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PATENT DUCTUS ARTERIOSUS IN EXTREMELY PRETERM INFANTS – CHARACTERISTICS, RISK FACTORS AND TREATMENT DECISIONS

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Patent ductus arteriosus in extremely preterm infants – characteristics, risk factors and treatment decisions
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Í minningu föður míns, Guðmundar (1942-2016) og frænku minnar Siggu (1948-2015)

quid est ergo tempus? si nemo ex me quaerat, scio; si quaerenti explicare velim, nescio: non vere dicamus tempus esse, nisi quia tendit non esse.

What, then, is time? If no one asks me, I know what it is. If I wish to explain it to him who asks me, I do not know. Thus, can we not truly say that time is only as it tends toward nonbeing.

Hvað er tíminn? Þegar enginn spyr mig veit ég það. Sé ég spurður og beðinn að skýra það veit ég það ekki. Í rauninni er ekki unnt að segja að tíminn sé til nema af því að hann stefnir í að vera ekki til.

Confessiones, Augustinus

English translation: Albert C. Outler
Íslensk þýðing: Hr. Sigurbjörn Einarsson

ABSTRACT

Survival rates in infants born extremely preterm, before 28 weeks of gestation, are increasing and the focus has shifted towards decreasing morbidites after extremely preterm birth and promoting life-long health for the survivors.[1-3]Approximately 50-60% of extremely preterm infants have the past decade been treated for patent ductus arteriosus (PDA) to induce ductal closure.[4,5]In the abscence of ductal closure, hemodynamic changes follow which can result in significant systemic hypoperfusion and excessive pulmonary perfusion which has been associated with an increased risk of the neonatal morbidities such as intraventricular hemorrhage (IVH), necrotising enterocolitis (NEC), renal failure and and bronchopulmonary dysplasia (BPD).[6] Cyclooxygenase (COX) inhibitors, ibuprofen or indomethacin, are used as first line of treatment to promote ductal closure.[7,8] Surgical closure may be indicated if pharmacological therapy fails, or when contraindications to COX inhibitors are present.[9] The theoretical rationale for PDA treatment is unquestionable, but trials showing long-term benefits from PDA treatment are scarce.[10-12] In this thesis, paper I-III are cohort studies conducted in Sweden and in Europe. The study in paper IV is a hospital-cohort study in Stockholm. The overall aim of the studies is to investigate PDA incidence, neonatal characteristics associated with PDA closure or treatment, variation in treatment strategies, and association with neonatal outcomes. Furthermore, to evaluate the role of cardiac biomarkers in predicting PDA closure.

In paper I, the aim was to investigate if timing of pharmacological PDA treatment (at 0-2 days, 2-6 days or ≥7 days of age) was associated with risk of later PDA surgery or death; or risk for BPD at 36 weeks postmenstrual age (PMA). This was investigated in the population-based prospective Extremely Preterm Infants in Sweden (EXPRESS) cohort (infants born at <27 weeks of gestation during 2004-2007).[13] Two hundred ninety of 585 children were treated pharmacologically, of whom 102 later underwent PDA surgery. In a model stratified on GA and adjusted for clustering on region, hazard ratios (HR) for late and intermediate vs early start were 1.10 [CI 0.53–2.28] and 0.89 [CI 0.57–1.39] respectively. Compared to early start, the risk of BPD after late start of PDA treatment was associated with a significantly lower risk of BPD odds ratio (OR) 0.29 [CI 0.13-0.61] in a model stratified on gestational age (GA) and adjusted for sex and small for gestational age (SGA).

In paper II, the aim was to investigate incidence and variation in PDA treatment and association with BPD at 36 weeks PMA or death; and survival without major neonatal morbidity. This was performed in a large European cohort (the EPICE study) of infants born at <32 weeks of gestation during 2011-2012, including 6898 infants.[14] The results show that there is significant variation of 10% to 39% in PDA treatment between the different regions ($p<0.001$). The regions were categorized according to low (<15%, n = 6), medium (15–25%, n = 9), or high (>25%, n = 4) proportion of PDA treatment. The difference in PDA treatment could not be explained by differences in perinatal characteristics between these regions. Infants treated for PDA, compared to those not treated, were at higher risk of BPD or death in all regions, with an overall propensity score adjusted risk ratio of 1.33 [95% confidence interval 1.18–1.51]. Survival without major neonatal morbidity was not related to PDA treatment.

In paper III, the neurodevelopmental outcome after PDA treatment was studied. In the EXPRESS cohort (see paper I), the survivors at 6.5 years of age had an extensive neurodevelopmental follow-up.[15] Four hundred and thirty five of 486 children had available data on both PDA treatment and neurodevelopmental outcome. PDA treatment as an exposure was categorized as no PDA treatment; pharmacological PDA treatment; PDA surgery after prior pharmacological treatment; and primary PDA surgery. The outcomes studied were NDI (by the definition of Moore[16]) and the full-scale intelligent quotient (FSIQ) as measured by Wechsler Intelligent Scale for Children (WISC-IV[17]). No increased risks of adverse neurodevelopment were found among children treated pharmacologically for PDA, regardless of whether they later had surgical PDA closure or not. The risk of moderate to severe NDI was higher among children treated with primary PDA surgery in the adjusted model than in extremely preterm children not receiving PDA treatment, IRR 1.62 [95% CI 1.28-2.06] $p<0.001$ and a lower FSIQ, adjusted mean difference -7.1 [95% CI -11 till -3.2] $p<0.001$. Timing of PDA surgery was investigated as an exposure in the children undergoing PDA surgery. Children having PDA surgery at <10 days of age (irrespective if primary or after prior pharmacological treatment), compared to at >20 days of age, had increased risk for moderate to severe NDI, IRR 3.26 [95% CI 2.40 to 4.42] $p<0.001$; and adjusted mean difference of FSIQ -15 [95% CI-19 till -12] $p<0.001$.

In paper IV, the perinatal characteristics associated with spontaneous PDA closure were investigated in a hospital-based cohort of extremely preterm infants in Stockholm. The association of the biomarkers N-Terminal fragment-pro-Brain Natriuretic Peptide (NT-proBNP) and cardiac Troponin T (cTnT) with spontaneous closure and all types of PDA treatment was investigated. Fifty-eight of 98 infants were treated for PDA with a median age at start of treatment of 8 days (interquartile range, IQR 5-11). Six (6%) infants closed their PDA at ≤7 days of age. All infants who closed their duct spontaneously were born at ≥25 weeks of gestation. Higher NT-proBNP values on day 3 were associated with later need of PDA surgery and lower levels were associated with PDA closure without any PDA treatment.

In conclusion, there is large variation in PDA treatment across Europe which is not associated with perinatal characteristics. This indicates need of standardization of diagnostics and treatment. In all four studies, the strongest predictor for PDA treatment is GA and the spontaneous closure rate in extremely preterm infants born at ≥25 weeks is relatively high. A more conservative approach with later start of PDA treatment is not associated with increased risks of morbidity and PDA treatment is not associated with decreased risk of survival without major morbidities. PDA pharmacological treatment with or without later surgery is not associated with adverse neurodevelopment. PDA surgery at <10 days of age and primary PDA surgery are associated with increased risk of adverse neurodevelopment. More precise PDA diagnostic criteria and are needed in future studies. NT-proBNP may be useful as an additional parameter in combined scores of clinical and echocardiographic markers of ductal severity. Optimally designed, blinded placebo-controlled studies with a clear definition of the PDA exposure and related outcomes are needed to understand the PDA in extremely preterm population

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers. The papers will be referred to by their Roman numerals (I-IV).

I. A Gudmundsdottir, S Johansson, S Håkansson, M Norman, K Källen, AK Bonamy.Timing of pharmacological treatment of patent ductus arteriosus (PDA) and risk of secondary PDA-surgery, death or bronchopulmonary dysplasia-a population-based study of extremely preterm infants. Neonatology. 2015;107(2):87-92.

II. AK Bonamy, A Gudmundsdottir, RF Maier, L Toome, J Zeitlin, EM Boyle, M Norman and the EPICE Research Group. Patent ductus arteriosus treatment in very preterm infants – a European, Population-based Cohort Study (EPICE) on variations and outcomes. Neonatology; 2017; 111(4):367-375

III. A Gudmundsdottir, L Broström, B Skiöld, K Källén, F Serenius, M Norman, U Ådén, A-K Bonamy. Treatment for patent ductus arteriosus and neurocognitive outcome at 6.5 years of age in children born extremely preterm.

Submitted.

IV. A Gudmundsdottir, M Bartocci, O Picard, J Ekström, K Bohlin, C Attner, G Printz, M Karlsson, L-A Mohlkert, A-K Bonamy. Diagnostics and treatment strategies for hemodynamically significant patent ductus arteriosus (hsPDA) in extremely preterm infants – perinatal, echocardiographic and biomarker factors associated with closure.

Manuscript.

Related publication:

A Gudmundsdottir, S Johansson, S Håkansson, M Norman, K Källen, AK Bonamy.The Importance of echocardiography and an individual approach to patent ductus arteriosus treatment in extremely preterm infants. Neonatology. 2015 Feb 20;107:257.

CONTENTS

LIST OF ABBREVIATIONS

Ao	aorta
BPD	bronchopulmonary dysplasia
CI	confidence interval
CPAP	continuous positive airway pressure
CRIB	The Clinical Risk Index for Babies
cTnT	cardiac Troponin T
DA	ductus arteriosus
EPICE	Effective Perinatal Intensive Care in Europe
EXPRESS	Extremely Preterm Infants in Sweden Study
FSIQ	Full-Scale Intelligence Quotient
GA	gestational age
HR	hazard ratio
hsPDA	hemodynamically significant Patent Ductus Arteriosus
IQR	interquartile range
IRR	incidence rate ratio
IVH	intraventricular hemorrhage
La:Ao	left atrial:Aortic
LPA	left pulmonary artery
NDI	neurodevelopmental impairment
NO	nitric oxide
NSAID	non-steroidal anti-inflammatory drug
NT-proBNP	N-terminal pro b-type natriuretic peptide:
OR	odds ratio
PA	pulmonary artery
PDA	patent ductus arteriosus
PEEP	positive end expiratory pressure
PG	prostaglandin
PGE_2	prostaglandin E_2
PMA	postmenstrual age,
PNA	postnatal age

pPROM	preterm premature rupture of membranes
PVL	periventricular leukomalacia
RPA	right pulmonary artery
RR	risk ratios
SD	standard deviation
SDS	standard deviation score
SGA	small for gestational age
TNF-alfa	tumor necrosis faktor-alfa
Vmax	maximal velocity
Vmin	minimal velocity
WISC-IV	Wechsler Intelligence Scale for Children, Version IV

1 INTRODUCTION

Prematurity is defined by the World Health Organization as birth before 37 weeks of gestation.[18] It is further categorized into: late or moderately preterm infants, born at 32-36 weeks; very preterm born at less than 32 weeks of gestation and extremely preterm born at less than 28 weeks.[19] Preterm birth rates vary between countries but a global estimation rate is approximately 11%.[19] In Europe, a rate of 5-11% has been established and in Sweden preterm birth accounts for 5-6% of all births and extremely preterm births account for 0.3-0.4% of all births.[19-21] In the last decades, advances in neonatal intensive care have greatly increased the survival in this group, particularly in countries with a highly developed health care system. Infants born extremely preterm have a higher risk of morbidity and mortality compared to those born at term.[4,13] With increasing survival rates in this group, the focus has shifted more towards decreasing morbidity and promoting life-long health for the survivors of extremely preterm birth. Patent ductus arteriosus (PDA) is one of the factors that can lead to morbidity as it is the most common circulatory problem in extremely preterm infants.[6] Historically 50-60% of infants born before 28 weeks of gestation have been treated for PDA to induce ductal closure.[4,5] In the abscence of ductal closure, hemodynamic changes follow where blood is shunted from the systemic circulation to the pulmonary circulation. In severe cases this results in significant systemic hypoperfusion and excessive pulmonary perfusion which has been associated with an increased risk of the neonatal morbidities such as intraventricular hemorrhage (IVH), necrotising enterocolitis (NEC), renal failure and and bronchopulmonary dysplasia (BPD).[6,7] Until recently, active PDA closure was considered beneficial.[10,11,22] Cyclooxygenase inhibitors, ibuprofen or indomethacin, are used as first line of treatment to promote ductal closure.[7,8] Surgical closure may be indicated if pharmacological therapy fails, or when contraindications to cyclooxygenase inhibitors are present.[9,23] The theoretical rationale for PDA treatment is unquestionable, but trials showing long-term benefits from PDA treatment are scarce.[10] Moreover, surgical closure has been associated with adverse outcome though it has been debated that these studies have had problems with confounding by indication.[24-28]On the other hand, spontaneous PDA closure has recently been shown to be high, even among the most immature infants.[29-32] Early treatment of PDA might thus expose infants, in whom ductus ultimately would close spontaneously, to unnecessary treatment with potent and potentially toxic drugs. The overall aim of this project is explore the incidence of PDA treatment in cohorts of very and extremely preterm infants, risk factors for treatment and associations with neonatal outcomes. Furthermore, the aim is to explore clinically relevant and objective diagnostic criteria for PDA in infants born extremely preterm. The purpose is to better characterize infants in whom benefits of treatment might outweigh the risks with treatment, both in the short- and long-term.

2 BACKGROUND

2.1 PATENT DUCTUS ARTERIOUSUS

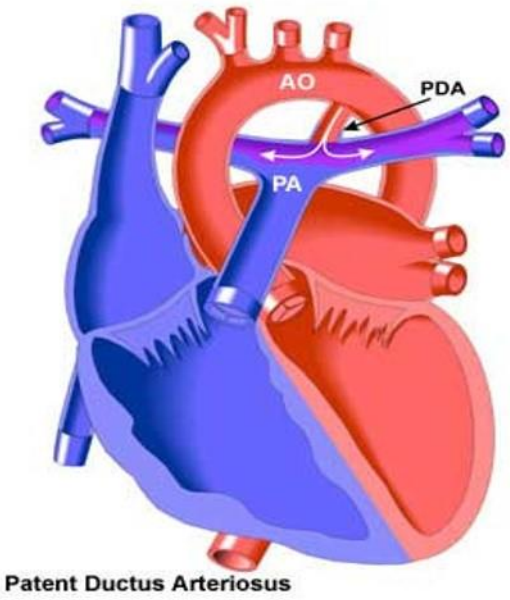

Figure 2.1. A four chamber view of the heart with the right and left atriums and right and left ventricles communicating with the great vessels, the pulmonary artery (PA) and aorta (Ao). The ductus arteriosus (DA) is a vessel connecting the PA and the Ao.
Published with permission from ©Stefan Johansson.

The ductus arteriosus (DA) is a fetal vessel that connects the main pulmonary artery (PA) with the descending aorta (Ao) and is necessary for the fetal circulation.[33] In fetal life, the diameter of the DA is similar to that of the the descending Ao.[6] Functionally, the DA provides a bypass of the pulmonary blood flow to the descending Ao. i.e. the blood streaming from the placenta to the right atrium through the foramen ovale, to the left atrium and further to the left ventricle and out to the Ao. From the right ventricle the blood flows to the PA and as the resistance is high there, it flows through the open duct to the descending Ao.[6] In the newborn infant the DA usually closes in the first one to three days after birth.[33,34] A patent ductus arteriosus is when the DA closure is delayed (PDA). Ductal closure may be delayed in infants born with duct dependent congential heart diseases or persistent pulmonary hypertension for example due to meconium aspiration, congenital sepsis or asphyxia.[6] Furthermore, a reason for a PDA can be congenital, i.e. an anatomical PDA.[34] When the PDA has hemodynamic consequences it is defined as a hemodynamically significant PDA (hsPDA).[34,35] It has become more evident that genetics are a contributing factor in ductal patency in term and probably in preterm infants also.[33,36] In term infants an incidence of

approximately 0,5% has been described and it is more common in females than males.[37] Finally, ductal closure is delayed in preterm infants compared to full term infants.[6,33,34]

- Ductus arteriosus (DA): It is normal to be born with an open DA and during normal transition it closes spontaneously within the the first few days.
- Patent ductus arteriosus (PDA): The delayed process of closure results in a PDA.
- Hemodynamic significant ductus arteriosus (hsPDA): A PDA with hemodynamic consequences on the heart and circulation is defined as a hsPDA.

2.2 MECHANISMS OF DUCTAL CLOSURE IN FULL TERM AND PRETERM INFANTS

Fetal life

During later stages of pregnancy, the vasodilating prostaglandins (PG), especially prostaglandin E_2 (PGE_2)maintain the ductus arteriosus (DA) open.[6]

At birth in full term infants

Ductal closure is described as occurring in two phases: functional and anatomical, with the former taking place during the first hours of life and the latter in the first week(s) of life.[6] The mechanisms conducting the closure are initiated and balanced by many factors.

• Functional closure occurs when the smooth muscle cells in the vessel wall constrict and the lumen narrows. This is determined by balancing factors such as oxygen tension, levels of vasoconstrictors (such as endothelin and catecholamines) and levels of vasodilators (such as PGs and nitric oxide (NO)), and finally the intraluminal pressure in the DA.[33,38,39] After birth there is a decrease in circulating PGE_2 due to increased removal by the lungs together with the cease in supply from the placenta once the umbilical cord is clamped.[6] Also, the PGE_2 sensitivity in the ductal wall decreases.[6,33,34] Furthermore, in the initial functional closure, platelets are believed to have a role in "sealing" the DA, which further promotes closure.[33,40]
• Anatomical closure is the remodeling of the lumen and vessel wall tissue, which follows the initial functional constriction of the duct.[41,42]

At birth in preterm infants

In preterm infants, especially the most immature group, the normal signals impaired and the mechanisms for ductal closure are delayed (discussed later).[6,33,34,41,43,44] The circulatory transition after birth together with the immature lungs is part of the explanation for this.[33]

Furthermore, it has been shown that the DA in preterm infants is more sensitive to the vasodilating effects of PGE_2 as well as NO.[45] This impairs the anatomical closure, as the vasoconstriction of the vessel is not prominent enough to induce the anatomical changes necessary for permanent closure.[33]

2.3 TREATMENT STRATEGIES FOR PDA CLOSURE

In term infants with an anatomical hemodynamically significant patent ductus arteriosus (hsPDA) intervention to close the duct is often indicated. The standardized procedure is to close the duct with percutaneous catheterization. This is done after a time of observation, and often when the child is up to 18-24 months of age or even older.[46]

In extremely preterm born infants (<28 weeks) the recommendation to close the PDA pharmacologically has almost developed to become standard of care (even in infants born at 28-32 weeks).[8] Indomethacin and ibuprofen are cyclooxygenase (COX) inhibitors leading to a reduction of PG-mediated vasodilation in the ductal wall.[7] The efficacy of these two drugs is comparable but the side effect profile is different as ibuprofen is believed to have less side-effects on renal and mesenteric circulation.[8,47] Paracetamol as a treatment for PDA has evolved recently. Moderate evidence suggest that it is as effective as ibuprofen.[48] Studies in infants <28 weeks of age are lacking. Furthermore, concerns have been raised on prenatal and postnatal exposure to paracetamol and neurodevelopment and further studies are recommended.[48]

In case of pharmacological treatment failure for hsPDA, surgical strategies are usually warrented. This has mostly been performed with an open thoracotomy, but video-assisted thoracoscopy techniques have evolved.[9,49] It has been the general recommendation to close the PDA after prior pharmacological treatment in infants with a hsPDA.[23] Primary PDA surgery has been a strategy but is no longer recommended, unless pharmacological treatment is contraindicated.[9,23] These strategies have been subject to discussion in recent years. Percutaneous catheterization techniques for PDA closure have evolved as a treatment option recently. This technique is still in its research stadium for infants with very low birthweight (1000-1500g).[9,46]

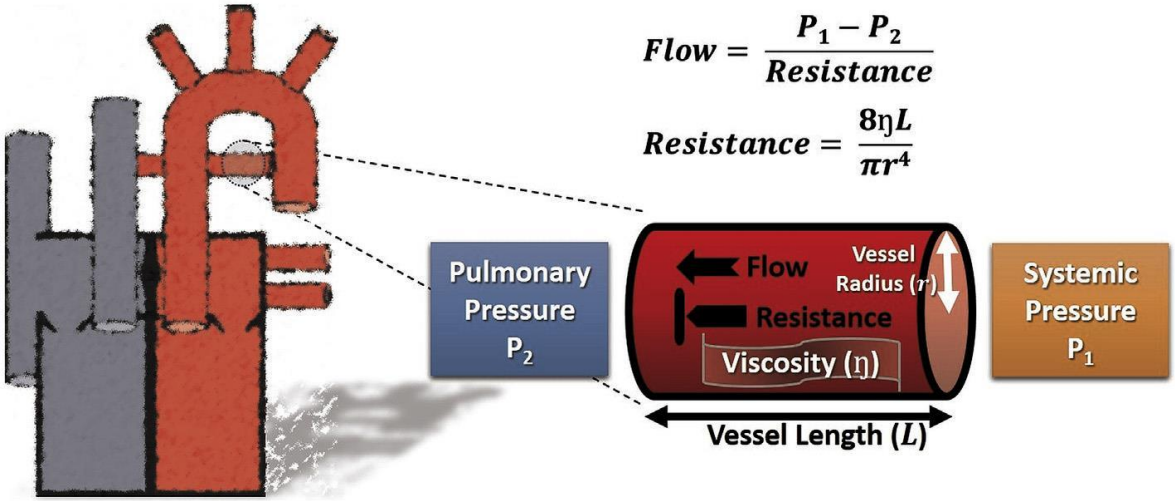

Figure 2.2. Determinants of the flow through the duct. Printed with permission from ©Elsevier. Ref. Smith et al. Seminars in Fetal and Neonatal Medicine. 2018.(23)245-249.

2.4 DETERMINANTS OF THE FLOW THROUGH THE DUCT AFTER BIRTH

Flow through vessels is determined by Poiseuille´s law as illustrated in Figure 2.2.[50] Resistance is dependent on the vessel radius, the length, and the viscosity of the blood that flows through the vessel.[50] For the DA, the pulmonary and systemic pressures are the governing factors for the pressure difference and flow direction through the DA.[6] Furthermore, vasoconstriction and vasodilation of the DA change the radius.[6] These constantly changing dynamics factors make estimation of the shunt through the duct difficult.

- The postnatal decrease in pulmonary resistance results in changes of ductal flow from purely right to left in utero, to bidirectional during the transitional period, and purely left to right there after.[6]

- In full term infants, smooth muscle cells in the ductal wall react to the oxygen tension changes postnatally, where higher oxygen tension triggers ductal closure.[33]

Infants born extremely and very preterm are often very sick after birth and the postnatal transition can differ from full term infants. Here are a few examples of the dynamic nature of the flow through the duct in this group.

- Directly after birth or during the first hours of life, many preterm infants develop respiratory distress syndrome and are treated with surfactant. This has not only effect on the ventilation mechanics, but also on the pulmonary and systemic circulation.[6,51] As the lung opens and aerates, the pulmonary resistance decreases and the shunt, often bidirectional or right to left, changes to left to right through the duct.[51]

- The ventilator strategy on invasive and non-invasive ventilation such as continuous positive airway pressure (CPAP) with the degree of positive end-expiratory pressure

(PEEP) can modulate the ductal shunt, i.e. higher PEEP decreases the shunt from left to right with increased pulmonary pressure.[52] The shunt through the duct can be modified by changing the respiratory mode.[6]

- Vasoactive mediators affect the ductal constriction.[33] It has been described that in context with infections, the vasoactive inflammatory mediator; tumor necrosis factor alfa (TNF-alfa) is associated with delayed duct closure and even re-opening.[53]

For the clinicians that care for the extremely preterm infants, the constantly changing "dynamic nature of the ductal shunt" due to these factors, becomes evident.

When is the shunt through the duct of such a magnitude that it negatively impacts the infant? In approaching this, the definition of a hsPDA evolved.[11,35] As shown in **Figure 2.4** and described above, all infants are born with an open DA but not all develop pathological symptoms related to it. It has been intensively debated which predictors are most relevant of a significant ductal shunt in the population of extremely preterm infants. Clinical signs have been shown to evolve late, when the infant has already developed a significant shunt.[54] The role of echocardiography as a diagnostic tool in confirming non-closure of the duct and staging the ductal severity has been shown to be of importance. Echocardiographic markers are as a proxy of the ductal flow and shunt magnitude but do not always account for the dynamic changes.[6,55]

2.5 ECHOCARDIOGRAPHIC DIAGNOSTICS OF THE PDA

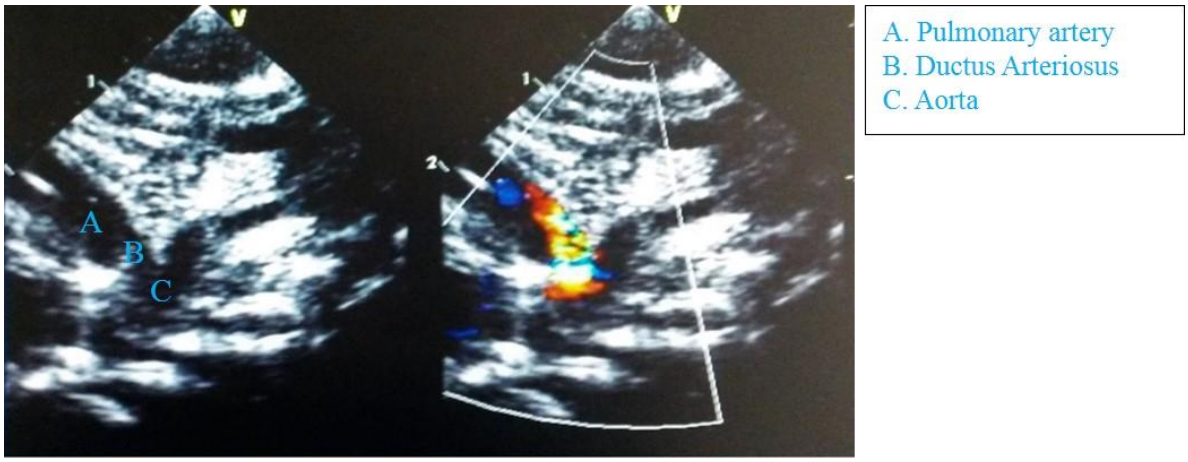

Figure 2.3. Ductal view on two dimensional echocardiography and color Doppler. ©Anna Gudmundsdottir.

Echocardiography remains the golden standard for confirming the presence of a PDA.[56] Furthermore, by using echocardiographic surrogate markers, it is possible to estimate the excessive pulmonary circulation and systemic hypoperfusion due to the ductal shunt.[35,55] In **Table 2.1** the most common echocardiography markers and the method used to acquire them are detailed. This is the base for the definition of the echocardiographically significant PDA, i.e. a moderate to large duct with significant signs of shunt and increased left ventricular

output.[35] With more advanced echocardiography, further estimations of left ventricular output and myocardial performance are possible.[57]

The markers can be categorized into ductal size and flow characteristics, signs of excessive pulmonary circulation and systemic hypoperfusion (end-organ steal), as well as heart function and size of left atrium and ventricle.[35,55,58-63] The studies underlying these criteria are mostly from the first week of life in extremely and very preterm infants (born at less than 30-32 weeks of gestation). In a newly published study, the conventional echocardiographic PDA markers only showed a fair correlation with a significant ductal shunt at more than 7 days of age so further investigation is needed.[64]

In Table 2.1 the most common echocardiographic markers are described

Table 2.1. Commonly used echocardiographic markers for hemodynamic significant PDA (hsPDA)			
	Echocardiographic criteria and cut-off values	Echocardiographic projection	Ref.
Ductal characteristics			
Diameter	Diameter of the duct (>1.5mm)	Ductal diameter acquired with two dimensional (2D) mode at the narrowest diameter of the ductal pulmonal end, in high parasternal short axis (ductal view)	[35,59]
Direction of flow through duct	Left to right shunt (L->R)	Colour Doppler (CD) used to detect the PDA and the direction of the flow (L->R, bidirectional or R->L)	[35,58]
Flow characteristics	Pulsatile flow through the duct with a non-restrictive flow profile	Duct visualized from ductal view. Velocity through duct measured either with Pulsed Wave Doppler (PWD) or Continuous Wave Doppler (CWD)	[35,58]
Excessive pulmonary flow			
Left atrium	Left atrial to Aortic root (La:Ao) ratio (>1.5)	Left atrial diameter determined in 2D-mode according to guidelines and standards in a parasternal long axis view	[55,61]
Pulmonary flow characteristics	End-diastolic flow in LPA/RPA (>0.2m/s)	Diastolic forward flow (velocity) with PWD in RPA and LPA from a high parasternal short axis view	[35,55]
Mitral valve function	Mitral valve early phase to atrial phase velocity ratio (E:A) (≥1 signifies increased pulmonary venous return and left atrial pressure loading)	Colour Doppler and PWD obtained in apical view.	[35]
End-organ ductal steal			
Ductal steal signs	Reversed diastolic flow in descending aorta /reversed or absent flow in mesenteric artery and/or cerebral arteries (ACA, MCA)	PWD used to detect reversed diastolic flow in descending thoracic aorta and images acquired from a high parasternal view. The subcostal view used for abdominal aorta or mesenteric diastolic reversed or absent flow. PWD from cerebral arteries (ACA) assessed for reversed or absent diastolic flow.	[35,65]
Heart failure and ventricular enlargement			
Left ventricle	Signs of enlargement	Left ventricle diameter was determined in 2D-mode according to guidelines and standards in a parasternal long axis view	[62]
Cardiac output	Left ventricle output (LVO) = Stroke volume (SV) x heart rate/minute If >300ml/kg/min considered significant	Measured in apical 4 chamber view and parasternal long axis view. SV = π x AoV annulus^2/4 x VTI $_{LVOT}$ (AoValve annulus hinge point).	[35]
Abbreviations: hsPDA: hemodynamically significant Patent Ductus Arteriosus, 2D: Two dimensional, La: Left Atrial, Ao: Aorta root, LPA: left pulmonary artery, RPA: right pulmonary artery, PWD: pulse wave Doppler, CWD: continuous wave Doppler, ACA: anterior cerebral artery; MCA: middle cerebral artery. LVO: left ventricle output, VTI: Velocity time integral. AoV: Aorta Valve Annulus. LVOT: Left ventricular outflow tract.			

2.6 CLINICAL DIAGNOSTICS OF THE PDA

Signs of a significant PDA are not always evident during the first days but appear later. In the definition proposed by McNamara and Sehgal in 2007 a hemodynamic significant PDA fulfills both echocardiographic and clinical criteria.[35] The problem is that the symptoms may differ depending on the infant´s age, i.e. not being the same during the first 24 hours of life, at 3 days of age or at >7 days of age. In Table 2.2, the clinical criteria most often used are detailed.

Table 2.2. Clinical signs of a hemodynamic significant Patent Ductus Arteriosus (hsPDA)

	Comment
Heart murmur	
Bounding femoral pulses	Non-specific
Number of apneas and bradycardias	Non-specific
Need for respiratory support, failed extubation	Both invasive (ventilator) and non-invasive (CPAP, nasal cannula)
Fraction of inspired oxygen (FiO_2)	No specific cut off limits. For PDA surgery
Signs of pulmonary congestion on X-ray	Need of diuretics
Circulatory failure	Hypotension, need of inotropes
Feeding intolerance	Proportion of enteral to parenteral nutrition
Renal failure, oliguria	Creatinin and urea value, diuresis in mL/kg/day or hour
Ref.	[7,35,66]

2.7 BIOMARKERS AS DIAGNOSTIC TOOLS FOR PDA

Recently, cardiac biomarkers have been investigated and proposed as complementary tools in PDA diagnostics.[67,68] The biomarkers of most interest are N-Terminal fragment-pro-Brain Natriuretic Peptide (NT-proBNP) and cardiac Troponin T (cTnT).[68]In adult and pediatric cardiology, they are used to evaluate heart failure and myocyte injury, such as in myocardial infarction.[69-71] The most well-studied biomarkers are the peptide NT-proBNP which is biologically inactive which is secreted together with the biologically active natriuretic peptide BNP.[67] They are released in response to ventricular and atrial wall stretching and used generally as[67] markers of congestive heart failure. Of particular interest is how BNP is used in determining the effect of mitral regurgitation on left ventricular dysfunction and predicting symptom onset.[72] This in some ways resembles how the effects of the left to right shunt through the duct affects the left ventricle and atrium in infants with a hemodynamically significant shunt.[73] NT-proBNP is the biomarker preferably used. In term healthy infants,

NT-pro-BNP has been shown to rise during the transition period after birth, peaking in the first 48 hours and then decreasing the following days.[74]

cTnT is a protein in the cardiac muscle that is secreted in response to myocyte injury.[67] It has been associated with respiratory disease morbidity (pulmonary hypertension, RDS) and circulatory failure in infants, caused by for example PDA, both in term and preterm infants.[75,76] It is used as a marker of perinatal asphyxia in term infants.[77] It has been postulated that the correlation between PDA and cTnT levels stems from myocardial ischemia due to the ductal steal of blood flow affecting the coronary arteries especially in preterm infants.[78]

Both NT-proBNP and cTnT have been associated with myocardial function in the preterm population and correlated with echocardiographic markers.[76] The analysis methods are based on immunochemistry methods and are in general the same for cTnT between laboratories, but not for NT-proBNP where the analysis may differ between laboratories.[67,68]

The use of biomarkers as a complement to scoring systems is an attractive approach, as they are neither dependent on echocardiographic resources, nor associated with inter-examiner variability. Studies on NT-proBNP values in infants with hsPDA have detected associations with PDA treatment or not as well as decreased values after treatment.[79,80] As the immunochemistry assay methods for NT-proBNP can differ between laboratories, it has been difficult to compare the levels in different studies in infants with a hsPDA.[67] Furthermore, few of these studies have included the most immature infants, born at ≤ 25 gestational weeks, although these infants have the most complex hsPDA.[67] Higher levels of NT-proBNP have also been correlated to BPD.[81] However, these studies suffer from the lack of consistent definitions of hsPDA, as well as the variation in laboratory methods used.[67,68]

Interestingly, NT-pro-BNP and cTnT have been shown to predict both short term adverse outcome (IVH grade 2 or higher /cPVL or death) and neurological outcome at two years of age in preterm infants with PDA in the neonatal period.[82]

2.8 PATHOPHYSIOLOGIC CONSEQUENCES OF THE DUCTAL SHUNT AND RELATION TO OUTCOMES

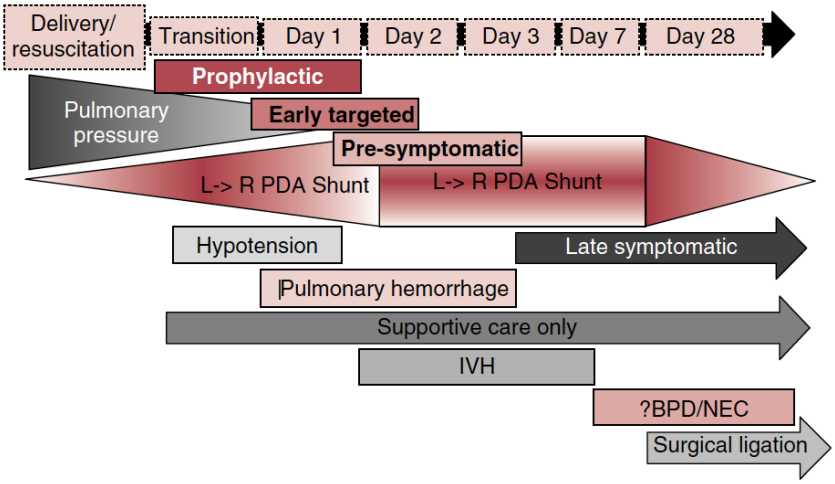

Figure 2.4. **The physiological effects of the shunt on the preterm circulation are shown and where the potential treatment strategies are applied.** Printed with permission from M. Kluckow and ©Elsevier. From Hemodynamics and Cardiology Elsevier series. Editors I. Seri and M. Kluckow. 2018 Fig 25.3 in Ch 25.

The changes after birth and during the transition with the decrease in pulmonary pressure and the changes from bidirectional to mainly left to right shunt are shown in **Figure 2.4**. In extremely preterm infants, already during the first day the shunt is mainly left to right. The hemodynamic effects are an excessive flow (hyperperfusion) in the pulmonary circulation and hypoperfusion in the brain and systemic circulation with risk for pulmonary hemorrhages and IVH's respectively.[6] Furthermore, the ductal shunt is a factor in the hypotension often seen in extremely preterm infants during the first 24-48 hours though the abrupt stop for placental flow is probably the main factor.[6]

Later, with unlimited left to right shunt through the duct, as a result, reversed flow (steal) may occur in the systemic circulation both in the mesenteric and brain circulation, both theoretical risk factors for NEC and PVL respectively.[6] The excessive lung flow has been associated with the risk of BPD.[6,83]

2.9 PDA TREATMENT STRATEGIES IN CONTEXT WITH DUCTUS PHYSIOLOGY

It is debated how the treatment strategies are associated with the outcomes, and which strategies prevent (if possible at all) the outcomes of interest. The neonatal morbidities most studied in association with PDA are: IVH, pulmonary hemorrhage, necrotizing enterocolitis (NEC) and BPD. Later neurodevelopmental outcome is always a concern in the extremely preterm population and the association with such a common exposure as PDA is important.

Prophylactic treatment (without an echocardiography) with indomethacin was applied but as it has not been shown to have long-term benefits it is generally not applied.[84]

- Early targeted treatment, where an early echocardiography is performed to confirm a PDA) has been applied in Australia and has been shown to reduce pulmonary hemorrhages.[85]
- Pre-symptomatic treatment before 72 hours of age, where echocardiographic classification is the cornerstone of the selection of infants for treatment. This is probably the most commonly applied strategy the past years.[7]
- Late symptomatic start of treatment, at about one week of age or later has been gaining more interest.[7] The questions have been raised if the defined echocardiographic criteria are adapted to this strategy.[11,64]
- Surgical treatment is reserved for infants not responding to pharmacological treatment or having contraindications to it. It is most often performed at a few weeks of age (3-6 weeks)[9]
- Conservative treatment, with nasal CPAP, diuretics and volume restriction is widely practiced but so are far not fully evaluated.[50]

2.10 PDA AS AN EXPOSURE AND ASSOCIATED OUTCOMES

In a paper by Zonnenberg et al the diagnostics underlying the PDA exposure in trials are discussed.[86] Wide variation was found, as some are based solely on clinical symptoms (for example heart murmur), others on echocardiographic markers and still more have a comprehensive approach with the combination of echocardiographic markers and clinical signs.[86] The diagnosis is the underlying factor for the decision to treat, which is the most common PDA exposure used.

In many randomized-trials and cohort studies on PDA in the preterm population, the exposure is defined as PDA treatment or not (yes/no). This dichotomized exposure does not account for the ductal severity, which is more of a spectrum. Most studies do have information on PDA treatment categories, pharmacological with or without PDA surgery, but very few until now have information on conservative treatment of the PDA.

The "true" incidence of PDA in the very and extremely preterm population is yet to be established. Publications on the spontaneous PDA closure rate in very and extremely preterm infants are appearing.[30-32] In a European cohort study, with low rates of PDA treatment, the median time to ductal closure varied from 6 days in infants born at ≥30 weeks GA, 8 and 13 days for infants born at 26-30 weeks to 71 days for infants born at <26 weeks.[29]

The incidence of PDA treatment in the very and extremely preterm population has been changing the past 15 years. In the EXPRESS Swedish cohort study, 61% of the infants born at <27 weeks of gestation were treated for PDA, either pharmacologically with or without later surgery or primary PDA surgery.[4] Similar treatment rates from Europe, North-America and Japan have been described for the same period.[3,87-89] Many studies have reported variation and changes over time in treatment strategies. The NICHD Neonatal Research Network in the USA has described variation between participating centers in PDA diagnosis and all types of PDA treatment during the years 2003-2007.[3] There was three and four-fold

variation in pharmacological PDA treatment and PDA surgery respectively during this period.[3] From the Canadian Neonatal network declining rates of PDA treatment have been reported between 2012 and 2016.[90] This is confirmed by Bixler et al where changes in PDA treatment strategies over time in the US from 2006 to 2015.[5] The most pronounced changes have been in the PDA surgery strategy, with rates falling from 8.4% to 2.9%.[5] Further analyses of associations with morbidities was not described in detail.[5]

PDA surgery strategies are controversial, as it is a complicated procedure in a vulnerable population. Strategies have varied from early surgery after failed pharmacological treatment to later surgery after a long observational period of the infant. Prophylactic and early surgery at <10 days of age have been associated with poor outcomes, such as BPD and adverse neurodevelopment.[91-94] The clinical discussion now is focused on selecting the right infants for surgery. Standardization, and a system for categorizing and triaging infants in a referral center in Toronto Canada resulted in improved outcomes after PDA surgery.[95]

PDA and association to neonatal mortality and morbidities

A causal relationship has not been confirmed between PDA or hsPDA and short- and long-term outcomes. The following outcomes will be discussed: mortality, IVH, pulmonary hemorrhage, NEC, BPD and neurodevelopmental outcome.

Mortality

Survival after extremely preterm birth has increased the past decades. In the EXPRESS 2004 to 2007 cohort of infants born at <27 gestational weeks, one-year survival among live-born infants was 70%.[13] In a newly published article from the same group, the survival has increased to 77% in a population based Swedish cohort from 2014-2016.[1] Similar reports are published from other countries.[2,3] Survival is strongly associated with gestational age (GA) as earlier reported.[13] Infants having a hsPDA on day 3 have been shown to have increased risk of later mortality.[96,97] As mortality is highest in extremely and very preterm infants during the first week, it has to be accounted for when examining PDA outcomes in this group.[98]

IVH

The association of significant ductal steal shortly after birth followed by brain hypoperfusion, with risk of IVH is known.[6,99]

Pulmonary hemorrhage

In a randomized trial of early targeted PDA treatment, where an early echocardiography is performed to confirm a PDA there was a decrease in pulmonary hemorrhages in the group receiving early PDA treatment with indomethacin.[85]

NEC

Theoretically the ductal steal leads to ischemia in the gut of infants with a hsPDA. The association of PDA and later NEC has not been easy to study, but an association has been described.[100,101]

Bronchopulmonary dysplasia (BPD)

BPD is most commonly defined in PDA studes as oxygen need at 36 weeks postmenstrual age (PMA).[102] Previous studies have suggested that early PDA treatment leading to PDA closure is beneficial in terms of decreased pulmonary morbidity and development of BPD.[103,104] It is still unclear if there is a causal relationship between PDA and BPD. In a randomized trial of early versus later ibuprofen treatment, Sosenko et al. did not find any differences in BPD incidence or mortality between the two groups.[105] Moreover, in the Trial of Indomethacin Prophylaxis in Preterm infants, indomethacin prophylaxis reduced the incidence of PDA, but did not change the BPD incidence[93]. PDA surgery has often been associated with BPD, but in the same manner, it has been complicated to disentangle the association.[83]

Neurodevelopmental outcome

Various cohort studies have shown an association between PDA treatment and later adverse neurodevelopmental outcome.[24,25,27] PDA surgery, especially, has been associated with adverse neurodevelopmental outcome but this is constantly debated.[98] Neurodevelopmental outcomes are most often determined with Neurodevelopmental Impairment (NDI) as defined by Moore et al.[16] NDI is categorized into none, mild, moderate or severe and includes hearing impairment, visual impairment, presence of cerebral palsy (as defined by Gross Motor Function Classification System) and cognitive assessment according to the appropriate test for age.[16] A common age to investigate neurodevelopmental outcome is 18-24 months using Bayley Scales of Infant Development.[106] Evaluation of cognitive development is more comprehensive at school-age compared to two years of age. Assessment with for example Wecshler Scales of Child Development (WISC) at 6.5 years of age is often used.[17] The overall results are presented as full-scale intelligent quotient (FSIQ) and usually compared with a standard population or matched controls (distribution of the mean and standard deviations).

2.11 EPIDEMIOLOGICAL CONCERNS IN PDA STUDIES

Epidemiologic terminology of importance for the studies on PDA in this thesis.

1. Confounding: As defined by Rothman is a mixing of effects that is most often imbalanced between the the groups being compared (exposed and non-exposed).[107] A confounder must be associated with the disease but may not be an effect of the disease. A confounder must also be associated with the exposure.[107]

2. Bias: A systemic error in studies is called bias. It can be introduced when designing the study, by choosing the measurement for the outcome or if a confounding factor is not completely controlled for.[107]

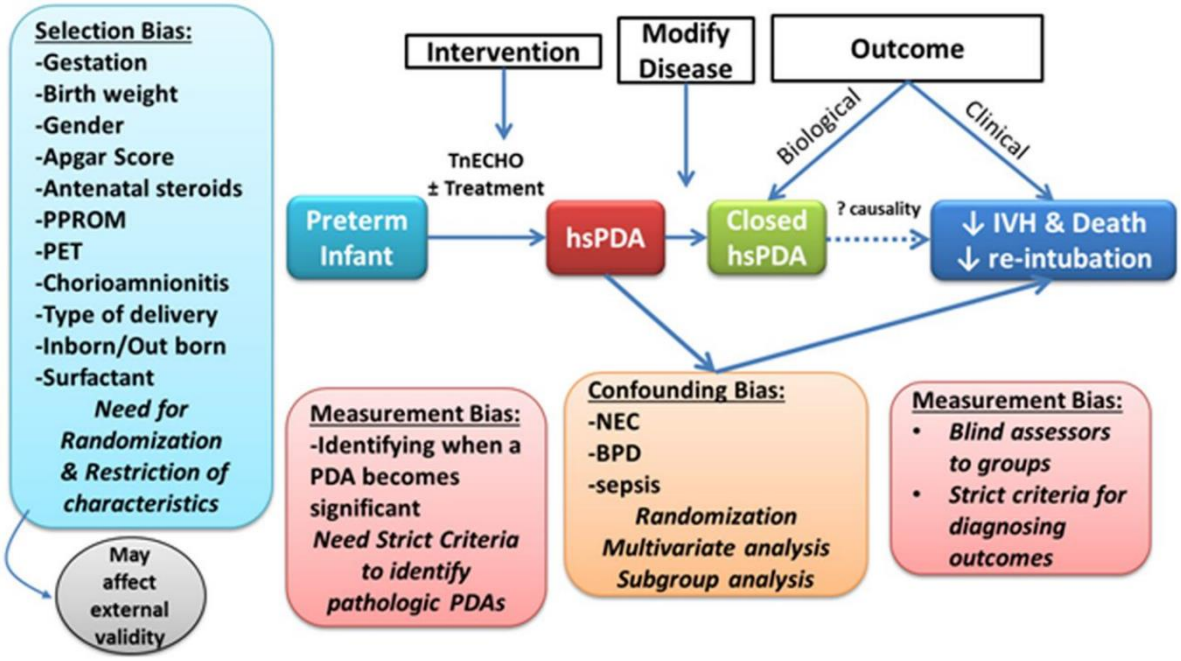

Figure 2.5. In this flow chart possible clinical outcomes in an extremely preterm infant developing a hsPDA are shown and where multiple factors can introduce bias. Causality has not been confirmed in any studies of PDA and outcome, but associations have been detected. Possible timepoints for intervention and modifying disease are noted as well as possible methods to handle bias in future studies on PDA. Published with permission from ©Elsevier. Ref. El-Khuffash et al. Arch Dis Child Fetal Neonatal Ed. 2016.101(5):F474-8.

Abbreviations: hsPDA: hemodynamically significant patent ductus arteriosus. pPROM: preterm premature rupture of the membranes. PET: pre-eclampsia. TnECHO: targeted neonatal echocardiography. IVH: intraventricular hemorrhage, NEC: necrotizing enterocolitis. BPD: bronchopulmonary dysplasia.

In Figure 2.5 by El-Khuffash et al.[108], epidemiologic factors associated with PDA are explained. In preterm infants, mainly extremely preterm infants the best way to study PDA as an exposure is to use predefined echocardiographically and clinical criteria of when it is pathological.[11,108] In many published studies as well as the studies in this thesis the exposure is PDA treatment and the exact criteria for treatment are not always clear. It is important to have a well characterized cohort with optimal, predefined perinatal and early neonatal data as well as the temporal relation to morbidities such as sepsis.[28] This is important when

understanding the associations with the outcomes measured. Possible entries of bias are many. Selection bias, which can be handled with randomized trials or by restricting the studies to the population most representative for PDA problems (i.e. <26-28 weeks).[107,108] Measurement bias is for example when ductal significance is measured using different criteria. This leads to heterogeneity in the classification and can be called a misclassification bias.[109] Bias due to confounding can be handled with thourough data collection of potential confounders and in selecting appropriate statistical analyses.[107,108] Lastly, choosing the appropriate outcomes and the best method to measure them are equally important.[108]

Following is an example of the PDA exposure and the outcome in question in paper III where possible covariates and confounders are illustrated.

Example

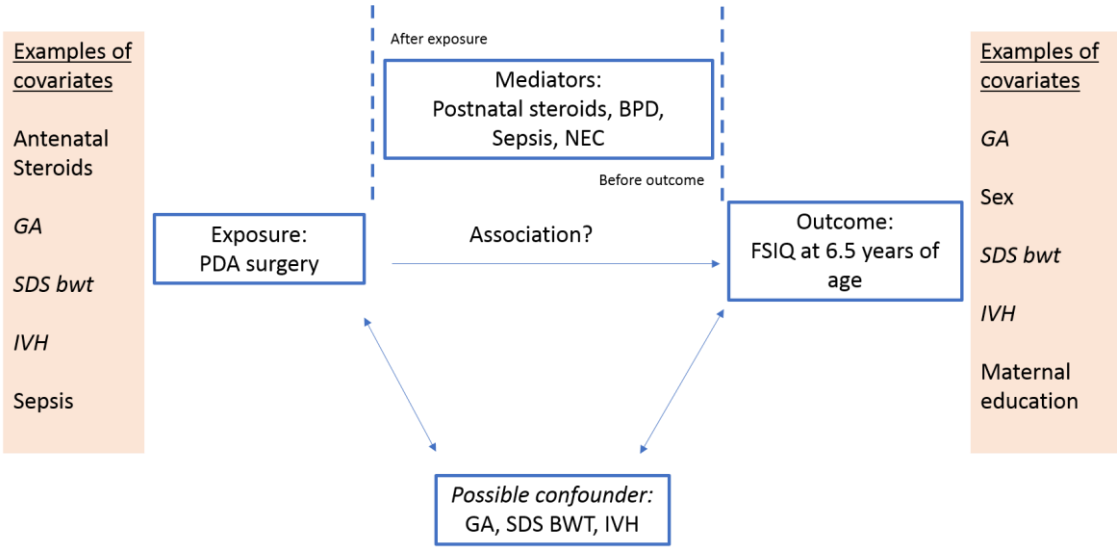

Figure 2.7. PDA surgery and association with FSIQ at 6.5 years of age as measured by WISC IV[17].

Abbreviations: GA: gestational age. SDS: standard deviation score. BWT: birth weight PDA: patent ductus arteriosus. IVH: intraventricular hemorrhage. BPD: bronchopulmonary dysplasia. NEC: necrotizing enterocolitis. FSIQ: full-scale intelligent quotient: WISC-IV: Wechsler intelligence scale for children, version IV.

As shown in **Figure 2.7** there are many possible covariates, which are related to the exposure or the outcome. Some are true confounders, as they are related to both the exposure and the outcome as shown. GA is strongly associated with both the need of PDA treatment and neurodevelopmental outcome.[15] Presence of IVH is also of importance as it is related to both GA and risk of primary PDA surgery (IVH is a contraindication for NSAID treatment of PDA) as well as neurodevelopmental outcome.[110] This is an example, other associations than those illustrated are possible.

3 AIMS AND RESEARCH QUESTIONS

The aim of this thesis is to explore the incidence and type of PDA treatment and the association with short- and long-term outcome in extremely preterm infants. Furthermore, the aim is to study the role of cardiac biomarkers in assessing PDA severity in extremely preterm infants.

The research questions are:

1) Is age at start of PDA treatment with Non-Steroidal Anti-Inflammatory Drugs (NSAID´s) associated with later risk of surgical closure of the PDA or death before 3 months of age; or with later risk of BPD in infants surviving to 36 weeks post-menstrual age? (Paper I)

2) How does PDA treatment vary after very preterm birth in Europe, and are different approaches to PDA treatment associated with BPD or survival without major morbidity after very preterm birth? (Paper II)

3) Is the type and timing of PDA treatment (e.g. surgery, pharmacological or both) associated with neurodevelopmental outcome at early school-age in children born extremely preterm? (Paper III)

4) How are the cardiac biomarkers Troponin T and NT-proBNP correlated to different PDA treatment types of an echocardiographically hsPDA in extremely preterm infants? (Paper IV)

5) What is the probability of spontaneous PDA closure in extremely preterm infants and are the cardiac biomarkers helpful in predicting spontaneous closure? (Paper IV)

4 MATERIALS AND METHODS

4.1 OVERVIEW OF THE STUDIES

	Study I	Study II	Study III	Study IV
Design	Prospective cohort study	Prospective cohort study	Prospective cohort study	Prospective cohort study
Population	**EXPRESS cohort** All infants born in Sweden	**EPICE cohort** Infants born in 19 regions in 11 countries in Europe	**EXPRESS cohort** All infants born in Sweden	**DEDUCT cohort** Level III county hospital. Inborn at the Karolinska University Hospital, Stockholm
Gestation weeks	<27 weeks of gestation	<32 weeks of gestation	<27 weeks of gestation	<28 weeks of gestation
Number of infants included	290	6896	435	98
Study period	2004-2007	2011-2012	2004-2007	2012-2014
Exposure	Timing of NSAID pharmacological PDA treatment	PDA treatment, pharmacological and/or surgical	PDA treatment (pharmacological and/or surgical), both mode and timing	Presence of an echocardiographically verified hsPDA
Outcome	a. PDA surgery or death b. BPD in survivors at 36 weeks PMA	BPD or death at 36 weeks PMA. Survival without major neonatal morbidities (IVH≥3, PVL, surgical NEC, ROP≥3)	NDI and FSIQ at 6.5 years of age	PDA closure: spontaneous, after ibuprofen treatment or with PDAsurgery
Follow-up time	a. 90 days of age b. 36 weeks PMA	36 weeks PMA	6.5 years of age	40 weeks PMA
Main research question	Timing of PDA treatment and association with death, need of PDA surgery and BPD at 36 weeks of age.	Variation of PDA treatment and association with outcomes at 36 weeks PMA.	Mode and timing of PDA treatment and association to cognitive outcomes at 6.5 years of age.	Association of gestational age with PDA closure. Association of markers of ductal severity to treatment. Association of markers of ductal severity with PDA closure.

4.1.1 Papers I and III

Study design

In papers I and III, infants were born at less than 27 weeks of gestation in Sweden from April 1st 2004 to March 31st 2007 and included in a prospective national population-based cohort (EXPRESS).[13] The main purpose of the EXPRESS cohort study was to explore factors of

significance for survival and morbidity after extremely preterm birth.[13] Inclusion criteria for the studies in papers I and III are displayed in **Figure 4.1.1.1**

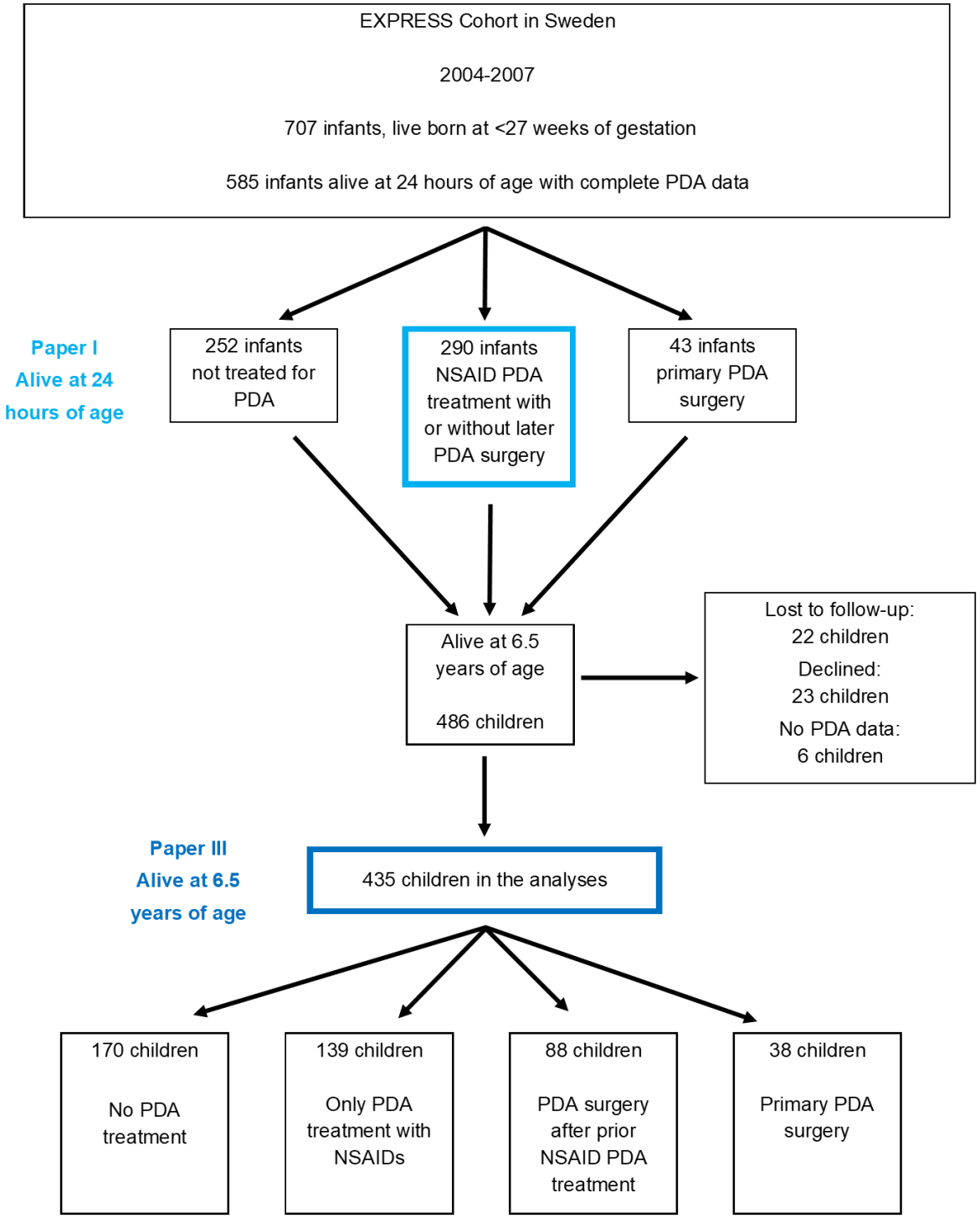

Figure 4.1.1.1 Flow chart of inclusion for studies in paper I and paper III. Exclusion criteria, missing data and lost due to follow-up is detailed.

Data collection

Data in the EXPRESS study was collected from all of the 42 delivery departments where the infants were born. Of all included infants, 82% were born at the 7 regional level III hospitals in Sweden, as the intention has been to centralize extremely preterm deliveries to these hospitals. The data was acquired at each local hospital prospectively from mother´s and infant´s medical charts. Data was collected on demographic information, maternal pregnancy factors, birth and resuscitation, selected procedures in the neonatal unit, common exposures and outcomes of the extremely preterm infant. All data was collected on standardized study forms in accordance with a manual defining the variables. Regional data was then electronically transferred to a central database, in collaboration with the Swedish Perinatal Quality Register (MedSciNet AB, Stockholm, Sweden). Data on live-born infants, admitted to the neonatal department, was collected prospectively until discharge, even if the infant was transferred to another hospital or till death occurred. Quality controls were conducted, both for missing or erroneous data, and internal and external validity was checked.[13]

In the EXPRESS cohort, PDA was defined as pharmacologically treated, surgically treated or both. PDA was diagnosed with echocardiography, but the details of the ductal echocardiographic severity were not collected.[111] Infants having a conservatively treated PDA were not defined as having a PDA in the study. The exposures, outcomes and statistical methods for papers I and III are shown in **Table 4.1.1.1** and **4.1.1.2**

Table 4.1.1.1 Exposures, outcomes and analysis methods used in Paper I

Exposure	**Outcome**	**Analysis method***
Timing of pharmacological PDA treatment categorized (according to tertiles of distribution) into: -0-2 days of age (reference) -3-6 days of age -≥7 days of age	A composite outcome of PDA surgery or death	COX regression analysis with stratification on gestational age and hazard ratio (HR). Follow-up time was from start of pharmacological treatment and up to 90 days of age, date of PDA surgery or death, whichever occurred first.
	BPD at 36 weeks postmenstrual age in surviving infants	Logistic regression analysis, (Odds ratio, OR) stratified on gestational age and adjusted for sex, SGA status and duration of mechanical ventilation.
*To take intra-regional correlation between observations into account, the cluster statement in STATA was used in the regression models. Introducing the cluster statement into the regression model does not change the point estimate of the hazard ratio but influences the standard error.		
Abbreviations: PDA: patent ductus arteriosus. BPD: bronchopulmonary dysplasia. SGA: Small for gestational age (>-2SD below the mean birthweight).		

Table 4.1.1.2 Exposures, outcomes and analysis methods used in paper III.		
Exposure	**Outcomes**	**Analysis method***
Type of PDA treatment -NSAID treatment -PDA surgery after prior NSAID treatment -Primary PDA surgery	Neurodevelopmental Impairment (NDI) categorized into: no or mild and moderate or severe NDI[a] Full-Scale Intelligence Quotient[b] (FSIQ) as a continuous variable.	A mixed-effects regression modified Poisson model, presented as incidence rate ratios (IRR) with [95% confidence intervals (CI)]. Adjustments made for GA, sex, birthweight, SDS birthweight, IVH grades 3 or higher and maternal education. In a mixed-effects generalized linear regression model and presented as adjusted mean difference in FSIQ with with [95% confidence intervals (CI)] Adjustments made for GA, sex, birthweight, SDS birthweight, IVH grades 3 or higher and maternal education.
Timing of PDA treatment categorized as: Pharmacological: -0-2 days of age (reference) -3-6 days of age -≥7 days of age Surgical: -≤10 days of age -11-20 days of age ->20 days of age (reference)	Neurodevelopmental Impairment (NDI) categorized into: "no or mild" and "moderate or severe" Full-Scale Intelligence Quotient (FSIQ) as a continuous variable	A mixed-effects regression modified Poisson model, presented as incidence rate ratios (IRR) with [95% confidence intervals (CI)]. Adjustments made for GA, sex, birthweight, SDS birthweight, IVH grades 3 or higher and maternal education. In a mixed-effects generalized linear regression model and presented as adjusted mean difference in FSIQ with with [95% confidence intervals (CI)]. Adjustments made for GA, sex, birthweight, SDS birthweight, IVH grades 3 or higher and maternal education.
* To account for potential clustering, the models included region and mother as the random effect variables except in the model investigating the association of timing of PDA treatment and FSIQ, where only region was included because of limitations in power.		
[a]Neurodevelopmental Impairment as defined according to Moore et al 2012. [16] [b]Full-Scale Intelligent quotient (FSIQ) as determined by Wechsler Intelligence Scale for Children (WISC-IV) results.[17]		

4.1.2 Paper II

Study design

In paper II, the Effective Perinatal Intensive Care in Europe (EPICE) Cohort Study was a prospective European cohort study, including 19 regions in 11 countries in Europe. It was conducted during a 6 to 12 months period (depending on region) in very preterm infants (born at <32 gestational weeks) from 2011-2012. Included in paper II were a total of 6896 infants, all surviving to ≥24 hours of age.[14] A flow chart of the study is shown in **Figure 4.1.2.1**

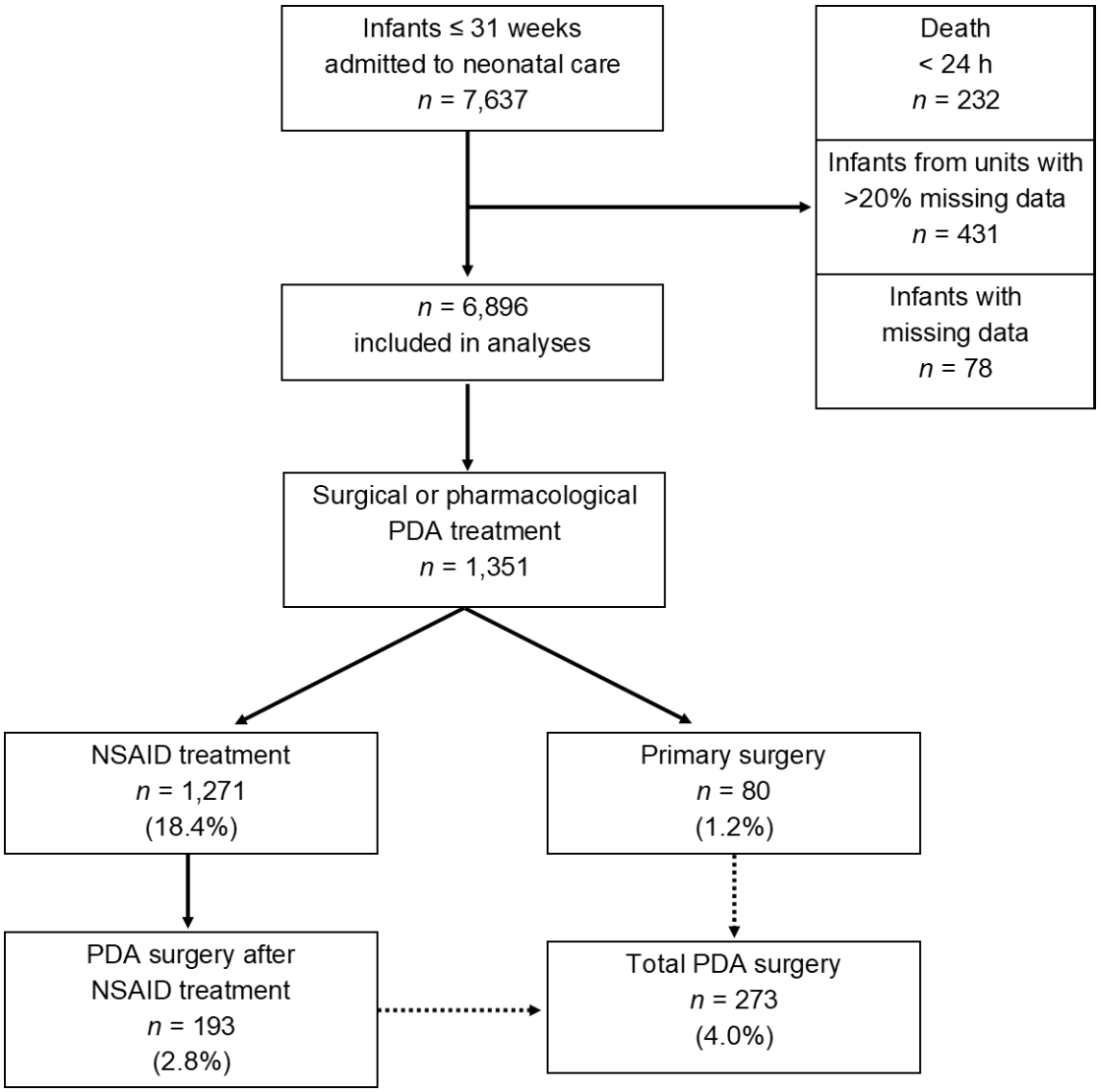

Figure 4.1.2.1 Flow chart of inclusion for the study in paper II. Exclusion (death before 24 hours of age) and missing data are detailed. © 2017 S. Karger AG, Basel. Published with permission.

Data collection

In all regions, information on type of PDA treatment and date of start of treatment were collected. The diagnosis of PDA differed between regions but was based on a clinical and/or echocardiographic assessment. Echocardiographic PDA diagnosis was available on site for 6768 infants (98.1%). Data was collected about each infant until death or hospital discharge. The exposures, outcomes and statistical analysis methods are shown in Table 4.1.3.1.

Table 4.1.2.1. Exposures, outcomes and statistical analysis methods used in Paper II.

Exposure	Outcome	Statistical analysis method**
PDA treatment: -NSAID (ibuprofen or indomethacin) treatment -PDA surgery after prior NSAID treatment -Primary PDA surgery	Composite outcome of BPD or death before 36 weeks gestation[a]	Mixed-effects generalized linear regression models presented as risk ratios (RR) with [95% confidence intervals (CI)]. Adjustments for gestational age (GA) separately and then for the propensity score* (which included GA).
	Survival without major neonatal morbidity[b]	As above with further adjustments for total duration of mechanical ventilation and number of confirmed septicemias.

*Propensity score for PDA treatment: A single index variable which summarizes the pre-treatment perinatal characteristics. It was estimated from presence of preeclampsia/eclampsia, spontaneous onset of labor, preterm premature rupture of membranes (pPROM), maternal infection as indication for delivery, antenatal corticosteroid treatment, mode of delivery, gestational age, birth weight, infant sex, small for gestational age (>-2SD below the meanbirth weight) and use of mechanical ventilation on first day of life.

**Clustering: It was accounted for in the analyses, where the neonatal unit was added as the random effects variable in the multi-level mixed effects model. In a separate analysis, the identity of the mother was added as a second random effect, as multiples could have more similarities.

[a]Bronchopulmonary dysplasia (BPD) defined as any oxygen treatment at 36 weeks postmenstrual age (PMA)
[b]Major neonatal morbidity defined as presence of any of the following: intraventricular hemorrhage (IVH) grade ≥ 3, cystic periventricular leukomalacia (cPVL), necrotizing enterocolitis (NEC) requiring surgery or peritoneal drainage, or retinopathy of prematurity (ROP) stage≥ 3

4.1.3 Paper IV

Study design

In paper IV, a prospective hospital-based cohort study was conducted at the neonatal intensive care unit at the Karolinska University Hospital Solna, from February 14th 2012 to July 31st 2014. All infants born at this hospital at less than 28 weeks gestational weeks and admitted to the NICU were eligible for inclusion. A flow chart of the inclusion criteria is shown in **Figure 4.1.3.1**. Reasons for non-inclusion were cardiac or other major malformation, death before 72 hours, critically ill infants or mothers during the first 72 hours and non-availability of study personnel. Ninety-eight infants were included in the final analysis.

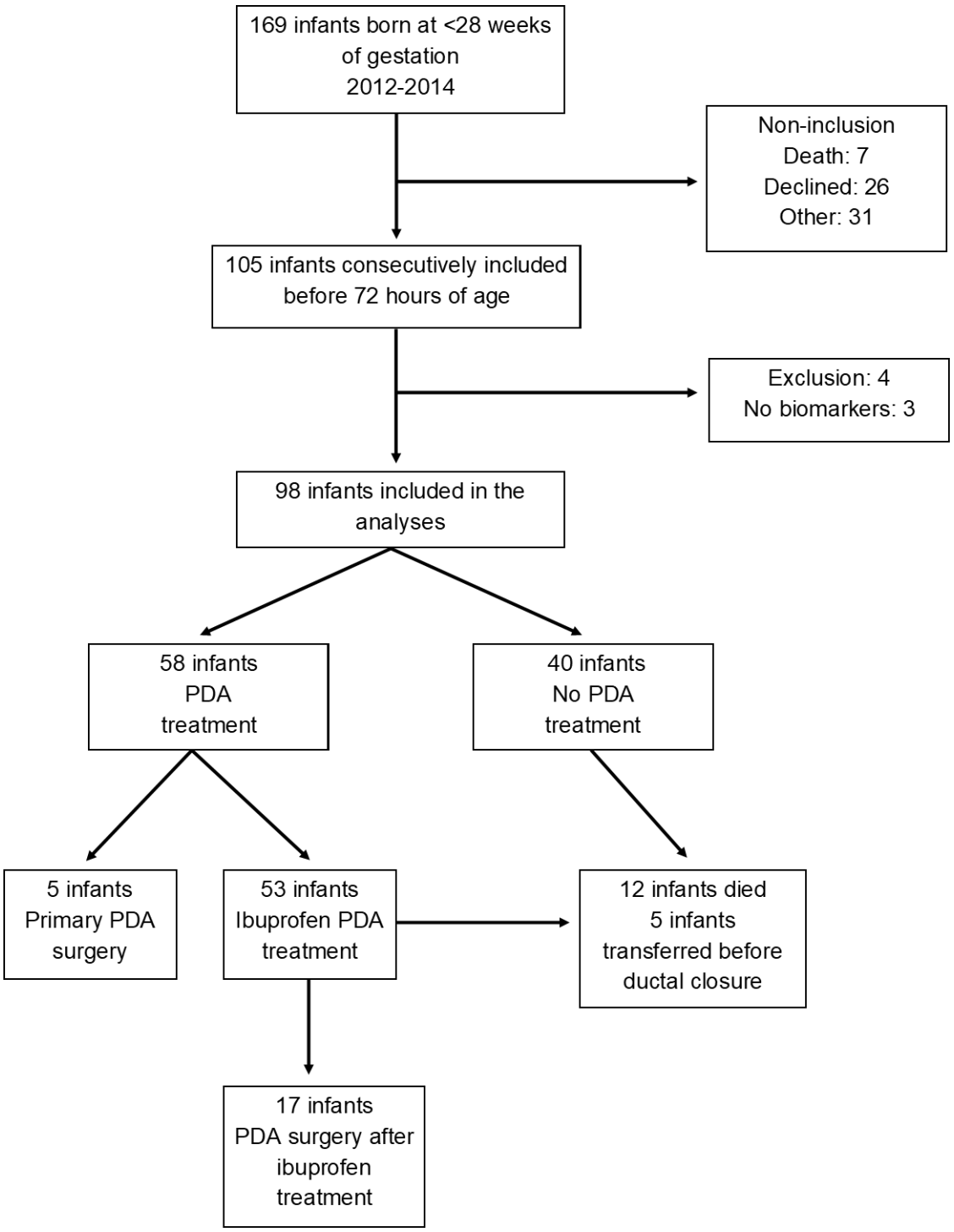

Figure 4.1.3.1 Flow chart of inclusion and exclusion critera for the study in paper IV.

Study protocol

Echocardiography was performed for all infants on day 3, day 7, and day 14 (for infants not treated), before PDA treatment and following PDA treatment. Neonatologists trained in echocardiography performed the echocardiography according to a clinical protocol shown in **Table 4.1.3.1.** The cardiac biomarkers NT-proBNP and cTnT were measured in blood samples collected in 0.5 ml lithium heparin tubes and analysed at the Karolinska University Hospital Accredited Laboratory. The results were registered in the electronic medical record, so the neonatologist in charge of the infant was not blinded to the results. The timepoints chosen for further analyses of the echocardiography and cardiac biomarker measurement wereday 3 and day 7. The predefined age ranges accepted for the day 3 measurement was 2-4 days, and 5-9 days for the day 7 measurement.

Table 4.1.3.1. Clinical local guidelines for hemodynamic Patent ductus arteriosus (hsPDA) at the Karolinska University Hospital during 2012-2014	
• The PDA treatment decision was at the discretion of the neonatologist in charge. • Local guidelines for PDA treatment combined echocardiographic and clinical criteria. • Clinical criteria were: respiratory failure, FiO2, cardiorespiratory instability and signs of heart congestion on x-ray, signs of kidney failure and feeding difficulties. • The echocardiography was Neonatologist Performed Echocardiography (NPE) according to a revised clinical study protocol.	
	Echocardiographic criteria (at least the ductal characteristics and one other had to be present)
Ductal characteristics	
Diameter	Diameter of the duct >1.5mm
Direction of flow through the duct	Left to right shunt (L->R)
Flow characteristics	Pulsatile flow through the duct with a non-restrictive flow profile
Excessive pulmonary flow	
Left atrium	Left Atrial to Aortic root (La:Ao) ratio >1.5
Pulmonary flow characteristics	End-diastolic flow in LPA/RPA >0.2m/s
End-organ ductal steal	
	Signs of ductal steal (reversed diastolic flow in descending aorta /reversed or absent flow in mesenteric artery and/or cerebral arteries (ACA)
Heart failure and ventricular enlargement	
	Visual signs of left ventricular enlargement (Left ventricle end-diastolic diameter was not routinely measured)
Abbreviations: hsPDA: hemodynamically significant Patent Ductus Arteriosus, 2D: Two dimensional, La: Left Atrial, Ao: Aorta, LPA: left pulmonary artery, RPA: right pulmonary artery, PWD: pulse wave doppler, CWD: continuous wave doppler, ACA: anterior cerebral artery	
References: [35,55,58,59,62]	

Data collection

Data on perinatal and neonatal characteristics as well as the results of the biomarker measurements were extracted from the infants' medical charts. Ultrasound studies were evaluated off-line for PDA related measurements by the study investigators according to a predefined protocol. If an echocardiographic image was missing for the evaluation, results from the medical chart were used to replace that measurement. For a complete description of all materials (ultrasound equipment and cardiac biomarker measurements), see manuscript paper IV.

The exposures, outcomes and statistical methods for study 4 are shown in **Table 4.1.3.2**

Table 4.1.3.2. Exposures, predictors and outcome and analysis method used in paper IV.

Exposure	**Outcome**	**Statistical analysis method**
Presence of an echocardiographically determined PDA -all infants in cohort -categorized into infants born at <25 weeks or ≥25 weeks -categorized according to treatment strategies	PDA closure (confirmed with echocardiography, defined as no flow through duct with color Doppler on ultrasound).	Survival curves (Kaplan-Meier) were used to describe the incidence of ductal closure over time, from birth to 40 weeks postmenstrual age (PMA). Infants were censored at death, transfer to hospital out of county or surgical closure of the PDA, whichever occurred first.
Presence of an echocardiographically determined PDA and infants categorized into born at gestational age <25 weeks or ≥25 weeks	PDA closure (confirmed with echocardiography, defined as no flow through duct with color Doppler on ultrasound).	As above. To compare Kaplan-Maier curves, the $\chi 2$ method using Log-rank and Wilcoxon non-parametric tests were performed.
Presence of an echocardiographically confirmed PDA and associated NT-proBNP and cTnT value on day 3 and day 7.	PDA treatment categories: -spontaneous closure≤7 days -no PDA treatment -only ibuprofen PDA treatment -all PDA surgery	Descriptive statistics with median and interquartile range. Comparison between treatment groups with Kruskal Wallis, Wilcoxon rank test and Mood Median test. For pairwise post-hoc comparison, Dunn´s test was used.

4.2 ETHICAL CONSIDERATIONS

All the investigations in the studies conform to the principles outlined in the "Declaration of Helsinki"; from 1964. Ethical approvals, from the Regional Research Ethics Boards were granted for all the four studies.

Paper I and III (Regional Research Ethics Board Lund Dnr 42/2004 and 2009/524):

For the EXPRESS study, the parents as the legal guardians of the infants, were informed about the purpose of the study (written and oral information) at the time of the admission of

the infant. The method of passive consent was applied and if the parents did not consent to the data collection an active "withdrawal" was required. At the time of the follow-up study at 6.5 years of age, the parents were again informed and a new written consent was acquired. The follow-up consisted of psychological testing, physiotherapy testing, ophthalmologist assessment, hearing assessment and pediatric evaluation. Furthermore, information on parental education was retrieved. No blood tests were included. The parents were informed of the results of all studies by the doctor, psychologist and physiotherapist.

There are certain ethical concerns on neurodevelopmental follow-up in this category. For the parents to be reminded of their stay in the NICU and raising questions on the child´s neurodevelopment can be stressful. The families with lower socioeconomic resources may not have as easy access due to inability or language issues. For children already diagnosed with disabilities it may be considered a discomfort for one more appointment.

Paper II: (Regional Research Ethics Board Stockholm Dnr 2011/209-31/1 and 2011/1356-32)

As this was a European collaboration with 19 regions in 11 countries, ethical approval was obtained in each region in each country, as required by national legislation. Therefore, the parental consent procedure differed between regions, from active written consent to passive consent (information received and consent assumed if not stated otherwise). In the Stockholm region, parents were informed on the study after the admission of their infant. Passive consent to the data collection was applied.

Paper IV: (Regional Research Ethics Board Stockholm Dnr 2011/772-31/4 and 2011/1253-32)

Parents, as the legal guardians, were informed both orally and with written information about the study protocol before or after birth of their child. Parental written consent for inclusion was acquired after birth.

Extremely preterm infants are a vulnerable population and their families are in a state of shock in relation to the birth of their infant. This became most obvious in the study for paper IV. Precautions were taken from the beginning in the study protocol to minimize both the infant´s discomfort and potential blood loss due to study samples. Echocardiography investigations were part of routine care of the infants. Blood samples were needed to measure levels of cardiac biomarkers. Initially, extra study blood samples were required as to fulfill blinding towards the clinician in charge of the infant. For all study blood tests, the upper limit of maximal 2.5mL blood per kg per month of included infants, was followed (taking into account other clinical studies requiring blood samples for research purposes). During the first 4 months of the study, parental concerns over additional blood loss due to study blood sampling became a hindrance as the parents declined study participation. Therefore, the protocol was revised and the ethical permission amended to include the study tests from

blood samples already taken for clinical purposes, thereby minimizing patient discomfort and blood loss.

4.3 STATISTICAL METHODS

Descriptive methods were used when appropriate: means (with standard deviations, SD), medians (with interquartile ranges 25^{th} to 75^{th} percentile, IQR) and numbers (with proportions) were used.

In paper IV, time to event curves (Kaplan-Meier estimator/curve) were used to describe the incidence of ductal closure over time, from birth to 40 weeks PMA. This allowed censoring at time of death, transfer to hospital out of county or surgical closure of the PDA, whichever occurred first.

To test for normality of continuous data, Shapiro Wilk's and Anderson Darling tests were used. T-test was used for comparison of normally distributed data. In cases where normal distribution of data was not confirmed, non-parametric tests were used for comparison, Wilcoxon rank-sum or Mood Median tests if only two groups were compared, and Kruskal Wallis test for group comparisons of more than two groups. For post hoc pairwise comparison, Dunn´s test was used. For comparison of proportions, χ^2 test or Fischer´s exact test was used. To compare Kaplan-Maier curves, the $\chi 2$ method using Log-rank and Wilcoxon non-parametric tests were performed.

To select covariates and confounders of interest for adjustment in the regression analysis, various methods were used in the papers (described separately in each paper).

Regression models used (dependent on type of data; continuous, categorical, dichotomous or time-dependent).

- COX proportional hazards model (time-dependent), presented as hazard ratio (HR) with 95% confidence intervals (CI).
- Logistic regression (binomial distribution) presented as odds ratio (OR) with 95% confidence intervals (CI),
- Linear regression models (Gaussian distribution) presented as risk ratios (RR) with [95% confidence intervals (CI)].
- Mixed-effects generalized linear regression models (with Poisson distribution) presented as risk ratio (RR) (paper II) and incidence rate ratio (IRR) (paper III) with [95% confidence intervals (CI)]

Clustering was accounted for in the analyses in papers I, II and III. This is due to the risk of similarities between 1) infants born in the same region or cared for in the same neonatal unit 2) siblings (multiple birth).

A p-value of <0.05 was considered significant in the analysis.

Statistical software used: All statistical analyses were performed using STATA SE13.1 and IC15 (http://www.stata.com/), Microsoft Excel version 1808 (https://products.office.com/en-us/excel) and Minitab Statistical Software Version 17 (http://www.minitab.com).

5 RESULTS

5.1 PAPER I

The median postnatal age (PNA) at start of PDA treatment was 4 days, (IQR 2-7). PDA surgery after prior pharmacological treatment (secondary PDA surgery) was performed on 102 infants (35%). The three categories did not differ regarding obstetric background variables, birth weight, GA, sex, CRIB score (Clinical Risk Index for Babies) or early surfactant administration. Twenty-eight infants (10%) died between the start of treatment and before 36 + 0 weeks of PMA. Of these 25 infants died before 28 days of age.

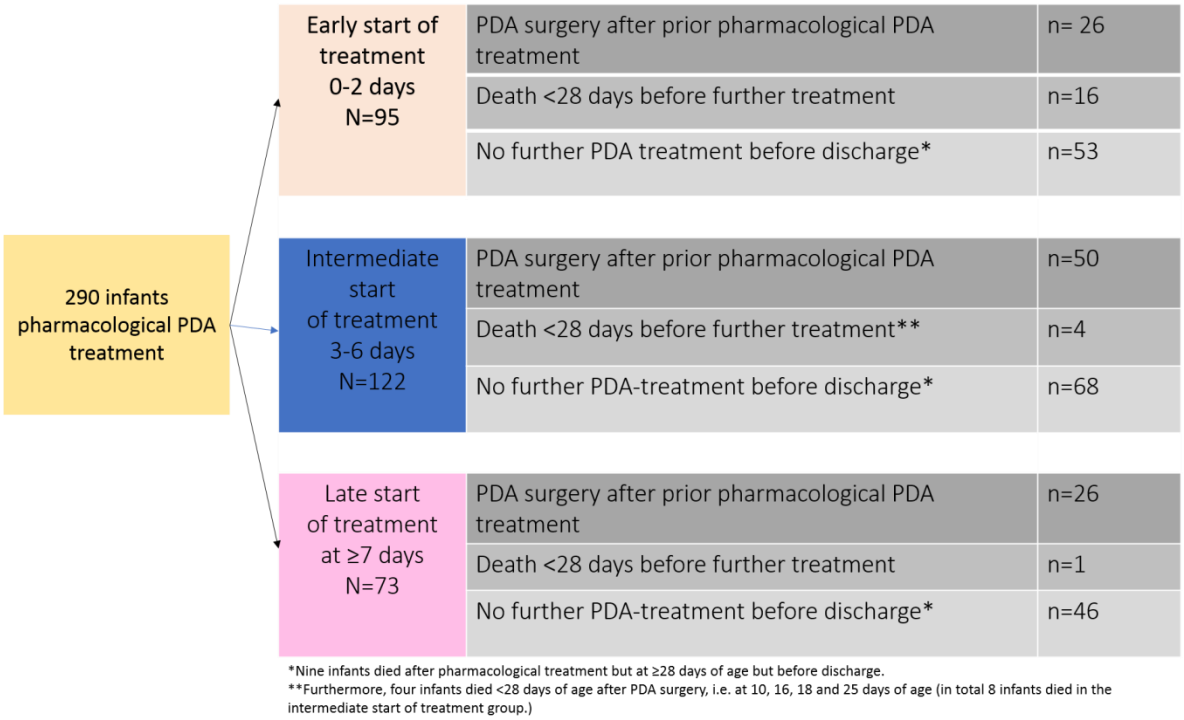

Figure 5.1.1 Overview of the 290 infants in the cohort and categorization according to timing of treatment. The outcomes, secondary PDA surgery, death <28 days or no further PDA treatament are detailed.

The proportion of death differed between the three treatment categories as shown in **Figure 5.1.1** above, with the highest proportion among those who started PDA treatment early.

Risk of PDA surgery

As shown in Figure 5.1.2, there were no significant differences in risk of PDA surgery or death detected between the three groups (early treatment as reference group). In the crude analyses, a higher GA was associated with a lower risk of the composite outcome, secondary PDA surgery or death (HR 0.61 with 95% confidence interval, [CI 0.52–0.72] per week increase in GA). In a model stratified on GA and adjusted for clustering on region, HR for late and intermediate vs early start was 1.10 [CI 0.53–2.28] and 0.89 [CI 0.57–1.39] respectively.

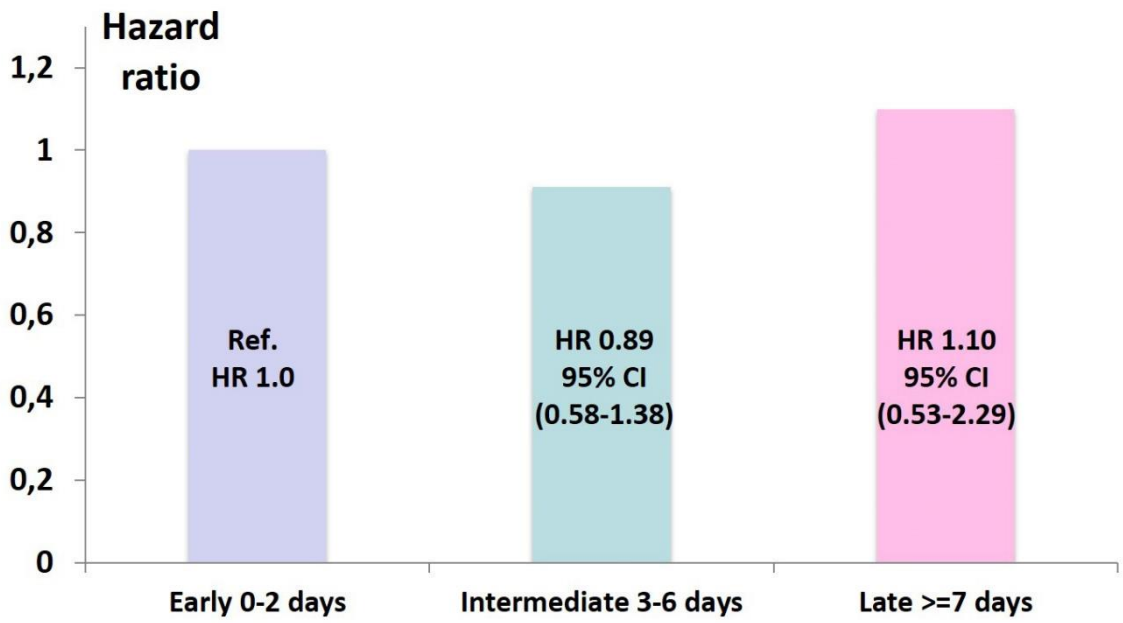

Figure 5.1.2. Risk of later PDA surgery after different timing of NSAID pharmacological treatment (categorized as early, intermediate and late start). The cox regressions model is stratified on GA and adjusted for clusterin on region.
Abbreviations: HR:hazard ratio. CI: confidence interval.

Association between timing of pharmacological treatment and risk of BPD

Two hundred and fifty-nine infants (89%) survived to 36 weeks PMA and had available data on oxygen treatment. Of these, 166 (64%) were defined as having BPD. The duration of mechanical ventilation differed between the categories of timing of PDA treatment. The duration was significantly shorter among infants with early start of treatment. In the late start group, fewer infants were treated with mechanical ventilation. However, when all treatment groups were compared on postnatal day 7, no significant difference in the proportion of infants on mechanical ventilation was found.

Risk for BPD according to early, intermediate or late start of pharmacological treatment is shown in **Figure 5.1.3**. Late start of PDA treatment was associated with a significantly lower risk of BPD OR 0.29 [CI 0.13-0.61] compared to early start of treatment.

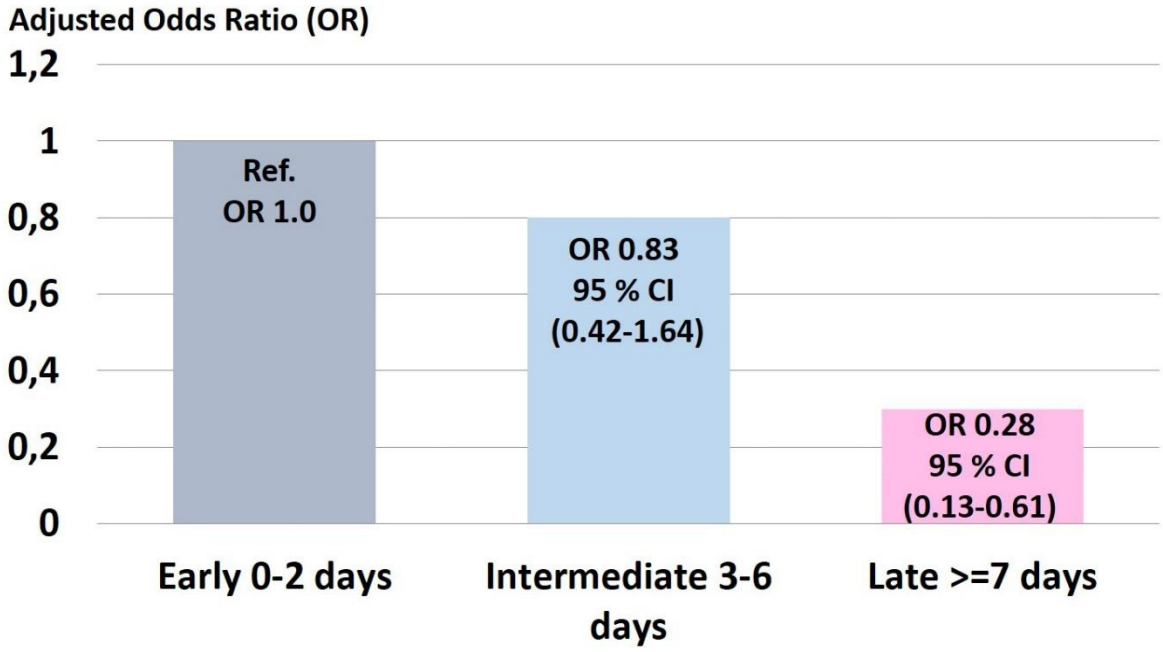

Figure 5.1.3. Risk of BPD according to timing of NSAID pharmacological PDA treatment (categorized as early, intermediate and late start). Logistic regression model stratified on GA and adjusted for sex and SGA (adjusting for duration of ventilation did not changes the estimates).

Abbreviations: OR: odds ratio. CI: confidence interval. GA: gestational age. SGA: small for gestational age.

Key points Paper I:

- Neither late start of pharmacological PDA treatment (at ≥7 days of age) nor intermediate (3-6 days of age) was associated with an increased risk for PDA surgery or death compared to an early start of treatment (0-2 days of age).
- A high proportion of extremely preterm infants died in the first weeks of life and this had to be accounted for in the analysis by a combined outcome of PDA surgery or death.
- Due to different age at start of pharmacological treatment as well as time at risk for surgery, the COX proportional hazard regression was chosen to account for the at-risk time (up to 90 days of age).
- Gestational age is an important predictor of PDA treatment.
- Later pharmacological PDA treatment seems to be a safe treatment option.

5.2 PAPER II

In the cohort of the study in paper II, the mean GA in the cohort was 29.1 weeks (SD 2.2) and mean birthweight 1223 grams (SD 384). The total proportion of any PDA treatment was 20%. Of infants born at less than 28 weeks, 44% were treated for PDA and 9.8% of infants born at 28 to 31 weeks. PDA treatment was strongly associated with GA, see figure 5.2.1.

Figure 5.2.1.

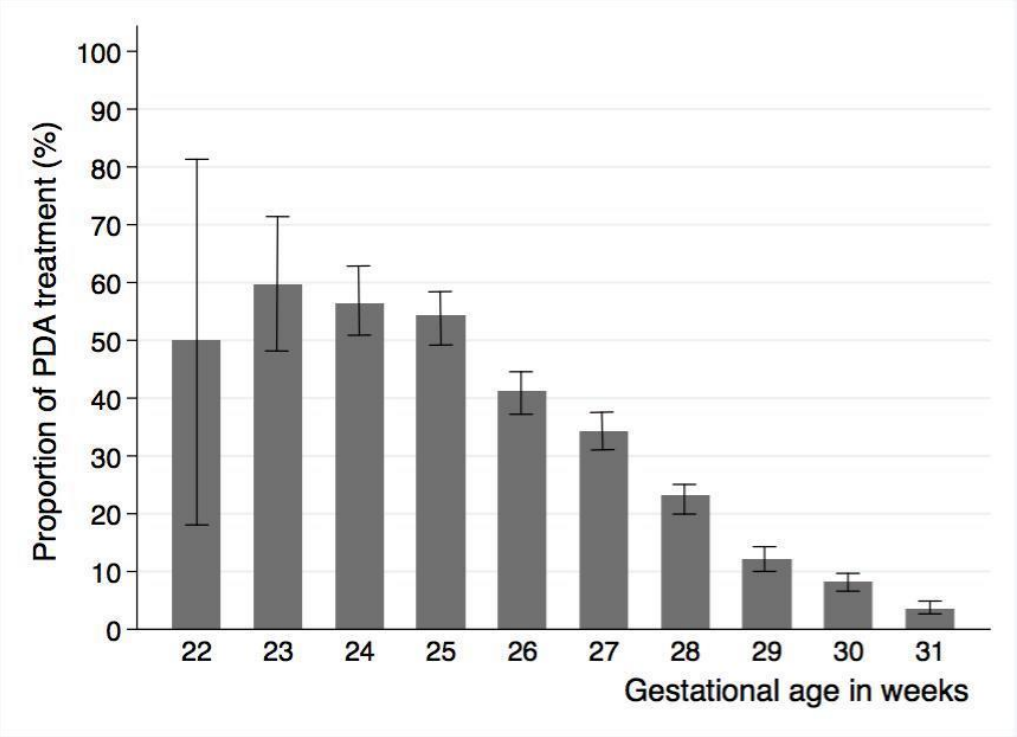

Figure 5.2.1 Proportion of PDA treatment according to gestational age in weeks in the EPICE cohort. © 2017 S. Karger AG, Basel. Published with permission.

Differences in infant characteristics were studied using a propensity score derived from perinatal risk factors for PDA treatment. When variation between regions was studied, the proportion of PDA treatment varied significantly, from 10 to 39% between regions ($p < 0.001$). The regions were categorized according to low (<15%, n = 6), medium (15–25%, n = 9), or high (>25%, n = 4) proportion of PDA treatment. The difference in PDA treatment could not be explained by differences in perinatal characteristics as displayed by propensity score (see **Figure 5.2.2**).

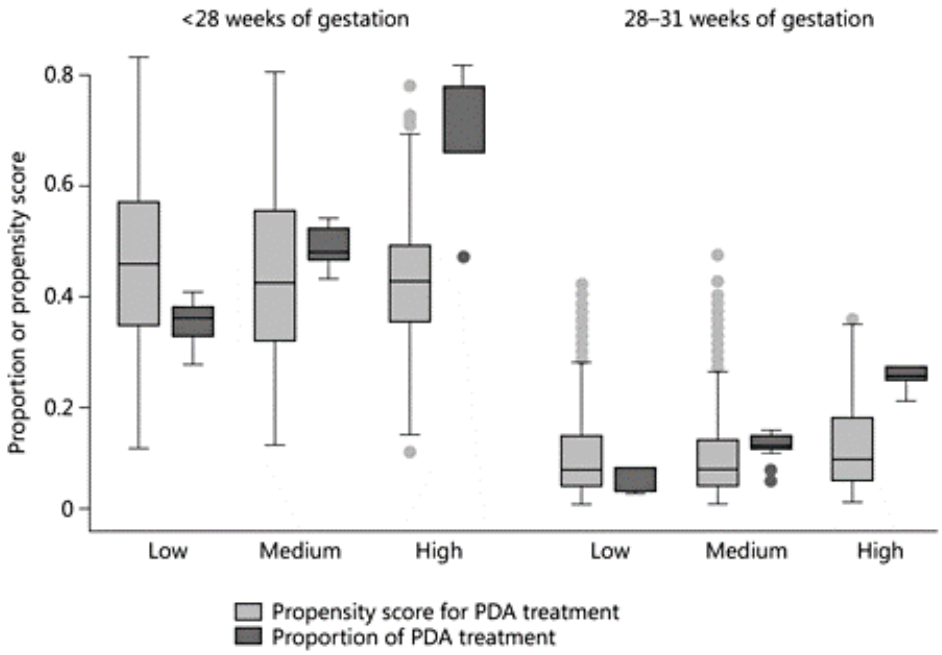

Figure 5.2.2. In the 2 categories of gestational age born at <28 weeks and at 28-32 weeks the proportion of PDA treatment in the regions (categorized into low, medium or high) are shown in relation to propensity score. © 2017 S. Karger AG, Basel. Published with permission.

Infants treated for PDA, compared to those not treated, were at higher risk of BPD or death in all regions, with an overall propensity score adjusted risk ratio of 1.33 (95% confidence interval 1.18–1.51), see **Table 5.2.1**. Survival without major neonatal morbidity (IVH grade ≥ 3, cystic periventricular leukomalacia (PVL), necrotizing enterocolitis requiring surgery or peritoneal drainage, or retinopathy of prematurity stage ≥ 3) was not related to PDA treatment.

Table 5.2.1. Numbers and adjusted risk ratios (95% confidence intervals) for the composite outcomes of BPD or death[a], and survival without major neonatal morbidity[b] according to PDA treatment (Y/N) or type of PDA treatment in 19 European regions with low (<15%), medium (15-25%) or high proportions (>25%) of PDA treatment.

	Low N=2,875		**Medium N=3,417**		**High N=604**	
	No. of events	**aRR (95% CI)[c]**	No. of events	**aRR (95% CI)[c]**	No. of events	**aRR (95% CI)[c]**
COMPOSITE OUTCOME OF BPD OR DEATH[1]						
No PDA treatment	523	1.00 (ref)	327	1.00 (ref)	45	1.00 (ref)
Any PDA treatment	228	1.26 (1.06-1.49)	294	1.34 (1.12-1.61)	96	2.20 (1.47-3.28)
By type of PDA treatment						
Pharmacological treatment only	166	1.21 (0.98-1.50)	205	1.27 (1.05-1.55)	68	2.11 (1.40-3.17)
Surgery after pharmacological treatment	36	1.36 (0.94-1.98)	72	1.51 (1.22-1.86)	17	2.66 (1.41-5.02)
Surgery without prior pharmacological treatment	26	1.45 (1.20-1.75)	17	1.79 (1.27-2.53)	11	2.76 (1.45-5.24)
SURVIVAL WITHOUT MAJOR NEONATAL MORBIDITY[2]						
No PDA treatment	2091	1.00 (ref)	2321	1.00 (ref)	349	1.00 (ref)
Any PDA treatment	223	1.03 (0.94-1.13)	522	1.03 (0.96-1.10)	165	0.98 (0.88-1.09)

[a]Any oxygen treatment at 36 weeks of postmenstrual age (PMA) or death before 36 weeks PMA.

[b] Survival to hospital discharge without intra-ventricular hemorrhage (IVH) grade≥3, cystic periventricular leukomalacia (cPVL), retinopathy of prematurity (ROP) stage ≥3, surgical NEC. Total N=6,335 survivors.

[c] Results from a generalized linear mixed model adjusted for propensity score for PDA treatment. The propensity score for PDA treatment, i.e., a single index variable summarizing the pre-treatment perinatal characteristics, was estimated from presence of preeclampsia/eclampsia, spontaneous onset of labor, preterm premature rupture of membranes (pPROM), maternal infection as indication for delivery, antenatal corticosteroid treatment, mode of delivery, gestational age, birth weight, infant sex, small for gestational age and use of mechanical ventilation on first day of life.

Abbreviations: aRR: Adjusted Risk Ratio

Key points Paper II:

- In infants born at <32 weeks of age, PDA treatment varied largely across Europe without associated variations in perinatal characteristics.
- PDA treatment was strongly associated with GA.
- Infants treated for PDA, compared to those not treated, were at higher risk of BPD or death in all regions.
- Survival without major neonatal morbidity was not related to PDA treatment.

5.3 PAPER III

The characteristics of the infants in paper III are described in the manuscript. Children treated with PDA surgery had lower GA and birthweight and higher CRIB II score. They had longer duration of mechanical ventilation and were more often treated with postnatal steroids than children not treated for PDA. Children who had primary PDA surgery were more often diagnosed with IVH grade 3 or higher and had the highest proportion of BPD. IVH grade 1-2 was evenly distributed between the categories.

Neurodevelopmental outcome by PDA category

The proportion of extremely preterm born children with moderate to severe NDI varied from 27% in the group not treated for PDA to 55% in children who had primary PDA surgery, see **Figure 5.3.1a**.

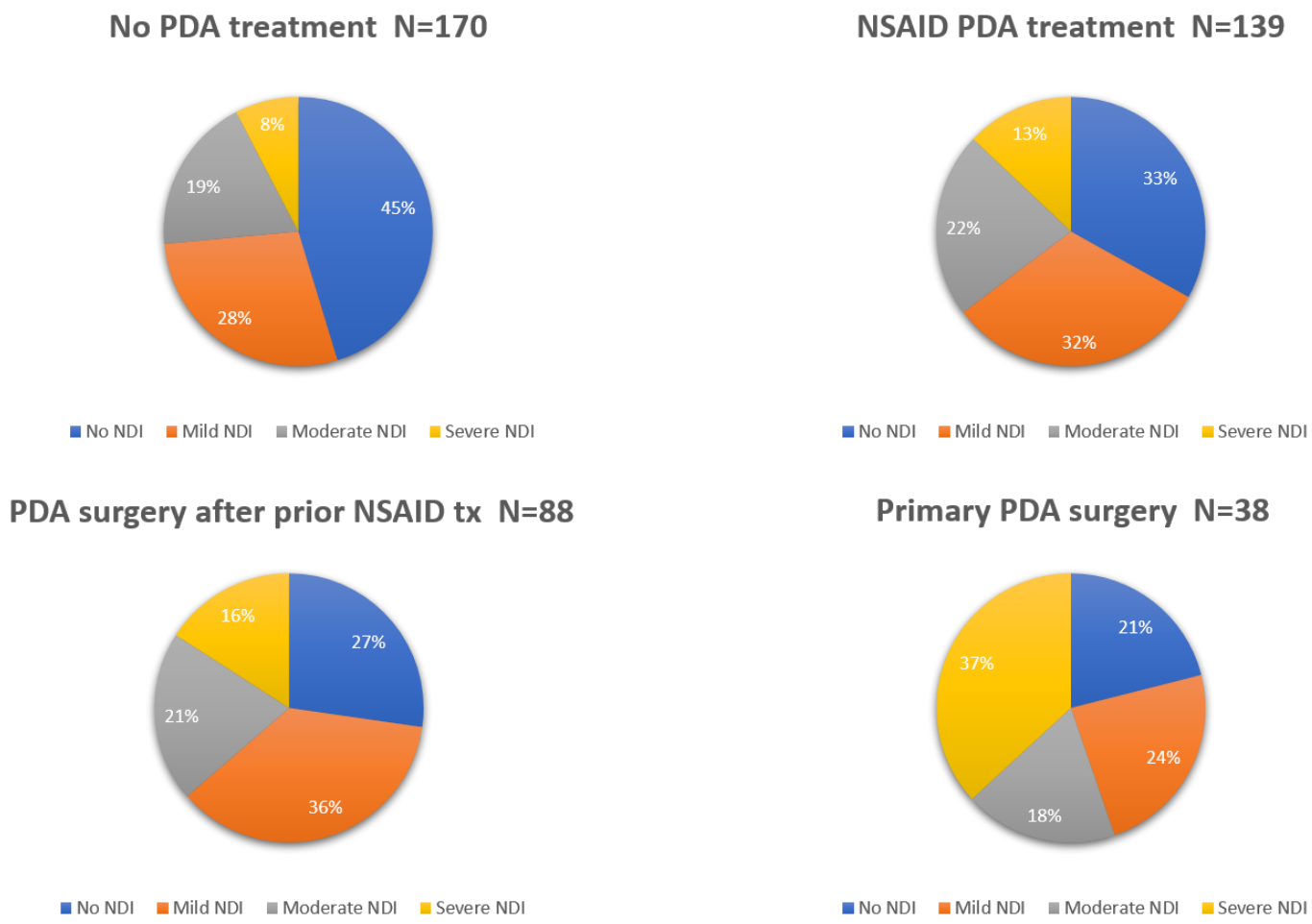

Figure 5.3.1a Distribution of NDI according to PDA treatment in 435 children, at 6.5 years of age, born at <27 weeks GA.

Abbreviations: PDA: Patent Ductus Arteriosus. NSAID: Non-Steroidal Anti-Inflammatory Drugs. NDI: Neurodevelopmental Impairment. Tx: Treatment. GA: Gestational Age.

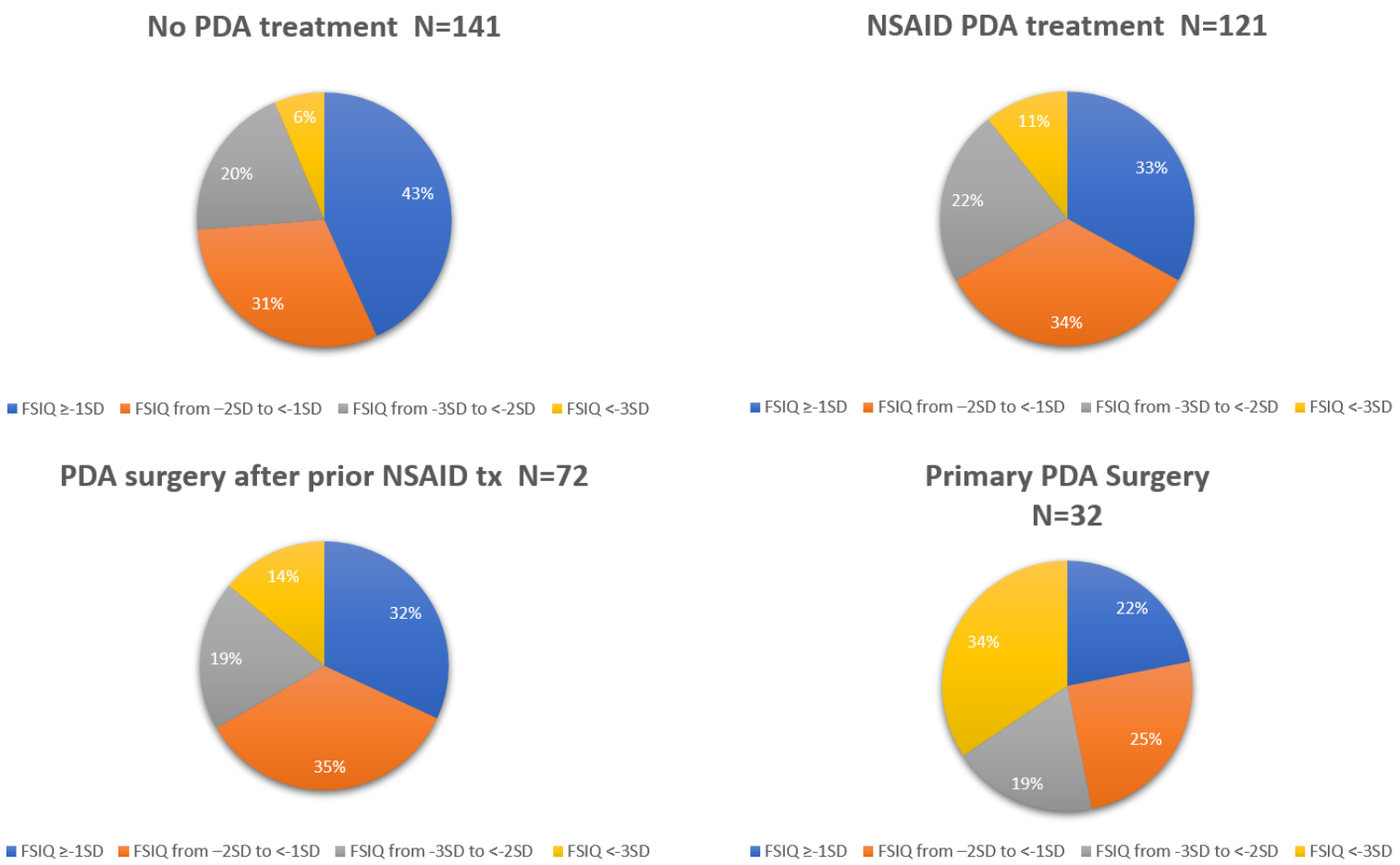

Figure 5.3.1b Distributions of FSIQ according to PDA treatment in 366 children, at 6.5 years of age, born at <27 weeks GA. Categorization according to results of term-born Swedish children tested with WISC-IV. FSIQ into SDs: <-3SD (FSIQ<66); From-3SD to below -2SD (FSIQ 66-76); from-2SD to below -1SD (FSIQ 77-88); ≥-1SD (FSIQ >88). Abbreviations: PDA: Patent Ductus Arteriosus. NSAID: Non-Steroidal Anti-Inflammatory Drugs. FSIQ: Full-Scale Intelligent Quotient. Tx: treatment. EXPRESS: Extremely Preterm Infants in Sweden Study. WISC-IV: Wecshler Intelligent Scales. SD: Standard Deviation.

In **Figure 5.3.1b,** the categories of FSIQ SD's, are shown for all categories of PDA treatment. Children undergoing primary PDA surgery had the highest proportion of FSIQ below -3 SD (34%).

The risk of moderate to severe NDI was 62% higher among children treated with primary PDA surgery in the adjusted model (see **Table 5.3.1**) than in extremely preterm born children not receiving PDA treatment. No increased risks of NDI were found among children treated pharmacologically for PDA, regardless of whether they later had surgical PDA closure or not.

In analyses of 366 children that had FSIQ measured, extremely preterm born children treated with primary PDA surgery had significantly lower total FSIQ in the adjusted model, compared to extremely preterm born children not treated for PDA. Pharmacological PDA treatment, with or without later PDA surgery, was not associated with lower FSIQ in extremely preterm born children (See **Table 5.3.1**, shown as adjusted mean difference in FSIQ).

Table 5.3.1 Association of PDA treatment category and NDI or FSIQ

	N=435	Risk of NDI Adjusted IRR* [95% CI] N=435	Adjusted mean difference in FSIQ* [95% CI] N=366
No PDA treatment	170	1.00 (ref)	Ref.
NSAID PDA treatment	139	1.23 [0.80-1.89]	-1.2 [-4.8 to 2.4]
PDA surgery after prior NSAID treatment	88	1.09 [0.70-1.70]	1.2 [-2.5 to 4.8]
Primary surgery	38	1.62 [1.28-2.06]	-7.1 [-11 to -3.2]

* adjusted for gestational age, sex, birth weight, SDS-score, maternal education, and intra-ventricular hemorrhage grades 3 or higher

Both postnatal steroids and BPD has been associated with risk of adverse neurodevelopmental but in the context of PDA they could be considered as mediators, when the association of PDA treatment to neurodevelopmental outcome is examined (they occur after PDA treatment which is the exposure). These adjustments were therefore made in a separate model for both NDI and FSIQ. The NDI risk estimates were slightly attenuated, but still highly significant for primary PDA surgery. The estimates for the association between the other PDA treatment categories and NDI remained unchanged. Furthermore, adjustment for postnatal steroids and BPD did slightly attenuate the association between primary PDA surgery and FSIQ, however, it also remained highly significant

Timing of PDA treatment

The median age for primary PDA surgery in the cohort was 17 days (IQR 12-26), and 20 days (IQR 13-26) for PDA surgery after prior NSAID treatment; p= 0.85. The risk of moderate to severe NDI was more than threefold, and the adjusted mean difference in FSIQ was -15 [95% CI: -19 to -12] among children operated at ≤10 days of age compared to children operated at ≥20 days of age. When all the children having PDA surgery were studied with timing of PDA surgery as exposure, the risk of moderate to severe NDI remained more than doubled in both early (at ≤10 days of age) PDA primary surgery and early PDA surgery

after prior NSAID treatment, while the FSIQ was no longer significantly associated with timing of surgery (see **Table 5.3.2**). The association of timing of surgical PDA treatment with moderate to severe NDI and FSIQ is shown in **Table 5.3.2**.

Table 5.3.2. Moderate to severe Neurodevelopmental Impairment (NDI) and cognitive outcome (measured as FSIQ[a]) by type and timing of PDA surgery at 6.5 years of age, after birth at <27 gestational weeks.

	≤10 days	**11 -20 days**	**>20 days**
PDA surgery, all (N=124)[b]	n= 23	n= 44	n= 57
Number of children with moderate or severe NDI, n	n=17	n=21	n=15
Moderate to severe NDI, IRR[c] (95% CI)	3.26 [2.40 to 4.42][c]	1.85 [0.99 to 3.44][c]	1.00 (ref)
Adjusted mean difference in FSIQ[d], (95% CI)	-15 [-19 to -12][d]	-1.4 [-6.2 to 3.3][d]	1.00
PDA surgery, primary (N=37)	n=7	n=15	n=15
Number of children with moderate or severe NDI, n	n=7	n=9	n=5
Moderate to severe NDI, IRR[c] (95% CI)	2.35 [1.11 to 4.96][c]	1.58 [0.77 to 3.24][c]	1.00
Adjusted mean difference in FSIQ[d], (95% CI)	-14 [-30 to 1.6][d]	-0.65 [-15 to 14][d]	1.00
PDA surgery, after NSAID treatment (N=87)	n=16	n=29	n=42
Number of children with moderate or severe NDI, n	n=10	n=12	n=10
Moderate to severe NDI, IRR[c] (95% CI)	2.90 [1.62 to 5.22][c]	2.07 [0.61 to 7.10][c]	1.00
Adjusted mean difference in FSIQ[d], (95% CI)	-10 [-17 to -3.1][d]	-2.0 [-8.5 to 4.6][d]	1.00

[a]FSIQ: Full-Scale Intelligent Quotient measured by the Wechsler Intelligence Scale for Children, fourth edition (WISC-IV)
[b]Of the 126 children having PDA surgery, only 124 had a date of surgery and were included in the analysis.
[c]Mixed effects Poisson model adjusted for GA, SDS birth weight, sex and IVH 3 or higher and maternal education with region and mother as random effect variable.
[d]Mixed effects linear model adjusted for: GA, SDS birth weight, sex and IVH 3 or higher and maternal education, with region as random effect variable.

Abbreviations: NDI: Neurodevelopmental Impairment; FSIQ: Full-Scale Intelligent Quotient, WISC: Wechsler Intelligence Scale for Children, IRR: Incidence Rate Ratio; CI: Confidence Interval; SD: Standard Deviation. NSAID: Non-Steroidal Anti-Inflammatory Drugs, GA: Gestational Age, SDS: Standard Deviation Score. IVH: Intraventricular Hemorrhage

Key points Paper III:

- No association was detected between pharmacological PDA treatment or PDA surgery following prior pharmacological treatment in the neonatal period, and neurodevelopmental outcome at 6.5 years of age.
- An increased risk of moderate to severe NDI and lower FSIQ was detected in children having primary PDA surgery.
- When timing of PDA surgery was examined as an exposure, children who were operated at ≤10 days of age, compared to children operated >20 days of age, had the highest risk for moderate to severe NDI and lowest FSIQ.

5.4 PAPER IV

Characteristics

There were no differences in GA, birthweight and proportion of PDA treatment between the included and non-included infants. The mortality rate was similar, but infants not included died at an earlier age than infants included. In total, 12 included infants later died, at a median age of 14 days (IQR 14-16) and no deaths occurred in the group of infants having PDA operation. Median age at start of any PDA treatment was 8 days (IQR 5-11). Of the 24 infants born at <25 weeks of gestation, 20 (83%), received PDA treatment, the remaining four were not treated and died before PDA closure. Of the infants having PDA surgery, 9/22 (41%) were born at <25 weeks.

PDA closure

Eighty-two (84%) infants had a PDA closure date registered, which was echocardiographically confirmed or due to PDA surgery in 79 (81%) of the infants. Three infants were assigned a closure date at full term (40 weeks). The remaining infants either died or were transferred before confirmed PDA closure. All infants with spontaneous closure of the PDA were born at ≥25 weeks of gestation. In **Figure 5.4.1a**, the probability for PDA closure for every day of life up to 40 weeks of PMA in infants born at <25 and at ≥25 weeks GA. (Kaplan Meier curve with censoring on death, transfer out of county or on day of PDA surgery) at ≥25, see **Figure 5.4.1a**. Four infants born at less than 25 weeks did not have PDA surgery, i.e. closed their PDA after pharmacological treatment only. In **Figure 5.4.1b** the cumulative probabilities of PDA closure in infants treated for PDA or not are shown, after exclusion of infants who later died, were transferred or had PDA surgery (a total of 38 infants). There was a significant difference in the 50% cumulative probability of PDA closure for treated vs not treated; by day 28 for infants spontaneously closing their duct and by day 51 for ibuprofen treated (p-value= 0.03).

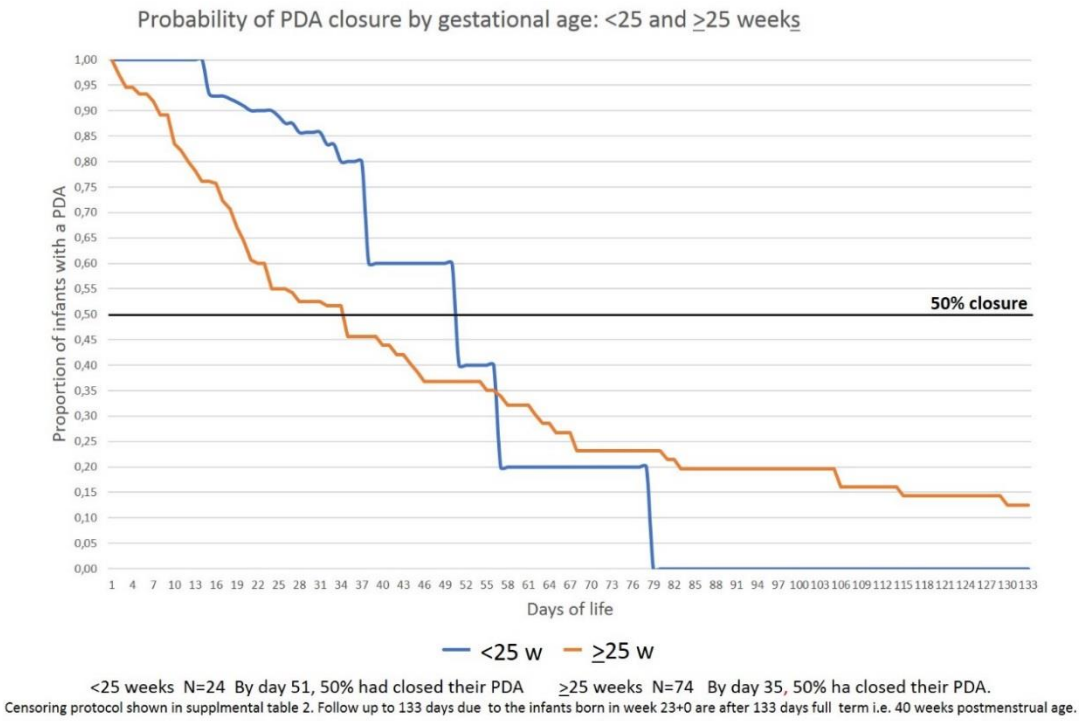

Figure 5.4.1a. Probability of PDA closure by gestational age. Censoring protocol is shown in paper IV.

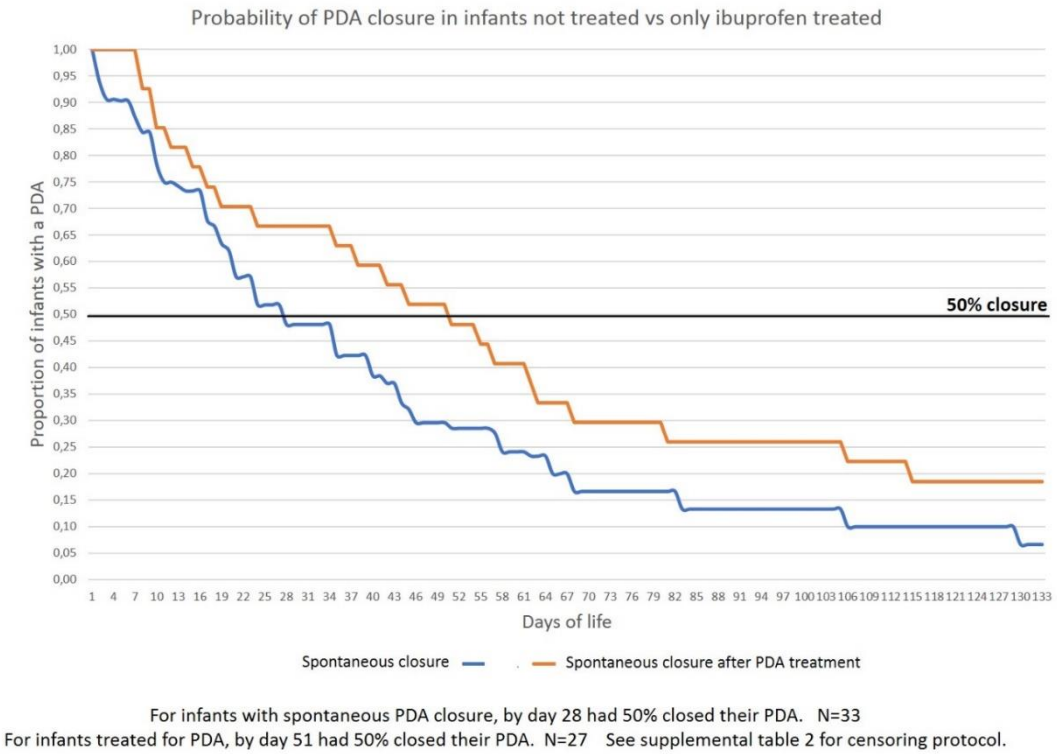

Figure 5.4.1b Probability of PDA closure in infants not treated vs only ibuprofen treated. Censoring protocol is shown in paper IV.

Echocardiographic markers of ductal severity

In table **5.4.1** the echocardiographic characteristics on day 3 and day 7 are shown.

Table 5.4.1. Echocardiographic markers at different times in different PDA treatment categories.				
98 infants in the cohort	**No PDA treatment**	**Ibuprofen only PDA treatment**	**Surgical PDA treatment**	
	N=40	**N=36**	**N=22**	
On day 3 (range 2-4)[a]				**P-value[g]**
Bidirectional shunt, or right to left through duct, n (%)	N=26	N=21	N=16	
	8 (30.8)	1 (4.8)	3 (18.8)	0.078
Ductal diameter, mm, median (IQR)	N=22	N=23	N=15	
	1.3 (1.2-1.7)	1.9 (1.7-2.0)	1.7 (1.6-1.9)	0.002
Vmax through duct, m/s, median (IQR)[b]	N=16	N=20	N=12	
	1.6 (1.2-2.4)	1.3 (1.1-1.9)	1.3 (1.2-1.6)	0.327
Vmax-Vmin through the duct, m/s, median (IQR)[b]	N=16	N=20	N=12	
	0.9 (0.6-1.2)	0.9 (0.7-1.0)	0.9 (0.7-1.0)	0.675
Resistance index, n (%), median (IQR)[b]	N=15	N=20	N=12	
	0.7 (0.6-2.8)	1.1 (0.7-1.6)	1.2 (0.8-2.2)	0.213
La:Ao ratio, median (IQR)	N=27	N=18	N=16	
	1.4 (1.2-1.6)	1.6 (1.4-1.9)	1.7 (1.6-1.8)	<0.001
Pulmonary (LPA or RPA) end-diastolic flow >0,2m/s, n (%)	N=26	N=18	N=16	
	4 (15.4)	4 (22.2)	11 (68.8)	0.001
Signs of ductal steal[c], n (%)	N=31	N=21	N=16	
	2 (6.5)	8 (38.1)	7 (43.8)	0.005
On day 7 (range 5-9)[e, f]				**P-value[g]**
Bidirectional, or right to left flow through duct, n (%)	N=29	N=33	N=20	
	6 (20.7)	4 (12.1)	2 (10.0)	0.506
Ductal diameter, mm, median (IQR)	N=25	N=31	N=17	
	1.6 (1.4-1.9)	1.6 (1.4-2-0)	1.8 (1.5-2.0)	0.411
Vmax through duct, m/s, median (IQR)[b]	N=19	N=29	N=17	
	1.8 (1.5-2.7)	1.7 (1.5-2.2)	1.3 (0.9-1.6)	<0.001
Vmax-Vmin through the duct, m/s, median (IQR)[b]	N=19	N=29	N=17	
	1.2 (1.0-1.4)	1.1 (0.9-1.3)	0.8 (0.6-1.0)	0.016
Resistance index[c], n (%), median (IQR)	N=19	N=29	N=17	
	0.8 (0.7-8.0)	2.0 (0.8-2.3)	0.9 (0.7-3.0)	0.481
La:Ao ratio, median (IQR)	N=31	N=29	N=16	
	1.5 (1.3-1.5)	1.5(1.4-1.8)	1.6 (1.4-1.7)	0.037
Pulmonary (LPA or RPA) end-diastolic flow >0,2m/s, n (%)	N=27	N=28	N=15	
	8 (29.6)	11 (39.3)	0 (0)	0.021
Signs of ductal steal[d], n (%)	N=32	N=30	N=19	
	7 (21.9)	15 (50.0)	6 (31.6)	0.064
[a]5 infants had closed their duct on the day 3 echocardiography. [b]When bidirectional shunt through duct, Vmax and Vmax-Vmin were not registered. [c]Resistance index calculated as: (Vmax-Vmin)/Vmax. Vmax and Vmin measured through the duct. [d]Ductal steal was defined as any of: reversed diastolic flow in descending aorta, absent or reversed diastolic flow in mesenteric arteries, absent or reversed diastolic flow in anterior cerebral artery. [e]4 infants had closed ducts on day 7 echocardiography, i.e. a total of 9. [f]Some had already started ibuprofen treatment. [g]Differences between treatment categories tested with Kruskal Wallis test.				
Abbreviations: PDA: patent ductus arteriosus. IQR: interquartile range. Vmax: maximal velocity. Vmin: minimal Velocity.. La:Ao: Left atrial to Aortic root. LPA: left pulmonary artery. RPA: right pulmonary artery.				

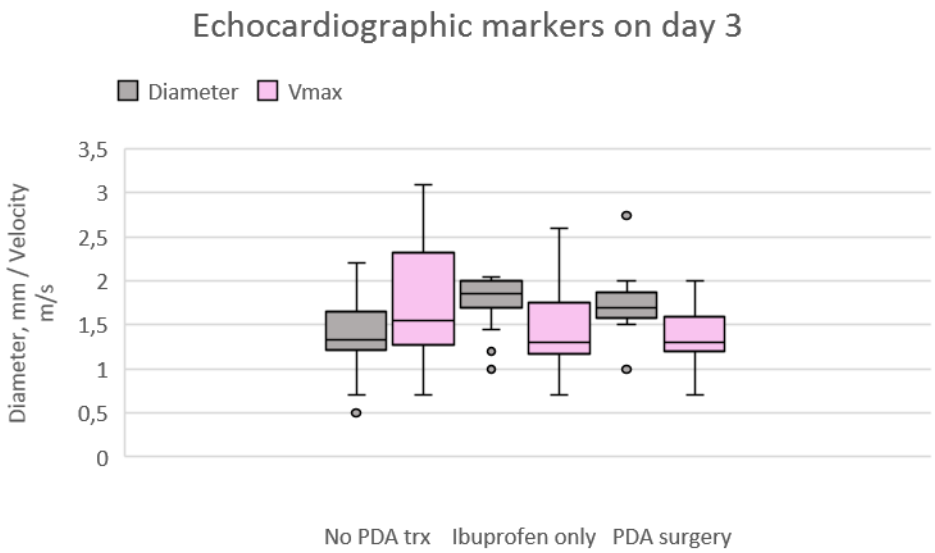

Figure 5.4.2. The echocardiographic markers ductal diameter and maximal velocity through duct (Vmax) on day 3 in the three PDA categories.

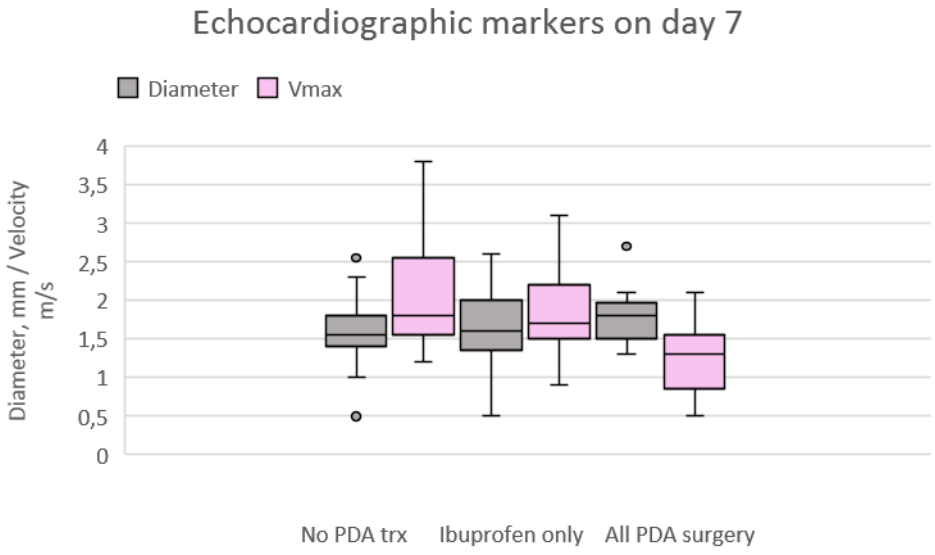

Figure 5.4.3. The echocardiographic markers ductal diameter and maximal velocity through duct (Vmax) on day 7 in the three PDA categories.

Biomarkers

cTnT values were not associated with spontaneous PDA closure or treatment for PDA.

Among 6 infants closing their duct spontaneously at ≤7 days, NT-proBNP was significantly lower on day 3, median 1810 ng/L (IQR, 1760-6000) compared to infants closing spontaneously at >7 days, 10900 ng/L (6120-19200); treated for PDA either with ibuprofen only, 14600 ng/L (7740-28100); or undergoing PDA surgery, 32300 ng/L (29100-35000).

Infants who later received PDA surgery had significantly higher NT-proBNP values than all the other groups on day 3. This is illustrated in **Figure 5.4.4**.

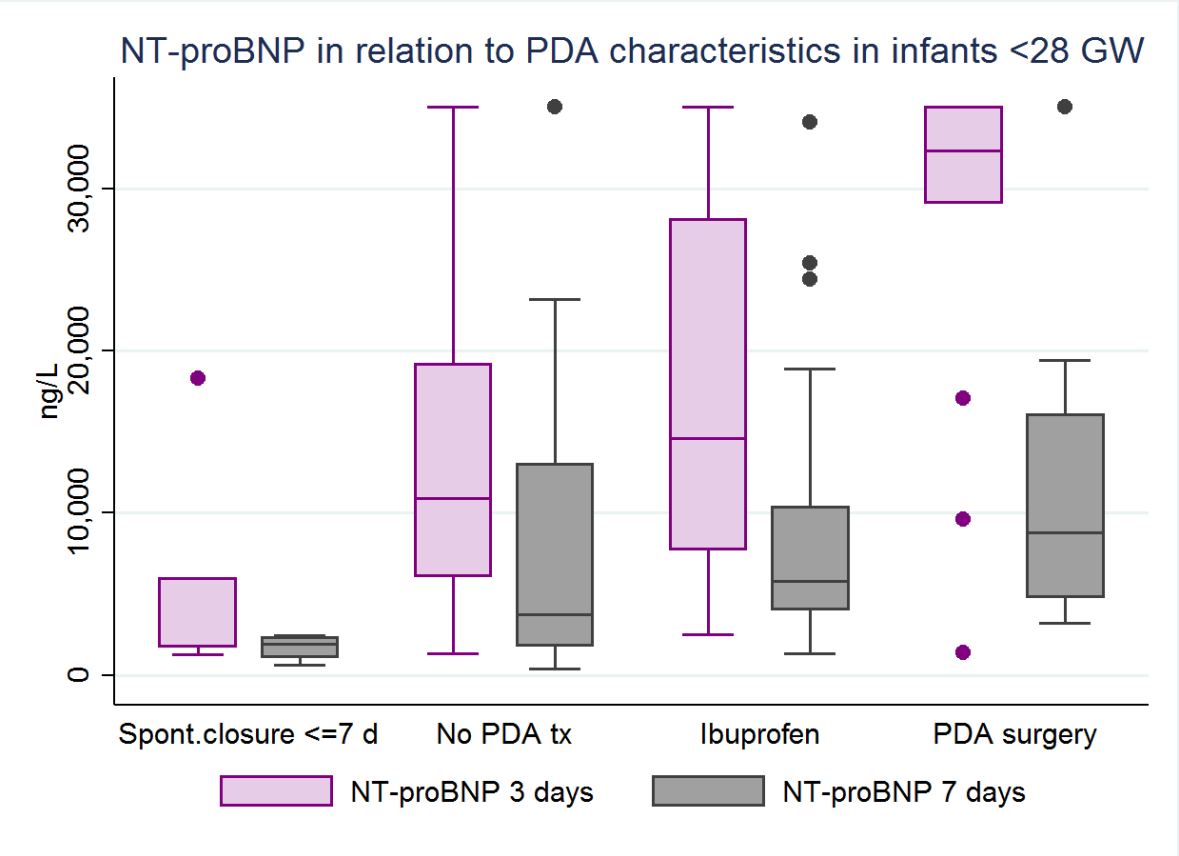

Figure 5.4.4 NT-proBNP in relation to PDA characteristics in infants born at <28 weeks of gestation.

Overall comparison between the groups on both day 3 and day 7 showed significant differences (p=0.001 and p=0.003 respectively) On day 3: In pairwise comparisons (Dunn's test): $p<0.05$ between spontaneous PDA closure ≤ 7 days and all other PDA categories, and between all other PDA categories and any PDA surgery. There was no significant difference between no PDA treatment (with open duct beyond first week of life) and ibuprofen treatment only. On day 7: $p<0.05$ for all pairwise comparisons, except for the difference between ibuprofen only and any PDA surgery.

Key points Paper IV:

- 59 % of the infants received any PDA treatment at a median age of 8 days (IQR 5-11) and 26% of infants received ibuprofen at an age of >7 days.
- One third of infants, of whom all were born at ≥25 weeks, closed their duct spontaneously, with a 50% probability of closure by day 28.
- Infants closing their duct spontaneously at ≤7 days had significantly lower NT-proBNP levels on day 3 and 7 compared to all other PDA categories.
- Infants who later had PDA surgery had significantly higher NT-proBNP levels on day 3 and 7 compared to all the other PDA categories.
- cTnT values were not associated with spontaneous PDA closure or treatment for PDA.

6 DISCUSSION

6.1.1 Importance of studies

The studies described in this thesis are cohort studies of extremely and very preterm infants conducted over a ten-year period. Two of the three cohorts are large population-based cohorts and include 2501 infants born at less than 28 weeks of gestation. Thereby, the studies are representative for the population in today´s neonatal units as the most complex PDA problems arise in infants born at less than 28 weeks of gestation. With the variation in treatment strategies the last decade, well designed observational cohort studies are informative, even though the PDA treatment strategies investigated are changing.[5,10] The design of the studies and the extensive prospective data collection make it possible to investigate the association between PDA and short and long-term outcomes after extremely preterm birth. The European EPICE cohort is very large and represents European PDA treatment well.[14]Although the infants in the EXPRESS cohort were born 15 years ago, the results from the cohort are still relevant, as the mean GA is 25.3 weeks and they received antenatal steroids to a high degree (90%), which is associated with optimal care and the best chances of survival.[13,112] Furthermore, the extensive neurodevelopmental assessment at school-age in paper III is informative, as it is important to evaluate neurodevelopment at an age that is sufficiently advanced to be predictive, yet young enough to benefit from modifying interventions (such as behavioral interventions and intensive physiotherapy). The EXPRESS and the EPICE cohorts do not have information on the echocardiographic diagnostics of the PDA, but the DEDUCT cohort in paper IV has that information, which makes it an informative study on diagnostics of the PDA, with combination of echocardiography and biomarkers.

6.1.2 Variation in PDA treatment

In the population-based EPICE cohort of very preterm infants, there were large differences in PDA treatment without associated variations in perinatal characteristics. Local treatment guidelines, if existing, varied (*personal communication Anna-Karin Edstedt Bonamy*). At the time of the EPICE study, the evidence for PDA treatment and association with outcomes was debated just as it is today. Different treatment strategies were practiced and in certain centers PDA treatment was abandoned.[113-115] Rates of both pharmacological and surgical PDA treatment have been declining worldwide. In the USA, rates of PDA treatament were compared from 2006 to 2015 with significant decreasing rates of PDA diagnosis, pharmacological treatment and PDA surgery.[5] In Sweden, the PDA treatment rates have also declined, from a rate of 60% during the EXPRESS study period 2004-2007 to approximately 40 % during 2016-2017.[4,21] The associations of these changes, if any, to incidence of neonatal morbidity is not fully examined or understood.

All studies described in papers I-IV are limited due to heterogenous data. In paper I-III the PDA treatment decision is based on PDA diagnosis which has low precision due to lack of data on echocardiographic details of the ductal severity and certain clinical variables. In paper

I, this may introduce a bias in the group treated at ≥7 days, as they may solely have been treated in the window of opportunity for ibuprofen, and not due to the ductal severity. In paper II, this introduces uncertainty in the interpretation of the variation in PDA treatment and especially in understanding of the association between PDA treatment and BPD. Evaluation of the PDA severity and the duration of the ductal shunt with echocardiography are very important factors in understanding the association between PDA and outcomes after treatment in extremely preterm infants.[73] In paper III, the untreated infants are likely a mix of infants not treated who closed their ducts, and infants who had a significant ductal shunt, but were never actively treated (conservative treatment was not registered). In paper IV, the echocardiographic severity is known, but due to methodological complications, data is heterogenous because of different timepoints of echocardiographies and biomarker values. Furthermore, there were >10 neonatologists who performed echocardiography of the included infants, which may have introduced an inter-operator variability.

Biomarkers as a complement to scoring systems of echocardiographic and clinical PDA severity is an attractive approach, as they are not dependent on echocardiographic resources or associated with inter-operator variability.[67] In our study, we found that NT-proBNP on day 3 is predictive of later PDA surgery in infants born at <28 weeks. Furthermore, infants with very low levels of NT-proBNP on day 3 closed their ducts spontaneously at ≤7 days of age. Infants, who later were treated with either ibuprofen only or later surgery had on day 3 an echocardiographically hsPDA (as defined by local guidelines as well as international scoring systems).[35]This indicates that the PDA treatment decision in the cohort is in line with the echocardiographic severity of the duct but the associations of NT-proBNP and the echocardiographic markers need to be further studied. In the group of infants not treated, the echocardiographic markers on day 3 were more varied, which can be explained by the fact that the group included both infants closing the duct spontaneously and infants who later died. In studies using the same NT-proBNP laboratoy analysis method, an association between NT-proBNP levels and PDA treatment were seen in cohorts of more mature infants.[79,80] This could support the role of NT-proBNP as a proxy for ductal severity.

6.1.3 Covariates

All three cohorts in the papers had information on many covariates and confounders, mostly perinatal and early neonatal. In general, the cohort characteristics did not differ substantially. The one characteristic of most importance for PDA treatment was GA, which confirms what other studies have shown.[29] Other covariates of importance for the specific outcomes were expected, such as SGA and mechanical ventilation for BPD and IVH and maternal education for neurodevelopmental outcome.[110,116,117] We have taken clearly defined perinatal and early neonatal factors into account, but the more detailed temporal relation of morbidities, which mostly are between the exposure and the outcome and therefore determined as mediators, are not as well defined in our cohorts. An example of such is sepsis which is related to long-term outcomes, such as neurodevelopmental outcome.[118]However, in these studies factors such as nutrition in context with PDA treatment has not been examined. It has been confirmed in the

EXPRESS cohort that infants undergoing PDA surgery had suboptimal nutrition.[119] This is a factor that needs consideration. Furthermore, retinopathy of prematurity (ROP) was not studied, but is an interesting morbiditet both as a mediator for later neurodevelopmental outcome and as an outcome after PDA treatment.

6.1.4 Gestational age

In paper I, the contribution of every week increase in GA was substantial in decreasing the risk of PDA surgery or death. In paper II, the risk for PDA treatment was also strongly associated with GA. Studies on spontaneous closure of PDA support this, but due to high treatment rates these studies are scarce.[29-32] In paper IV, all infants spontaneously closing their duct were born at ≥25 weeks.

Gestational age is strongly associated with both the need of PDA treatment and neurodevelopmental outcome, as is confirmed in our study in paper III, and therefore the most important confounder.[15,120] The exact contributions of IVH, GA, and PDA to neurodevelopmental outcome remain to be explored, as GA is a strong predictor of both IVH and PDA.

6.1.5 PDA closure

PDA closure has been the goal of PDA treatment and has been the outcome used for evaluating the efficacy of pharmacological treatment and later need for PDA surgery. Nevertheless, studies that can confirm the time of PDA closure are lacking in the literature (except when closed with PDA surgery). In paper IV, we could study PDA closure as longitudinal echocardiographies were performed. Spontaneous closure rate was similar and even higher in paper IV than earlier reported in extremely preterm infants.[29] Studies have confirmed that even infants born in the lowest GA's can close the duct spontaneously, but information on conservative treatment such as diuretics are lacking.[30,32] Spontaneous ductal closure rate in infants born <25 weeks is still difficult to study due to high PDA treatment rates.[121]In paper II, it is noted that infants born in weeks 28-32 of gestation are still being treated for PDA to a high extent in some regions. Current evidence argues that in most cases this is unnecessary.[29,31]

6.1.6 Timing of PDA treatment

Early studies on PDA treatment with NSAIDs showed that prophylactic or early treatment was associated with higher PDA closure and lower rates of PDA surgery.[122,123] These studies did not account for probability of spontaneous PDA closure.[29] Trials on early treatment compared with expectant management (day 3 vs day 7 to 11) did not show differences in frequency of later PDA surgery.[105,124] Furthermore, efficacy of NSAIDs on PDA closure is highest within the first 2-3 weeks of life.[125] It can be deducted that this has been a driving factor in the PDA treatment strategies studied and practiced through the years. In our studies, we had the opportunity to examine the timing of treatment, both pharmacological and surgical as an exposure. In paper I, in the Swedish EXPRESS cohort, infants treated at > 7

days of age compared to 0-2 days or 3-6 days did not have higher risk of PDA surgery or death, or later BPD. In a newly published study where start of treatment at more than 6 days of life was investigated, later PDA treatment were not associated with higher rates of PDA surgery or presence of PDA at discharge compared to earlier start of treatment.[126] Furthermore, there was no difference in the incidence of BPD between the two groups. In paper III, school-aged children undergoing PDA surgery at ≤10 days of age, compared to at >20 days of age, had the highest risk for moderate to severe NDI and lowest FSIQ. In the models, adjustments were made for many of the important confounders such as maternal education. Wickremashinge et al have earlier discussed that timing of PDA surgery is of importance when studying later neurodevelopmental outcome and physiologically the immature infant has less resilience to the hemodynamic challenge of PDA surgery early in life.[94]

6.1.7 PDA and BPD

BPD is studied as an outcome in papers I, II and as a mediator in paper III. The association between PDA and BPD is complex with many common risk factors. In theory, a shorter duration of the PDA shunt and thus shorter period of excessive pulmonary circulation could be beneficial. Results of trials and cohort studies have been contradictory; PDA treatment has been associated with both decreased and increased pulmonary morbidity and development of BPD.[103,127] In paper I, early pharmacological PDA treatment was not associated with lower risk of BPD compared to late start of PDA treatment. Other cohort studies have supported these findings.[128] In a few contemporary randomized trials, one of them using prophylaxis with indomethacin (TIPP trial) and two on early vs expectant management, there was no difference in BPD incidence or mortality between infants receiving early NSAIDs and later treatment or placebo.[93,105,126] In the EPICE study, paper II, any PDA treatment was associated with the risk of BPD. Furthermore, infants born in regions with high proportion of PDA treatment had higher BPD risk compared to low proportion of PDA treatment. This can be due to a more pro-active surgery approach in the high proportion regions, or to suboptimal care. PDA surgery has been associated with higher risk of BPD, though it is debated that it is due to confounding of indication.[127] Studies on prophylactic PDA surgery described an increase in BPD, despite shorter mechanical ventilation.[9,103,129] A limitation of our studies in paper I and II, is the lack of knowledge on exact ductal shunt time, as the exact date of ductal closure is not known (unless when date of surgery). This information would be helpful for understanding the association of PDA and BPD and can be explored in the cohort in paper IV as the data is collected for further analyses.

6.1.8 PDA and neurodevelopmental outcome

Active PDA closure in extremely preterm infants has not been convincingly shown to improve long-term neurodevelopmental outcome.[11] In fact, PDA treatment has been associated with adverse neurodevelopmental outcome at two to three years of age compared to untreated children.[24,25,27] It is debated what the benefits of PDA surgery are and if they outweigh the risk with surgery in such a vulnerable population as extremely preterm infants.

It has been suggested that the associations between PDA treatment and adverse neurodevelopmental outcome may result from confounding by indication or survival bias as well as early timing of surgery.[98,130] In paper III, an association could not be detected between pharmacological PDA treatment or PDA surgery following prior pharmacological treatment in the neonatal period and later neurodevelopmental outcome. Children having primary PDA surgery, on the other hand, had increased risk of moderate to severe NDI and lower FSIQ compared to children not treated. This was supported by a recent cohort study, in which PDA surgery was not associated with NDI at 18-24 months of age when adjusted for both perinatal and preligation neonatal risk factors.[106] In that study, all PDA surgery was investigated as one exposure, regardless of whether primary or not.[106] In our study in paper III it was possible both to explore PDA surgery with and without prior ibuprofen treatment and to explore the association between timing of PDA surgery and later outcome. In all children undergoing PDA surgery at ≤10 days of age, compared to surgery at >20 days of age, the highest risk for moderate to severe NDI and lowest FSIQ was in the group operated early (<10 days). The increased risk for adverse neurodevelopmental outcome observed after primary PDA surgery in our study was explored. Earlier timing of the PDA operation was one of them and incidence of IVH grade 3 or higher, in the group having primary PDA surgery was another but even after adjustment were made for these and other several potential confounders the association remained significant. Confounding, either residual or by indication, is still possible though, as the temporal relationship of comorbidities was not known and lack of power for IVH cannot be excluded. It has been discussed that BPD is a risk factor for adverse neurodevelopment. This was confirmed in a recent a meta-analysis of cohort studies.[131] In the analyses in paper III, this was approached as we adjusted for the potential mediators, postnatal steroids (given to treat pulmonary morbidity) and BPD, but that did not change the association between PDA treatment and risk for adverse neurodevelopmental outcome. Future studies should elucidate the role of PDA and its treatment in the evolvement of BPD, and further examine their role on neurodevelopmental outcome.

6.2 EPIDEMIOLOGICAL CONSIDERATIONS

Table 6.2.1 Overview of epidemiologic and statistical considerations in all the studies in the thesis

	Study I	*Study II*	*Study III*	*Study IV*
Design	Prospective cohort study	Prospective cohort study	Prospective cohort study	Prospective cohort study
Population, period and gestational age	**EXPRESS** Sweden 2004-2007 <27 weeks	**EPICE** Europe 2011-2012 <32 weeks	**EXPRESS** Sweden Born 2004-2007 <27 weeks Follow-up 2010-2013	**DEDUCT** Stockholm 2012-2014 <27 weeks
Number of infants included	290	6896	435	98
Power consider-ations	In a sample of 290 infants having NSAID PDA treatment, the power was 80% to detect a HR of 1.45, as 127 infants had later PDA surgery or died. (alfa-level of 0.05).	Large population-based cohort so assumed that power is not an issue.	In a sample of 435 children, with 4 treatment categories, the power was 80% to detect differences in FSIQ of 4 in overall analysis and of 11 in analysis for timing of surgery. (mean FSIQ 100 and SD 15 in the comparison group) (alfa-level 0.05)	In a sample of 80 infants where 50% have a hsPDA, the power is 80% to detect a difference in biomarkers with SD <0.5, between infants with hsPDA and minimal or closed DA (alfa-level of 0.05)
Age at inclusion	Alive at 24 hours of age	Alive at 24 hours of age	Alive at 6.5 years of age at follow-up.	Alive at inclusion (up to 72 hours of age)
Definition and diagnostics of the exposure	Echocardiographically verified PDA, treated with NSAIDs. Echocardiographic data not available (i.e. ductal severity not known)	PDA treated with NSAIDs and PDA surgery. Echocardiographic data not available (ductal severity not known)	Echocardiographically verified PDA treated with NSAID´s and/or PDA surgery. Echocardiographic data not available (ductal severity not known)	Echocardiographically verified hsPDA. Ductal severity known.
Heterogenous population in exposure categories	Ductal severity not known due to non-availability of echocardiographic data and therefore uncertainty about the indication for treatment			Due to delayed or deferred blood sampling or echocardiography, the biomarkers and echocardiography markers results can be heterogenous
Outcomes	Composite outcomes: 1) PDA surgery or death 2) BPD at 36 weeks PMA in survivors	Composite outcome 1) BPD or death 2) Survival without major neonatal morbidity	NDI and FSIQ at 6.5 years of age in survivors	PDA closure -spontaneous -ibuprofen treatment only -PDA surgery
Examples of bias	Survival bias Selection bias	Survival bias Selection bias	Selection bias	Survival bias Selection bias
Clustering	Addressed	Addressed	Addressed	Not addressed
Other analytical considerations	At risk time-period handled with the COX proportional hazards regression model (from treatment start and up to 90 days of age). Temporal relationship of morbidities, such as sepsis would have been optimal to account for.	Perinatal factors in the propensity score and adjusted for one factor instead of many covariates. Temporal relationship morbidities, such as sepsis, would have been optimal to account for.	Power concerns in primary PDA surgery group. BPD and postnatal steroids in the mediator pathway and therefore "not correct" to adjust for. Mediator analysis tested, but no difference in results compared to usual regression analysis.	What is the most optimal outcome in this study? Echocardiographically verified PDA closure was chosen. Treatment of PDA was at the discretion of the neonatologist in charge. What is the gold standard?

6.2.1 Bias

The role of bias in studies on PDA is debated and many have pointed out the importance of taking the risk of bias to account in the study design and the analyses strategy.[26,130]

1. *Survival bias:* In all the studies, there is an inclusion criterion of surviving up to 24 hours of age (and up to 72 hours of age in paper IV) to be included in the analysis. This introduces a survival bias as the infants have to survive to be included. In paper I, we have used a composite outcome of PDA surgery or death and a time-to-event approach. This aids in approaching the high mortality rate in extremely preterm infants the first few days and weeks as well as accounting for the time the infant was at risk for PDA surgery after pharmacological PDA treatment, until they were operated or 90 days of age. By this approach, underestimation of the risk of surgery in the early treatment group (having the highest mortality, see Figure 5.1.1 in methods) is avoided. In paper II, the same approach is applied with the composite outcome of BPD or death to account for mortality. In paper III, only survivors to school-age were included and survival bias was not an issue. In paper IV, Kaplan Meier curves allowed for censoring at time of death.[132] Here the data show that infants need to survive to have PDA treatment, as the infants not treated who later died had an echocardiographical PDA severity which was similar to the severity seen in infants that were later treated for PDA. The infants not treated who later died, would probably have been treated if they had survived. Furthermore, it becomes evident that infants have to survive to undergo PDA surgery, as none of the operated infants in the cohort in paper IV died before 40 weeks of gestation. In paper I, there were infants who died after PDA surgery, indicating that pre- and post- operative care may have improved between 2004-2007.

2. *Selection bias:* In paper I, the risk of BPD was lower in the infants treated later (<7 days) compared to infants treated early (0-2 days), despite the early group having a shorter duration of mechanical ventilation. This can be explained by selection bias, infants treated late are treated in the window of opportunity for NSAID treatment as discussed earlier. In paper II, the differences in access to PDA surgery may have introduced a bias which can be associated with the increased risk of BPD in regions with higher proportion of PDA treatment and higher PDA surgery rates. In paper I and III, region of birth was associated with timing of PDA treatment as well as incidence of PDA surgery. In paper III, the outcome of neurodevelopmental outcome was not associated with region. Nonetheless, clustering was accounted for in the analyses in both studies. (se methods section). In paper IV, selection bias may have been introduced as the clinician in charge of the infant was not blinded to the biomarker results.

3. *Misclassification bias:* By definition[107] the studies in this thesis (paper I-IV) do not have a misclassification bias as the categories of exposures are clear, i.e. pharmacological PDA treatment in paper I, pharmacological and/or surgical PDA

treatment in paper II and in paper IV an echocardiographically determined hsPDA. In papers II and III, the comparison group are infants not treated for PDA. As the severity of the PDA is not accounted for in papers I-III due to non-availability of echocardiographical data, there is a risk of misclassification from the point of view of PDA severity. The control groups of infants not treated are hetergenous, there are probably infants that had significant PDA shunts, not treated or treated conservatively (CPAP or diuretics). This is a methodological problem in all the studies in this thesis. In paper IV, the echocardiographic data reveals this and furthermore reveals another problem, i.e. the definition of the hsPDA is unclear.

6.2.2 Confounding

The data sets allowed for adjustments for various covariates and confounders in the analyses of all the four studies. There were limitations though. In paper II, the propensity score only included perinatal and early neonatal factors, and not information on the severity of the respiratory distress and time sequence of neonatal morbidities (such as infections) as this was not collected. The propensity score has to include factors occurring before PDA treatment and as the detailed temporal relationship was not known, these mentioned factors could not be included. This is a limitation in understanding the association of PDA treatment outcomes and especially BPD and residual confounding or confounding by indication cannot be excluded in that study. In paper III, confounding by indication or residual confounding cannot either be excluded as a contributing factor to the association between primary PDA surgery and later risk of NDI and lower FSIQ. Confounding by indication has in other papers been suggested as a potential explanation of the association between PDA surgery and adverse developmental outcome.[130]

6.2.3 Power

As shown in table 6.2.1 above, the calculated power for studies I, III and IV was appropriate for the hypotheses tested. In paper III, there were issues that may be explained by limited power. In the analysis, IVH grade 3 or higher was neither significantly associated with NDI, nor with FSIQ at 6.5 years of age, which might be explained by limited power especially in the case of FSIQ. In paper IV, the subgroup analysis is limited due to lack of power and posthoc analysis not possible on the echocardiographic data.

6.2.4 Time-dependencies and temporal relationships

In all the papers the importance of time dependencies and the time sequence of morbidities is emphasized. In paper I, the time at risk for PDA surgery has to be identified and accounted for in the analysis. This is fulfilled with the COX proportional hazards regression model. In paper II, it is a clear limitation that the temporal relationships were not known for certain neonatal morbidities. This was of methodological reasons not possible, firstly the exact dates of diseases were not known, such as sepsis, or start and stop date of each episode of mechanical ventilation. Therefore, these factors were not included in the propensity score, which predicted PDA treatment as they have to occur before treatment. Furthermore, this can

be a source of confounding. In paper III, the same methodological problem of temporal relationship was encountered and furthermore, the confounders were more of mediators as they encountered after the exposure but before the outcome (example of postnatal steroids and BPD described in background-epidemiology chapter). In paper IV, the longitudinal echocardiographies enabled us to measure shunt time and verify PDA closure. In the analysis of the PDA closure rate, the Kaplan Meier curves allow for censoring because of death or loss to follow-up.[132] As extremely preterm infants have a high mortality rate during the first weeks, this is advantageous. All these factors have been discussed in previously published papers on methodological consideration in PDA studies.(see above)

6.2.5 Validity

The internal validity of the studies in this thesis is relatively high, if the considerations described in the discussion earlier on the methodological concerns is taken into account. External validity, that is how the results can be generalized, is appropriate. The cohorts are representative for modern neonatal intensive care population which strengthens the external validity, although the PDA treatment strategies are changing. In paper III, the cognitive results (FSIQ) of the extremely preterm infants were categorized according to the distribution (SD´s of FSIQ) of the matched control children recruited for the original EXPRESS study.[15] This increases the generalizability of the results to other similar highly educated populations. The external validity of paper IV is strengthened by the number of immature infants in the cohort with both cardiac biomarkers and echocardiographic evaluations of ductal severity as 24% are born < 25 weeks.

7 CONCLUSIONS

PDA treatment incidence

- There is a large variation in PDA treatment strategies in very preterm infants, in Europe which is not explained by associated variation in perinatal risk factors for PDA.

Immaturity

- Gestational age is the most important predictor for PDA treatment.
- Spontaneous PDA closure rate is higher than earlier observed in extremely preterm infants (from 25 weeks of gestation).
- Infants born <25 weeks of gestation have high PDA treatment rates and therefore the spontaneous closure rate was not possible to establish.

Timing of PDA treatment

- Timing of pharmacological treatment is not associated with risk of later need for PDA surgery or death.
- Later PDA treatment is not associated with increased risk for BPD.
- Later start of PDA treatment, possibly reduces the risk of unnecessary NSAID treatment and thereby exposure to a potentially toxic drug.
- Early PDA surgery (at <10 days of age) may be a contributing factor in the association between PDA surgery and adverse neurodevelopmental outcome.

Outcomes after PDA treatment

- Infants treated for PDA, compared to those not treated, are at higher risk of BPD or death in all regions.
- Survival without major neonatal morbidity is not related to PDA treatment. The potential association between PDA and BPD is complicated and not easy to disentangle in cohort studies.
- PDA surgery after prior pharmacological treatment and at a later age in life is not associated with adverse neurodevelopmental outcome.

Biomarkers

- NT-proBNP biomarker is a possible proxy for ductal severity and can be helpful in combination with clinical and echocardiographic markers in evaluating need of PDA treatment.
- cTnT is neither associated with ductal severity, as estimated with echocardiographic markers, nor PDA treatment in the neonatal period.

Methodological considerations

- Defining the PDA as an exposure is of importance and the most relevant exposure is probably the magnitude and duration of the PDA shunt.
- Time-dependent analysis strategies, and analysis of sequential disease burden is important in studies on PDA as an exposure for later morbidities.
- Conservative treatment should be distinguished from no treatment and evaluated in the same manner as pharmacological and surgical PDA treatment.
- There is a need to standardize PDA treatment, but first optimal placebo-controlled trials on PDA management are needed for further optimizing evidence-based care of extremely preterm infants.

8 IMPLICATIONS

The studies in this thesis have identified several areas of concern in the diagnostics and treatment of the PDA. There is large variation in PDA strategies between units and data was not collected on ductal severity in papers I-III. Neither our cohort studies, nor randomized trials on PDA treatment have been able to support a causal relationship between PDA and adverse outcomes. Nevertheless, the theoretical rationale and clinical experience support that the PDA should be respected and not treated as an innocent bystander, but more understanding is needed. PDA diagnostics and management need to be studied in optimally designed trials. Furthermore, this should be conducted as non-invasively as possible as extremely preterm infants belong to a very vulnerable population.

The first step is to be taken in routine clinical care. We need to start registering the diagnostic markers and diagnoses with more precision, to help distinguishing between infants with a non-significant PDA that closes spontaneously and infants having a PDA that is treated conservatively. In the current studies, the PDA exposure has seldom taken into consideration the duration of the shunt. Therefore, longitudinal echocardiographies are needed to confirm closure of the duct and to determine the shunt time. This will strengthen the clinical understanding and pave the way for future studies. Only a few of the most commonly used echocardiographic markers are strongly related to shunt magnitude. In fact, most of the markers are correlated to an open duct in the first days of life and not beyond the first week. Gestational age is the strongest predictor of PDA treatment and future studies should focus on infants born at less than 26 weeks. Other diagnostic methods could be helpful, one of them is Near-Infrared Spectroscopy, measuring regional saturation which is important in evaluating signs of ductal steal. Optimal scoring systems of PDA severity, where clinical and echocardiographic, as well as biomarkers are combined are attractive since PDA severity is likely multifactorial. With more precise diagnostics of the PDA, it is probably easier to define the risk population and sharpen the inclusion criteria in future studies. What are the optimal outcomes to study in context of the PDA? It can be argued that aiming for absolute PDA closure is not the most important outcome, rather how the ductal shunt can be modified and minimized to reduce clinical morbidities such as IVH, NEC and pulmonary hemorrhage and further on BPD. For example, if we modify the shunt with pharmacological treatment without closing the PDA, does the likelihood of extubation increase and thereby reducing the duration of mechanical ventilation, which in turn is a strong riskfactor for BPD?

Understanding the pathophysiology of the PDA and the hemodynamic changes with the usual treatment applied in the NICU are central to this. What do long periods of ductal shunting with negative effects on the circulation have for consequences? Is it possible to modify these effects to decrease risks of IVH, pulmonary hemorrhage, NEC, BPD, ROP and long-term adverse neurodevelopment? An example, of the complexity in understanding the clinical course and the vulnerability of the population in question, is in infants undergoing PDA surgery. They comprise a high-risk population, born at low gestational ages with a complex respiratory and circulatory clinical course which could be related to the PDA. Imaging studies

have found an association between surgical treatment for PDA and smaller brain volumes at term age in this group of infants.[133] Smaller brain volumes are indicators of disturbances in white matter maturation and associated with adverse neurodevelopmental outcome.[134] Plausible explanations for this could be related to circulation disturbances in association to surgery, as both changes in regional cerebral oxygenation and blood flow have been described.[135-137] But these changes could also be related to the duration and magnitude of ductal shunt.[138] This understanding of the hemodynamic changes is maybe a key issue in the infants and how to reduce morbidities for example NEC.[139] The hemodynamic consequences of a long standing PDA shunt are not fully understood and studies on long-term cardiac outcome are needed.

The future goal must be to design optimal trials as well as observational studies, with a clear definition of the PDA exposure and all treatment modalities considered. In a randomized trial, a placebo-arm with either no rescue treatment or at least not before two weeks of age, is warranted.

Moreover, in designing future trials measures to minimize the discomfort of the immature infants due to examinations and interventions need to be taken. Further innovation of non-invasive methods to evaluate the hemodynamics in extremely preterms is needed.

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