therefore, CSOR values increased following feedings. This would make sense from a physiologic standpoint as the body diverts blood flow to carry away digested nutrients. Doppler ultrasonography has previously shown that superior mesenteric blood flow increases postprandially,[40,41] supporting these findings. Given this data and the results of Fortune's study,[5] CSOR measurements could be beneficial in analyzing infants who may ***not*** be tolerating feeds prompting further investigation into possible factors related to intolerance. Because this technology can be used at the bedside without interrupting routine care, infants can be easily monitored for perfusion changes before incurring ischemic insult.

In addition to monitoring splanchnic perfusion, NIRS is also capable of simultaneously monitoring renal perfusion; however studies focused on this aspect are limited. In one study, desaturation episodes (SaO2 70-80%) not accompanied by bradycardia in mechanically ventilated patients were associated with renal compromise but not cerebral, and renal recovery to baseline was incomplete at desaturation levels below 76% after one minute following hypoxic episodes.[2] This study supported prior findings that renal saturations decreased while preserving cerebral saturations during cardio-pulmonary bypass surgery.[42] These findings suggest that cerebral autoregulation

likely exists in newborn infants.[2,43] Berens et al. found that regional (somatic) oxygenation decreased following aortic coarctation repair in infants less than 1 year of age due to inadequate collateral circulation.[44] The benefit of two-site NIRS monitoring is clearly exhibited in these studies to monitor for the risk of somatic ischemia even while cerebral preservation is attempted through autonomic nervous system control.

NIRS measurement in infants with respiratory compromise

Cerebral tissue oxygenation is influenced by blood flow, volume and consumption and cerebral blood flow can be altered by pCO_2 levels. Theoretical concerns about wide fluctuations in cerebral perfusion, accentuated by oscillations in pCO_2 levels, could place premature infants at greater risk for intraventricular hemorrhage (IVH).[45] Hypocapnia causes decreased neuronal perfusion thus potentially increasing the risk for periventricular leukomalacia (PVL).[46,47] For infants requiring respiratory support, including mechanical ventilation, maintaining normal pCO_2 levels becomes challenging, especially in the first days of life as pulmonary vascular resistance changes. Furthermore, the capability of the premature brain to maintain protective autoregulation remains compromised in a number of studies[9,10,38]; however, this topic remains in debate. With the use of NIRS, the influence of pCO_2 levels on cerebral hemodynamics demonstrates a positive correlation during the first few days of life in premature infants.[48] As pCO_2 levels decline, cerebral blood flow and oxygenation also decline increasing the risk for ischemia and PVL; conversely as pCO_2 levels rise, cerebral blood flow increases which could lead to IVH.[48] Mean arterial blood pressure has been shown to have no effect on cerebral oxygenation,[48] even in those with respiratory distress syndrome

(RDS)[49] suggesting that pCO_2 changes may have a greater impact. In addition to fluctuating pCO_2levels, cerebral pressure-passivity vacillates during the first few days of life[9] and NIRS reveals that cerebral autoregulation may be lost as much as 50% of the time.[9] Sick preterm infants frequently exhibit impaired cerebral autoregulation, in addition to having an associated higher mortality.[10] It is evident that wide variations for hemodynamic regulation exist in the preterm population requiring reliable and continuous trend analysis to assist with therapeutic interventions. This appears to be an area NIRS could provide more reliable data to effectively guide prompt intervention strategies.

Future Direction

Many avenues exist for the use of NIRS in neonatology. Because of the ability to non-invasively monitor at the bedside without interrupting routine care, NIRS provides continuous tissue oxygenation monitoring which permits assessment of perfusion status. Although research has demonstrated its benefit in cerebral perfusion, PDA, and NEC, further studies are needed to establish the use of this technology within the preterm population. In addition to its perioperative use, bedside NIRS monitoring provides a potential mechanism to reduce risks associated with impaired perfusion that may lead to ischemia and organ damage. NIRS can augment current physiologic monitoring in terms of organ perfusion status in the preterm population; and in certain disease states characterized by altered perfusion patterns (NEC, IVH, and PVL),may eventually be useful in the prediction, prevention and treatment of these disease states.

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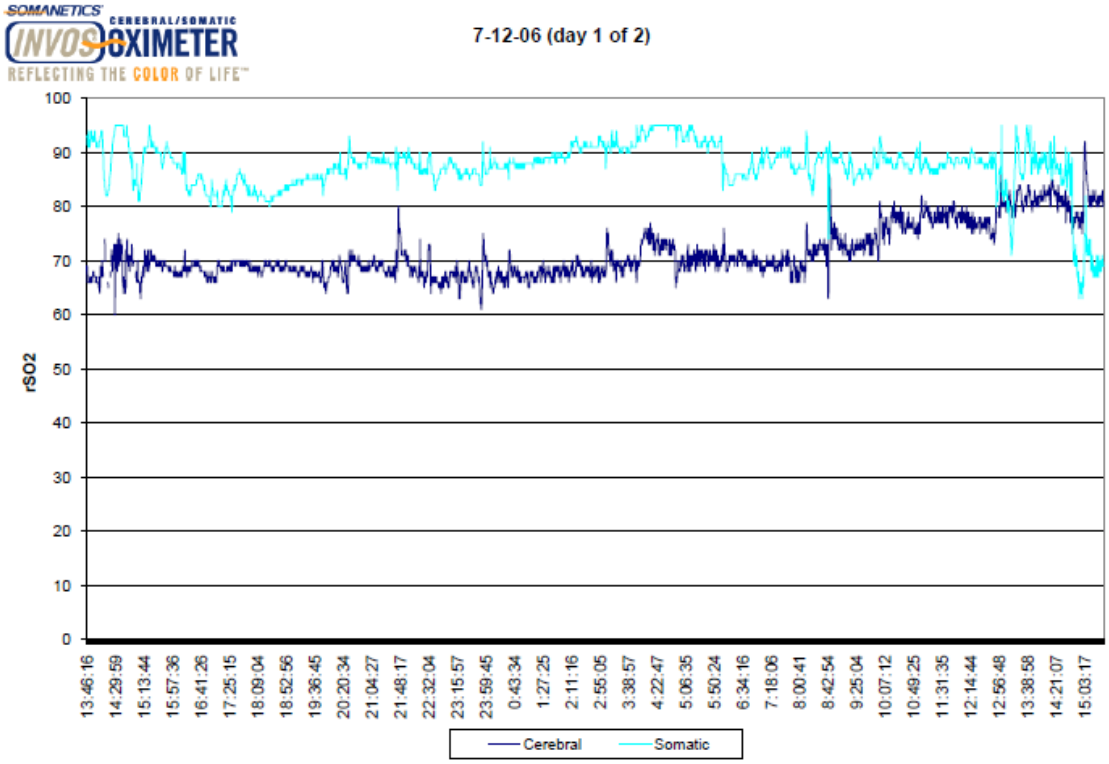

Figure 1 Legend: *Figure 1: NIRS graph depicting normal cerebral and somatic tracings. Image used by permission from Nellcor Puritan Bennett, LLC, Boulder, Colorado, doing business as Covidien.*

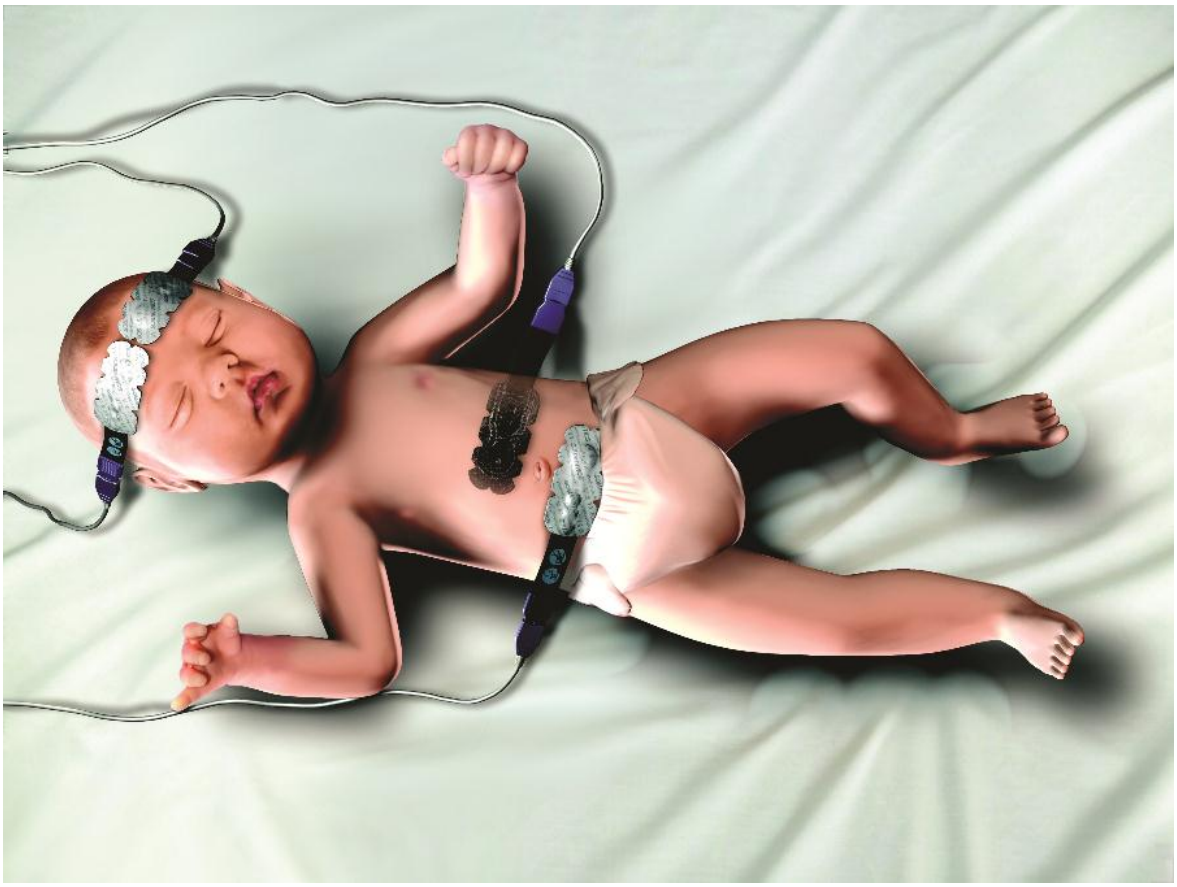

Figure 2: Illustration depicting NIRS probe placement on different areas of infant body to simultaneously measure perfusion. Image used by permission from Nellcor Puritan Bennett,LLC, Boulder, Colorado, doing business as Covidien.

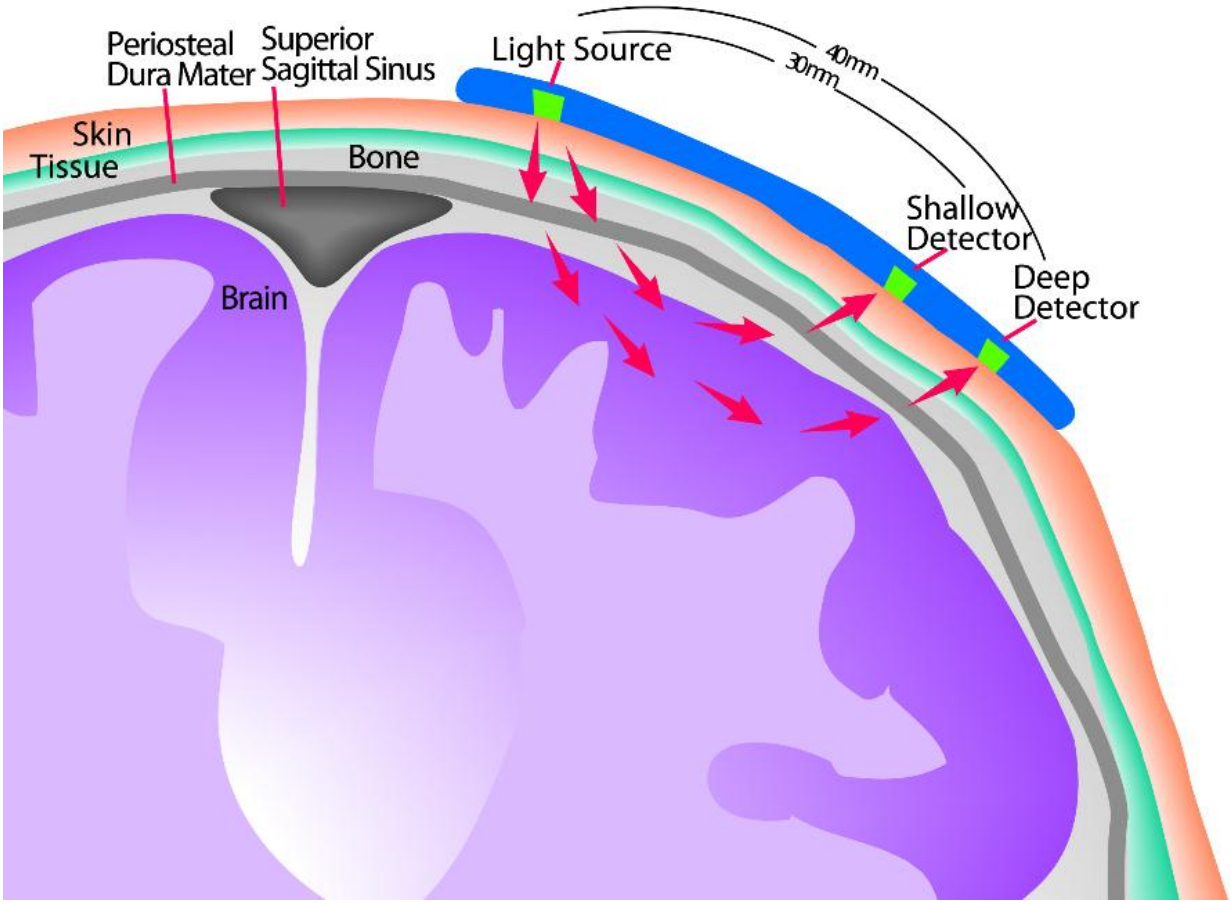

Figure 3: Illustration depicting cerebral sensor probe depth measurement: rSO2 value represents the deep measurement minus the surface measurement. Image used by permission from Nellcor Puritan Bennett, LLC, Boulder, Colorado, doing business as Covidien.

Publication 2:

Transfusion-Related Necrotizing Enterocolitis: A Conceptual Framework

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Transfusion-Related Necrotizing Enterocolitis: A Conceptual Framework

Abstract

Necrotizing enterocolitis (NEC) is a disease primarily of prematurity that is characterized by partial or entire gut necrosis related to impaired tissue perfusion. Surgery is required in about half of those affected and mortality rates of 30 to 50% occur in those requiring surgery. Recent studies report approximately 25-35% of infants receiving packed red blood cell (PRBC) transfusions develop temporally associated NEC. Although there are many known risk factors for NEC, this paper will focus on three contributing factors: packed red blood cell transfusions, enteral feedings and gastrointestinal immaturity. Previous data suggest that these factors may interact to affect neonatal intestinal tissue oxygenation which may lead to tissue ischemia resulting in intestinal injury. This paper will review previous research examining these variables and how they their interaction may increase the risk for NEC development. Additionally, incorporation of the proposed framework to guide research will be discussed.

Keywords: Transfusion-related necrotizing enterocolitis, premature infants, blood transfusions, near infrared spectroscopy, perfusion

Introduction

Necrotizing enterocolitis (NEC) is a disease common in premature infants that results in bowel necrosis[1] which affects approximately 4500 infants annually in the United States.[2] It is associated with high mortality and morbidity rates resulting in over $600 million in-hospital costs annually. Approximately half of the preterm infants with NEC undergo surgery with associated mortality rates ranging 30 to 50%. Infants that survive are at high risk for complicated morbidities.[2]

The pathogenesis of NEC is most likely multifactorial, but the specific processes involved are unclear. Multiple risk factors that predispose preterm infants to NEC have been identified, however prematurity is the only clear predictor, and is inversely related to gestational age with the highest incidence occurring in infants < 1500 grams within the first six weeks of hospitalization.[3] Despite four decades of extensive research, specific associations and causal factors for the disease remain elusive, prevention strategies unclear, and its incidence unchanged.[1,4]

Based on prior research, clinical reports, and pathophysiologic principles, this conceptual framework was developed to combine specific factors that may explain changes in tissue oxygenation patterns that reflect mesenteric perfusion status during and subsequent to packed red blood cell (PRBC) transfusions given to preterm infants and how these changes may contribute to NEC development. These factors are divided into three categories: 1) transfusion, 2) feeding, and 3) gastrointestinal immaturity factors. Previous studies suggests that these factors may act singly or interact together to affect tissue perfusion in the neonate's gut.[5-12] This article presents a review of transfusion-related NEC research, offers an explanatory framework that may be used to guide exploratory research to identify the nature in which these contributing factors may lead to the development of NEC and offers a feasible method for measuring mesenteric perfusion patterns.

Overview of Conceptual Framework

This conceptual framework was based upon a theoretical perspective originally developed by Lin and colleagues describing pathophysiologic components related to NEC; [13] however, the current model introduces specific variables relevant to transfusion-related NEC. Based on previous research, three major components emerged for one pathway of NEC

development that relate to the preterm infant's immature gastrointestinal system, blood transfusions and feedings. Although each of these explanatory factors may independently relate to NEC pathogenesis, the combination of their effects on gut perfusion patterns in the neonate have not been previously specified.

Although the pathogenesis of NEC is most likely multifactorial, one important aspect involves bacterial colonization and resultant stimulation of the inflammatory cascade. The premature intestine has an ineffective immune response, impaired barrier, slow motility and inefficient circulatory autoregulation that increase susceptibility to epithelial surface injury.[13] Proinflammatory and counterinflammatory mediators are released in response to pathogenic bacterial colonization and translocation, and the interaction of these mediators is key in maintaining intestinal homeostasis.[14] Recent evidence suggests that in the presence of ineffective circulatory gut regulation and immature immune response, a preponderance of proinflammatory mediators may occur increasing the likelihood for vasoconstriction and potential mesenteric ischemia.[13,15] It is unclear whether ischemia is actually the initiating event or the result of this process.[16] Many factors have been identified as risks for NEC development, but prematurity is the only uniform predictor as NEC follows no clear pattern for incidence, prevalence, or specific causative pathogenic agent. [2,17]

The transfusion of packed red blood cells (PRBCs) has been identified as a possible factor for NEC development in premature infants. Several studies have temporally linked PRBC transfusions with NEC in preterm infants,[6,7,9,18] and one factor may be related to increased storage time of blood.[7] During storage, red cells undergo changes that can potentially cause sludging in the microvasculature of the preterm gut which may impair tissue perfusion.[19,20] Enteral feedings may further exacerbate this problem by reducing blood flow to the gut. Doppler

studies suggest superior mesenteric flow is reduced postprandially following a blood transfusion increasing the risk for possible perfusion impairment.[5] Lack of gut maturity may further aggravate the scenario.

In sum, altered tissue perfusion may disrupt intestinal mucosal surfaces allowing for bacterial colonization which stimulates an inflammatory response and subsequent vasoconstriction which further impairs perfusion, leading to ischemia and possible NEC. [1,13,16] Therefore, gastrointestinal immaturity factors, PRBC transfusion factors, and feeding factors are purported to potentially interact and contribute to the development of NEC, however; the duration and degree to which this insult may be related to actual bowel necrosis is unknown.

Gastrointestinal Immaturity Factors

The central element of this conceptual framework relates to the pathophysiology of the immature gut. Gastrointestinal immaturity is inversely related to gestational age and these factors may work singly or together to contribute to the development of NEC. These factors include a compromised gastrointestinal barrier, dysmotility, immature immune response, and impaired intestinal circulation – all of which can increase susceptibility to injury and alter intestinal perfusion.

Compromised Gastrointestinal Barrier.

Any factor that impairs the integrity of the immature gut can contribute to gut injury in the preterm infant. It has been postulated that the integrity of the epithelial intestinal barrier is enhanced by the introduction of enteral feedings, but compromised by conditions such as stress, indomethacin administration, glucocorticoids and enteral fasting.[21] Animal studies indicate that formula feedings are associated with cellular and intestinal epithelial injury[22,23] and breast milk feedings may offer greater protection. Recent evidence suggests early trophic feedings of breast

milk may stimulate the development of intestinal barrier function in preterm infants. [24,25] In general, feeding delay or intolerance may further increase susceptibility of the immature gut barrier to injury.[24,26] Once the mucosal layer is damaged, bacterial colonization can occur increasing the risk for NEC development.

Dysmotility of the Gut.

The immature gut of the preterm infant is often characterized by poor motility because adequate gastrointestinal motility does not mature until the third trimester, or around 34 weeks gestation.[27,28] Dysmotility can lead to accumulation of harmful substances and further predispose the immature gut barrier to injury.[29] Enteral feedings have been implicated in the pathogenesis of NEC due to inefficient digestive capability and dysmotility of the immature intestine which possibly lead to mucosal injury.[28] Data suggest a relationship with feeding advancements, feeding intolerance and NEC.[28,30] Further increasing the likelihood of epithelial damage is the inadequate absorption and digestion of nutrients which may cause acid production, accumulation and subsequent injury. [23,31,32] Bacterial translocation following the introduction of feedings has been postulated as the catalyst for this occurrence.[33]

Immature Immune Response.

The role of the preterm infant's innate immune system and NEC has been the focus of numerous research studies over the past several years. Recent evidence suggests there may be an overexaggerated immune response to invading pathogenic bacteria [34] increasing the risk for cellular apoptosis[35,36] and subsequent intestinal epithelial injury. Cytokines are released in response to invading organisms following inflammatory response stimulation. The cytokine cascade involves the release of proinflammatory and counterinflammatory mediators.[14] Proinflammatory mediators capable of potent vasoconstriction, such as tumor necrosis factor-α

(TNF-α), platelet activating factor (PAF) and interleukins (IL-1β and IL-6), are elevated in preterm infants with NEC. [37-42] However, recent evidence suggests vasodilatory anti-inflammatory mediators (IL-8 and IL-10) are also present in infants with NEC, and may be reflective of an attempt to regulate the effect of proinflammatory cytokines following the initial inflammatory stimulus.[43] Studies indicate that the extent of the anti-inflammatory response may be insufficient favoring greater vasoconstriction with proinflammatory cytokine predominance. [15,43] Further research is needed to establish definitive evidence and increase our understanding of the preterm infant's immune response to infection, and how these responses vary in different clinical situations.

Impaired Intestinal Circulation.

Ineffective intestinal circulatory regulation may play a significant role in NEC development.[44] This factor is associated with hypoxic episodes that may compromise intestinal circulation. During periods of hypoxia, the immature gut is deficient in maintaining adequate blood supply and tissue oxygenation through ineffective autoregulatory mechanisms, thereby increasing the risk for ischemic insult.[44,45] A hypoxic-ischemic insult that occurs at birth is unlikely to be associated with the NEC development;[46] however, repeated hypoxic episodes during the postnatal period are likely to place the preterm infant in jeopardy for reduced intestinal circulation.[47] Further, nitric oxide endothelial production may be altered in the preterm gut predisposing the mesenteric vasculature to vasoconstriction and decreased perfusion.[16,44] Nitric oxide helps regulate vascular tone through vasodilatory effects; therefore, depletion of its supply disrupts vessel homeostasis.[48] Animal models suggest that nitric oxide production and supply may be lower in the presence of NEC.[49,50] Other studies have shown associations

between NEC development and genetic polymorphisms of nitric oxide precursors such as vasoendothelial growth factor[51] and carbamoyl phosphate synthetase.[52] These findings are extremely interesting, however; further research is needed to examine processes related to immature autoregulation of blood flow to the preterm gut and its relationship to NEC development.

Transfusion Factors

Research related to adverse outcomes associated with transfusions among preterm infants is limited; however, data suggest increased mortality rates if a transfusion is received.[53] Retrospective studies examining transfusion-related NEC report between a 25-35% incidence, [8,54,55] and that a temporal association exists between transfusions and NEC onset occurring immediately and up to 96 hours post transfusion.[8,11,12,54] Further, these studies have found that infants with low birth weights < 1500 grams, low gestational ages at birth and greater intensive care needs were more likely to develop NEC following transfusions.[8,54] However, effects of specific factors related to transfusion practice (volume and duration of transfusion, age and length of irradiation) on mesenteric perfusion are unknown in stable, growing preterm infants receiving blood transfusions for anemia of prematurity. Suggested mechanisms involved in transfusion-related NEC include perfusion-reperfusion injury,[56,57] blood hyperviscosity,[58] ineffective or overexaggerated immune response,[59] and prolonged storage of donor blood.[7,60]

It has been purported that there is a relationship between the length of blood storage and the effect of "storage lesion," a phenomenon related to cellular degradation of banked blood.[19,61-63] To minimize multiple donor exposure and reduce infection risks, preterm infants may receive multiple transfusions from one unit of stored blood.[64] Stored RBCs undergo biochemical, metabolic, and molecular changes that may alter microcirculation, potentially causing

microsludging in capillary beds.[19,59,62] While administering stored blood may decrease exposure to multiple donors, it may increase the risk for altered perfusion. Currently, there are no guidelines in the United States specifying the maximum age of blood to be administered to high-risk preterm infants.[65]

Prolonged storage of red blood cells can have a devastating effect on cell structure and functional capacity. Although the life span of in vivo RBCs is 90 to 120 days, the life span of stored blood is shortened by half, with most red cells only surviving 45 days.[63] One study found that after 14 days of storage, RBC deterioration is much greater suggesting that giving older blood may increase the risk for adverse outcomes. The mechanisms involved in accelerated aging seem to be related to loss of intracellular constituents; mainly adenosine triphosphate (ATP), 2, 3 diphosphoglycerate (2,3-DPG), and nitric oxide.[61,66] Additionally, irradiation of blood may accentuate this process.[67] Around day eight of storage, levels of ATP and 2,3-DPG rapidly decline[68] causing loss of membrane deformability, degeneration and cellular death. Stored RBCs incur significant membrane damage due to earlier apoptosis, with near complete destruction by day 42, even when stored in a preservative solution.[63] Increased oxygen-hemoglobin affinity results from the loss of 2,3-DPG which may impair oxygen release at the tissue level.[61,69] The loss of ATP and 2,3 DPG is associated with decreased membrane deformability potentiating cell hemolysis.[59,68] The senescent red blood cell loses the ability to effectively maneuver through constricted blood vessels which may cause microsludging, micro-occlusion and eventual hemolysis especially in the microvasculature. Hemolysis releases plasma free hemoglobin which rapidly depletes nitric oxide supply.[60] The net effect is reduced oxygen delivery to the tissue bed due to reduced flow, nitric oxide depletion and increased red blood cell oxygen affinity. Administration of stored blood may also stimulate an inflammatory response, as

cytokines and other proinflammatory mediators accumulate during storage. The degradation of RBCs as they age may induce leukocyte influx causing endothelial damage, tissue destruction and possible organ failure. [70] Preterm infants frequently receive blood transfusion due to anemia of prematurity,[71] and for those who receive stored blood, especially greater than 14 days,[62] such changes in the red blood cell may contribute to reduced intestinal perfusion. Therefore, future research is needed to analyze samples from transfused blood for age, length of irradiation, ATP, 2,3-DPG, plasma free hemoglobin levels, volume and rate in relation to intestinal perfusion.

Feeding Factors

Feeding factors including the timing of feedings related to blood transfusions, volume and type of feedings have been found to play a role in the development of NEC. [11] There is evidence of decreased intestinal blood flow following blood transfusions, and when feedings are given, flow is further impaired.[5] Doppler technology revealed diminished superior mesenteric artery flow in the postprandial state in preterm infants following blood transfusions, which suggests a predisposition to ischemia and possible risk factor for NEC.[5] Rapidly increasing both the volume and density of enteral feedings has long been considered a risk factor for NEC.[28,29,72] Although debates regarding optimal feeding regimens continue, infants fed breast milk have a lower incidence of NEC compared to preterm infants fed formula.[29,32,72]

Introduction of bacteria into the intestinal lumen through enteral feedings coupled with gut dysfunction in the premature neonate increases the risk for NEC.[29] If the tenuous epithelial lining of the intestine is disrupted or damaged, the risk for enteric bacterial colonization is enhanced triggering an inflammatory response. Additionally, enteral feedings elevate metabolic demands required for digestion which increase oxygen extraction at the tissue level. In the presence of a blood transfusion and factors previously discussed, this process may be further

complicated. Enteric bacteria ferment in the intestinal lining producing intraluminal gases that cause distention and further impair blood flow potentially worsening mucosal surfaces. When inflammatory mediators are released, severe vasoconstriction ensues significantly increasing the risk for impaired perfusion.[37]

To fully understand the role of feedings in contributing to NEC, it is imperative to examine perfusion patterns following a transfusion when a feeding is given, regardless of type or amount. If significant changes in intestinal tissue oxygenation are found to be associated with feedings, then type, volume and temporal relationship of feedings to blood transfusions should be investigated to determine the potential role of each to reduced intestinal perfusion.

Intestinal Perfusion

Adequate intestinal perfusion is necessary for nutritive delivery of arterial blood to the premature infant's gut. Intestinal ischemia is a predominant finding in NEC and may relate to the inability of the immature vasculature to regulate blood flow coupled with the effects of immune response mediators.[16,44] Since transfusion of stored blood may be related to altered microcirculation, and gut immaturity is vulnerable especially when enteral feedings are given, the risk for NEC may be heightened given these events.

As previously discussed, NEC occurs when the intestinal lining is compromised and pathogens enter through the disrupted mucosal barrier allowing for infection and inflammation to occur. Altered gut circulation and tissue oxygenation may cause further mucosal disruption and bacterial colonization. Ischemia may occur and can either precede or result from this disruption.[16] The inflammatory response releases vasoconstrictive mediators that may compromise the integrity of the bowel wall further reducing blood supply. Therefore, perfusion

worsens and a vicious cycle of tissue ischemia, further mucosal disruption, bacterial colonization, release of inflammatory mediators and vasoconstriction ends in NEC. [73]

Measurement of Variables

In order to conduct research using this conceptual framework, accurate operationalization of key variables is important. Prematurity is defined as an infant born < 37 weeks completed gestation. Level of gastrointestinal immaturity is inversely related to gestational age (greater immaturity is present with lower gestation age). Feeding factors include volume, timing and type of feeding as formula, breast milk or mixture of both. Transfusion factors include the age of blood, date of irradiation, quality (assessed by ATP, 2,3 DPG and plasma free hemoglobin levels), volume and duration of transfusion. However, the central concept from this model that needs accurate operationalization is intestinal perfusion.

Measurement of Perfusion

Given the central role that intestinal perfusion plays in the development of NEC, precise and accurate measurement is important. Simultaneous measurement of tissue oxygenation which reflects perfusion in the brain, intestines, and kidneys may be helpful in assessing the risk for the NEC development before, during and after a blood transfusion is given and in relation to feedings in order to evaluate differential oxygen uptake between cerebral and somatic vascular beds. Near infrared spectroscopy (NIRS) technology provides a direct measurement of tissue oxygenation in different organ beds.[74] Evaluating changes from baseline NIRS values during these events will aid in detection of independent or combined effects on tissue oxygenation patterns.

Near infrared spectroscopy (NIRS) was first introduced in 1977, and is an accurate approach to non-invasively measure tissue oxygenation.[75] NIRS differs from pulse oximetry in that it

measures the difference between oxygenated and deoxygenated hemoglobin at the tissue level, while pulse oximetry only measures arterial oxygenation. NIRS real time measurements provide regional saturation (rSO_2) of capillary beds where oxygen extraction occurs,[76] and studies have shown that pulse oximetry and other bedside monitoring devices are unable to delineate this regional difference.[77] By applying sensor probes to various parts of the body (e.g., head, abdomen, and back), NIRS provides simultaneous monitoring of different tissue beds (brain, gut and kidney) by revealing differential oxygen extraction reflecting perfusion to various organs.

Studies using NIRS have shown that while cerebral perfusion can be maintained during hypotension, mesenteric circulation may be diminished.[78,79] Simultaneous monitoring of the brain and gut analyzed in ratio format provide a measure of differential tissue bed oxygenation. During hemodynamic stability, cerebral tissues have increased tissue oxygen extraction due to higher metabolic demands generating lower rSO2 values compared to splanchnic tissues which extract less oxygen due to lower metabolic demand. [57,80] This difference can be represented in ratio format known as cerebral-splanchnic oxygenation ratio (CSOR) which provides a direct evaluation of splanchnic vs. cerebral perfusion. One study suggests that CSOR values ≤ 0.75 have been associated with increased risk for intestinal injury.[79]

Neonatal studies using NIRS to evaluate splanchnic perfusion have established reference ranges in stable, growing preterm infants,[80] those tolerating feeds,[81] and not tolerating feeds.[57] It was found that splanchnic rSO_2 values, but not cerebral, increased following feedings[81] and rSO_2 values were diminished in infants not tolerating feedings.[57] It was further found that preterm infants that developed NEC had significantly diminished rSO_2 values, with frequent "signal drop out" indicating substantial tissue oxygenation impairment.[57] One small study evaluated tissue oxygenation patterns in preterm infants receiving transfusions and found that cerebral values

immediately increased at the beginning of the transfusion, and splanchnic values gradually elevated midway and immediately post-transfusion; however; values began to decline at 12 hours post-transfusion.[10] However, no studies have evaluated CSOR or rSO_2 values related to the combination of RBC transfusions and effects of feeding, or impact of increased storage time on perfusion patterns. Therefore, to operationalize perfusion patterns during and subsequent to a transfusion event, rSO2 patterns used in conjunction with CSOR values will be used to identify differential tissue bed oxygenation patterns.

Previous studies have demonstrated wide perfusion variability of mesenteric tissue beds.[57,80] Therefore, to accurately evaluate mesenteric perfusion pattern changes, it is essential to establish baseline values, preferably over a considerable amount of time (at least 1-2 hours). NIRS records rSO_2 values in 30 second time points; therefore, mean rSO2 values calculated in 30 minute intervals allows for appropriate interpretation of changes over time. Changes from baseline evaluated as percent change may effectively capture significant alterations in perfusion. McNeill[80] et al found that stable preterm infants tolerating feeds frequently exhibited ~15% decreases; therefore it seems plausible to consider reductions greater than this—possibly 25-30% below baseline—to accurately evaluate meaningful mesenteric fluctuations. Additionally, it is important to identify the duration of these reductions and correlate to concurrent events, such as enteral feedings, PRBC transfusions, medications and changes in clinical status.

CSOR values may augment absolute rSO_2 value interpretation. In ratio format, derived by dividing mesenteric rSO2 by cerebral rSO2, CSOR values represent improvement or impairment of mesenteric perfusion in the presence of stable cerebral perfusion. However, if cerebral perfusion is highly variable or impaired, CSOR values may elevate. Therefore, CSOR values used independently of known absolute values may be misleading and should always be

interpreted within the context of absolute cerebral and mesenteric rSO_2 measurements. As previously discussed, research has shown that CSOR values < 0.75 have been associated with an increased risk for intestinal injury and this cut-off value is often used as a marker for impaired perfusion.[79] More recently, researchers postulate that preterm infants with CSOR values < 0.73 prior to PRBC administration demonstrated greater clinical improvement than those with CSOR values > 0.73.[82]

Implications for Research

This conceptual framework of NEC Development specifies key factors that have been associated with the development of NEC based on research and clinical experience. This is the first conceptual framework that specifies the interrelationship of variables important for perfusion alteration in premature infants related to blood transfusions. The authors are currently conducting a prospective, observational study examining factors outlined in this conceptual framework to evaluate perfusion patterns in preterm infants before, during and 48 hours subsequent to PRBC transfusions. Near-infrared spectroscopy technology will be employed to evaluate changes in mesenteric means over time related to transfusion and feedings events. Additionally, NIRS CSOR values of ≤ 0.73 will be used as a measurement to evaluate differential tissue oxygenation between cerebral and somatic vascular beds. If NEC should develop, onset timing will be documented to temporally relate the occurrence to the transfusion and/or feeding event. Further, perfusion pattern changes during and following the onset of transfusion-related NEC will be examined. Other factors that occur during transfusions (feedings, medications and procedures) and laboratory analysis of the blood unit (ATP, 2,3-DPG and plasma free hemoglobin) will be included in a relational analysis. Finally, perfusion changes will be examined as they relate to the age of blood infused. This framework will guide all

aspects of the study including research design, methodology, data analysis and interpretation.

Prior to our current study, no studies have evaluated somatic tissue perfusion changes in preterm infants during and following blood transfusions, with and without feedings. There are also no perfusion studies using NIRS technology evaluating the effect of the age and quality of RBCs given to preterm infants. These factors in combination with gastrointestinal immaturity theoretically increase the risk for perfusion alterations that could lead to NEC. This conceptual framework for transfusion-related NEC incorporates specific factors that provide a context for evaluating perfusion changes surrounding a blood transfusion utilizing NIRS technology and provides a foundation to address gaps in the current body of scientific knowledge concerning the impact of perfusion change related to blood transfusions, quality of blood being given and the presence of enteral feedings. The results of this study may influence medical and nursing practice and could lead to the establishment of safe guidelines for the administration of transfusion and feedings to preterm infants.

Summary

With added knowledge regarding the development of NEC based on the factors described in this article, there is the potential to reduce the risk for tissue oxygenation alteration that may lead to ischemia and possible NEC development subsequent to PRBC administration. By identifying mechanisms to detect pre-NEC pattern trends utilizing NIRS technology in addition to traditional physiologic monitoring, the potential to intervene sooner to prevent NEC is possible, feasible and may reduce costs associated with prolonged hospital stays while improving overall outcomes in the premature infant population. Therefore, this conceptual framework has both research and clinical relevance that may impact current packed red blood cell transfusion protocols in neonatal practice.

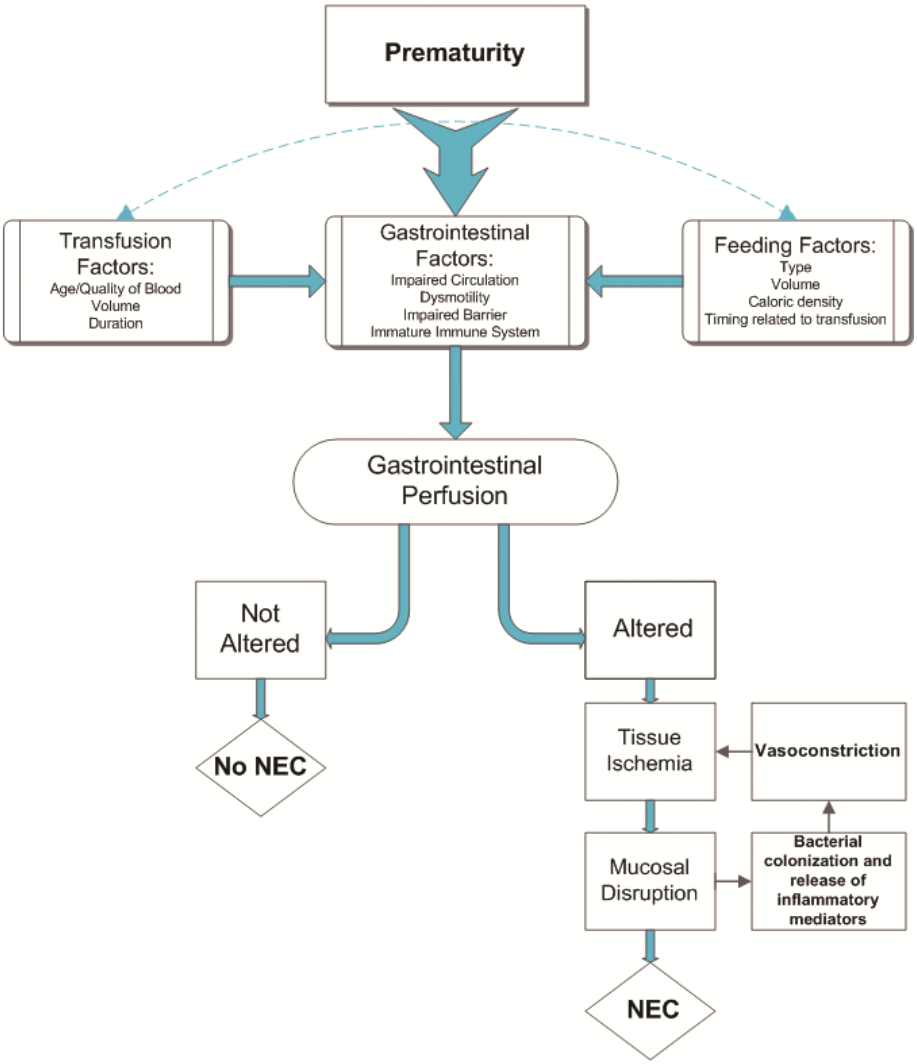

Figure 1: Conceptual Framework; (adapted from framework originally published by Lin et. al., 2008). This diagram illustrates the theoretical relationships between factors associated with blood transfusions that either alter or do not alter perfusion patterns in preterm infants.

Prematurity is directly associated with gastrointestinal immaturity factors that include impairment of autoregulation (circulatory), barrier function, mobility (peristalsis) and immune response. When a blood transfusion is given, factors associated with length of storage (storage lesion), duration and volume may couple with gastrointestinal immaturity factors to affect gastrointestinal perfusion. Feeding factors (type and volume) and gastrointestinal immaturity may affect perfusion status. When feedings and transfusions are combined (represented by dashed line) with gastrointestinal immaturity factors, further impairment of perfusion may result. When perfusion is altered, several outcomes can ensue including tissue ischemia, mucosal disruption, bacterial colonization and release of vasoconstrictive immune mediators which culminate in further tissue ischemia and damage potentially leading to NEC development.

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Publication 3:

Red Blood Cell Transfusion-Associated NEC in VLBW Infants:

A Near-Infrared Spectroscopy Investigation (NIRS)

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Red Blood Cell Transfusion-Associated Necrotizing Enterocolitis in Very Low Birth Weight Infants: A Near-Infrared Spectroscopy Investigation

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Sources of support: Florida Association of Neonatal Nurse Practitioners; Sigma Theta Tau International Honor Society of Nursing

There are no conflicts of interest of any authors

Abstract

Background: Necrotizing enterocolitis (NEC), the most common gastrointestinal emergency encountered by very low birth weight (VLBW) infants, is temporally associated to packed red blood cell (PRBC) transfusion. The underlying mechanism for this association is not known. Poor perfusion in the mesenteric vasculature during PRBC transfusion has been hypothesized to contribute to NEC development and is being investigated in this study.

Study design and methods: Perfusion patterns among four VLBW infants who developed transfusion-related NEC (TR-NEC) were compared to four VLBW infants with similar gestational age who did not develop NEC (non-NEC). Cerebral and mesenteric perfusion patterns were recorded before, during and 48 hours subsequent to PRBC transfusion using near-infrared spectroscopy technology (NIRS). Percentage change from mean baseline regional saturation (rSO2) and cerebro-splanchnic oxygenation ratio (CSOR) means were analyzed.

Results: All TR-NEC infants demonstrated greater negative percentage change in mesenteric perfusion from baseline prior to TR-NEC onset than non-NEC infants. CSOR were similar in both. TR-NEC infants received larger volumes of blood (mean 27.75 ± 8.77) than non-NEC infants (mean 15 ± 0); p=0.27 (95% CI: 2.02-23.48). TR-NEC infants were more likely fed during transfusion (75%) than non-NEC infants (25%).

Conclusion: Negative fluctuations in mesenteric perfusion patterns are more pronounced in TR-NEC infants, especially prior to TR-NEC onset, as compared to non-NEC infants. Transient increases in mesenteric perfusion subsequent to TR-NEC development in two infants may have been related to volume resuscitation. Greater volumes of infused blood and concurrent enteral feeding may increase the risk for TR-NEC in preterm infants.

Keywords: Transfusion-related NEC, necrotizing enterocolitis, near-infrared spectroscopy

Introduction

Necrotizing enterocolitis (NEC) is a process characterized by mesenteric ischemia that results in an inflammatory cascade which leads to bowel necrosis.[1] This complication of prematurity is a leading cause of neonatal morbidity and mortality, especially in those very low birth weight (VLBW) infants weighing < 1500 grams.[2] The pathogenesis of NEC is unclear, however leading hypotheses implicate a multi-factorial pathophysiology with specific causal factors not identified, prevention strategies imprecise, and its incidence therefore has remained unchanged over several decades.[2,3]

Several recent studies have suggested that packed red blood cell (PRBC) transfusions are temporally related with NEC onset, occurring immediately and up to 48 hours post-transfusion (TR-NEC).[4-12] The underlying mechanism of this relationship is unknown; however, suggested processes include ischemic-reperfusion injury,[13,14] blood hyperviscosity,[15] ineffective or overexaggerated immune response,[16] enteral feedings[6,17] and prolonged storage of donor PRBCs.[18] Enteral feedings introduce bacteria which may colonize in the intestinal tract, and in the presence of compromised or injured mucosal surfaces, the risk for an inflammatory response is increased. The effect of transfusions, feedings and age of blood on mesenteric perfusion has not previously been identified.

Our prospective study endeavors to demonstrate mesenteric perfusion patterns, using Near Infrared Spectroscopy (NIRS) technology, of VLBW premature infants receiving PRBC transfusions and concurrent enteral feedings. Doppler studies have demonstrated preterm infants have diminished superior mesenteric artery blood flow subsequent to PRBC transfusions in the postprandial state.[17] Additionally, animal studies have shown that the immature gastrointestinal vasculature lacks appropriate autoregulation response to changes in blood flow, revealing a predominance of vascular constriction subsequent to nitric oxide depletion in low-flow states.[19-21] Therefore, we hypothesize that mesenteric perfusion diminishes during and subsequent to PRBC

transfusions and further reduces when enteral feedings are administered concurrently and subsequent to a transfusion event. This case series compares mesenteric perfusion patterns exhibited by a subset of VLBW infants from a larger ongoing study who developed TR-NEC to perfusion patterns of four VLBW infants of similar gestational age in the same study who did not develop NEC (non-NEC) subsequent to PRBC transfusion.

Materials and Methods

Study population

Premature infants were recruited into the Emory institutional review board approved study from November 30, 2010 to December 31, 2011. Infants recruited were gestation age (GA) < 37 weeks, received a PRBC transfusion and were admitted to Emory level IIIB neonatal intensive care unit (NICU). Infants with congenital anomalies, intraventricular hemorrhage Grade III or greater, hemodynamically significant patent ductus arteriosis, requiring vasopressor support or previous NEC were excluded. Demographics on each infant were collected including: gender, race, birth weight (BW), current weight and medications, feeding status, birth history, ABO/Rh type, and pertinent previous and current diagnoses. All routine care, enteral feedings, laboratory values, vital signs, ventilation status and clinical changes during the study period were recorded from nursing flow sheets. Routine neonatal care was determined by the attending physician. All infants were followed until discharge, death or transfer for the development of NEC. NEC diagnosis was based on Bell's Staging criteria.[22] TR- NEC was defined as infants diagnosed with NEC within the 48 hour period post-transfusion. Medical TR-NEC was defined as infants who were medically managed with antimicrobial therapy and did not require surgical intervention due to complications associated with NEC progression. Surgical TR-NEC was defined as infants who required surgical intervention for complications directly resulting from TR-NEC onset and/or progression of disease.

As part of a larger ongoing study examining perfusion patterns during and subsequent to PRBC transfusion using NIRS, this case series describes four VLBW infants who developed TR-NEC and were compared to four VLBW infants who received a transfusion that did not develop this complication. The non-NEC infants were selected from other VLBW infants on study with the closest corrected GA at the time of transfusion. Only 4 of the 19 infants enrolled in the larger study met this criterion.

Near-Infrared Spectroscopy Monitoring

Cerebral and mesenteric regional oxygen saturation (rSO_2) values were measured using an INVOS 5100C Cerebral/Somatic Oximeter (Covidien, Boulder, CO), an FDA approved NIRS device. Measurements were recorded every 30 seconds in real-time prior to, during and 48 hours subsequent to receiving a PRBC transfusion. NIRS measures oxygen uptake in tissue beds which reflects perfusion status reported as rSO_2 value. NIRS sensor probes were placed on the forehead and lower abdomen to obtain cerebral and mesenteric measurements. Upon completion of the monitoring period, data were downloaded from the NIRS device and transferred to a research computer for data analysis.

To identify meaningful fluctuations in mesenteric tissue beds, total percent changes (positive or negative) from baseline mesenteric mean were calculated. Cerebro-splanchnic oxygenation ratios (CSOR) evaluate differential tissue bed perfusion and were obtained by dividing mesenteric rSO_2 by cerebral rSO_2 measurements. Perfusion changes were analyzed by evaluating mean mesenteric and CSOR patterns as they temporally related to concurrent events including enteral feedings, apnea, bradycardia, desaturation and TR-NEC onset. For infants that developed TR-NEC, perfusion means were analyzed relating to resuscitation measures including mechanical ventilation initiation, antimicrobial therapy and any surgical intervention required within the study period.

Packed Red Blood Cell Data and Characteristics

All PRBC units transfused to infants were stored in a citrate-phosphate-dextrose-adenine (CPDA-1) solution. In this case series, all infants received cytomegalovirus negative, irradiated, leukoreduced, type O PRBC units. Irradiation storage time and age of PRBCs were recorded. Infant hemoglobin values were recorded prior to each PRBC transfusion per routine NICU care prior to transfusion.

For each infant, the number of PRBC transfusion events received before and after the study transfusion event were recorded, as were the volume and duration of the study PRBC transfusion. Enteral feeding events were recorded during and after transfusion events including type, volume, duration, route, tolerance and timing related to the transfusion event.

Data Analysis

SAS statistical software (SAS/STAT Software, version 9.2 Cary, NC: Institute; 2000-2008) was used to calculate mean mesenteric and cerebral rSO_2 values in 30-minute intervals for all infants. Baseline mesenteric values were calculated from raw rSO_2 mesenteric readings for the 30-minute period preceding the first transfusion event for all infants. Mean mesenteric rSO_2 values that demonstrated greater than 25% reduction from baseline measures were calculated (mean value x 0.75). Cerebro-splanchnic ratios (CSOR) were calculated from raw rSO_2 cerebral and mesenteric values by dividing mesenteric rSO_2 by cerebral rSO_2, and then 30-minute interval means were calculated. Cut-off values for CSOR raw and mean scores were set at 0.75. Mesenteric and CSOR mean perfusion patterns were descriptively interpreted as they related to concurrent events during transfusions and over time subsequent to each transfusion event.

Results

Infant Characteristics

This case series is part of a larger on-going study examining perfusion patterns during and subsequent to PRBC transfusions and concurrent enteral feedings in preterm infants. During the study, four VLBW infants developed TR-NEC and were compared to four VLBW infants also enrolled in this study that did not develop NEC, and were the closest GA to the TR-NEC infants.

Infant characteristics are listed in Table 1. Mean birth GAs of the TR-NEC group were 26.5 (± 2.1) weeks with mean BW 897 (± 173) grams. Non-NEC group mean birth GA was 28.6 (± 1.07) weeks with mean BW 1102 (± 102.6) grams. Respiratory support was required in two infants in the TR-NEC group and three in the non-NEC group. Excluding those on respiratory support, the mean inspired fraction of oxygen (FiO_2) was 0.27 for the NEC group versus 0.44 for the non-NEC group. All infants in both groups were receiving enteral feedings prior to their study transfusion event; 3 in the TR-NEC group and 1 in the non-NEC group received enteral feedings during the transfusion event. Caffeine citrate treatment for apnea of prematurity was given to 3 infants in the TR-NEC group and two in the non-NEC group. Antimicrobial therapy was ongoing prior to transfusion event for two infants in the TR-NEC group (one with confirmed sepsis and one with clinical sepsis) and no antimicrobial therapy was administered to non-NEC infants.

Transfusion data

Table 2 includes information regarding transfusion data for both study groups. All four infants in the TR-NEC group had two transfusion events prior to NEC onset. The mean volume of the first transfusion was 14.4 ml/kg (± 5.2) and 13.4 ml/kg (± 3.9) for the second transfusion. Three infants received two full volume transfusions (15ml/kg or greater) in < 72 hours. The fourth infant received a full volume transfusion split into two 7.5 ml/kg aliquots administered in a

12 hour period. For the non-NEC group, only one infant received two transfusions which were 7.5 ml/kg each in a 12 hour period. Two infants (2 and 4) in the TR-NEC group received PRBC aliquots from the same donor unit.

The mean age of transfused blood for the TR-NEC group was 7 (± 0) days for the first transfusion and 8.0 (± 1.4) days for the second transfusion. The mean age of transfused PRBCs for the non-NEC group was 9.5 (± 3.5) days. PRBC was irradiated for a mean time of 3.5 (± 0.6) days for the TR-NEC group for the first transfusion event and 4.5 (± 1.7) days for the second transfusion event. The mean irradiation time for the non-NEC group was 5 (± 4.1) days for the first transfusion, three of which received single full volume transfusions. The only non-NEC infant who received a second transfusion received 13 day old blood that had been irradiated for 8 days.

Hemoglobin levels were collected per routine NICU policy prior to the first transfusion event for all infants. Mean hemoglobin levels were only slightly lower for TR-NEC group (8.1± 1.5) compared to the non-NEC group (8.4 ± 1.2) days. All PRBC transfusion in this case series were administered for anemia of prematurity.

Enteral Feeding Characteristics

Enteral feeding characteristics are listed in Table 3. All infants were receiving enteral feedings prior to the transfusion event. In the TR-NEC group, enteral feedings were held during the transfusion event for 1 of 4 infants; whereas in the non-NEC group, 1of 4 infants received enteral feedings during their study transfusion events. Volume, caloric density, type of milk, duration and route of feedings received were similar between groups. If feedings were held, time until feedings resumed ranged between 2.5 to 20 hours post-transfusion. Infant 7 in the non-NEC group experienced multiple episodes of apnea, bradycardia and desaturation necessitating

mechanical ventilation 15 hours post-transfusion; therefore, feedings for this infant were resumed 20 hours post transfusion.

TR-NEC Onset

The onset of Bell's Stage IA or greater occurred within 48 hours of the second transfusion for all four TR-NEC group infants (see Table 1). Two were medically managed and two required surgical intervention. Infant 1 acutely developed medical TR-NEC within 30 minutes, and infant 2 developed medical TR-NEC within 11.5 hours subsequent to the second transfusion event. Both infants required mechanical ventilation for respiratory distress, and were placed on antimicrobial therapy, NPO status and gastric decompression. Following a 10-day course of antimicrobial therapy and bowel rest, both infants recovered without further problems. Infant 3 developed gastrointestinal perforation 38.5 hours subsequent to receiving a second full volume transfusion (15ml/kg). Peritoneal drains were placed with transient improvement in clinical status over the next 14 days; however, bowel resection and ileostomy were required for bowel necrosis two weeks subsequent to this event. Infant 4 developed symptoms of TR-NEC during the second transfusion event, including abdominal distention, green gastric residuals, dilated loops of bowel on abdominal radiograph without pneumatosis. Although enteral feedings were resumed 12 hours later, a pattern of feeding intolerance continued and was again placed on NEC precautions four days subsequent to the second transfusion event. Eight days later, pneumatosis was evident on abdominal radiograph confirming Bell's Stage IIB NEC. Bowel resection related to stricture development was required five weeks after this event.

Near-Infrared Spectroscopy Data

Mean mesenteric baseline values were calculated for the 30 minute period prior to the first transfusion received. Two infants (1 in the TR-NEC group and 1 in the non-NEC group) did

not have mean baselines calculated because the NIRS device was placed after the first transfusion had begun. Therefore, baseline data for these infants during the first hour of the first transfusion event were used for subsequent perfusion pattern comparison. Baseline means are listed in Table 1. Overall, the TR-NEC group had higher initial mesenteric baselines (37.5 ± 26.7) than the non-NEC group (22 ± 20.24).

TR-NEC Group Mesenteric Perfusion Patterns

During the first transfusion, mesenteric means slightly fluctuated for medical TR-NEC infant 1 and 2 (figure 1). However, one hour prior to receiving the second transfusion during an enteral feeding, the means dropped to 36% below baseline for infant 1. Infant 2 had a similar drop in mesenteric mean to 22% below baseline one hour prior to the second transfusion also during an enteral feeding. Both infants demonstrated approximately 30-50% increased mesenteric means at the time of TR-NEC diagnosis. Both infants were placed on mechanical ventilation within 6 hours of TR-NEC onset for and mesenteric means gradually drifted back towards baseline values approximately 24 hours later. By 36 hours subsequent to TR-NEC onset mesenteric perfusion patterns in both infants declined following intubation to 49% (infant 1) and 31% (infant 2) below baseline. Both were medically managed and recovered without further problems.

Mesenteric perfusion patterns differed for surgical TR-NEC infants 3 and 4. Both demonstrated severe drops in mesenteric means immediately subsequent to the first transfusion event (Figure 2). Infant 4 did not have a mean baseline calculated due to NIRS placement after the start of the first transfusion event; therefore, we observed changes relative to baseline data generated during the first hour of the first transfusion event. The severe drop in this infant's mesenteric perfusion of 47% coincided with a bolus enteral feeding given within ten minutes subsequent to commencement of the first transfusion event. There were some perfusion

elevations from baseline six hours later, but values again dropped following bolus feeds. This pattern persisted until the second transfusion was administered 24 hours later at which time symptoms of Bell's Stage IA necrotizing enterocolitis developed (abdominal distention, green gastric aspirate, dilated loops of bowel evident on abdominal radiograph). The infant was placed on antimicrobial therapy and made NPO. During this time, mesenteric means elevated to 11% above values exhibited during the first transfusion event. Feedings were subsequently resumed 12 hours after the transfusion was complete, and mesenteric means initially fell by 48%, but then increased to 45% above baseline by the end of the study period. Symptoms consistent with Bell's Stage IB NEC developed 4 days later including feeding intolerance, bloody stools, and abdominal wall erythema. The infant was made NPO again and placed on NEC precautions for the next 4 days. He was definitively diagnosed with Bell's Stage IIB NEC eight days following the second transfusion event and required multiple transfusions over the next several weeks. Surgical intervention was eventually required for stricture repair including an ileal resection 5 weeks following our study.

Infant 3 incurred a severe drop of 74% below baseline (Figure 2) with minimal variation midway through the first transfusion event and this perfusion pattern persisted throughout the second transfusion event given 67 hours later. Bolus enteral feedings were resumed 4.5 hours subsequent to the first transfusion event and continued every three hours until the infant was made NPO during and following the second transfusion. There was a mild increase above baseline of 12% six hours after the second transfusion event, but values again dropped to 76% below baseline at which time a gastrointestinal perforation was identified on abdominal radiograph. Saturations measured by pulse oximetry (SaO_2) remained steady between 90-99% during this period of low rSO_2 means with no required changes in FiO_2. Mesenteric perfusion patterns greatly improved to 50% above baseline subsequent to peritoneal drain placement 12

hours following perforation. Prior to receiving the first transfusion, this infant required antibiotic therapy for confirmed Serratia sepsis. Peritoneal fluid cultures were positive for enterococcus and candida albicans. Two weeks after this event, the infant underwent bowel resection and ileostomy placement for necrotic bowel.

TR-NEC group Cerebro-splanchnic Ratio (CSOR) Patterns

Cerebro-splanchnic ratio (CSOR) means for the TR-NEC group are shown in Figure 3. Infant 1 demonstrated an increase from baseline of 0.67 during the first transfusion to 0.71, decreased during the second transfusion and increased during volume resuscitation measures subsequent to TR-NEC development (0.8). Infant 2 showed a slight improvement following the first transfusion and marked improvement in CSOR (1.14) following the second transfusion despite TR-NEC onset. CSOR means for infant 3 sharply declined during and following the first transfusion but increased above baseline values six hours following the second transfusion and remained elevated for 12 hours. At the time of gastrointestinal perforation diagnosis, CSOR decreased to 0.21 with elevation following peritoneal drain placement. Infant 4 showed elevation of CSOR during both transfusions, drops following transfusions and stabilization to above baseline mean 36 hours after the second transfusion. CSOR values overall showed wide fluctuation, with 3 of the 4 TR-NEC infants demonstrating occasional means > 0.75 during and subsequent to TR-NEC development.

Non-NEC Group Mesenteric Perfusion Patterns

The non-NEC group demonstrated less negative fluctuations in mesenteric patterns during and following transfusions (figure 4). Infant 5, the fraternal twin of TR-NEC Infant 1, received two transfusions in a 24-hour period, and was the only infant in the non-NEC group to receive enteral feedings during and following both transfusions. This infant's mesenteric

perfusion patterns overall demonstrated a gradual rise in means following the first transfusion, with one drop back to baseline 3 hours subsequent to the first transfusion and below baseline 6 hours post transfusion during an enteral feed. Infant 6 exhibited a 15% drop in the first hour of the transfusion event, which then rose to 35% above baseline midway, and remained 33% above baseline at the conclusion of the transfusion. This infant had a significant drop to 54% below baseline 6 hours post transfusion and one hour post enteral feed. Large gastric residuals were obtained and enteral feedings were held for 3 hours. Subsequently, mesenteric patterns improved, feedings were resumed and by the end of the study period were 37% above initial baseline mean. Infant 7 was NPO for the transfusion event and developed significant apnea, bradycardia and desaturation episodes 15 hours following the transfusion necessitating mechanical ventilation placement and antimicrobial therapy. Feedings were resumed 20 hours post transfusion which resulted in brief increases in mesenteric means (7-88%) above baseline); however, overall patterns remained consistent at 16-25% below baseline for the remainder of the study period. Infant 8 demonstrated great improvement in mesenteric means six hours following the transfusion, but had small negative drops (13-17%) during and immediately following the transfusion all occurring either during or immediately following enteral feeds. At the conclusion of the study period, this infant's mesenteric means increased to 182% above baseline.

Non-NEC Cerebro-splanchnic Ratio (CSOR) Patterns

CSOR means for the non-NEC group are shown in Figure 5. Infants 5, 6 and 8 demonstrated overall improvement in CSOR throughout the study period with only slight fluctuations. These infants CSOR means were > 0.75 for the majority of the study period. However, infant 7 did not show much improvement throughout the study period with ending CSOR below baseline. However, during episodes of profound apnea, bradycardia and desaturation, raw CSOR values rose (from 0.36 to 1.00) and frequently mesenteric rSO_2 signal

drop out was recorded which sharply decreased cerebral values (data not shown). The elevation in CSOR means was related to sharp decreases in both cerebral and mesenteric values. Once this infant was placed on mechanical ventilation, cerebral values stabilized, but mesenteric means remained low generating overall low CSOR values (0.19-0.24).

Conclusion

This novel study demonstrated mesenteric tissue oxygenation pattern changes using NIRS technology during TR-NEC onset in VLBW infants. Although previous studies have used NIRS to prospectively examine tissue oxygenation patterns following PRBC transfusions, the actual occurrence of TR-NEC was not observed.[23,24] Further, previous studies did not observe or include the effect of enteral feedings during and following the transfusion event. The results of this study demonstrated unique tissue oxygenation pattern variations for infants experiencing medical versus surgical TR-NEC temporally related to PRBC transfusions. Medical TR-NEC infants had decreased rSO_2 patterns just prior to disease onset with subsequent rises during the acute phase of TR-NEC onset. Surgical TR-NEC infants demonstrated severe drops from rSO_2 baseline means immediately following their first transfusion event. One surgical infant demonstrated significant improved mesenteric perfusion patterns following peritoneal drain placement to relieve pneumoperitoneum. The other surgical TR-NEC infant exhibited persistent feeding intolerance for weeks following the transfusion events requiring eventual surgical repair to relieve intestinal strictures. When compared to non-NEC preterm infants with similar characteristics, variations in tissue oxygenation was less pronounced with greater $rS0_2$ stability throughout and subsequent to the transfusion event. These differences may be related to withholding feedings during transfusions and less volume of blood infused in the non-NEC group.

Previous retrospective studies suggest that PRBC transfusions are an independent risk

factor for TR-NEC,[12,25] with a 25-35% incidence in VLBW preterm infants.[4,7-9,12,26,27] Furthermore, TR-NEC occurs immediately and up to 48 hours post PRBC transfusion in VLBW infants.[4-6,8,11,12,27] In addition, studies have shown that TR- NEC occurs more frequently in infants with lower GA at birth, lower BW and older postnatal age (> 4 weeks).[4,9,28] In contrast, one study reports that PRBC transfusions may be protective against NEC.[29] Compared to previous studies, 21% (4/19) of the VLBW infants in our study developed Bell's Stage IA NEC or greater following a PRBC transfusion, with all cases occurring in < 48 hours subsequent to the transfusion event. Similar to previous findings, our TR-NEC group had low birth weights (mean 897 ± 173 grams) and earlier gestational ages at birth (26.5 ± 2.1 weeks). However, in contrast to prior studies, the mean age of these infants in our study was younger (18 ± 10.6 days).

NIRS technology simultaneously measures real-time regional tissue oxygenation producing rSO_2 values which reflect differential organ perfusion. The actual rSO_2 reading measures changes in tissue concentration of oxyhemoglobin and deoxyhemoglobin, or the balance of oxygen that is delivered minus the amount extracted at the tissue level.[30] There are several reasons for decreased rSO_2 values: increased consumption of oxygen at the tissue level, diminished or absent blood flow, or altered and/or decreased oxygen carrying capacity.[30] A combination of any or all of these factors may also be present. Therefore, it is vital that infant factors be closely monitored for reasons contributing to low rSO_2 measurements.

Normal rSO_2 values have not been formally established for the preterm population; however, studies describe cerebral, mesenteric and peri-renal rSO_2 reference values demonstrated by preterm infants in the first few weeks of life.[14,31] According to these studies, cerebral variation is minimal and mesenteric variations are large. We found similar findings of wide mesenteric variability in all infants and minimal cerebral variability in all but two occasions. Infant 7, a non-NEC infant, developed profound apnea, bradycardia and desaturation episodes subsequent to the

transfusion event which produced numerous sharp declines in cerebral, mesenteric and renal rSO_2 values. Infant 4 in the TR-NEC group demonstrated improved mesenteric rSO_2 values which coincided with reduced cerebral and renal values during the last hour of the first transfusion event (prior to developing TR-NEC symptoms). This loss of cerebral variability could be related to the onset of a septic event and associated interventions producing loss of cerebral autoregulation.[32,33]

Other studies evaluate rSO_2 values in a cerebro-splanchnic ratio (CSOR) format which is calculated as mesenteric rSO_2/cerebral rSO_2.[24,34,35] Fortune et al found that mesenteric perfusion alteration associated with NEC occurred in preterm infants when CSOR values were < 0.75.[34] Bailey et al reported recently that infants with pre-transfusion CSOR values < 0.73 are more likely to demonstrate clinical improvement following a PRBC transfusion than those with CSOR values > 0.73.[24] Our study findings related to CSOR values were not in agreement with these previous studies. CSOR values are beneficial when cerebral autoregulation is intact as the change in value directly reflects mesenteric changes. However, if cerebral autoregulation is lost or impaired, an improvement in the CSOR value may be reflective of decreased cerebral tissue oxygenation with little or no change in mesenteric values. Cerebral autoregulation may be impaired or lost in critically ill low birth weight preterm infants;[33,36] therefore, CSOR values are only meaningful when interpreted within the appropriate context. It is essential to evaluate absolute cerebral and mesenteric rSO_2 values to ensure an improved CSOR value reflects mesenteric, not cerebral changes. For these reasons, we chose to evaluate absolute mesenteric measurements as a percentage of negative or positive fluctuations from baseline mean to accurately describe the effects of related transfusion and enteral feeding events. We then analyzed CSOR means within the context of absolute rSO_2 pattern changes. Our study demonstrates that CSOR values < 0.75 are not always associated with ischemic bowel and NEC development. We further illustrate that infants who develop TR-NEC may exhibit CSOR values

> 0.75. To increase the generalizability of our study findings, we did not exclude infants with confirmed or suspected sepsis as did the Bailey study.

The effect of enteral feedings on mesenteric tissue oxygenation patterns during and following a transfusion event has not been previously identified. However, the relationship between higher enteral feeding volumes, formula and the onset of NEC is well documented.[37-40] Christensen et al found that TR-NEC infants were more often fed during transfusions, and were more likely to have been fed a bovine formula product.[9] Doppler technology has shown that superior mesenteric artery blood flow diminishes subsequent to PRBC transfusions and is further impaired when enteral feedings are given.[17] In stable growing preterm infants, it has been shown that mesenteric rSO_2 values increase following enteral feedings.[35] Two small studies report that withholding enteral feedings during PRBC administration reduces the incidence of subsequent TR-NEC.[6,27] In this case series, 75% of the infants who developed TR-NEC were fed during their transfusions, and 25% of the non-NEC group received concurrent feedings. Similar to previous findings, formula feedings were more prevalent in our TR-NEC infants, but volumes of feeds did not differ. Further research regarding the effect of enteral feeding continuation during PRBC transfusions, feeding intolerance post transfusion and the development of TR-NEC is warranted.

Maturational deficits of the gastrointestinal system related to prematurity may have played a substantial role in mesenteric oxygenation pattern variation for all infants in this case series.[3] Factors related to dysmotility, impaired barrier function, immature circulatory regulation and deficient immune response of the preterm infant may contribute to the susceptibility for NEC development in the presence of PRBC transfusions and enteral feedings. Mesenteric peristalsis does not mature until approximately 32-34 weeks gestation producing delayed transit time, and potential accumulation of noxious substances that may interfere and damage immature mesenteric epithelial barriers.[41,42] In the presence of impaired immune response and ineffective circulatory

regulation, VLBW infants are greatly vulnerable to the effects of impaired mesenteric blood flow.[43,44] The increased metabolic demand related to enteral feedings given during and subsequent to transfusions may have contributed to gastrointestinal compromise in the infants who developed TR-NEC in our study. The potential for PRBC transfusions triggering an immune response exacerbated by enteral feedings requires further investigation.

Inflammation has also been proposed to play a central role in NEC pathogenesis. In the presence of sepsis, proinflammatory and anti-inflammatory mediators such as tumor necrosis factor alpha (TNFα) and interleukins (IL), which are known to be involved in the pathogenesis of NEC, and may increase the risk for intense vasoconstriction further impairing mesenteric blood flow.[45,50, 51] In this case series, two infants were receiving antibiotics prior to the first transfusion event; one for confirmed Serratia sepsis and one for clinical sepsis. Both infants developed NEC after PRBC transfusion and required surgery related to complications following TR-NEC development.

The unique tissue oxygenation patterns exhibited between medical and surgical TR-NEC infants in this study may relate to different pathogenic mechanisms. Studies have shown that sustained decreased blood flow in the superior mesenteric artery followed by reperfusion may disrupt mesenteric circulatory regulation mechanisms and increase susceptibility to intestinal barrier injury.[19] The low perfusion patterns with subsequent increases at the time of TR-NEC onset in the medical TR-NEC infants may have been related to perfusion-reperfusion injury.[19,46] It is also possible that the increase in rSO_2 following TR-NEC onset in these infants was the result of volume resuscitation.[47] The sharp decline in mesenteric oxygenation in our infant who developed pneumoperitoneum may have resulted from distortion of infrared light path length, ischemic bowel or absence of mesenteric perfusion. These findings are consistent with previous NIRS studies during NEC onset.[14] The contrasting patterns exhibited in infant 4 may have been

related to perfusion-reperfusion injury.[19,48,49] the consequence of enteral feedings immediately post transfusion,[17] or combined effect.

Previous studies have examined the age of blood and potential adverse outcomes related to the "storage lesion" phenomenon.[50-52] The relationship between administration of older stored blood and transfusion-related NEC remains in debate.[4,8,9] Kriebardis et al report that blood stored for two weeks or longer increases the possibility of detrimental effects when transfused due to cellular degradation, even when stored in a preservative solution such as CPDA.[52] In our case series, all infants received < 14 day old donor blood, and the mean age of blood for the non-NEC group was greater than for the TR-NEC group for both transfusion events. The mean length of irradiation (days) was also greater for the non-NEC v. TR-NEC group. However, the TR-NEC group received larger mean volumes of blood for both transfusions than the non-NEC group. Although studies have shown multiple transfusions are significantly related to late-onset TR-NEC,[4] further research is needed to identify relationships between transfusion volume, duration, age of blood given and days of irradiation to TR-NEC development.

There were several limitations to this study, the first being a small sample size. We recognize our comparison group differed maturationally as compared to the TR-NEC group, and these differences may have influenced the risk factor for disease development, feeding intolerance and mesenteric tissue oxygenation changes. Additionally, our findings would be strengthened if perfusion patterns were known prior to transfusion administration in the presence of enteral feedings. This would allow direct comparison of pre- and post- transfusion perfusion changes and to what extent enteral feedings further affect perfusion patterns. A third limitation relates to NIRS technology, in that probe displacement interferes with continuous trend monitoring, and ability to calculate CSOR values if cerebral and/or mesenteric values are missing. Continuous mesenteric monitoring is challenging, given the large surface are of the

intestines, peristalsis, and increased infrared path length in the presence of pneumoperitoneum, abdominal distention or increased fluid/gas surfaces. These limitations may lead to "signal drop out" which was observed on our infant with pneumoperitoneum. However, it is more likely that persistent low rSO_2 readings coupled with frequent signal drop is associated with a substantial decrease in tissue oxygenation.[14]

In conclusion, this prospective observational case series demonstrates actual changes in mesenteric oxygenation patterns in infants who developed TR-NEC. Further distinct differences in these perfusion patterns were demonstrated in the TR-NEC infants as compared to similar infants that did not develop NEC. The major differences between our groups were the TR- NEC infants were more likely to receive enteral feedings during and subsequent to transfusions and larger volumes of infused blood. We further demonstrate that analyzing percent changes from baseline quantifies the magnitude of baseline changes, and may be more beneficial when used in conjunction with CSOR values, rather than CSOR values alone.

We recognize that establishing rSO_2 patterns during enteral feedings prior to the transfusion event would strengthen our understanding of pattern changes during and subsequent to transfusions, illustrating the need for further research. Additionally, the effect of PRBC transfusions on immune response stimulation is unknown and requires further investigation. This study demonstrates that severe and sudden decreases in mesenteric tissue oxygenation patterns may increase the risk for TR-NEC onset, especially if low readings persist and are accompanied with loss of mesenteric perfusion pattern variability. Enteral feedings may have a compounded impact on mesenteric perfusion during and following PRBC transfusions. NIRS is a useful diagnostic tool to directly observe tissue bed perfusion in real-time without interrupting routine bedside care and may elucidate compromised mesenteric perfusion before changes in routine physiologic monitoring are evident, primarily SaO_2 measurements. Future studies utilizing this

technology to analyze mesenteric perfusion pattern changes relating to risk factors for NEC development seems promising and feasible with the capability to improve prediction and prevention strategies especially when modifiable risk factors are present.

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	TR-NEC Group				Mean±SD	Non-NEC group				Mean±SD
Infant	**1**	**2**	**3**	**4**		**5**	**6**	**7**	**8**	
GA Birth (weeks)	29	27	24	26	26.5 ± 2.1	29	30	27.6	28	28.6± 1.07
cGA (weeks)	33.4	30.1	25.6	27.1	29.1 ± 3.4	33.4	33.4	28.7	30	31.4 ± 2.4
Birth weight (g)	1080	1000	705	803	897.0 ± 173	1210	1160	980	1060	1102.5 ± 102.6
Current weight (g)	1635	1196	710	797	1084 ± 423.6	1655	1417	880	1000	1238.0 ± 360.9
PNA (days)	31	22	11	8	18 ± 10.6	31	24	8	14	19.3 ± 10.2
Gender	F	M	F	M		F	M	M	M	
Ethnicity	B	B	W	B		B	B	W	B	
1 minute Apgar	6	7	3	8		1	2	5	6	
5 minute Apgar	7	8	5	9		6	6	7	7	
10 minute Apgar	8	-	4	-		8	7	-	8	
FiO2	0.21	0.21	0.28	0.25-0.35		1.00	0.21	0.21-0.30	0.30	
Ventilation Mode	RA	RA	AC/VG	NCPAP		NC	RA	SiPAP	NC	
Mean mesenteric baseline (rSO_2)	41	46	63	-	37.5 ± 26.7	-	49	21	18	22 ± 20.24
Time to NEC Onset (hours)	0.5	11.5	38.5	0	12.6 ± 18	-	-	-	-	

Antibiotics prior to 1st transfusion	No	No	Yes	Yes	No	No	No	No
Caffeine therapy	No	Yes	Yes	Yes	No	No	Yes	Yes

Table 1: Infant Demographics and Clinical Data. GA, gestational age at birth; cGA, corrected gestational age; PNA, postnatal age; FiO2, Fraction of inspired oxygen; RA, Room air; AC/VG, Assist Control/Volume Guarantee mechanical ventilation; NCPAP, nasal continuous positive airway pressure; NC, nasal cannula; SiPAP, continuous positive airway pressure with delivered respiratory rate and intermittent sigh;

	TR-NEC group					Non-NEC group				
Infant	**1**	**2**	**3**	**4**	Mean±SD	**5**	**6**	**7**	**8**	Mean±SD
Hemoglobin (g/L) prior to transfusion	6.5	7.3	9.3	9.6	8.1 ± 1.5	7.1	7.7	9.6	9.3	8.4 ±1.2
Infant Blood Type/Rh	A+	O+	O+	O+	-	A+	AB-	O+	B+	-
Unit Blood Type/Rh	O+	O+	O+	O+	-	O+	O-	O+	O+	-
Volume of 1st transfusion (ml/kg)	7.5	15	20	15	14.4 ± 5.2	7.5	15	15	16	13.4± 3.9
Duration of transfusion (hours)	3	4	4	4	3.75 ± 0.5	3	4	3	3	3.25 ± 0.5
Age of Blood 1st transfusion (days)	7	7	7	7	7 ± 0	13	5	14	6	9.5 ± 3.5
Length of Irradiation 1st transfusion (days)	4	3	4	3	3.5 ± 0.6	8	2	10	2	5 ± 4.1
Volume of 2nd transfusion (ml/kg)	7.5	15	15	16	13.4 ± 3.9	7.5	-	-	-	-
Duration of transfusion (hours)	3	4	4	4	3.75 ± 0.5	3	-	-	-	-
Age of Blood 2nd transfusion (days)	7	7	10	8	8.0 ± 1.4	13	-	-	-	-
Length of Irradiation 2nd transfusion (days)	4	3	7	4	4.5 ± 1.7	8	-	-	-	-
Time between transfusions (hours)	12	21	67	24	-	12	-	-	-	-

Table 2: Transfusion Characteristics

	TR-NEC group					Non-NEC group				
Infant	**1**	**2**	**3**	**4**	Mean±SD	**5**	**6**	**7**	**8**	Mean±SD
Feedings held for transfusion?	No	No	Yes	No	-	No	Yes	Yes	Yes	-
NPO prior to transfusion	No	No	No	No	-	No	No	No	No	-
Time feedings resumed post transfusion if held (hours)	-	-	4.5	-	-	-	4	20	2.5	-
Feeding Volume (ml/kg/day)	181	147	56	100	121.0 ± 54.6	145	175	16	136	118 ± 70.0
Feeding type	PEF	PEF	BM	PEF/BM		PEF	PEF	BM	BM	
Feeding caloric density (calorie/ounce)	24	24	20	20	22 ± 2.3	24	24	20	22	22.5 ± 1.9
Duration of feedings (minutes)	30	90	60	BOLUS	46.25 ± 36.8	30	60	30	60	45 ± 17.3
Frequency of feedings (hour interval)	Q3	Q3	Q3	Q3	-	Q3	Q3	Q3	Q3	-
Route of feeds	OG	OG	OG	OG	-	OG/PO	OG/PO	OG	OG	-

Table 3: Feeding Characteristics. PEF, premature Enfamil formula; BM, breast milk; Q3, every 3 hours; OG, orogastric tube feeding; PO, per os (bottle) feeding.

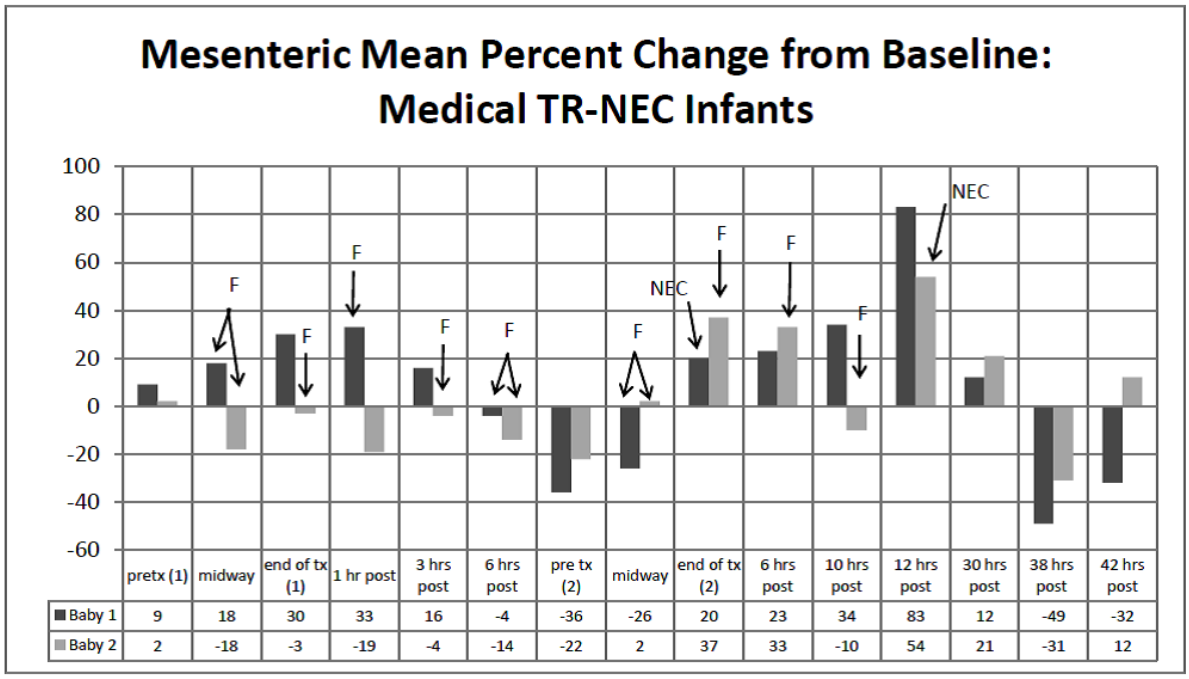


Figure 1: Medical TR-NEC Infants Percent Change from Baseline Means. Infant 1 had NIRS device removed during resuscitation measures, and transfer from Intermediate care to NICU. "F" denotes enteral feeding given during this specific time frame. "NEC" denotes onset of necrotizing enterocolitis.

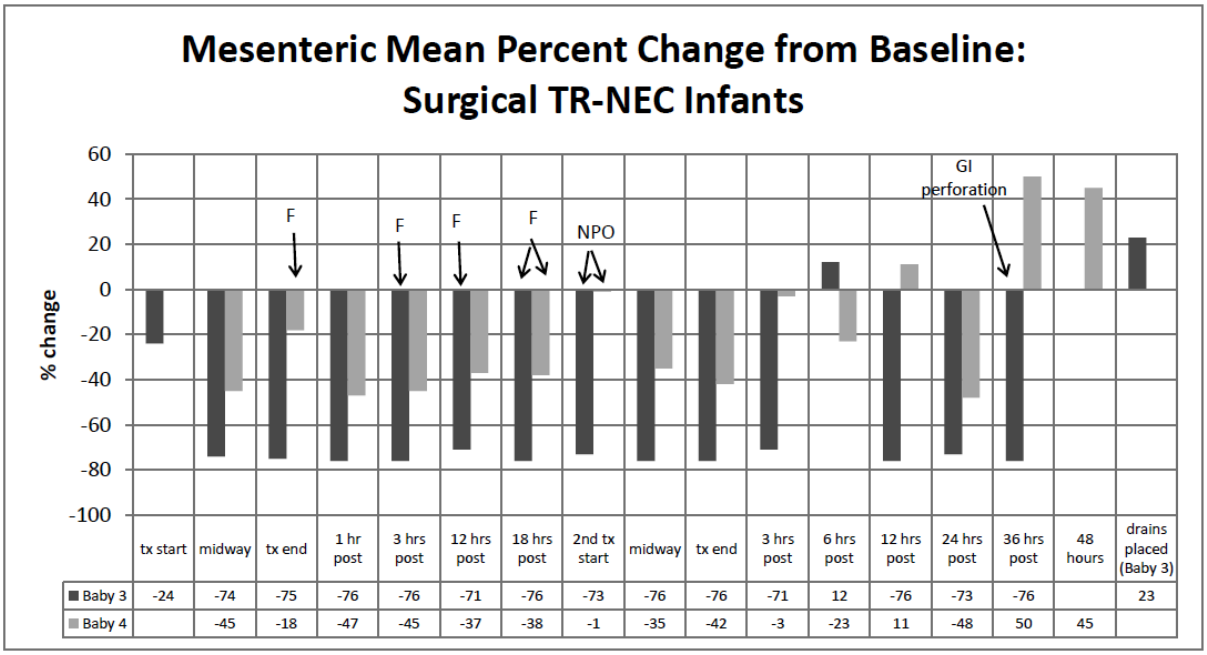
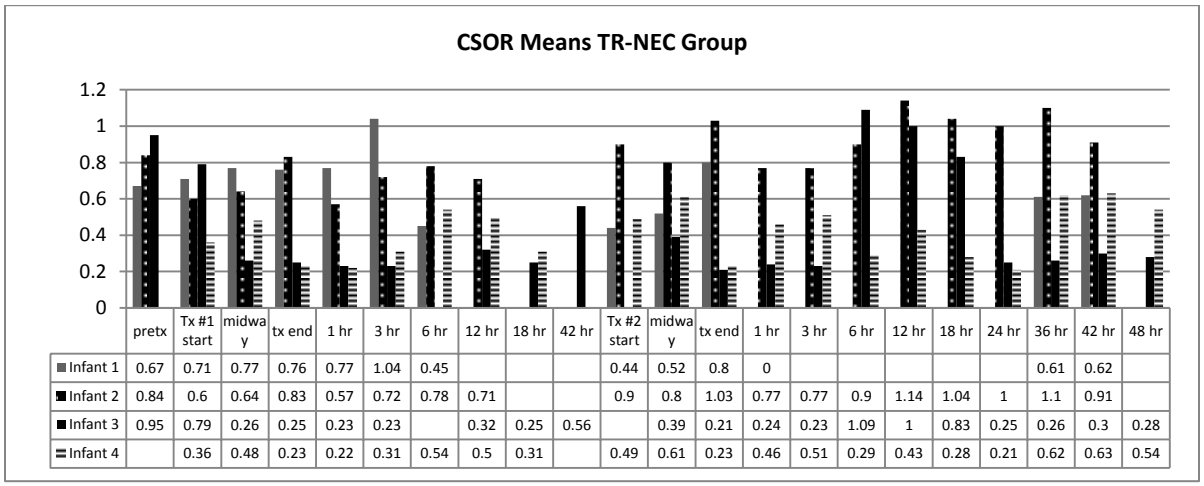
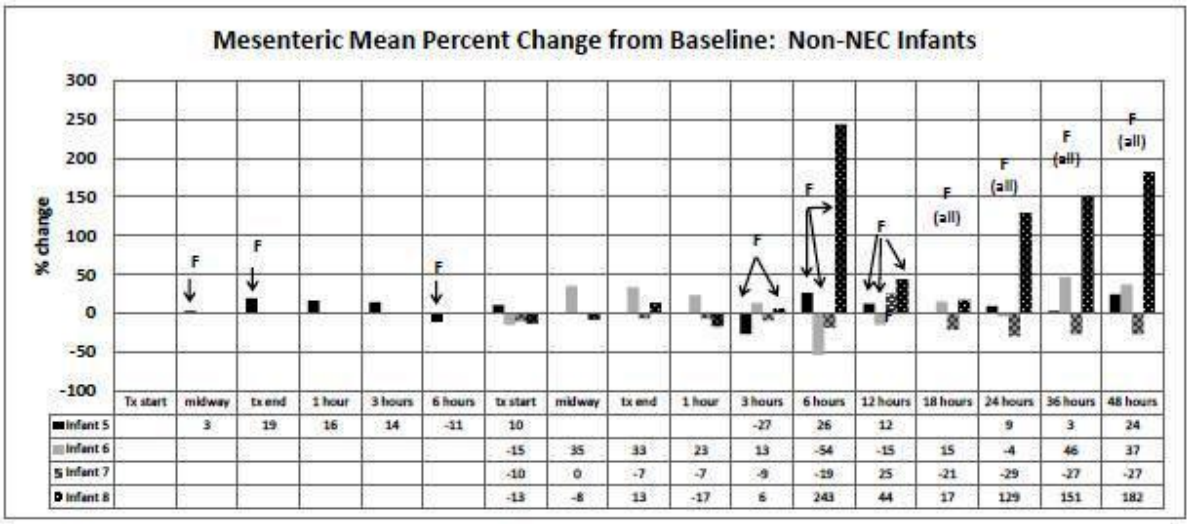


Figure 2: Surgical TR-NEC Infants Percent Change from Baseline Means. Infant 4 did not have NIRS monitor placed until after the start of the first transfusion event; developed NEC IA during second transfusion event. Infant 3 developed pneumoperitoneum 38.5 hours post second transfusion event. "F" denotes enteral feeding event during this specific time frame.



	pretx	Tx #1 start	midway	tx end	1 hr	3 hr	6 hr	12 hr	18 hr	42 hr	Tx #2 start	midway	tx end	1 hr	3 hr	6 hr	12 hr	18 hr	24 hr	36 hr	42 hr	48 hr
Infant 1	0.67	0.71	0.77	0.76	0.77	1.04	0.45				0.44	0.52	0.8	0						0.61	0.62	
Infant 2	0.84	0.6	0.64	0.83	0.57	0.72	0.78	0.71			0.9	0.8	1.03	0.77	0.77	0.9	1.14	1.04	1	1.1	0.91	
Infant 3	0.95	0.79	0.26	0.25	0.23	0.23		0.32	0.25	0.56		0.39	0.21	0.24	0.23	1.09	1	0.83	0.25	0.26	0.3	0.28
Infant 4		0.36	0.48	0.23	0.22	0.31	0.54	0.5	0.31		0.49	0.61	0.23	0.46	0.51	0.29	0.43	0.28	0.21	0.62	0.63	0.54

Figure 3: CSOR Means for all TR-NEC infants



	Tx start	midway	tx end	1 hour	3 hours	6 hours	tx start	midway	tx end	1 hour	3 hours	6 hours	12 hours	18 hours	24 hours	36 hours	48 hours
Infant 5		3	19	16	14	-11	10				-27	26	12		9	3	24
Infant 6							-15	35	33	23	13	-54	-15	15	-4	46	37
Infant 7							-10	0	-7	-7	-9	-19	25	-21	-29	-27	-27
Infant 8							-13	-8	13	-17	6	243	44	17	129	151	182

Figure 4: Mesenteric percent change from baseline mean for Non-NEC Infants. "F" denotes feeding event during specific time frame.

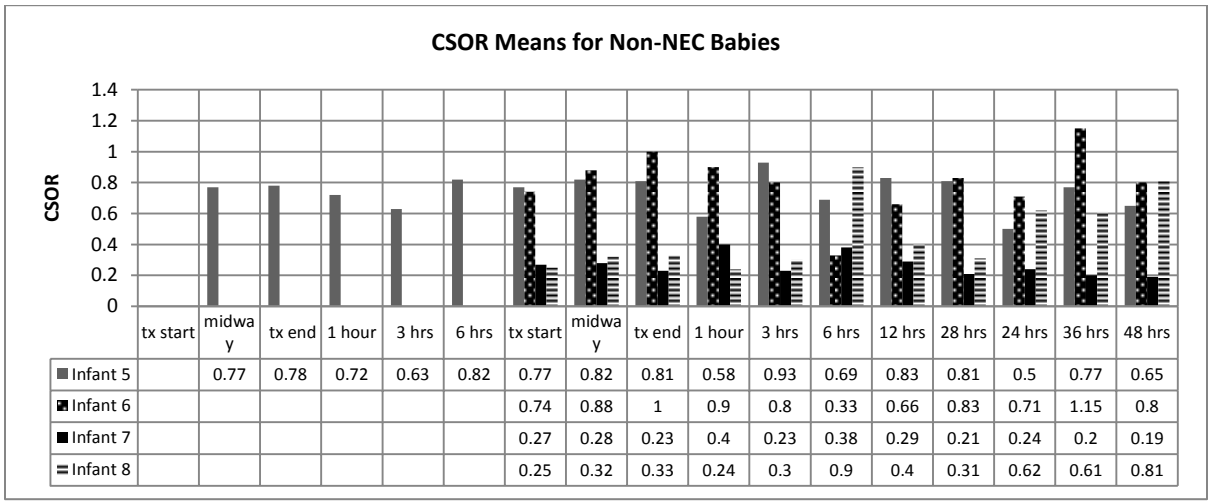


Figure 5: CSOR Means for Non-NEC Infants. Infant 5 received two transfusions.

CHAPTER V

Discussion, Implications and Future Direction

This chapter includes discussion of study results and implications for practice and policy changes. Future direction for scientific advancement related to study findings is also presented, including planned future analyses of collected data relevant to this study. Identified avenues for further research related to components of this study that require additional inquiry are also discussed.

Discussion of Study Findings

Similar to previous retrospective studies, the incidence of TR-NEC in this study was 21% (4/19 subjects or 8/33 transfusion events) (Blau et al., 2011; Christensen et al., 2010; Josephson et al., 2010; Paul et al., 2011) and was temporally associated with PRBC transfusion, as all cases occurred within 48 hours subsequent to a transfusion event (El-Dib, Narang, Lee, Massaro, & Aly, 2011; Singh et al., 2011). Gastrointestinal immaturity associated with lower gestational age may alter mesenteric response to packed red blood cell administration in preterm subjects.

Previous studies suggest that low perfusion followed by high perfusion in preterm infants alters nitric oxide production disrupting the delicate balance required for normal vessel homeostasis (Nowicki, 1998; Reber & Nowicki, 1998). Our findings suggest that although a transient increase was observed in perfusion patterns during transfusion events, declines in MP in the post-transfusion state and lower values overall for the most immature neonates may be reflective of this phenomenon. The risk for perfusion alteration as a function of gestational age may be heightened following blood flow changes through the immature intestinal vasculature subsequent to PRBC administration.

Similar to findings presented in Bailey et al (2010) study which observed mesenteric perfusion patterns during PRBC transfusions, our findings were similar in that perfusion patterns transiently increased during and immediately post-transfusion, with subsequent decline 12 hours post-transfusion (Bailey, Hendricks-Munoz, Wells, & Mally, 2010). The major difference between their study and ours was the length of time perfusion patterns were examined post-transfusion. We were able to establish that after 12 hours, patterns continued to decline for approximately 24 hours and were highly related to gestational age; however, final overall MP were mostly above beginning baseline values by the end of our study period 48 hours post-transfusion. We also found that if a second (split) transfusion is administered, MP do not increase, but actually decline over time with no improvement.

Although previous studies examining the effect of enteral feedings during transfusion are extremely limited, Krimmel et al (2009) found that superior mesenteric artery blood flow is diminished following PRBC transfusions and is further diminished when enteral feedings are given in the post-transfused state in subjects < 1250 grams (Krimmel, Baker, & Yanowitz, 2009). An unexpected finding of our study revealed that enteral feedings given during a transfusion event were not associated with diminished MP during the first transfusion event; however, those who received a second transfusion exhibited lower overall perfusion values. Because the majority of subjects < 30 weeks were not fed during the transfusion event, and no second transfusion event occurred for this group, we cannot establish the effects of enteral feedings during transfusion for the most immature subjects in our study. However, our findings did reveal that subjects > 30 weeks gestation who received enteral feedings during the transfusion event demonstrated higher overall MP than subjects of this same age group who did not receive feedings. Interestingly, the slopes over time for events associated with concurrent enteral feedings decreased, and slopes over time for events not associated with concurrent feedings

increased suggesting that holding feedings during a transfusion are associated with greater positive response than continuing feedings during a transfusion. Furthermore, 75% of the subjects in our study who developed TR-NEC received feedings during their transfusion events. This study also demonstrated that large perfusion variability occurs during feeding events and continues for up to 48 hours post-transfusion illustrating the need for close monitoring of feeding tolerance subsequent to PRBC administration. Therefore, the associated risk related to enteral feeding continuation during and following PRBC administration cannot be dismissed as a potential risk factor for perfusion alteration that may be related to TR-NEC onset. However, other possibilities for this occurrence must be explored.

To our knowledge, this is the first study that found a statistically significant effect between MP patterns and the age of blood transfused. Events associated with the administration of blood > 6 days old demonstrated significantly greater decreases in MP patterns over time than events associated with the administration of blood ≤ 6 day old blood. This effect may be related to storage lesion which causes cellular degradation and increased release of plasma free hemoglobin subsequent to senescent cellular hemolysis (Gladwin & Kim-Shapiro, 2009; Kim-Shapiro, Lee, & Gladwin, 2011). Although this is an important finding, the implications must be interpreted with caution. There was no statistical difference between the average age of blood given to TR-NEC v. non-NEC cases. However, we did observe that perfusion patterns were decreased over time when older blood was given. It remains to be determined if TR-NEC pathogenesis is caused by perfusion alteration alone or if a combination of factors related to an inflammatory response and vessel vasoconstriction in the presence of proinflammatory mediators precedes disease onset (Chan, Wong, & Luk, 2009; McElroy et al., 2011; Reber, Nankervis, & Nowicki, 2002). Because previous studies have shown that cytokines accumulate during blood storage (Stack, Baril, Napychank, & Snyder, 1995), this effect combined with cellular

degradation and depletion of endogenous nitric oxide may be related to the perfusion alterations seen events associated with older blood administration in our study. Given our study findings and others regarding the potential detrimental effects related to prolonged storage of blood, further investigations are imperative.

The purpose of this study was to observe perfusion changes in preterm subjects during and following PRBC transfusions, and if the presence of enteral feedings further impacted perfusion patterns. We found that lower gestational age subjects are at greater risk for MP alteration following PRBC transfusions. Administering older blood may accentuate this risk. Whether perfusion alteration is directly associated with TR-NEC remains to be determined, as factors associated with the quality of blood infused may interact with gastrointestinal immaturity factors to heighten this risk. Furthermore, the issue surrounding enteral feeding continuation during the actual transfusion event needs further exploration, as this study was not able to assess related effects in preterm subjects < 30 weeks gestational age. However, the negative impact of feedings on MP for several hours in the post-transfused state may indicate an increased risk for perfusion alteration and may explain the temporal association of TR-NEC. We also demonstrated that conventional physiologic monitoring was incapable of capturing differential tissue bed perfusion in cases where TR-NEC onset is imminent. Furthermore, accurate interpretation of CSOR values is essential and requires the presence of stable cerebral measurements. The findings of our study demonstrate the need for further large multi-centered studies examining these factors as they relate to gestational age, enteral feedings and the age of blood transfused to the association of MP pattern changes that may precede the development of TR-NEC.

Limitations

The major limitation of this study is the small sample size which limits generalizability. In addition, all subjects were enrolled from one institution with similar population characteristics.

To improve the generalizability of our findings, it would be necessary to repeat this study with a focus to enroll a larger number of subjects in a multi-center setting. Enrollment should focus on acquiring equal numbers of subjects for each corrected gestational age category for accurate statistical interpretation of findings and conclusions. This approach would allow greater applicability of finding to guide PRBC transfusion practice protocol development for the preterm population.

Another limitation of this study regarded the inability to place the near-infrared spectroscopy device in a timely manner to capture perfusion patterns during enteral feedings prior to PRBC infusion. Unfortunately, the study design did not allow for early parental consent to achieve this goal. Study team members were notified by medical team personnel when the decision was made to administer a blood transfusion. A major ethical stipulation of this study was to not delay PRBC transfusion administration related to subject enrollment; therefore, once parental consent was obtained, the NIRS device was applied and PRBC transfusion was began by nursing personnel.

Sensor probe displacement and/or artifact of near-infrared spectroscopy measurement were another limitation of this study. CSOR measurement depends on accurate measurement of both cerebral and mesenteric values simultaneously, and there were instances where both readings were not acquired. In one case where pneumoperitoneum developed, possible increased infrared path length due to free peritoneal air accumulation may have distorted accurate mesenteric rSO_2 measurements and may have attributed to the frequent signal "drop out" obtained and persistent low rSO_2 measurements.

To appropriately interpret CSOR ratios as a reflection of improved or impaired MP, cerebral values must be stable without significant fluctuation and variability. Subject 19 accurately demonstrated this principle. Because this particular infant demonstrated significant decreases in cerebral, mesenteric and renal values related to multiple apnea, bradycardia and

desaturation episodes, CSOR values actually increased. However, the increase in CSOR values was related to low cerebral values—not increased mesenteric values. Therefore, in the presence of impaired cerebral perfusion, CSOR values may be misleading. This demonstrates the importance of evaluating absolute values when interpreting CSOR values to accurately extrapolate differential tissue bed perfusion.

Implications for Practice

This study has enormous implications for both medical and nursing practice. First, the use of near-infrared spectroscopy (NIRS) as a supplement to conventional physiologic monitoring has been demonstrated. Current methods (pulse oximetry, heart rate, respiratory rate and blood pressure) do not effectively capture diminished MP in the presence of stable cerebral perfusion. Therefore, NIRS may be extremely beneficial to augment current physiologic monitoring, especially for preterm subjects of younger gestational age, to identify direct, real-time changes in MP subsequent to PRBC transfusions that may precede ischemic insult or TR-NEC development. Although specific practice regarding continuation of enteral feedings during transfusion events cannot be concluded from this study, we have demonstrated that NIRS offers a feasible reliable method to identify feeding intolerance and MP pattern reduction before clinical symptoms of TR-NEC present.

This study highlights the importance for clinicians to ascertain the age of blood being transfused to preterm subjects. For subjects of lower gestational age, it may be beneficial for prescribing clinicians to request $\leq$ 6 day old blood to be administered to subjects < 30 weeks gestation. This practice may substantially decrease the potential for decreased MP related to storage lesion and associated effects. This change in practice may not be feasible for many institutions and further research is needed to substantiate this potentially advantageous change in practice.

Implications for Policy

Policy changes related to this study are centered on the age of blood allocated to premature subjects from a blood bank perspective. For some institutions with high blood demand and distribution, it may not be feasible to allocate fresher blood to neonates only. However, in institutions with lower blood demand, this may be a reasonable request. Until larger studies are conducted determining that the age of blood is a significant risk factor for TR-NEC in preterm subjects leading to increased mortality, morbidity and economic burden, this policy change is not likely to occur. This study has demonstrated the potential increased risk for associated TR-NEC development in younger gestational age subjects subsequent to blood infusion, and has further illustrated the potential for MP alteration when given older blood. The impact of these two variables needs to be further elucidated in larger cohorts before these policy changes can be implemented at the national level.

Smaller institutional policy changes could be implemented given the findings of this study. There are no universally accepted policies for PRBC administration in the preterm population (Strauss, 2010). Perhaps the results of this study could guide policy regarding hyper-vigilance in cases where feeding intolerance is present, when older blood is administered, or in all preterm subjects < 30 weeks given a PRBC transfusion. If NIRS monitoring were utilized in these cases, detection of MP changes prior to TR-NEC onset may be identified with earlier therapeutic intervention to decrease the risk for TR-NEC onset or disease progression.

Future Direction

To appropriately evaluate changes in MP patterns of preterm subjects before, during and subsequent to PRBC transfusion events, a large multi-center investigation is needed. Establishing pre-transfusion baseline values of all subjects over a substantial amount of time would further

understanding of changes in perfusion patterns observed during and following the transfusion event. This would require application of at least two-site (cerebral and mesenteric) near-infrared spectroscopy technology for at least 24 hours prior to the PRBC transfusion in order to effectively ascertain pre-transfusion patterns for subjects receiving enteral feedings in the anemic state. This approach would allow for direct observation and analysis of perfusion changes (mesenteric and CSOR values) as a result of the transfusion event. It would also be extremely beneficial to observe a large number of subjects of various gestational ages to determine MP baseline values related to gastrointestinal immaturity, and how the transfusion event impacts perfusion changes during and subsequent to PRBC infusion. Furthermore, the effect of continuing and withholding feedings must be examined in subjects of all gestational ages to determine how MP changes are related to the presence or absence of feedings when a transfusion is administered.

The effect of the age of blood on perfusion changes needs to be investigated in a large cohort of preterm subjects with variable gestational ages. The effects of perfusion, using near-infrared spectroscopy technology, would demonstrate direct changes related to the age of blood infused. In addition, the effects of other mechanisms that may be associated with tissue perfusion alteration should be explored. These include the effects of many medications commonly prescribed to premature subjects, such as caffeine, indomethacin and antimicrobial agents. Preferential blood shunting to major organ systems during periods of hemodynamic instability associated with conditions such as sepsis, intraventricular hemorrhage and patent ductus arteriosis commonly seen in prematurity may have devastating effects on MP status increasing the risk for TR-NEC (Reber, et al., 2002). Because these conditions are often associated with anemia and subsequent blood transfusions, the need to examine these occurrences are paramount in preventing a potential onset of mesenteric ischemia and TR-NEC development.

Secondary analyses of other information collected during this dissertation study are planned. Because the age of blood infused was found to be significantly related to decreased perfusion patterns over time, further analysis of blood quality will be performed. This includes evaluation of plasma free hemoglobin, adenine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) levels that were collected and measured prior to blood transfusion administration for each event in this study. This analysis will shed light regarding possible cellular degradation that may occur during blood storage and may further explain the diminished MP pattern associated with older blood infusion. To further elucidate the storage lesion phenomenon, future studies are also planned to examine the presence of cytokines (proinflammatory and counterinflammatory mediators) in various ages of stored blood and will be conducted simultaneously with near-infrared spectroscopy monitoring of perfusion patterns before, during and subsequent to the administration of this blood to preterm subjects. Analyses are planned from information collected during this study related to medication administration and effects on perfusion patterns, specifically caffeine administration and antimicrobial therapy. This may help to further explain the differences seen in perfusion pattern variation related to ineffective gastrointestinal vasculature autoregulation and the presence of clinical or confirmed sepsis.

Finally, renal perfusion measurements were collected during all transfusion events, and these patterns will be analyzed as they relate to PRBC administration, and simultaneous comparison to changes observed in mesenteric and cerebral tissue beds. Our understanding of renal perfusion during transfusions is non-existent. Therefore, this analysis will provide an initial understanding of differential oxygenation of cerebral, mesenteric and renal tissue beds during and subsequent to PRBC administration in preterm infants and will further elucidate relative perfusion changes in each organ bed during the development of TR-NEC.

This study has added to the existing scientific body of knowledge by increasing awareness that MP changes are associated with gestational age in the presence of PRBC transfusions. Further, the age of blood may be instrumental in accentuating these changes increasing the risk for substantial perfusion alteration which may lead to the development of TR-NEC. Although it remains questionable if ischemia is the inciting or end result of these changes, our data demonstrate that decreased MP patterns precede the development of TR-NEC subsequent to PRBC transfusions, and that volume of transfusions and volume of feedings given during the transfusion seem to moderate this risk factor.

Summary

Preterm subjects are at increased risk for TR-NEC enterocolitis because of the immature response of the gastrointestinal vasculature to changes in blood flow, age of blood administered and potential interaction of enteral feedings. Further investigation is warranted in larger cohorts to extrapolate the interrelated effects of gestational age, age of blood infused and effects of enteral feedings administered during and subsequent to blood transfusions. Near-infrared spectroscopy is a reliable technology to accurately detect changes in mesenteric and cerebral tissue oxygenation as a result of PRBC transfusion. This study demonstrates the benefit and feasibility of this technology to aid in the detection of differential tissue oxygenation which may increase awareness of mesenteric tissue oxygenation depletion subsequently increasing the potential for ischemia and potential onset of TR-NEC in the preterm population.

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Appendix A

Institutional Review Board Approval

Study Protocol

Informed Consent for Subject Enrollment

Standard Operating Procedure, Blood Bank

Standard Operating Procedure, Roback Laboratory

TO: Terri Marin
Principal Investigator

CC: Kosmetatos Niki Neonatolog
Moore James Neonatolog

DATE: July 23, 2010

RE: **Notification of Expedited Approval**
IRB00024067
Cerebral and Somatic Tissue Perfusion Changes in Preterm Infants as a Result of the Age of Transfused Packed Red Blood Cells

This is your notification that your above referenced study was reviewed and APPROVED under the Expedited review process per 45 CFR 46.110 and 21 CFR 56.110. The approval is valid from 7/21/2010 until 7/20/2011. Thereafter, continued approval is contingent upon the submission of a continuing review request that must be reviewed and approved by the IRB prior to the expiration date of this study. The use of children in the subject population has been approved under 45 CFR 46.404, and one parent's permission is sufficient for participation. A partial waiver of HIPAA authorization is included with this approval.

Any reportable events (serious adverse events, breaches of confidentiality, protocol deviation or protocol violations) or issues resulting from this study should be reported immediately to the IRB and to the sponsoring agency (if any). Any amendments (changes to any portion of this research study including but not limited to protocol or informed consent changes) must have IRB approval before being implemented.

All correspondence and inquiries concerning this research study must include the IRB ID, the name of the Principal Investigator and the Study Title.

Approved Consent Documents
Consent: *version 3/29/2010*
Consent, verbal script: *version 3/29/2010*
HIPAA Authorization: *version 3/28/2010*

Sincerely,

Sarah K. Clark, CIP
Senior Research Protocol Analyst
This letter has been digitally signed

Cassandra
Josephson
Principal
Investigator
Pathology -
Main

DATE: July 20, 2011

RE: **Continuing Review Expedited Approval**
CR1_IRB00024067

IRB00024067
Cerebral and Somatic Tissue Perfusion Changes in Preterm Infants as a Result of the Age of Transfused Packed Red Blood Cells

Thank you for submitting a renewal application for this protocol. The Emory IRB reviewed it by the expedited process on 7/19/2011, per 45 CFR 46.110, the Federal Register expeditable category F(4). This reapproval is effective from **7/21/2011** through **7/20/2012** . Thereafter, continuation of human subjects research activities requires the submission of another renewal application, which must be reviewed and approved by the IRB prior to the expiration date noted above. Please note carefully the following items with respect to this reapproval:

- Subpart D: 46.404, one parent signature required

Any reportable events (e.g., unanticipated problems involving risk to subjects or others, noncompliance, breaches of confidentiality, HIPAA violations, protocol deviations) must be reported to the IRB according to our Policies & Procedures at www.irb.emory.edu, immediately, promptly, or periodically. Be sure to check the reporting guidance and contact us if you have questions. Terms and conditions of sponsors, if any, also apply to reporting.

Before implementing any change to this protocol (including but not limited to sample size, informed consent, study design, you must submit an amendment request and secure IRB approval.

In future correspondence about this matter, please refer to the IRB file ID, name of the Principal Investigator, and study title. Thank you.

Sincerely,

Andrea Goosen, MPH
Research Protocol Analyst
This letter has been digitally signed

CC:

Castillejo	Marta	Pathology - Main
Kosmetatos	Niki	Neonatolog
Marin	Terri	Graduate Nursing

Cerebral and Somatic Tissue Perfusion Changes in Preterm Infants as a Result of the Age of Transfused Packed Red Blood Cells

Cassandra Josephson, MD
Terri Marin, MSN, NNP-BC
Niki Kosmetatos, MD
Emory Department of Pediatrics, Division of Neonatology

Background: Recent studies suggest that red cell transfusions may be associated with Necrotizing enterocolitis (NEC),[1-3] a disease primarily of prematurity and occurs as a result of decreased intestinal perfusion and ischemia. The suggested mechanism of transfusion related NEC may be related to increased storage time of blood; however, the exact cause is unknown. During storage, red cells undergo changes that can potentially cause sludging in the microvasculature[4-8] which may impact tissue perfusion, thereby increasing the risk for NEC. In addition, enteral feedings may further exacerbate this problem. Studies have demonstrated reduced superior mesenteric flow postprandially which would suggest further perfusion impairment.[9] Further in-depth understanding and measurement of somatic tissue perfusion changes during events associated with NEC will enhance prediction and prevention therapies to reduce mortality and morbidity associated with this disease.

In addition to RBC deformity of stored blood, studies suggest that the loss of other constituents of stored blood may also play a role in producing altered microcirculation. Stored blood has decreased levels of 2, 3 DPG impairing hemoglobin affinity for oxygen and decreasing oxygen delivery to tissues. [4, 6-8, 10, 11] Nitric oxide bioavailability is also decreased in stored blood, producing a lack of vasodilatory capability of mesenteric circulation that potentially impairs adequate blood flow. [12-14] Currently we do not know the safe storage period of blood for preterm infants, or to what degree these RBC changes affect microcirculation and subsequent perfusion states when given to premature infants. Investigation regarding length of blood storage and its association to altered microcirculation and tissue perfusion will enlighten an important an unrecognized predisposition to NEC development.

Near infrared spectroscopy (NIRS) was first introduced in 1977 and is a non-invasive device that detects regional oxygen saturation changes when applied to various tissue beds of the neonate (cerebral, splanchnic and renal).[15] NIRS differs from pulse oximetry in that it measures the difference between oxygenated hemoglobin and deoxygenated hemoglobin at the tissue level. These real time measurements provide regional perfusion measurements (rSO2) of capillary beds where oxygen extraction occurs.[16] Studies using NIRS have shown that while cerebral perfusion can be maintained during hypotension, mesenteric and renal circulation is usually diminished. Pulse oximetry and other current bedside data values are not able to reflect this difference.[17] Cerebral-splanchnic ratios (CSOR) are reliable indicators of decreased splanchnic perfusion

during episodes of hemodynamic instability when preferential shunting of blood to vital organs occurs. CSOR values ≤ 0.75 have been associated with NEC development.[18] Currently there are no data that evaluates CSOR during red cell transfusions, the effects of feeding, or the impact of increased red cell storage time on tissue perfusion patterns. Preliminary piglet studies have shown that the severity of gut and renal perfusion changes increases with longer red cell storage time with minimal changes to cerebral perfusion.[19] These associations must be evaluated in the preterm population to establish safe guidelines for red cell transfusions. Studies using NIRS to monitor cerebral and intestinal perfusion in cardiac patients are numerous; however, studies monitoring somatic perfusion associated with NEC are limited. [16, 17, 20-23]

Objective: The objective of this investigation is to examine perfusion patterns associated with red cell transfusions in preterm infants using near infrared spectroscopy (NIRS) and determine if the age of red cells given further alters these perfusion patterns. The proposed study has three aims:

1. Evaluate cerebral, splanchnic and renal tissue perfusion patterns during blood transfusions with and without feedings. This is to identify variations in tissue oxygenation delivery that may suggest relative ischemia or variations in oxygen delivery in preterm infants during these two conditions.
2. Examine the association between length of time blood is stored and changes in splanchnic perfusion patterns in preterm infants resulting from this blood transfusion.
3. Determine if perfusion patterns differ in infants who develop NEC vs. those that do not following red cell transfusion.

Significance: This investigation will address gaps in the current body of scientific knowledge regarding the association between ischemia and the administration of red cell transfusions in preterm infants. Additionally, the association between age of red cells given and perfusion pattern change is unknown. Further, the impact of feeding on tissue perfusion during transfusions is also unknown. The preterm gastrointestinal system is extremely vulnerable, and when stressors are encountered within this system, the potential for injury and ischemia escalate. Presently, there are no studies evaluating somatic tissue perfusion changes during and following blood transfusions. Therefore, investigation of tissue perfusion changes during blood transfusions is urgent and warranted. NIRS provides a non-invasive mechanism to observe tissue perfusion changes and further our understanding of associated factors. This study will influence clinical practice and establish safe guidelines for the administration of transfusion and feeding practice around transfusions to preterm infants. With this knowledge we can potentially reduce the risk for NEC, decrease overall hospital stay and costs, and potentially improve outcomes of the premature population.

Methods

General Study Design and Overview: The proposed study will use a longitudinal descriptive correlational design to examine changes in perfusion patterns before, during and following red cell transfusions in preterm infants specifically evaluating:

- Pattern comparison between infants receiving feedings and those that do not
- Pattern comparison between age (older v. newer) of red cells being transfused
- Pattern comparison of infants that developed necrotizing enterocolitis vs. infants that did not after red cell transfusion

Components of the red cells to be collected and measured prior to transfusion include 2,3-DPG, nitric oxide, RBC smear, hematocrit, and electrolytes. These values will be recorded for future analysis if significant relationships are found between age of blood and altered perfusion patterns. Duration of blood transfusion, age of stored blood, and amount given will be recorded. Perfusion patterns will be analyzed using Near Infrared Spectroscopy (NIRS) before, during and up to 48 hours following the transfusion. Feedings will be recorded according to type, route, infusion rate, volume, caloric density, and time given in relationship to transfusion.

Our population will be preterm infants who have been admitted to Emory University Hospital Midtown, Grady Memorial Hospital or Egleston Children's Hospital Neonatal ICU who meet inclusion criteria. We will measure cerebral and splanchnic perfusion on infants who receive a red cell blood transfusion. Monitoring of perfusion will not affect the management of the infant, and will not influence therapeutic options. Routine neonatal care will be entirely determined by the attending physician in the NICU.

NIRS Data Collection and Monitoring: Regional tissue oxygen saturation of the cerebral and somatic regions will be prospectively recorded in 1 minute intervals for 1 hour prior to blood transfusion and up to 48 hours following the transfusion. If the infant is receiving feedings, a baseline recording will be taken during the feeding given prior to the transfusion. An investigator will place the two sensors on the head and abdomen, and lateral flank areas and will remove them at 48 hours. If the attending neonatologist feels that the sensors are interfering with medical care in any way, the sensors will be removed immediately. If skin irritation develops, the sensors will be removed immediately and the skin will be treated according to physician discretion.

Data Collection Procedures During NIRS Monitoring from patient records: During the study period, all vital signs will be collected from the patient record. These values are measured routinely and documented in the chart/computer. If the value has not been recorded, it will not be measured specifically for the purposes of the study. If the patient is on a ventilator, the settings the patient is on will be monitored as will the blood gases (Pa02, PaC02, pH, base excess). If the patient is receiving other forms of oxygen support (nasal cannula, continuous positive airway pressure), this data will also be collected as recorded in the chart. Other procedures or medications the patient receives during the transfusion and 48 hours following will be collected as recorded in the patient record. This data will be stored and analyzed in conjunction with the NIRS data.

We will also collect blood samples from the blood unit being transfused, before the transfusion is started. The blood sample will be analyzed for 2,3 DPG, nitric oxide, electrolytes, RBC smear and hematocrit. These values will be collected by the investigating team and analyzed in conjunction with NIRS data.

We will also document from the infant's chart the following information: gestational age at birth, current age, birth weight, current weight, sex, race, hospital number, apgar scores at 1 and 5 minutes, date of onset of NEC (if applicable), and current medications the baby is on. Feedings type, amount, duration, caloric density, route and time given will be recorded. Length, volume and age of blood transfused will be recorded. All infants will be followed until hospital discharge for the development of NEC.

Subject Inclusion/Exclusion Criteria: The sample will include premature infants of all races and gender who are admitted to the study NICUs, who meet the Inclusion/Exclusion criteria after obtaining informed consent from the parent or legally authorized representative. The sample will consist of 80 patients to achieve power of .80, effect of 20% and allowing for 10% attrition rate due to transfer or withdrawal from the study for any reason. There will be no postnatal age restrictions for inclusion in the study. The daily average census of the three sites for data collection is 29, 28 and 35 with approximately 3-5 transfusions ordered per week.

Inclusion criteria:

1) < 37 weeks corrected gestation
2) Receiving packed red blood cell transfusion for anemia as determined by primary care team.
3) Not receiving intravenous vasopressors
4) Patients with patent ductus arteriosis (PDA) that has been previously treated and/or ligated and no longer hemodynamically significant can be included.
5) English-speaking

Exclusion criteria:

1) ≥37 weeks corrected gestation
2) Does not receive transfusion
3) On intravenous vasopressors
4) Infants diagnosed with a major congenital anomaly, trisomy, genetic anomaly, hemodynamically significant PDA or necrotizing enterocolitis will not be included.
5) Non-English speaking

Rationale for inclusion/exclusion criteria: Any infant with a condition that is considered hemodynamically unstable may confound the measured perfusion status and alter the results being analyzed. Excluding those that are unstable for the above reasons will control for these confounders. Non-English speaking participants will not be included; however our targeted enrollment includes a small percentage of Hispanic patients. The majority of the Hispanic population at the three clinical sites typically speaks English and therefore will be able to fully comprehend the study purpose, risks, benefits and procedures and provide consent for their child. The recruitment efforts will focus on providing a diverse minority and non-minority sample based on the normal distribution percentages of ethnicity/race representative of the population in the State of Georgia

and Dekalb county.

Data Analysis: Data will be analyzed using the most current SPSS-PC statistical software under the direction of a biostatistician. Methods for statistical analysis will include repeated measures analysis, correlations, and multiple logistic regression. Each specific aim and research question will be analyzed according to the stated outcome of interest. General descriptive statistics on sample characteristics will include gender, ethnicity, mean corrected gestational age, mean current weight, mean birthweight and postnatal age. Initial analysis will also include distribution of the data, type and extent of missing data, and determination that statistical assumptions are met. Underlying assumptions of logistic regression and correlation analysis will be examined; normality visually assessed using frequency distributions, scatter plots, kurtosis, and skewness.

Perfusion patterns will be analyzed using repeated measures design examining rolling averages and percent change in fluctuating patterns. Comparisons of pattern change will be examined pre- and post-transfusion. This data will be specifically examined looking for changes associated with older vs. newer red cells administered during transfusion and in relation to feeding status. Duration and frequency of pattern change will be examined as it relates to accompanying events: transfusion, feeding, medication treatment, and change in clinical status. Duration and frequency of pattern changes will be correlated with age of red cells transfused, the presence or absence of feeding, and the subsequent incidence of NEC development.

Perfusion patterns will be examined for changes according to CSOR ratio value ≤0.75, or a 20% or more change from baseline, time length of change, and associations of change to concurrent events. Frequency of pattern changes over time will be evaluated and correlated with concurrent event and age of red cells given.

Instrumentation: For this study we plan to use the FDA approved IN Vivo Optical Spectroscopy (INVOS) System (Somanetics, Troy, MI) to provide continuous real-time monitoring of changes in somatic and cerebral oxygen saturation. The INVOS sensor has a light emitting diode that emits NIR light of two wavelengths 730 nm and 810 nm and two optodes that subsequently receive the scattered light. The proximal (shallow) detector receives a signal from the peripheral tissue and the distal (deep) detector receives a signal from the deep tissues; by subtracting the proximal from the distal value, tissue specific rSO2 is obtained. The rSO2 is reported as a percentage on a scale from 15% to 95%. The SomaSensor is connected to a preamplifier which is placed close to the patient and amplifies the rS02 signal which is then carried to a display unit where the values and trends are visually displayed on the screen. The display unit controls all functions of the system with selections made by keys with onscreen labels. The data from the Somanetics USB port can be transferred and stored to a computer for offline analysis via the USB Flash Drive component within the INVOS system. Two types of neonatal sensors are available for use, and we will use both the cerebral and somatic (splanchnic and renal) sensors for this investigation. This instrument is precalibrated by the manufacturer and does not require user calibration. It has safety rating of BF Class I, and complies with U.S. regulatory standards for medical equipment: IEC 60601-1, UL 60601-1 and CSA 22.2.601-1.

NIRS monitoring will be utilized to measure cerebral and somatic perfusion. Near infrared spectroscopy (NIRS) is a noninvasive measurement device that provides real-time recordings of regional tissue perfusion (rSO2). It operates on the principle that near-infrared (NIR) light (700–1000 nm) passes easily through human tissue and is

absorbed by chromophores in an oxygen-dependent manner that include hemoglobin and cytochrome aa3.[24-26] NIRS differs from pulse oximetry in that it provides real-time measurement of tissue oxygenation (rather than measuring only arterial oxygen saturation as in pulse oximetry) and is not dependent on pulsatile blood flow for accurate monitoring. [27] NIRS measures changes in tissue concentration of oxyhemoglobin (HbO2) and deoxyhemoglobin (HHb), and derives changes in regional tissue oxygen saturation (rSO2) expressed in the formula:[25, 28-30]

$$rSO2 = \frac{HbO2}{HbO2 + HHb}$$

To detect splanchnic ischemia after transfusion, NIRS will be used to evaluate the cerebral-splanchnic ratio (CSOR). This variable is an expressed ratio between cerebral and splanchnic oxygenation rSO2 values[18] as expressed below.

$$CSOR = \frac{\text{splanchnic rSO2}}{\text{cerebral rSO2}}$$

Current data in both the literature and studies conducted by Dr. James Moore indicate CSOR measurements ≤ 0.75 are consistent with the development of splanchnic ischemia and NEC development.[18] Preliminary piglet studies conducted by Dr. Moore have demonstrated a 20-35% change in baseline somatic perfusion patterns occur after transfusion of packed red cells. This data and previous work in preterm infants are why a 20% effect change was chosen for this study and sample size calculation.

The validity of NIRS as an accurate measurement of regional perfusion is its comparison to S_VO_2[31, 32] and gastric tonometry measurements. [33] These studies have demonstrated that abdominal NIRS rSO2 measurements correlate with invasive SvO2 indwelling monitoring systems, [27, 31, 32] as well as highly invasive gastric tonometry which measures intraluminal PCO2 and Ph as determinants for hypoperfusion.[33] NIRS has also been extensively utilized to monitor cerebral perfusion during cardiothoracic surgery. [21, 27, 32, 34-38] Data have shown that rSO2 measurements were good correlates of neuronal perfusion and accurately detects cardiac shunting pre- and post-operatively.[34] Therefore, NIRS has been proven as an acceptable instrument to measure tissue perfusion, providing indices that can be analyzed in predicting circulation shunting.

Vital signs will be collected and recorded using Hewlett-Packard cardiovascular monitoring devices. Heart and respiratory rates will be recorded for 1 hour prior to transfusion, every 15 minutes for the first hour of the transfusion, every 30 minutes for the duration of the transfusion, and every 1 hour for the remainder of the monitoring period of 48 hours post transfusion. Mean arterial pressure will be recorded and time synced with the NIRS data as a measure of adequate cardiac output. Pulse oximetry monitoring will also be recorded at the same intervals using Sensormetics devices. Feedings will be recorded to include volume, type, infusion rate, time of onset, route, and caloric density. These data will be simultaneously entered into the NIRS monitoring system and time sync will be verified by the study team. The NIRS data will be downloaded by the research PI/team member 48 hours following the transfusion, and analyzed using current SPSS statistical software.

Limitations: Participants recruited through convenience sampling may not be representative of the total population of premature infants ≤ 36 weeks corrected gestation, thus limiting the generalizability of the study findings and may threaten

external validity. Because this is a descriptive study which will only analyze patterns, cause and effect relationships cannot be determined. Specific types and caloric densities of feedings will not initially be delineated during data analysis, therefore limiting the study findings to feedings vs. NPO status only. However, if patterns are found to significantly differ during the initial data analysis phase, further investigation is planned to analyze these specific relationships. Other limitations of this study include any event or intervention that occurs during the monitoring period that is not accounted for in this study that influences perfusion patterns may potentially skew results. To address this limitation, these variables will be considered confounders and controlled for during statistical analysis through risk adjustment measures. Probe placement may affect rSO2 measurements if not uniformly applied among the sample population. To address this limitation, the PI will ensure that all personnel that will be applying these probes are properly trained during competency checks for proper location of probe placement based on literature and manufacturer recommendations. Furthermore, the PI/research team members will strive to apply the NIRS probes for all infants recruited for the study if feasible. Finally, genetic predisposition to NEC has been cited as a potential precursor for disease development,[39] and this study will not collect data to identify genetic markers, therefore limiting this aspect of prediction from the model. Due to the design of this study, this variable will not be controlled.

Potential Risks: Risks to human participants are low and poses no greater than minimal risk in the proposed study. Participation in the study will be purely voluntary and will be determined by the parent(s) of the neonate. No intervention or change in patient care practices will be instituted upon recruitment. Only infants that are to normally receive transfusions for uncomplicated anemia of prematurity will be recruited. The potential risks of participating are related to probe placement with adhesive material that is placed on the infant's skin on the forehead and abdomen. Abrasions from the probes are unlikely, but possible, and if they occur will be treated as directed by the attending MD/NNP. Prevention of skin abrasions will be instituted by applying a protective skin covering (tegaderm) underneath probes on all infants and probes will be closely monitored by nursing staff every hour during monitoring, and daily by the research staff. If skin irritation is observed, the probe will be removed and replaced with tegaderm protective covering at a different location.

Treatment and Compensation: Although this is a non-invasive procedure and we are confident that no risks are involved in it, any injuries related to this study will be treated by the NICU team. There will be no compensation for injuries associated with this study.

Cost and Reimbursement: There will be no cost to the patient associated to the study, and no reimbursement to the families.

Expected/Alternative Outcomes: The expected outcomes of the proposed study is to show a negative impact on perfusion patterns in preterm infants receiving transfusions, and these patterns will be further impaired when the age of blood increases and feedings are given. Further, we expect to find an association between altered perfusion patterns and the development of NEC. However, we may not find these associations and altered perfusion patterns may not be significant. This will provide us information that transfusions, regardless of the age of the blood being given, do not affect perfusion patterns in preterm infants and therefore no restriction needs to be placed on the age of

blood transfused. Additionally, if no changes in perfusion are seen when feedings are given during a red cell transfusion, recommendations can be made to safely administer feedings during a transfusion. It should be noted that larger studies may be warranted if there are borderline findings suggesting possible associations between these variables. Regardless of the outcomes, the results of this study will have implications for the direct care of preterm infants.

Confidentiality: The name and medical record number for each patient will be part of the original data gathering form. The patient will be assigned a study number with an identification link that will be available in the Principal Investigator's office. This identification-link will be kept in a locked cabinet. The name, medical record number, or any other characteristic which may lead to the identification of a patient will not be part of any publication resulting from the study.

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Emory University School of Medicine

Consent to be a Research Subject
July 201, 2011

Title: Cerebral and Somatic Tissue Perfusion Changes in Preterm Infants as a Result of the Age of Transfused Packed Red Blood Cells

Principal Investigator*:* Cassandra Josephson MD

Terri Marin NNP-BC

Niki Kosmetatos MD

Sponsor: Florida Association of Neonatal Nurse Practitioners, Sigma Theta Tau International

Introduction

You are being asked to consent for your child to be in a medical research study. This form is designed to tell you everything you need to think about before you decide to consent (agree) for your child to be in the study or not to be in the study. **It is entirely your choice. If you decide for your child to take part, you can change your mind later on and withdraw from the research study.** The decision to join or not join the research study will not cause you to lose any medical benefits. If you decide for your child not to take part in this study, your doctor will continue to treat him/her.

- Please carefully read this form or have it read to you
- Please listen to the study doctor or study staff explain the study to you
- Please ask questions about anything that is not clear
- Feel free to take home an unsigned copy of this form and take your time to about it and talk it over with family or friends

After talking about the information in this consent form with the study team you should know:

- Why this research study is being done

- What will happen during the research
- What parts of the study are experimental and what parts are standard medical care
- If this study uses a drug or device, whether the US Food and Drug Administration has approved it or not
- Any possible benefits to you. Most research is done to learn things that will help patients in the future. No one can guarantee that a study will help your child.
- The possible risks to you. Consider these carefully.
- What other medical care you could seek instead of being in this research study and
- How problems will be treated during the study and after the study is over.
- Who will have access to your study information

If you agree to join this research study, you will receive a copy of this consent form with your signature and the date, to keep. Do not sign this consent form unless you have had a chance to ask questions and get answers that make sense to you. Nothing in this form can make you give up any legal rights. By signing this form you will not give up any legal rights.

Purpose:

You are being asked to give permission for your infant to take part in a research study. The purpose of the study is to look at changes in blood flow to your infant's gut, brain and kidneys during the transfusion of blood ordered by your infant's doctor. We will take this measurement using an instrument that shines light on the belly or forehead and measures how much comes back out. This procedure does not use radiation. Instead, it uses a light source in the near infrared range. We want to measure the blood flow in your baby's gut, brain and kidneys continuously for 48 hours following the blood transfusion. We also want to take a measurement 1 hour before the transfusion is given, or starting at the time the last feeding is given just prior to the transfusion if your baby is receiving feedings. This will help us see if there are changes in blood flow patterns when a transfusion is given. We hope to enroll 85 infants in this study.

Procedures:

Routine care: Your child's doctor has ordered a blood transfusion for your child. This will happen whether or not your child takes part in the study. No blood will be drawn from your child during the study. Before the blood transfusion, we will take a sample of the blood that is going to be given to your child. This blood sample will be drawn from the unit using sterile technique. Your child's care will not be affected in any way if you would prefer that they did not participate in the study**.** Also your child's care will not be changed in any way if they do participate in the study.

Experimental: If you agree to allow your child to take part in the study, we will attach small pads to your child's belly, forehead and back. The pads are attached to wires that lead to the instrument for us to make recordings. The pads are held in place with a sticky material. To avoid skin irritation, we will check the skin every 24 hours and remove the pads if we see any change in your child's skin. We will then measure and record the blood flow patterns for about 48 hours (or 2 days). After 48 hours, we will remove the pads. After that, we will record information being collected for routine care of your infant. No extra tests will be done because your child is in the study. Your infant will be in the study until he or she leaves the neonatal intensive care unit. All research will be done in the neonatal intensive care unit.

The experimental measurement for this study will not interfere with the care of your child. It will be stopped immediately if your child is not considered to be clinically stable in the opinion of the care team. Your child will not have any responsibilities or expectations before, during or after the procedure.

Risks*:*

We do not believe that there are any serious to your child by taking part in this study. The pads that we will put on your child's belly have some sticky material and may cause some irritation when in place and when removed. If the investigators or your child's doctors feel that the pads are in the way of examining your child, he or she will not be included in the study, or the pads will be removed immediately. If skin irritation develops, the pads will be removed immediately and the skin will be treated by the attending medical team. Previous research has shown that using the light source in the near infrared range has not been associated with any damage to the infant or to cause any discomfort to the infant. The light source is similar to the light used by a pulse oximeter. This is used to measure your infant's oxygen saturation level.

Benefits:

Taking part in this research study may not benefit your child personally, but we may learn new things that could help other patients in the future.

Alternatives:

This is a non-therapeutic study, and the alternative is to not participate.

Confidentiality:

Certain offices and people other than the researchers may look at your medical charts and study records. Government agencies, Children's Healthcare and Emory employees overseeing proper study conduct may look at your study records. Study sponsors may also look at your study records. These offices include the Office for Human Research Protections, the sponsor(s), the Emory Office of Research Compliance, the Office for Clinical Research, and the Emory IRB. Children's Healthcare and Emory will keep any research records produced private to the extent they are required to do so by law. A study number rather than your/child's name will be used on study records wherever possible. Your/child's name and other facts that might point to you/your child will not appear when we present this study or publish its results.

Study records can be opened by court order or produced in response to a subpoena or a request for production of documents unless a Certificate of Confidentiality is in place for this study.

Since your child is a patient at an Emory Healthcare facility or CHOA, then they will have an Emory Healthcare or CHOA medical record.

Children's Healthcare and Emory Healthcare may create study information about you/your child that can help Emory Healthcare take care of you/your child. For example, the results of study tests of procedures. These useful study results **will** be placed in your/child's Children's Healthcare or Emory Healthcare medical record. If you agree to be in this study, a copy of the consent form and HIPAA patient form that you sign will be placed in your/your child's Children's Healthcare or Emory Healthcare medical record. Anyone who has access to your/child's medical record will be able to have access to all the study information placed there. The confidentiality of the study information in your/child's medical record will be protected by laws like the HIPAA Privacy Rule. On the other hand, some state and federal laws and rules may not protect the research information from disclosure.

Children's Healthcare and Emory do not control results from tests and procedures done at other places. So these results would not be placed in your/child's Children's Healthcare or Emory Healthcare medical record. And they will not likely be available to Children's Healthcare or Emory Healthcare to help take care of you/your child. Children's Healthcare and Emory also do not have control over any other medical records that you/your child may have with other healthcare providers. Children's Healthcare and Emory will not send any test or procedure results from the study to these providers. So if you decide to be in this study, it is up to you to let them know.

The researchers will review the results of certain study tests and procedures **only** for the **research**. The researchers will **not** be looking at these results to make decisions about your/child's personal health or treatment. For this study, those things include:

Near Infrared Spectroscopy (NIRS***)*** data on blood flow to the intestines, brain and kidneys.

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Compensation:

You will not be paid for allowing your child to participate in this study.

In Case of Injury

Emory and Children's Healthcare have not set aside any funds to pay for urgent health care. Also, Emory and Children's Healthcare have not set aside any funds to pay you if you/your child become/s ill or injured from being in this study. The only exception to this policy is if it is proven that the negligence of an Emory or Children's Healthcare employee directly caused your/child's injury or illness. "Negligence" means the failure to follow a standard duty of care.

If you believe your child has been injured by this research, you should contact Dr. Cassandra Josephson (Phone 404-785-4553.)

Costs:

There is no cost to the patient associated with this study

Questions

Contact Terri Marin at 678-358-9940:

- if you have any questions about this study or your part in it,
- if you have questions, concerns or complaints about the research

Contact Dr. Cassandra Josephson at 404-785-4553

- if you feel you have had a research-related injury or a bad reaction to the study drug, or

If you have questions about your rights as a research subject or if you have questions, concerns or complaints about the research, you may contact:

The Emory Institutional Review Board

1599 Clifton Rd.

Atlanta, GA 30322

404-712-0720

Toll Free: 1-877-503-9797

If you are a patient receiving care at Children's Healthcare of Atlanta and have a question about your rights, please contact Kristine Rogers, Director of Clinical Research at 404-785-1215.

New Findings*:*

We may learn new things during the study that you may need to know. We can also learn about things that might make you want to stop your child participating in the study. If so, you will be notified about any new information.

Voluntary Participation and Withdrawal

Your child's participation is completely voluntary and you have the right to refuse your child to be in this study. You can also stop your child being in the study at any time after giving your consent. This decision will not affect in any way your child's current or future medical care or any other benefits to which your child is otherwise entitled.

If you wish to withdraw your child from the study, please write a letter stating that you wish your child to be withdrawn from the study. This letter should be sent to:

Dr. Cassandra Josephson

1405 Clifton Road NE

Atlanta,

GA 30322

You can call Dr. Josephson (404-785-4553) or Terri Marin (678-358-9940) if you have any further questions about your wishing your child to withdraw from the study.

The study doctor may stop your child from taking part in this study at any time if they decide it is in your child's best interest.

We will give you a copy of this consent form to keep.

Consent

I have read this consent form (or it has been read to me). All my questions about the study and my part in it have been answered. I freely consent to be in this research study.

By signing this consent form, I have not given up any of my legal rights.

_____	_____	_____
Subject's name (Please Print)		
_____	_____	_____
Subject's legally authorized representative	Date	Time
_____	_____	_____
Person Obtaining Consent	Date	Time
_____	_____	_____
Initials of person obtaining consent	Date	Time
_____	_____	_____
Principal Investigator	Date	Time

Standard Operating Procedure: Roback Lab

Contact PI: Terri Marin, NNP-BC, PhD student Co-PI: James Moore, MD, PhD
Home: 770-631-9447 Cell: 678-358-9940 Office: 404-727-5285 Cell: 404-275-3021

Subject ID

#_____

Delivered to Core Lab

by_____

Date_____Time_____

_

Segment Collection

Date_____Time_____

Procedure:

1) Remove segments (2) from bag—**DO NOT CENTRIFUGE, SHAKE or SQUEEZE SEGS**. The tests will be performed on only one segment. Two segments are provided in case of insufficient quantity.
2) Perform following tests on one segment:
    a. ATP
    b. 2,3-DPG
3) Record results on this form (see below). If Quantity not sufficient on either specimen, please indicate below and notify one of the above study personnel.
4) Place this completed form in the **GREEN** notebook labeled "NIRS Transfusion Study".

Results:

ATP_____

2,3-DPG_____

Plasma Free Hgb_____

*QNS_____

*Study Personnel notified

_____Date/Time_____

Blood Bank Packed Red Blood Cell Unit Segment Collection and Log

Standard Operating Procedure

Cerebral and Somatic Perfusion Pattern Changes as a Result of the Age of Transfused Packed Red Blood Cells Study

Principal Investigator: Cassandra Josephson, MD

Purpose: To prepare two (2) serum/plasma segments or samples from parent unit of PRBCs prior to release of unit to be transfused to preterm infant enrolled in the NIRS transfusion study for the measurement of plasma-free hemoglobin, ATP and 2,3 DPG testing.

Procedure:

1. Once a patient is enrolled, the PI will notify the Blood Bank by email and confirm with phone call. The infant's name, medical record number and study ID number will be communicated to the Blood Bank director. The PI will also place a piece of paper in the blood bank study notebook labeled "NIRS Transfusion Study" with the same information that was communicated by email to the Blood Bank director.

2. Blood Bank staff will enter this study information into the patient's laboratory information system file so that each time this enrolled infant is ordered to receive a transfusion, the blood bank staff will be alerted to prepare two (2)segments or samples from the RBC parent unit prior to transfusion.

3. There is a study log form in the **"NIRS Transfusion Study" notebook (blue)** in the blood bank for the medical technologist to complete that will include the subject ID number, blood unit number, unit collection date (ARC invoice), irradiation date and date and time that the segments were prepared from each ***parent*** unit of blood before it is sent to the Neonatal Intensive Care Nursery to be transfused to the enrolled patient. Each

entry should be initialed by the blood bank staff. Our study requirement is that two (2) RBC segments or samples will be prepared from the ***parent unit*** prior to transfusion that is prepared for the purpose of plasma-free hemoglobin, ATP and 2,3-DPG testing.

4. **If multiple babies are to receive transfusion from ONE parent unit on the SAME DAY**, segment preparation will only be done **ONCE** from the parent unit—i.e. only two (2) segments will be prepared from this parent unit and the medical technologist will note this combined parent unit collection on the study log. See attached "Example of Log for multiple patients receiving transfusion from SAME PARENT UNIT".

5. Prepare two (2) segments or samples of blood in red top tubes without preservatives (2cc of blood in each tube) from parent unit label with subject ID# on supplied labels found in study notebook. If multiple patients are receiving blood from this parent unit **ON THE SAME DAY**, note on study log with brackets (per example provided), and **only** attach the 1st patient's subject ID # label.

6. If the parent unit of blood is prepared for a subsequent transfusion to the same or different patient ***on a different day***, two (2) segments or samples should be prepared and labeled.

7. Segments should be stored in Blood Bank refrigerator in basket labeled "NIRS Transfusion Study". Each segment will be labeled with patient's subject ID #. Terri Marin will de-identify patient sample prior to transport.

8. The PI or designee will pick-up the collected segments and transport to laboratory for testing.

Appendix B

Subject Data Form

Blood Bank Subject Data Form

Subject Data Form

Study ID #_____ MR #_____

Date of Transfusion_____Infant current age_____

Gestation age at birth_____Corrected gestational age_____

Infant birthweight_____Current weight_____

Ethnicity_____gender_____

First transfusion received? Yes No If no, which # transfusion?_____

Infant: ABO type_____ Rh type_____ Unit: ABO type_____ Rh type_____

Donor Unit Number_____

Volume of transfusion: _____cc/kg to be given over _____hours Divided? Y N

Reason for transfusion_____

Hct before each tx_____Date/time collected_____

Time transfusion started_____Time ended_____

2nd transfusion start_____end_____

NPO ☐ Feedings* ☐ Type_____Amount_____

Frequency_____Time of feeding prior to transfusion:_____

**Times of feeding during/following transfusion to be documented on flowsheet data form*

Ventilation status at start of transfusion:
Mode_____FiO2_____rate_____PIP/PEEP_____

NIRS Information:

Channel 1_____Channel 3_____Channel 4_____

Date/Time applied_____

Date/Time removed_____

Time on machine_____

Time on Clock_____Difference_____

Pertinent diagnoses/medications:

Laboratory Data:

Date	Time	pH	pCO2	pO2	HCO3	Base	Na	K	Cl	CO2	BUN	Cr	Glucose	Ca

Vital Signs:

Date	Time	HR	RR	BP	Sat	Apnea	Brady	Comments

Feeding Data:

Date	Time	Type	Duration	Residual amount

Medication Data:

Date	Time	Medication	Dosage	Duration	Route

Procedures:

Date	Time	Procedure	Length of procedure	Comments

Blood Bank Collection Log

Date: (*mm/dd/yy*)
Time: (*ex: 0900; 1900*)
Initials: (*F/M/L*)

Unit ID:

Component: ☐ RBC ☐ Platelet ☐ FFP ☐ Gran ☐ Cryo

Volume of parent unit: (*mL*)

Unit donation date: (*mm/dd/yy*)

Date irradiated @ Red Cross: (*if applicable*) (*mm/dd/yy*)

Direct Donor? Y N

Subject ID#

Date: (*mm/dd/yy*)
Time: (*ex: 0900; 1900*)
Initials: (*F/M/L*)

Unit ID:

Component: ☐ RBC ☐ Platelet ☐ FFP ☐ Gran ☐ Cryo

Volume of parent unit: (*mL*)

Unit donation date: (*mm/dd/yy*)

Date irradiated @ Red Cross: (*if applicable*) (*mm/dd/yy*)

Direct Donor? Y N

Subject ID#

Date: (*mm/dd/yy*)
Time: (*ex: 0900; 1900*)
Initials: (*F/M/L*)

Unit ID:

Component: ☐ RBC ☐ Platelet ☐ FFP ☐ Gran ☐ Cryo

Volume of parent unit: (*mL*)

Unit donation date: (*mm/dd/yy*)

Date irradiated @ Red Cross: (*if applicable*) (*mm/dd/yy*)

Direct Donor? Y N

Subject ID#

Date: (*mm/dd/yy*)
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Initials: (*F/M/L*)

Unit ID:

Component: ☐ RBC ☐ Platelet ☐ FFP ☐ Gran ☐ Cryo

Volume of parent unit: (*mL*)

Unit donation date: (*mm/dd/yy*)

Date irradiated @ Red Cross: (*if applicable*) (*mm/dd/yy*)

Direct Donor? Y N

Subject ID#

Date: (*mm/dd/yy*)
Time: (*ex: 0900; 1900*)
Initials: (*F/M/L*)

Unit ID:

Component: ☐ RBC ☐ Platelet ☐ FFP ☐ Gran ☐ Cryo

Volume of parent unit: (*mL*)

Unit donation date: (*mm/dd/yy*)

Date irradiated @ Red Cross: (*if applicable*) (*mm/dd/yy*)

Direct Donor? Y N

Subject ID#

Date: (*mm/dd/yy*)
Time: (*ex: 0900; 1900*)
Initials: (*F/M/L*)

Unit ID:

Component: ☐ RBC ☐ Platelet ☐ FFP ☐ Gran ☐ Cryo

Volume of parent unit: (*mL*)

Unit donation date: (*mm/dd/yy*)

Date irradiated @ Red Cross: (*if applicable*) (*mm/dd/yy*)

Direct Donor? Y N

Subject ID#

Appendix C

Blood Characteristics

Subject ID	Subject Blood Type/Rh	Donor Blood Type/Rh	Received two transfusions?	Split Volume	Full Volume	Volume 1st Transfusion (cc/kg)	Age of Blood (Days)	Irradiation Length (Days)	Volume 2nd Transfusion (cc/kg)	Age of Blood (days)	Irradiation Length (Days)	TR-NEC	Comments
1	B+	O+	•	•		7.5	6	1	7.5	6	1		Same donor unit
2	A+	O+	•	•		7.5	13	8	7.5	13	8		
3	A+	O+	•	•		7.5	7	4	7.5	7	4	•	
4	O+	O+	•	•		7.5	10	6	7.5	10	6		
5	B+	O+			•	10	7	3					
6	B+	O+			•	15.5	7	3					
7	B+	O+			•	16	6	2					Same donor unit
8	O+	O+			•	15	6	2					
9	O+	O+			•	15	4	1					Same donor unit
10	O+	O+			•	15	5	2					
11	B+	O+			•	15	6	3					
12	O+	O+			•	15	7	3					Same donor unit
13	O+	O+			•	15	7	3					
14	B+	O+	•	•		10	6	2	10	6	2		
15	A+	O+	•		•	20	7	4	15	10	7	•	
16	O+	O+	•		•	15	7	3	15	7	3	•	Same donor unit
17	O+	O+	•		•	15	7	3	16	8	4	•	
18	A+	O+			•	15	14	10					
19	AB-	O-			•	15	5	2					
20	O+	O+			•	15	5	2					
21	O+	O+			•	15	6	2					
22	A+	O+			•	15	4	0					
23	O+	O+			•	20	7	4					
24	O+	O+	•	•		10	6	2	10	6	2		