Exploration of the Linkage between the A: a Gradient and

Oxygen Transfer Efficiency

As a Measure of Response to Treatment with Inhaled Nitric Oxide

in a Heterogenous group of

Newborns Treated for Acute Hypoxic Respiratory Failure

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by

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DEDICATION

This dissertation is dedicated to my family, whom I love with all my heart and soul, now and forever. To Richard Hull, CRRT, my friend, and mentor who unknowingly sparked my interest in pulmonary shunting in babies, and who unselfishly taught me more than I ever wanted to know about neonatal respiratory care… we all miss Richard terribly. Lastly, I cannot neglect to dedicate this dissertation to my second “family” at the study hospital NICU, who have devoted their careers to the care of our tiny and dearly loved babies and their families; without you all, this would not have been possible.

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I believe “To be great, one does not have to do great things; only to do small things with ***great love***… we are blessed because “we work where Angel’s live!”

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Abstract

Acute hypoxic respiratory failure, (AHRF) is a severe condition associated with high morbidity and mortality rates in neonates. While much is known about the treatment for AHRF, less is understood about the types or degree of physiological shunting which occurs in this condition. Therefore, the purpose of this study was to explore the relationship between intrapulmonary and extra pulmonary shunting and oxygen transfer efficiency in the newborn treated for AHRF using inhaled nitric oxide. The aims of this study were to identify linkages between shunting and oxygenation prior to and during inhaled nitric oxide (iNO) therapy and to determine whether gestational age is predictive of infants' response to iNO therapy.

A secondary data analysis was conducted on 74 infants who received a treatment protocol of iNO over a one-week period. A:a gradient measures were examined to determine the correlation prior to treatment for baseline measures. Infants' data were grouped by mode of ventilation to determine whether shunt differed between groups. Infants were then grouped by responders and non responders to examined differences in the A:a gradient over the treatment period. Finally gestational age was examined as a possible predictor of response, followed by examination of other possible predictive variables.

The findings suggest there was a negative moderate relationship between the A:a gradient ($r = .33$, $p=.004$) but there was no difference in the degree of shunt based on the use of high frequency vs. conventional ventilation ($t = .07$, $p =.944$). Additionally, there were significant main effects for time ($F=4.94$, $p=.009$) and groups ($F=13.74$, $p=.001$). Although the less mature infants failed to respond twice as often, they failed to reach statistically significant levels ($\chi^2=2.79$, $df=1$, $p=.095$). Race, ventilator type, nor early onset-sepsis was a predictor in response

to iNO. In conclusion, the A:a gradient provides useful information relative to oxygen transfer efficiency and it appears useful in trending infant's response to treatment and degree of shunt. Although the more premature infant is most likely to fail the first iNO course, many survived without serious sequelae. Therefore, gestational age should not be used for exclusion criteria when designing treatment protocols.

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CHAPTER 1: INTRODUCTION

This chapter presents an introduction to acute hypoxic respiratory failure (AHRF) with a discussion of the background and significance of the problem. An introduction to the theoretical framework that guided the study design and descriptions of the concepts and variables of the study are presented next, followed by the two specific aims and hypotheses for each aim.

Acute hypoxic respiratory failure (AHRF) of the newborn is associated with mortality rates in excess of 50% (Fanaroff & Martin, 1997). This disease process represents one of the most challenging acute care issues in the Neonatal Intensive Care Unit (NICU) for health care providers. In the United States (US) acute hypoxic respiratory failure affects an estimated 80,000 neonates yearly. The rate of occurrence is 18 per 1000 live births or 2% of all live births. The cost of treatment is over $4.4 billion dollars yearly. There are 8,500 deaths per year representing one third to one-half of all neonatal deaths in the United States (Fanaroff & Martin, 1997). The etiology of respiratory failure depends on the underlying pathophysiology. Generally, neonates with respiratory failure fall into two categories: 1) conditions affecting ventilation and or oxygenation, and 2) mechanisms affecting pulmonary perfusion and oxygenation. Issues of lung development and function negatively affect the preterm infant with pulmonary hypoplasia. Most of these issues of the preterm infant are related to immaturity and are expressed differently in the term infant. Therefore, the potential risks for future respiratory morbidity and mortality differ substantially in preterm and term

infants. AHRF involves two or more criteria reflective of clinical or laboratory outcomes. Clinical criteria for diagnosis of acute hypoxic respiratory failure include retractions, respiratory rate > 60 breaths/minute, cyanosis, and intractable apnea. In contrast, laboratory criteria consists of arterial blood gas results with a pH < 7.25, $PaCO_2$> 60 mm Hg, PaO_2 < 50mm Hg and, or O_2 saturation < 80%, with 1.0 FiO_2 (Goldsmith and Karotkin, 1999, 2002).

Despite recent treatment modalities such as surfactant replacement and antenatal steroids, infants experience rapid, life threatening deterioration presenting as a true emergency. The hallmark symptoms of this deterioration are prolonged and severe hypoxemia, hypotensive crisis, hypercarbia, and acidosis eventually leading to multiple organ failure and death. Rapid evaluation and response can make the difference between life and death or irreversible organ failure and damage. In most cases these infants require immediate intensive care measures including, but not limited to intubation, mechanical ventilation, umbilical or central line placement, surfactant replacement, vasopressor support, volume expanders, blood transfusions, fluids and electrolytes, antibiotics, multiple bolus infusions and a full array of medications and other treatments for stabilization. Term and near term infants with uncomplicated meconium aspiration, persistent pulmonary hypertension of the newborn (PPHN) or pneumonia generally recover rapidly without severe long term consequences, whereas premature infants usually require long-term ventilation and chronic use of oxygen therapy (Wessel et al., 1997).

When infants fail to respond to traditional supportive care, oxygenation indicators are essential in making life saving decisions. Clinicians must quickly determine whether alternative intensive rescue interventions are appropriate. These interventions may include therapies such as changes in ventilation strategies, use of pharmacologicals, high frequency ventilation, inhaled nitric oxide (iNO) when available, extra corporeal membrane oxygenation (ECMO) or a combination of any of these therapies.

While a great deal of research has been conducted on life saving interventions such as intubation, mechanical ventilation, and surfactant replacement, less is understood about the effects of inhaled nitric oxide (iNO) on long term respiratory outcomes. Clinicians must have readily available data to assess the oxygen transfer ability of infants in respiratory crisis in order to make rapid and effective adjustments to treatments. These decisions can ultimately improve the morbidity and survival of the infants.

Current techniques for assessment and evaluation of the respiratory status commonly include arterial blood gas sampling and monitoring of oxygenation indices such as the PaO_2 and SaO_2 at frequent intervals. While the PaO_2 and SaO_2 of the infant are readily available and the most frequently used indices, they are dependent on many factors and can change very rapidly. These measures while useful do not indicate the degree of oxygen transfer efficiency. Oxygen transfer efficiency is important to determine the severity of respiratory illness and identify the degree and type of extra-pulmonary or intrapulmonary shunting. Care of the neonate with AHRF should include close analysis of these

indices to assess frequently changing parameters in order to make adjustments in treatment to decrease the shunt. The A:a (Alveolar: arterial) gradient and A:a ratio are readily available though seldom used indicators of the severity of illness associated with intrapulmonary or extra-pulmonary shunting. The A:a gradient quantifies the partial pressure oxygen tension difference or “gradient” between oxygen which is present at the end (A) alveolus and that which diffuses across the (a) alveolar-capillary junction as demonstrated by the PaO_2. This exchange is essential for all metabolic cellular activity (Goldsmith & Karotkin, 2002).

Although most centers are capable of sampling arterial blood gases to monitor ventilation and oxygenation of the patient in respiratory distress, these indices represent only a part of the clinical picture and cannot predict the trend as to how the next few hours or days will unfold in terms of the infants response to therapy. In fact, there are no known reliable indicators of the patient’s response to iNO treatment in this population, leading to transfer of the unstable infant to a higher level of care. Trending of the A:a gradient and PaO_2 values may be useful to determine the response to iNO therapy. Significant improvement of this disease process is indicated by a decreasing A:a gradient (shunt) and stable or increasing PaO_2 levels. PaO_2 levels may remain unchanged or steady, but the FiO_2 (fraction of inspired oxygen) delivered to the infant may have been weaned, or adjustments in ventilator settings may alter the PaO_2 values. These changes indicating the ability to wean an infant in AHRF are positive signs of improved oxygen transfer efficiency. Oxygen uptake is essential for vital organ function. Because of the high mortality rates associated with this disease, it is important

for neonatal clinicians to have *every* diagnostic and treatment resource available for the care of this vulnerable population of newborns.

Theoretical Model

Two pulmonary physiology theories are used to guide the study: 1) the oxygen demand theory, and 2) specific components of the vasodilator theory. These concepts combined with extensive knowledge of the physiological and pathophysiological characteristics of the newborn and premature neonate will provide guidance into the exploration of the relationships among the variables of interest. Acute hypoxic respiratory failure is a complicated pathological respiratory condition. Persistent hypoxemia results from a severe mismatch of oxygen supply to oxygen demand. This is important because critically ill neonates die or become severely impaired neurologically when oxygen demands are not met resulting in prolonged hypoxia. There are many factors related to ventilation-perfusion mismatch occurring at the local capillary end-alveolar pathway. Two theories for the local regulation of blood flow are the vasodilator theory and the oxygen demand theory.

The vasodilator theory states that as organ tissues become metabolically active, they produce vasodilator substances (i.e. CO_2, adenosine, lactic acid, endothelium-derived relaxation factor or nitric oxide, potassium ions, hydrogen ions, and phosphate compounds) that dilate pre-capillary sphincters and arterioles. The specific substance of interest related to the pulmonary bed is epithelial derived relaxing factor (EDRF) or Nitric oxide (NO). More recently, EDRF has been of great interest and promoted in many studies as an important

factor in regulating local vascular resistance. Controlled release of NO is believed responsible for maintaining a continuous state of vasodilatation, and is released by the endothelium increasingly in hypoxic states (Beachey, 1998).

The oxygen demand theory is similar in principle, in that this theory states that a lack of oxygen and other nutrients dilates precapillary sphincters and arterioles. The length and number of cycles is related to tissue metabolic rate. When blood then delivers oxygen and nutrients to the tissues, pre-capillary sphincters close again. For this reason, blood flow through capillaries is not continuous, rather it is cyclic, fluctuating because pre-capillary sphincters periodically relax and contract. This cyclic process is interrupted in persistent pulmonary hypertension as is seen in acute hypoxic respiratory failure, resulting in blood flow bypassing the lungs or being “shunted” from the lungs capillary bed. The duration of the open phases of the cycle is proportional to oxygen requirements of the tissues. This cyclic opening and closing of the pre-capillary sphincters is called vasomotion, which is responsible for auto regulation of blood flow to local tissues. According to this theory, under normal conditions, arterial blood pressure can vary widely yet blood flow to tissues remains constant when these mechanisms are functional.

These theoretical principles lend support to this research because during acute hypoxic respiratory failure of the neonate, the normal physiologic mechanisms are severely compromised by multiple differing factors. Factors such as surfactant deficiency, air leak syndrome (pneumothorax), meconium aspiration syndrome, septicemia, pneumonia, persistent pulmonary hypertension.

These factors create differing degrees of shunting at the local pulmonary level resulting in a disparity between demand and supply of oxygen. These processes result in an inevitable diversion of blood flow from the lungs through patent fetal circulatory mechanisms forming a right to left shunt. In some cases an additional left to right shunt (extra-pulmonary) occurs, again complicating management decisions. Diversion of blood flow from the left side of the heart through a septal defect, patent foramen ovale, or from systemic circulation to the pulmonary circulation through the patent ductus arteriosis worsens the ventilation perfusion relationship.

Specific Aims

Since acute hypoxic respiratory failure results in hypoxemia, examination of the relationship between shunt indices and oxygen transfer efficiency may provide useful information to determine the etiology of hypoxemia, degree, and type of shunting and the response to respiratory management being delivered.

Improvement in shunting is indicated by decreasing A:a gradients in conjunction with improvement in or at least maintenance of adequate blood oxygen levels. While, these variables maybe directly influenced by ventilation management strategies, pulmonary blood flow, pulmonary edema, maturity of lung development, cardiac output and hemoglobin's oxygen carrying capacity, this study examined only relationships between intrapulmonary shunting and oxygen transfer efficiency before the initiation of and at specified intervals during iNO therapy. Thus, the aims and the associated hypotheses of this study were to:

Aim 1: Identify the linkages between shunting (extra pulmonary and/ or intrapulmonary) and oxygenation in infants with Acute Hypoxic Respiratory Failure (AHFR) prior to and during inhaled nitric oxide (iNO) therapy.

Hypothesis 1-There is a negative linear relationship between shunting (extra pulmonary and/ or intra-pulmonary; A:a gradient [A:a DO_2]) and oxygenation (PaO_2) in infants with AHRF prior to the initiation of iNO therapy.

Hypothesis 2- Prior to initiation of iNO therapy, most infants in severe AHRF will exhibit severe respiratory compromise with extra or intrapulmonary shunting despite the ventilation strategy. Therefore, there will be no significant difference in the A:a gradient dependent upon the type of ventilator, i.e., high frequency or conventional ventilator used.

Hypothesis 3- During treatment with iNO the difference in the mean A:a gradients will be significantly higher in the responder group (survivors with successful wean from iNO per the protocol design) versus the non-responder group (survivors who fail to wean from primary iNO course and require second iNO course or expired during primary treatment course) over three specified time intervals.

Time 1- mean A:a gradient at 1hour, 8 hours, and 24 hours after initiation of treatment.

Time 2- mean A:a gradient at days 2, 3, and 4

Time 3- mean A:a gradient at days 5, 6, and 7

Aim 2: Determine whether gestational age is predictive of responsiveness to iNO therapy.

Hypothesis 1 – Infants nearest the age of viability 23 0/7-26 6/7 weeks gestation will fail to respond to iNO therapy significantly more frequently than infants 27 0/7 -42 0/7 weeks gestation.

Hypothesis 2- Most prevalent confounding causative factors of acute hypoxic respiratory failure were controlled for through inclusion and exclusion criteria; however, septicemia is a condition that can occur during treatment and needs to be controlled for in the analysis. Thus after controlling for septicemia, gestational age will remain a valuable predictor of response to iNO treatment.

Definition of Terms

a. Acute Hypoxic Respiratory Failure is defined by Goldsmith and Karotkin in 1999 and in the edited version in 2002 the respiratory condition reflective of the following clinical or laboratory criteria. A diagnosis of acute respiratory failure is made when infants meet two or more of the following clinical or laboratory criteria. Clinical criteria are defined by retractions, respiratory rate equal to or exceeding sixty breaths per minute, cyanosis, and intractable

b. Intrapulmonary Shunting is the process by which normal pulmonary venous blood flow between the alveolar/arterial unit is interrupted, or shunted away from the alveolar unit resulting in the inability to adequately exchange gasses resulting from pulmonary origin, such as atelectasis or severe pulmonary edema, air block etc. This type of shunt does not improve with increasing oxygen therapy, which is described as a hallmark finding of this process (Beachey, 1998).

c. Ventilation: Perfusion Mismatch (V/Q mismatch) is the result of two possibilities. Hypoxemia occurs when under ventilated alveoli with normal perfusion or normally ventilated alveolar units with decreased perfusion. V/Q mismatch is unlike intra pulmonary shunting because this process responds well to oxygen therapy. (Beachey, 1998).

d. Extrapulmonary Shunting occurs when blood flow is diverted from the left side of the heart to the right side of the circulation via alteration of the cardiovascular flow through normal fetal channels, or through septal defects. Examples of the aforementioned pathways include patent ductus arteriosis, atrial septal defects, ventricular septal defects, or patent foramen ovale (Beachey, 1998).

More serious conditions resulting in intracardiac shunting occur in cyanotic heart defects (i.e., right to left shunting) such as transposition of the great vessels, or hypoplastic left heart syndrome. These anomalous conditions require surgical intervention to correct absolute shunting away from the pulmonary vasculature resulting in intractable hypoxemia. Infants with these diagnoses were not included in this study.

e. Responders: Those surviving infants treated with inhaled nitric oxide for AHRF whose respiratory statuses improve over the course of treatment. Improvement criteria was determined by a 10% improvement in one or more measures of oxygenation, an ability to wean the FiO_2, inspiratory, or end expiratory pressures, or a decrease in the mean airway pressure over the course of treatment.

f. Non-responders: Those surviving infants treated with inhaled nitric oxide for AHRF that fail to respond to the first course and require re-entry into the iNO protocol, or those that experience severe deterioration and expired.

g. Inhaled Nitric Oxide: Inhaled nitric oxide was first demonstrated as beneficial in treatment of term infants with persistent pulmonary hypertension by specifically dilating the pulmonary vasculature without any measurable systemic effects (Wessel et al., 1997). Nitric oxide is non-organic free-radical gas as well as an important

h. Oxygenation: Oxygenation is the process in which available oxygen molecules efficiently transfer via diffusion onto the hemoglobin portion of the red blood cell and are available for delivery to the tissues for necessary metabolic functions. Clinically, when infants are well oxygenated they appear active, responsive, well perfused, with normal respiratory and cardiac functions. Infant's skin tones and mucous membranes are pink in color. (Avery et al, 2004).

i. Alveolar: arterial Gradient: P(A:a DO_2); the best known index of oxygen-transfer efficiency. This measurement quantifies the oxygen tension difference between PAO_2 (Alveolar oxygen tension) and arterial PaO_2 oxygen tension. The A:a gradient ($PA–PaO_2$) in the absence of shunt is equal, with no difference. The normal value is about 7-14 mmHg during breathing of room air and increases to 30-56 during breathing 100% FiO_2. Higher values reflect severity of shunting.

The remainder of this study is organized into four chapters, followed by references, and appendices. Chapter two presents a review of the literature and includes an introduction to the major concepts of the study including acute hypoxic respiratory failure, (AHRF), developmental anatomy and physiology of

the fetal lung, clinical pathophysiology of AHRF, chemical and physical properties of inhaled nitric oxide (iNO), clinical trials of infants utilizing iNO, and an introduction to oxygenation indicators, particularly the alveolar to arterial O_2 gradient. Chapter Three presents the research design and methodology of the study, including and introduction to the purpose and study design, description of the sampling techniques, sample determination and criteria, subject recruitment and consent procedures, data collection, storage and analysis, staff recruitment, responsibilities and training procedures. Additionally, study assumptions, supervision of researcher, facility, and protection of human subjects are discussed. Chapter four presents the study findings, while Chapter five contains a discussion of the findings, summary, conclusions, and recommendations for further research.

CHAPTER 2: LITERATURE REVIEW

Introduction

In this chapter, a comprehensive review of the relevant literature for this study is presented. First, anatomy of the fetal lung, acute hypoxic respiratory failure, clinical pathology, and the chemical and physical properties of nitric oxide are discussed. Next is a synthesis of the current and past research focused on the physiologic development of the neonate relative to acute hypoxic respiratory failure (AHRF). Other major concepts and variables discussed in this chapter include intra and extra pulmonary shunting, conventional and high frequency ventilation, and the alveolar-arterial gradient as an indication of degree and presence of a shunt. Additionally, clinical research trials pertinent to the oxygen demand theory and the vasodilator theory were synthesized and are presented.

Review of Developmental Anatomy of the Fetal Lung

In order to differentiate the severity of acute hypoxic respiratory failure from other respiratory diseases of the newborn, the clinician must understand the normal developmental anatomy as well as associated pathological processes and conditions commonly treated in the neonatal intensive care unit. Adaptation from intra-uterine to extra-uterine life involves complex processes requiring physiologic changes affecting the respiratory and circulatory systems (Avery et al., 1999).

Under normal conditions, the mature or “full term” newborn is well prepared to undergo the normal processes occurring during the transition period (Nelson, 1999). The normal gestation period in humans is approximately 40 weeks plus or minus 2 weeks. Infants born at earlier gestations are at higher risk for respiratory complications such as respiratory distress syndrome and pulmonary insufficiency, which inherently complicate their capacity to respond to traditional rescue therapies.

Normal embryonic lung development consists of five stages: 1) embryonic phase 2) pseudoglandular period 3) canalicular period 4) saccular period and 5) alveolar period. The primordial lung bud appears on the 26th day of gestation as a ventral epithelial outgrowth from the foregut. This is followed by progressive caudal progression of the primitive lung by continuous dichotomous branching (Avery et al., 1999).

The embryonic phase of development occurs from the fourth through the sixth weeks of gestation. By the fourth week, the laryngotracheal groove develops from the ventral wall of the primal esophagus, and then divides to form the two primary bronchial buds. The primary bronchi divide further to form bronchial lobes with extension of lung buds forming laterally into the primitive lung. Separation from the esophagus occurs by a band of tissue called the tracheoesphageal septum (Whitsett et al. 1999). By the sixth week, completion of the proximal airway is achieved from segmental bronchi. Pulmonary malformations are rarely dated to this period, because insults during this stage of development generally have widespread damage resulting in death of the

embryo. However, tracheoesphageal malformations such as tracheoesphageal fistulas and laryngeal clefts can occur during this phase (Avery et al., 1999).

The pseudo glandular period includes the next ten weeks resulting in development of all the conducting airways. This period is from the 7th to the 16th week of gestation. After that time, these tissues merely grow in size, not in number. Approximately twenty generations of bronchi develop by the end of this phase. The most proximal twelve of these generations contain cartilage rings with the last eight generations devoid of these structures, thus being named bronchioles. Between the eight and tenth weeks the pleural membranes and pulmonary lymphatics develop congruently. Insults or injury during this period may result in abnormal numbers, position, or malformations of bronchi and bronchioles. If injury is severe further normal develop may not occur. Additionally this period includes the separation of the pleural and peritoneal cavities, development of the diaphragm and rotation of the intestinal tract into the abdominal cavity. If by the tenth week the diaphragm is not completely closed, the intestines have access to the thoracic cavity and may interfere with pulmonary development. Reduced numbers of bronchial generations are frequently associated with diaphragmatic hernia and agenesis (Avery et al.,1999).

The following stage occurs between the 17th to the 28th weeks. This period is the called the canalicular stage of fetal lung development. Therefore, Infants delivered after 23 weeks gestation would fall within this stage of development. According to the American Academy of Pediatrics (2000)

Recommendations for Neonatal Resuscitation, infants are considered viable and resuscitation should be considered when they have completed 23 weeks gestation and with estimated birth weights of greater than or equal to 400 grams. Infants who fall within this phase of development are commonly afflicted with pulmonary insufficiency and respiratory distress syndrome. Delivery during this developmental stage is not always lethal, yet afflicts approximately 20,000 to 30,000 infants each year in the United States resulting in higher morbidity rates for this group (Avery et al., 1999). During this stage, acinar development gives rise to the gas-exchanging component of the lung. The basic structure of the first intra-acinar bronchioles appears at seventeen weeks along with a distinct increase in capillary numbers aligned adjacent to the air spaces, establishing the crude connections between overlying epithelial cuboidal tissues forming the air saccules. These saccules form the acina units, and begin to arise from the last generation of bronchioles at eighteen weeks. As the acina grow, the saccules elongate and the distance between the pleura and the terminal bronchioles increase. By three years of age, these crude units will develop into adult alveolar units.

Function of fetal lung gas exchange units depend on two types of epithelial cells differentiated during this stage of development. Type I pneumocytes are formed simultaneously with developing capillary cells forming the air-blood barrier. Type II cells, on the other hand, form the tissues responsible for surfactant production, and are distinguishable at twenty weeks. When either type of cell is absent or there are insufficient numbers present,

infants present with respiratory distress. Injuries or malformation at this point in development manifest as a deficiency in radial count and may be severe enough to produce pulmonary insufficiency. When complete arrest of development occurs, pulmonary hypoplasia develops. A deficiency in surfactant production is hallmark in respiratory distress syndrome (RDS). A 50% incidence is commonly found in infants between 26-28 weeks. Infants between 30-31 weeks usually present with less than twenty to thirty percent (20-30%) incidence (Avery et al., 1999). While respiratory distress syndrome commonly affects the premature infants, infants of diabetic mothers, multiple gestations, intrauterine or birth asphyxia, infection and maternal-fetal hemorrhage are also associated with higher risk for RDS.

The saccular period begins at twenty-eight weeks gestation. The lung takes on a different appearance due to changes in the interstitial tissue decreasing while the airspace walls become narrower and more compact. There is a sudden increase in lung volume and surface area. This is the prenatal phase of alveolar development. The alveoli appear as early as thirty-two weeks in some infants and are present in all by thirty-six weeks (Avery et al., 1999).

The alveolar period can start as early as thirty weeks, but is nearly always before thirty-six weeks. This stage is predominantly noted by the formation of secondary alveolar septa, with division and development of actual alveolar ducts. Development of more mature alveolar units greatly increases the surface area for gaseous exchange. Previously formed subsaccules become alveoli multiplying to reach approximately 50 million at term gestation. After birth, this rapid growth

slows for approximately three months, and then increases rapidly during the first year of life. The adult lung contains approximately 300 million alveoli all of which are developed by the age of three (Avery et al, 1999).

Fetal lung fluid is another essential component in the development of the pulmonary system. During airway development, tissues are in contact with amniotic fluid when the glottis is open. Secretions from buccal and nasopharyngeal cavity as well as the lung itself make up the tracheal exudates. Unless the fetus is distressed, rarely is this fluid found within the developing lung. Lung fluid itself is different from amniotic fluid. Potential air spaces are filled with liquid containing large amounts of chloride, small amounts of bicarbonate and is nearly absent of protein. Concentrations of potassium are similar to serum levels until near term when surfactant secretion increases**.** The fetal lung contains approximately 20-30 ml/kg of fluid within the air spaces, which are gradually removed from the lung by lymphatics and pulmonary blood flow. This process occurs during normal transition beginning with the newborns descent through the vaginal canal and through the initiation of breathing stimulus at the time of birth. Disruption of this process, manifested by a delay in the removal of fetal lung fluid, results in transient tachypnea of the newborn (TTN).

Acute Hypoxic Respiratory Failure

The clinical presentation of acute hypoxic respiratory failure can vary widely in the neonatal period. Criteria were described previously in Chapter one including both physiologic and lab components for a definitive diagnosis. Retractions and increased work of breathing are compensatory mechanisms of

the infant indicating loss of lung volume, but it is often futile due to the overly compliant chest wall of the neonate. Rather than providing a supportive sub-structure, the thorax collapses freely, resulting in negative intra-pleural pressures and worsening atelectasis. As segments of the lung collapse further, gaseous exchange is significantly impaired, resulting in acidosis, desaturation, hypoxemia and cyanosis. The rapid progression of this cycle produces intractable apnea, particularly in more premature infants since they have less metabolic reserve to sustain the increased workload imposed.

Clinical Pathology of Acute Hypoxic Respiratory Failure

AHRF is triggered by a number of acute respiratory complications. Extreme immaturity, severe respiratory distress syndrome, pulmonary hypoplasia, persistent pulmonary hypertension, diaphragmatic hernia, neonatal pneumonia, meconium aspiration, sepsis, and trauma are among the most common (Fanaroff and Martin, 1997). While each of these differential diagnoses varies in the pathophysiologic changes and mechanisms, the result is destruction of the immature, fragile newly forming lung tissue of the neonate.

Primary respiratory distress typically affects preterm infants below 35 weeks due to immature development, lack of surfactant and poor function of the fetal tissues of the pulmonary system (Avery et al., 1999). Gestational age of the infant and associated co- morbidities directly affect physiological properties necessary for adequate gas exchange and ventilation. Surfactant deficiency, cardiac lesions, perinatal infection, pneumonia, perinatal asphyxia, meconium aspiration, and maternal diabetes can have severe pulmonary implications with

requirement of supportive measures affecting both preterm and mature infants. Hypoxemia associated with respiratory distress syndrome is predominantly the result of intrapulmonary shunting, extra-pulmonary shunting, or poor ventilation-perfusion matching (Fanaroff & Martin, 1997; Beachey, 1998). In many infants, pulmonary hypertension with significant right to left extra pulmonary shunting may also contribute to arterial hypoxemia (Beachey, 1998).

Under normal conditions, pulmonary artery pressure and resistance decrease over time following physiological transition from intrauterine life. The fetus begins to reabsorb fetal lung fluid and increase surfactant production upon receiving signals before birth. At birth, the first breath results in filling the alveoli with air, displacing the balance of lung fluid (Avery et al., 1999). This simple action initiates the fall in pulmonary artery pressure. The effect of oxygen decreases pulmonary artery pressure in addition to endothelial derived relaxing factors (EDRF's) that signal pulmonary vasculature to continue to relax and dilate to accept the full volume of the newborn's cardiac output (Avery et al.1999). Under normal circumstances, intrauterine shunt pathways, the patent ductus arteriosis, the foramen ovale and intra-pulmonary pathways begin to subside and eventually dissipate so that by 3-7 days of age they are nearly absent. Pulmonary artery pressures reach near adult values by eight weeks of life (Hansen et al., 1996).

Infants with acute hypoxic respiratory failure experience abnormal transition resulting in persistent pulmonary hypertension of the newborn (PPHN). Pulmonary hypertension is the result of both active and passive factors. Passive

factors include lung volume, effects on alveolar and extra-alveolar blood vessels, vascular pressure and blood volume. Active factors include neurogenic stimuli, humoral agents, nitric oxide, and chemical factors, most importantly oxygenation and blood pH after birth (Beachey, 1998).

Pulmonary vasculature of the premature infant is fragile, poorly innervated and highly resistant, resulting in ineffective oxygenation and gas exchange due to ventilation/ perfusion mismatch (V/Q mismatch). Additionally infants with RDS, pneumonia, MAS and other diagnoses exhibit inflammatory reactions further compromising ventilation and pulmonary circulation with the release of pro inflammatory cytokines. Infants diagnosed with primary pulmonary hypertension of the newborn have reduced amounts of cyclic guanylate monophosphate (cGMP) circulating, secondary to inadequate NO production needed to relax and dilate the pulmonary vasculature after birth (Wessell et al., 1997; Avery et al., 1999).

Pulmonary vessels are classified in two categories; those exposed to alveolar pressure (alveolar vessels) and those not in contact with alveoli, exposed to intra-pleural pressure (extra-alveolar vessels). Alveolar vessels are mainly pulmonary capillaries in intimate contact with alveolar walls. As the terminal air sac inflates, these vessels are compressed and resistance increases. Extra-alveolar vessels include all pulmonary arteries, arterioles, venules and veins not in contact with alveoli. These vessels stretch to larger diameters as the lung inflates (Beachy, 1998). Vascular pressures are also affected by cardiac output, stroke volume, and pressure gradients at the alveolar-arterial unit. When

pressures are excessively elevated in the lung parenchyma, such as in multiple atalectatic areas, blood flows from the right side (the venous), to the left side (arterial) without contacting alveolar gas. Intrapulmonary shunting is complex, and challenging to manage, particularly when complicated by surfactant deficiency, sepsis, increased chest wall compliance, patent foramen ovale, and ductus arteriosus, as is common in the premature infants (Avery et al., 1999).

Medications have been used in previous attempts to dilate the pulmonary capillary bed to improve perfusion. Unfortunately, using vasodilators resulted in hypotension secondary to the non-selectivity of the pharmacological agents. Atelectasis in the premature infants with surfactant deficiency results in alveolar surface area collapse with tidal volume losses requiring higher pressures for re-inflation. Increased pressures distend the distal airways and alveolar structures resulting in barotrauma and volutrauma, which ultimately increase the risk of pneumothoraces and structural damage of the immature lung. Prolonged trauma to the tissue results in scarring, and chronic lung disease (Beachy, 1998). By determining the degree of shunting as indicated by the A:a gradient and monitoring changes in these values, clinicians will be better able to judge the effect of respiratory management in real time. The improvement in oxygen transfer efficiency can be seen by declines in the A:a gradient with increases in PaO_2 values.

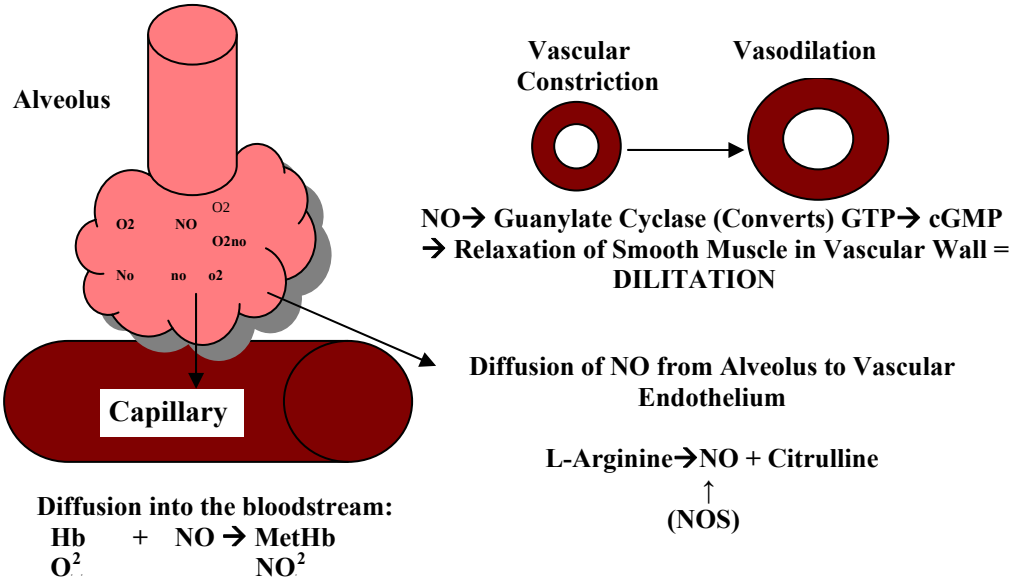
Nitric Oxide: Chemical and Physiological Properties

Nitric oxide is a non-organic free-radical gas as well as an important messenger molecule involved in nearly every organ system in the human body

(Palmer et al.,1987). Nitric oxide is produced in cells and diffuses into underlying vascular smooth muscle (Roberts, 1992). The oxidation product of the terminal guanidine-nitrogen atom of L-arginine, NO has been studied extensively since its discovery in 1980 as reported by Palmer et al. (1987). In 1987, Palmer et al., and Ignarro et al., discovered endothelial derived relaxing factor actually is nitric oxide according to an article by Vyas et al. (1999). Previously termed endothelium-derived relaxing factor (EDRF), NO has preferred properties desirable to dilate specifically only pulmonary vasculature without compromise to the systemic vascular system. Nitric oxide selectively dilates pulmonary arteries through release of cyclic guanosine monophosphate (cGMP). Relaxation of the vascular smooth muscle occurs by binding to the heme moiety of cytosolic guanylate cyclase, activating guanylate cyclase and increasing intracellular levels of cyclic guanosine 3', 5'-monophosphate, which leads to vasodilation (Evgenov et al., 2007). Uptake occurs through absorption systemically after inhalation. During inhalation of iNO, most of it traverses the pulmonary capillary bed where it combines with hemoglobin which is 60-100% saturated with oxygen. At this level of oxygen saturation, nitric oxide combines predominantly with oxyhemoglobin to produce methemoglobin and nitrate. Methemoglobin (MetHb) is hemoglobin (Hb) with iron in the ferric (Fe^{+++}) state rather than the ferrous (Fe^{++}) state. When oxygen combines with this form, it is rapidly oxidized, rather than oxygenated, and thereby cannot release oxygen or combine with more oxygen. Methemoglobinemia (high levels of serum MetHb) can be caused by nitrate

poisonings or toxic reactions to oxidant drugs, and must be monitored closely during iNO use (Stamler et al., 1997). See Figure 2.1 below.

Figure 2.1 Nitric Oxide: Mechanisms of Action



Palmer et al used a chemiluminescent technique to demonstrate bradykinin-induced NO release from endothelial cells (Palmer et al., 1987). The amount of NO detected was sufficient to explain the degree of relaxation observed in the bioassay tissues. The half-life of NO ranges from 3 to 90 seconds, depending on local conditions (Moncada et al., 1991). Vayas et al. (1999) report NO is a diverse molecule, playing a role in relaxation of smooth muscle in blood vessels, immune system functions, neurotransmission, and platelet function, with a half life of less than one second in blood. Bloch et al. (2007) reviewed recent research aimed at iNO as a therapeutic agent. Unanticipated systemic effects provide hopes for treating a variety of disorders

including prevention of bronchopulmonary disease in neonates, and ischemia related reperfusion injuries (Bloch et al., 2007). Nitric oxide diffuses freely between cells, therefore it does not require an elaborate transport mechanism. It is inhibited by hemoglobin and inactivated by super-oxide. It remains difficult to quantify NO due to its lability. However, if the NO synthase (NOS) inhibitors are used, NO synthesis can be studied indirectly, via oxidation products of NO, such as nitrates and nitrites (Palmer et al, 1987).

Nitric oxide synthase is present in airway epithelium and pulmonary vascular endothelium, which effect lung function and perfusion. Functions of NO in the lung airway include bronchomotor tone regulation, bacteriostasis, regulation of mucin secretion, ciliary motility, plasma exudation, and regulation of airway branching in the developing lung. Pulmonary circulatory functions of NO include regulation of pulmonary vasomotor tone and regulation of vascularization in the developing lung (Adams et al., 2005).

Potential toxicity of NO is associated with three effects; methemoglobinemia, nitrogen dioxide (NO_2) formation and hemorrhagic complications such as intraventricular hemorrhage. Methemoglobinemia arises from the reaction between NO and hemoglobin particularly in premature infants who have lower levels of methemoglobin reductase. Levels must be measured at baseline and again at periodic intervals. Nitrogen dioxide (NO_2) is a toxic by-product of NO combined with oxygen that caused pulmonary edema and bronchiolitis obliterans. It can be formed with NO source cylinder contamination or errors in the delivery system and ventilator set-up. The admixture of NO and

oxygen must have limited dwell time to minimize nitric dioxide formation. Delivery systems have standardized methods to enhance safety with constant monitoring of NO_2 for the safety of infants receiving this therapy. NO mediates hemostatic thrombotic balance regulation of vascular tone. Hemorrhagic complications are concerning because of the disturbances in platelet aggregation and prolongation of bleeding time in both adults and neonates treated with iNO. Platelet aggregation was reduced significantly in infants receiving 2-20ppm iNO. Premature infants are particularly susceptible to development of intracranial hemorrhages and iNO could enhance that risk. Close monitoring for intraventricular hemorrhage (IVH) via ultrasonography is necessary while undergoing treatment (Van Meurs, 2005).

Early reports of treatment with inhaled nitric oxide (iNO) have been particularly promising in term infants with persistent pulmonary hypertension (PPHN). Inhaled nitric oxide was first demonstrated as beneficial in treatment of term infants with persistent pulmonary hypertension (Wessel et al., 1997). We have seen marked improvements in this population as well, with significant immediate responses. It would seem the response is less remarkable in smaller infants, due to pulmonary insufficiency, adrenal insufficiency, and overall immaturity, yet evidence is limited due to the low number of studies of this sub-group. This study will explore whether there are differences in response to iNO based on gestational age (maturational lung development) as well as disease process.

iNO Clinical Research Trials

In a search and review of the literature, four major studies were cited repeatedly. These study authors were Subhedar (1999); Mercier (Franco-Belgium Trial 1999); Kinsella (1999), and Schreiber (2003). A meta analysis of iNO for respiratory failure in infants born at or near term was conducted by Barrington and Finer of the Cochrane Review Group in 2005. The Cochrane reviewers analyzed and reported on three of the four previously mentioned trials. These trials are considered sentinel in the literature and are discussed in greater detail in this chapter following the Cochrane analysis.

Cochrane Analyses

Dr. Keith Barrington and Dr. Neil Finer are well known reviewers for the Cochrane Review Group. They published the first review of iNO for respiratory failure in 1998 with subsequent edited revisions in 2001 and most recently in 2005. The edited reviews (2001 and 2005) will be covered in this literature review since they are the most recent and relevant to this study. In 2001, Barrington and Finer analyzed all available trial reports for methodological quality and selected 12 trials for inclusion of the meta analysis. The objectives of the analysis were to determine whether treatment of hypoxemic term and near term neonates with inhaled nitric oxide 1) improves oxygenation and reduces mortality rates, 2) reduces the requirement for ECMO and 3) affects long-term neurodevelopmental outcomes. The meta analysis included only randomized trials with participants who were term or near term newborns, greater than 34 weeks gestation at birth, less than one month of age when enrolled and received iNO after adequate

treatment with surfactant and developed hypoxemia due to either primary lung disease or pulmonary hypertension with right to left shunting. Infants with extra pulmonary shunting due to structural congenital heart disease were excluded. One trial (Ninos, 1997) included infants with congenital diaphragmatic hernias, which is a known confounding condition with significantly higher morbidity when compared to affected infants.

Outcome measures of each of the selected trials included 1) death prior to hospital discharge, 2) death or requirement for ECMO, 3) intraventricular hemorrhage or periventricular dysplasia at 30 weeks corrected age, 4) improvement in oxygenation as a dichotomous variable within 30-60 minutes, 5) effects on oxygenation index after 30-60 minutes of therapy, 6) effects on Pa02 after 30-60 minutes of therapy, 7) neurodevelopmental disability at 18-24 months, 8) cerebral palsy, 9) cognitive impairment and 10) deafness (Finer & Barrington, 2001).

Trials for inclusion were thoroughly described and compared for quality, treatment protocols, and outcome criteria. Studies included had similar inclusion criteria for hypoxic respiratory failure. Only one study enrolled both term and preterm infants but reported findings by two groups - preterm (<33 weeks and term (> or = 33 weeks gestation). While the quality of each of the studies varied, those considered most rigorous were blinded, multi-centered randomized controlled studies with adequate powered. These included the Nino's (1996) and Clark (2000 studies. Mercier (1999) Davidson (1997) and Roberts (1996) were rated as intermediate quality while Barfield (1996), Cornfield (1999), Day (1996),

and Wessel (1996) contained small samples without randomization, and were conducted in single centers without blinding. Of particular interest, Kinsella's (1999) study comparing high frequency ventilation with iNO was designated of high quality, with a complex protocol. This study demonstrated that near term infant's response to iNO was not significantly different to those treated with HFOV only. However, when combining therapies, 32% of infants had improved responses using a combination of these therapies. This suggested that the use of both iNO and HFOV might be more efficient than either therapy alone. The results of the review implied that infants who failed to respond to traditional therapies should have a trial of iNO, since the studies demonstrated a significant reduction in the need for ECMO. However, studies did not imply safe and effective doses or most appropriate lengths of treatment. This remained greatly variable, yet some safety measures for toxicity were brought forward in terms of monitoring methemoglobin levels after an accidental overdose at >135 ppm of NO (Heal, 1995) were reported.

The updated edit of the Cochrane Review (2005) consisted of eight relevant studies. Inclusion criteria remained the same as the first review as per protocols established by the group. The objectives differed however in that the follow up review update focused on whether preterm infants (<35 weeks) with AHRF, treated with iNO 1) improved oxygenation within 2 hours, 2) reduction of mortality, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage, (IVH) or neurodevelopmental disability. Seven randomized controlled trials were selected for criteria with the quality and comparison of trials performed as in the

first review. Five studies consisted of infants with highly predicted mortality based on oxygenation these are Kinsella (1999), Hascoet (2005), INNOVO (2005), Van Meurs (2005), and Mercier (1999); Subhedar (1997) explored the risk of developing BPD. Finally, Schreiber (2003) studied the routine use of iNO in all preterm ventilated infants. Findings were not significant in terms of mortality or developing BPD, however, there were no increased risk of IVH, and short-term improvement was demonstrated with the use of INO. This analysis did not support the use of iNO on all premature infants being ventilated, and suggest further study for the potential benefit of use in milder cases of respiratory failure be designed in the future. Although the Cochrane Data Base presents a great deal of information and is highly regarded as a scientific gold mine of information, these and other sentinel studies relevant to this study were reviewed and are discussed in detail the following paragraphs.

Both the Kinsella (1999) and Mercier (1999) trials had similar admission criteria, both requiring an oxygenation deficit in ventilated preterm infants in the first week of life. Additionally, both studies randomized the infants to low dose iNO. Direct comparisons were not possible due to the different methods of determining oxygen deficits. Kinsella's control group had higher mortality rates (53%) compared to 35% in Mercier's study. Further, the rate of bronchopulmonary dysplasia in Kinsella's study exceeded that of the Mercier study with 80% of the controls compared to 29% of controls respectively (Finer & Barrington, 2001). These outcomes might suggest a "higher acuity" in this population at baseline in the Kinsella study or differing ventilation strategies. In

both studies, the majority of patients entered the study before 3 days of age. The major differences in design between the studies were the lack of blinding of the intervention in the Mercier study, and the availability of backup treatment of controls with iNO if the oxygenation index worsened. Subhedar's (1999) study was unlike Kinsella (1999) or Mercier (1999), using a factorial design. The trial investigated dexamethasone therapy as well. Infants were selected at 96 hours for a high risk of bronchopulmonary dysplasia and almost universally developed bronchopulmonary dysplasia at 36 weeks.

In 1997 Subhedar reported a randomized clinical trial using iNO in premature infants less than 32 weeks post conceptual age. Subjects were selected if still intubated at 96 hours of age. The entry criteria included having a "high" risk for developing broncho-pulmonary dysplasia, based on criteria developed by Ryan, (1996) which was not reported or available. Infants were randomized in a 2 x 2 factorial design to either iNO only (n =10), iNO plus dexamethasone (n = 10), dexamethasone only (n = 11) or neither (n = 11) (Finer & Barrington, 2001).

The infants were treated with 20 ppm of iNO with a reduction to 5 ppm according to the response obtained. The dexamethasone dose was 1mg/kg/d for 3 days, then 0.5 mg/k/d for another 3 days. 42 infants were studied, with a mean birth weight of 882g (range 416 - 1354g); mean gestational age 27 weeks (range 24 - 30 wk) for the 20 iNO infants compared with 762 g (range 520 - 1320 g) and 27 weeks (range 22 - 31 wk) for the 22 non-iNO infants. This trial had unmasked interventions, but the randomization was adequately blinded by sealed envelopes

(Finer & Barrington, 2001). There were four groups in this study with 10 or 11 infants per group. Inhaled NO, steroids and the combination were all compared to "standard therapy." The planned sample size was for 88 subjects, but the study was terminated before this sample size was reached because the incidence of death or BPD, at a pre-determined 12-month period, was much higher than expected. Oxygenation was not well matched at baseline between the groups, despite randomization. The median oxygenation index in the control infants was 3.9, the range was 1.2 to 11.5, in the nitric oxide group was 7.9, with a range 1.6 to 46.7 (Finer & Barrington, 2001). There were also a greater proportion of males in the iNO group, 12 males out of 20 total, than the controls, 5 males of 22 total infants. Other baseline characteristics were similar (Finer & Barrington, 2001).

Results were not separately described for each of the four groups randomized in this study. Almost all data are presented as inhaled nitric oxide vs. no nitric oxide, regardless of the use of dexamethasone. Therefore, it is not possible to assess possible interactions, or compare the effects of inhaled nitric oxide solely in the infants who did not receive steroids. Outcomes reported were death, chronic lung disease and both. The results demonstrated no significant decrease in chronic lung disease or death (Finer & Barrington, 2001).

Kinsella (1999) developed trial criterion to include neonates with an expected mortality of at least 50%.He randomized 80 preterm infants who each had a baseline head ultrasound prior to the administration of iNO to scan for intraventricular hemorrhage. All infants received surfactant prior to enrollment,

with one exception. Neonates were randomized to blinded administration of 5 ppm inhaled NO or no additional gas. The study‘s main outcome was survival to discharge and therefore was powered to detect a 30% reduction in mortality with iNO which led to a required sample size of 210 infants.

Seven patients were excluded from the acute oxygenation response data because of early protocol violations but were included in all other presented data. There were 48 infants assigned to the iNO group, and 32 were controls. There were no differences between groups for birth weight, 1040 SD +/- 461 grams in the inhaled NO group vs. 988 SD 387 grams in controls. Gestational age was 27.1 +/- SD 2.5 weeks in the inhaled NO infants and 26.8 SD 2.5 weeks in the controls. Male to female ratio was 28/20 in the iNO infants and 20/12 in controls (Kinsella, 1999). There were no differences in the 1 and 5 minute Apgar scores or the presence of intraventricular hemorrhage at the start of the study. PaO_2/FiO_2 was 42 SD 18 in the iNO and 42 SD 16 in controls (Kinsella, 1999). There were also no differences in antenatal steroid use (52 vs. 53%) or in high frequency ventilation (56% in each group). Treatment was continued for 7 days, after which "trials off" were attempted; study gas was restarted for an increase in oxygenation index of 15% or more. A maximum duration of treatment of 14 days was allowed (Kinsella, 1999). The article does not describe what happened to iNO therapy in infants who were extubated, or why 7 days was chosen for the treatment duration. No weaning of the iNO concentration was allowed. The diagnosis of intraventricular hemorrhage was extensively reported in infants who died and in infants who survived, but had no influence in criteria for selection or

exclusion (Finer & Barrington, 2001). This was a blinded, multi-center (10) study, with a careful investigation of intraventricular hemorrhage results, though no exclusion criteria were used. The study was terminated after 80 of the planned 210 infants were enrolled as an interim analysis suggested that significant benefit was unlikely to be detected "within a reasonable time frame". The interim analysis was planned at the start of the study, but it is not stated if slow enrollment was a pre-designated stopping criterion. Baseline characteristics of the groups were similar, apart from a greater number of iNO infants having no intracranial hemorrhage at the start of the study, 73%, vs. 59% in control group infants. Overall, the study demonstrated a significant increase in Pa02 after 2 hours, decrease number of ventilator days and decreased trend in chronic lung disease.

Although early studies initially only included more mature infants, the Franco-Belgium Collaborative NO trial group randomized near term and preterm infants into stratified groups. The results for the two strata were reported separately. The cut off between strata for this study was at 33 weeks gestation. Admission criteria included less than 1 week of age with oxygenation index values between 12.5 and 30. All except one of the 85 preterm infants had received surfactant with the majority (75%) receiving high frequency ventilation. The majority of the infants were enrolled on the first or second day of life. Infants were randomized to either treatment (using10 ppm iNO) or control groups. If the oxygenation index [OI, which is calculated as (mean airway pressure x FiO_2 x 100)/PaO_2)] exceeded 30 during the 2 hour study period then iNO at 20 ppm was

used. The median baseline OI was 18 in the control infants and 20.2 in the NO group.

The entry criteria for the three studies reported by Finer and Barrington were somewhat different, with enrollment in the first 2 days of life in most of the infants in Kinsella (1999) and Mercier (1999) compared to an entry at 96 hours for Subhedar (1997). The main eligibility criterion was a score predicting a high risk of BPD in Subhedar 1997, whereas Kinsella required an arterial to alveolar oxygen ratio of less than 0.10 predicting a 50% mortality, and Mercier 1999 requiring an OI between 12.5 and 30 (Finer & Barrington, 2001). Thus, the infants in the study of Subhedar were entered after the major risk period for the development of IVH, which was not the case for most of the infants in the Kinsella (1999) or Mercier (1999) studies.

The Mercier trial was a multi-center international trial in which the intervention was unmasked, but the randomization was adequately concealed by the use of sealed envelopes at the co-coordinating center. The study was designed with the primary outcome being assessed after 2 hours. Later treatment with iNO was allowed if the infant's oxygenation worsened such that the upper limit of OI was reached; this limit was 30 for the preterm infants. Five of the control infants eventually received iNO. The availability of back up treatment for the control infants with iNO limits the ability of the study to address long-term outcomes. The study was designed to enroll 360 infants across both gestational age strata, but was terminated because of slowing enrollment after 2 years and 3 months; it is not stated whether the early analysis or the cessation criteria of the

study were pre-designated. Analysis was based on “intention to treat.” All the baseline characteristics were similar between groups. Mercier’s study demonstrated a significant decrease in the oxygenation index after 2 hours on inhaled nitric oxide therapy using 10-ppm initial dose in premature infants.

In contrast to the short come outcome of oxygenation at 2 hours in the Mercier trial, Schreiber initiated a single center, masked, randomized control trial in 2003 focused on decreased incidence of chronic lung disease, death, and rates of incidence of intraventricular hemorrhage or periventricular leukomalacia (Adams et al., 2005). Reports detailed primary outcomes in subgroups by weights, < 750 grams, 750-1000grams, >1000-1500grams and > 1500 grams in iNO and control groups. Outcome measures were: 1) Survival with no CLD and 2) Death or CLD (Adams et al., 2005). The overall importance of the study was that the iNO group had a significant difference in decreased incidence of CLD or death. Decreases in incidences of severe IVH or PVL in the iNO group were also observed. The study was adequately powered with 207 participants. Infants with RDS received treatment with surfactant followed by iNO x 12-24 hours at 10ppm, then 5 ppm for 6 days. Infants were < 34 weeks, and <2kg and < 12 hours of age (Adams et al., 2005). These findings were promising for the smaller and more immature infants. Though all were important, the studies covered previously were most often cited and blazed the trail for other trials.

This current study differs from the above cited trials since the current study: 1) includes extremely low birth weight infants as well as term infants and 2) none of the previous studies examined the correlation between the A:a

gradient and PaO_2 in response to iNO therapy or the relationship between these two specific variables.

A:a Oxygen Gradient in AHRF as an Oxygenation Indicator

Tamburro, Bugnitz, and Stidham (1991) studied the Alveolar-arterial oxygen gradient as a predictor of outcome in patients with non-neonatal pediatric respiratory failure in 1991. These authors concluded the A:a gradient to be a valid predictor for outcomes in severe respiratory failure. However, other authors challenged this finding by citing flaws in this study (McLaughlin et al., 1992). Tamburo et al. concluded that the A:a gradient is useful as an early predictor of death in a population of pediatric patients who were not newborns. However the assertion that barotrauma was excluded as a significant factor associated with increased risk of death was vigorously challenged McLaughlin et al. in a peer editorial. According to Moler and Cluster (1991), Tamburro et al. also failed to describe the methods of ventilator management, which was also argued to be a major design flaw. The editorial states the A:a gradient is highly influenced by the physician's respiratory management strategy (McLaughlin et al., 1992). The critiques of the study make good points regarding the differences in neonatal and pediatric respiratory care. Neonatal respiratory management principles differ from those of children. Ventilator management principles such as the use of higher positive end expiratory pressure (PEEP) are not equal in both populations. Neonatal ventilation modes differ greatly in the type of ventilation as well as the strategies used. For example, one study demonstrated improvement in

respiratory outcomes with decreased barotrauma and volutrauma, while other studies have shown the A:a gradient to be reliable and sensitive indication of predicting response to HFOV (p=0.024 at 2 hours, and p=.005 at 6 hours) (Ko et al., 2000). Equally important is the need to control for influential variables, particularly management decisions and changes in variables that directly affect oxygenation. Neonatal respiratory care of infants in respiratory failure involves strategies involving close monitoring and frequent adjustments in inspiratory and expiratory pressures, flow, mean airway pressures, inspiratory time, expiratory time, rate of respirations, FiO_2 and selection of ventilator type and mode. HFOV or conventional time limited or pressure-controlled ventilators are selected dependent on the disease process and response to therapy and experience of the operator. Differences in conventional versus high frequency mode of ventilation in conjunction with iNO has not been studied extensively. Moler and Custer, 1992 criticized the study's small sample size statistical analysis, rebutting the small sample with inadequate power to demonstrate the reported trend.

According to McLaughlin's editorial response in 1992, the A:a gradient in a patient supported with mechanical ventilation is a variable directly influenced by the physicians ventilation management strategy. Tamburro and colleagues investigation of whether the A:a gradient is a useful predictor of death in a population of non-neonates did not control for physician directed variables related to oxygenation changes such as adjustments in FiO_2 delivery and PEEP, according to this argument. Although this is true, it is not the only variable influencing the intrapulmonary shunt. This editorial was a criticism of another

study, but was included because it supports the idea of the A:a gradient as a marker of efficacy of the care provider's management strategies and will add to the cache of information for the bedside clinician. In most neonatal intensive care units (NICU), neither the A:a gradient, nor other shunt indicators are typically used at the bedside, though readily available on most blood analysis reports. Understanding these values could prove an important tool for management in infants with AHRF. This study proposal is designed to explore the relationship between pulmonary shunt indicators, PaO_2, levels and the response to iNO. This information could provide additional knowledge about the infants' ability to oxygenate in real time, thus providing support for management decisions.

In 1984, a study by Krummel and associates was undertaken whereby the A:a gradient was investigated in neonates with pulmonary insufficiency for prediction of mortality in ECMO candidates (Krummel et al., 1984). This study compared the A:a gradient to the Neonatal Pulmonary Insufficiency Index (NPII) for predictability of death. The NPII is described as a recommended tool for selection for ECMO at the time. Unfortunately, the instrument was not included in the report. Fifty (50%) of newborns with severe hypoxic respiratory failure received maximum mechanical and pharmacological support with poor response, thus were considered possible candidates for ECMO. Serial arterial blood gas measures were evaluated. The A:a gradient demonstrated much more reliable predictability than the NPII. Over a 12 hour period, serial A:a gradients were analyzed, demonstrating 100% predictability of mortality in sustained values greater than 620 torr over 12 hours. A:a gradients greater than 600 torr for 12

hours demonstrated 93.8% mortality rate. Diagnoses varied from meconium aspiration, congenital diaphragmatic hernias, or persistent pulmonary hypertension. ECMO candidates have strict selection criteria and at this period in time did not include premature infants less than 35-36 weeks. Early literature is interesting, as it demonstrates the enormity of change over time, as now even many preterm infants are surviving with A:a gradients of this magnitude sustained for greater lengths of time.

Alternatives to ECMO were demonstrated useful in 1995 with HFOV an iNO decreasing the need for ECMO in about 40%-50% of neonates. It is unclear from the reports which therapy should be used first and which should follow, however, more ECMO centers have seen decreases in the number of cases since these alternatives have been trialed (Katchel et al., 1995). A study by Hintz and colleagues (2000) demonstrated that ECMO was used less frequently when HFOV, survanta and iNO was more commonly used at Stanford in the mid 1990's. Although the need for ECMO has been reduced in infants with AHRF, ECMO continues to be required as a rescue treatment for infants who suffer from AHRF because of congenital diaphragmatic hernia.

The A:a gradient has not only been explored in different conditions causing respiratory failure as reported above but studies also investigated the A:a gradient in comparison to other oxygenation indices. For example, changes in the A:a oxygen difference (gradient) and oxygenation index (OI) were studied in Europe in 1996 in a small group of 15 infants with severe respiratory insufficiency during low dose iNO (Stranak et al., 1996). The mean length of

treatment was 51 hours. Four infants died in the study, with the remaining 11 demonstrating significant reduction in the oxygen index and A:a gradient in the first 6 hours of treatment. Again, the study was single centered, non-randomized group, but was heterogeneous in diagnosis. Most of the candidates were term or near term. At the time of the study, most centers were not using iNO for infants less than 33 weeks. The authors concluded that survival or necessity for ECMO therapy may have been influenced by iNO. They describe significant decreases in both the A:a gradient and the OI within the first 6 hours of iNO ($p = <0.001$) and 11 remaining survivors with significant decrease in the next 24 hours ($p = <0.0001$). No comparison was made between the A:a gradient and OI as to the sensitivity of prediction. Bleeding complications occurred in two infants who underwent surgery, and ECMO, was believed to be responsible for the problem, rather than iNO therapy.

Another such comparative analysis was undertaken by Atanasov and Despotova-Toleva (1997) to assess the real-time convenience, reliability and accuracy of the changes in the oxygenation index (OI), A:a ratio (a/A PO_2) and A:a gradient in ventilator dependent neonates with RDS. An analysis of the feasibility and potential information yield in O_2 delivery and ventilator therapy was undertaken. Additionally, the prognostic implications and predictive value was explored related to these indices. The study included a group of 20 neonates with RDS. Serial OI, A:a gradient and a:A ratio values were analyzed hourly and calculated to determine the utility in ventilator and oxygen therapy. The study results showed that the combination of the three indexes were indeed useful

discriminating predictors of neonatal lung maturity reflecting arterial blood gas status in ventilator dependent neonates with RDS. The indices also functioned well *individually* to assess the efficacy of conventional ventilator treatment with real-time convenience and reliable accuracy, forming a foundation of clinical decision making in RDS. The author's suggest that use of these measurements could provide more timely corrections, and real time data for analysis. When used together however, the three indexes improved the predictive value as compared to any single test of lung maturity with important implications in the management of neonates with RDS (Atanasov, & Despotova-Toleva, 1997).

Atanasov and Despotova-Toleva (1997) studied the oxygenation indices in infants treated with conventional ventilators, in contrast, Ko et al. (2000) compared respiratory indices for predicting response to high frequency oscillatory ventilation in 2000. The Ko study was a comparison of the A:a gradient, arterial to alveolar ratio (a:A ratio) and the oxygenation index, (OI) was examined in prediction of response to HFOV. Twenty-three very low birth weight infants with respiratory distress syndrome were ventilated using high frequency ventilators. Clinical records were reviewed and data analyzed comparing each index individually over several time points up to 24 hours. Mean A:a gradients demonstrated significant differences earlier in the responder group than the a:A ratio values. At 2 hours after treatment, responders A:a gradient was reduced significantly ($p=0.024$) and an even more remarkable difference at 6 hours ($p=0.005$). Death in the patient with A:a gradient over 350 at 2 hours after HFOV therapy was 100% in sensitivity and 80% in specificity. The earliest a:A ratio

mean difference was significant at six hours after HFOV treatment ($p = 0.019$). The oxygen index showed no significant differences between the two groups. They concluded the A:a gradient was the most reliable indicator of responsiveness with earlier results than other indices studied.

High frequency ventilation has been demonstrated to improve patient outcomes by improving gas exchange with lower pressures, thereby reducing volutrauma and barotrauma. This breakthrough has effectively reduced the development of chronic lung disease in infants with RDS (Clark et al., 2000). Additional studies have demonstrated decreased morbidity and mortality with this mode of ventilation including decreased rates of air leak syndrome associated with use of higher pressures in infants with severe RDS. HFOV improves oxygenation with less risk of chronic lung disease (Clark et al.. 1992; The HiFO Study Group, 1993; Chan, et.al., 1994). This study of HFOV in very low birth weight infants with RDS compared three oxygenation indices for predicting response to HFOV. In a group of 23 infants with RDS, 12 responded to HFOV and 11 died. The two groups were studied comparing the A:a gradient, PaO_2, and Oxygen index (OI) for predicting infants response to treatment. A retrospective review was performed on the medical record with findings showing significantly lower A:a gradient in responders than in non-responders. The A:a gradient was the most effective and sensitive respiratory index for predicting responsiveness to HFOV with severe RDS. The researchers found using an A:a gradient cut off value of 350 after two hours of HFOV therapy predicted death with 100% sensitivity and 80% specificity (Ko et al., 2000) .

In contrast, Subhedar et al. (2000) found no difference in the performance of these same oxygenation indices in a study of 150 preterm infants less than 34 completed weeks gestation. Subjects were enrolled in this study comparing oxygen indices of respiratory failure in ventilated preterm infants. Infants had no major congenital anomalies, at least three arterial blood gasses were collected and analyzed within the first 24 hours of life from an indwelling arterial line, and all received mechanical ventilation within six hours of life for a minimum of 24 hours (Subhedar et al., 2000). The author's explanation of ventilation management, use of surfactant, type of ventilation was well described. HFOV was not used in the study. FIO_2 was delivered to maintain PaO_2 levels between 50-70 mm Hg, and SaO_2 greater than 94 in the acute phase of illness. Infants received sedation and neuromuscular blockade when asynchronous respirations were observed. These practices are aligned with the current study proposal methods of management. Data collected included information about PaO_2, $PaCO_2$, FiO_2, and mean airway pressure (MAP). Simultaneously, alveolar: arterial gradient (difference) (A:a gradient = (A-a DO_2), a/A ratio (arterial/Alveolar O_2 ratio), and OI were collected. Patient characteristics were compared between groups using univariate analysis. Mann-Whitney and Chi Square test were used for continuous and categorical data. This study found no evidence of significant differences between the performance of the a:A ratio, A:a gradient and OI. Use of the OI was recommended due to the ease of calculation in the clinical setting (Subhedar et al., 2000). It is unclear whether the blood gas analyzers used during the study period provided the calculations for the A:a gradient and the a:A

ratio or whether these had to be hand calculated. This might explain the difficulty in calculation referred to in the recommendations, while in contrast today, blood gas machines with co-Ox capability provide these readily on the blood gas printout; however this is not true of the oxygenation index.

In summary, the literature review resulted in developing ideas for further exploration. While reports on term and near term infants are available, research indicates little is known related to the use of iNO for acute hypoxic respiratory failure in very early, very small infants. Furthermore, studies have yet to indicate the safest, most effective dosage or treatment protocols. Predictors for response to treatment have been studied in terms of very short-term response, along with outcomes such as death and chronic lung disease, but this research does not indicate what variables or measurements are the best indications of immediate, real time response. Additionally, few studies examined the A:a gradient or other oxygenation indicators in a group including all ages and birth weights while receiving iNO in addition to conventional treatments. There is still much to explore to better understand the relationships between the oxygenation indices and degrees and types of intra or extra pulmonary shunting, as well as the response to treatments applied to save these very sick infants. The use of inhaled nitric oxide has recently received regulatory approval in the United States for term and near term infants and it is already widely used in neonatal intensive care units around the world. Neonatal nurses, as well as practitioners will benefit from participation in research and education related to iNO to enhance their practice. With clinical trials underway, this new intervention is promising for term

infants and may be beneficial for preterm infants at gestational ages of 23 weeks and above. Nurses, physicians, and respiratory care therapists will certainly benefit by understanding the different types of pathophysiology related to pulmonary diseases of the neonate, as well as development of evidence based effective treatment modalities.

CHAPTER 3: RESEARCH DESIGN AND METHODS

Purpose and Study Design

This chapter presents the research design and methodology used in the study, including sample, sampling procedures, instrumentation, data collection and data management procedures, assumptions and limitations. This study is a secondary data analysis which expands upon a Federal Drug and Administration (FDA) approved single center parent study, which examined inhaled nitric oxide as an anti-inflammatory agent in the prevention of chronic lung disease in infants with respiratory failure. “Secondary analysis involves studying data previously collected in another study. Data are re-examined using different organizations of the data and different statistical analyses than those previously used, to validate reported findings, examine dimensions previously unexamined, or redirecting the focus of the data to allow comparison with data from other studies” (Burns & Grove, 1997). The parent study involved treatment of eligible neonates of any gestational age with 20 ppm of nitric oxide (iNO) for 24 hours and 5 ppm iNO for up to 7 days with two serum samples of cytokines-collected at two intervals during the treatment period. The purpose of the parent study was to determine whether anti-inflammatory properties of inhaled nitric oxide effectively prevented chronic lung disease in premature infants. During the study period, secondary questions were developed for the purpose of this study. Thus, the current study examines dimensions previously not examined in the parent study. Data collected from the parent study was identified as useful to answer clinical

questions not originally posed, resulting in the development of specific aims and formation of the hypotheses for this study.

The purposes of this secondary analysis were to (a) examine the linkages between intrapulmonary, V/Q mismatch and/or extra pulmonary shunting and oxygenation in infants with acute hypoxic respiratory failure (AHRF) prior to initiation of iNO therapy, and (b) to determine whether inhaled nitric oxide therapy is associated with improvement of intrapulmonary shunting and oxygenation in infants with AHRF and (c) determine whether differences in the degree of shunting existed based on the type of ventilator selected for treatment of acute respiratory failure prior to initiation of iNO therapy, (d) to explore whether lung maturation based on developmental age (gestational age) is predictive of response to iNO therapy.

The main outcome variables of interest of this study were (a) oxygenation, measured by the partial tension of oxygen (PaO_2), (b) intrapulmonary and/or extra pulmonary shunting; measured as the difference between alveolar oxygen tension and arterial oxygen tension (A:a gradient), (c) functional lung maturity, measured by the gestation of weeks completed at time of birth, and (d) inhaled nitric oxide, (iNO), an experimental gas administered for treatment of acute hypoxic respiratory failure was the treatment variable.

It is important to understand the relationships and the differences between and among the aforementioned variables so the underlying physiological problem is addressed in the clinical setting utilizing all relevant clinical data. The outcomes of this study are expected to expand clinical knowledge that can be

used to: a) further assess and enhance current treatment modalities and b) improve upon existing and or develop new management strategies focused on oxygenation and ventilation occurring during AHRF.

Setting, Sample Size and Determination

The sample size and setting for the parent study are outlined below. Since the current study is a secondary data analysis, the sample size and setting were determined by these parameters.

The setting for the parent study was a 42 bed neonatal intensive care unit with 20 Level III beds and 22 level 2 beds. The NICU at the treatment hospital (NICU) admits approximately 400-500 infants each year. The NICU is staffed by three (3) neonatologists and four (4) full-time neonatal nurse practitioners (NNP) approximately 60 full time registered nurses and 10 respiratory therapists. Newborns admitted to the NICU vary in gestational age from 23 to 42 weeks, with birth weights greater than 400 grams. The infants requiring intensive care are a heterogeneous group with many different diagnoses and co-morbidities, as well as varying socio-economic statuses.

The sample was a non-randomized, heterogeneous convenience sample of infants inborn or referred to a single center within a 100 mile radius of the level three neonatal intensive care unit at the study hospital. Only specific data related to the variables of the current study were used for hypotheses testing.

A power analysis was not conducted since this study expands on a larger clinical trial and the length of study trial was determined and fixed by the original trial. However, for non-significant results, post-hoc power analyses were

conducted to determine whether the sample size was sufficient to detect significant differences. At the very least, this study provided data necessary to estimate the standard deviation for the variables of interest, which could be used to power further studies.

Sample Selection Criteria

The sample for the parent study was based on the criteria outlined below. Since the data for the current study were based on the data of the subjects from the parent study, the subjects for the current study met the same criteria:

Inclusion criteria: Infants were included in the study if: a) their birth-weights were greater than 401 grams, b) their gestational ages were between 23-42 6/7 weeks at time of birth and/ or c) they were less than 1 month of age at time of entry into the study. Other inclusion criteria included a diagnosis of acute hypoxic respiratory failure, being treated with inhaled nitric oxide, endotracheal intubation and mechanical ventilation with oxygen requirements of >30% FiO_2 to maintain an arterial saturation greater than 90%.

Exclusion Criterion: Infants whose birth-weight were less than or equal to 400 grams or on oxygen less than 30% and were expected to wean quickly from ventilator support were excluded from the study. Also, subjects were excluded if diagnosed with major structural, congenital anomalies including, but not limited to, congenital heart disease, pulmonary malformations, Potter's sequence, Hydrops Fetalis, trisomies, monosomies, and/or non-viable infants (i.e. infants <23 weeks gestation and less than or equal to 400 grams).

IRB-approval for this secondary data analysis was obtained from the University of Texas Medical Branch and the treatment hospital following approval of the proposal from the dissertation committee. Additionally, approval from the Medical Director of the NICU and the principle investigator (PI) at the treatment hospital was obtained.

Subject Recruitment Procedure

Parents of infant's with severe hypoxic respiratory failure were approached for consent of treatment and study enrollment as soon as the diagnosis was confirmed. The admitting neonatologists or NNP obtained consent for enrollment. Interpreters were provided to present the information in the parent's native language whenever necessary. Early identification and consent were obtained to allow the investigator to provide parents with information related to the protocol and allow time for parents to ask questions to assure their understanding. It was necessary to obtain consent during a highly stressful and emotional time for the parents. The NNP and neonatologists were sensitive to the nature of the situation and took time to visit with each parent. All attempts were made to provide full explanations immediately in order to rapidly initiate therapy and avoid any possibility of less than optimal outcomes.

Infants who met criteria and did not respond to conventional therapy were recruited for the iNO study. Discussions with parents included a thorough description of the diagnosis of AHRF, the seriousness of the condition, a complete update of their infants' existing condition and treatments provided for the infant. An introduction to the iNO study gas protocol was then explained to

the parents as an option for treatment. All benefits and risks were thoroughly explained to the parents. Once the Neonatologist or NNP were satisfied, the parents understood the explanation and had no further questions, parents were asked to sign the consent form with a witness present. Parents were given the option to decline study participation, without coercion or prejudice and were informed they may discontinue participation at any time during the treatment. Once the parents agreed to enroll their infants in the study, the parent's signatures were obtained and witnessed, parents were given a copy of the consent form, and the original form was placed in the infants' medical record.

Data Collection

Staff Recruitment, Training, and Responsibilities

Recruitment of care providers was unnecessary; rather the study was presented and integrated in the natural NICU setting. Nursing and respiratory care department administrators were committed to the parent study. Clinical training for all level three staff nurses and respiratory therapists was provided by clinical educators several months in advance under the direction of the primary investigator.

Responsibilities were delineated among the staff as follows. Nurses were responsible for collecting all blood gas samples using routine procedure guidelines. They were also instructed on the procedures for sampling the cytokine, (IL-6, IL-8) serum levels. This included collection of blood samples in red top micro-tubes without gel or additives, labeling samples appropriately and

assuring samples were sent to the laboratory efficiently to maintain sample integrity. Other nursing responsibilities were to: maintain medical records per routine, assist in assuring signed consents were located on the medical chart prior to initiation of the protocol, and to assist the respiratory therapists during initial set up and during the course of treatment as needed. Nurses and therapists were given a checklist to follow for each patient enrolled in the study for quick reference.

Respiratory therapists, (RT) were trained to be responsible for setting up the iNO ventilator circuit, maintaining cylinders and dispensing logs (form 16.0). Additionally, they were responsible for special bagging equipment, running and recording blood gas samples and the entering of data on the iNO flow sheet (form 22.0). Consistency in RT staffing familiar with the study protocol was ensured by assigning two licensed RT's to the neonatal intensive care area 24 hours a day seven days a week. These RT's were trained in the procedures and in the study protocol. This assignment was necessary due to the unpredictability of enrollment and the need to have an RT available twenty-four hours per day.

As infants completed the study, forms 16.0 and 22.0 were collected by the respiratory therapist supervisor to review for accuracy and completion. Once all data were checked and completed by the respiratory care supervisor, they were given to the respiratory department manager for review. Forms were then delivered to the data monitor. The data monitor was the PI of this study. Responsibilities of the data monitor were to identify and retrieve missing data on all forms, screen, and clean data, create and manage an electronic database in

Excel, maintain the integrity of the study records and assist in writing a paper upon completion of the parent study.

The data monitor acted as director of the data management center. All data were maintained confidential, in a locked file cabinet in the neonatology offices located adjacent to the NICU. Access to the area was prohibited without knowledge of several locked doors, coded for entry, and a key was only provided to the data monitor and the parent study primary investigator, Dr. Bedi. Security measures were maintained throughout the study period.

The case report forms (Form 17.0) were completed through review of the medical records by the parent study data monitor. Since records are archived after a set period, special permission was granted by administration to access archived electronic records for research purposes. All records were kept confidential, and a private password was necessary to access data. The Case Report Form was initiated by the data monitor when infants were enrolled in the parent study and completed after 30 days of study enrollment, since some data were required after a thirty-day period. At that time, the data was double-checked against the infant's flow sheets in the electronic medical record to verify the data for accuracy. Data collected on the dispensing log (form 16.0), adverse event form (18.0) and the iNO flow sheet (form 22.0) underwent thorough review by the data monitor prior to final inspection by the PI of the parent study. Several meetings between the PI and data monitor took place prior to final review. Meetings generally involved communication related to process issues, adverse effects, reporting of status of the clinical trial and problem solving.

Instruments and Forms

Three instruments and five forms were used in treatment and recording of the data for infants enrolled in the parent study. Three of the forms, the consent form, the case report form and the iNO Flow Sheet provided the necessary data for this secondary data analysis. The other two forms, the Adverse Event Form (18),appendix A.6 and the Dispensing Log (16) appendix A.4 were not used as a part of this study and are not discussed below, but are located in Appendix A for reference.

Blood Gas Analyzer-(Appendix B.1)

The Bayer Rapid Point Blood Gas Analyzer Model 405 was used for blood gas analysis. The analyzer required approximately 0.2-0.3 milliliters of serum to analyze blood gases. The Bayer analyzer is capable of accurate analysis of serum sampling from arterial, venous, or capillary samples and was used without problems throughout the parent study period. The results of the blood gas samples were transcribed onto data collection iNO (form 22.0), and were recorded by respiratory therapists in the electronic medical record, using Meditech Systems at the treatment hospital. Nurses routinely recorded all blood gas results on the patient flow sheet at the bedside.

Bear Cub 750 PSV-(Appendix B.2)

During the study period, the Bourne Bear Cub 750 model ventilator was the primary conventional ventilator used for ventilation of all neonates admitted to the study. These ventilators proved to be effective and reliable throughout the

clinical trial; however, specific reliability, specificity or sensitivity data are not available. Purchase of the ventilators was done with approval from the medical director after review of the available specifications prior to initiation of the study. The Bourne Bear Cub 750 was used consistently for an extended period prior to the study, and all clinicians were confident in the reliability of the system. Respiratory therapists assigned to the NICU were trained and passed competency testing related to the use of this ventilator. The Bourne Bear Cub 750 was consistently used with the parent study sample in lieu of two other ventilators also used in the NICU. These ventilators, Siemens Servo 1000 (appendix B.3) Adult/Infant Ventilator or the Puritan Bennett 840 model (appendix B.4) ventilators were not used, because they could not support the iNO infusion system used in this study.

SensorMedics 3100 High Frequency Ventilator-(Appendix B.5)

The SensorMedics 3100 High Frequency Ventilator was used during the study period for rescue therapy of infants who failed conventional ventilation. Approved in 1991, this ventilator is used in adults and pediatrics for acute respiratory failure. It was later used in infants in 1995 for the treatment of neonatal respiratory failure. The philosophy behind utilizing this form of ventilation is to protect lung tissue from volutrauma and barotrauma associated with conventional distending pressures delivered by conventional ventilators (Appendix B).

Forms

The following forms were used to collect data for the parent study. These forms also included the variables of interest for the current study. The Parent Study Protocol Abstract AIA-NO-1 (Appendix A.2) and the IRB approval (Appendix A.1) are located in the appendix section for reference.

Parent Consent (Form 15.0) (Appendix A.3)

The consent form was provided by the parent study investigators, is written in lay terms, and describes the protocol procedures. A copy of this form was provided to the parents of infants enrolled in the study. The consent form was co-signed by a witness at the time of consent. It was assembled as a stapled packet and included additional forms specific to the parent study that were not utilized for this study. When possible, information associated with the infants that could be used as identifiers, was not collected in this current study, to further protect the confidentiality of the subject.

Case Report Form 17.0 (Appendix A.5)

This form is a form was designed for the parent study and only demographic information from the form were extracted for the proposed study, including gestational age, coded identification number, and other pertinent data for the parent study. Data extracted for the current study included maternal and infant data, number of study treatment, entrance and exit dates, associated complications, deaths, and the related cause.

iNO Flow Sheet (Form 22) (Appendix A.7)

This flow sheet is the primary flow sheet used to extract data for this study. It included the arterial blood gas analysis data, the type of ventilator and its settings at the time of sampling including fi02, pressures, inspiratory time, mean airway pressure, amplitude and the A:a gradient. This form was developed by the primary investigator of the parent study to capture data over the study period, (see Appendix A).

Data Collection Procedures

Data collection forms were provided in pre-assembled packets within a blue envelope. Blank packets were stored in the neonatal intensive care unit respiratory care workstation and the nurse's station. Each envelope contained a checklist provided for RN/RT team caring for the infant. The packet documents included a complete Parent study protocol (P), consent form, dispensing log, case report, adverse event form, and the iNO flow sheet. When an infant was identified as a study participant, the packet was opened, and patient labels affixed to consent, case report and iNO flow sheet. Packets were disassembled and the consent form was placed in the medical record after it was signed and witnessed. A copy of the protocol and consent form was given to the parents. The case report form and the iNO flow sheet remained at the bedside for ready access to record data.

Data collected on the iNO flow sheet obtained primarily by a respiratory therapists providing direct care to the infants participating in the study. Data was recorded on the iNO flow sheet by the respiratory therapist at the time of

collection. Blood gas samples with related treatment information, study serum samples, as required were taken and recorded at specified times designated. Data collection did not require scoring or agreement on phenomena; therefore establishing interrater reliability was not necessary. However, consistency in data collection and transcription were essential and four checkpoints were established to ensure this consistency.

Before establishing these checkpoints, training sessions were provided by the respiratory department supervisor and the parent study primary investigatory prior to the study initiation. Respiratory therapists were instructed in collecting data on iNO flow sheet accurately and thoroughly in a classroom setting with examples. All forms were double-checked against daily flow sheets used in the NICU, as well as in the electronic record archives for transcription errors by the shift supervisor at completion of infants' protocol. Continued support and supervision were available by the RT shift supervisor, data manager, respiratory care manager and primary investigator throughout the study period. Satisfactory data collection techniques were established, with four (4) checkpoints, in the following order: 1) daily checks of forms by therapists before, during and after study is completed. The respiratory therapist discontinuing the iNO gas was responsible for making sure the dispensing log and iNO flow sheets were complete and accurate, then promptly turned over to the supervisor, 2) a weekly review of all data collected was performed by the respiratory care unit supervisor, to establish accuracy, and locate any missing data, then turned packets in to the respiratory department manager, 3) the respiratory department manager again

reviewed packets and provided feedback to individuals, missing data or errors corrected at this time, and finally 4) the data monitor and PI looked at all forms for completeness and accuracy. Data were then entered into an Excel database, and approved by the data monitor and primary investigator.

Each study participant had a possibility of 12 entries on form 22.0, the iNO flow sheet upon completion of the treatment protocol. If participants expired or were transferred out for ECMO prior to completion of the protocol, the forms were checked by the RT supervisor for completeness and then given to the respiratory care department manager/director. Therapists were given constant feedback and corrections were made if the therapist neglected to record data properly, as each packet was screened by the shift supervisor and again when turned in to the respiratory department manager. Additionally, training or explanation was provided by the data manager or RT supervisor when problems with data collection were identified.

Serum blood gas samples were collected by nursing staff and then run by respiratory therapist's using blood gas analyzer Bayer Rapid Point Blood Gas Model 405 located within the NICU. The analyzers perform auto calibration's every five minutes, as well as quality control analysis with control substrates every eight hours. If mechanical problems occurred a back up unit of the same make and model was available for analysis. Quality control records of blood gas analysis machines were kept by the respiratory care department.

Data Analysis

Once the forms were satisfactorily completed, the data was transferred to an Excel spreadsheet .Data was coded for statistical analysis and imported into SPSS statistical analysis program. Data was again screened for errors and cleaned for missing or encoded data. Sample demographic data was displayed and summarized using tables and graphs when possible for ease of explanation and to enhance understanding of the sample characteristics. Descriptive statistics were used to summarize sample characteristics and reconstruct data for reporting. Ranges, frequencies, standard deviations, percentages, and means were used for this purpose. Analysis for each hypothesis is explained below.

To address Hypothesis 1 that there is a negative linear relationship between the A:a gradient and PaO_2, the Pearson r correlation was used. The Pearson r was used for interval data and assumes a normal distribution and homoscedasticity, (i.e., for every value of X, the distribution of Y must have approximate variability). In light of the multitude of confounding factors likely influencing the outcome variables, moderate correlations 0.3 - 0.5, $p < 0.05$ were identified to find support for the hypothesis.

The second hypothesis for Aim 1 of no difference in the A:a gradient dependent on type of ventilator(conventional or high frequency) used was tested using the t-test. The t-test assumes the independent variable is categorical and contains two groups, there is a normal distribution, and the variance of the dependent variable for the two groups is similar. Data are at the interval level and significance levels were set at .05, with confidence interval of 0.95.

To address the third hypothesis of Aim 1, the A:a gradient will be significantly lower in the responder group versus the non-responder group over three specified time intervals during treatment, both separate t-tests at each time point as well as a mixed model repeated measures ANOVA was utilized. Within interval multiple assessments (three assessments each) were averaged to produce grand mean A:a gradients for each time interval as detailed below.

Time 1- mean A:a gradient at 1hour, 8 hours and 24 hours after initiation of treatment.

Time 2- mean A:a gradient at days 2, 3, and 4

Time 3- mean A:a gradient at days 5, 6, and 7

If the hypothesis is supported, responders to treatment would demonstrate greater improvement in the A:a gradient at the various time points and over time. While the difference from baseline A:a gradient and interval means for the nonresponder group may also decrease over time, we would have expected to see significantly less difference over the course of treatment.

To test the first hypothesis in Aim 2 - whether functional lung maturity based on gestational age is predictive of responsiveness to iNO therapy, chi-square analyses assessed the distribution of groups (gestational age) across response categories (responders versus nonresponders). The dependent variable is dichotomous, responder or non-responder, and the independent variables are categorical [group 1: 23-26 6/7 weeks and group 2: 27-41 weeks].

The second hypothesis in Aim 2 states that after controlling for septicemia, the remaining prevalent confounding causative factor of acute hypoxic respiratory

failure, functional lung maturity (based on gestational age) would remain a valuable predictor of response to iNO treatment. Backward stepwise logistic regression was used for this analysis. In this analysis, seven predictor variables were regressed upon a responder/nonresponder criterion. Variables included were gestational age, gender, A:a gradient before the treatment, ventilator type at start of treatment, PaO_2 before treatment, early onset septicemia (yes/no) and race (white/nonwhite). Backward regression enters all variables into the model initially and then systematically removes the least important factor at each step until the model reaches significance. The benefit of backward stepwise regression versus forward stepwise regression is that synergistic interactions between variables are examined that may not have been included in the more conservative forward approach. This analysis was chosen to determine whether septicemia or demographic variables contribute or impact the likelihood of response or non-response.

Assumptions

Six assumptions formed the basis for this study and are as follows:

Assumption**:** There is a strong correlation between the degree of oxygen transfer efficiency (A:a gradient) and the PaO_2 as indicated in the literature and experienced in clinical practice. Typically, academic and non-academic clinicians do not use the A:a gradient for evaluation and assessment of respiratory status or treatment effectiveness.

Assumption: Saturations above 96% are not as sensitive to changes in the PaO_2 and are therefore less reliable as an indicator of oxygenation.

Therefore, the saturation level or SaO_2 is not a sensitive measurement variable of oxygenation for this study. For example, saturations of 96-100% are seen whether the paO_2 is 65 or 350, as long as the hemoglobin is fully saturated. Most infants in the study will require higher levels of oxygen delivery for some duration of the study.

Assumption: Responders: Improvement of the PaO_2 or stability of the PaO_2 during manipulation of ventilation and oxygenation parameters delivered to the infant indicates oxygen transfer to the serum from the alveoli. These measurements indicate a positive response to treatments, including (iNO) in infants with acute hypoxic respiratory failure. Treatments are consistent among and between providers of the group due to strict protocols and training.

Assumption: Continuing failure to demonstrate improvement or worsening PaO_2 values usually indicate inadequate or poor oxygen transfer to the serum from the alveoli indicating a poor response or failure to respond to the initial course of treatment of (iNO) in infants with acute hypoxic respiratory failure.

Assumption**:** Infants enrolled in the study met criteria for acute hypoxic respiratory failure, and all respiratory care treatments were delivered similarly by each clinician according to protocols agreed upon by all providers among the medical staff associated with Neonatal Consultant's.

Assumption: The A:a gradient was not previously utilized as a predictor or explanation of respiratory outcomes in the neonatal critical care area within the group.

Supervision and Facilities

The student research was directly supervised by Alice Hill Ph. D., F.A.A.N., dissertation Chairperson. Dr. Hill is the Director of the Nursing Ph. D. Program in the Graduate School of Biomedical Sciences. The data collection occurred at the treatment hospital Neonatal Intensive Care Unit, and in the Medical Records department under the Supervision of Dr. Harvinder Bedi, Principal Investigator of the parent study, and Medical Director of the Neonatal Intensive Care Unit at the treatment hospital. Other facilities include the University of Texas Medical Branch Library, and the neonatal nurse practitioner office at the treatment hospital. All data were locked in a file cabinet in the offices of Neonatal Consultant's at the treatment hospital. Access to data was limited to the investigator, IRB, and dissertation committee members on a need to know basis only. Protection of infants' identities was maintained by removing all identifiers from the data.

Protection of Human Subjects

The participants were the premature and term infants admitted and treated in the NICU recruited for participation in the iNO study. The parent study was approved by the institutional review board at the study center and UTMB. Participation in the parent study was by written informed consent of the parents. All study materials were coded so that participants' names were not on the data that could be linked to participants. The code detail was kept in a locked file in the investigator's office separate from all other study materials. Any participant could discontinue or withdraw from the study at any time without penalty or harm,

which was written on the consent form that the parents signed. No records were reviewed that were not included in the iNO study. Socio-demographic data collected had an IRB-approved form created for the study and was coded as all other study materials to protect the anonymity of the participants. Data collection was conducted within the NNP office, treatment unit, or medical records department at the treatment hospital.

This study proposal is designed as a secondary data analysis. The Principal Investigator for this current study served as the data monitor for the parent study and reviewed all records for accuracy of data collection and transcription. Records were examined for completeness to eliminate incomplete or missing data at multiple instances. Findings of the study were reported in the aggregate so that individual participants could not be associated with isolated instances of data. The current study involved no manipulation of patient care or treatments. The study caused no harm, as it examined data extracted for analyzing relationships among specific variables, without conflicting interest concerning the parent study. Infants enrolled in the study had informed Consent by Proxy due to the minor statuses of the infants. Proxy consent was legally obtained from the parents and was confirmed in the medical records. Consent obtained included permission to use anonymous data in the iNO parent study for secondary study analysis.

CHAPTER 4: RESULTS

As stated in Chapter 1, this study examines linkages between intra and extra pulmonary shunting and oxygenation in infants diagnosed with Acute Hypoxic Respiratory Failure (AHRF). This chapter will first present a description of the sample characteristics, including maternal and infant data, extracted from the records relevant to the study. The remainder of the chapter is then organized around two specific aims and five associated hypotheses. The first aim and three associated hypotheses, identifies linkages between intra and extra pulmonary shunting and oxygenation in infants with AHRF prior to and during iNO treatment. The second aim and the two associated hypotheses, explore whether gestational age is associated with and predictive of responsiveness to inhaled Nitric Oxide treatment.

Descriptive Data Analysis

All descriptive data were obtained from the infants and mothers' medical records. In some instances, not all descriptors were available for each variable on each subject.

Infant Descriptive Data

Of the 74 infants enrolled in the study, 45 (60.8%) were male and 26 (39.2%) were female. The majority of males were Hispanic (40%, n = 18), with Caucasian males representing 38% (n = 17) and black males representing 11% (n = 5). The remaining five males (11%) did not have race reported. Of the 29

females the majority were Caucasian (62%, n = 18), followed by Hispanic (28%, n = 8).Unexpectedly, there were no black females identified; three females were missing ethnic assignment (10%). Race information was established by self-reported data collected at the time of mother's hospital admission. Information was entered into the computer database by admitting personnel.

Gestational age

Infants' gestational ages ranged from 23 to 40 completed weeks (m = 28.5 weeks, sd =5.4; md = 26 weeks). Completed weeks are reported as infants whose gestational age was rounded down to the former week, thus completed weeks of development. For example, if mother's data reports she is 24 weeks and four days (by verified dates), the infant has *completed* 24 weeks gestation and is thus assigned 24 weeks as his or her gestational age at the time of birth. A good portion of the sample consisted of the youngest and most vulnerable in the sample. Ten of the infants' gestations were 23 weeks (14%) and eighteen infants completed 24 weeks gestation (38.9%). Interestingly, more than half, (52.8%) of the subjects' (n = 28) gestational age were less than or equal to 26 weeks gestation. Seventy-five percent (n = 54) were less than or equal to 33 weeks. The remaining 25% (n = 18) completed between 33-40 weeks. Seventy-two (97.6%) cases in total were reported with two missing (2.4%). Full descriptive statistics on frequencies, percent of cases in that category, and the cumulative percent of the total number of cases of reported gestational age and birth weights assigned at birth is found in Appendix C.1.

Birth weights

Birth weights ranged from 480 grams to 5420 grams (m = 1472 grams, sd = 1.1; md = 865 grams). Interestingly 58.1% (n = 43) of the infants were less than 1,000 grams at birth. In the clinical setting, these infants are deemed the most vulnerable “micro-preemies.” Approximately 67.6% (n = 51) weighed less than 1500 grams at birth. This weight is considered low birth weight in clinical neonatology. It is important to recognize that a majority of the sample consisted of extremely premature and very low birth weight infants. Table 4.1 exhibits birth weights at quartile points.

Table 4.1 Birth weights at quartile points

Birth weight Range	N	Quartiles CUM %
480 -660	19	25.7
670-860	18	50.0
870-2220	19	75.7
2420-5426	18	100

Infant Delivery Data:

While all infants were treated with inhaled nitric oxide within the treatment facility, not all were delivered there, but rather transported in. Forty-nine infants were inborn (67%) while 24 (32.8%) were transferred in from referring hospitals. Table 4.2 details the breakdown of infants transferred to the treatment hospital for higher level of care enrolled in the study.

Table 4.2 Birthplace

Place	N	%
BMC	11	14.9
SJM	8	10.8
MCH	1	1.4
SEM	4	5.4

It is important to report that the same neonatology group actively practiced within the referral hospitals and provided stabilization and transfer management to all subjects. The group personnel remained stable throughout the study period. Referrals were received either from the obstetrician for high-risk deliveries, the pediatric hospitalist, or from the pediatrician selected by the parents prior to the birth. Once a consult was made, these infant's were assigned to Neonatal consultants' service, who then provided stabilization, transport management and continuous medical care throughout the infants' hospitalization.

Method of Delivery

Sixty-five percent (n = 48) were delivered by cesarean section and 27% (n = 20) delivered by vaginal delivery. Approximately 20.3% (n = 15) maternal membranes were prematurely ruptured more than eight hours prior to delivery.

Resuscitation

Resuscitation of infants incorporated standard procedures based on American Academy of Pediatric "STABLE "guidelines and the American Heart Association neonatal resuscitation program guidelines. Of the 68 records with data (91.8%) only one infant (1.4%) required no oxygen at delivery or respiratory

assistance, 79% (n = 59) of the infants required intubation and intermittent positive pressure ventilation at delivery, 10.8% (n = 8) required oxygen delivery by blow with the remaining 8.1% (n = 6) missing data. One infant was diagnosed with birth asphyxia representing 1.4% of the total infants.

Apgar scores were collected as a standardized, yet subjective evaluation of the newborn's physical condition at one minute following birth and again at five minutes after birth. Scores are directly related to the infants' physiologic response at the designated time. This method includes a brief assessment and assignment of a score to the heart rate, respiratory effort, color, reflex irritability, and muscle tone. Total scores range from 0 (indicating still-birth) to 3 represent severe distress, a score of 4-7 represents moderate distress, and score of 7-10, indicates an absence of difficulty in adjusting to extra-uterine life at a five minute interval. The mean Apgar score at 1 minute was 5.9 and the mean score at 5 minutes was 7.73. Apgar scores assigned at delivery at one and five minutes are presented in Table 4.3 below.

Table 4.3 Apgar Scores Assigned after Delivery

VARIABLE		
	N	**%CASES**
Apgar 1 minute	67	
	M=5.9	Sd=2.08
2	2	2.7
3	10	13.5
4	11	14.9
5	5	6.8
6	5	6.8
7	14	18.9
8	16	21.6
9	4	5.4
Missing	7	9.5
Apgar 5 min	N	%CASES
	67	
	M=7.73	Sd=1.29
4	2	2.7
5	3	4.1
6	5	6.8
7	13	17.6
8	22	29.7
9	22	29.7
Missing	7	9.5

Infant's Major Diagnoses and Co-morbidities:

A number of major diagnoses were reported in the sample records and transferred to a database established by the group practice for the purpose of statistical analysis in the evidence-based practice. Practice patterns and protocol

development is ongoing using the data from patients outcomes. Pertinent descriptive variables are included in order to describe the subjects' major health conditions encountered. Some of the most notable findings were: 26% (n = 37) infants were diagnosed with persistent pulmonary hypertension, 14/% (n = 10) with congenital pneumonia, and 1.4% (n = 1) with meconium aspiration syndrome. Early onset septicemia was diagnosed in 26.4% (n = 19) with late onset septicemia diagnosed in 44.1% (n = 30). Intraventricular hemorrhages occurred in 36.6 % infants (n= 26), with 10 infants with grade III and IV hemorrhages. Retinopathy of prematurity occurred in 36.8% (n = 25) and necrotizing enterocolitis was diagnosed in 5.7 %(n = 4) of infants. Patent ductus arteriosis occurred in nearly one third of these infants with 29.7% (n = 22). These co-morbidities are mentioned because they raise the acuity of the infants, making management difficult, and may have a negative impact on the outcomes for these infants. This data is displayed by systems in Appendix C.2. Records without variable data are reported as missing, with accompanying percentiles of total cases for the specific variable.

The co-morbidities identified represent the degree of the challenges associated with caring for infants with acute hypoxic respiratory distress. As indicated, these are often the most acutely ill infants with extensive intensive care needs. However difficult, it should be made clear that while some infants suffered from co-morbidities during the period of respiratory failure, not all occurred congruently with AHRF, or during the treatment protocol. Since co-morbidity data was not collected concurrently during the study period, there is no way to

ascertain which occurred during the period of respiratory failure and which occurred at other points during the hospitalization. Since these are the most critically ill infants in the NICU, the incidence of number and severity of co-morbidities is much higher.

Infant Mortality

Of the 74 infants enrolled, 20.3% (n = 15) died during the hospitalization, 8.1% (n = 6) of which occurred during the primary iNO treatment period. Cause of death and gestational ages on those who expired during the treatment are exhibited in Table 4.4 below.

Table 4.4 Infant Mortality (Sample)

Expired during Treatment	N =6	% (8.2)
GA died during TX 1		
23	2	2.8
24	1	1.4
26	1	1.4
29	1	1.4
30	1	1.4
Cause of Death		
AHRF	3	4.1
Con. Anom	0	0
NEC	1	1.4
Late onset Sepsis	0	0
Early Onset Sepsis	2	2.7

Maternal Descriptive Data

Maternal ages ranged from 17 to 43 years (mean age = 27, sd = 6.9, median = 28). The majority of mothers were prima gravida's and most were singleton births while there were six mothers with twin gestation and one with triplets (see Table 4.5). The majority of deliveries were by cesarean section compared to vaginal delivery.

The majority of the mothers received a minimum of four prenatal visits prior to delivery, with only five who had no prenatal care. In regards to ethnicity, again, it is important to note black mothers were under represented in this sample (n = 5), necessitating a reduction of data into white and non-white categories for equality of variance considerations in some statistical analyses found in later discussions.

Table 4.5 Maternal Descriptive Data

VARIABLE	N
AGE	67
17-22	15
23-27	17
28-31	19
32-43	16
Missing	7
GRAVIDA	66
G1	27
G2	19
G3	7
G4	6

G5		3
G6		2
G8		1
G10		1
Missing		8
PARITY		66
P0		33
P1		20
P2		7
P3		4
P5		1
P6		1
Missing		8
ABORTIONS		66
AB0		46
AB1		12
AB2		5
AB3		2
AB6		1
Missing		8
PRENATAL		
CARE(MIN	4	N
VISITS)		68
YES		63
Missing		6
MODE	**OF**	
DELIVERY		68
C-SECT		48
VAGINAL		20
Missing		6

PROM	69
(prem.rupture of membranes)	
YES	15
Missing	5
No. babies this Pregnancy	71
Singleton	64
Twin (2)	6
Triplet (3)	1

Payor Source

Mothers' and infants' funding sources were extracted from the records providing information related to the socio-economic status of the sample. Of sixty-nine completed records, 51% (n = 38) of the mothers were insured by commercial carriers. The remaining 42% (n = 31) were Medicaid recipients; thus, the infants coverage were the same. Cross tabulations were run on race and funding source to determine whether differences existed among the groups. Violations related to cell size on the race variable precluded chi-square analyses across the initial various categories resulting in a decision to collapse the variable into two categories; white and non-white. Chi square results indicated a significantly different proportional distribution across race and funding source, (χ^2 = 8.9, df = 1, p = .003). There were twice as many non-white mothers and infants in the Medicaid group (n =20, 66.7%) compared to white mothers and infants receiving Medicaid (n =10, 33.3 %). Infant's transferred from referral centers had no significant differences in funding when compared to inborn infants; (χ^2 = 3.04, df = 1, p = 0.81).

Results of Major Hypotheses

Aim 1: Identify the linkages between shunting (extra pulmonary and/ or intrapulmonary) and oxygenation in infants with Acute Hypoxic Respiratory Failure (AHFR) prior to and during inhaled nitric oxide (iNO) therapy.

Hypothesis 1- There is a negative linear relationship between shunting (extra pulmonary and/ or intra-pulmonary; A:a gradient) and oxygenation (PaO_2) in infants with AHRF prior to the initiation of iNO therapy.

For the first hypothesis within Aim 1, Pearson's r correlation was conducted to determine whether a relationship exists between the A:a gradient and the PaO_2, before and during treatment with inhaled nitric oxide. Results indicated a moderate significant negative correlation ($r = -.33$, $p = .004$) between the A:a gradient and the PaO_2 which supports the hypothesis indicating that high A:a gradient, ($n = 74$, $m = 515.76$, $sd = 138.43$) is related to low PaO_2 ($n = 72$, $m = 64.97$, $sd = 51.58$).

Hypothesis 2- Prior to initiation of iNO therapy, most infants in severe AHRF will exhibit severe respiratory compromise with extra or intrapulmonary shunting despite the ventilation strategy. Therefore, there will be no significant difference in the A:a gradient dependent upon the type of ventilator, i.e., high frequency or conventional ventilator used.

In the second hypothesis, t-test analyses were used to determine whether the degree of shunt, indicated by the A: a gradient differed between two different modes of ventilation. A bivariate analysis was conducted with two groups; infants treated with 1) conventional ventilators versus 2) high frequency ventilators

before treatment with inhaled nitric oxide. Analysis indicated no significant differences between the two groups (t = -.07, p = .944; conventional vent group, n = 32, m = 513.63, sd = 133.04 versus high frequency group, n = 42, m = 515.95, sd = 145.47). Equal variance between the groups was confirmed using the Levene's test for equality of variances (F = .041, p = .840). Therefore, support for the hypothesis is demonstrated since the mode of ventilation made no difference in the degree of the shunt prior to treatment.

Hypothesis 3- During treatment with iNO the difference in the mean A:a gradients will be significantly higher in the responder group (survivors with successful wean from iNO) versus the non-responder group (survivors who fail to wean from primary iNO course and require second iNO course or expired during primary treatment course) over three specified time intervals.

Time 1- mean A: a gradient at 1 hour, 8 hours, and 24 hours after initiation of treatment.

Time 2- mean A: a gradient at days 2, 3, and 4

Time 3- mean A: a gradient at days 5, 6, and 7

The third hypothesis additionally explored the A:a gradient during treatment over time. The analysis required several steps to analyze the data. First, infants were assigned to two groups using criteria for response to treatment. Infant's were assigned to the responder group if they remained on iNO with adequate oxygenation as demonstrated by stable PaO_2 measurements or improvement by the ability to wean ventilator pressures, or FiO_2 support 10% or more over the duration of the treatment course. Non-responders either died

during the treatment or failed to wean on the ventilator, or FiO_2, or required a second course of treatment within 24-72 hours of end of the first course. Secondly, data was collected at three intervals at each time point. Grand means were calculated across the three intervals for each time point for analyses. Independent t-tests compared responder and non-responder groups at each time point (see Table 4.6). There were no significant differences in heterogeneity between groups at any time point (as measured by Levene's test of homogeneity). Results indicated marginally significant differences at Time 1 and significant differences at Times 2 and 3. In all cases, nonresponders had significantly higher A:a gradient scores indicating failure to respond to treatment.

Table 4.6 Independent T-tests / Comparison across groups on A:a gradient at 3 time points.

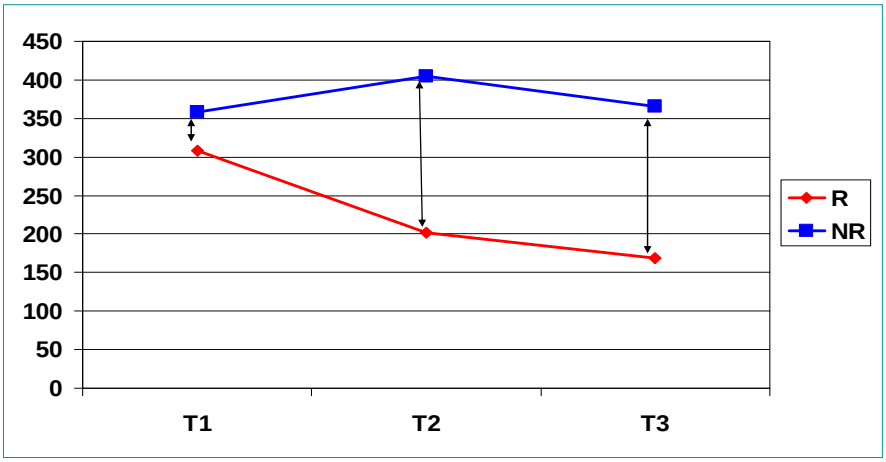
	Time 1	Time 2	Time 3
Responders			
N	48	44	35
Mean A:a grad	311.9	187.6	169.1
Sd	147.8	136.2	138.6
Non Responders			
N	22	17	14
Mean A:a grad	386.5	385.3	366.3
Sd	158.7	167.6	155.7
P <	.06	.000	.000

To control for the impact of test-wise error and evaluate change across time within and between each group, a repeated measures ANOVA was

conducted to determine whether changes in the A:a gradient differed between groups over T1, T2, and T3 (see Figure 4.1).

Figure 4.1: A:a gradient changes over time in responder vs. non-responder

Figure 4.1



There were significant main effects for both time ($F(2) = 4.94$, $p = .009$) and groups ($F(1) = 13.74$, $p = .001$) indicating significant change across time within groups as well as significant differences between groups. However, there was also a significant interaction between time and group ($F(2) = 8.57$, $p < .001$) indicating a clearly different pattern of response for the nonresponder group compared to the responder group (see Figure 4.1). Nonresponder A:a gradients worsened (A:a gradient values increased) from Time 1 to Time 2 and then recovered slightly but failed to return to Time 1 levels. Responders improved dramatically (A:a gradients decreased) from Time 1 to Time 2 and continued to improve to Time 3.

Aim 2: Determine whether gestational age is predictive of responsiveness to iNO therapy.

Hypothesis 1– Infants nearest the age of viability 23 0/7 - 26 6/7[*] weeks gestation will fail to respond to iNO therapy significantly more frequently than infants 27 0/7 - 42 0/7 weeks gestation.

To test the first hypothesis in the second aim, infants were assigned to two groups based on gestational age at birth. Group 1 included infants between 23 and 26 6/7 weeks, where Group 2 included infants between 27 - 40 weeks. Chi-square analyses assessed the distribution of groups (gestational age) across response categories (responders versus nonresponders). A clinically noteworthy trend in the distribution lends some support to the hypothesis however, the small sample size provided insufficient power to demonstrate statistical significance (χ^2 = 2.79, df = 1, p = .095). Interestingly, our results indicated that twice as many infants less than 27 weeks (67%, n=16) failed to respond to iNO treatment compared to infants 27 weeks and greater (33%, n = 8) (total n non-responders = 24).

Hypothesis 2- Most prevalent confounding causative factors of acute hypoxic respiratory failure were controlled for through inclusion and exclusion criteria; however, septicemia is a condition that can occur during treatment and

[*] Fractions represent days beyond the completed week, i.e., 25 2/7 signifies 25 weeks + 2 days

needs to be controlled for in the analysis. Thus after controlling for septicemia, gestational age will remain a valuable predictor of response to iNO treatment.

The second hypothesis was tested using a backward stepwise logistic regression model using the gestational age, gender, A:a before treatment, ventilator mode before treatment, PaO_2 before treatment, early onset sepsis (yes/no), and race (white/nonwhite) on response (responder=1) as variables for the equation. Backward stepwise regression enters all the variables initially and then eliminates variables one at a time from least to most contributing stopping at the point in which subsequent deletions would reduce the fit of the model. None of the predictors significantly contributed to predicting the likelihood of response thus support was not found for the hypothesis. Interestingly, gestational age remained in the model until the next to last step with race being the last variable removed.

CHAPTER 5: CONCLUSIONS, IMPLICATIONS, AND DISCUSSION

This chapter presents a discussion of the findings and conclusions drawn from the data presented in Chapter five relative to the framework of the study and associated literature. Additionally, it provides a discussion of the limitations, implications for nursing, and recommendations for future research.

Discussion of Sample Characteristics

Although a majority of the infants in this sample demonstrated characteristics (weight and gestational age) suggestive of extreme fragility and potentially higher morbidity and mortality rates, it appears that deaths occurred less frequently in the study sample. While it is not possible to make exact comparisons between the current study and the literature on infant survival rates, it is possible to draw some limited conclusions about the survival rates of infants who were similar in weight and gestational age. For example, when compared to infants in a study conducted by Pediatrix, the infants in the current study appeared to have better survival rates than the Pediatrix sample. Further, when the current outcomes are viewed in the context of similar morbidity and mortality findings (Appendix C.4) the infants in this current study appear to have a better survival rates (Appendix C.5; Clark et. al., 2005).

Additionally it is unclear why there were so few black infants in the current study. Other researchers have shown that black females have the highest survival rates and the lowest morbidity and mortality rates of all low birth weight infants (Avery et al., 1999). Perhaps the robustness of the black preterm infant,

especially the female black infant, decreased their need for this treatment modality. Another possibility may be that fewer black infants are admitted to the hospital based on the overall population of the region. It would also appear that resource allocation differed by race, with more black and Hispanic mothers covered by state funded programs than the Caucasian mothers. This phenomenon was not explored as part of the study, but provides some insight about the socio-economic status of these sub-groups.

Aim One and Associated Hypotheses

The first aim of the study was to identify linkages between shunting and oxygenation in the sample before and then during treatment with inhaled nitric oxide. Three hypotheses were associated with this aim.

Aim one; Hypothesis One

The first hypothesis was designed to determine the relationship between the A:a gradient and PaO_2 measures before the initiation of iNO treatment. Theoretically, a negative relationship is expected; that is, a low PaO_2 is expected to correlate with an elevated A:a gradient. The results demonstrated a weak to moderate negative correlation between the A:a gradient and PaO_2 at baseline, and continued to show a negative relationship in the infants who responded during treatment . While it is unclear why a stronger correlation was not found, one explanation might be that intra pulmonary and extra pulmonary factors may have influenced oxygenation in this AHRF sample. In other words, factors affecting oxygenation and diffusion (e.g., lung maturity, hemoglobin affinity,

concentration of inspired oxygen, atelectasis, air leaks, airway pressures, and tidal volumes) may have affected the degree of oxygen transfer from the alveoli to the arterial blood. Likewise, the extra pulmonary factors such as cardiac anomalies (resulting in intra cardiac shunting bypassing lung circulation) may have affected oxygenation and transfer efficiency. Since these factors were not measured in this study, it is possible they may have affected the study outcomes.

The findings of this study are supported by both neonatal and non-neonatal literature (Wally, 1997; Beachey, 1998; Nemerovskii et al.,1998). Researchers have shown that the A:a gradient is a reliable indicator of oxygen transfer efficiency in the neonate with respiratory failure treated with high frequency ventilation in addition to traditional treatment methods (Tamburro et al., 1991; Ko et al., 2000). The findings from this study extend these findings in the literature and suggest that the degree of oxygen transfer for infants on iNO therapy is also negatively correlated with the degree of the shunt. Although previously not described in the literature in infants' receiving iNO therapy, a high A:a gradient indicated fewer oxygen molecules were transferred across the Alveolar-arterial unit into the bloodstream, thus a low PaO_2 measure resulted. As the shunt index decreased, PaO_2 values increased, in spite of ventilator management changes or other medical treatments concurrently provided to the infants. Given the findings in this study, it may be concluded that if the A:a gradient values decrease the PaO_2 levels will rise, if all other factors are equal.

Aim One; Hypothesis Two

The second hypothesis was designed to determine whether the degree of the shunt (A:a gradient) was affected by the mode of ventilation prior to the introduction of the study gas. The findings showed no significant differences in the groups' severity of shunt based on the ventilator type prior to treatment with inhaled nitric oxide, suggesting that neither conventional (time cycled pressure-limited) nor high frequency oscillation methods made a difference in the magnitude of shunt. Early studies were conducted on infants with persistent pulmonary hypertension, (PPHN) when high frequency ventilation techniques were being introduced in neonatology for the treatment of severe respiratory distress and failure. These earlier studies suggested that HFOV might provide favorable responses compared with conventional methods, by maintaining lung volume with decreased tidal volume reducing barotrauma (Hoen et al, 1998; Goldsmith & Karotkin, 2002). Moreover, other researchers have suggested that use of HFOV in combination with iNO or liquid ventilation augmented the response to iNO therapy (Buden et.al, 1997; Kinsella et al., 1997). However, the findings of this study showed no difference in terms of the severity of the shunt regardless of the ventilation method. It is unclear why the findings of the current study differed from the earlier research. Perhaps one explanation may be that the higher number of very immature and very low birth weigh infant's in the current study had some affect on the response to trials of high frequency ventilation. This age and weight factor is thought to be a plausible explanation since the infants in many of the earlier studies were older and weighed more than the infants in this

current study. Thus, given these findings it may be concluded that there is no difference in the severity of shunting (A;a gradient means) regardless of the ventilation method.

Aim One; Hypothesis Three

The third hypothesis explored the A:a gradient during treatment over the duration of iNO treatment. While the A:a gradient of responders decreased in a negative linear fashion, surprisingly, the gradient pattern in the group of non responders actually worsened at Time 2, then improved only slightly at Time 3. Both oxygen demand and vasodilator theory provide the conceptual explanation for these findings. Since inhaled nitric oxide is a potent vasodilator that specifically targets prolonged dilatation of the pulmonary circulatory bed, a failed response would indicate continued vasoconstriction of the vascular structures, decreased perfusion, or severe atelectasis. This process then results in the absence of efficient diffusion across the alveolar surface into the arterial blood, thus resulting in persistently low PaO_2 levels. PaO_2 levels are one of the determining factors indicating the ability to wean ventilator support parameters relative to oxygenation, and indeed, non- responders were unable to tolerate weaning.

In addition, the findings are supported by theory selected for the study framework. Under normal circumstances, oxygen demand theory specifies that in a state of low oxygen, capillary sphincters dilate and vaso motor processes result in relaxation of the pulmonary vasculature. The persistently high shunt (A:a gradient) seen in the non responder group would indicate a failure to respond to

the therapy, while responders demonstrated a persistent and steady decline in the shunt. This is clinically relevant, and indicates further management strategies aimed at reducing the shunt. Although it is difficult to ascertain without further information as to the type and degree of shunt, these measures seem to give the clinician a good indication that the infant is or is not responding to treatment in real time. Results suggest that if no improvement in the shunt measurement is seen by 24-72 hours (Time 2) the clinician should regard these findings with a high level of suspicion for a failed response and further explore alternate treatment options or adjustments in the current therapy provided.

It can be concluded that in infants who respond to iNO treatment, the shunt decreases overtime with non-responders becoming worse initially and improving only slightly within 24-72, with no further improvement over the 7-day treatment protocol.

Aim Two and Associated Hypotheses

The second aim of the study was to determine whether developmental lung maturity is predictive of responsiveness to iNO therapy. Two hypotheses were associated with this aim.

Aim Two; Hypothesis One

The first hypothesis of the second aim of the study explored infants' developmental lung maturity based on gestational age with their response to iNO therapy. The assumption that extremely immature infants would more likely fail to respond to the first course of treatment was supported in the overall trend,

although the chi- square analysis failed to reach statistical significance. This is most likely explained due to insufficient power relative to the small sample size. What is more clinically relevant here is that in the younger group (less than 27 weeks), 16 (42%) out of 38 infants did not respond, while the remaining 22 responded to the treatment. On the other hand, only 8 (23.5%) out 34 infants failed to respond in the older group (27 weeks and older). Proportionately more of the smaller infants failed to respond to the first treatment.

This portion of the study was important to determine if the study gas is of benefit for application to very young infants. Controversy continues in terms of criteria for utilization of iNO. INO treatment is incredibly expensive and requires specialized training and equipment for appropriate safe delivery. Extremely immature infants make up a sub-group of infants who typically utilize proportionately higher resources, and have the highest risk of morbidity and mortality of than any other group of newborns. Very often, they will reach the preset lifetime insurance limits during the initial hospital course alone. In order to defend the utility of this treatment, it is important to explore whether there is a true benefit in this group. In conclusion, the hypothesis that extremely immature infants would more likely fail to respond to the first course of treatment was supported in the overall trend, although the chi- square analysis failed to reach statistical significance.

Aim Two; Hypothesis Two

The second hypothesis of the second aim explored whether gestational age was a predictive factor of response to treatment when sepsis was controlled.

The assumption was that infants with early onset sepsis would be more likely to fail to respond to therapy. Findings unexpectedly did not support this hypothesis. Indeed none of the clinical factors entered into the equation provided insight as to predicting response to therapy. Still, no criteria exists whether to include or exclude infants for use of the gas. While this question remains unanswered, we found that within this study, five of the ten infants born at 23 weeks survived and only two of the ten 23 weeker's enrolled died during the first treatment, with the remaining three expiring from complications unrelated to the initial episode of AHRF later in the hospital stay. Additionally of the eighteen infants enrolled in the study at 24 weeks gestation, only one died during the treatment period with 4 expiring during hospitalization after they were no longer on the study protocol. The results suggest a much better mortality and morbidity rate than expected. In 2000, Hoehn et al. reported a meta-analysis of three randomized controlled trials involving 210 infants below 33 weeks gestation who received iNO treatment for AHRF. While the role of iNO is firmly established in term infants with persistent pulmonary hypertension, the use in premature newborns remains controversial. Hoehn et. al, concluded that while iNO may temporarily improve oxygenation, it did not improve survival in preterm infants with AHRF. The study further reported odds ratios, which were not in favor of iNO with respect to mortality or chronic lung disease. Although the hypothesis is not supported, the data supports the opinion of the investigator and the neonatology group, that without iNO, it is highly likely that these very fragile infants with AHRF would more than likely expired during the critical stages, since ECMO is not an alternative therapy for

this group. In conclusion, statistical support for the hypothesis was not found using the variables thought to be predictive of response. Gestational age is not predictive of response to treatment, which suggests age is not a valid criterion to exclude very small infants from the treatment. Indeed, the gas may have further beneficial results not yet identified, such as reducing the risk of chronic lung disease. Further study is indicated to explore if factors can be identified for criteria development.

Limitations

First, because this study involved, in part, a chart review, the demographic information was limited relative to the study design. Chart review of data over several years resulted in missing demographic and study variable data. Secondly, not all relative variables affecting oxygenation and oxygen transfer efficiency could be realistically explored. Although results indicated a severe shunt-process was present, no conclusion could be drawn about whether ventilation changes or other management decisions may have affected the shunt indicator and or the PaO_2 values.

The study was also limited by not exploring whether physician/NNP directed variables, influenced the response to therapy. Determining whether management strategy differences existed would have been a difficult challenge, and was believed to be controlled for prior to the study commencement. It was assumed that within the group practice, management would not vary significantly since protocols were agreed upon and astringently adhered to in the parent study. We hold this to be true overall, yet, as in all practice groups, there were

subtle differences noted in terms of comfort levels and experience with different medications, i.e., steroids, diuretics, epinephrine drips and ventilator management strategies that might have affected infant's responses. Exploration of these factors would provide insight to develop "best practice methods" to be used in future protocols. Understanding the relationship between the PaO_2 and the A:a gradient may provide further insights into management strategies directed at shunt closure. Other physiologic factors such as cardiac output, stroke volumes, pulmonary artery pressures, systemic pressures were also not explored and were a limitation of the study. In adult studies, the use of the Swan-Ganz's catheter provided vast information about the circulation and intra cardiac pressure gradients along with other vital circulatory shunt variables. This technology is not available in neonates to date; however, vital circulatory information may be available using the echocardiogram and is a potential area for inclusion in the design of future studies.

The second hypothesis determined there were no differences in ventilator type and magnitude of shunt prior to the initiation of iNO treatment. We still do not know if any differences were present during the treatment course, which may also be a limitation. Clinically, one might expect more vulnerable, less mature infants to be affected differently, yet the data supported the hypothesis, providing confidence that all infants were equal in terms of severity before the start of treatment. We remain unclear whether if any difference existed between vent groups over the course of the treatment. The parent study data did not provide controls resulting in mixed groups over the course of treatment. While some

infants remained on the same ventilator over the course of treatment, many infants mode of ventilation changed during the treatment period. No conditions were established in the parent study to maintain a selected ventilator type throughout the gas infusion protocol. Therefore, during the treatment period, when infants' respiratory status improved or deteriorated, ventilators were changed in response to infant's respiratory needs for support.

Another limitation was that, because the study was retrospective in nature, we were able to use only a small amount of gross data as it related to blood gas sampling. The blood gas data available represented only one of many samples during a 24 hours period, limiting the statistical analysis. To truly see the trend in the relationship between the A:a gradient and the PaO_2 and its trend over time, all A:a gradients, and FiO_2 measures over the treatment course would have provided much more information than one sample per day.

Another limitation of this study was the sample size and relative power especially as it relates to Aim 2 Hypothesis 1. A power analysis was not conducted since this study was part of a larger clinical trial and the sample size was set by the original trial. The study also did not explore outcomes of infants who repeated the treatment protocol.

For the first hypothesis, one must also recognize limitations regarding using gestational age as definite criteria of developmental milestone in terms of pulmonary functionality. The researcher was unable to use a more definitive measure of lung maturity such as phospholipid levels or amniotic fluid surface because they were not recorded in the parent study. While clinicians cannot

ascertain that at 27, 34, or 40 weeks a newborn would not be expected to require intubation, supplemental oxygen, continuous positive airway pressure (CPAP), nor that the lungs are capable of a specific level of function, it can be argued that gestational age is simply an *estimate* of maturation. Evaluation of fetal lung maturity may be determined by direct amniotic fluid phospholipids levels of indirectly by evaluating amniotic fluid surface tension properties. The lethicin to sphingomyelin of more than 2:1 is associated with low risk of neonatal respiratory distress syndrome in non- diabetic mothers. Unfortunately, this data was not collected and available in all patients. While there is an array of other fetal testing available such as phosphatidylglcerol, disaturated phosphatidylchonin lung profile, and others, but again were not available in the study design. Indeed, no exact tests for functional lung maturity were available (Spitzer, 2005). In the practice of neonatology, GA is one of the most important and useful indicators as predictors of infants physical functionality. Gestational age is commonly accepted for use as a guideline for reference for expected developmental capabilities the neonate, relative to developmental maturity. Gestational age is utilized as soft criteria for many developmental milestones and limitations in daily management. An example of this would be, whether an infant is expected to be able to feed orally or not. We would not expect a 24-week infant to be developmentally mature enough to perform this function whereas we would a 34-week infant. These developmental milestones have been studied and well documented over the years. However, in relation to functional lung maturity, there are little

developmental criteria available; therefore, we cannot make any concise claims with confidence related to pulmonary functional maturity.

Nursing Implications

There are many implications for nursing derived from this study. For one, demographic sample analysis provides and understanding of the characteristics and nature of the patients analyzed from descriptive data. This is useful information in terms of holistic nursing practice because knowledge of the problems related to the physical, psychological, spiritual, and social issues surrounding our patients is important to providing family centered nursing care. For example, we know that we had a great number of Hispanic infants. We must be mindful of the fact that many of our parents may not speak English as a primary language and will require educated interpreters to communicate with this population regarding the medical terminology. Demographic data related not only to infants within the study sample, but all infants within the scope of nursing practice can provide understanding of the cultural, physical, economic and health care needs of our patients. Evidence based research provides information aimed at the specific problems and issues of patients within our practice.

An additional implication for nursing is that data analysis through chart review presents many challenges and limitations, but also provides insights for improvement in collection techniques. Research provides an opportunity to educate the staff regarding the importance of charting accurately and timely. Documentation is often regarded by the bedside nurse as a task performed to

preserve information and data about the individual patient, less often is it realized as a source research analysis.

In terms of the research variables, neonatal nursing education should include in-services and educational programs to enhance knowledge specific to understanding shunt index results on the arterial blood gas data, and what these measurements signify clinically. The A:a gradient and the PaO_2 were selected as variables of study to begin laying a foundation for nursing knowledge development and theory construction. Nurses within the study center are beginning to explore the shunt indicator, noting whether the A:a gradient is associated with improvement, or deterioration of the infant. Using the indicator to wean or change strategies cannot be implied, only its use as an indication to response in a general sense.

Infants with acute hypoxic respiratory failure make up a sub-set of the most acutely ill neonates admitted to the NICU. Astute observation, care practices, and monitoring are necessary, with a total understanding of ventilation modes, and delivery of support. Nursing and respiratory staff must be equipped to provide different modes of ventilatory support during treatment with iNO gas. As critical care providers, ongoing nursing assessments are essential in determining how infants respond to respiratory support and ventilator management. The blood gas analysis is subject to error, and infants with AHRF do not tolerate weaning well, particularly infants with PPHN. Tolerance to weaning is labile during the critical period, and nursing intuition and knowledge play an important role in decision making when physicians and practitioners

make adjustments in ventilator settings in this population. Trust and communication are very important among the team members. “Knowing” an infant only comes from constant care and observation of how infants respond to stimulus and therapies ordered for them. Nurses must be able to communicate whether they believe an infant is capable of weaning, or need additional support based on the clinical observations.

Although not statistically significance trends were established, this showed that smaller, younger infants were twice as likely to fail to respond to the first treatment. With this understanding, nurses caring for these infants will have some insight in terms of the different patterns of response relative to the very immature infant; and will be able to answer parents’ questions as they relate to the very immature infants. While we are unable to predict which infants will and will not respond to therapy, we have shown some trends in terms of age, race and birthweights. Since iNO criteria have not been established, nurses can expect to continue to provide iNO therapy to very young infants at the cusp of viability until sufficient evidence is available to form protocols based on the evidence.

Further Research Implications:

Further research implications include examining whether race, especially in black female infants with AHRF, makes a difference in there need for iNO treatment. Are survival rates better for infants with AHRF since the inception of iNO?

Additionally, follow up, to determine the long-term effects of post-treatment with iNO, is also recommended. Maternal data indicating statistical differences in funding sources and race is worthy of exploration as well. It would be interesting to study whether the level of education, socio-economic status or income levels influence the risk of diagnosis with AHRF.

While the neonatal intensive care nurse is prepared to collect and analyze the arterial blood gas, nursing education has been deficient relative to shunt indices. Though a relationship was demonstrated in the study findings, the A:a gradient can give us an indication as to how the infant if responding. Unfortunately, this index alone does not define the type of shunt. Further research to distinguish which factors influence the type and degree of shunt is warranted in this area. More importantly, if we are able to use these indicators along with other diagnostic information, to determine the type of shunt, we can then design specific management strategies aimed to effectively reduce shunt and improve oxygenation.

Additionally, other shunt indicators, such as the oxygenation index, a:A (arterial to Alveolar) ratio, and the oxygenation factor should be explored in relation to the A:a gradient to determine whether these are sensitive or reliable indicators of oxygenation transfer efficiency and shunt. The a:A ratio is expressed as a percentage of oxygen transferred to the arterial blood, and may give more clues into the pulmonary function and relative shunt. In theory, if lung function remains unchanged, this index is more stable regardless of the amount of FiO_2 available, and is therefore more stable than the A:a gradient when an

intrapulmonary shunt is present. The oxygenation factor is a newer formula introduced which takes into account the usual PaO_2/FiO_2 but also takes into consideration some important mechanical ventilatory support variables such as peep, inspiratory time, and tidal volume. This index has been studied in adults with open-heart surgery to assess the intrapulmonary shunt, but not in neonates yet.

Further study could also include actual ventilation modes during treatment with iNO to compare infants' response to treatment with different ventilator types. The literature suggests high frequency oscillation in conjunction with iNO improves the response to treatment in cases of intra-pulmonary shunting. While this dissertation study findings did not demonstrate differences prior to treatment, testing was not done to determine whether differences in response to treatment occurred dependent of ventilation mode. Additionally, it remains unclear whether differences occurred among or between subjects with the combination of surfactant, steroids, or ventilator type. This is another focus area for future research that could provide important information.

Further methods should also be explored to determine which factors which would most likely influence improvement in the shunt index as well as the type of shunt. The use of the echocardiogram may provide important relevant data to determine shunt etiology. Shunt indexes could be evaluated for correlations associated with common intra-cardiac shunts such as the patent ductus arteriosus, foramen ovale or across septal defects in non-AHRF patients. Further relationships should be explored in between shunt indicators, and intra- cardiac

pressures. These may have been examined in prior studies, but no research on infants treated with iNO was found making this a fertile area for further research.

While the small sample size provided insufficient power to demonstrate statistical significance (χ^2 = 2.79, df = 1, p = .095), regarding gestational age and prediction of response, this finding also raises further questions. This study should be repeated to determine whether the trends that were found still hold. Further study of infants who received a second trial will yield additional important information. For example, if non-responders received a second course, and then responded to the 2nd course, might imply that the treatment duration should be extended beyond the 7-day period to those infants. Further examination into secondary treatments could include exploring patterns in terms of length of treatment, and at what point infants begin to respond. For example on day 8 or 13 might be when these infants improved, this is unclear.

Additionally, further information related to factors such as steroid use, sepsis, hypotensive crisis, and adrenal insufficiency might provide clues as to factors relative to the infants' response to treatment. One must consider whether responses to therapy were affected by the differences in treatment protocols applied to this sub-group. For example, one might argue that the smaller group received the minimal stimulation protocol during the first 5 days of life, where infants 27 weeks and older did not. It is important to clarify that while all infants meeting criteria for the minimum stimulation protocol (MSP) were treated as such; all infants in this sub-group did not experience acute hypoxic respiratory distress, nor received iNO treatment. Although the minimal stimulation protocol,

(MSP) was used over the study period, it is not believed to have any effects on response to therapy, particularly since all infants with AHRF also required similar modified minimal stimulation techniques during the acute states. The minimal stimulation protocol is applied to infants born between 23 0/7 and 26 6/7 weeks gestation. The protocol includes a five-day regimen beginning immediately at birth. This regimen includes administration of medications used for neuromuscular blockade, sedation, and prevention of intraventricular hemorrhages. Additionally all efforts are made to reduce any noxious physical and environmental stimulation. The MSP is applied over the first five days of life to reduce environmental stressors that might induced blood pressure fluctuations and undesired stress responses in these infants. These interventions are applied to all infants in this sub-group due to the high risk of IVH, which occurs most frequently in the first 72 hours of life. The MSP has shown great benefit in reduction of severe IVH within the practice, which in turn produces less risk of less than optimal long-term developmental outcomes.

Another factor to be considered in terms of response to treatment is steroid utilization. Whether steroids played a part in the response of the groups is undeterminable in the present study. The extreme immature infant is more likely to exhibit signs of adrenal insufficiency and more often succumb to refractory hypotension requiring stress doses or replacement therapy of glucocorticosteroids. Very low dose hydrocortisone (0.5mg/kg/dose) was administered for refractory hypotension in later years in many of the infants under going iNO therapy, in addition to catecholamine drips when necessary during the

treatment protocol in some of the infants. No data was available for analysis for any of the infants, but this raises suspicion as to the confounding effects on the response to iNO therapy, and may have affect on the response. While health care costs increase, with further restrictions of experimental therapies, prudent use of the iNO therapy begs for evidence-based criteria. Additional investigation is also warranted to explore factors among subjects that might provide insight into responders to treatment.

Summary

The results of the study add to the body of nursing knowledge and provide a starting point for further investigation. Although very young, very small infants are more vulnerable and are at much higher risks of mortality and morbidity, these infants have benefited from iNO treatment. It cannot be concluded that when iNO was added to traditional rescue methods survival rates of these infants were improved or enhanced, however, when compared to national statistics, the overall survival rate of the study sample appears to be better than that of the general population.

Additionally, the A:a gradient is a useful indicator of the infants in acute hypoxic respiratory failure's oxygen transfer efficiency. Furthermore, it gives a good indication of how infants are responding to treatments relative to the shunt. Although multiple factors influence infants' response to treatment with iNO, the study demonstrated that even without controlling for these co-variables, we can confidently utilize the A:a gradient as an additional tool to assess the oxygenation status of the infant. The A:a gradient indicated the high degree of severity before

treatment was not affected by the type of ventilator used for treatment. Gestational age was not demonstrated statistically as a predictor of response, however, younger infants responded less often to the first course of treatment, as was hypothesized.

Unexpected Outcomes

An unexpected, yet important outcome of this study is that research, which had not been formally conducted within the private practice group within this hospital, has been embraced. During the research process, nursing and medical staff have had the opportunity to directly participate and be a part of the research process. A newfound interest, support, and respect for the importance of research are now evident in the staff members and administration. Since the initial parent study, and follow up with this study began, the primary investigator of this study has been approached by other researchers to participate and assist in future studies. Importantly, the path has been cleared for the development of further research within the community hospital with support from the Medical Director, administration and Institutional Review Board. This newly developed milieu will hopefully support continued opportunities for future research questions to be developed and a foundation for an evidence based and outcomes research model within the group practice.

Thanks to the vision and commitment of the medical director and the support of administration and staff who participated, an extensive database has been established and other studies are under consideration. This study provided

a launching pad for many other opportunities for nursing research, building on the foundation now being established.

APPENDIX A-

IRB Permission #07-143

Parent Study Protocol (Abstract)

And Parent Study

Forms

A.1: IRB Permission

UTMB
The University of Texas Medical Branch

OFFICE OF RESEARCH SUBJECT PROTECTIONS
Institutional Review Board

July 23, 2007

MEMORANDUM

TO: Alda Hill, PhD/Leah Best, RNC, MSN, NNP
School of Nursing / 1132

FROM: Wayne R. Patterson, PhD
Senior Assistant Vice President for Research
Office of Research Subject Protections
Institutional Review Board 0158

SUBJECT: IRB #07-143 - Final Approval of Expedited Protocol
Exploration of the Linkage Between the Aa- Gradient & Oxygen Transfer Efficiency as a Measure of Response to Treatment with Inhaled Nitric Oxide in a Heterogenous Group of Newborns Treated for Acute Hypoxic Respiratory Failure

Having met the requirements set forth by the Institutional Review Board by an expedited review process on April 18, 2007, your research protocol is now approved. I am therefore, pleased to inform you that you may proceed with this project immediately.

This project will require annual review by the IRB and will be due by **March 31, 2008.**

Comments: The UTMB IRB has approved your request to collect and use protected health information (PHI), for research purposes, i.e. (medical record review). In addition, the UTMB IRB waives the requirement to obtain subject authorization for use and disclosure of PHI and alters or waives the requirement to obtain prior consent of the individuals affected. The review was completed **in accordance with expedited review procedures as described in 45 CFR 46.110(b) on (04/18/07).** The IRB further determined that: 1) The use or disclosure of PHI involves no more than minimal risk to the subjects; 2) the alteration or waiver will not adversely affect the rights and welfare of the subjects; 3) the research could not practicably be conducted without the alteration or waiver; 4) the research could not practicably be conducted without access to the or the use of the PHI; 5) the privacy risks to individuals whose protected health information is to be used or disclosed are reasonable in relation to anticipated benefits, if any, to individuals and the importance of the knowledge that may reasonably be expected to result from the research; 6) there is an adequate plan to protect the identifiers from improper use and disclosure; 7) there is an adequate plan to destroy the identifiers at the earliest opportunity consistent with the conduct of the research, unless there is a health or research justification for retaining the identifiers or such retention is otherwise required by law; and 8) there are adequate written assurances that the PHI will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research project, or for other research for which the use or disclosure would be permitted by IRB policy.

WRP/cv

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A.2: Parent Protocol Abstract

1.0 Abstract

Protocol Number:	AIA-NO-1A
Protocol Title:	Inhaled nitric oxide as an anti-inflammatory agent in the prevention of chronic lung disease in infants with respiratory failure
Study Drug Name:	ViaNOx-H (Nitric Oxide)
Route of Administration:	Inhaled
Study Phase:	Phase 2
Study Design	In a prospective controlled trial, with a historical control, we will compare 20 ppm of iNO for 24 hours and 5 ppm iNO for up to 7 days in the prevention of acute and chronic lung disease.
Duration:	12-30 months
Study Population:	Neonates presenting with respiratory failure that require assisted mechanical ventilation.
Data Collected:	Primary Measures include: Dose of iNO Methemoglobin levels Serum Interlukin-8 (IL-8) levels FiO_2
Safety Criteria:	NO, NO_2, FiO_2 Levels Incidence and types of reported adverse events

A.3: Parent Consent (Form 15)

15.0 Consent Form

1. **TITLE:** A comparison of inhaled nitric oxide to historic controls in the prevention of chronic lung disease in infants in respiratory failure.

2. **PURPOSE OF THE STUDY:** You are being asked to allow your baby to participate in a research study to find out if a new treatment is best for your baby's illness. Your baby has a problem called respiratory failure. Respiratory failure causes the lungs to respond to the respiratory problems by releasing or trapping chemicals that can inflame the tissues. The result of this is that your baby may end up with chronic changes to their lungs that may persist for months or years and result in the need for them to receive oxygen for longer periods. In this consent form, we are asking your permission to treat your baby with a medicine which may reduce the inflammation of the lungs.

   The new medicine being studied is a gas called nitric oxide which would be blended with the oxygen gas your baby is already receiving. Nitric oxide is a gas which is normally produced in the airways and tissue of the blood and lungs and may decrease the inflammation. The use of nitric oxide gas is meant to provide that substance directly to the airways and blood vessels of the lung to decrease the inflammation.

   Your baby is eligible for this study because he/she has respiratory failure. Nitric oxide is an experimental treatment and can only be use as specified in this study. In this study we want to determine if nitric oxide is safe and effective.

3. **DESCRIPTION OF THE STUDY:** If you agree to allow your baby to participate, we will treat your baby with nitric oxide gas. For treatment with the nitric oxide gas, we will use a low concentrations of the nitric oxide (up to 0.002%) for up to 7 days or until your baby no longer requires a mechanical ventilator to assist their breathing.

4. **RISKS AND DISCOMFORTS:** At high concentrations, nitric oxide gas can injure the lungs, but we believe that these problems are highly unlikely to occur at the low concentration of nitric oxide gas that will be used for your baby. Studies in term newborns show that nitric oxide can be used safely at the concentration that we will use to treat your baby. These concentrations are approved by the Food and Drug Administration for treating newborns with diseases that affect the blood vessels in term newborns. Nitric oxide can also cause the production of methemoglobin, an abnormal form of hemoglobin in the blood. Elevated levels of methemoglobin can worsen the blood oxygen level. The methemoglobin level will be closely monitored during nitric oxide treatment and the treatment will be discontinued if the level increases above normal. We have not had to stop nitric oxide treatment due to problems with methemoglobin. No discomfort should be caused by breathing this gas. However, we can make no guarantees to you regarding the effectiveness or safety of either therapy with regard to your child's disease.

   In addition, a small amount of blood will be drawn (1/5 of a teaspoon) two to three times during treatment (in addition to usual blood tests and those to measure methemoglobin levels) to measure substances in the blood which could be related to your baby's problem. The extra blood will be drawn at the same time as routine blood tests. The total amount of extra blood for this study will amount to 1 teaspoon.

5. **BENEFITS:** I understand that my child may receive no direct benefit from participation in this project. The major benefit of the program is to assist in establishing the most effective method of treating infants with lung disease, thereby improving the level of care provided to patients in the future. I understand that my child can only be offered nitric oxide under the guidelines of this study protocol.

6. **ALTERNATIVES TO THE STUDY:** I may choose not to let my child be in this study. This will not cause problems with his/her medical care. If my child is not in this study, he or she will receive the usual medical care for a baby with respiratory failure. This care includes, but is not limited to conventional or high frequency ventilation as appropriate.

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7. **QUESTIONS ABOUT THE STUDY:** I understand that I may make inquiries concerning this study by contacting H. S. Bedi, M. D. at phone 281-338-3381. If you have any questions about my child's rights as a research subject, please contact the hospital institutional review board (IRB) at phone 281-338-3110.

8. **MEDICAL TERMINOLOGY:** All the medical words in this document that I don't understand have been explained to me.

9. **CONFIDENTIALITY:** The people doing this study will keep the facts about my child private. If the results of this study are published, my child's confidentiality will be maintained, and his/her name will be withheld from publication. I understand that there is a possibility that the Food and Drug Administration, as well as other governmental agencies, may inspect my child's records to evaluate the safety and efficacy of nitric oxide. I give my permission for release of my child's records for this purpose.

10. **VOLUNTARY PARTICIPATION AND OPTION TO WITHDRAW:** My child is part of this study because I want him/her to be. I understand that I am free to withdraw my consent and to discontinue participation in the project at any time. I also understand that my decision to participate or not participate will not affect my child's treatment. No guarantees have been made to me regarding the safety and efficacy of either therapy used in this study.

11. **FUNDING AND COMPENSATION:** Although the investigator will make available or arrange for appropriate management and treatment for any physical injury resulting from this project, I understand that Clear Lake Regional Medical Center, H. S. Bedi, M. D., or their associates, employees &/or agents have made no provision for payment of cost associated with any injury resulting from my child's participation in this study. The drug and all the associated lab tests will be provided to your child free of any charge to you.

12. **AUTHORIZATION**: I have read and understand this paper about the study. I know what will happen, both the possible good and bad (benefits and risks). I hereby consent to have my child volunteer to participate in this study.

Signature: _____ Date: _____
Parent or Guardian

Consent form explained by: _____ Date: _____

Witnessed by: _____ Date: _____

Patient's Name: _____

Parent's Name: _____

Address: _____

City, State, Zip: _____

Phone: _____

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A.4: Dispensing Log (Form 16)

DISPENSING LOG FOR INO

This record must be filled out for each cylinder used in the INO protocol.
The pressure gauge reading must be taken by the RCP at the beginning of each shift.

Site Name: Clear Lake Regional Medical Center
Site Location: _____
_____
Cylinder Serial #: _____

Patient MR #	Random #	Date of Reading	Time of Reading	Pressure Gauge Reading	Signature

Note: Cylinders MUST be changed when the pressure is below 200 psi.

If this cylinder is used for more than 1 patient, a photocopy must be made for each patient's folder

A.5: Case Report Form (Form 17)

Infant Respiratory Failure iNO Trial
Case Report Form

Field	Circle or write in	Definition
Center Name	Clear Lake Regional Medical Center	PI Institution
Maternal Data		
Antenatal Steroids	Complete, Incomplete None Unknown	Incomplete = if delivery occurred < 24 hours after first dose of steroids or > one week after the last dose. Complete = if delivery occurred > 24 hours and less than one week after a dose of steroids.
Infant Data		
Patient Last Name		
Medical Record ID Number		
Gender	Female, Male, Unknown	
Race	White, Black, Asian, Native American, Hispanic, Other, Unknown	Biological mother's Race
Birth Date		Date of Birth (day/month/year)
Best Estimated Gestational Age	22-40 wks, Unknown	Estimate from Revised Dubowitz / Ballard - 1991
Location of Birth	Inborn, Outborn, Unknown	Any infant requiring ambulance transfer is outborn.
Birth weight		Record in grams
Intubation Date		Date patient was first intubated. (day/month/year)
Treated with Surfactant.	Exosurf, Infasurf, Survanta, Curosurf, KL4, None	
If yes, total number of doses.	1, 2, 3, 4 Unknown	
Congenital Anomaly	None, Cyanotic Congenital Heart Disease, Major Heart Defects, Brain Anomaly, Major Renal, Major Genetic, Major GI, Major Metabolic, Multiple, Unknown	Heart defects do not include PDA, small VSD or small ASD.
Early Sepsis/Meningitis	NO, YES, Unknown	Less than 72 hours of age with a positive blood or CSF culture.
Study Gas Treatment		
Treatment Course 1	NO, YES, Unknown	
If yes,	Start Date _____, Start Time_____	
	Stop Date _____, Stop Time_____	
Methemoglobin	Before Treatment_____ 1 hour after Treatment_____ 8 hours after Treatment_____ 24 hours after Treatment_____ Maximum Level_____	
Treatment required for Methemoglobin >5%?	NO, YES, Unknown	
If yes, what?	Wean Treatment, Stopped Treatment, Vitamin C, Methylene Blue	
Did the patient require more than 1 course of treatment?	NO, YES, Unknown	
Treatment Course 2	Start Date _____, Start Time_____	
	Stop Date _____, Stop Time_____	
Methemoglobin	Before Treatment_____ 1 hour after Treatment_____	

A.5: Case Report Form (Form 17) page 2

	8 hours after Treatment_____ 24 hours after Treatment_____ Maximum Level_____	
Treatment required for Methemoglobin >5%?	NO, YES, Unknown	
If yes, what?	Wean Treatment, Stopped Treatment, Vitamin C, Methylene Blue	
Pulmonary Intestinal Emphysema	NO, YES, Unknown	Radiographic evidence of perivascular and peribronchial dissection of air.
Pneumomediastinum	NO, YES, Unknown	Radiographic evidence of free air in mediastinum.
Pneumothorax	NO, YES, Unknown	Radiographic evidence of free air in thorax.
Pulmonary Hemorrhage	NO, YES, Unknown	Yes, if acutely 5 ml/kg or more of bright red blood is retrieved from the ET tube with suctioning and there are X-ray showing radiopacification.
Steroids for Hypotension	NO, YES, Unknown	Steroids used to treat adrenal insufficiency and associated hypotension.
Steroids for Lung Disease	NO, YES, Unknown	Postnatal systemic steroids used to treat lung disease, anytime, dose or duration. This should include intratracheal steroids.
Total days treated with steroids		1 to 42
Inflammatory		
Serum sample day 1	Yes No	Label & send to Texas
Serum sample day 3	Yes No At extubation	Label & send to Texas
Date of extubation		(day/month/year)
Support		
FiO2 (prior to start of gas 1)		Record last Fi02 value before starting treatment gas. (.21 - 1.0)
P_aO_2 (before start of gas)		Record P_aO_2 at start of therapy. (0 – 600)
FiO2 (day 7)	RA, > 0.21-0.3, .31-.6, >.6, Unknown	Highest level used for more than 12 hours during the 24-hour period.
FiO2 (day 30)	RA, > 0.21-0.3, .31-.6, >.6, Unknown	Highest level used for more than 12 hours during the 24-hour period.
Support (before start of gas)	IMV HFOV (Sensor Medics) SIMV	Record type of ventilators support being used at start of treatment gas.
Support (day 7)	None, Nasal Cannula Low Flow, Nasal Cannula High Flow, CPAP, Ventilator, Unknown	Highest level used for more than 12 hours during the 24-hour period. Low = < 0.5 liter / minute High = ≥ 0.5 liter / minute CPAP = independent of the route Vent = IMV, high frequency
Patient Exit	Completed Study; Exclusion noted after treatment; Parent withdrew consent; Protocol Violation	Discharged home or foster care (out of the hospital). Died; because this is a study end point, the patient is classified as completed study.
Exit Date		Date (day/month/year)
30 Day Outcome		
Status at 30 days or 36 weeks PCA	Alive off oxygen Alive on oxygen Dead	Alive off oxygen - Home off O_2 prior to 36 weeks PMA, Alive on O_2 - Home on O_2 prior to 36 weeks PMA, Dead - Dead Prior to 36 weeks PMA
If Died, Cause of Death	Pulmonary Hemorrhage, Lung Hypoplasia, Pneumonia, Sepsis, Genetic Syndrome, Malformation, NEC, Cardiac Disorder, IVH, Renal Failure, Liver Failure, Hydrops, Viral Illness, Hypoxic Ischemic Encephalopathy, Other Respiratory, Deceleration of Care, Unknown	Primary event leading to cardio-pulmonary failure. Pick one. If the patient died, record the date and time of death in the study exit date and time field.

A.5: Case Report Form (Form 17) page 3

If Alive on O_2, reason for O_2 use	Lung disease Apnea without lung disease Recent surgery Growth ROP prevention Other, Unknown	Oxygen need for more than Room Air (sea level) Support to maintain a Sat > 90%. Sea Level Room Air means P_IO_2<160, $P_IO_2=FiO_2*(P_B)$. Recent surgery < 1 week
Date of first successful extubation		Off Ventilator > 48 hours (day/month/year)
Respiratory Support at 30 days or 36 weeks PCA	None, Nasal Cannula Low Flow, Nasal Cannula High Flow, CPAP, Ventilator, Unknown	Highest level used for more than 12 hours during the 24-hour period. Low = < 0.5 liter / minute High = ≥ 0.5 liter / minute CPAP = independent of the route Vent = IMV, high frequency

Data Entry Person _____ **Date** _____

PI _____ **Date** _____

A.6: Adverse Report Form (Form 18)

Version #2

ADVERSE EVENT FORM

Inhaled Nitric Oxide Infant Trial

Hospital: Clear Lake Regional Medical Center **Patient MR#:** ____________

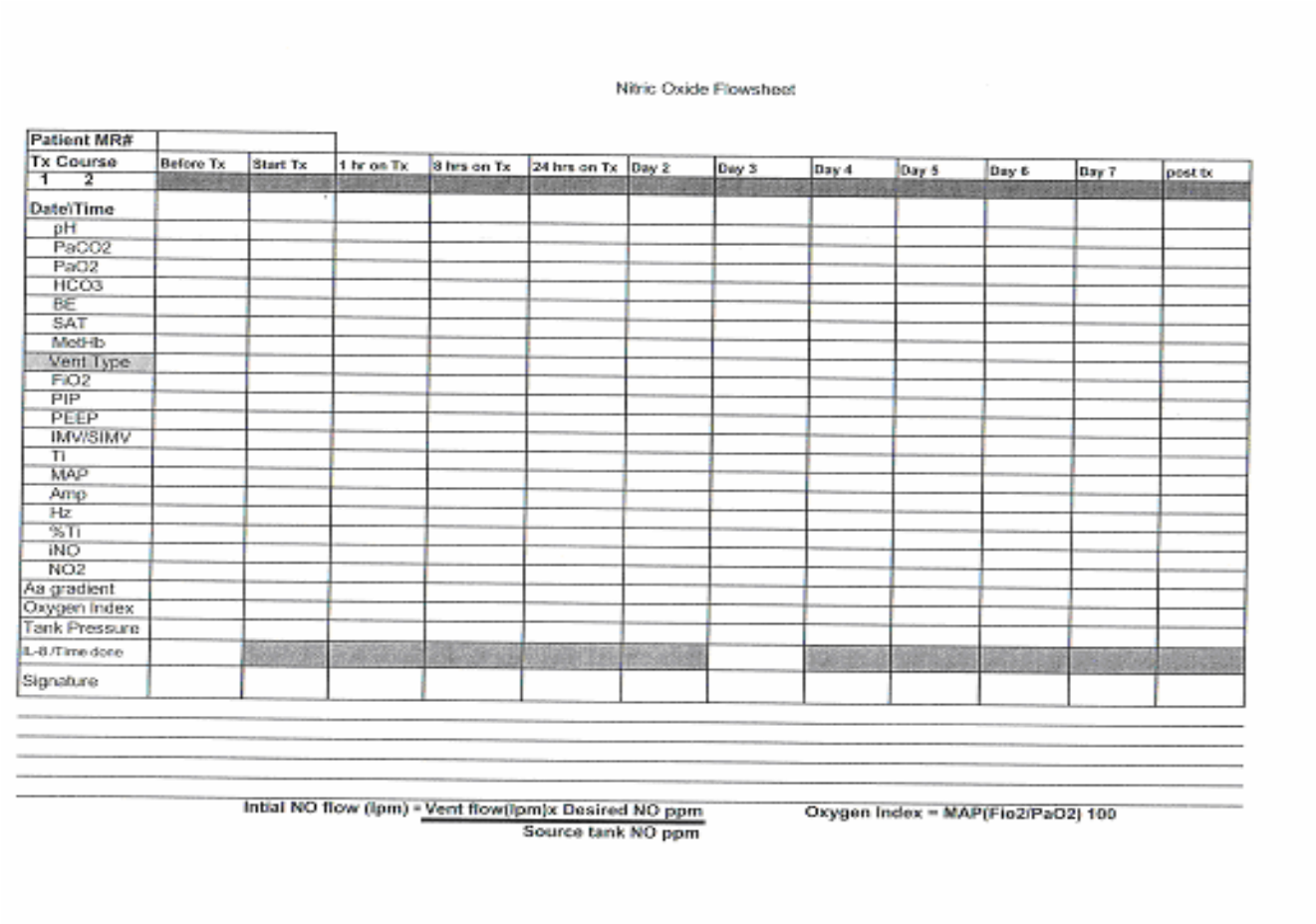
1. ADVERSE EVENT FORM

Adverse Event	Date of Onset (mm/dd/yy)	Time of Onset 24-Hour Clock (hours – minutes)	Duration Date and Time	Severity	Study Drug Relationship	Action	Outcome	SAE
			Indicate: M = Minutes H = Hours D = Days C = Continuing (Please fill in the blank and circle)	1 = Mild 2 = Moderate 3 = Severe (Check One)	1 = Not Related 2 = Possible 3 = Related (Check One)	1 = RxTherapy 2 = Prolongs Hosp. Stay 3 = Interrupt Study Drug 4 = D/C Study Drug 5 = None (Check ALL that apply from #1 to #4 or check #5 for None)	1 = Recovered 2 = Improved 3 = Unchanged 4 = Worse 5 = Died (Check One)	YES or NO
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	
	__/__/__	__:__	____ M H D C	1 2 3 ☐ ☐ ☐	1 2 3 ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	1 2 3 4 5 ☐ ☐ ☐ ☐ ☐	

Signature ______________________ Date ____________

Copy to Study Coordinator at Hospital **Fax to H. S. Bedi, M. D.** at 281-338-3380

A.7: iNO Flow Sheet (Form 22)



Nitric Oxide Flowsheet

Patient MR#												
Tx Course 1 2	Before Tx	Start Tx	1 hr on Tx	8 hrs on Tx	24 hrs on Tx	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	post tx
Date\Time												
pH												
PaCO2												
PaO2												
HCO3												
BE												
SAT												
MetHb												
Vent Type												
FIO2												
PIP												
PEEP												
IMV/SIMV												
Ti												
MAP												
Amp												
Hz												
%Ti												
iNO												
NO2												
Aa gradient												
Oxygen Index												
Tank Pressure												
IL-8 /Time done												
Signature												

Intial NO flow (lpm) = $\frac{\text{Vent flow(lpm)x Desired NO ppm}}{\text{Source tank NO ppm}}$

Oxygen Index = MAP(Fio2/PaO2) 100

APPENDIX B-

Blood Gas Analyzer and

Ventilator Specifications

B.1: Bayer Rapid Point Blood Gas Analyzer

Point of Care with the New Rapidpoint® 405 Critical Care Analyzer

Only Point of Care Blood Gas System to Offer Fully Integrated CO-oximetry Module

March 12, 2003

TARRYTOWN, NY - March 12,2003: The Diagnostics Division of Bayer HealthCare LLC, a member of the Bayer Group (NYSE: BAY) announced today the worldwide launch of the new Rapidpoint® 405 critical care analyzer. The Rapidpoint 405 is the first blood gas system specifically designed for point of care testing to offer a fully integrated CO-oximetry module. Physicians will now have real-time access to the oxygenation status of their critically ill patients enabling more precise therapeutic management decisions and better patient care.

"In the critical care setting, fast access to crucial results is essential when physicians rely on up-to-the minute information to determine a patient's status," said John Sperzel, vice president marketing, Near-Patient Testing, Bayer Healthcare Diagnostics Division. "By bringing CO-oximetry results closer to the patient, the Rapidpoint 405 provides physicians with immediate access to laboratory quality results so they can make critical and quality management decisions."

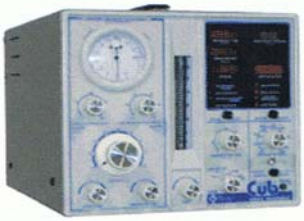
The fully integrated CO-oximetry module offers five new hemoglobin tests -- tHb, O2Hb, COHb, MetHb and HHb -- to assist in the assessment of medical emergencies including hypoxia, burns, sepsis, poisoning, acute respiratory distress syndrome, multiple organ failure, septic shock, blood loss, toxicology screening, and post-op recovery from surgery.

The Rapidpoint 405 analyzer offers customers all the benefits that they have come to expect from the Rapidpoint 400 including maintenance free operation, intuitive sample management system, and on-board quality management. Capable of reporting results within 60 seconds, the Rapidpoint 400 series systems offer the fastest response of any point of care blood gas system and the most complete point-of-care menu available -- pH, blood gas, electrolytes, glucose and hematocrit, and now CO-oximetry -- all with the capability to be monitored remotely by the central laboratory.

B.2 Bear Cub Ventilator

Bear Medical Ventilators

Bear 750vs Pediatric Critical Care Ventilator
• Neonatal to pediatric ventilator
• Integrated synchronized ventilation and tidal volu
monitoring
• Internal back-up battery
• Exclusive volume limit feature
• Optional graphics display (not shown)



Bear Cub Infant Critical Care Ventilator
• Infant ventilator
• Time cycled, pressure limited operation with CPA
and IMV modes
• Calibrated selection of Ventilator Rate from 1 - 15
and Inspiratory Time from 0.1 - 3.0 seconds
• Digital Display
• Audible and visual alarms
• Optional accessories: Humidifier, pedestal stand,
circuit, etc.

Bear 33 Critical Care Ventilator
• Compact, portable device
• Provides volume ventilation for adults and some p
patients
• Designed for long-term use in home, nursing hom
hospital setting
• Three modes of ventilation: Control, assist Control
SIMV

B.3 Seimens Servo 300 Ventilator

SIEMENS® SERVO 300 VENTILATOR

- Adult/Infant Ventilator
- Supported and controlled ventilation modes
- Customized respiratory patterns
- Context sensitive controls

Typical Manufacturer's Picture

Specifications

Front Panel Controls	Patient type (adult, pediatric, neonate); mode of ventilation; pressure or flow triggering; pressure limit; baseline pressure; minute volume; ventilator frequency; start breath; pause hold; inspiratory time (%); inspiratory pause time (%); rise time of inspiratory pressure; FIO2; deliver present group of 100% oxygen breaths.
Power Requirements	Electrical power: 115 VAC, 60 Hz; pneumatic power: air and oxygen at 29 to 100 psig.
Alarm Controls	Airway pressure; pressure > upper limit or set PEEP level plus 15 cmH2O for more than 15 seconds; O2 concentration: FIO2 above or below 6% of set alarm value or if the oxygen fuel cell is not connected; expired minute volume alarm; minute volume > set value or <0.3 L/min for adults and children or 0.06 L/min for neonates.
Non-Adjustable Alarms	Battery; main electric power has failed and the ventilator has switched to battery backup; gas supply; gas pressure < 29 psig or > 94 psig; apnea; time between breaths > 10 seconds for adults, 15 seconds for children, or 20 seconds for neonates.
Monitored Variables	Pressure; peak, mean, pause, baseline, and an analog bar graph of instantaneous airway pressure; respiratory rate; tidal volume; inspired and expired; and minute volume.
Waveforms	Pressure; rectangular, adjustable slope; Flow; rectangular, adjustable slope.
Operating Range of Controls	Trigger sensitivity level; pressure 0 to 20 cmH2O below PEEP, flow - 0.6 to 2.0 L/min adult, 0.3-1.0 L/min pediatric, 0.15 to 0.5 L/min neonate. CMV frequency: 0.5 to 40 breaths/min. Pressure control levels below PEEP: 0 to 100 mmH2O. Pressure support level above PEEP: 0 to 100 H2O. Pause time: 0% to 30%. Minute volume: 0.2 to 60 L/min. Inspiratory time: 10% to 80% of the ventilatory period. Baseline pressure: 0 to 50 cmH2O.
Triggering	Pressure, flow, time, manual
Graphic Display	Red digital display
Power	AC 110V/60Hz
Pressure Controlled Modes	Pressure control; pressure support; SIMV, pressure regulated; volume control; volume support, and CPAP
Flow Controlled Modes	Volume control; SIMV, pressure support.
Dimensions	Patient unit: 242 mm W x 370 mm D x 240 mm H. Control unit: 431 mm W x 150 mm D x 325 mm H.
Weight	~26kg

SOMA TECHNOLOGY, INC.

1486 Highland Ave Unit #3 Cheshire, CT 06410 U.S.A.
Tel: (203) 272-2300 Fax: (203) 272-2250 Email: soma@somatechnology.com

Rev 1 1/03

B.4 Puritan Bennett 840 Ventilator

PURITAN BENNETT® 840 VENTILATOR

- Dual view, color, touch screen display
- Offers pressure- and volume-based delivery
- Precise breath delivery for infant, pediatric and adult patients
- SmartAlert® alarm system

Typical Manufacturer's Picture

Specifications

Modes	Assist/Control, synchronous intermittent mandatory ventilation, or spontaneous
Respiratory Rates	1.0 to 100/min
Inspiratory Pressure	5 to 90 cm H2O
Inspiratory Time	0.2 to 8.0 seconds
I:E Ratio	≤ 1:299-4.00:1
Expiratory Time	Te ≥ 0.2 second
PEEP	0 to 45 cm H2O
Apnea Insp. Pressure	5 to 90 cm H2O
Apnea Insp. Time	0.2 to 8.0 seconds
Apnea Interval	10 to 60 seconds
Apnea Resp. Rate	2.0 to 40/min
Apnea I:E Ratio	≤ 1.00:1
Apnea Exp. Time	≥ 0.2 second
Alarm Limits	
High Circuit Pressure	7 to 100 cm H2O
High Exhaled Min. Vol	0.1 to 99.9L or OFF
High Exhaled Tidal Vol	50 to 3000mL or OFF
High Respiratory Rate	10 to 110/min or OFF
Low Exhaled Mandatory Tidal Vol	5 to 2500 mL or OFF
Low Exhaled Min. Vol	0.01 to 60.0L
Low Exhaled Spontaneous Tidal Vol	5 to 2500 mL or OFF
Monitored Data	Breath type, delivered O2, end expiratory pressure, end inspiratory pressure, exhaled minute volume, exhaled tidal volume, I:E ratio, maximum circuit pressure, mean circuit

SOMA TECHNOLOGY, INC.
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Rev 1 1/05

B.4 Puritan Bennett 840 Ventilator (page 2)

pressure, spontaneous minute volume, total respiratory rate, rapid shallow breathing index, spontaneous respiratory time.

Weight	
Breath Delivery Unit (BDU)	40.1 lb (18.2 kg)
Graphic User Interface (GUI)	12.6 lb (5.7kg)
Back up Power Source (BPS)	14.6 lb (6.6 kg)
Cart	34.2 lb (15.5 kg)
Compressor	55 lb (25 kg)
Dimensions (H x W x D)	
BDU	13" x 18" x 10" (330 mm x 457 mm x 254 mm)
GUI	18.1" x 15.5" x 6.7" (460 mm x 394 mm x 170 mm)
BPS	3.25" x 9.6" x 10" (83 mm x 244 mm x 254 mm)
Cart	39.3" x 22.9" x 23.7" (998 mm x 582 mm x 602 mm)
Compressor	16.4" x 18" x 14.25" (417 mm x 458 mm x 362 mm)

SOMA TECHNOLOGY, INC.

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Rev 1 1/05

B.5 SensorMedics 3100 A High Frequency Ventilator

CardinalHealth

About us Careers Contact us
Products & Services Events Investors Custor

Products & Services

Language Selector
3100A HFOV Neonate/Pediatric
Deutsch
Español
Français

Locate Products

Product Locator
You are just 3 clicks away to find product information. GO

Featured Products
Select a Product

Locate Products by Brand Name
Select a Brand

Literature & Educational Materials

3100A Brochure.pdf
3100A Spec Sheet.pdf

Contact Information

Product Info Request

Critical Care Directory

Sales & Tech Support:
Toll-Free: 800-231-2466
Phone: 714-283-2228
Fax: 714-283-8439

Related Links

Events
National Perinatal Association: 2008 Annual Conference - November 19-21, 2008

Reference Information
Reference Material

HFOV FAQ's

Rental Information
HFOV Rentals

Training
Education Program: 3100A Neonatal

Archive Webcast: HFOV in Pediatrics: What You Need to Know- Featuring Ira Cheifetz, MD, FAARC - 11:00am Pacific August 9, 2006

Organizations
National Perinatal Association

Ventilator Warranty Request

3100A HFOV

Achieving a delicate balance in mechanical ventilation.

Providing the ultimate in lung protection.

The SensorMedics 3100A High Frequency Oscillatory Ventilator was first approved for use in 1991 and is the only HFV approved for early intervention in the treatment of neonatal respiratory failure. The scope of application was broadened in 1995 to include selected pediatric patients failing conventional mechanical ventilation. The 3100A provides the ultimate in lung protection by inflating the lung with a continuous distending pressure and superimposing very small pressure and volume swings. Numerous publications, including clinical, animal and bench studies have reported improved benefit and outcomes associated with the use of HFOV. There are over 3500 SensorMedics High Frequency Oscillatory Ventilators in use worldwide today. The 3100A is the standard of care in more than 90% of Level III nurseries and 75% of the Pediatric Intensive Care Units in the US.

The Alliance of Children's Hospitals, Inc. has awarded their prestigious "Seal of Acceptance" to VIASYS for the 3100A HFOV. The Alliance is an affiliate of Child Health Corporation of America (CHCA) and is comprised of over half of the children's hospitals in the US, representing nearly one quarter of the country's leading pediatricians. The purpose of the seal is to establish Standards of Excellence for pediatric products and create a new standard of expectations for pediatric product users.

Return

APPENDIX C

ADDITIONAL TABLES FOR REFERENCE

C.1 Infant Descriptive Data

Gestational Age

(Completed Weeks at Delivery)

Value	N	%CASES	CUM %	TOTAL N	MISSING	MISS%	Range	
				72	2	2.4	17	
23	10	13.9	13.9					
24	18	25	38.9	Mean	Median	Mode	St.Dev.	Var.
25	6	8.3	47.2	28.5	26.0	24.0	5.40	29.21
26	4	5.6	52.8					
27	3	4.2	56.9					
28	5	6.9	63.9					
29	2	2.8	66.7					
30	1	1.4	68.1					
31	1	1.4	69.4					
32	2	2.8	72.2					
33	2	2.8	75					
34	3	4.2	79.2					
35	2	2.8	81.9					
36	3	4.2	86.1					
37	4	5.6	91.7					
38	3	4.2	95.8					
39	2	2.8	98.6					
40	1	1.4	100					

C.1 Infant Descriptive Data (Birth weights)

Birth weights

Value	N	%CASES	CUM %	TOTAL N	MISSING	MISS%	Range	
480	1	1.4	1.4	74	0	0	4946	
520	1	1.4	2.7					
540	1	1.4	4.1					
550	1	1.4	5.4					
567	1	1.4	6.8					
570	3	4.1	10.8	Mean	Median	Mode	St.Dev.	Var.
590	1	1.4	12.2	1472	865	660	1.116	1.25
610	1	1.4	13.5					
620	1	1.4	14.9					
630	1	1.4	16.2		25%ile	50%ile	75%ile	100%ile
640	1	1.4	17.6		>/= 660	>/= 865	>/= 2270	>/= 5426
650	2	2.7	20.3					
660	4	5.4	25.7					
670	1	1.4	27					
680	1	1.4	28.4					
684	1	1.4	29.7					
690	1	1.4	31.1					
700	1	1.4	32.4					
710	3	4.1	36.5					
721	1	1.4	37.8					
740	2	2.7	40.5					
760	1	1.4	41.9					
770	1	1.4	43.2					
780	1	1.4	44.6					
830	1	1.4	45.9					
850	1	1.4	47.3					
860	2	2.7	50					
870	1	1.4	51.4					
900	1	1.4	52.7					
930	1	1.4	54.1					
944	2	2.7	56.8					
950	1	1.4	58.1					
1050	1	1.4	59.5					
1134	1	1.4	60.8					
1150	1	1.4	62.2					
1155	1	1.4	63.5					
1280	1	1.4	64.9					
1365	1	1.4	66.2					
1417	1	1.4	67.6					
1733	1	1.4	68.9					
1932	1	1.4	70.3					
1950	1	1.4	71.6					
2022	1	1.4	73					
2080	1	1.4	74.3					
2220	1	1.4	75.7					

C.1 Infant Descriptive Data (Birthweights continued)

Value	N	%CASES	CUM %
2420	1	1.4	77
2465	1	1.4	78.4
2550	1	1.4	79.7
2842	1	1.4	81.1
2948	1	1.4	82.4
2950	1	1.4	83.8
3010	1	1.4	85.1
3040	1	1.4	86.5
3094	1	1.4	87.8
3175	1	1.4	89.2
3215	1	1.4	90.5
3320	1	1.4	91.9
3380	1	1.4	93.2
3410	1	1.4	94.6
3565	1	1.4	95.9
3605	1	1.4	97.3
3670	1	1.4	98.6
5426	1	1.4	100

C.2 MAJOR INFANT DIAGNOSES/CO-MORBIDITIES

RESPIRATORY SYSTEM:

VARIABLE	N (freq)	%CASES	CUM %	TOTAL N	MISSING	MISS%
				72	2	2.7
RDS						
Yes	63	87.5	87.5			
No	9	12.5	100			
PPHN				71	3	4.1
Yes	26	36.6	36.6			
No	45	63.4	100			
CONG PNEUMONIA				71	3	4.1
Yes	10	14.1	14.1			
No	61	85.9	100			
MAS				71	3	4.1
Yes	1	1.4	1.4			
No	70	98.6	100			
Diaphragmatice Diaphragmic Hernia				50	24	32.4
Yes	0	0	0			
No	50	100	100			
Pulmonary Hypoplasia				50	24	32.4
Yes	0	0	0			
No	50	100	100			
Tracheal Stenosis				50	24	32.4
Yes	0	0	0			
No	50	100	100			
Apnea				65	9	12..2
Yes	38	58.5	58.5			
No	27	41.5	100			

C.2 MAJOR INFANT DIAGNOSES/ CO-MORBIDITIES (cont)

IMMUNOLOGIC SYSTEM:

VARIABLE	N (freq)	%CASES	CUM %	TOTAL N	MISSING	MISS%
SEPSIS						
Early Onset				72	2	2.7
Yes	19	26.4	26.4			
No	53	73.6	100			
Late Onset				68	6	8.1
Yes	30	44.1	44.1			
No	38	55.9	100			

CENTRAL NERVOUS SYTEM:

Intraventricular Hemorrhages	N (freq)	%CASES	CUM %	TOTAL N	MISSING	MISS%
				71	3	4.1
Yes	26	36.6				
No	45	63.4	63.4			
Classification:						
Grade I	9	12.7	76.1			
Grade II	7	9.9	85.9			
Grade III	5	7	93			
Grade IV	5	7	100			
Meningitis				69	5	6.8
Yes	3	4.3	4.3			
No	66	95.7	100			
	N (freq)	%CASES	CUM %	TOTAL N	MISSING	MISS%
Retinopathy of Prematurity				68	6	8.1
Yes	25	36.8	36.8			
No	43	63.2	100			

C.2 MAJOR INFANT DIAGNOSES/ CO-MORBIDITIES (cont)

GASTROINTESTINAL SYSTEM:

	N (freq)	%CASES	CUM %	TOTAL N	MISSING	MISS%
*NEC /SIP				71	3	4.1
Yes	4	5.7	5.7			
No	66	93.4	100			

*Necrotizing enterocolitis (NEC) / Spontaneous Intestinal Perforations (SIP)

CARDIOVASCULAR SYSTEM:

	N (freq)	%CASES	CUM %	TOTAL N	MISSING	MISS%
Patent Ductus Arteriosus				66	8	10.8
Yes	22	29.7	33.3			
No	44	59.5	66.7			

C.3 Procedures & Treatments

PDA surgery				66	8	10.8
Yes	22	33.3	33.3			
No	44	66.7	100			
Umbilical Arterial line placement				73	1	1.4
Yes	65	91.5	91.5			
No	6	8.5	100			
Umbilical Venous Line Placement				72	2	2.8
Yes	70	97.2	97.2			
No	2	2.8	100			
Apnea Treated (Theophylline or Caffeine)				70	4	5.4
Yes	44	62.9	62.9			
No	26	37.1	100			
Central or Percutaneous. Line placement				67	7	9.5
Yes	12	17.9	17.9			
No	55	85.1	100			
Surgery for ROP				68	8	10.8
Yes	20	30.3	30.3			
No	46	69.7	100			
Vent, INO, O_2 Therapy				74	0	0
Yes	74	100	100			
No	0	0	0			

C.4 Sample Mortality Rates

Mortality

Expired during Treatment 1				74	0	0
Yes	6	8.2	8.2			
No	68	91.9	100			
GA died during TX 1				6	0	0
23	2	2.8	2.8			
24	1	1.4	4.2			
26	1	1.4	5.6			
29	1	1.4	7			
30	1	1.4	8.4			
Cause of Death						
AHRF	3	4.1	4.1			
Con. Anom	0	0	0			
NEC	1	1.4	5.4			
Late onset Sepsis	0	0	0			
Early Onset Sepsis	2	2.7	8.2			
Expired during Hospitalization				73	1	1.4
Yes	15	20.5	20.5			
No	58	79.5	100			
Cause of Death						
AHRF	4	5.5	5.5			
Combined/other	3	4.1	9.6			
NEC	1	1.4	11			
Late onset Sepsis	5	6.8	17.8			
Early Onset Sepsis	2	2.7	20.5			
Gest & Birthweight Deaths During Hosp Stay				15	0	0
Case No.	GA at birth	BWT				
750569	30	1280				
705616	23	610				
817460	26	944				
754883	29	1730				
822902	24	684				
883317	23	579				
843425	27	540				
711756	24	760				
759056	23	520				
736357	24	480				
753712	33	3565				
705553	23	630				
851024	24	660				
707559	23	620				
764233	25	650				

C.5 Pediatrix Outcomes Data (2003-2004): Survival by gestational age and birth weight/Survival without severe IVH or ROP

	Estimated Gestational Age							
Birth weight(gm)	23	24	25	26	27	28	29	30
250-500 (	23/9%	28/10%	53/20%	59/41%				
501-750	33/12 %	57/29%	73/46%	82/58%	80/68%	86/75%	84/84/%	
751-1000		64/32%	80/52%	86/68%	93/85%	92/88%	95/89%	99/96%
1001-1250				91/77%	94/85%	97/93%	97/93%	99/96%
1251-1500						97/96%	98/95%	98/96%
1501-1750						-/95%	95/97%	98/97%
1501-1750		%	%	%	%	%	97%	98%

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VITA

Leah Michaelle Best is the daughter of deceased parents, Robert J. and Fredina L. Lusby of Galveston Texas. Leah was born in Galveston, and has lived in Galveston County for most of her life. After graduating from O'Connell High School in Galveston, Mrs. Best enrolled in and graduated from Galveston College with an associate degree in nursing. Following her marriage to Andrew L. Best III and the birth of three children, Andrew, Lindsay and Shawn, she entered the work school program at the University of Texas Medical Branch where she earned her Bachelor's and Master's degree in Nursing, graduating with high honors. She continued her pursuit of higher education by enrolling and completing the Ph.D. nursing program with the Graduate School of Biomedical Sciences in 2003. As a Neonatal Nurse practitioner, she is board certified by the National Certification Corporation for the Obstetric, Gynecologic, and Neonatal Nursing Specialties in both Level 3 Neonatal Intensive Care and Neonatal Nurse Practitioner certifications.

Mrs. Best has been employed in numerous clinical and administrative roles involving neonatal nursing. Her career started at the University of Texas Medical Branch at Galveston where she was employed for more than fifteen years in various nursing and administrative roles. She founded a nursing staffing agency in Destin Florida, which is still thriving today. In 1999, Mrs. Best took a full time position with Neonatal Consultants, L.L.P. As the first practitioner to join the neonatology group, Mrs. Best was instrumental in developing the neonatal nurse practitioner practice model where she continues to practice full time today.

She provides clinical expertise, leadership, education, research, and administrative assistance to the group. Mrs. Best is dedicated to caring for neonates and continued to practice in the level three NICU full time while completing her doctoral studies.

Mrs. Best has been bestowed with many honors over her 25 year nursing career including: Good Samaritan Foundation Scholarship recipient, Who's Who in American University College Students, Who's Who in Nursing Entrepreneurs, and International Professionals and recipient of Salute to Nursing Scholarships. She is currently a member of the Texas NNP organization, and Sigma Theta Tau International Honor Society, as well as former member of Phi Theta Kappa honor society. She is an active participant in her community. She actively ministers to others as the lead singer, guitarist, and leader of a local non-denominational praise and worship band, and is a youth group leader and sponsor. Mrs. Best has also opened her home as a host family to international high school exchange students from Germany, Mexico, and Central America. She participates in many non-profit charity organizations and performs at events such as the March of Dimes Walk America program.

Mrs. Best is dedicated to continuing her commitment to quality care of the sick neonate utilizing and conducting research while continuing to practice full time in the clinical setting. She is currently working on a research study with Dr. Helen Mintz-Hittner involving intravitreal injection of Bevacizumab (Avastin) for treatment of severe retinopathy, and will work on writing grants, collecting,

managing, analyzing and reporting data, and was invited to co-author a paper for publication in the journal “Opinions in Pediatrics” for the spring edition of 2009.