

THE ROLE OF PLATELETS IN THE SUCCESSFUL CLOSURE
OF THE DUCTUS ARTERIOSUS IN PREMATURE INFANTS
AFTER INDOMETHACIN TREATMENT

by

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Abstract

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Premature birth (birth before 37 weeks gestation) remains a global problem with negative consequences profoundly affecting the infants, their families, and society. Persistent patent ductus arteriosus (PDA) is a significant complication seen in preterm infants. Premature infants with persistent PDAs are at increased risk for morbidity and mortality. Indomethacin is reported to successfully close the PDA in only 20 to 40 percent of infants whose birth weights are less than 1,000g. Common side effects of Indomethacin include renal dysfunction, gastric perforations, and decreased cerebral blood flow and perfusion. Several studies have shown thrombocytopenia is associated with persistent PDA in premature infants who have received Indomethacin. The purpose of this cohort study was to compare circulating platelet counts in premature infants with persistent PDA versus those with DA closure after Indomethacin treatment.

This retrospective cohort study compared the platelet counts of two groups of premature infants after Indomethacin treatment for a hemodynamically-significant PDA (hsPDA). The data for this study were obtained from electronic medical records of infants in a large, Level III neonatal intensive care unit. The sample consisted of 63 premature infants who were diagnosed with a patent ductus arteriosus and received Indomethacin.

Nineteen percent of the infants treated with indomethacin had successfully closed ductus arteriosus. No associations were found between platelet counts and successful closure of the DA after receiving Indomethacin. There were also no significant differences between the two (closed-PDA and open-PDA) groups with regards to gender, race, birth weight, gestation at birth, size of PDA, or respiratory support. Further research is needed to examine possible predictors of successful PDA closure and Indomethacin.

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Abbreviations

AA	Arachadonic acid
BPD	Bronchopulmonary dysplasia
DA	Ductus arteriosus
DOL	Day of life
FDA	Food and Drug Administration
hsPDA	hemodynamically-significant PDA
IVH	Intraventricular hemorrhage
NEC	Necrotizing enterocolitis
NO	Nitric oxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
PDA	Patent ductus arteriosus
PFA	Platelet function analyzer
PG2	Prostaglandin G2
PGE2	Prostaglandin E
PGH2	Prostaglandin H2
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
TXA2	Thromboxane A2

Chapter 1

Introduction

Premature birth (birth before 37 weeks gestation) remains a global problem with negative consequences profoundly affecting the infants, their families, and society. Persistent patent ductus arteriosus (PDA) is a significant complication seen in preterm infants. All of the current treatment options present risks as well as limited success. Some believe platelet adhesion and aggregation enhance the success of ductus arteriosus (DA) closure with pharmacological therapy.

Background information on the problem of prematurity and therapies used to treat patent DA will be addressed. An understanding of the anatomy and physiology of the term and premature infant's DA and the potential role of platelets will provide scientific background and framework for this study. The chapter will end with the study purpose, hypothesis, and assumptions.

Background and Significance

The preterm birth rate in the United States in 2011 was 11.72% with a low birth weight rate of 8.10% (Hamilton, Hoyert, Martin, Strobino, & Guyer, 2013). While the survival rate of preterm infants in the U.S is comparable with other industrialized countries, prematurity continues to contribute to the United States' infant mortality rate of 6.5 infant deaths per 1000 live births. In 2007 the Committee on Understanding Premature Birth and Assuring Healthy Outcomes estimated the costs associated with preterm birth (medical/educational expenditures in addition to lost productivity) were more than \$26.2 billion in the United States.

Persistent PDA is a significant complication seen in preterm infants with the incidence inversely related to gestation. It is estimated that 55 to 70 percent of infants born less than 30 weeks gestation or weighing less than 1000 grams have significant

PDA (Chiruvolu & Jaleel, 2009a; Hermes-DeSantis & Clyman, 2006). Persistent PDA results from either initial failure (no response) to medical treatment, or reopening of a previously closed PDA. Complications of persistent PDA include exacerbated respiratory distress syndrome (RDS), pulmonary hemorrhage, prolonged assisted ventilation, broncho-pulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), renal dysfunction, periventricular leukomalacia (PVL), cerebral palsy, and death. Noori et al. (2009) found an eightfold increase in mortality in infants with a persistent PDA (failed to close either spontaneously or with medication). Unfortunately, the most premature and smallest infants have the greatest risk for failed treatment and adverse outcomes.

No therapy currently exists that is free from potential adverse side-effects. Current treatment options include conservative medical management, pharmacologic therapy, or surgical ligation. Conservative medical management involves fluid restriction, watchful waiting, and ventilator support. Medical or pharmacological treatment is initiated when the PDA becomes clinically significant/symptomatic. Indomethacin or ibuprofen are two cyclo-oxygenase (COX) inhibitors currently approved for use in neonates by the United States Food and Drug Administration (FDA). Surgical closure essentially guarantees ductal closure, but is generally reserved for those infants who do not respond to medical therapy or for whom such medications is contraindicated.

In 2010 Echtler et al. reported findings that improved scientists' understanding of the biological process required to produce permanent closure of the DA in premature infants. In studies of mice they found permanent closure required platelet aggregation and the formation of occlusive thrombi in the DA lumen. The authors went on to provide evidence that a low platelet count in preterm human infants was associated with an increased risk of a persistent patent ductus arteriosus. This supports a study by Boo,

Mohd-Amin, Bilkis, and Young-Junina (2006) which found low a platelet count was predictive of indomethacin failure in the treatment of PDAs.

Further research is required to validate these findings. If platelet aggregation and thrombus formation are able to completely occlude the DA lumen and promote permanent closure, new therapies should be directed in this area.

Framework

An understanding of the differences of the anatomy and physiology between the premature and newborn ductus arteriosus will provide the scientific background for this research. Histologic differences of the DA and other arteries will be presented.

Because the placenta provides gas exchange for the fetus, fetal and newborn circulations differ. Oxygenated blood from the placenta enters the fetus through the umbilical vein where it travels to the right atrium. From there, the majority of blood flows through the foramen ovale to the left atrium where it is pumped into the left ventricle. Thereafter blood is sent to the systemic circulation via the aorta. The ductus arteriosus, a small vessel that connects the pulmonary artery and aorta, shunts deoxygenated blood away from the fetal lungs, into the aorta where it is dispersed to the body tissue. A small amount of blood flows from the right atrium to the left ventricle and into the pulmonary arteries to provide the nutrition required for adequate growth of the lungs. See Figure 1-1.

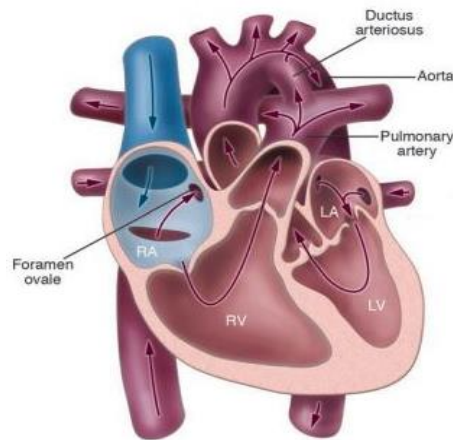


Figure 1-1 Fetal Heart

Note: DA patent between pulmonary artery & aorta
 From: "The paradoxical patent ductus arteriosus" by K. Ivey & D. Srivastava. 2006.
 Journal of Clinical Investigation, 116(11), 2864.

Birth signals the onset of newborn circulation. Clamping the umbilical cord increases the systemic vascular resistance, while the first few breaths clear the lungs of fluid and decreases the pulmonary vascular resistance. This combination increases pulmonary blood flow, which decreases the amount of blood shunted through the foramen ovale and ductus arteriosus. Shortly after birth, pressure on the left side of the heart is greater than on the right, forcing the foramen ovale to close. Normal permanent closure of the ductus arteriosus occurs over several weeks. Figure 1-2 depicts the normal newborn heart.

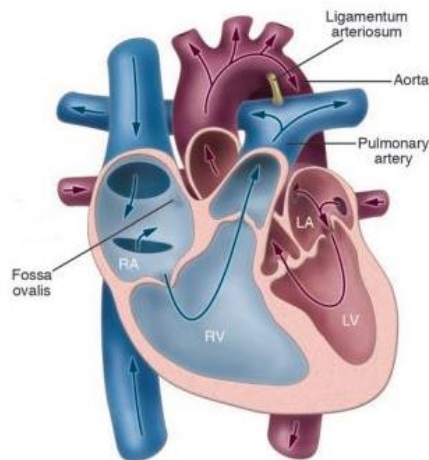


Figure 1-2 Newborn Heart

Note: No DA between pulmonary artery and aorta.

From: "The paradoxical patent ductus arteriosus" by K. Ivey & D. Srivastava. 2006. Journal of Clinical Investigation, 116(11), 2864.

Histology of Arteries and Ductus Arteriosus

The histological structure of the DA is vastly different from the aorta and pulmonary arteries. The DA consists of a thicker middle tissue layer comprised mainly of smooth muscle cells with only a limited amount of elastin. These smooth muscle cell layers of the DA are arranged spirally in opposing directions, forming the thick muscular wall. As a result the DA is muscular in nature, while the aorta and pulmonary arteries are extremely elastic.

The inner-most layer of the artery is the intima. During the second trimester a distinctive remodeling process unique to the DA occurs. Endothelial cells lining the interior of the DA lift to form mounds, known as intimal cushions (Gittenberger-de Groot, Strengers, Mentinik, Poermann, & Patterson, 1985). The space created by the intimal mounds will be filled with migrating smooth muscle cells and other material at the end of gestation. These intimal mounds play a significant role in DA closure (see below in

anatomic closure of the ductus arteriosus). Table 1-1 describes the four stages of DA histological maturity developed by Gittenberger-de Groot, van Ertbruggeten, Moulaert, & Harinck, (1980).

Table 1-1 Histological States of Ductus Arteriosus

Stage	Appearance	Gestational Age (Approximate)
Stage I	Appears as thin muscular artery with 3 thin layers	16-20 weeks gestation
Stage II	Local formations of intimal thickenings or cushions begin to develop	22 - 32 weeks gestation
Stage III	Extensive and pronounced intimal cushions are present	32 - 40 weeks gestation
Stage IV	Intimal cushions fuse	

From Gittenberger-de Groot et al., 1980

Larger arteries, including the DA, have a vasa vasorum, a network of small vessels, which supply the vessel oxygen and nutrients. Delivery of oxygen and nutrients to the muscular inner layer of the DA vessel wall occurs by filtration from the vessel lumen as well as by the vasa vasorum. The arterial wall's highest level of oxygenation lies directly adjacent to the vessel lumen while the lowest is near the middle of the vessel wall. Oxygenation then steadily increases toward the outer layer, where the vasa vasorum lie (Barker, Talbert, Cottam, Baskerville, & Martin, 1993) This middle area is believed to be especially vulnerable to changes in oxygen and plays a key role in the patency of the ductus arteriosus after birth. The vasa vasorum also plays a significant role in closure of the DA. The roles of this vulnerable middle area of the DA vessel wall and vasa vasorum are discussed below in anatomic closure of the DA.

Patency of Ductus Arteriosus

Patency of the DA is maintained by high pulmonary vascular resistance and potent endogenous vasodilators, prostaglandin and nitric oxide (NO). High pulmonary

vascular resistance in the fetus is achieved by several mechanisms. Because the placenta is responsible for the gas exchange, the oxygen tension in the fetus is low. This low oxygen tension vasoconstricts the lung tissues and pulmonary vessels, increasing pulmonary vascular resistance. The amniotic fluid that surrounds the fetus also fills the fetal lungs, further increasing pulmonary vascular resistance.

Nitric oxide, an endogenous and potent vasodilator, is present in the endothelial cell-lining of the DA lumen as well as in the vasa vasorum in the ductus' outer layer (Hermes-DeSantis & Clyman, 2006). Animal studies have shown NO contributes to fetal DA relaxation (Baragatti, et al., 2003; Richard et al., 2004).

Prostanoids, fatty acid derivatives of the cyclo-oxygenase pathway, are important regulators of DA patency. The prostanoids most actively influencing DA activities are prostaglandins (Coceani, Olley, Bishai, Bodach, & White, 1978; Hermes-DeSantis & Clyman, 2006). Prostaglandins are produced throughout the body by one of two enzymes, cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2). COX-1 is predominant in the fetal DA and plays a major role in ductal tone. Inhibition of COX-1 constricts the DA by reducing the circulating levels of prostaglandin E₂ (PGE₂). COX-2 is found only in term DA and thus maintains DA patency during late gestation.

Prostaglandin E₂, present throughout the body, is the most influential in maintaining ductal patency with its four specific receptors: EP₁, EP₂, EP₃ and EP₄. PGE₂ is produced in the fetal ductus arteriosus, with peak production occurring between weeks 10 and 23 of gestation (Falkay, Herczeg, & Sas, 1980; Yokoyama, Minamisawa, & Ishikawa, 2010). Additional PGE₂ is produced in the uterus and crosses the placenta to join the fetal circulation. Placental production of prostaglandins and decreased catabolism in the fetal lungs (due to the majority of fetal blood by-passing the lungs

through the DA) contribute to the elevated circulating levels of prostaglandin in the fetus (Schneider & Moore, 2006).

Closure of the Ductus Arteriosus

Normally, the ductus arteriosus is closed in two stages. The first step, functional closure occurs through a series of events shortly (several hours) after birth by smooth muscle contraction. Anatomic closure, the second step, occurs two to eight weeks later (Chiruvolu & Jaleel, 2009a; Gilberti, Paolo; Leonibus, Giordano, & Gilberti Paola, 2009).

Functional Closure

Smooth muscle constriction functionally closes the ductus arteriosus. Four events contribute to smooth muscle constriction in the DA immediately after birth: (1) an increase in arterial oxygen tension, (2) a dramatic decline in concentration of PGE₂, (3) a loss PGE₂ receptors in the DA wall, and (4) a decrease in pulmonary vascular resistance that leads to decrease pressure within the DA lumen (Bouayad, et al., 2002; Clyman, 2006). Studies by Quinn, Cooper, and Clyman (2002) found the degree of DA constriction determines the rate of anatomic remodeling and permanent closure.

Compared to other vascular smooth muscle that contracts in response to increasing oxygen tension, the DA's response is exaggerated. The DA has more calcium channels than in the aorta and researchers speculate an influx of calcium through these calcium channels constricts the DA (Reeve, Tolarova, Nelson, Archer, & Weir, 2001).

In utero, the patency of the DA is maintained by PGE₂ that is produced in the DA and the placenta. Once born, circulating levels of PGE₂ plummet because placental production is no longer available and the lung actively breaks down PGE₂ (Smith, 1998). Loss of sensitivity to PGE₂ within the DA has been linked to fewer receptors within the DA lumen. In animal studies, Bhattacharya et al. (1999) found the decrease in PGE₂ sensitivity was due to a loss of EP₃ and EP₄ receptors in DA tissue.

Onset of respiration at birth clears amniotic fluid from the infant's lungs and results in a decrease in pulmonary vascular resistance. Pressure within the DA lumen dramatically falls, collapsing the vessel walls. As a result, blood flow through the DA is significantly reduced.

Anatomic Closure

A cascade of events results in permanent anatomic closure where the DA becomes the ligamentum arteriosum. Two key actions are integral to DA anatomic closure: intimal cushion formation and the development of hypoxia within the DA vessel walls.

Intimal cushion formation. During the second trimester, the intimal lining (inner-most layer) of the DA begins its remodeling process with the formation of intimal cushions or mounds. Hyaluronic acid accumulates between the endothelial cells and subendothelium which promotes smooth muscle cell migration into the subendothelial layer to form intimal thickening (Coceani & Baragatti, 2012; Yokoyama, et al., 2006; Boudreau, Turley, & Rabinovitch, 1991; Gittenberger-de Groot, et al., 1980). These intimal cushions continue to expand over the first few days after birth and will eventually permanently fill and occlude the DA lumen.

In addition to promoting DA relaxation, research has found the relationship between PGE₂ and EP₄ plays a role in intimal cushion formation (Yokoyama, et al., 2010; Coceani & Baragatti, 2012; Akaike et al., 2009). PGE₂ promotes hyaluronan deposits within the gaps formed between the endothelium and the subendothelium cells. PGE₂ then stimulates smooth muscle cells to migrate to replace hyaluronan within the intimal cushions. This process mainly occurs during the last trimester of pregnancy (after 28 weeks gestation).

Hypoxia. In the term DA with fully formed intimal cushions, the width of the vessel wall is large and requires the vasa vasorum to provide nutrients (oxygen) to the outer muscle media. Smooth muscle constriction compresses the vasa vasorum, causing it to collapse. A hypoxic zone within the DA develops which inhibits the productions of PGE2 and nitric oxide. The ischemic/hypoxic zone leads to a cascade of events which promotes local production of growth factors and produces smooth muscle apoptosis. Permanent closure ensues. Because the preterm DA is structurally different, permanent closure occurs less frequently. This structural difference, indomethacin, and the role of platelets in the premature DA are discussed below.

Platelets

Hemostasis (the clotting process) is a complicated procedure involving two phases with two different body systems. In phase one, the cellular system (platelets) form a platelet clot over the injured blood vessel. Platelets passing through the blood vessel attach to the injured epithelial cells lining the vessel wall. The adhered platelets release chemicals to attract additional platelets to the damaged area (aggregation). In phase two, the coagulation cascade, is activated and proteins known as clotting factors work together to form a fibrin clot. This phase stabilizes the clot to prevent excessive bleeding and/or excessive clotting.

The developing fetus begins platelet production as early as five weeks gestation (Parker, Jr., Hauth, Goldenburg, Copper, & Dubard, 2000). By 18 weeks gestation the mean platelet count is less than the term newborn but is still within normal range for adults of 150 to 450 X 10⁹/L (Israels, Rand, and Michelson, 2003).

Platelets primarily produce thromboxane (TXA₂) which is another type of prostanoid. Thromboxane is produced by both COX-1 and COX-2 enzymes. COX-1 is produced by mature platelets, while COX-2 expression is temporary and limited to newly

formed platelets (Patrono & Baigent, 2014). Like the PGE₂, TXA₂ is affected by indomethacin. The significance of the PGE₂, TXA₂ and indomethacin will be discussed in the indomethacin and platelets section.

Preterm DA

The preterm DA differs from the term DA in several ways. The thickness of the preterm DA is much less because of absent or poorly developed intimal cushions. Consequently, the vasa vasorum is not required to provide oxygen and nutrients to the vessel wall. Luminal blood flow must be completely absent before the preterm DA can develop a comparable degree of hypoxia as seen in the term infant.

The mild to moderate degree of hypoxia in the preterm DA does not inhibit prostaglandin and nitric oxide production. The immature vessel continues to respond to vasodilators. The presence of more prostaglandin receptors in the immature DA increases its sensitivity to PGE₂ and nitric oxide which impedes constriction as well.

Table 1-2 summarizes the physiologic differences among term and preterm DAs.

Table 1-2 Summary of Physiologic Differences of Preterm and Term DAs

Characteristics of ductus arteriosus	Pre-term	Term
Distance between lumen & effective vasa vasorum perfusion during postnatal DA constriction		Marked increase
Intramural vasa vasorum	Absent	Present
Degree of reduction of DA luminal blood flow	Same	Same
Avascular zone of DA vessel	Decreased or absent	Present
Prostaglandin receptors present in DA tissue	EP ₂ , EP ₃ , EP ₄	EP ₂
Platelet thrombi	Present	Absent

Because the full-term DA is thick-walled, it requires blood flowing through its lumen and the vasa vasorum to maintain adequate oxygen delivery to its cells. After birth, profound hypoxia in the vessel wall, cell death, and permanent closure are simply achieved by constriction of the DA. Not so with the premature DA. The DA of the premature infant is a much thinner vessel and capable of extracting all the oxygen it needs from merely the blood flow within its lumen. As a result, the premature DA is much less likely to become profoundly hypoxic when it constricts at birth. The under-developed intimal cushions are sparse and the lumen of the DA is never fully occluded. The DA remains patent. Figure 1-3 depicts anatomical differences of the term newborn and fetal DA.

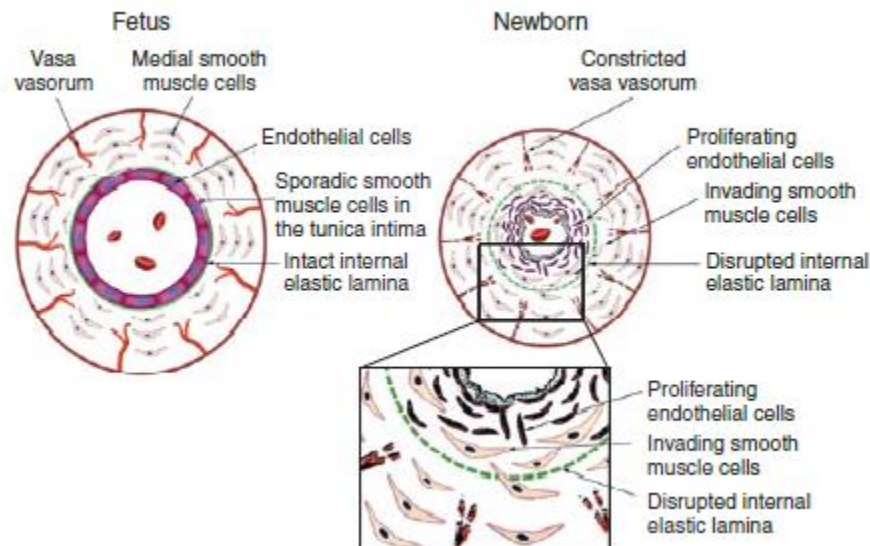


Figure 1-3 Anatomical Differences of Premature and Term Ductus Arteriosus

From "Vessel remodeling in the newborn: Platelets fill the gap" by R. Clyman & S. Chemtob, 2010. Nature Medicine, 16(1), 33-35.

Indomethacin

Indomethacin, a medication that facilitates closure of the fetal ductus, was the first COX-inhibitor drug approved for closure of the PDA by the FDA. COX inhibitors block

the production of various prostaglandins. While indomethacin blocks both COX-1 and COX-2 production, it has a stronger action on COX-1 (Sekar & Corff, 2008). The reported effectiveness of indomethacin in premature infants less than 32 weeks gestation ranges between 70 to 80 percent (Andriessen, et al., 2009). It is most effective if given by one week of age, but may be given as late as two weeks of age (Chiruvolu and Jaleel, 2009b). The infant's improved renal clearance and decreasing sensitivity to circulating prostaglandins diminishes its effectiveness.

Indomethacin has been studied extensively since it was first introduced in the early 1980s. The many side effects of indomethacin on preterm infants are most commonly related to the vasoconstrictor effect from inhibition of prostaglandin production.

Indomethacin and Platelets

Indomethacin inhibits not only PGE₂, which dilates the DA, but also TXA₂ a powerful inducer of platelet aggregation (clumping). Prolonged bleeding times have been documented in premature infants two hours after receiving the first indomethacin dose which persisted up to 48 hours after treatment was completed (Corazza, Davis, Merritt, Bejar, & Cvetnic, 1984). Brash et al. (1981) report decreased platelet function for seven to nine days after indomethacin treatment.

Platelets and the Preterm DA

In studies of mice pups, Echtler et al. (2010) found platelet accumulation was vital to completely seal the constricted DA. The DA of full term newborn mouse and the preterm human infant are similar in several ways: 1) it is thin-walled, 2) it lacks the vasa vasorum, and 3) neointimal cushions do not develop. Much like the preterm DA in humans, initial constriction of the DA alone was not able to adequately eliminate blood flow within the lumen. Under microscopic examination, the researchers detected platelets were actively recruited to the lumen of the constricted DA. Those adhered platelets

continued to recruit additional circulating platelets and formed a thrombus. The process continued for 30 to 50 minutes after birth and resulted in complete blockage of the constricted DA. Mice with disrupted platelet function or platelet adhesion given indomethacin improved DA constriction but did not experience permanent vessel closure.

The authors propose a sequence of events that contribute to postnatal DA occlusion in preterm infants. During DA constriction endothelial cells lining the DA detach. This triggers recruitment of platelets passing the constricted DA and a platelet plug forms. The plug seals the residual lumen of the constricted DA and facilitates luminal remodeling. Their model is shown in Figure 1-4.

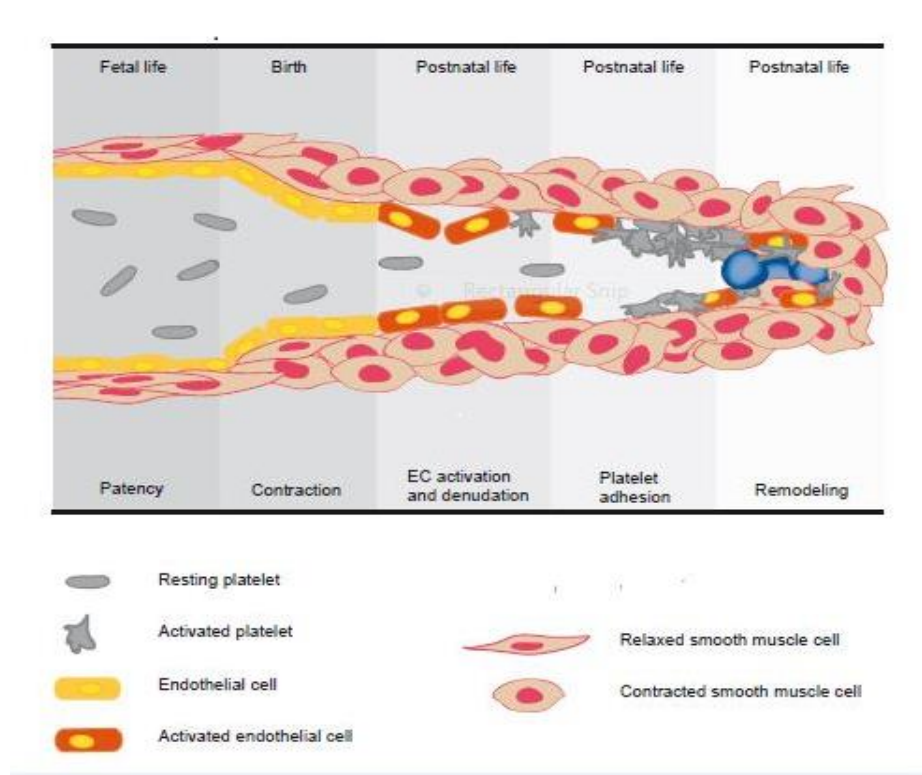


Figure 1-4 The Role of Platelets Sealing of the Contracted DA

Schematic drawing of the role of platelets in sealing in the contracted DA from "Platelets contribute to postnatal occlusion of the ductus arteriosus" by K. Echtler, K. Stark, M. Lorenz, S. Kerstan, A. Walch, L. Jennen, ... & S. Massberg, 2010. Nature Medicine 16(1), Supplementary Figure 9.

Propositions:

1. The anatomical and biological differences between term and preterm infants alter the responsiveness of the DA to hypoxia.
2. The altered response of the premature infant's DA to hypoxia increases the risk of failed closure of the ductus resulting in the need to treat.

Purpose

Premature infants with persistent PDAs are at increased risk for morbidity and mortality. Several studies have shown thrombocytopenia has been associated with persistent PDA in premature infants. Other studies report conflicting results. The purpose of this cohort study is to compare circulating platelet counts in premature infants with persistent PDA versus those with DA closure after indomethacin treatment.

Research Question

The research question examines the relationship of circulating platelet counts and successful closure of a hemodynamically-significant PDA (hsPDA) in premature infants treated with indomethacin. Specifically, the research question is:

1. What is the relationship between circulating platelet counts and ductus arteriosus patency after indomethacin treatment?

The primary hypothesis is premature infants with platelet counts greater than or equal to 100,000 μ L will have a significantly higher rate of ductal closure than those premature infants with platelet counts less 100,000 μ L after indomethacin treatment.

Assumptions

Assumptions of permanent closure of the DA in preterm infants are:

1. Preterm infants have normal anatomy and physiology except for a patent ductus.

2. Infant's musculature will have the ability to contract.
3. Indomethacin, the right drug, is given to the right patient, with the right dose, by the right route, and at the right time.
4. Platelet counts will be accurate.

Summary

PDA is a significant and common complication seen in premature infants. The smallest and most premature infants are at the greatest risk for persistent PDAs from either lack of response from treatment or reopening of a partially closed DA. Platelets have been proposed to fill the gap of the preterm DA when intimal cushions are lacking.

Several studies report thrombocytopenia is associated with persistent PDAs (Dani, Poggi, & Fontanelli, 2013; Echtler et al., 2010; Dizdar, 2012; and Boo, et al., 2006), while others did not (Sallmon et al., 2012 and Shah et al., 2011). Further research is needed to determine the role of platelets in successful closure of the DA in preterm infants.

Chapter 2

Critical Review of Relevant Literature

Introduction

Persistent hemodynamically-significant PDA (hsPDA) is a significant complication seen in preterm infants. It is estimated that 55 to 70 percent of infants born less than 30 weeks gestation or weighing less than 1000 grams have significant PDA (Chiruvolu & Jaleel, 2009a; Hermes-DeSantis & Clyman, 2006). Persistent hsPDA results from either initial failure (no response) to medical treatment or reopening of a previously closed PDA. Complications of persistent hsPDA include exacerbated respiratory distress syndrome (RDS), pulmonary hemorrhage, prolonged assisted ventilation, broncho-pulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), renal dysfunction, periventricular leukomalacia (PVL), cerebral palsy, and death.

Recently the role of platelets in the closure of the DA in premature infants has been examined. Several studies report thrombocytopenia is associated with persistent PDAs (Dani, et al., 2013; Echtler et al., 2010, Dizdar, et al., 2012, and Boo, et al., 2006), while others did not (Sallmon et al., 2012, and Shah et al., 2011). The purpose of this review is to describe the production of the vasodilators involved in DA patency and the role of indomethacin in pharmacologic closure of the DA. An in-depth review of platelet function and their proposed role in permanent DA closure in preterm infants is provided. Finally, a review of four studies that examine the relationship of platelets and indomethacin in DA closure is reviewed.

Review of Relevant Literature

Fetal and newborn circulations differ because the placenta provides gas exchange for the fetus. The ductus arteriosus (DA), a small vessel that lies between the aorta and pulmonary artery, diverts blood away from the fetal lungs into the aorta where it

is dispersed to the other body structures. The patency of the DA is maintained in utero by high pulmonary vascular resistance and potent vasodilators.

Arachadonic Acid Cascade

The arachadonic acid cascade has been studied at length and flows as follows:

- 1.) arachadonic acid (AA) is released from the phospholipids within the cell membrane.
- 2.) a COX enzyme interacts with AA to form first prostaglandin G2 (PG2), then 3.) prostaglandin H2 (PGH2), and finally 4.) depending upon the type of cell and stimuli, a prostacyclin, prostaglandin, or thromboxane is produced. Prostaglandins are the prostanoids most involved in regulation of DA patency. Thromboxane A2 (TXA2) plays a predominate role in platelet aggregation and will be reviewed in the platelet section.

Figure 2-1 depicts the AA cascade.

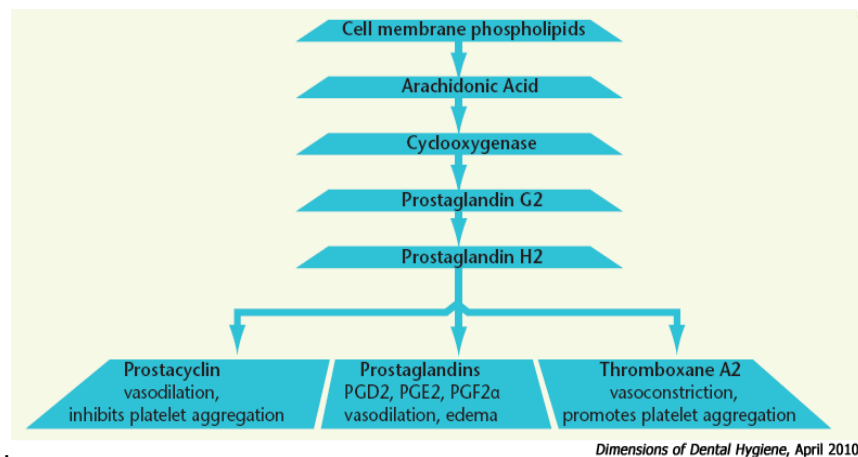


Figure 2-1 Arachidonic Acid Cascade

Prostaglandins

A number of fatty acid derivatives, prostaglandins, have been identified to have profound effects on the DA. Synthesized in numerous tissues and cells, prostaglandins regulate inflammation, fever, and pain. They are also involved in fertilization, ovulation, nidation (early implantation of an embryo into uterine mucosa), platelet aggregation, and renal function (Tanabe & Tohnai, 2002).

Prostaglandins are produced by two cyclooxygenase enzymes (COX1 and COX2) and the AA cascade. Discovered in 1976, COX1 enzyme is produced and present in nearly all cell types throughout the body. It is present in constant amounts (constitutive) (Vane, Bakhle, & Botting, 1998). COX2 enzyme was discovered in 1991 (Vane et al., 1998). While similar in structure to COX1, COX2 behaves very differently. It is an inducible enzyme, usually present in low amounts unless production is increased by one of its many stimuli.

Animal studies have shown production of COX1 and COX2 develop unevenly in the DA. COX1 is predominant in fetal/preterm DA. At term COX2 is upregulated so that both enzymes are present in equal amounts (Bouayad, et al., 2002; Coceani, Ackerly, Seidlitz, & Kelsey, 2001). In the future these findings may be used to develop treatments that specifically target only the COX1 enzyme and/or both enzymes. Table 2-1 summarizes COX1 and COX2 differences.

Table 2-1 Characteristics of COX1 and COX2

Cox1	Cox2
Continuously stimulated by body	Produced in response to body stimuli
Constitutive (it's concentration in body remains stable)	Built only in special cells
Creates prostaglandins used for basic housekeeping throughout body	Used for signaling pain & inflammation
Prostaglandins stimulate normal body functions (protect stomach mucosa, aid platelet aggregation, and kidney water excretion)	Produces prostaglandins for inflammatory response
	Stimulated only as part of immune response
<i>Predominant in fetal tissue</i>	Production is stimulated by inflammatory cytokines & growth factors
<i>Expression plays major role in ductal tone</i>	<i>Found only in term DA tissue</i>
<i>Inhibition reduces circulating levels of PGE2 and constricts DA</i>	<i>Maintains ductal patency during late gestation</i>

Shaded areas/***Bold-Italics*** reflect COX enzymes effect on DA

Review of the Pathophysiology of a PDA

Birth signals the onset of separate pulmonary and systemic circulation systems with closure of the DA and foramen ovale. The pulmonary and systemic circulation systems are placed in series with each other and are meant to be balanced (equal). Blood flows through the pulmonary system, then on to the systemic circulation, back through the pulmonary system etc. A PDA between the pulmonary artery and aorta (a left-to-right shunt) "leaks" blood back from the systemic to the pulmonary circulation. Blood volume and pressure become abnormally elevated in the pulmonary system. If the

left-to-right shunt persists, eventually systemic blood flow decreases. Blood flow to the skin, bone, and skeletal muscles are the first affected by the left-to-right shunt through the PDA (Clyman, 2006). Decreased blood flow from the localized vasoconstriction and decreased diastolic pressure contribute to the morbidities of the PDA.

Mechanical ventilation of the preterm infant with lung disease increases pulmonary vascular resistance which can effectively (and temporarily) prevent blood from leaking back from the systemic to the pulmonary system. When lung disease improves and mechanical ventilation is weaned, pulmonary vascular resistance drops and a left-to-right shunt develops.

Indomethacin

Indomethacin was the first COX inhibitor to be approved by the FDA in the treatment of PDA. Indomethacin decreases prostaglandin synthesis by inhibiting both COX1 and COX2 enzymes. Indomethacin is metabolized in the liver and kidneys where its inactive compounds are excreted in the urine and feces. Serum half life is approximately 30 hours with a range of 15 to 50 hours (Brash, 1981). Clearance depends on postnatal age; the older neonate the faster the clearance. The DA's response to indomethacin directly correlates with the plasma concentration (Brash). Smaller, more immature infants have higher total body water and higher volumes of distribution. As a result, infants less than 1,000g have been found to have plasma levels less than those infants that weigh more than 1,000g (Austen, Pairaudeau, Hames, & Hall, 1992).

Because there are no universally accepted criteria that predict which PDA will become hemodynamically significant, treatment protocols and dosing regimens are as varied as the physicians who prescribe them. Three treatment strategies exist for dosing indomethacin: prophylaxis, asymptomatic, and symptomatic. Prophylactic dosing is generally defined as treating all at-risk infants within the first 24 hours of birth.

Asymptomatic dosing typically involves confirmation of a PDA with an echocardiogram within the first few days of life. The DA remains patent, but the infant remains asymptomatic. Regardless of their clinical status, the infants are treated with the drug. The most conservative approach used with pharmacologic treatment involves watchful waiting. Treatment begins when the infant becomes symptomatic (requires increased oxygen or ventilator support, etc). This treatment strategy is often less successful because the infant is older and has improved renal clearance and decreased sensitivity to the medication.

Indomethacin Side Effects

Indomethacin has many effects on preterm infants other than ductal constriction. Most are related to the vasoconstrictor effect from inhibition of prostaglandin production and primarily involve the kidneys and the gastrointestinal tract. Indomethacin also affects platelet function due to suppression of COX1.

The most commonly reported side effect is renal dysfunction. While usually temporary, decreased glomerular filtration, decreased urine output, and decreased fractional excretion of sodium and chloride occur (Carey, 2003). Hyponatremia or hyperkalemia may be the result of these renal changes.

Because prostaglandins protect the stomach mucosa, necrotizing enterocolitis, gastric hemorrhage, and gastric perforations are a potential side effect of indomethacin. Most practitioners discontinue feedings during the indomethacin course.

Decreased cerebral blood flow and cerebral tissue oxygenation are documented side effects of indomethacin (Austen et al., 1992; Liem, Hopman, Kollee, & Oeseburg, 1994; Van Bel, Van de Bor, Stijnen, Baan, & Ruys, 1989). Prolonging the infusion time lessens this side effect, In fact, Lemmers, Toet, and van Bel (2008) found infusing indomethacin over sixty minutes actually increased cerebral blood flow in preterm infants

(less than 32 weeks gestation) who were treated with indomethacin for a PDA. They speculate closing the DA improves organ perfusion that occurs with the left-to-right shunt of the PDA.

Platelets aggregation occurs when platelets clump together and is a vital part of hemostasis. Indomethacin has been reported to prolong platelet aggregation because it suppresses the cyclooxygenases and thus the production of thromboxane A₂ (TXA₂). TXA₂ enhances platelet aggregation and is covered in depth below in the platelets section. Indomethacin has been reported to double the bleeding time in preterm infants and remained elevated for up to 48 hours after a three day course (Corazza, et al., 1984).

Table 2-2 presents a summary of studies reporting indomethacin side-effects.

Table 2-2 Summary of Studies of Indomethacin Side Effects

Side Effect	Supporting Studies
Decreased cerebral blood flow	Clyman, (1996) Mosca, et al., (1997)
Oliguria	Van Overmeire, et al., (2001)
Increased serum creatinine clearance	Clyman, (1996) Gersony et al., (1983)
Decreased splenic and renal blood flow	Mosca et al., (1997)
NEC	Grosfeld et al., (1996)
Isolated bowel perforation	Van Overmeire et al., (2001)
Bronchopulmonary dysplasia	Schmidt et al., (2006)
Platelet aggregation	Brash et al., 1981

Platelets

A single layer of endothelial cells line the inner surface of all blood vessels. Under normal circumstances these cells control blood fluidity by providing a thrombo-resistant surface. When a vessel is injured, the thrombo-resistant properties of the endothelial cells are damaged or lost and activates hemostasis.

Hemostasis occurs in two phases. Platelets are active in the first stage and perform three functions: adherence, aggregation, and support. When a blood vessel is injured, the endothelial cells lining its wall become sticky. Platelets flowing through the injured vessel attach (adhere) to the sticky wall. The attached platelets release chemicals to attract other platelets to form a platelet plug (aggregation). These initial two steps stop bleeding. The collection of platelets at the injury site then provides support for the second phase of hemostasis, the coagulation cascade.

Platelet Function Measurement

Platelet function testing can be measured by four parameters: bleeding time, aggregometry (clumping), platelet function analyzer (PFA), and flow cytometry. Bleeding time measures the interaction between platelets and the blood vessel wall and simply determines the time it takes for bleeding to stop after the skin is punctured (usually with a small lancet).

Platelet aggregometry was developed in the 1960s and remains the most commonly used test of platelet function today (Harrison, 2005). When platelet-rich plasma is exposed to a platelet agonist (platelet stimulator) various parameters of the platelets are monitored as they cluster together (aggregate). Platelet aggregation studies have shown newborn platelet aggregation is significantly decreased when compared to adult platelets (Israels, Rand, & Michelson, 2003). These differences were more

pronounced in the platelets of preterm infants (Israels, Odaibo, Robertson, McMillan, & McNicol, 1997).

The PFA also measures bleeding time. A very small sample of blood (microgram) is placed in a cartridge that contains a tiny aperture that has been coated with a platelet agonist. The platelet agonist induces platelet activation within the blood sample as it flows across the opening. The time required to fully occlude the aperture is the closure (or bleeding) time. The PFA has become the preferred measurement of bleeding time because it requires a minute blood sample, can be used by non-skilled personnel, the results are quick, and the test is automated. Higher hematocrits are associated with shortened (faster) closing times. Thus term newborns have shortened bleeding times when compared to adults and older children.

Platelet flow cytometry is the newest and most advanced device available to measure platelet function. Flow cytometry measures the physical and chemical characteristics of platelets as they flow single-file through a stream of light. Using minute volumes of whole blood taken from the infant, platelets are examined. Flow cytometry has become the measurement of choice because platelets are measured while in their 'natural' circulating state.

Platelets and Newborns

Studies have established neonatal platelets have a blunted response (platelets are hyporeactive) when compared to adult platelets. This hypo-reactivity is thought to be a developmental phenomenon (Grosshaupt, Muntean, & Sedlmayr, 1997). Research has demonstrated that the hypo-reactivity is due to several factors. Newborn platelets have decreased aggregation, decreased secretion and production of TXA₂ and fewer adhesive receptors on their membrane after activation (Israels et al., 1997). This blunted response

has been reported to last between three and fourteen days (Israels et al., 2003; Ucar, Gurman, Arsan, & Kemahli, 2005).

Preterm infants were found to have significantly decreased platelet adhesion when compared to full term infants and adults and did not reach full term levels during a 10-week follow up period (Linder et al., 2002). Decreased platelet adhesion was more pronounced in infants with hyaline membrane disease (Linder).

Israels et al. (1997) studied platelet aggregation, secretion and production of TXA₂ in cord blood obtained from thirty five preterm infants (mean gestational age 32±3.2wk). Compared to adults and healthy term newborns, the researchers found the preterm infant's platelet function was more significantly compromised than in the full-term infant. Premature infants had lower amounts of coagulation proteins and significant impairment of platelet activation. Preterm infants were also found to produce less TXA₂. Other researchers report similar findings (Rajasekhar, Barnard, Bednarek, & Michelson, 1997; Ucar et al., 2005).

In a review by Sola-Visner (2012) research findings of the preterm infant's platelet functioning tests (i.e. bleeding time, aggregometry, PFA, and flow cytometry) were reported and support the work by Israel above. Bleeding time of infants born less than 30 weeks gestation were almost twice as long as those from full-term neonates. Aggregometry and PFA studies showed platelet hyporeactivity was more pronounced in preterm infants and inversely proportional to gestational age. Finally flow cytometry clearly demonstrated a presence of platelet hyporeactivity in preterm infants.

Platelets and Cyclooxygenases

COX1 is constitutive while COX2 is induced. COX1 is present in the platelet cell wall in a steady amount. COX2 is present in megakaryocytes (precursor for platelets found in bone marrow) and immature platelets (Smyth, Grosser, Wang, Yu, & FitzGerald,

2009). During times of stress, COX2 activity may increase and decrease in a matter of hours after a single stimulus (Vane et al., 1998). Both forms produce the prostanoid, thromboxane A2 (TXA2) (see Figure 2-1above).

TXA2, a powerful agonist, enhances platelet aggregation. During hemostasis, activated platelets manufacture TXA2 which intensifies additional platelet activation and recruitment. Inhibition of COX1 (and COX2) reduces production of TXA2, and consequently decreases platelet aggregation.

Depending upon the medication used to suppress COX activity, inhibition may be permanent or temporary. Aspirin irreversibly inhibits production of COX, while other nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, does not. Thus, aspirin inhibits TXA2 production for the platelet's lifetime of eight to ten days in the circulation (Vane et al., 1998). With other NSAIDs (including indomethacin) however, inhibition is temporary and TXA2 function is restored as the drug is cleared from circulation (Schafer, 1995). The serum half-life of indomethacin is approximately 30 hours and decreases with postnatal age (Brash et al., 1981). Therefore decreased platelet aggregation can be expected to last a minimum of a little more than a day.

Platelets' Role in the Term PDA

In term infants, platelet thrombi have a minimal role in ductus closure (Silver, M.M., Freedom, Silver, M.D., & Olley, 1981). In histologic studies of term nonhuman primates adhered platelets have not been found during early ductal closure (Waleh et al., 2005). Nor have platelet thrombi been detected in the DA lumen of term human infants during either the first few days or during the first weeks after birth (Waleh). Further evidence that platelets are not necessary for permanent DA closure in term infants is that otherwise healthy term newborns with severe alloimmune or autoimmune

thrombocytopenia do not have a higher incidence of PDA (Sallmon, Weber, von Gise, Koehn, & Hansmann, 2011).

In a MEDLINE search combining the terms, "patent ductus arteriosus" and "platelets" and "closure" six relevant study were retrieved. Results of these reports were conflicting. Four studies reported thrombocytopenia was associated with persistent PDAs (Dani et al., 2013; Echtler et al., 2010; Dizdar et al., 2012; and Boo et al., 2006). Studies published by Sallmon et al. (2012) and Shah et al. (2011) found low platelet counts were not associated with persistent PDAs. Infants in the studies by Dani et al. and Dizdar et al. received ibuprofen, so those studies are not reported here.

Thrombocytopenia Associated with PDA

Boo et al. (2006) did a prospective observational study of 60 preterm infants weighing less than 1,750g with symptomatic PDA. Diagnosis was confirmed by echocardiography. At a median age of 7 days, all received indomethacin, 0.1mg/kg/dose, intravenously daily for six days. PDA closure was reassessed after completion of therapy with echocardiography. Forty percent of the treated infants continued to have a PDA. Forward logistic regression analysis revealed three significant predictors of failed PDA closure: PDA size, birth weight, and platelet count ($p = .05$).

Echtler et al. (2010) report a three-pronged analysis: (1) the role of platelets in the DA of neonatal mice, (2) the presence of platelets in histological sections of human DA specimens, and (3) a retrospective analysis of preterm infants with PDAs and thrombocytopenia.

The researchers observed platelets adhered to the lumen of the contracted DA shortly after birth in neonatal mice. These adhered platelets recruited additional circulating platelets to form a platelet plug within the DA lumen. They found the constricted DA of neonatal mice were fully occluded (no blood flow detected) within 30-50

minutes of age. The researchers then examined the DAs of neonatal mice with defective platelet adhesion and defective platelet biogenesis. Fifty four percent of the DAs of neonatal mice with defective platelet adhesion and 70 percent of the neonatal mice with defective platelet biogenesis remained patent at 12 hours of age. Indomethacin did not decrease the incidence of PDA in mice with defective platelet biogenesis. This suggests the constriction of the DA wall, even when enhanced by indomethacin, does not negate a loss of platelets.

To examine whether platelets were also involved in DA closure in human newborns, the researchers examined histological sections of DA specimens obtained from human neonates who underwent cardiac surgery for congenital heart disease. They found adhered platelets in the constricted DAs, but not the patent DAs.

To determine the possible clinical relevance of their findings, the researchers completed a retrospective study of preterm infants to assess the impact of concurrent thrombocytopenia on the risk of PDA. The study included 123 infants born at 24-30 weeks gestation admitted to a neonatal unit between the months of January 2003 through November 2008. Thrombocytopenia was defined as a platelet count less than $150 \times 10^3 \mu\text{l}^{-1}$. The thrombocytopenic group (N=19) had a median platelet count of $125 \times 10^3 \mu\text{l}^{-1}$, while the median platelet count in the non-thrombocytopenic group was $232 \times 10^3 \mu\text{l}^{-1}$ (N=104). A duct was considered hemodynamically significant if the end diastolic flow in the celiac trunk was 0, or the left atrium to aorta diameter (LA/AO) was 1.5, or if there were any clinical signs of hemodynamic relevance (hemodynamically clinical signs were not specified). There was no significant differences between the two groups in gestational age, sex or birth weight. The overall frequency of PDA was 71 percent. Compared to the thrombocytopenic group, the non-thrombocytopenic group had higher rate of DA closure (35 percent versus none) and a lower incidence of hemodynamically

significant PDAs (15 percent versus 68 percent). Using multivariate analysis, confounders were also examined (maternal chorioamnionitis or preeclampsia/HELLP syndrome, sepsis, or steroid treatment). Thrombocytopenia remained the only predictor of a hemodynamically significant PDA ($p < .0001$, OR = 13.1[3.2-49.6]).

Conflicted results were reported by Shah et al. (2011). In a cohort of 497 infants who received prophylactic indomethacin to close the DA, thrombocytopenia was not associated with permanent ductus closure or ductus reopening. In the study, all infants born less than 28 weeks gestation received indomethacin according to the institution's PDA care-oriented protocol. Infants were given two doses of indomethacin (administered at 24-hour intervals) starting within 15 hours of birth. If an echocardiogram, performed after the second dose, revealed DA closure, infants received one additional dose of indomethacin (the short three-day course). If the echocardiogram demonstrated any evidence of DA patency, the indomethacin course was extended to a total of six doses. A second echocardiogram 24 -36 hours after the final dose of indomethacin determined the DA response to the prophylactic indomethacin. The researchers examined three groups: (1) Infants with DA patency after two doses of indomethacin (34 percent), (2) Infants with DA patency after six doses of indomethacin (19 percent), and (3) DA reopening after initial indomethacin-induced closure (14 percent)

Platelet nadirs were categorized into quintiles to better characterize the platelet nadir effect. The lowest quintile was the reference group. Each quintile was examined for its effect on the incidence of patency of the DA. They found infants whose platelet counts were among the highest quintile had a significantly lower incidence of PDA at three and seven days ($p = .05$). They report no association between platelet counts (high or low) and the incidence of the DA reopening after successful closure with indomethacin. This

finding questions the theory that platelets are involved in "filling the gap" of the premature ductus and promoting the permanent closure of the DA.

Sallmon et al. (2012) examined the association of thrombocytopenia and PDA in a retrospective cohort study of 758 very low birth weight and 592 extremely low birth weight infants. Inclusion criteria consisted of a complete blood count within the first 24 hours of life and an echocardiogram on day of life four or five. A PDA was considered hemodynamically significant if there was an increase of respiratory support (supplemental oxygen greater than 30 percent and/or mechanical invasive or non-invasive ventilation), LA/AO ratio greater than 1.4, ductal diameter greater than 2.5mm, and evidence of ductal steal. There was no significant differences in platelet counts, the incidence of PDA, or specific therapeutic intervention (indomethacin, ibuprofen, surgical ligation) in either the very low birth weight or extremely low birth weight infants ($p < 0.05$). Birth weight and lower gestational age were significantly associated with the prevalence of PDA, the need for treatment, and the rate of pharmacologic treatment failure ($p < .05$).

Summary

Patent ductus arteriosus remains a significant complication of prematurity. Less than 30 percent of infants born prior to 28 weeks gestation will spontaneously close their PDA (Narayanan, Cooper, Weiss, & Clyman, 2000). Persistent PDA results from either initial failure (no response) to medical treatment, or reopening of a previously closed PDA. Research has shown when successfully treated, PDAs in ELBW infants did not contribute significantly to chronic lung disease, ROP, or increased age at discharge (Adrouche-Amrani, Green, Gluck, & Lin, 2012). Failure of a repeat course of a COX inhibitor to close a PDA was a risk factor for developing chronic lung disease in ELBW (Adrouche-Amrani et al., 2012).

The term DA is a thick muscular vessel that when constricted develops a hypoxic zone. Cell death occurs which results in anatomic remodeling and permanent closure of the DA. In contrast, in preterm infants, the DA is a much thinner vessel, lacks the vasa vasorum and is able to obtain all of the oxygen it needs from blood flow within the lumen despite vessel constriction. Thus, the preterm DA is much less likely to become hypoxic when it constricts after birth. The under-developed DA fails to occlude and the DA remains patent.

Platelets are theorized to collect within the narrowed lumen of the preterm DA and develop thrombi. The thrombi organize to progressively occlude the DA lumen at its narrowest point. Hypoxia occurs leading to cell death, anatomic remodeling and permanent closure of the DA, just as in the term DA.

Recently, the role of platelets in successful (permanent) closure of the PDA has been examined with conflicting results. Further research is needed to determine whether platelet aggregation and thrombus formation can substitute for the lack of intimal mounds in preterm infants.

Chapter 3

Methods and Procedures

Introduction

This retrospective secondary analysis compared the platelet counts of two groups of premature infants after indomethacin treatment for a hemodynamically-significant PDA (hsPDA). A description of the research design, sample, and setting is presented. Measurement method, procedure, and ethical considerations are then addressed. Data preparation, statistical analysis, and research assumptions conclude the chapter.

Research Design

This research design was a secondary analysis that examined the successful closure of hsPDA in premature infants. According to Doolan and Froelicher (2009) the advantages of a secondary analysis include the capacity to conduct the study in less time and at a lower cost. The major disadvantage of a secondary analysis is that the investigator has no control over the original data collection, the measures used, nor how well data collection was done (Doolan & Froelicher, 2009). The available data may be inaccurate, incomplete, or inconsistently measured (Hulley, Cummings, Browner, Grady, & Newman, 2007).

Research published by Echtler et al., (2010) and Boo et al., (2006) defined thrombocytopenia as platelet counts of less than 150,000/ μ L. This retrospective study compared the response of two groups: Group 1 consisted of infants' whose platelet counts were greater than or equal to (\geq) 100,000/ μ L, while Group 2 were those infants' whose platelet counts were less than ($<$) 100,000/ μ L. The platelet value of 100,000/ μ L was selected because platelet counts less than 100,000/ μ L are considered moderately thrombocytopenic. Additionally, infants with platelet counts of less than 100,000/ μ L are

not treated with indomethacin without first receiving a platelet transfusion. The Babysteps® database was used for this study. Babysteps® software program and database are owned by Mednax, a national medical group made up of neonatal, maternal-fetal, and pediatric healthcare providers as well as anesthesia services.

Echtler et al., 2010 reported an OR of 13.1 (3.5-49.6). This estimation was based on a two-tailed test, $\alpha = .05$, power = .80. Boo et al. (2006) reported an OR of 0.987 (0.975, 1.000). A power analysis was done using G*Power 3.1.0 (Faul, Erdfelder, Buchner, & Lang, 2009) for a Chi-square test to measure the difference in successful closure of hsPDA among two group of premature infants. A probability level of .05, power of .80 and small effect size (0.2) were used to calculate the a priori sample size of 210.

Sample

The data for this study was abstracted from the Babysteps ® database from a large metropolitan, 80-bed NICU with approximately 900 admissions per year. The sample included inborn premature infants (born at less than 32 weeks gestation) with a diagnosis of PDA who received indomethacin.

Setting

The setting for the study was a large urban Level III NICU in Dallas, Texas. The 80-bed NICU is part of a 1,079-bed, not-for-profit hospital. The majority of the infants in the NICU are inborn, but transport services to outlying hospitals are offered.

Measurement Methods

The method of data collection was a review of existing electronic medical records from the Babysteps ® database. Infants diagnosed with PDA who received indomethacin and had a documented platelet count within seven days of receiving indomethacin were included. Data were also collected on gestational age at birth, birth weight, gender, race,

number of indomethacin doses, size of PDA prior to treatment, DOL at time of treatment, and mode of ventilation at time of treatment.

Exclusion criteria were congenital malformations, congenital heart disease, persistent pulmonary hypertension, neonatal sepsis, neonatal death, and infants with incomplete data. Operational definitions of the variables are in Table 3-1.

Table 3-1 Conceptual and Operational Definitions of Study Variables

Study Variable	Conceptual Definition	Operational Definition
Gestation at birth	Length of time in the womb between conception and birth	Documented in Babysteps® in weeks plus days
Gender	Male or female	Male = 0 Female = 1
Maternal race	A group with similar physical characteristics or origin	White = 0 Black = 1 Hispanic = 2 Other = 3
Birth weight	The first weight of an infant obtained after birth	Documented in Babysteps®; expressed grams
Day of Life (DOL) at time of treatment	Length of time an infant has lived; expressed in days.	Documented in Babysteps®
PDA-Size (mm)	A persistent opening of fetal ductus arteriosus that connects the pulmonary artery to the descending aorta allowing unoxygenated blood to bypass the lung and flow to the placenta. Normally, the ductus is closed shortly after birth.	Documented in Babysteps®; expressed in millimeters
Number of indomethacin doses	Indomethacin, a medication used in the treatment of persistent PDA which decreases prostaglandin synthesis.	Documented in Babysteps®;

Table 3-1—Continued

Platelet Count	The concentration of platelets in blood	Documented in Babysteps®; expressed as thousands per microliters (μL)
Platelet Group	The concentration of platelets in blood	Documented in Babysteps®; expressed as thousands per microliters (μL) 0 = $>100,000/\mu\text{L}$ 1 = $<100,000/\mu\text{L}$
Mode of ventilation	The method of movement of air between the environment and the lungs through inhalation and exhalation	Documented in Babysteps®; Ventilator = 0 Continuous positive airway pressure (CPAP) = 1 Nasal Cannula (NC) = 2 Room air (RA) = 3
	Presence or absence of blood flow through the ductus arteriosus after receiving indomethacin.	Documented in Babysteps®; Closed = 0; follow-up echo demonstrated DA closure, Open = 1; follow-up echo demonstrated persistent blood flow through DA, OR indomethacin treatment extended or repeated, OR infant underwent surgical ligation
PDA Ligation	Surgical procedure used to permanently close PDA	0 = No 1 = Yes

Procedure

Following institutional review board (IRB) approval from the hospital and the University of Texas at Arlington, a review the Babysteps® database was performed to collect a sample of premature infants with a diagnosis of PDA and treated with indomethacin. To be included in the sample, subjects must also have had a documented platelet count within seven days of receiving their first dose of indomethacin. Those infants were then subdivided into two groups: group 1: low platelets ($<100,000/\mu\text{L}$) or group 2: high ($\geq 100,000/\mu\text{L}$) platelets. Because data was obtained from the same

database, comparability of the data between the two groups was ensured. Since gender, gestation, and birth are required by the database before an admission note can be generated, all patient records had data entered for those variables. Babysteps® alerts the user if the gestational age or birth weight is out of a reasonable range, improving accuracy of the data. The daily note automatically provides the infant's day of life. Providers must also enter type of respiratory support, provided from a drop down menu, to generate daily progress notes. The variable platelet counts was dependent upon the provider entering the lab values in the lab section of the note. The number of indomethacin doses was also dependent upon provider entry. Repeat echocardiogram which demonstrate persistent patent DA after receiving three doses of indomethacin, an extended indomethacin course (more than three doses), and/or surgical ligation were considered treatment failure (non-closure).

To ensure accuracy of data collection and entry, all subjects' data entry were checked a second time. A random selection of 10% of the records was audited for data collection. Any errors were corrected for the study variables. A copy of the data collection tool is provided in Appendix A.

Ethical Considerations

Because the study was a review of existing information in a database, the only risk to participants was loss of confidentiality. The protected health information, the medical record number, was de-identified in order to protect subject confidentiality. Subjects were assigned a unique study identifier. The list of medical records associated with the study identifier was kept in a file separate from the study data. The flash drive and a hard copy of the record were kept in the investigator's safe. Instead of birth dates, their day of life was recorded with day of life 1 as their day of birth (automatically generated in Babysteps® database).

Data Analysis

SPSS Statistics (22.0) was used to analyze the study data. Data were prepared for analysis by checking the frequencies, distribution, and measures of central tendency of the variables. Descriptive statistics were computed for the categories of birth weight, gestation at birth, gender, and race. The two groups of infants (those with closed hsPDA versus those with open hsPDA after indomethacin) were compared on variables identified as predictors of successful closure of the hsPDA from previous studies. The variable 'size of hsPDA' was the only variable with missing values (40 entries for a total of 63 infants). Non-parametric data analyses (Fisher's exact test and Mann Whitney U) were used as the variables did not have a normal distribution.

Delimitations

The delimitations of this study were that the study sample included only infants less than or equal to 32 weeks gestation who were treated with indomethacin for PDA and only those who were included in the BabySteps® Database from a Level III NICU in Dallas, Texas. Thus, the findings of this study cannot be generalized to all preterm infants treated with Indomethacin for PDAs in other hospital settings.

Summary

This retrospective secondary analysis examined data obtained from Babysteps® database. Two groups of infants were studied: group one consisted of infants diagnosed with hsPDA who were successfully treated with indomethacin, while group two was made up of those infants whose hsPDA remained open after indomethacin treatment. Analysis was performed using SPSS 22.0.

Chapter 4

Findings

Introduction

The purpose of this retrospective cohort study was to determine if there was an association between circulating platelet counts in premature infants with persistent patent ductus arteriosus (PDA) versus those with ductal arteriosus (DA) closure after indomethacin treatment. Descriptive statistics for the demographic and study variables as well as analyses of results will be presented.

Results

Sample Demographics

Sixty-three infants met inclusion criteria for this study. The data were collected from the Babysteps® database of a Level III NICU in a metropolitan hospital in Dallas, Texas. The demographics of the study population are presented in Table 4-1. The sample was racially diverse with approximately equal numbers of male (n= 33) and female (n=30) infants. The mean birth weight of the sample was 833.54g (SD=218) with a mean gestation (at birth) of 25.89 weeks (SD=1.64). Racial ethnicity characteristics of the population were white 28 percent, Black 35 percent, Hispanic 27 percent, and all others ten percent.

Analysis of Demographic Variables

The demographic variables examined in this study were gender and maternal race. Because of the small sample size (n = 63) Fisher's exact test was computed. Twenty one percent of male infants responded to indomethacin while seventeen percent of females did so. There was no significant difference between the male and female infants who's hemodynamically significant PDAs (hsPDA) closed after treatment

($p = .76$). The ductus arteriosus (DA) closed in thirty five percent of Hispanic mothers, twenty three percent of infants of black mothers, eleven percent of White mothers, while seventeen percent of infants of mothers of all other racial groups did so ($p = .36$).

Table 4-1 Demographic Characteristics of Infants (N = 63)

		PDA open n (%)	PDA closed n (%)	p
Gender	Male	26 (78)	7 (21)	.76
	Female	25 (81)	5 (17)	
Maternal Race	White	16 (89)	2 (11)	.36
	Black	17 (77)	5 (23)	
	Hispanic	11 (65)	6 (35)	
	Other	5 (83)	1 (17)	

Description and Analysis of Predictor Variables

The variables found to be predictive in previous studies of successful closure of the hsPDA with indomethacin were gestation (at birth), birth weight, age when indomethacin treatment begun, size of PDA, level of respiratory support, and number of circulating platelets. The descriptive statistics of predictive variables are presented in Table 4-2.

Table 4-2 Reported Predictive Variables of Successful PDA Closure (N = 63)

	N	Mean	Std. Deviation
Birth Weight	63	833.54	218
Birth gestation (weeks)	63	25.89	1.64
PDA size (mm)	39	2.42	.47
Platelet count (μ L)	63	225.19	91.82
DOL at treatment	63	8	3.53

Results of the comparison of infants who did not respond to Indomethacin (hsPDA remained open) to those who did (hsPDAs closed) are presented in Table 4-3. Because Shapiro Wilk test indicated the variable PDA size had a normal distribution an independent t-test was calculated. There was no significant difference in size of PDA between the open PDA and closed PDA groups ($p = .60$). The respiratory support variable consisted of two values (ventilator and continuous positive airway pressure, CPAP) so Fisher's exact was calculated and was not significant ($p = .73$). The variables birth weight, gestation, platelet count, and day of life (DOL) when Indomethacin began were skewed and did not meet the assumptions of normal distribution. Therefore Mann Whitney tests were computed to compare the open PDA and closed PDA groups. There was no significant difference between the open and closed PDA groups for birth weight, gestation, platelet counts, respiratory support, and DOL Indomethacin was initiated ($p=.42, .53, .56$, and $.16$, respectively).

Table 4-3 Reported Predictor Variables with Treatment Outcomes

	Status of PDA after treatment	N	Mean Rank	Sum of Ranks
Birth Weight	Open	51	32.9	1678
	Closed	12	28.17	338
	Total	63		
Gestation	Open	51	32.71	1668
	Closed	12	29	348
	Total	63		
Platelet Counts	Open	51	32.66	1665.5
	Closed	12	29.21	350.5
	Total	63		
DOL at time of Indocin treatment	Opened	51	33.56	1711.5
	Closed	12	25.38	304.5
	Total	63		

Discussion

Nineteen percent of infants in this study who were treated with indomethacin responded to treatment (closed PDA). In this sample of 63 infants, no associations were found between platelet counts and closure of the DA after receiving indomethacin. Analyses of other variables reported in the literature to be associated with closure of the DA and indomethacin were also examined. No associations were found between birth weight, gestation at birth, size of PDA, and respiratory support. Demographic analyses of race and gender revealed no associations with failed treatment of PDA with indomethacin. There were also no significant differences between the two (closed-PDA and open-PDA) groups with regards to gender, race, birth weight, gestation at birth, size of PDA, respiratory support.

No findings from this study supported findings published by Echtler et al., (2010) or Boo, Mohd-Amin, Bilkis, & Yong-Junina (2006). The study by Echtler et al. was a retrospective analysis of an existing data set of preterm infants, while Boo's was a prospective observational study. Both reported low platelet counts were associated with failed closure of the PDA in infants treated with indomethacin. Boo also reported lower birth weight and larger PDA size were significant predictors of increased failure in indomethacin treatment. A summary comparing the studies is presented in Table 4-4.

Gestational Age and Birth Weight

It is estimated that 55 to 70 percent of infants born less than 30 weeks gestation or weighing less than 1,000 grams have significant PDA (Chiruvolu & Jaleel, 2009a; Hermes-DeSantis & Clyman, 2006). Boo et al. reported an incidence of only 15 percent. Echtler et al. reported an incidence of 71 percent while the present study had an incidence rate of 57 percent.

Indomethacin is reported to successfully close the PDA in 20 to 40 percent of infants whose birth weights are less than 1,000g (Adrouche-Amranim, Green, Gluck, & Lin; 2012). The persistence of the PDA in preterm infants is inversely related to gestational age and birth weight. Successful closure varied between the three studies with Boo at 60 percent, followed by Echtler et al. at 35 percent, and 19 percent in this research. The low incidence and high successful closure rate reported by Boo et al. are most likely due to the larger birth weight and gestational age of his sample. However, infants in that study were treated with an extended six day course of indomethacin, compared to a three day course the infants in Echtler et al. and the present study received. This lengthened course could also have played a role in the high closure rate. Successful closure rate in the present study was slightly less than the rate commonly reported in literature. A possible reason for this may be due to the smaller sample size.

Day of Life when Treated

Successful closure of the PDA with indomethacin can also be affected by the infant's age (DOL, days of life) when indomethacin treatment is begun. Clearance of indomethacin depends on postnatal age; the older neonate the faster the clearance. An echocardiogram to diagnose and/or confirm a hsPDA was routinely done on subjects in Boo et al. and Echtler et al. studies. The age (DOL) when infants in these studies were begun was earlier than the study reported here. In the present study, infants who developed signs and/or symptoms of hsPDA (tachycardia, murmur, bounding pulses, widened pulse pressure) received an echocardiogram for confirmation. Consequently, the DOL (mean) infants began indomethacin was 7.9 (SD = 3.2, range 2 to 17). This later date of treatment initiation may have affected (decreased) closure rate found in this study.

Table 4-4 Comparison of Studies' Variables and Outcomes

	Boo et al. (2006)	Echtler et al. (2010)	Heilskov (2015)
N (Total)	60	123	63
Open PDA (after treatment)	24	19	12
Closed PDA (after treatment)	36	104	51
Mean Gestation Age (week)			
Open PDA	28 (2.5)	26	26
Closed PDA	29.6 (2.7)	28	25.5
Mean Birth Weight (g)			
Open PDA	850	1,027	851
Closed PDA	1,070	1,175	777
Mean Platelet Count (1,000)			
Open PDA	125**	183.4 (38.8)	197 (96.7)
Closed PDA	232**	137.1 (69)	223 (87.2)
Day of life treated	3-5 (range)	7 (\bar{X})	8 (\bar{X})
Incidence PDA (per cent)	15.4	71	57
Closure Rate (per cent)	60	35	22

() Std. Deviation ** Median

Platelets

Neither study by Boo et al., or Echtler et al. defined low platelet groups. Rather, they computed correlations between low platelet counts and the successful closure of hsPDA with indomethacin. No correlation between platelet counts or any other variable (birth weight, gestational age, gender, maternal race, size of PDA, or DOL treatment

began) was found in this study. The small sample size of this study was under-powered and contributed to the failure to detect correlations or differences in the two groups (closed-PDA and open-PDA).

Traditionally, infants' whose platelet counts are less than 150,000/ μ L are considered to be thrombocytopenic (Roberts, Stanworth, & Murray, 2008). This value was derived because infants reach a platelet mean of 150,000/ μ L by the end of the first trimester and proceed to develop levels within the normal adult range by 22 weeks gestation (Sola-Visner, 2012). Consequently, even the most immature infant (at 22 weeks gestation, the lower end of viability) in a neonatal intensive care unit (NICU) has platelet count of 150,000/ μ L.

This idea was recently challenged by a large ($N = 47,000$) population study of neonates in a multi-hospital healthcare system. (Wiedmeier, Henry, Sola – Visner, & Christensen, 2009). The platelet counts of neonates from as young as 22 weeks gestation to term (41 weeks) were collected. Researchers found platelet counts increased with advancing gestational age, but the platelet counts of those infants born before 35 weeks gestation were significantly lower than late-preterm or term infants. Wiedmeier reported the mean platelet count for infants born at 26 weeks gestation and at one week of age (7 days) was $\sim 220,000/\mu$ L which is similar to the mean platelet counts of the open-PDA and closed-PDA groups in this study.

Limitations

The major limitation of this study was the small sample size. A power analysis for a study with a probability level of .05, power of .80 and small effect size (0.2) was calculated. The a priori sample size was 210 (105 infants/group). A total of 63 subjects were obtained.

Another limitation of the study was the data storage. Data prior to 2013 had recently been archived and not available for the study, which severely limited the sample size.

A secondary analysis of an existing data set limited the number of variables and data collected. For example, the variable day of life the platelet count was obtained would have been useful. Also the size of the PDA variable was documented in only 63 percent of the cases, limiting the usefulness of this data.

Finally, this convenience sample was obtained from the neonates admitted to an NICU in an urban hospital in north Texas limiting the study's generalizability.

Conclusions

Nineteen percent of the 63 infants in this study who were treated with indomethacin responded to treatment (closed PDA). Unlike two previous studies reported in the literature, no associations were found between platelet counts and closure of the DA after receiving indomethacin. Analyses of other variables reported in the literature to be associated with closure of the DA and indomethacin were also examined. No associations were found between birth weight, gestation at birth, size of PDA, and respiratory support. Demographic analyses of race and gender revealed no associations with failed treatment of PDA with indomethacin. There were also no significant differences between the two (closed-PDA and open-PDA) groups with regards to gender, race, birth weight, gestation at birth, size of PDA, and respiratory support.

Implications for Nursing

Premature babies who's PDA remain open are at an increased risk for pulmonary edema and prolonged ventilator support, broncho-pulmonary dysplasia, and heart failure. Understanding the mechanisms of functional closure in the DA is also important for those

term infants born with ductal-dependent congenital heart disease. While low platelet counts were not found to be associated with or a predictive indicator of failed indomethacin treatment for a PDA, lower platelet counts might be used as a predictor of hsPDA

Recommendations for Future Research

It has been suggested that impaired platelet function (adhesion and aggregation) due to immaturity and acute illness rather than platelet number contribute to PDA. Studies that examine platelet number *and* function are needed. Alternative approaches to the treatment/manipulation of a PDA, such as medications that target platelet function is needed. Further research should also examine other possible predictors of significant PDAs and their relationship(s) with successful treatment.

Summary

Patent ductus arteriosus remains a significant complication of prematurity. Less than 30 percent of infants born prior to 28 weeks gestation will spontaneously close their PDA (Narayanan, Cooper, Weiss, & Clyman, 2000). Persistent PDA results from either initial failure (no response) to medical treatment, or reopening of a previously closed PDA. Complications of persistent PDA result from the increase in pulmonary blood flow and decrease in systemic perfusion. Because none of the available treatment options are not without risk it is imperative to optimize successful closure. Further research of the role of platelets in the closure of the PDA may prevent unnecessary exposure to indomethacin.

Appendix A
Data Collection Tool

Demographic Data

Study ID #	Birth weight in grams	Gestation in weeks	Gender	Maternal Race
1				
2				
3				
4				
5				

Gender: Male =0, Female =1

Maternal race: White =0, Black =1, Hispanic =2, Other =3

Study Variables

Study ID #	BW (grams)	Gestation at birth in weeks	DOL at time of treatment	Size of PDA in mm	Number of indocin doses	Platelet count	Mode of ventilation	Status of PDA
1								
2								
3								

Platelet count: $\geq 100,000/\mu\text{L}$ = 0 < 100,000/ μL = 1

Mode of Ventilation: Ventilator = 0, CPAP = 1, NC = 2, RA = 3

Status of PDA: Closed = 0, Open 1

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Biographical Information

Joan Heilskov completed her undergraduate nursing education at the University of Iowa in 1979. She happily found her niche with her first job as a new graduate in the neonatal intensive care unit. She obtained a Master of Arts in Nursing degree and certification as an advanced practice pediatric nurse practitioner from the University of Iowa in 1992. In 1996 she received a post-graduate certification as an advanced practice neonatal nurse practitioner from St. Catherine's University in St. Paul, MN. While at St. Kate's she found the positive and supportive learning environment that would eventually lead her to pursue teaching. She received a Master of Science in Health Care Administration Degree from the University of Texas at Arlington in 2005.

Joan began her doctoral studies at the University of Texas at Arlington in 2008. While there she received the Graduate Dean Fellowship. Joan is a member of the international nursing honor society, Sigma Theta Tau and the international honor society of business programs, Beta Gamma Sigma.

After graduation Joan plans maintain her clinical practice as a neonatal nurse practitioner and to pursue teaching opportunities of graduate students. She hopes to continue her research into predictors of successful closure of patent ductus arteriosus in premature infants.

Joan has been married to her husband, Tom Wasolaskus for 14 years. They enjoy fly-fishing in New Mexico and Alaska. She enjoys cycling with her sister, Jane and hanging out with her hairdresser, David whenever possible. She is an ardent Iowa Hawkeye and Texas Ranger fan.