Haemodynamics in preterm infants with Patent Ductus Arteriosus

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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Abstract

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The primary aims addressed by my thesis were a multimodal, integrated assessment of influences of patent ductus arteriosus (PDA) on cerebral haemodynamics in extremely preterm infants using echocardiography, cerebral near infrared spectroscopy, amplitude integrated electroencephalography (aEEG) and cerebral ultrasonography, and the evaluation of a pre-defined two-step model for the causation of brain injury by PDA shunt (Chapter 3). The dataset was rich and provided an opportunity to characterise components of the multimodal assessment including: influences on the aEEG (Chapter 4) and antenatal influences on elements of the multimodal assessment (Chapter 5). Moreover, I investigated further existing biomarkers of aortic haemodynamics [pulse transit time (PTT) and pulse wave velocity (PWV)] and related them to the PDA and cerebral biomarkers and devised a novel biomarker [phase difference (PD)] (Chapter 6).

The analysis demonstrated that severe intraventricular haemorrhage (IVH) was associated with the presence of large PDA and lower cerebral oxygenation. Furthermore, there was a significant association between lower cerebral oxygenation, lower cerebral electrical activity and higher resistivity index on anterior cerebral artery with larger PDA size, but not in interaction of these variables with IVH severity. This means that separate steps of a proposed two step model were valid, but not the entire two-step model. IVH happens early during extrauterine life or even during pregnancy and during the study there were more observations reflecting the aftermath following the IVH. Hence, it is uncertain whether the observed relationships are the cause or the epiphenomenon of brain injury. The strong association between PDA size and all the cerebral biomarkers implies that PDA has important and simultaneous effects on global cerebral function. PDA in some preterm infants has a different natural course and maybe linked to cerebral injury. However, many preterm infants with large PDAs did not develop severe IVH and had normal cranial ultrasound at discharge. This supports the ongoing controversy regarding the definition of the haemodynamically significant PDA. Whether and at which critical point the PDA becomes a disease is still uncertain and it is very possible this goalpost to be shifting depending on the rest of the neonatal comorbidities.

Chapter 4 demonstrated that cerebral electrical activity increases with postnatal age and baseline gestational age, but decreases with morphine administration and the presence of a larger PDA.

Chapter 5 investigated the neonatal cardiovascular and cerebral function following antenatal maternal $MgSO_4$ administration. Maternal BMI and duration of $MgSO_4$ infusion (and their interaction) appeared to have significant effect on neonatal Mg^{2+} that may merit changes to the dosing regimen. No associations were demonstrated

between neonatal serum Mg^{2+} with PDA score, cerebral oxygenation and cerebral electrical activity. Future dose finding studies should be based on multicompartmental population pharmacokinetic studies that include maternal and neonatal pharmacodynamic measures and sample size calculation was performed to inform these future studies.

Chapter 6 demonstrated that PDA has significant effects on aortic haemodynamics as measured by PTT, PWV and PD. In a small population of extremely preterm infants a strong positive correlation was found between PDA and PD which renders further investigation with validation studies. A continuous predictive biomarker for PDA size can be a helpful screening tool for infants needing echocardiography in the neonatal unit in an effort to use human resources more effectively and reduce the disturbance of the vulnerable preterm infants by repeated echocardiograms. Moreover, the analysis demonstrated the clinical potentials of combining PWV and blood pressure data to predict left ventricular cardiac output in the descending aorta without the need for echocardiographic assessment, which can be useful clinical information for the management of this population.

“As you set out for Ithaka
hope the voyage is a long one,
full of adventure, full of discovery.
Laistrygonians and Cyclops,
angry Poseidon—don’t be afraid of them:
you’ll never find things like that on your way
as long as you keep your thoughts raised high,
as long as a rare excitement
stirs your spirit and your body.
Laistrygonians and Cyclops,
wild Poseidon—you won’t encounter them
unless you bring them along inside your soul,
unless your soul sets them up in front of you.

Hope the voyage is a long one.
May there be many a summer morning when,
with what pleasure, what joy,
you come into harbours seen for the first time;
may you stop at Phoenician trading stations
to buy fine things,
mother of pearl and coral, amber and ebony,
sensual perfume of every kind—
as many sensual perfumes as you can;
and may you visit many Egyptian cities
to gather stores of knowledge from their scholars.

Keep Ithaka always in your mind.
Arriving there is what you are destined for.
But do not hurry the journey at all.
Better if it lasts for years,
so you are old by the time you reach the island,
wealthy with all you have gained on the way,
not expecting Ithaka to make you rich.

Ithaka gave you the marvellous journey.
Without her you would not have set out.
She has nothing left to give you now.

And if you find her poor, Ithaka won’t have fooled you.
Wise as you will have become, so full of experience,
you will have understood by then what these Ithakas mean.”

Konstantinos Kavafis: Ithaka

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List of Abbreviations

ACA	Anterior Cerebral Artery
AIC	Akaike's Information Criterion
aEEG	Amplitude Integrated Electroencephalogram
ANP	Atrial Natriuretic Peptide
ANS	Autonomic Nervous System
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
BPD	Bronchopulmonary Dysplasia
BW	Birth Weight
CBF	Cerebral Blood Flow
cFOE	Cerebral Fractional Oxygenation Extraction
CK	Charalampos Kotidis
CLD	Chronic Lung Disease
CPAP	Continuous Positive Airway Pressure
CO	Cardiac Output
CONS	Coagulase Negative Staphylococcus
CRF	Case Report Forms
CRT	Capillary Refill Time
CrUSS	Cranial Ultrasonography
cTOI	Cerebral Tissue Oxygenation Index
CWD	Continuous Wave Doppler
DA	Ductus Arteriosus

Dao	Descending Aorta
DW	David Wertheim
E:A ratio	Mitral valve E:A ratio
ECG	Electrocardiogram
EDFV	End-Diastolic Flow Velocity
EEG	Electroencephalogram
EPI	Extremely Preterm Infants
GA	Gestational Age
HFOV	High-Frequency Oscillatory Ventilation
HIE	Hypoxic-Ischaemic Encephalopathy
hsPDA	Haemodynamically Significant Patent Ductus Arteriosus
IQR	Interquartile Range
IRAS	Integrated Research Application System
IUGR	Intrauterine Growth Restriction
IVH	Intraventricular Haemorrhage
IVRT	Isovolumic Relaxation Time
LA	Left Atrium
LA:Ao	Left Atrium To Aortic Root Ratio
LPA	Left Pulmonary Artery
LV	Left Ventricle
LVEDD:Ao	Left Ventricular End Diastolic Diameter To Aortic Root Ratio
LVO	Left Ventricular Output
LWH	Liverpool Women's Hospital
$Mg2^{+}$	Neonatal Serum Ionised Magnesium Concentrations
$MgSO_4$	Magnesium Sulphate

MRI	Magnetic Resonance Imaging
N	Number Of Patients
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
NIRS	Near Infrared Spectroscopy
NO	Nitric Oxide
NPV	Negative Predictive Value
NSAID	Non-Steroidal Anti-Inflammatory Drug
NT-proBNP	Amino-Terminal Pro-B-Type Natriuretic Peptide
PA	Pulmonary Artery
PD	Phase Difference
PDA	Patent Ductus Arteriosus
PFO	Patent Foramen Ovale
PK	Pharmacokinetics
PSFV	Peak Systolic Flow Velocity
PNS	Parasympathetic Nervous System
PPV	Positive Predictive Value
PROM	Prolonged Rupture Of Membranes
PTT	Pulse Transit Time
PVL	Periventricular Leukomalacia
PWD	Pulsed Wave Doppler
PWV	Pulse Wave Velocity
RA	Right Atrium
RCT	Randomised Controlled Trial
RDS	Respiratory Distress Syndrome

RI	Anterior Cerebral Artery Resistivity Index
ROP	Retinopathy Of Prematurity
RVO	Right Ventricular Output
SaO2	Transcutaneous Arterial Oxygen Saturation
SNS	Sympathetic Nervous System
SVC	Superior Vena Cava
SVD	Spontaneous Vaginal Delivery
TOI	Tissue oxygenation index
TPN	Total Parenteral Nutrition
UAC	Umbilical Arterial Catheter
UK	United Kingdom
VLBW	Very low birth weight infant
VTI	Velocity Time Integral

Chapter 1: Introduction

Prematurity is the leading cause of mortality and morbidity in children less than 5 years of age with approximately 1.1 million deaths in 2010 (1). A common feature among babies born at extreme prematurity is a patent ductus arteriosus (PDA). The impact of PDA has not been identified completely, but there is concern that PDA alters the amount of blood flowing into the brain. This thesis explores the relationship between PDA and blood flow into the brain including multiple methods to assess, and identify the implications of that relationship.

Prematurity

Definition

Prematurity, according to the World Health Organisation, is defined as birth before 37 weeks gestational age (GA) are completed (2). Preterm infants can be further subdivided according to GA into the following groups: extremely preterm (less than 28 weeks), very preterm (28 to 32 weeks) and moderate to late preterm (32 to 37 weeks). Of the births that were preterm in the United Kingdom (UK) 5% were extremely preterm, 11% very preterm and 85% moderately preterm (3).

Epidemiology

The exact burden of this condition globally is unknown, but it is estimated that 15 million infants are born earlier than normal which is 1 in 10 live births (4). Preterm birth rates vary widely among European countries, ranging from 5.4 to 12% (5). The rates in UK were above the European average and consisted of 7.6% in England and Wales, 8.2% in Scotland and 7.4% in Northern Ireland (6).

Preterm babies born before 28 weeks of gestational age constitute over one-third of all deaths, but data are not comparable between countries. About one-third of all fetal deaths and 40% of all neonatal deaths were of infants born before 28 weeks of gestational age (5). The geographical distribution of preterm births among live births in Europe can be further assessed in Figure 1.

Survival and Morbidity

Advances in understanding of prematurity and medical treatment led to improved outcomes in the recent decades (Table 1). The survival rates of preterm infants in France were 94% at 27-31 weeks GA and 99% between 32-34 weeks (EPIPAGE-2 study) (7). In the same cohort survival without severe neonatal morbidity was 0% at 23 weeks, 12% at 24 weeks, 30% at 25 weeks, 48% at 26 weeks, 81% at 27-31 weeks, and 97% at 32-34 weeks. Between 1997 and 2011 the proportion of infants surviving without severe morbidity increased by 14% at GA between 25 and 29 weeks and 6% at 30-31 weeks, but did not change significantly for infants born at less than 25 weeks (7).

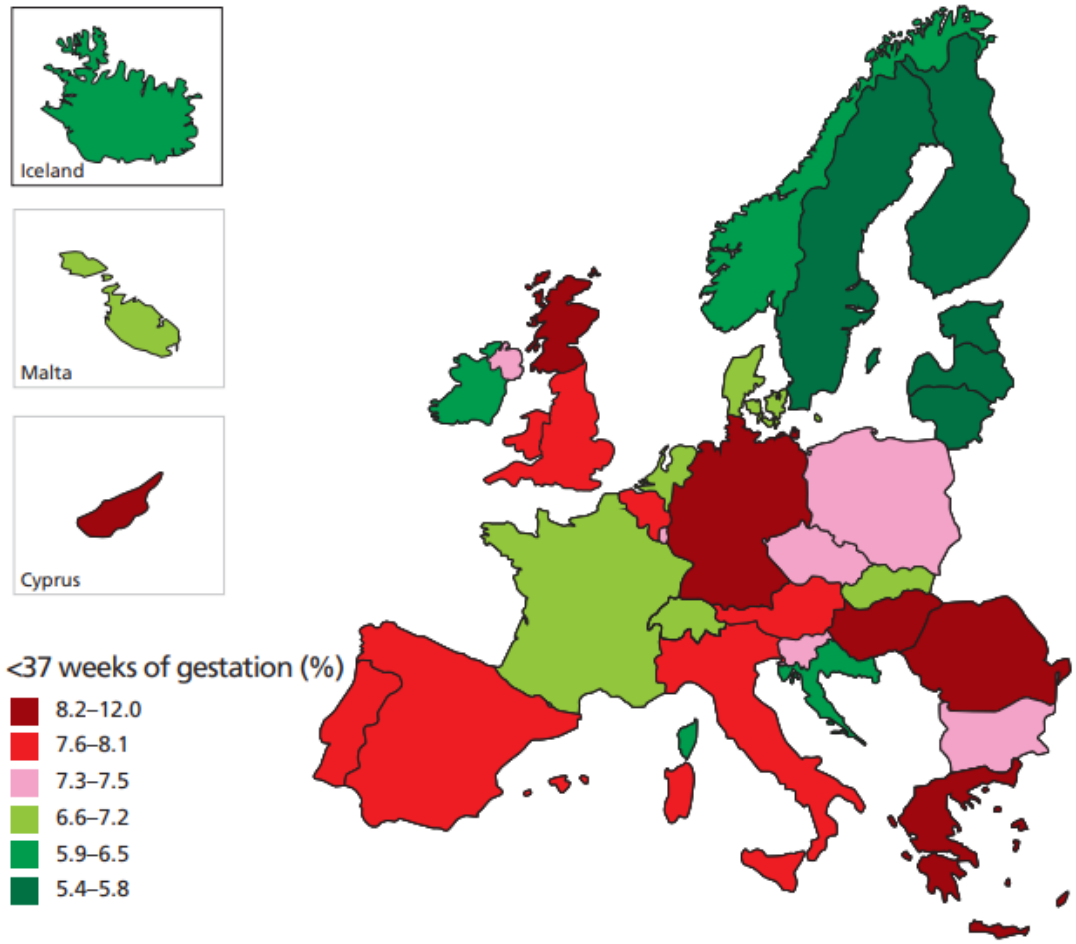

Figure 1: Geographical distribution of preterm births among live births in Europe. Adopted from EU report: Babies' health: mortality and morbidity during pregnancy and in the first year of life (5).

Innovation	Time
CPAP, Mechanical Ventilation	1980s
Exogenous Surfactant	Early 1990s
Antenatal Steroids	Mid/Late 1990s
Avoiding Postnatal Steroids	Early 2000s
Targeted Oxygen Therapy	Mid 2000s
Systematic Care/Experience	Continuous

Table 1: Innovation of medical treatment of extremely preterm infants over the last decades.

Costs

The total cost of preterm birth to the UK public sector was estimated to be 2.95 billion pounds and an inverse relationship was identified between gestational age at birth and the average public sector cost per surviving child. The incremental cost per preterm child surviving to 18 years compared with a term survivor was estimated at £22,885. The corresponding estimates for a child born as very or extremely preterm infant were substantially higher at £61,781 and £94,740, respectively (8). There are also other costs that are difficult to quantify precisely; non-medical direct cost (transportation, accommodation, home or car remodelling, childcare/babysitting for other siblings, special education/schooling, home help and higher insurance premiums), indirect cost (income losses, missed working days and time losses-opportunity costs) and intangible costs (quality of life aspects, physical and emotional burden on parents or other caregivers) (9).

Morbidity associated with prematurity

Preterm infants have immature organ systems and need a lot of support until they can regulate independently their function and react appropriately to the challenges the extra-uterine environment poses on them. The level and duration of the support is inversely related to GA (10).

Neurodevelopmental outcome

Neurodevelopmental outcome in preterm infants can be affected by many factors including antenatal, perinatal and post discharge from the neonatal unit by family and social factors (Table 2) (11, 12). These factors may be amenable to intervention for improving long-term outcomes in this population (13). There are also different

methods to assess neurodevelopmental outcome and scores are not often comparable (14).

Period	Factors
Antenatal	• Inflammation • Infection • Intrauterine growth restriction • Maternal nutrition • Substance misuse • Preterm rupture of membranes
Postnatal	• Gestation age • Birth weight • Hypoxia • Hypoperfusion • Congenital anomalies affecting haemodynamics • Nutrition
Infancy to adulthood	• Low socioeconomic status • Low education • Divorced parents • Parental mental illness • Maternal substance misuse • Bad parental attitudes

Table 2: Risk factors of adverse neurodevelopmental outcome in preterm infants.

Brain injury in preterm infants

Brain injury is common in preterm infants and has often long-term devastating consequences for individuals and their families. It can be divided in haemorrhagic lesions and white matter damage, but these lesions often coincide and overlap.

Periventricular and intraventricular haemorrhages (IVH) occur usually in the first three days after birth and can be categorised with cranial ultrasonography (CrUSS) according to Papile criteria: Grade I: bleeding restricted to germinal matrix, Grade II: intraventricular bleeding without ventricular dilatation, Grade III: intraventricular bleeding with ventricular dilatation, Grade IV: parenchymal echodensity which represents periventricular haemorrhagic infarction (15). However, this system is imperfect as there is often uncertainty on the degree of ventricular dilatation and whether Grade III IVH is actually Grade II followed by ventricular dilatation, which is often transient. Moreover, Grade IV IVH includes a wide spectrum of disease severity, which ranges from one small lesion of periventricular infarction in an area known to have negligible clinical impact to multiple large lesions involving both hemispheres. However, the advantage of Papile classification is the ability to perform quantitative statistical analysis and relate classes of brain injury to specific risk factors and clinical outcomes.

Periventricular leukomalacia (PVL) refers to the damage of the white matter around the lateral ventricles (16). PVL mainly occurs around border zones between long penetrating branches from middle cerebral artery and anterior or posterior cerebral arteries called watershed areas (centrum semiovale and the optic and acoustic radiations) (16). Cerebral ultrasonography is not particularly sensitive to diagnose PVL (sensitivity 26-28%) (17). The prognostic value of echolucencies and ventricular

dilatation, detected by ultrasonography, has been assessed by multiple studies and is relatively good (18, 19). As with IVH there is spectrum of PVL disease severity. The severe form of cystic PVL is easily diagnosed with CrUSS and has a very low prevalence, whereas diffuse non cystic PVL is very difficult to diagnose and quantify with CrUSS, but magnetic resonance imaging (MRI) studies have demonstrated prevalence up to 50% in extremely preterm infants (20).

Mechanism

The immaturity of three fundamental components of cerebral anatomy and function are the substrate for the higher risk of cerebral injury in preterm infants: 1. immature brain structure and cerebral vasculature, 2. immature cardiovascular system and 3. immature autonomic nervous system with limited ability to handle the challenges of transitional circulation with open fetal channels and poor pulmonary and cardiac reserves.

During fetal life, neurons and glial cells migrate outwards from the germinal matrix and sub-ventricular zone towards the cortex. This process is supported by a rich capillary bed in the germinal matrix. However, blood vessels in the germinal matrix are thin walled, lack pericytes and have few glial fibers making them very fragile. Moreover, cerebral circulation is provided by terminal arteries and the venous drainage also ends up in terminal veins (21). Hence, any disruption on the provision or drainage of blood flow can affect a wide area of cerebral tissue and leads to a haemorrhage of an area crucial for cerebral development and maturation. The pathogenesis of IVH involves two postulated mechanisms (22). The first involves hypoxia reperfusion injury due to fluctuations in arterial blood pressure as result of various factors (perinatal hypoxia, sepsis, fluid boluses or inotropes), which leads to

bursting of the fragile cerebral vessels. The other mechanism involves obstruction of the venous return (secondary to pneumothorax, ventilator asynchrony or just collapsing veins due to head position) causing an increase in venous pressure and leading to the rupture of capillary vasculature (22).

The “dose” of haemodynamic insult required to cause brain injury in preterm infants is not known and is possibly fluctuating depending on the background risk factors and the mechanism of the injury (23). The dose also depends on the duration and the pattern of the insult. Hence, insults can be brief and severe, mild but prolonged and/or repetitive of variable duration and all these insults can have a cumulative effect. The identification of temporal relationship between the haemodynamic insult and brain injury is challenging not only because the onset of brain injury is often unknown, but also because there is no reliable and portable continuous measurement of cardiac performance and brain perfusion.

There are several risk factors associated with IVH. There is negative correlation between GA and IVH incidence (24). The immature vasculature of periventricular germinal matrix makes them prone to injury during fluctuations of cerebral blood perfusion, which are common during the period of transitional circulation (25, 26). The immaturity of autonomic nervous system can further exacerbate the fluctuations of cerebral blood flow (21). Hypercapnia is associated with cerebral vasodilatation and higher risk of IVH (27), but hypocapnia is associated with cerebral vasoconstriction, poor perfusion in water shed areas and higher risk for PVL (28). Sepsis, coagulation problems and systemic inflammation are all associated with increased risk of IVH (29, 30). PDA is also a known risk factor causing

prolonged diastolic hypotension and its impact on cerebral circulation will be further discussed in Chapter 3.

The study of brain injury in preterm infants is challenging due to the unique anatomy, physiology and pathophysiology in this age group. The exact brain injury onset is often unknown and, in many cases, impossible to determine as it may occur antenatally (31, 32). Brain injury in preterm infants is not accompanied by the usual neurological sequelae observed in older children as they often remain asymptomatic, which makes the timing of the brain insult more difficult to determine. The mechanisms of brain injury due to haemodynamic compromise are variable and hence the period of the insult may extent to several weeks and the insults may have cumulative effect. In these settings, determining a temporal relationship between a possible insult and the resultant brain injury can be challenging. Moreover, the neurodevelopmental deficits often become apparent years after the initial insult and there are many other contributing factors (biological and social) which can ameliorate or enhance the impact of the initial insult. As preterm labour occurs at a period of rapid cerebral growth, our knowledge is limited on the impact of brain injury on developing preterm brain and the possible reprogramming. On the other hand, the premature brain has increased neuronal plasticity and potential of ameliorating the impact of brain injury (33).

IVH results in a wide spectrum of neurodevelopmental deficits in preterm infants ranging from asymptomatic patients to severe cerebral palsy and global developmental delay. Behavioural, cognitive, emotional, motor and sensation problems can coincide in the same individual with variable severity depending on

the localisation and the extent of brain injury. Previous studies found that Grade 1-2 IVH are not associated with significantly different neurodevelopmental outcome compared to infants without IVH (34-36). This is in contrast with other studies which found opposite results even in extremely preterm infants without PVL (37, 38). These contradictory results may be due to the underlying wide interobserver variability on classification of IVH grade 2 and 3 (39) and variability in cohort demographics, clinical exposures, treatments and evaluation methods. In the current thesis I focus on IVH as PVL is more difficult to precisely quantify with CrUSS and use Papile criteria with dichotomisation of our cohort into mild (Grade 0-2 IVH) and severe (Grade 3-4 IVH) brain injury.

Between the onset of the potential insult and the occurrence of irreversible brain injury there is a compensatory phase with disturbed brain perfusion and loss of brain function, which maybe a compensatory mechanism to decrease cerebral metabolic demands. Cerebral hypoperfusion can be detected with cerebral near infrared spectroscopy (NIRS) and loss of brain function with amplitude integrated electroencephalogram (aEEG) before the anatomical evidence of IVH becomes apparent (40, 41). Metrics of autonomic function may also predict the occurrence of IVH as shown in previous studies (42).

Each of these physiological processes could be included in the causal pathways leading to adverse outcomes of premature birth. These processes can influence each other. Accordingly, simultaneous multiple assessments are needed to identify the nature and extent of physiological determinants of adverse outcome.

Antenatal maternal magnesium sulphate administration for neuroprotection

One of the significant breakthroughs for neonatal neuroprotection came from observations of infants born to mothers given magnesium sulphate ($MgSO_4$) to prevent eclamptic seizures or as tocolysis which found a reduction in rates of cerebral palsy (43). Evidence from subsequent RCTs and metanalysis led to low dose antenatal $MgSO_4$ (a bolus of 4 g followed by a maintenance infusion 1g/hr until the birth or discontinued if not delivered by 24 hours) to become a recommended practice for neonatal neuroprotection (44, 45).

The exact underlying mechanism which mediates $MgSO_4$ actions is unknown. However, there are several hypotheses. Blockage of NMDA receptors by $MgSO_4$ is believed to ameliorate the effects of hypoxic brain injury and magnesium is known to be a calcium antagonist and reduces calcium influx into the cells (46, 47) 10, 11. $MgSO_4$ is considered also to have antioxidant (47) and vasodilative effects (48), stabilises vascular function, prevents hypoxic damage (49) 12, ameliorates cytokine or excitatory amino acid induced cell damage (49)12 and has anti-apoptotic properties (50). Magnesium is essential for the function of many membrane transporters, ion pumps and enzymes (47).

However, the optimal dosage regimen for maternal $MgSO_4$ treatment remains unknown (51, 52). Animal studies showed that high dose bolus $MgSO_4$ (250mg/kg, equivalent to 17.5 g for the average weight woman) affects fetal brain organogenesis (51, 53). Neonatal adverse reactions to maternal administration of $MgSO_4$ before birth may include cardiovascular or neurological sequelae (54, 55). Increased maternal serum magnesium concentrations have been associated with lower Apgar scores, intubation in the delivery room, hypotonia and admission to

special care suggesting that neonatal safety should be balanced against efficacy during dose selection (54).

There is limited evidence regarding $MgSO_4$ pharmacokinetics (PK) and pharmacodynamics. The materno-placento-fetal unit is a multicompartment system that reflects multiple physiological influences (56). Among pregnant women a two-compartment model for magnesium concentrations is appropriate, although a one compartment model has a better fit during pre-eclampsia (57). Brookfield et al. developed a population PK model of maternal concentrations and cord blood (58). They found that maternal weight and a diagnosis of preeclampsia significantly affected maternal and neonatal serum magnesium levels ($Mg2^+$). Maternal exposure was associated with significantly higher magnesium concentrations in neonatal serum samples within 48 hours of birth (59). These descriptive data are not sufficient to develop dosing recommendations in the absence of a PK model and information about the relationship between neonatal $Mg2^+$ and effects on the neonate. In order to support the design of a study that integrates maternal and neonatal PK and pharmacodynamic data, we conducted a perinatal feasibility study.

Ductus arteriosus

A common feature among babies born at extreme prematurity is a PDA. PDA is associated with many adverse outcomes (60), but it is unclear whether a PDA directly causes adverse long term outcomes. One issue with assessing the effects of PDA on target organs is the lack of clarity about the measurement of PDA and the biomarkers that may be surrogate endpoints for clinically meaningful outcomes. This thesis explores these measurement issues so that the next section describes the ductus arteriosus (DA) before methods are reviewed and the purpose, aims, and objectives of the thesis are presented.

Historical considerations

There is evidence that Galen, in his 2nd century A.D. Greek texts, noticed the presence of DA and patent foramen ovale (PFO) and the significant changes they undergo after birth, although he did not realise the blood flow across it and made incorrect conclusions for its use and significance (61). DA, known also as ductus Botalli, was named wrongly after the Leonardo Botallo after misinterpretation of his manuscripts due to bad translations (62). Fabrizi d' Acquapendente, a surgeon in Padua described the DA (Figure 2) in 1600 in his detailed manuscripts (63) as:

> 'a branch of the great artery (aorta) to the arterial vein (pulmonary artery). It appears large in the fetus, but is cord-like after birth... Shortly after the birth of the fetus, the arrangement is changed..... both vessels are occluded. For nature takes the little valve (ostiolum) situated on the inside at that right orifice in the vena cava, and glues shut and completely conceals the opening. But she dries up the left

arterial branch so that it becomes a cord, without any lumen whatsoever' (62).

William Harvey, who was a student of Fabrizi d' Acquapendente and trained in Padua, was the first to conceptualise active blood flow by rejecting Galen's assumptions and the established beliefs. Harvey was aware of the DA and described also the DA changes during the transition from intrauterine to extrauterine life (62). It was not until the 19th century when the Berlin pathologist Rudolf Virchow suggested that the DA closure results from the formation of a blood clot (64), while the Lille anatomist G. Gérard based on post-mortem examinations of infants proposed the concept of two-stage closure, in which functional constriction is followed by anatomical closure (65).

The first successful surgical PDA closure by Robert Gross in Boston opened the new era for congenital heart surgery (66). Subsequently, transcatheter PDA closure allowed interventional cardiology to evolve into a separate paediatric cardiology subspecialty (67).

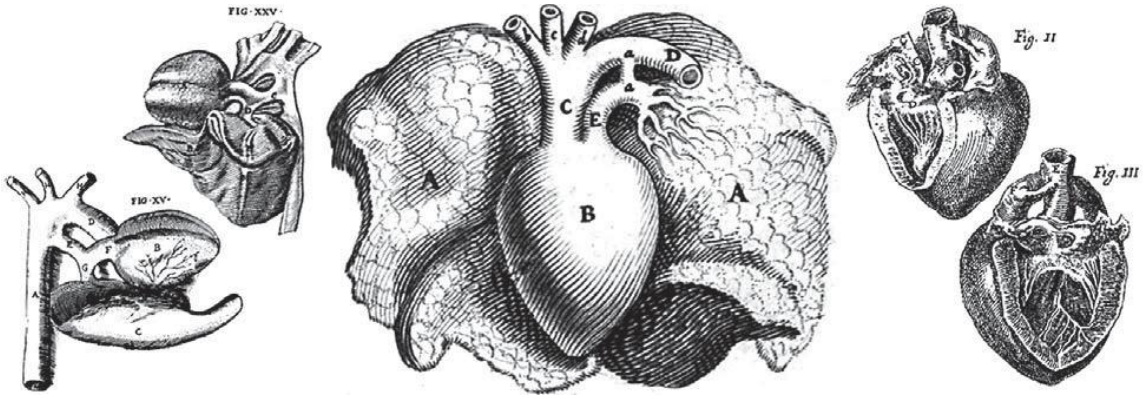

Figure 2: Schematic representation of the ductus arteriosus from 17th century illustrations. Left: tab. XV and XXV of Fabricius ab Aquapendente in 'De formatio foetu' 1606. Centre: Johann Vesling in 'Syntagma anatomicum' 1651. Right (fig. II and III): Johan van Horne's addition to Botallo's 'Opera Omnia', 1660. Adapted by (62).

Embryology

During normal fetal development, the proximal portions of the sixth pair of embryonic aortic arches persist as the proximal branch pulmonary arteries, and the distal portion of the left sixth arch persists as the DA, connecting the left pulmonary artery with the left dorsal aorta (68). The transformation is completed by the eighth week of fetal life. The distal right sixth aortic arch normally disconnects from the dorsal aorta and undergoes an apoptotic process and disappears. Persistence of the right duct is always associated with other cardiac anomalies. Several teratogens are known to affect the DA development including alcohol, rubella, amphetamines and the anticonvulsant hydantoin, with the duct being most sensitive during the first trimester (69).

Anatomy

The DA is a large artery connecting the left pulmonary artery near its origin to the descending aorta just distal to the left subclavian artery. It is present in all mammalian species and it is an important haemodynamic difference between intrauterine and extrauterine circulation. The connection of the pulmonary and systemic circulation through the DA makes the fetal cardiac ventricles work in parallel compared to the serial connection in extrauterine life. The DA is slightly smaller than the main pulmonary artery and equal in size to the ascending and descending aorta. This signifies the distribution and amount of blood flow carried out by each of the main fetal arteries. Two thirds of the fetal cardiac output is ejected by the right ventricle and only 5-10% of the combined ventricular output flows to the lungs. Although the macroscopic appearance of DA is similar to the neighbouring aortic and pulmonary tissue, there are many histological differences

(70). There are different types of DA anatomy and the premature DA is often elongated and constricts distally close to the pulmonary end (71). This can affect the accuracy and reproducibility of the PDA size measurement as the size varies across the PDA lumen.

PDA innervation

Evidence for PDA innervation comes from old studies and is limited. In vitro experiments in DA obtained from human fetuses of GA between 10 and 24 weeks demonstrated DA constriction from both noradrenaline and acetylcholine (72). Opposite to other studies the sympathetic nerve terminals were seen penetrating into the media. The same study reported that the nerve terminals were similar to that of adult tissues, which suggests an earlier maturation of the fibres and this may play a role in the DA closure. Aronson et al. found a rich supply of adrenergic nerve terminals in the human DA from fetuses between 10 and 21 weeks GA (73). The same study found acetylcholinesterase only in scattered thick bundles in the adventitia of the peripheries of DA indicating that there is no parasympathetic innervation in stages of the development assessed.

Animal studies have demonstrated parasympathetic nerve fibres in DA. Silva and Ikeda demonstrated more extensive acetylcholinesterase-containing nerve fibres in the DA in near-term fetal lambs (74). Moreover, observations by Barcroft et al. showed that stimulation of the left vagus constricted the DA of the guinea-pig foetus (75).

The presence of a cross talk between ANS and DA through ductal innervation may have significant implications in DA closure (76). The long observed association between PDA and brain injury is viewed usually as one way causation. There is the assumption that PDA causes cerebral dysfunction due to cerebral hypoperfusion. However, the opposite could also be possible and should not be de facto excluded. Perinatal events could compromise cerebral function and the already immature ANS, which could lead to disturbed autonomic control of DA closure and a vicious cycle of prolonged and larger PDA and further exacerbation of the cerebral dysfunction. This coupled with the compromised cardiac reserves, impaired cerebral autoregulation and the vulnerable cerebral structures can lead to higher risk of brain injury. Furthermore, established brain injury could further disturb the misbalance between SNS and PNS and the normal maturation process leading to prolonged and larger PDA. In conclusion, it is uncertain whether a haemodynamically significant PDA can be the cause or the epiphenomenon of brain injury/dysfunction in a complex interaction between ANS and control of DA closure.

Physiology of DA closure and prolonged patency

Birth is accompanied by significant events which promote DA closure. The removal of placental effects decreases the circulating prostaglandin (PG) levels and their potent vasodilatory effects on the DA. The combined effects of ventilation and higher arterial oxygen levels lead to further reduction in pulmonary resistance. This increases pulmonary flow which leads to increased PG metabolism and further reduction of their levels (77). Moreover, the increase of arterial oxygen levels triggers DA vasoconstriction through different molecular mechanisms, which causes the functional DA closure (78). This causes ductal tissue hypoxia which activates a

cascade of events leading to permanent anatomical closure and transformation of DA into a ligament between the aorta and the pulmonary artery (79).

Premature DA and prolonged patency

The premature DA has different physiology and often remains patent for a longer period. The anatomical structures, the receptors and the underlying molecular pathways are developmentally immature. Hence, the incidence of PDA is inversely related to the GA at birth with up to 90% of infants born at 24 weeks GA having a PDA in the end of the first week after birth (80). The muscular media of the DA is not fully formed and the myosin isoforms of the smooth muscle cells are immature resulting in weaker contractile response (81, 82). The premature DA is also less responsive to high oxygen levels (83) and, in contrast to term DA, nitric oxide has a significant vasodilator effect (84). Moreover, premature infants have increased circulating PG levels due to reduced efficacy of premature lungs to metabolise PG (85). Infection is very frequent in preterm infants and can lead to PDA reopening as circulating PG levels can reach pharmacological levels (86). In recent years, genetic factors were found involved both on prolonged patency of DA and the efficacy of different medications for PDA treatment (87).

PDA pathophysiology in preterm infants

PDA diverts blood flow from the systemic circulation to the pulmonary circulation. If the shunt volume is large, PDA can cause systemic hypoperfusion and pulmonary overcirculation. This is the hallmark of the PDA steal phenomenon. In this situation, PDA shunting causes increased preload and decreased afterload in the left chambers and this has multiple short- and long-term effects. The left atrium and ventricle become dilated and this causes increased atrial natriuretic peptide (ANP)

and brain natriuretic peptide (BNP) production which are potentially good candidates for biochemical biomarkers indicating a haemodynamically significant PDA (hsPDA) (88). However, the premature myocardium has impaired diastolic function (89) and the extra strain induced by the increased preload in combination with increased pulmonary perfusion can lead to raised intrapulmonary pressures and higher risk of pulmonary haemorrhage (90). There is an established opinion that preterm infants cannot handle well the increased preload by increasing stroke volume and they compensate with tachycardia. However, this is not supported by some studies (91, 92). Cardiac output is often increased in the presence of a PDA as left ventricle contracts against lower peripheral resistance (93). However, end organ perfusion maybe compromised depending on the magnitude of the PDA shunt, the ability of the premature heart to compensate for that, and the ability of vascular control to distribute appropriately the cardiac output (94). Infants with perinatal complications (ischemia, hypoxia, infection) may not have the reserves and the ability to handle peripheral organ metabolic demands. There are several studies demonstrating the impact of hsPDA on cerebral (95, 96), bowel (97, 98) and renal perfusion (99). PDA connects the high resistant systemic circulation to the low resistance pulmonary circulation resulting in diastolic hypotension as there is a continuous runoff from the descending aorta. The premature left ventricle is developmentally adapted to pump against a low resistant circulation (placenta), but following preterm birth it has to work against raised peripheral resistance. PDA and the associated diastolic hypotension can compromise coronary blood flow to the immature myocardium which is already working at its limits and has elevated

myocardial oxygen demands (100). This can lead to a vicious cycle of myocardial ischemia and reduced cardiac function with multiple organ failure.

Assessing the haemodynamically significant PDA

There is no consensus among the neonatal community for the definition of hsPDA. During the last decades the definition has changed and evolved as medical and technological advances improved our ability to study PDA haemodynamics in more sophisticated ways and changed the population demographics as they allowed the survival of more premature infants. Initially, clinical trials used mostly clinical criteria for the diagnosis of hsPDA that were neither specific nor sensitive (101, 102) and they could only detect the symptomatic PDA. Subsequently, with echocardiographic advances a lot of hope was invested on early detection of the hsPDA before it could "cause" organ damage and especially IVH. Most of the clinical trials ended up using solely echocardiographic criteria to define the hsPDA and recruit patients (103). However, this approach also did not result in beneficial PDA treatment long-term outcomes. Most recent RCTs combine clinical and echocardiographic parameters to assess the impact of PDA on target organs (104).

Most of the studies in the literature consider PDA as a uniform entity that is either present or absent. However, there is accumulating evidence that PDA can be considered as a spectrum from biological normality to a pathological disease state with clinical instability and varying effects on organs (105, 106). The lack of precise characterisation of the hsPDA might partially explain the controversial results from the studies that deal with causality and treatment. There have been attempts to devise PDA scoring systems to predict future outcomes which combine risk factors, clinical characteristics and echocardiographic measurements to define and predict

the outcomes of a hsPDA. Seghal et al created a PDA scoring system using solely echocardiographic parameters which was predictive of the development of chronic lung disease (107). El-Khuffash et al combined biochemical (troponin and B-type natriuretic peptide) with echocardiographic parameters at 48 hours of life in premature infants with a PDA and found this to be predictive of death before discharge or neurodevelopmental outcomes at 2 years of age (108). The current thesis utilised the scoring system devised by Seghal et al.

The haemodynamic impact of PDA on target organs is affected by many parameters and this makes a comprehensive assessment in RCT settings challenging. The following aspects should be considered when assessing the PDA haemodynamic impact:

1. The magnitude of the left to right shunt is determined by the Hagen–Poiseuille law which indicates that flow through a tube is proportional to the fourth power of the tube radius (PDA diameter) and the pressure gradient (BP difference between the pulmonary and systemic circulation), and inversely proportional to the PDA length and the blood viscosity (hematocrit). The pressure gradient is affected by the balance between pulmonary and systemic vascular resistances. The underlying lung disease disturbs gas exchange and can lead to hypoxaemia, hypercapnia and acidosis, all known to affect the pulmonary resistance (109). Systemic vascular control can be affected by perinatal hypoxia, infection and other inflammatory processes. Vasoconstricting drugs have variable effects on the

systemic and pulmonary vasculature of preterm infants and there is limited evidence relating to their pharmacodynamics (110).

2. The ability of the preterm heart to handle the excessive workload induced by the PDA shunting depends on myocardial performance. The preterm heart has limited capacity to increase contractility as the contractile apparatus is immature (111). The preterm myocardium has a high content of total collagen and type I collagen, which predisposes to diastolic dysfunction and impaired ability to handle the increased preload (112).
3. The general wellbeing of the infant and important antenatal and perinatal clinical factors (GA, antenatal steroids, IUGR, inflammation) can affect the infant's ability to handle the PDA shunt (113). Hence, it is important to consider the clinical settings when assessing PDA hemodynamic importance. This approach can possibly improve the identification of neonatal sub-populations for which the treatment risk-benefit ratio leans towards treatment.

PDA treatment

PDA treatment can be conservative, medical or surgical with ligation. There is large variation between neonatal centres in the management of PDA (114, 115).

Conservative treatment includes optimisation of ventilation (reduced inspiratory time and increased positive end expiratory pressure), fluid restriction and diuretics (116). Conservative management has attracted more interest in the recent years as PDA closes spontaneously in the majority of preterm infants (117). Moreover, an increasing number of observational reports have shown that PDA closure can be achieved without significant increase in morbidity and mortality (118, 119). A

multicentre, randomised, non-inferiority trial (BeNeDuctus trial), which compares conservative PDA management to early PDA treatment with ibuprofen, is expected to provide more evidence for optimal PDA treatment (120).

PDA treatment with non steroidal anti-inflammatory drugs (NSAIDs) (indomethacin and ibuprofen) is an effective intervention leading to high success rates of DA closure depending on the background GA (121, 122). However, despite intensive research over the last four decades with many clinical trials, there is no clear evidence that treatment has any beneficial long-term effects (121, 122). Medical treatment seems to decrease the incidence of severe IVH and increase retinopathy of prematurity (ROP) without any significant effect on mortality and neurodevelopmental outcome (123).

There is an established culture and already shaped opinions amongst clinicians in neonatal and paediatric cardiology regarding the detrimental effect of PDA on neonatal outcomes that has impacted on the design of clinical trials. This has resulted in clinical trials with high contamination rate from open label treatment, which has led to the absence of a real control group. Moreover, NSAIDs can cause significant side effects in preterm infants, which may counterbalance their beneficial effects. NSAIDs are associated with increased incidence of NEC, gastrointestinal perforation, renal impairment, coagulopathy, ROP and potentially reduced cerebral perfusion (observed only in indomethacin treated infants) (124-127). Advances also in the treatment and management of preterm infants in the last two decades have also rendered many of the previous clinical trials out-dated. Hence, the results of an ongoing RCT (Baby-OSCAR trial, EudraCT No.: 2013-005336-

23), which assesses the impact of early targeted treatment PDA treatment with ibuprofen on asymptomatic infants with 'significant' PDA in the first 72 h of life, will be very important (128). This study has included methodological approaches to minimise the contamination rate from open label treatment and introduced medical equipoise of PDA treatment in the inclusion criteria.

PDA ligation has become less attractive in the recent years after concerns were raised that it is associated with worse neurodevelopmental outcome (129, 130). However, a more recent study found that PDA ligation does not affect neurodevelopmental outcomes at 18-24 months, chronic lung disease, or retinopathy of prematurity after adjustment for confounders (131). Patients who were treated medically had higher overall risk-adjusted mortality rates attributed to higher incidence of deaths related to sepsis and brain injury. However, the results may have been skewed by contraindication confounding as sick patients were deemed unsuitable for surgical operation.

A large population study from United States demonstrated a significant decrease over a ten year period (2006-2015) in the diagnosis of PDA (from 51% to 38%), use of NSAIDs (from 32% to 18%) and PDA ligation (from 8.4% to 2.9%), while mortality decreased with no increase in morbidity outcomes (132). This realisation emphasises the importance of taking a step back to reassess the actual causal relationship between PDA and comorbidities before the initiation of another round of clinical trials with different methodological designs. The identification of at-risk groups with a particular focus on infants born at less than 26 weeks GA and finding

new more precise methods to assess the haemodynamic significance of PDA maybe a way forward.

After 50 years of intensive research in PDA molecular biology, physiology and clinical trials, we are still debating the optimal PDA management. This can be attributed to a number of reasons:

1. Neonatal population and epidemiology are constantly changing. The better understanding of neonatal physiology, the improved medical interventions and advances in technology have pushed the limits of viability at 22 weeks GA and this trend will further change in the forthcoming years. Although birth GA is a continuum, infants born at 30 weeks have significantly different physiology, pharmacology and epidemiology compared to 23 weeks.
2. The improved knowledge of physiology raises new questions and makes previous knowledge debatable or outdated.
3. Advances in technology as in echocardiography provided better tools for phenotyping our patients and changed the eligibility criteria for clinical trials.
4. We are entering into the era of precision medicine and individualised approach based on consideration of multiple parameters (genomics, imaging and analytics) is changing the way we appraise previous evidence, conduct research and treat patients.

Controversy may not be resolved in the next years as more treatment options are becoming available in the clinical setting without robust evidence of clinical efficacy. Controversy is due to number of reasons, but the main contributors are:

- High open label treatment rate in previous RCT studies that contaminate the comparing groups.
- Small sample sizes.
- Wide mix of gestation groups <32 weeks.
- Changing population due to improvement of neonatal care.
- Improved diagnostic tools for PDA screening and monitoring (echocardiography). The ideal echocardiographic biomarker is still in debate.

Background theory of the techniques used in the thesis

As noted above, multiple physiological processes are relevant to the outcomes of preterm birth. These processes need to be assessed in parallel. The next sections provide background about each of the techniques used in this study.

Echocardiography

Echocardiography can provide quantitative data to study PDA pathophysiology (133). PDA diameter as described earlier is an important component of PDA significance. Since it is difficult to assess PDA shunt volume directly, echocardiography provides surrogate biomarkers for the better approximation of shunt volume. The direction of flow and the maximum velocity of transductal flow provide information regarding the pressure gradient across the PDA. The increased preload dilates the left atrium (LA) and left ventricle (LV). As aortic root dimensions stay relatively stable and are not affected by preload, the ratio between LA and LV dimensions and the aorta (La:Ao and LVEDD:Ao respectively) are considered good biomarkers of PDA shunt volume (134). The ratio between the right and left outflow ventricular output can provide information regarding the transductal flow. However, the invariably present flow through the open PFO can make this

measurement imprecise (135). The diastolic velocities in the left and main pulmonary arteries have been used more recently for the same reason (136). Echocardiographic parameters of diastolic function [mitral valve E:A ratio and isovolumic relaxation time (IVRT)] have been also utilised for the assessment of hsPDA (137). The PDA steal phenomenon and its impact on target organs can be assessed by flow characteristics in the descending aorta and on peripheral arteries. Reduced diastolic or retrograde flow in the arteries has been proposed to be predictive of hsPDA (138). Further description of the echocardiographic biomarkers used in the current thesis can be found in Methods (Chapter 2) and Appendix 2.

Echocardiography has been validated against MRI measurements (139) and measurements with thermodilution, Fick or dye dilution methods (140). The reproducibility of the PDA echocardiographic biomarkers has been studied and was found to be variable and for some of the aforementioned biomarkers poor (141). Table 5 provides an overview of the reproducibility indices used in the current thesis. This is an important consideration when designing multicentre clinical trials, where the local investigators have variable echocardiographic skills and an already poorly reproducible biomarker can become a highly unreliable biomarker to use as inclusion criterion or surrogate short-term outcome.

The observed variability can be contributed to the intrinsic method imprecision, the simplifications applied for the creation of calculation formulas and the altered haemodynamics induced by the examiner during his interaction with the baby (chest pressure, altered temperature, crying, tachycardia). To achieve higher

reproducibility and accuracy it is important to undergo rigorous training and gain experience with neonatal echocardiography.

Cranial ultrasonography

Aspects of cerebral ultrasonography and IVH were previously discussed (page 31).

Near infrared spectroscopy in preterm infants

NIRS technologies utilise the property of near infrared (NIR) light to penetrate human tissue and being differentially absorbed by specific pigmented compounds; specifically in medical applications by oxygenated and deoxygenated haemoglobin. There are many types of NIRS devices using different methodologies. In the current thesis NIRS refers to spatially resolved spectroscopy. This technology uses three optodes, one emitting and two detecting NIR light of specific wavelengths at different distances from the emitting optode to measure the ratio between absolute concentration of oxygenated haemoglobin and total haemoglobin. This is called the tissue oxygenation index (TOI) (142). However, this value is the weighted average of arterial, capillary and venous blood oxygenation across the path length, but, as up to 75% of blood in tissue sits in the venous compartment, TOI mainly represents venous saturation and hence the normal values are lower than the pulse oximetry, which represents arterial oxygen saturation (143). Preterm infants have small heads and relatively thin layers of skin and bone overlying brain tissue. This reduces the noise to signal ratio during NIRS monitoring and allows measurement of brain tissue oxygenation up to 3 cm in depth. This was the reason why the first application of NIRS for clinical research was performed in preterm infants (144). NIRS has the advantage of continuous non-invasive cerebral monitoring without significant interference with infants wellbeing even in the sickest.

There are reference values for preterm infants (145), but these vary depending on the different devices used (146, 147). Reproducibility of the measurements is fair [4.4% (CI 3.5-5.2)] with the device used in the current thesis (NIRO-200NX, Hamamatsu, Japan) (147). Animal studies have validated the use of cerebral fractional tissue oxygen extraction against central cerebral venous saturation (148). Cerebral NIRS has shown strong correlation to perfusion assessment with MRI (149, 150). True validation is difficult as cerebral tissue oxygenation index (cTOI) reflects a weighted average as mentioned before (151, 152). In addition, the presence of extracranial or subdural haemorrhages can artificially influence measured cTOI values (153). The cost of the technology and the consumables is still considerably high. Moreover, the lack of proved clinical efficacy in improving clinical outcomes has restricted its use in neonatal practice mainly to research.

Despite the presence of cerebral oxygenation reference values for extremely preterm infants, the optimal range is unknown. This is because the fetal brain develops in a hypoxic environment and following preterm birth the limits of cerebral oxygenation leading to a better neurodevelopmental outcome later in the life have not been extensively investigated. Cerebral oxygen demand, oxygen extraction and oxygen delivery are variable during hypoxia reperfusion cerebral injury, hence cerebral oxygenation maybe misleading when viewed in isolation (154). Hyperoxia and hypoxia can both be detrimental (155) constituting a double-edged sword. The SafeBoosC II study showed that cerebral oxygenation monitoring coupled with a specific protocol of medical interventions can significantly reduce the burden of hypoxia during the first days of life in extremely preterm infants

(156). The study found a trend towards reduced severe brain injury and mortality in the group of patients monitored with NIRS. However, this came with the burden of a higher incidence of bronchopulmonary dysplasia and retinopathy of prematurity. This emphasises the potential detrimental effects of hyperoxia when treating extremely preterm infants. The next study (SafeBoosC III) is currently recruiting patients with a primary outcome the composite of death or severe brain injury at 36 weeks' postmenstrual age (157) and will shed more light on the impact of cerebral oxygenation on neonatal outcomes.

NIRS and PDA

Different regions of the brain have differential blood flow with higher values observed in temporal and parietal areas in comparison to frontal as well as higher values in right hemisphere compared to the left (158). PDA may explain part of these observations as DA inserts closer to the left carotid artery and possibly affects more the left hemisphere haemodynamics. There are inconsistent data in the literature regarding the impact of PDA on cerebral oxygenation (159, 160). PDA treatment with indomethacin, unlike ibuprofen, was proved to decrease cerebral oxygenation (127). Studies investigating the impact of PDA ligation on cerebral haemodynamics have conflicting results (161, 162). The results of the existing studies appear inconsistent mainly due to the small study population of each study and differences in the study populations with studies involving relatively stable or more mature infants failing to find an association between cerebral oxygenation and PDA.

NIRS, brain injury and neurodevelopmental outcome

There is evidence that application of NIRS during the first days after birth can predict brain injury and long-term neurodevelopmental outcome in extremely preterm infants (155, 163, 164). However, cerebral NIRS monitoring in SafeBoosC II clinical trial did not show long-term benefits or harm on neurodevelopmental outcome at two years corrected age (165). Perioperative cerebral NIRS monitoring was found to be predictive of survival and neurodevelopmental outcome in patients with a variety of congenital heart disease (166, 167).

aEEG in preterm infants

aEEG is a monitoring technique derived by a time-compressed tracing of encephalogram (EEG) using a single or two channel bipolar EEG montage. A single channel montage consists of three wires placed over the bi-parietal regions and one reference electrode placed anywhere on the body. The machine filters, rectifies and compresses the electrical signals from brain activity. Signals with frequencies <2 and >15Hz are filtered out to reduce the artefacts caused by external electrical interference, muscle activity and cardiac function. The signal is also displayed on an x-axis using a slow chart speed (6cm/h) and an y-axis using a semi-logarithmic display (linear at 0-10µV and logarithmic display above this voltage) in order to detect changes in low amplitude background activity (168). This display enables spatial assessment of long-term changes of cerebral function. An EEG trace can be assessed simultaneously to discriminate artefacts and seizure activity. A biparietal montage is preferred as it represents the watershed areas between the posterior and middle cerebral arteries. Moreover signals derived from this area are less likely to be affected by scalp muscle activity and eye-movements artefacts. Impedance is

a measure of signal quality control and should be as low as possible. The Olympic Brainz monitor (Natus, UK), used in the current thesis, alarms if impedance is more than 10kΩ. The skin of premature infants is thinner than that of more mature infants with a higher concentration of water and thinner stratum corneum (169), so it exerts a lower resistance to electrical conduction and consequently needs less skin preparation. With increasing chronological and postnatal age the skin matures and adapts to the new environment and more intensive skin preparation is required.

Background cerebral electrical activity seems to increase and become more continuous during the first days of life (170). Extrauterine maturation differs from intrauterine maturation. Maturation of aEEG background activity is accelerated during postnatal life and in more mature infants (171). This might be attributed to accelerated postnatal brain maturation or may be the effect of environmental stimulation like noise, light, pain and stress (172).

There are different methods for aEEG assessment using qualitative, semi-quantitative and quantitative methods (173, 174). In the current thesis the Burdjalov score was used to assess brain function. This is a semi-quantitative method assessing continuity, cyclicity, degree of voltage amplitude depression, and bandwidth amplitude (174). Each variable receives a score and the total score ranges from zero to a maximum of thirteen with lower scores representing higher immaturity.

Several studies have demonstrated the ability of aEEG to assess brain function and predict short- and long-term outcomes. Acute changes in aEEG background are

powerful, but not specific markers of brain dysfunction (175). Depressed amplitude, increased discontinuity, seizure activity and loss of sleep-wake pattern have all been used for qualitative and quantitative cerebral function monitoring (176). Although the acute and chronic aEEG changes are non-specific regarding the type of brain injury, they correlate with later neurological and cognitive function (175). PVL associated chronic EEG abnormalities are characterised by dysmaturity and disorganisation of the EEG signal (176). aEEG monitoring during the first two weeks of life is both sensitive and specific for the prediction of future developmental outcome in preterm infants and its predictive value is equivalent to a cranial ultrasound scan (172). In future counselling of families of preterm infants will possibly include aEEG assessment in addition to CrUSS in order to combine morphological and functional cerebral parameters when trying to predict future outcomes.

There are many known parameters affecting aEEG measures: degree of prematurity, postnatal age (174), presence of brain injury (177), blood gas parameters (178), medication (179), blood glucose levels (180), infection (181, 182) and compromised systemic flow (183). There are limited data from studies assessing the impact of the PDA and its treatment on cerebral function (184-186). It seems that PDA has negative effect on the Burdjalov score and this is possibly due to lower cerebral perfusion (184). However, there is lack of data from large populations of preterm infants and there is, consequently, a need for more studies assessing the relationship between long-term monitored aEEG features, neonatal morbidity and long-term outcome.

Pulse transit time and pulse wave velocity in preterm infants

Vascular haemodynamics determine organ perfusion and information for them can be obtained through biomarkers conceptualised to measure some aspects of vascular physiology. Pulse transit time (PTT) and pulse wave velocity (PWV) are validated vascular biomarkers in adult medicine with predictive value useful in cardiovascular risk stratification (187). Cardiac contraction generates a pulse pressure wave which travels through the entire arterial tree with a velocity much greater than the forward movement of the blood itself. PTT is the time it takes the pulse pressure wave to travel through a specific segment of the arterial tree. PTT has been shown to be of value in monitoring BP changes in adults, where trend monitoring without disturbance of the underlying physiological process is necessary, as in sleep studies (188). Different methods can be used to measure PTT, but typically the time difference between the ECG R-wave and an element of the pulse oximetry plethysmographic trace is determined with the oximeter probe being placed on a finger or toe; the difference in time between the ECG R-wave and the midpoint of the following plethysmograph trace upswing is usually measured (189), but there can be methodological differences. PTT depends on the site from which the measurement is obtained as the distance between the heart and the specific site of measurement is variable. Murakami et al. found that PTT is strongly correlated with heart rate (190).

PWV partially corrects this variation and can be calculated from the physical distance between the heart and the oximeter probe divided by PTT. PWV is considered a surrogate marker of arterial stiffness (187). Aortic PWV is considered a useful prognostic marker for cardiovascular events in adults (191), as well as

monitoring changes of BP (192). PWV is affected by many recognised cardiovascular risk factors both in adults and in children, including body mass index, waist circumference, waist-hip ratio, systolic and diastolic blood pressure, insulin measures, triglyceride levels, C-reactive protein levels and homocysteine levels (193). There are established reference values in adults (194) and children (195). PWV is a highly reproducible biomarker (196). According to the Moens–Korteweg equation PWV is proportional to the square root of the incremental elastic modulus of the artery wall given constant ratio of wall thickness, to the vessel radius and blood density assuming that the artery wall is isotropic and experiences isovolumetric change with pulse pressure (197). PWV is affected by the pre-ejection period that is also dependent on HR (198). However, there has been little reported use of PWV or PTT in preterm infants probably in part due to the much higher HR and smaller physical distances affecting the precision of the measurements. As high resolution digital recording of ECG, BP waveforms, and pulse oximetry plethysmographic traces is now available, PWV and PTT can be more easily determined in neonates.

PDA has significant effects on cardiac and aortic haemodynamics. PDA connects the high resistance systemic circulation to the low resistance pulmonary circulation and overall reduces left ventricular afterload. The observed left to right shunt leads to increased preload on the left chambers and a wide pulse pressure that is described as a hyperdynamic circulation. These changes have potentially significant effects on both PTT and PWV (189).

As mentioned before there are available and effective treatments for PDA closure, but so far this has not been translated into meaningful long term beneficial outcomes (199). There is no consensus for the definition of the haemodynamically significant PDA and the decision to treat is generally based on a variable combination of echocardiographic and clinical criteria (200). The presence and severity of PDA are difficult to assess continuously in preterm neonates. Echocardiography is the gold standard. However, echocardiography is an assessment at a single time-point. It requires expertise that is not always readily available in the neonatal unit, is subject to considerable variation and sometimes is not well tolerated by critically ill preterm infants. The majority of extremely preterm infants have an indwelling umbilical artery catheter for BP monitoring, the tip of which lies in the descending aorta close to the PDA insertion. Hence, measuring PTT and PWV is feasible and may detect altered haemodynamics due to the PDA steal phenomenon. Moreover, combined information from PWV and BP can possibly provide information for the cardiac output as it is known that cardiac output is proportional to BP and the systemic vascular resistance (Cardiac output = Blood pressure/Arterial Resistance).

Summary of uncertainties

Therefore, there are some key uncertainties:

1. Demographics and the natural history of disease processes in extremely preterm infants are continuously changing and evidence from older studies is becoming outdated or not applicable in the new era. Up to date studies are needed to study this continuously changing population of vulnerable patients with high morbidity and mortality.
2. Despite the presence of an association between PDA and multiple co-morbidities a causal relationship has not yet been established. The study of the PDA effect on target organs in an integrated way is fundamental to elucidate a possible causal relationship.
3. An integrated approach to study PDA, cerebral haemodynamics and IVH includes simultaneous measurement of multiple biomarkers which assess different aspects of PDA and cerebral physiology. However, this approach is laborious and needs advanced statistical process. Hence, there is limited evidence in the literature.
4. The mechanisms of brain injury due to haemodynamic compromise are variable and the period of the insult may extent to several weeks and the insults may have cumulative effect. Moreover, the onset of brain injury is often unknown. There are few studies assessing the temporal relationship between a possible insult and the resultant brain injury.
5. There are inconsistent data in the literature regarding the impact of PDA on cerebral oxygenation.

6. There are limited data from studies assessing the impact of the PDA on cerebral electrical activity.
7. Few studies have reported the prognostic value of early aEEG. There is a need for more studies assessing the relation between long-term monitored aEEG/EEG background features, neonatal morbidity and long-term outcome
8. Cerebral electrical activity is affected by multiple factors following preterm birth including medication. The exact "weight" of each factor is not known. However, this information is important when designing clinical trials which use cerebral electrical activity as surrogate biomarker.
9. Antenatal magnesium sulphate administration is proved to be effective in reducing cerebral palsy incidence. However, the underlying mechanisms of action are not known.
10. PDA alters aortic haemodynamics. However, there is limited evidence in literature regarding the impact of PDA on PTT and PWV in extremely preterm infants.

Purpose of this project

To address key uncertainties in PDA pathophysiology by assessing influences on PDA status and cerebral haemodynamics.

In order to structure the assessment of these uncertainties I have postulated a model of the relationships between PDA and markers of cerebral haemodynamics. This model was tested in the studies presented in Chapter 3. I investigated the interaction between PDA diameter, cerebral oxygenation, cerebral electrical activity and cerebral blood flow and how these affect the severity of IVH using a two-step

model as shown in Figure 3. Other chapters consider alternative ways to assess relevant biomarkers.

The two-step model for the relationship between PDA shunting and brain injury was developed in the light of the literature summarised in this chapter. The central postulate of this model was that PDA shunting leads directly to abnormalities of cerebral oxygenation, electrical activity and blood flow to the brain (Figure 3). It postulates that these abnormalities lead directly to brain injury. The model allows for the evaluation of a direct effect of PDA on brain injury.

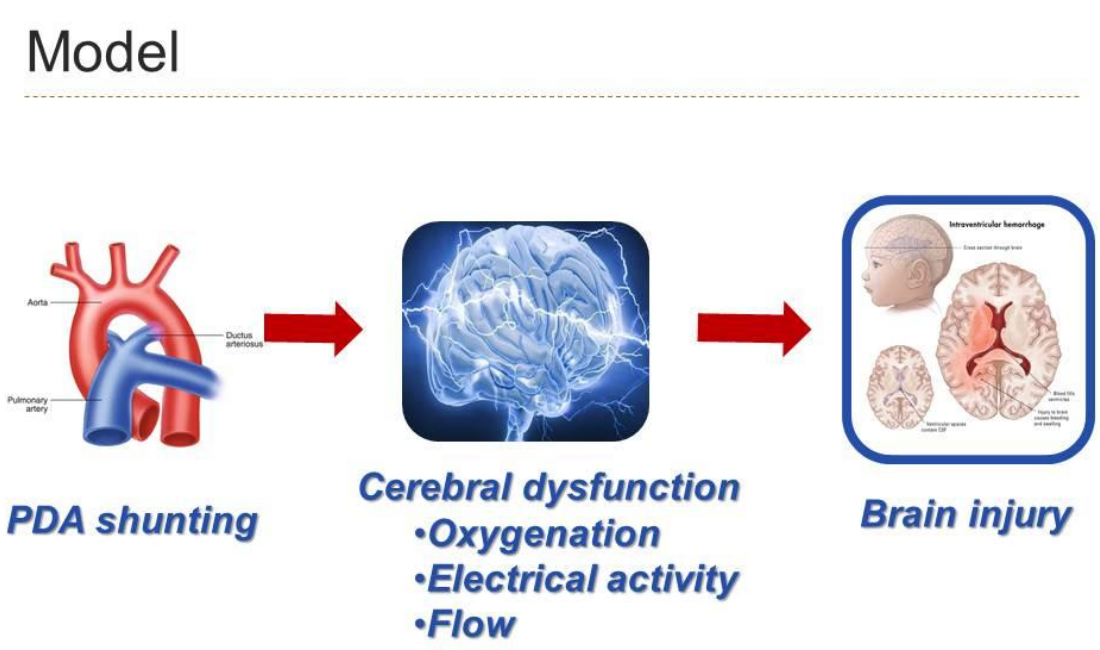

Figure 3: Two step model of interaction of PDA with brain injury through alterations in cerebral oxygenation, electrical activity and blood flow.

Aims of the project

The aim of this project was to conduct an integrated assessment of influences on PDA status and cerebral haemodynamics. This aim was addressed with a multimodal assessment of haemodynamic status that evaluated a pre-defined two-step model for the causation of brain injury by PDA shunt (Chapter 3). The dataset is rich and provides an opportunity to characterise components of the multimodal assessment including: influences on the aEEG score used in the multimodal assessment (Chapter 4); antenatal influences on elements of the multimodal assessment (Chapter 5); existing biomarkers and a novel biomarker for the transmission of the pulse pressure wave through the large vessels of the systemic circulation (Chapter 6).

PhD objectives

The primary objective of this thesis is an exploration of the relation between PDA, cerebral haemodynamics and intraventricular haemorrhage in extremely preterm neonates.

Chapter 3 investigates the relation between PDA, cerebral haemodynamics and intraventricular haemorrhage in extremely preterm neonates with the following objectives:

I. To investigate a possible causal pathway between PDA, cerebral haemodynamics and IVH. The research question was whether PDA has an impact on the incidence of IVH mediated by changes in cerebral oxygenation, electrical activity and blood flow. A two-step model was developed to explore the relationship between PDA shunting, cerebral dysfunction (cTOI), Burdjalov score and RI) and cerebral injury (IVH). Each step of the two-step model was assessed during the whole natural history of PDA disease.

II. To explore the associations between the different biomarkers used to build the previous model and understand better the pathophysiology associated with PDA.

    a. To explore further the ability of NIRS and aEEG to detect brain dysfunction in the presence of PDA.

Chapter 4 investigates in more detail the interactions between cerebral electrical activity (Burdjalov score), PDA size, use of morphine, postnatal age and gestational age with the following objective:

III. To build a linear mixed effect model to assess the impact of gestational age at birth, postnatal age/maturation, morphine administration and PDA diameter on cerebral electrical activity (Burdjalov score).

Chapter 5 analyses the neonatal cardiovascular and cerebral function following antenatal maternal $MgSO_4$ administration with the following objectives:

IV. To assess the feasibility of relating maternal exposure to MgSO4 to neonatal assessments that reflect the function of organs which are expected to be affected by MgSO4, namely the brain and heart.

V. To assess whether maternal characteristics has any impact on neonatal serum magnesium concentrations.

VI. To calculate a sample size required to detect differences in the cerebral biomarkers (cTOI and Burdjalov score) between subjects who have been exposed to MgSO4 and those who have not and to inform future studies which will assess the relationship between antenatal MgSO4 exposure and perinatal pharmacokinetics and pharmacodynamics to indicate the optimal dose and infusion duration of MgSO4.

Chapter 6 analyses the interaction of pulse transit time, pulse wave velocity and a novel biomarker [pulse phase difference (PD)] in extremely preterm infants with PDA with the following objectives:

I. To investigate whether measurements of PTT and PWV in extremely preterm infants are affected by PDA. The null hypothesis was that PDA diameter does not affect measures of PTT aortic PWV in the first days after birth in extremely preterm infants.

II. To explore the associations between PTT and PWV with other echocardiographic and haemodynamic parameters.

III. To explore the relationship between PD and the size of the PDA and test the null hypothesis that there is no relationship between these variables.

Chapter 2: Methods

Population

This study was conducted in Liverpool Women's Hospital (LWH) neonatal unit between November 2014 and January 2017. This is one of the larger units in UK and Europe with approximately 1,000 admissions annually and 110 admissions born < 29 weeks.

Inclusion criteria

Neonates admitted or transferred to Liverpool Women's Hospital between 24+0 and 28+6 weeks' gestation and postnatal age ≤72 hours were recruited during the period.

Exclusion criteria

Neonates were excluded from the study if one or more of the following parameters were present:

1. Non-viability (unlikely to survive more than 48 hours in the opinion of the attending physician).
2. Congenital hydrops, congenital heart disease or other malformations likely to affect cardiovascular adaptation.
3. Surgery planned within 72 hours of birth.
4. Chromosomal anomalies
5. IVH> grade 2 in the initial cranial ultrasound scan indicating antenatal cerebral events.
6. Informed consent form not signed.

Other competing studies at the same period

Baby-OSCAR study was a multicentre double blind randomised controlled trial that was assessing the outcome after selective early treatment for closure of patent ductus arteriosus in preterm babies using ibuprofen (128). We did not co-recruit as

the study intervention was blinded and there is uncertainty whether ibuprofen has any significant effect in cerebral perfusion and function.

However, we co-recruited with PAINT study (ClinicalTrials.gov Identifier: NCT02751437) which was investigating the effect of preterm arginine intake on biological pathways affecting immune function in infants requiring early parenteral nutrition. The increased arginine intake in those babies was considered to be very unlikely to affect PDA function or have any significant direct effect on cerebral oxygenation, electrical activity and flow.

Study procedures

Recruitment

Families were approached before birth if possible or during the first three days after birth, if the clinical team were happy with an approach being made. An information leaflet was specifically designed (Appendix 8) and was provided to parents before asking written consent. Consent covered all study procedures. Consent was obtained when convenient after admission. Parents were given time to consider the study, read the parent information leaflet and ask any relevant questions. Parents could withdraw consent at any point of the study without an explanation. All babies born at less than 29 weeks' gestation had a screening echocardiogram and cranial ultrasonography done as part of routine clinical care

Clinical data

Obstetric and intrapartum data were collected from hospital records. Neonatal data were collected prospectively. All clinical data of patients (vital signs, ventilator settings, laboratory blood results, medication and possible interventions) were

recorded in the full electronic patient record system (Badgernet, Edinburgh, UK) and subsequently retrieved and stored in the case report forms (CRF). The average of 5 sec intervals of physiological data is stored on Bagdernet (0.2Hz). The following data were entered in the CRF:

- Maternal history:
  - Age
  - Social history: job, education, income
  - Antenatal corticosteroid and $MgSO_4$ administration
  - Maternal hypertension and treatment
  - Chorioamnionitis defined as: fever (>38ºC), foul smelling amniotic fluid, tenderness of the uterus, maternal/fetal tachycardia, maternal leukocytosis, pathological diagnosis following assessment of the placenta.
  - Prolonged rupture of membranes (>24 hours to delivery).
  - Type of delivery
  - Previous medical history, medication and family history
  - Others (free text)

- Perinatal data:
  - Gestational age
  - Birth weight
  - Small for gestational age (defined by weight <3rd percentile according to neonatal centile charts)

- Multiple gestation (order)
- Gender
- Need for advanced resuscitation at birth (intubation ± assisted circulation)
- Placental transfusion procedures (e.g. delayed cord clamping, milking of the cord)
- 5 minute Apgar score
- Working weight and head circumference

- Neonatal outcomes:
  - Physiological data: heart rate, pulse oximetry, invasive or oscillometric blood pressure (systolic, diastolic and mean)
  - Biochemical data: blood gas parameters (pH, CO2, base excess, plasma lactate) haemoglobin, white cell count, neutrophil count, C-reactive protein, blood culture results, creatinine, electrolytes and serum glucose levels.

- Cranial ultrasound scan findings

- Major neonatal diagnoses/outcomes (at 40 weeks' gestation ± two weeks, or earlier if died or discharged)
  - Alive or deceased
  - Major CUS diagnoses: grade of IVH; cystic and non-cystic PVL, porencephalic cyst, ventriculomegaly

- Hypotension
- Days to reach full enteral nutrition
- Necrotizing enterocolitis or bowel perforation
- Oxygen dependence at 36 weeks´ gestation
- Oxygen dependence at discharge
- Nosocomial infection
- Ductus arteriosus patency duration and treatment (age)
- Retinopathy of prematurity (grade) and treatment

Obstetric management

Obstetric management was at the discretion of the attending obstetric team. The unit policy was that mothers with threatened premature labour or planned iatrogenic preterm delivery should receive antenatal steroids and $MgSO_4$. A full course of steroids should be delivered when there is sufficient time (betamethasone 12 mg given intramuscularly in two doses or dexamethasone 6 mg given intramuscularly when betamethasone was not available). When clinically appropriate, 4g of $MgSO_4$ were given by intravenous bolus over 20 minutes followed by a continuous infusion of 1g/hour until delivery in accordance with national guidelines (45). $MgSO_4$ was administered either for maternal and fetal neuroprotection in cases of severe preeclampsia or for fetal neuroprotection in the case of non-preeclamptic indications for preterm delivery, such as severe fetal growth restriction or spontaneous preterm labour.

Neonatal management

Resuscitation

Standard resuscitation guidelines were in use (201). Babies born in LWH were placed in a plastic bag and a transwarmer mattress was additionally used to prevent hypothermia. Babies who needed intubation received also immediately surfactant as per local guidelines though the endotracheal tube. Infants remained intubated with active ventilation weaning and aim for early extubation. Routine delayed cord clamping was introduced after the study was completed (202).

Fluid management

Preload has significant effect on many echocardiographic measurements (203) and previous studies have demonstrated that fluid management may have an impact on patency of DA (204). The majority of babies received fluid volumes according to the following guidelines shown in Table 3. However, the fluid management of each baby was reviewed and prescribed on an individual basis according to weight changes, GA, renal function, electrolyte and cardiovascular status.

All infants were commenced on total parenteral nutrition (TPN) as soon as possible after birth (policy is to start as soon as vascular access is available, usually within an hour). The policy of the unit was to give no electrolytes in the first two days of life and on day 3 to start TPN with sodium, potassium and other elements supplementation. When infants were reaching full TPN, usually by day 7, the content of electrolytes was: sodium 4 mmol/kg/day, potassium 2 mmol/kg/day, calcium 1.47mmol/kg/day and phosphate 2 mmol/kg/day.

Blood pressure management

There is no consensus among clinicians of the normal BP limits in extremely preterm infants. LWH threshold for diagnosing hypotension and instituting treatment in preterm infants was a mean BP (mmHg) below the infant's gestation (weeks) plus one or more of the following clinical/laboratory features: urine output < 0.5 mL/kg/hour, poor peripheral perfusion (capillary refill time > 3s) or lactate > 4 mmol/L. Treatment guidelines are shown in Figure 4.

Fluid management in extremely preterm infants in LWH	
Day 1	90 mls/kg/day
Day 2	120 mls/kg/day
Day 3-7	150 mls/kg/day
Day >7	165 mls/kg/day

Table 3: Fluid management in extremely preterm infants in Liverpool Women's Hospital.

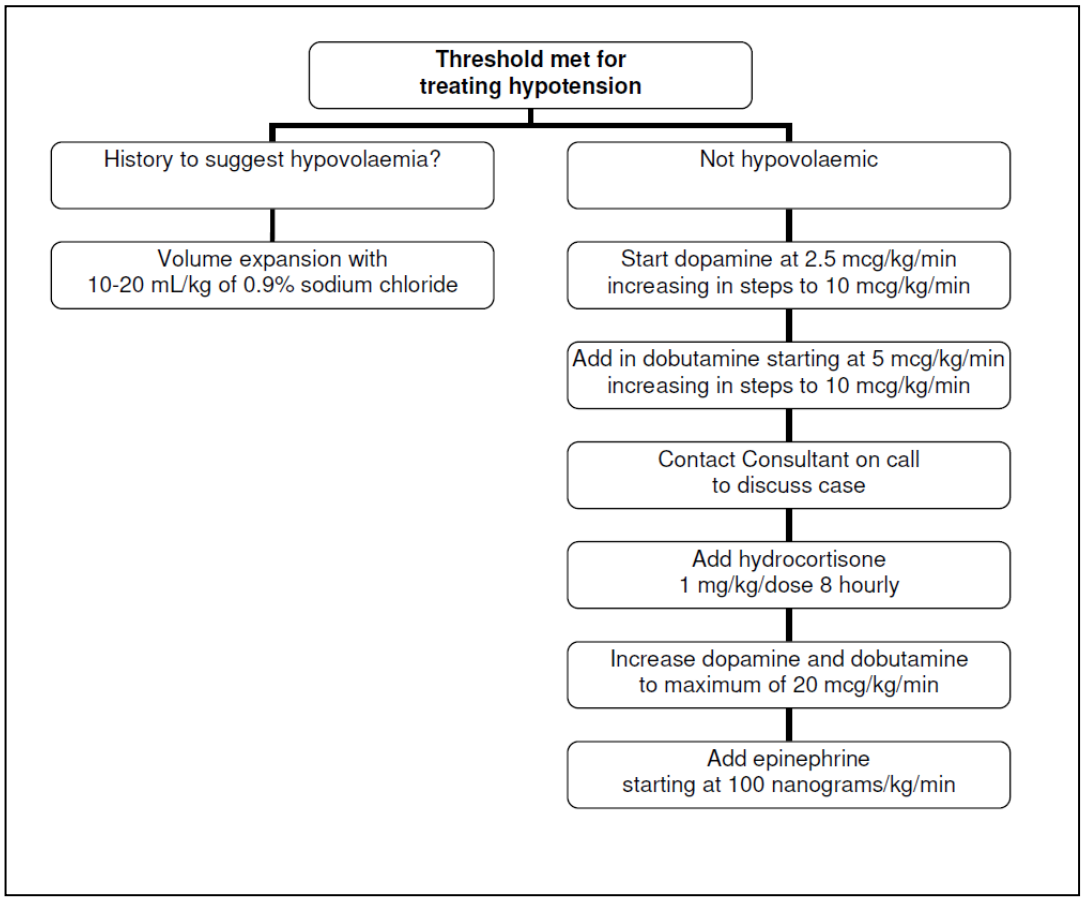

Figure 4: Liverpool Women's Hospital hypotension treatment guidelines.

PDA management

PDA management was at the discretion of the attending clinician. A significant number of babies were recruited to the Baby OSCAR study while this study was open. Accordingly, the sampling frame for this study was limited to babies who did not meet the inclusion criteria for the Baby OSCAR study (lack of equipoise whether PDA treatment is needed) or whose parents did not give consent to the Baby OSCAR study. PDA was considered haemodynamically significant and treated with ibuprofen based on a combination of echocardiographic and clinical criteria. Clinical practice in PDA management varied between clinicians in the unit. A symptomatic approach was used and infants were treated with ibuprofen, if two or more of the following echocardiographic criteria were present; 1. PDA diameter >2mm, 2. Left heart dilatation (e.g Left atrial:aortic ratio>1.8), 3. Pulsatile flow in the ductus arteriosus and 4. Absent or retrograde flow in the post-ductal aorta, in addition to being unable to be weaned on the ventilator. The therapeutic approach to achieve ductal closure was intravenous ibuprofen at 10 mg/kg on day 1, followed by 5 mg/kg daily for the next 2 days. Diuretics (furosemide and spironolactone) were also used in infants older than two weeks when ibuprofen was deemed unlike to be effective or there were contraindications for its use. PDA ligation was rarely used and was reserved for infants with haemodynamically significant PDA who were unsuccessfully treated with ibuprofen and required persistent or significant ventilatory support despite use of steroids for chronic lung disease (CLD). The echocardiographic staging used in the present study did not influence the decision to treat. The echocardiographic findings were revealed to the clinical team only

when a relevant question was asked by the clinical team. Findings from other assessments were not shared with the clinical team.

Near infrared spectroscopy

Cerebral oxygenation was assessed using a NIRO-200NX oximeter (Hamamatsu Photonics, Fukuoka, Japan) during aEEG monitoring. The optode was placed on the left fronto-parietal site and stabilised with an elastic bandage. Sampling frequency was set at 1 Hz. Recordings were performed for four hours per session. The cerebral TOI (cTOI) was used as a continuous estimate of cerebral venous oxygen saturation. The mean cTOI was calculated from the artefact free periods during the four hour monitoring period. NIRS monitoring preceded echocardiography and was combined with aEEG. NIRS application procedure and monitoring are specified in details in the standard operating procedure in Appendix 4.

Digital amplitude integrated electroencephalography

Recordings were performed for four hours per session using an Olympic Brainz Monitor (Natus Medical Incorporated, Munich, Germany). This approach was selected for logistic reasons (only one aEEG machine available) and the fact that echocardiographic measurements were taken at one time point per day and hence the correlation studies would only be meaningful for some period before or after the echocardiographic study. Hydrogel electrodes were applied to the neonatal scalp after skin preparation using the two parietal positions (P3 and P4) and a reference electrode placed on the shoulder. Impedance was kept at <10 kΩ. Recordings were performed whilst the infants were asleep and we aimed for minimal handling during the recording. The Burdjalov score was used to assess the aEEG trace as it has been shown to correlate with neurodevelopmental outcome

and does not require sophisticated software for signal analysis (174, 205, 206). Further details of aEEG application, monitoring and scoring are specified in details in Appendix 5.

Electrocardiography

The ECG data was recorded as part of the routine neonatal care from three lead electrodes forming the Einthoven triangle. Two electrodes were placed onto either side of the chest and a third on the outer aspect of one thigh. Recordings were performed whilst the infants were asleep and while they were not disturbed throughout the duration of the recording. Recordings preceded echocardiography and performed simultaneously with EEG and NIRS. Lead I was routinely selected for monitoring and recording. ECG and BP monitoring data were downloaded from IntelliVue MX800 monitors (Philips Healthcare, UK) using X2 module (Philips Healthcare, UK) and Massimo oximetry modules (Massimo, UK) (Figure 5) and stored as spreadsheet files on a laptop via a wireless connection using IxTrend software (Ixellence GmbH, Wildau Germany) (Appendix 6). The signal was sampled at 512 Hz.

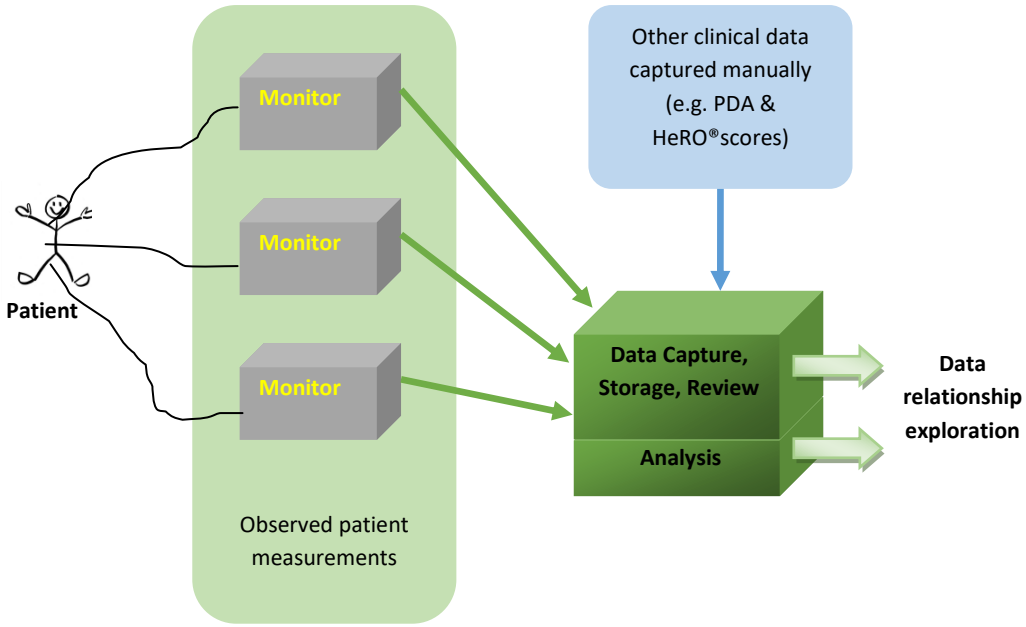

Figure 5: Data capturing and collection procedures from multiple patient-connected monitors during the study.

Cranial ultrasonography

A CrUSS was performed by a single operator [Charalampos Kotidis (CK)] as soon as possible after enrolment, preferably within the first 6 hours of birth to exclude antenatal cerebral events (IVH> grade 2) not related to postnatal PDA disease. CUS was then performed daily in the first 3 days after birth, then once weekly until the PDA closed and finally at discharge. CrUSS was performed after echocardiography. Sterile gel sachets were used and were previously warmed before the application to limit infant's disturbance. A Vivid E9 machine (General Electric, London, UK) with a 12 MHz probe was used. CUS was also part of the routine clinical care of premature infants especially in infants who were found to have significant cerebral pathologies. Eleven pre-specified views were recorded and stored for each scan. Cerebral blood flow velocity in the anterior cerebral artery was also measured by Doppler ultrasonography through the anterior fontanelle and recorded (207). The angle of insonation between the vessel and the Doppler ultrasound beam was kept below 20^{o} to ensure accuracy of the measurements. The Digital images were stored in the ultrasound machine and subsequently in CDs. The measurements were performed offline.

Technique

Each CrUSS comprised 11 standard views through the anterior fontanelle (6 coronal and 5 sagittal).

Coronal:

C1 frontal lobes

C2 anterior frontal horns of the lateral ventricles

C3 third ventricle, connections between the lateral and third ventricle (foramen of Monro)

C4 cerebellum largest diameter left-right

C5 level of the trigone of the lateral ventricles, posterior horns with plexus

C6 occipital lobes beyond the posterior horns

Sagittal:

S1 midline; measurement: Doppler flow measurements at the ACA

S2 left lateral ventricle

S3 left periventricular through the Sylvian fissure

S4 right lateral ventricle

S5 right periventricular through the Sylvian fissure

Method of classification

1. Normal brain scan

No cysts

No ventricular dilatation

No enlargement of extra-cerebral spaces

Normal cortical grey matter

2. Mild brain injury:

Grade 1-2 IVH

Persistent pathological non-decreasing flaring at days 7 and 14

3. Severe brain injury :

IVH grade 3-4

Parenchymal/periventricular haemorrhagic infarction

Local cystic lesions (unilateral)

Cystic periventricular leukomalacia (bilateral)

Cerebellar haemorrhage

Cerebral atrophy at term age (ventricular dilation and/or increased extra-cerebral spaces)

CrUSS -Doppler measurements:

1. Peak systolic flow velocity (PSFV) and end-diastolic flow velocity (EDFV).

2. Anterior cerebral artery resistivity index (RI): (PSFV-EDFV)/ PSFV.

Quality assurance

Doppler studies were performed at the beginning of the cranial ultrasonography to avoid the potential influence of baby handling on the cerebral haemodynamics. The pulse wave (PWD) gate was placed immediately after the origin of the ACA from the internal carotid artery and once the highest signal was found the average of three

consecutive measurements was captured. The angle of insonation was kept on minimal possible and always below 20^{o}.

Echocardiography

All neonates born at less than 29 weeks' gestation had a screening echocardiogram and cranial ultrasonography done as part of routine clinical care especially if they were symptomatic (need for ventilation or inotropic support). Echocardiography was performed by a single operator (CK) using a Vivid E9 machine (General Electric, London, UK) and 12 or 6-MHz probe depending on the gestational or postanatal age. The infant was kept in a comfortable environment in the incubator or cot and his/her welfare was the first priority at all times. Gel was warmed before it was applied. The infant was on supine position with the chest and abdomen exposed. The ultrasound machine was configured appropriately for the neonate, providing cross-sectional, M-mode, colour Doppler, PWD and continuous wave (CWD) Doppler and tissue Doppler imaging (TDI) were performed. Echocardiography was done every 24 hours in the first three days of life, once weekly until the PDA closed and then before discharge. If the PDA closed in the first week after birth a second echochardiogram was performed on second week to have a comparison group of patients with closed PDA. Infants without PDA or with a PDA closed in the first week after birth were used as a comparison group to assess "normality". The first echocardiogram was usually comprehensive to exclude any underlying congenital heart disease and confirm the eligibility for the study. The published guidelines for targeted neonatal echocardiography were followed for the measurement of echocardiographic biomarkers (203).

It is paramount to acquire good echocardiographic skills to minimise the intraobserver variability and ensure the observed variability reflects the altered neonatal haemodynamics and is not a measurement error. I was trained in echocardiography in LWH during a nine months placement in LWH as Academic Clinical Fellow under the direct supervision of Dr Nim Subhedar (Consultant Neonatologist and lead of Neonatal echocardiography in LWH, UK) and completed a logbook of 150 neonatal echocardiograms before the study recruitment commenced. I continued my echocardiographic training during a 9 months placement as Paediatric trainee in Paediatric Cardiology department in Alder Hey Children's Hospital, Liverpool. I was part of the NEOCIRCULATION consortium funded by Framework Programme 7 of the European Commission and received additional training for one week by Dr Maria Bravo (Consultant Neonatologist, La Paz University Hospital, Madrid, Spain) in SVC flow measurement in the trial specific training programme with other collaborators. I co-lead the formation of all standard operating procedures for echocardiographic and cerebral biomarkers used in the NEOCIRCULATION consortium studies.

PDA score

PDA score is a composite score that comprises ten echocardiographic biomarkers (Table 4) (107). Individual echocardiographic biomarkers are graded from 1 to 3 based on the measurement magnitude with a composite PDA score range 0-30. Echocardiographic assessment was not revealed to the clinical team unless specifically asked or umbilical vein catheter was found misplaced inside the heart. PDA score did not influence the decision for PDA treatment.

PDA size

PDA size was measured from the high parasternal view ("ductal cut") using colour Doppler after optimisation of colour gain (138). The 2-D view was used for optimisation to assess whether colour Doppler was "bleeding" into the tissue. The PDA size was measured at the narrowest part of the PDA usually at the pulmonary end of the DA. The measurement was taken ideally when the whole PDA lumen was visualised from the aortic to the pulmonary end. However, this was not always feasible as PDA tends to be tortuous in extremely preterm infants.

Transductal velocity

The transductal velocity was measured using pulse or continuous Doppler as appropriate from the ductal view (138). The cursor was placed in the narrowest point of the PDA and in the middle of the vessel. The maximum systolic velocity was measured.

Left pulmonary artery (LPA) size

The LPA internal diameter was measured from the high parasternal 2D ductal view with an angle so the three vessel view was obtained (208).

Antegrade pulmonary artery (PA) diastolic flow

Antegrade PA diastolic flow was measured from the parasternal long axis view angled so the pulmonary artery and flow were visualised. This allows the pulmonary flow to be parallel to the PWD cursor. The cursor was placed inside the main pulmonary artery and the diastolic velocity was measured (136).

Antegrade left pulmonary artery diastolic flow

Antegrade LPA diastolic flow was measured from the parasternal short axis view where the LPA lumen is parallel to the cursor of the PWD. The cursor was placed inside the left pulmonary artery and the diastolic velocity was measured (135).

Left atrium to aortic ratio (LA:Ao ratio)

PDA causes pulmonary overcirculation and increases preload in the left chambers. LA becomes progressively larger in hsPDA. However, the aorta does not change significantly size depending on different preload or afterload, so it can be a useful reference point that allows comparison of LA size for infants of different weight or gestation. LA:Ao ratio was measured from a long parasternal view when the whole left ventricle, LA and aorta were seen in the same view and measured with M-mode (LA in diastole and Ao root in systole). The cursor was placed at the plain of the aortic valve hinges to include the maximum LA diameter. The cursor was perpendicular to the aortic wall at the level of the aortic valve. Leading edge to leading edge was used for the measurements (138). There is no consensus regarding the clinically significant threshold. However, a ratio above 2:1 indicates a significant shunt.

Left ventricular: aortic ratio (LVEDD:Ao)

Similarly, the LVEDD:Ao ratio was measured to assess the preload changes induced by the PDA using the same view as LA:Ao adjusted so the M-mode cursor to be next to mitral valve hidges (209). The internal diameter of the LV in end diastole was measured and compared with the Ao size obtained from LA:Ao measurement.

Isovolumic relaxation time (IVRT)

IVRT is a marker of diastolic function. Prolongation of IVRT indicates poor myocardial relaxation. IVRT was measured from an apical 5-chamber view. PWD sample volume was positioned in the left ventricle between the mitral inflow and the left-ventricular outflow so as to visualise sequentially both the cessation of aortic outflow and the offspring of mitral flow from the Doppler shift 0 line (208). The time interval between aortic valve closure and mitral valve opening was measured. Measurements were averaged over three cardiac cycles. Preterm infants are considered having diastolic dysfunction and hence prolonged IVRT can be a valuable biomarker to assess diastolic dysfunction in this group of patients (210).

Mitral valve E:A wave ratio

E wave to A wave ratio (E/A wave ratio) is a marker of diastolic function. Cardiac diastole is divided into four phases: 1) isovolumic relaxation time, 2) early filling, 3) diastasis and 4) atrial contraction. We can assess these phases by studying the mitral flow pattern with PWD. Doppler was performed from the 4 chambers apical view. The PWD cursor was placed between the tips of the open mitral valve leaflets. Colour flow was used to assess the direction of flow and optimise the alignment. A dicrotic flow pattern is usually derived, consisting of E and A wave (208). E wave corresponds to early filling and A wave to atrial contraction. The peak flow velocities were measured and averaged over 3 cardiac cycles.

Left ventricular output (LVO)

LVO was derived by measuring blood flow across the aortic valve. The aortic diameter was measured from the parasternal long axis view at the level of aortic valve. The diameter was measured from the 2-D image at the insertion of the aortic valve leaflets, advancing frame by frame until mid- to end-systole, when the leaflets were in a parallel position. PWD was performed from the 4 chambers apical view modified in order to visualise the ascending aorta (5 chambers view). The range gate was positioned just beyond the valve leaflets. The angle of insonation was kept between 10-20o and no angle correction was used (208). The aortic velocity time integral (VTI) was measured (the average of three measurements) by tracing the area under the instantaneous velocity time curves on the screen using the relevant machine software. The heart rate was calculated from the distance between two consecutive aortic flow waves. The LVO was calculated based on the following formula:

LVO (ml/kg/min) = Aortic VTI x Heart rate x 3.14 x (aortic diameter/2)^2 / weight (kg)

VTI: velocity time integral

Right ventricular output (RVO)

RVO was derived by measuring blood flow in the main pulmonary artery (211). The pulmonary artery diameter was measured in the high parasternal long axis view by tilting the transducer slightly upwards. The diameter was measured from the 2-D image at the insertion of the pulmonary valve leaflets, advancing frame by frame until mid- to end- systole, when the leaflets were in a parallel position. A Doppler recording was performed from a low parasternal long axis view tilting the

transducer downwards. The range gate was positioned just beyond the valve leaflets to minimize any disturbance to the flow pattern from ductal shunting. The pulmonary artery is particularly good for Doppler studies because its posterior orientation takes the blood directly away from the transducer at a minimal angle of insonation from the low parasternal window. The pulmonary VTI was measured (the average of three measurements) by tracing the area under the instantaneous velocity time curves on the screen using the relevant machine software. The heart rate was calculated from the distance between two consecutive pulmonary flow waves (212). The RVO was calculated based on the following formula:

RVO (ml/kg/min) = pulmonary VTI x Heart rate x 3.14 x (pulmonary diameter/2)^2/weight (kg)

VTI: velocity time integral

Superior vena cava flow

SVC diameter was measured using the left mid-parasternal view in a true sagittal plane by positioning the transducer over mid sternum, a bit towards left side with the notch pointing upwards. The SVC usually funnels out as it enters the right atrium. The SVC diameter was measured at the base of the funnel by M-mode. As SVC diameter varies during the cardiac cycle, the maximum and minimum diameters were averaged over five cardiac cycles. SVC velocity was measured using the subcostal window. The transducer was positioned in the midline midway between the xiphisternum and umbilicus. The transducer was placed as close to the umbilicus as possible to decrease the angle of insonation. The PWD sample volume

was placed at the junction of the SVC and right atrium. SVC flow trace has a first peak corresponding to filling in ventricular systole, and a second peak representing positive ventricular filling in early diastole. Additionally, atrial systole is sometimes marked by a reversed flow. The Doppler velocity envelope was traced using the calculation package on the Echo machine. Reversed flow was incorporated and subtracted from the forward flow. Five cardiac cycles were measured and averaged (213).

The scoring system is given in Table 4.

Feature quantified	Modality/position of sample gate	Score 0	Score 1	Score 2	Score 3
Ductal features					
Transductal diameter (mm)	Two-dimensional, short axis view	0	<1.5	1.5–3	>3
Ductal velocity Vmax(m/s)	PWD at pulmonary end of duct	0	>2	1.5–2	<1.5
Magnitude of ductal shunt					
PDA:LPA diameter			<0.5	≥0.5–1	≥ 1
Antegrade PA diastolic flow (cm/s)	PWD within main pulmonary artery	0	0–20	>20	
Antegrade LPA diastolic flow (cm/s)	PWD within left pulmonary artery	0	<30	30–50	>50
Left atrial: aortic ratio	M-mode, long axis view	1.13 ± 0.23*	<1.4:1	1.4–1.6:1	>1.6:1
Left ventricular: aortic ratio	M-mode, long axis view	1.86 ± 0.29*	–	2.15 ± 0.39*	≥ 2.5
Features of myocardial performance					
LVO/SVC flow ratio	PWD of flow in superior vena cava	2.4 ± 0.3*	–	–	≥ 4
E /A wave ratio	Transmitral Doppler	<1		1–1.5	>1.5
IVRT (ms)	Between mitral and aortic valves	>55	46–54	36–45	<35

Table 4: Scoring system for the assessment of hemodynamically significant DA (107).

*Mean ± SD, Vmax: maximum velocity, LPA: left pulmonary artery, PA: pulmonary artery, PWD: pulse wave doppler, isovolumetric relaxation time (IVRT).

Biomarker validation and reproducibility

NIRS

Animal studies have validated the use of cerebral fractional tissue oxygen extraction against central cerebral venous saturation (148). Cerebral NIRS has shown strong correlation to perfusion assessment with MRI (149, 150). Reproducibility of the measurements is fair [4.4% (CI 3.5-5.2)] with the device used in the current thesis (NIRO-200NX, Hamamatsu, Japan) (147).

aEEG

It has been shown that EEG/aEEG recorded within the first 7 days of life in preterm infants may have potential as a predictor of a developmental quotient <70 points, cerebral palsy, or death, with a pooled sensitivity of 0.83 (95% CI, 0.69–0.92) and specificity 0.83 (95% CI, 0.77–0.87) (214).

Echocardiography and Doppler measurements

Echocardiography has been validated against MRI measurements (139) and measurements with thermodilution, Fick, or dye dilution methods (140). Previous studies have demonstrated that the reproducibility of some of the ultrasongraphic variables used for PDA diagnosis and characterisation is far from optimal and this should be considered when interpreting data and designing clinical studies. Table 5 provides an overview of the reproducibility indices used in the current thesis. The observed variability can be contributed to the inherited method imprecision, the simplifications applied for the creation of calculation formulas and the altered haemodynamics induced by the examiner during his interaction with the baby (chest pressure, altered temperature, crying, tachycardia). To achieve higher

reproducibility and accuracy it is important to undergo rigorous training and gain experience with neonatal echocardiography.

Author (Year):	Biomarker Assessed:	Results:							
		Within-Observer Repeatability:				Between-User Repeatability			
		RepC	95% CI	RepI	95% CI	Rep C	95% CI	RepI	95% CI
David van Laere et al. (2018) (215)	LVEDD	0.93	0.86–0.97						
	IVRT	0.84a	0.63–0.93						
	E/A wave ratio	0.9 a	0.77–0.95						
	LVO	0.97a	0.94–0.99						
Schwarz et al (2016) (141)	ACA RI					0.11	0.08-0.16	14	11-20
	LA:Ao					0.23	0.17-0.33	16	12-23
	PDA Diameter					0.28	0.15-1.47	21	12-112
Groves et al (2008) (216)	SVC Flow			30	17-43			85	35-136
	DAo Flow			60	34-86			80	33-127
Skinner et al (1996) (217)	PDA max	0.48	0.33-0.88	39	27-71	0.56	0.40-0.92	28	20-47
	PDA mean	0.39	0.27-0.71	47	32-85	0.58	0.41-0.95	36	26-60

Table 5: Repeatability of echocardiographic and Doppler parameters.

RepC: repeatability coefficient, 95% CI: 95% confidence interval, RepI: repeatability index, ACA RI: anterior cerebral artery resistance index, LA:Ao: left atrium to aortic root ratio, SVC: superior vena cava, DAo: descending aorta, IVRT: isovolumic relaxation time, LA: left atrium, Ao: aortic root, LPA: left pulmonary artery, LVEDD: left ventricular end-diastolic diameter, LVO: left ventricular output, a: Lin's concordance coefficient. Adopted and modified from (218).

Cranial ultrasonography

Previous studies have demonstrated that cranial ultrasonography has high diagnostic reliability and accuracy when assessing severe IVH (sensitivity 87–90%, specificity 92–93%), but sensitivity was poor to fair for grade 1 and 2 IVH (48–68%) and PVL (20–44%) (219). Reproducibility indexes for the Doppler interrogation of the anterior cerebral artery (ACA) are shown in Table 5.

Strategy for data collection

After parental consent was obtained, the newborn baby had a cranial ultrasound scan to exclude any pre-existing severe IVH (IVH> Grade 2). Subsequently, the study proforma with the background demographics and clinical history was filled in. The NIRS optode and aEEG electrodes were placed for four hours and in the end of this period echocardiography and CUS were performed. All the above procedures were mainly performed during the normal working hours 09:00 to 17:00.

Blood samples for biochemistry were usually obtained 06-09:00 am. Blood gases were performed as indicated by the clinical team and the closest to our observations was used for analysis. Hence, there was no significant clinical delay between blood sampling and specific neonatal biomarker measurement.

Measures to minimize pain and distress

All study related procedures were undertaken in line with local procedures to minimise pain and optimise welfare. All study-related procedures were conducted by professionals experienced in neonatal care who received study-specific training.

Sample size considerations

As this was a pilot study, power calculation was not applicable.

Ethical considerations

The study protocol (Appendix 1) was approved by North West-Lancaster ethics committee, REC reference: 14/NW/1274 (Appendix 10). Parental informed consent was obtained prior to any study procedures (Appendix 9). The study was conducted in accordance with the principles of Good Clinical Practice. Procedures were established to prevent and/or minimise risk of complication for participants, such as complications related to the device and the treatment guideline included only interventions that are commonly used during intensive care in this population.

Funding

The study was funded by the Neocirculation consortium (European FP7-Grant Number:282533).

Data Protection

The researchers involved in the present study complied with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and upheld the Act's core principles. Access to collated participant data was restricted to those clinicians treating the participants. Only designated university personnel had access to the department through a secure swipe card system. Extra encoded door protected the room where the paper files were stored. Paper and other manual files were filed and stored securely in a locked cabinet. Computers used to collate the data had limited access measures via user names and passwords. Encrypted external hard drives were used to transfer data from Echo, NIRS and EEG machines to the central database.

Statistical analysis

The statistical analysis was driven by hypotheses. For the main analysis in Chapter 3 the hypotheses were derived from a biological model. I developed the biological model

following a literature review that I performed. This review identified the gaps in knowledge and the needs for multimodal assessment of PDA pathophysiology in extremely preterm infants and its interaction with cerebral haemodynamics. The study methodology was subsequently adopted by the NEOCIRCULATION project, a European union FP7 research project and was meant to run in parallel with the main study (220). I co-led the biomarker group for NEOCIRC study and developed the biomarker capturing methodology that was similar to my PhD thesis methodology. I subsequently collected the data presented in Chapter 3 and analysed the data with time point analysis using SPSS. I presented my preliminary data at the Neonatal society meeting in 2015 (221) and the feedback from the panel was that I need advanced statistical support to deal with multiple comparisons, missing data and integrating the multiple biomarkers into a single biological model. I therefore approached Dr Antonio Eleuteri (Consultant Statistician-Honorary Lecturer, Department of Medical Physics and Clinical Engineering University of Liverpool, UK), who kindly agreed to support the statistical analysis.

Accordingly, statistical analysis was conducted using SPSS and R.

Characteristics of R

R is a powerful programming language that is very popular among statisticians and data scientists. It is popular in fields like science, health care, economics and finance, genetics research, and marketing. It is an open-source programming language and environment for statistical and data analysis, graphics generation and even report production. It compiles and runs on a variety of systems including Windows, Linux and MacOS. The main programming feature of R is CRAN (Comprehensive R Archive Network), which is a repository holding more than 10,000 packages available for download by the users. The packages typically include R functions, sample data, and compiled reusable code in a well-

defined format as well as documentation. These packages are created by statisticians to allow for specific functionality, often in conjunctions with peer reviewed publications, and to allow reproduction of the published results (222).

R offers solutions to complex data manipulation challenges as programmers can import data from different data sources including text files, Excel spreadsheets, statistical packages, and databases with relative ease. Probably the most time-consuming process is data cleaning, and this is supported by many R packages. It involves the selection of variables of interest, combining and deriving new ones, handling missing values through a coherent coding scheme, and correcting wrong data (e.g. wrong labelling or mixed units). Once the data are technically consistent, statistical analysis can be initiated. R contains a very rich repository of functions allowing the use of a variety of statistical techniques which can be adjusted to specific research questions. It allows for statistical techniques like regression modelling, classical hypothesis tests (parametric and nonparametric), classification, clustering, time-series analysis, data mining etc. The user retains full control of the statistical processes and can quickly assess the validity of the results through direct outcome comparisons and performance indicators.

Pattern recognition, trends, abnormal behaviour and exceptions (outliers) in the data can be easily identified through data visualisation techniques. The researcher can visually convey information in an efficient way, enhancing understanding and increasing the impact of the analysis. Additionally, the effect of different parameters can be examined, simultaneously improving the interpretability of the analysis. R offers robust data visualisation tools allowing the creation of high quality and elegant graphs ready for scientific publications. It contains packages for basic data representation elements, ranging from scatter plots,

charts, histograms, box plots to more advanced visualisations such as heat maps and detailed maps - the list is non-exhaustive.

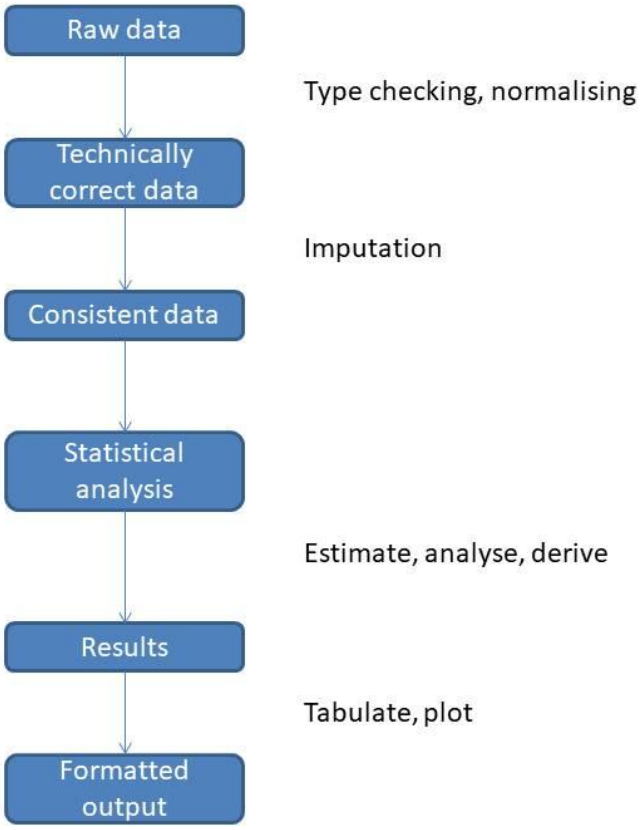

Figure 6: Statistical analysis flow chart

SPSS and R comparison

In the thesis both software packages have been used for statistical analysis and model development. SPSS has mainly been used to produce demographic analytics, simple correlation analysis and logistic regression models, while R has been used to allow for more complex and advanced techniques. As these functionalities were not available in SPSS, R was considered the only option to address these more complex tasks.

The main differences between the two software packages can be summarised below.

- Data manipulation: R packages support data manipulation in a flexible, but robust way allowing for reproducible and readily adjustable code to be created and tested on new datasets.
- Functionality: R packages contain a wide range of functions and advanced statistical methods that can be quickly implemented and adapted to the given problem. R provides powerful and flexible scripting. For computationally intensive tasks, C, C++ and Fortran code can be linked and called at run time. Advanced users can write C code to manipulate R objects directly. SPSS's scripting language has limited expressive power.
- Ease of use: SPSS is quick and easy to learn as it has a user-friendly interface while R is based on an object-oriented programming language and therefore is more challenging to learn.
- Data visualisation: R offers much more opportunities to modify and optimize graphs due to a wide range of available packages. Visualisations in SPSS are limited.
- Support: There is a large community of expert users, amongst them statisticians and the authors of the libraries, who can support in troubleshooting and provide freely

available resources (manuals and tutorials). SPSS support is costly and not so prompt.

- SPSS implementation of statistical methods is not transparent; the user cannot see how algorithms are implemented, and cannot verify the correctness of the implementations.
- Amount of data: R can handle large amounts of data while SPSS cannot.
- Automation and integration: R can be easily integrated with other software and frameworks like Git, LaTeX, ODBC, Apache Hadoop.

Contributions in study design and statistical analysis

When Dr Eleuteri joined the project, his task was difficult as the study methodology was already decided and the majority of data were already collected, thus not allowing him to amend the study methodology. We had multiple meetings and discussions to establish a common base in understanding the biological phenomena and the biomarkers used in the study from Dr Eleuteri's side; and understanding the benefits and the limitations of using different statistical methods from my side. This was a significant learning opportunity for me and I am grateful for Dr Eleuteri's guidance and support. The statistical analysis was refined collaboratively based on the results of the preliminary analysis and the feedback from the presentations of our work at multiple scientific meetings (Appendix 12: Publications). I performed the demographic analysis, time point correlation analysis and association studies and backward logistic regression using SPSS. Learning to program in the R environment to perform the necessary, complex and specialised statistical analysis beyond what SPSS offers was beyond the scope of the current PhD thesis and would have been also risky, based on the complexity and the experience needed to perform reliable statistical analysis.

Dr Eleuteri has a Ph.D. in Applied Mathematics and Computer Science, with a specialisation in Probability and Statistics. He is also a Chartered Scientist and is a member of the Institute of Physics and Engineering in Medicine. His research interests focus on statistical learning theory, survival analysis, machine learning, pattern recognition, information geometry, stochastic processes, probability theory and graphical models; with applications to medicine and health care. Dr Eleuteri had a major role in deciding the appropriate statistical methods for the analysis of the complete dataset once the clinical questions had been defined, and refined. He performed the advanced statistical analyses in Chapters 3, 4 and 5 (multiple imputation using additive regression, bootstrapping and predictive mean matching, robust estimation of the covariance matrix, smoothed local polynomial regression fits, addressing nonlinearities in regression, validation of models, power calculation for future studies). I defined the clinical issues and hypotheses for Chapters 4 and 5.

I performed independently the statistical analysis for Chapter 6 using SPSS. The work from this Chapter was recently published in the European Journal of Pediatrics and has attracted considerable attention as there have been 1190 accesses in 3 months (223).

Demographic factors were assessed using descriptive statistics. Continuous variables were compared using Spearman's correlation. Groups compared using Mann-Whitney. Categories were compared using Chi-squared. A P-value <0.05 was considered significant. Various regression techniques were used. Different models were constructed for assessing the relevant research hypothesises. An overview of each model is provided here. More detailed information about the development of each model is provided in the relevant section of the Results (Chapters 3, 4, 5 and 6).

Demographics and clinical outcomes

During the recruitment period there were other competing studies which may have led to selection bias. To assess whether the results of the present study can be generalised to other populations, I compared the HAPI-PDA population demographics and clinical outcomes with the local (LWH), national (National Neonatal Audit Programme online UK) and international neonatal populations (Vermont Oxford network) during the same period. After access was granted to the local electronic database, national (https://nnap.rcpch.ac.uk/unit-data.aspx) and international databases (https://portal.vtoxford.org/) relevant data was retrieved when possible. The local database had a large amount of missing data for some parameters. The amount of missing data varied between parameters. Percentages were reported and no statistical tests were done. The clinical relevance of the results was subsequently discussed along with the impact on generalisability of the HAPI-PDA study.

Timeline analysis of study biomarkers

Smoothed local polynomial regression fits (with 95% confidence intervals) of postnatal natural evolution of PDA score, PDA diameter, Burdjalov score, RI and cTOI in patients with and without severe IVH were performed to assess whether babies who developed severe IVH had different baseline and subsequently different temporal changes of the relevant biomarkers.

I performed Mann-Whitney test using SPSS for each variable for the first three days after birth to assess whether there was a statistically significant difference of the relevant biomarkers (PDA diameter, PDA score, cTOI, Burdjalov score, RI) between patients with and without severe IVH. Due to multiple comparisons (fifteen in total) the statistical significance was set at $P< 0.05/15=0.003$ (Bonferroni correction).

PDA, cerebral haemodynamics and intraventricular haemorrhage in preterm neonates: an investigation of possible causal pathway

The interaction between PDA diameter, cerebral oxygenation, cerebral electrical activity and cerebral blood flow was investigated as well as how these affect the severity of IVH by a 2 step model as shown in Figure 3. The results of this investigation are presented in Chapter 3.

Multiple imputation

In an effort to avoid discarding observations, a multiple imputation procedure (using additive regression, bootstrapping and predictive mean matching) was applied (224). A common approach to deal with missing data is casewise deletion, that is any subject having any predictor or outcome missing is excluded from regression analysis. This however results in regression coefficient estimates that are biased and/or imprecise and a general reduction of efficiency due to reduced sample size, which in turn causes standard errors to increase, confidence intervals to widen and reduction of the power of tests of association and lack of fit.

The primary alternative to casewise deletion is imputation (i.e. estimation) of missing data. One goal of imputation is to use as much information as possible from the other non-missing predictors and outcomes. This is typically done via a regression, so that if a predictor Z is missing for some subject, a regression model of Z (used as an outcome) vs. the outcome Y (used as predictor) and another predictor, say X, is used to predict the missing Z. This procedure is repeated for all missing variables (outcomes and predictors).

Single imputation produces a single prediction for each missing data entry; however, this can produce biased estimates, and underestimate the standard errors of the model parameters. The alternative is multiple imputation, where multiple data sets are produced;

the model parameters resulting from the fit of each data set are then averaged, to produce a final “average” model.

Robust estimation of the covariance matrix

The usual estimation of standard errors of a regression model’s parameters assumes that the observations in the data are statistically independent from each other, i.e. that each data point is maximally informative. However, when this assumption is violated (for example in the case of repeated observations) the standard errors will be underestimated. To solve this problem, alternative robust estimation methods are used, which account for violation of statistical independence. Since each subject in the current thesis had repeated measurements taken in time, potential intra-subject correlation of observations was taken into account by robust estimation of the covariance matrix of the model parameters (robust sandwich variance estimator) (224).

Smoothed local polynomial regression fits

The relationship between PDA vs. NIRS, aEEG and RI were assessed using smoothed local polynomial regression fits (with 95% confidence intervals). The purpose of smoothing methods is to estimate only the trends in a relationship between a predictor X and an outcome Y, i.e. not to completely model the (possibly large) variability observed in Y. As such, confidence limits on the estimated trend (e.g. by local polynomials) will typically be relatively small compared to the observed variability of Y. This method, instead of fitting a model for the entire dataset, assesses local data structure and applies polynomial regression multiple times locally in a weighted manner to produce a smoothed overall curve. It is normal for most of the data points to be outside of the confidence limits, as the method is designed to describe only the trends of the data and not the variations. The interaction of PDA with IVH severity (severe IVH) was also included in the regressions.

Logistic regression model

A logistic regression model was designed to estimate the association between the PDA and cerebral biomarkers (including interactions of PDA with the remaining factors, which seemed relevant) and the outcome. The model specified how the observed factors impact the separation of non-severe vs. severe IVH. The construction of this model was underpinned by the biological model and study population size restricted the selection of further variables. The relationship between IVH and the other variables was assessed using logistic regression, with a backward variable selection (using Akaike's information criterion to select the factors) applied on the fitted model (224).

Nonlinearities in regression

The simplest linear regression model assumes that the relationship between an outcome Y and a predictor X is linear. However, it is possible to nonlinearly transform a predictor X so that the relationship is nonlinear (though the model will still be linear in its parameters). A simple example of nonlinear relationship is provided by taking the square of X. Such relationship can describe a non-monotonic relationship between X and Y. It is possible to devise more general transformations of X which do not have a pre-defined shape (to the contrary of the fixed parabolic shape of X^2). An example of these more general transformations is provided by restricted cubic splines, i.e. local cubic polynomials for details (224).

Validation of classification models

The model was assessed both in terms of discrimination and calibration accuracy using bootstrapping techniques. The validation of a classification model (e.g. a logistic regression model) in terms of discrimination and calibration is an important element of the statistical analysis. Discrimination provides information whether a model correctly ranks individuals according to their risk (e.g. risk of IVH). Calibration provides information whether the model

accurately predicts a risk. Discrimination is measured by the C-index and it is the percentage of subjects correctly ranked according to their risk. Calibration is measured both by the shrinkage coefficient which is an estimate of overfitting and graphically by comparing predicted and observed risks (expressed as probabilities). Ideally, the predicted and observed risks should be equal. However, this is not always the case due to the limited sample size and possible model inadequacies. Moreover, the estimation of the shrinkage coefficient provides information whether the model may overfit the data, so the apparent calibration may be optimistic and biased. Therefore, it is useful to estimate a bias-corrected calibration relationship, where the bias is estimated by bootstrapping.

Backward logistic regression model

To assess whether inotropic support and sepsis had a significant effect on the interaction of the biomarkers of the original model (PDA diameter, cTOI, Burdjalov score, ACA RI) with the evolution of severe IVH, a backward logistic regression model was built in SPSS taking into consideration the biomarkers on day 3 after birth, which was the time point with the maximum number of observations and by when all the cases of severe IVH had occurred. A second backward logistic regression model was built substituting PDA score for PDA diameter. The default options for the backward regression model were used.

$MgSO_4$ specific methodology

This was a prospective convenience observational study recruiting infants 24-28+6 weeks' gestation and postnatal age ≤72 hours. The same cohort of patients as described in Chapter 3 was investigated.

Data collection

Data collection was limited to the first three days after birth and described in detail in Chapter 2 and Appendix 1. In addition, maternal data included: age; BMI; medicines (including steroids); indication and duration of $MgSO_4$ administration; reason for not receiving $MgSO_4$. Cerebral injury was defined by cranial ultrasonography and dichotomised into "mild" (IVH grade 0-2) or "severe brain injury" (IVH grade 3 or 4) according to Papile criteria (15).

Echocardiography

Echocardiographic assessment was described in detail in Chapter 2 (page 88). PDA score was used in this analysis instead of PDA diameter. PDA score was used in this analysis instead of PDA diameter. PDA score is a composite score generated by measuring ten echocardiographic biomarkers related to features of the ductal size, magnitude of the ductal shunt and myocardial performance and was previously described in Chapter 2.

aEEG and NIRS

Recordings were performed for four hours on each of the first three days after birth and previously described in Chapter 2 (page 82). The Burdjalov score was used to assess the aEEG trace. NIRS methodology was previously described.

Serum magnesium

Daily samples were obtained as part of routine daily clinical care. Neonatal Mg^{2+} was analysed by enzymatic assay (isocitrate dehydrogenase) on the Architect ci8200 System platform (Abbott laboratories, London, UK). Parenteral magnesium supplementation was

commenced on the third day after birth, so measurements reported for this study were not affected by postnatal magnesium supplementation. Blood samples for biochemistry were usually obtained between 06:00 and 09:00. Hence, there was not significant clinical delay between blood sampling and specific neonatal biomarker measurement.

Statistical analysis

The hypotheses for this chapter were defined after discussion with colleagues in Obstetrics. Statistical analysis was performed using the R statistical package and the rms library. Demographic factors were assessed using descriptive statistics. A linear multivariate regression model was fitted. The model linearly regressed neonatal serum Mg^{2+} against maternal $MgSO_4$ administration, maternal BMI and neonatal IVH severity, BW, cTOI, PDA score, Burdjalov score and the interaction of maternal $MgSO_4$ administration with the above covariates. Due to repeated measurements, intra-subject correlation of observations was a possibility and estimation of the covariance matrix of the model parameters was performed using Huber's nonparametric "sandwich" estimator (224).

There were 92 observations (of the potential 153) of which only 62 had a complete set of observations due to clinical reasons (delayed or no blood sampling, too clinically unwell to tolerate echocardiography or cerebral monitoring, some babies were not recruited on the first day of life and some babies had died by day three). Because of these considerations, a conditional multiple imputation algorithm was applied (Bayesian Alternating Conditional Expectations) (21). The imputation process was repeated 10 times, resulting in 10 different realisations of the data set. Based on the imputed data sets, 10 models were fitted and the parameters averaged to obtain the final model parameters.

Following model fit, graphical assessment of the relationship between neonatal Mg^{2+} levels (as a function of time) and $MgSO_4$ administration and BMI (dichotomised according to the median for ease of visualisation) was performed using robust nonparametric regressions by local second degree polynomials fitting (224)(21). Point-wise 95% confidence intervals were also calculated. The same technique was used to graphically assess the interaction between neonatal Mg^{2+} levels and $MgSO_4$ infusion duration for the subset of the data represented by those mothers who were administered $MgSO_4$.

Pulse transit time, pulse wave velocity and pulse phase difference methodology

Population

This was a nested cohort study within the previously described observational study looking for an interaction between PDA and biomarkers of aortic haemodynamics. Patients from the same cohort as in Chapter 3 were included if they had an umbilical artery catheter inserted for clinical reasons and their continuous physiological data were captured with IxTrend software (Ixellence, Wildau Germany) (Figure 5).

Echocardiographic and cerebral biomarkers

NIRS, aEEG and echocardiography methods were previously described in Chapter 2. A PDA with diameter >2mm was considered large in this analysis.

Clinical physiological monitoring

ECG, BP and SpO2 were monitored for clinical reasons in accordance with standard neonatal intensive care using Philips IntelliVue MX800 patient monitors (Philips Healthcare, UK). The data were recorded from the monitors by interfacing with a laptop via Bluetooth using IxTrend software (Ixellence GmbH, Wildau, Germany).

Invasive BP monitoring

The majority of extremely preterm infants on our NICU have invasive BP monitoring in the first days after birth for accurate measurement of BP. Invasive BP data were only captured when BP monitoring was clinically indicated. An umbilical arterial catheter (3.5F) was inserted soon after birth by the clinical team. The catheter (Vygon, Swindon, UK) was positioned between the sixth and tenth thoracic vertebra and connected to an electronic pressure transducer via a 38cm long rigid plastic extension catheter tubing. The distance from the BP transducer was thus made up of 37cm (umbilical artery catheter length) + 38cm

(extension length) giving a total of 75cm distance. The ECG and BP waveforms on the monitor screen were visually assessed to ensure good quality signal. Indicators of poor BP signal quality were an abnormal shape waveform (indicating dampening with air in the circuit between the UAC and the blood pressure transducer) or a low pulse pressure (indicating partially blocked UAC); in addition, the positioning of the BP transducer was checked to be at the level of the heart which is important for avoiding incorrect BP readings e.g. apparent low BP. A low pass filter cut-off frequency at 12Hz was applied by the monitor to the BP waveform and the output was exported with a sampling frequency of 125Hz.

Electrocardiogram

ECG was obtained through three electrodes attached to the infants' chest and left thigh forming the Einthoven triangle. Lead I was selected for the monitoring. ECG and BP monitoring data were downloaded from IntelliVue MX800 monitors and stored as spreadsheet files on a laptop via a wireless connection using IxTrend software. The sampling frequency for the ECG waveform was 500Hz and the filter passband was 0.5Hz to 55Hz. Recordings were performed while the infants were asleep and they were not disturbed throughout the duration of the recording. Recordings preceded echocardiography and performed simultaneously with EEG and NIRS, if possible. Otherwise, recordings were done as close to echocardiography as possible. Of note, this procedure did not cause any disturbance to the infant as it only involved recording data being acquired as part of standard monitoring.

Physiological signal data analysis

Professor David Wertheim (School of Computer Science and Mathematics, Kingston University, London) developed a software using MATLAB (The MathWorks Inc., Natick, MA, USA) to read, display and analyse the spreadsheet files. PTT was defined as the difference in

time between the ECG R wave and the following systolic BP wave peak (Figure 6). The systolic BP wave peak was used for analysis as this is easier to define precisely compared with the midpoint of the diastolic to systolic change; precise identification is important because of the higher HR in preterm neonates compared to adults.

In order to determine the PWV it was necessary to know the distance along the blood vessels from the aortic arch to the umbilical artery catheter tip. As the catheter tube connecting to the pressure transducer is rigid, it was assumed that this would have negligible effect on the calculation of PWV. The catheter position is always confirmed by X-ray imaging as per clinical protocol. The aortic valve is radio translucent and there is no clear landmark to identify it. The relation of the aortic valve to vertebral level varies and the average position, according to Eycleshymer and Schoemaker, is at the level of the middle third of the seventh thoracic vertebra (225). There are no corresponding data for preterm babies. Therefore, I reviewed with Dr Kaleem Mussa (Consultant Paediatric Radiologist, Alder Hey Children's Hospital, Liverpool, UK) the CT scans of ten term infants with underlying cardiac conditions. The relation of the aortic valve to the vertebral level varied, but the median observation was the 6^{th} thoracic vertebra (varied between 4^{th} and 7^{th} thoracic vertebra). Using the 6^{th} thoracic vertebra as a reference landmark for the aortic valve, the distance of the catheter tip from the aortic valve could be traced using PACS software (Phillips, London, UK).

As PTT and PWV are affected by HR (226), a novel variable (PD) was devised to take into account the variable HR. PD was calculated as follows: the difference in time between the ECG R wave and the following systolic BP wave peak was measured and expressed as phase, i.e. the proportion of one cardiac cycle expressed in degrees where one cycle is 360^{O} (Figure 7). The cardiac cycle duration length (L) was measured from the ECG as the distance

between the peaks of two consecutive R waves. PTT was measured as the time difference between the ECG R wave and the following BP systolic peak. The ratio PTT:L gives a dimensionless index that is HR independent and can be expressed as PD from 0 to 360° i.e. the PD= (PTT/L)*360°. PD was calculated by Prof David Wertheim. Breathing can cause beat to beat variability of the HR and hence the mean PD of two consecutive cardiac cycles was used for analysis.

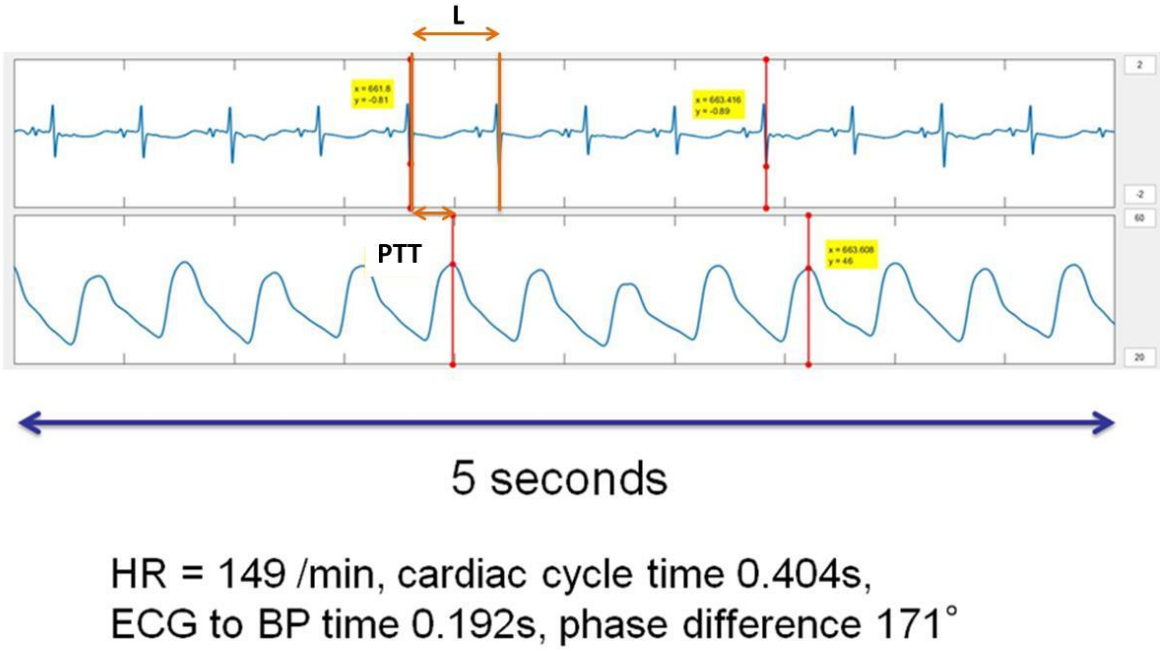

Figure 7: Cardiac cycle length (L) is measured from the ECG as well as the time difference PTT between the ECG R wave and the following BP systolic peak. The ratio PTT:L gives a dimensionless index that is HR independent and can be expressed as PD.

Statistical analysis

I defined the hypotheses for this chapter in discussion with Prof. Wertheim. Statistical analysis was performed using SPSS 25.0 (IBM, Chicago, IL, USA). Data were tested for consistency with a normal distribution and parametric or non-parametric statistics used as appropriate. Median and interquartile range were used to describe summary demographics. A Bland–Altman plot was used to assess the intra-patient repeatability of PD from two cardiac cycles (227)(13). The Spearman rank correlation coefficient was used to assess the correlation between aortic biomarkers and other continuous variables. Mann Whitney test was used to compare categories. Bonferroni correction was applied for multiple comparisons (twelve parameters as in Table 24) and statistical significance was set on P-value $<0.05/12=0.0042$.

Chapter 3: Patent ductus arteriosus, cerebral haemodynamics and intraventricular haemorrhage in preterm neonates: an investigation of a possible causal pathway

Introduction

This chapter describes the multimodal assessment of the relationships between PDA shunt, selected cerebral physiological measurements and an imaging biomarker of significant brain injury to meet objectives I and II in Chapter 1 (page 69). This chapter also describes the cohort study that underpins subsequent chapters.

Results

Data collection and demographics

Parents of seventy-nine extremely preterm neonates were approached for enrolment in the study. Fifty-two neonates were recruited (four twin pregnancies) with median GA 26.6 weeks [Interquartile range (IQR) (25.8-28.1)] and median BW 910 grams [IQR 0.91 (780-1,030)] (Table 7). Of the fifty-two, eight were twins. 268 observations were performed; (Figure 8). A complete dataset would have comprised 69 additional observations during the transitional circulation and observed ductal closure in the first two weeks after birth. Initially the plan was to discontinue the observations once the PDA closed. However, it became apparent that this would not allow a comparison group. Therefore, the protocol was amended after the recruitment of patient PDA012 to allow data collection from patients in whom the PDA had closed during the first two weeks.

Due to technical reasons or the critical condition of the infants, observations were not performed on nine occasions (3%) for PDA assessment, eleven (4%) for cTOI, 57 (21%) for RI and 15 (6%) for aEEG (Table 6). The most commonly missed biomarker was the RI (21% of the assessments) due to the presence of the hat used for the endotracheal tube stabilisation, the limited space for manoeuvres inside the cot, and the critical condition of some infants which did not allow a lot of handling. These constraints made it difficult to obtain the ideal Doppler alignment with the ACA which has a curved pathway around the

corpus callosum. Data were also lost when the ultrasound machine was reconfigured and stored data was lost. This was the main reason for the missed echocardiographic studies (3%). The critical condition of the patient, which made the placement of the relevant equipment challenging, were the main reasons for most of the missed NIRS and aEEG data.

Twenty-five patients had weekly assessments made until discharge from LWH, 29 patients were monitored until their PDA closed (median age of closure (days) 14, mean 28, minimum 2, maximum 105). Fourteen infants were transferred back to Level 2 neonatal units, where they came from, once they did not need intensive care. This was the main reason for missed observations beyond the first week after birth (Figure 8). The median number of occasions that each patient was assessed was five.

Table 7 summarises the maternal and perinatal demographics and their comparison with local (LWH), national and international populations when possible. Maternal age, alcohol consumption, use of antenatal steroids and $MgSO_4$ and incidence of IUGR were similar to the local neonatal population. Maternal $MgSO_4$ use was higher than national and international rates. The reported incidence of smoking was lower in our population compared to the local population. Pregnancy induced hypertension was higher in our population compared to local and national populations, but similar to European population. The incidence of offensive liquor and/or histological chorioamnionitis was high, but in line with the local, national and international populations. Only the incidence of histological chorioamnionitis was available for national and international populations. GA at birth, birth weight, mode of delivery, APGAR scores at 1^{st} and 5^{th} minute and cord gases values were all similar to the local population with no available data from national and international

populations. More babies in our cohort needed advanced resuscitation compared to the local population.

Variable	PDA	cTOI	RI	aEEG
Number missing	9 (3%)	11 (4%)	57 (21%)	15 (6%)

Table 6: Data completeness.

PDA: PDA diameter, cTOI: cerebral tissue oxygenation index, RI: anterior cerebral artery resistive index, aEEG: amplitude integrated EEG.

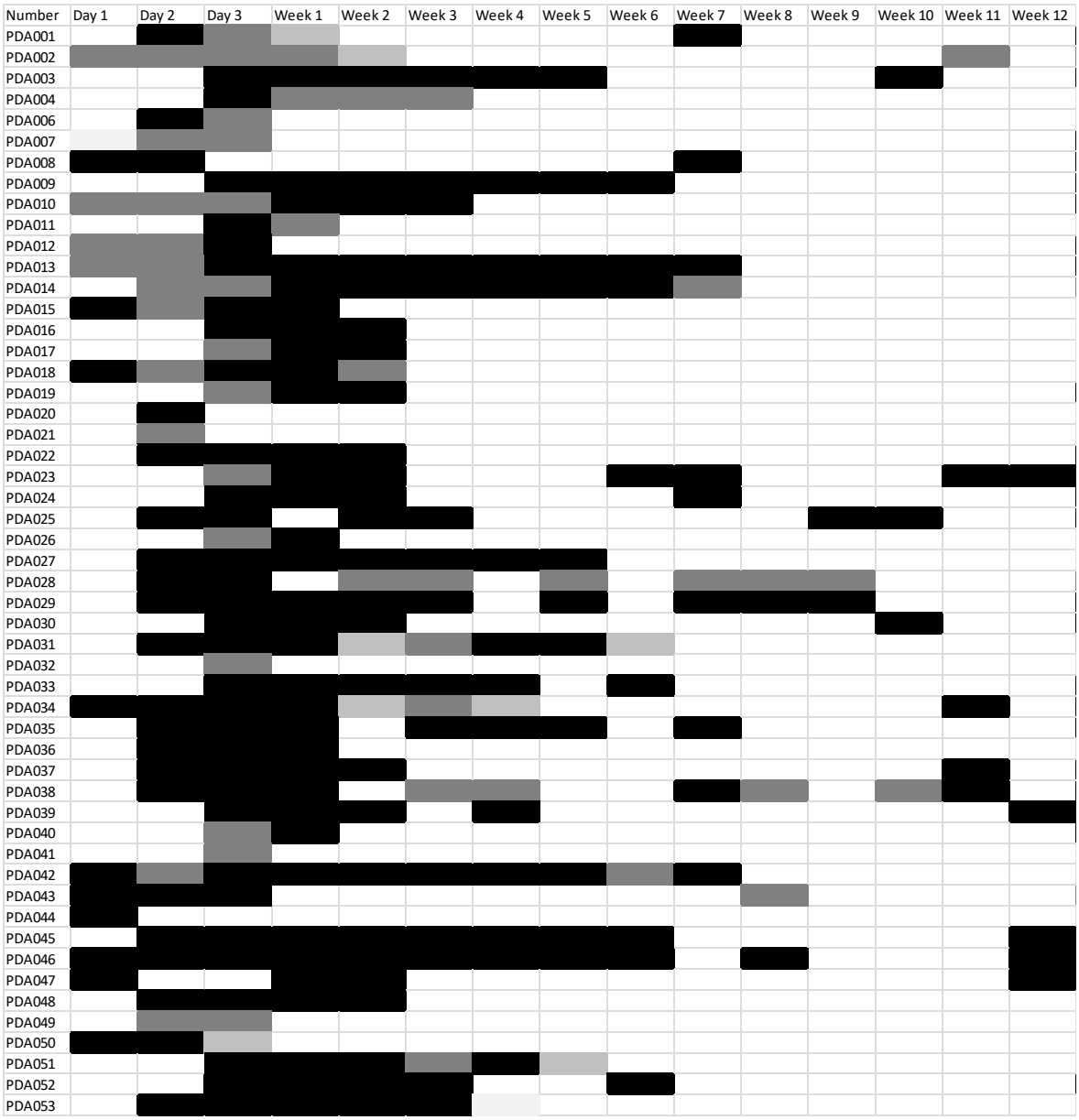

Figure 8: Visual representation of data collection and completeness.

The table columns were restricted to 12 weeks for better visualisation. Three patients had additional assessments which are represented as discharge assessment: PDA007 at week 15, PDA023: week 15 and week 16 and PDA040: week 14.

: all four assessments complete, : three assessments, : two assessments, : one assessment and : no assessment.

Table 8 summarises the incidence of the different clinical outcomes in my cohort and their comparison with LWH, national and international populations. Six infants (12%) developed severe IVH and nine died (17%). The median age of death was 21 days and the main reason was late onset sepsis. Inotropic support was needed at least once in 25 of them (48%). Eight infants (15%) needed PDA treatment with ibuprofen and one finally underwent PDA ligation. The incidence of severe IVH in my cohort, which was the primary focus of this thesis, was similar to the LWH, national and international populations. There were higher BPD rates compared to national and international populations, but similar to LWH population. Death rates were similar to national and international populations, but slightly lower than LWH population. The incidence of sepsis was similar to the European population, but higher than LWH and national populations. There was higher use of inotropes in my cohort (48%) compared to the local population (42/145, 29%).

Maternal demographics					
	HAPI-PDA study		LWH	Europe group	UK group
	Median/N	IQR/%			
Maternal weight (kg)	74	34			
Maternal BMI	26.2	9			
Maternal age	31	7	30 (7)		
Alcohol during pregnancy (Yes/No)	2	4%	5.6		
Smoking during pregnancy (Yes/No)	6	12%	25%		
Hypertension during pregnancy (Yes/No)	8	15%	6.6%	16.2%	11.7%
Chorioamnionitis/offensive liquor	16	31%	Offensive liquor 26.3% Chorioamnionitis 9.4%	- 23.4%	- 14.4%
PROM	24	46%			
Perinatal antibiotics	36	69%			
Indomethacin	5	10%			
Atosiban	5	10%			
$MgSO_4$	34	65%	62.1%	39.6%	57.3%
IUGR	5	10%	8.6%	11.2%	9.5%
Steroids					
Complete course	39	75%	84.8%		
Any dose	50	96%	92.9%	90.1%	92%
Perinatal demographics (N = 52)					
GA (weeks)	26.6	2.3	26.6 (2.4)		
Weight (gr)	910	250	880 (268)		
Mode of delivery					
SVD	27	52%	47%		
Emergency caesarean section	21	40%	48%		
Elective caesarean section	2	4%	3%		
Instrumental	1	2%	2%		
Advanced resuscitation	19	37%	10%		
Cord pH	7.31	0.10	7.3 (0.11)		
Cord base excess	-3.75	4.55	-2.8 (4.7)		
APGAR 1 min	5	4	5 (4)		
APGAR 5 min	7	3	7 (4)		

Advanced resuscitation: need for cardiac massage and/or adrenaline, BMI: body mass index, PROM: prolonged rupture of membranes, SVD: spontaneous vaginal delivery, IUGR: intrauterine growth restriction, N: number of patients. Data presented as number of patients or median and IQR or percentage as appropriate.

Table 7: Summary of the background maternal and perinatal demographics with comparison to local, national and international data. LWH: Liverpool Women's Hospital

Clinical outcomes	HAPI-PDA study	LWH	Europe group	UK group
Severe IVH	6 (12%)	12%	11.3%	11.2%
Death	9 (17%)	24.3%	17.9 %	17.2%
Death and/or BPD2	33 (64%)			
BPD2	27 (52%)	52.6%	28.1%	44.1%
BPD1	36 (69%)			
ROP	20 (38%)			
Sepsis	24 (46%)	32.2%	46.4%	35.8%
NEC	6 (12%)	8.1%	6.2%	6.4%
PDA treatment **Ibuprofen** **PDA ligation**	9 (17%) 8 (15%) 1 (2%)			

Table 8: Summary of the clinical outcomes with comparison to local, national and international data. LWH: Liverpool Women's Hospital

Causes of death in the study population		
Patient	**Age (days)**	**Cause**
04	33	CONS sepsis
06	30	Fungal sepsis
15	18	CONS sepsis
20	6	Respiratory failure
32	21	Fungal sepsis
36	22	Staph aureus sepsis
47	18	Severe RDS
49	43	Sepsis
50	10	Respiratory failure

Table 9: Cause of death of the individual study participants according to the discharge letter.

CONS: coagulase negative staphylococcus.

Because it is preferable to impute rather than discard data and there were missing data at different time points, statistical advice was taken from Dr Antonio Eleuteri and further statistical analysis was performed as described in Chapter 2 (page 103). A multiple imputation procedure was used (additive regression, bootstrapping and predictive mean matching) (224). Furthermore, since each subject had repeated measurements taken in time, potential intra-subject correlation of observations was taken into account by robust estimation of the covariance matrix of the model parameters (224). All the calculations were performed using the R statistical package and the rms library.

Cerebral markers vs. postnatal age

The effect of postnatal maturation on the biomarkers of interest was assessed. Figures 9-12 shows a scatter graph with local polynomial regression of the biomarkers of interest vs. postnatal age (weeks from birth). Each patient contributed variable amounts of data as indicated in Figure 8 and patients with persistent PDA are overrepresented after the second week as there is a continuous attrition of patient with closed PDA whom I stopped monitoring as per protocol. As patients had different gestational ages at birth, each time point consists of patients of the same postnatal age, but different corrected gestation age.

Inspection of Figure 9 suggests that PDA diameter decreases over time as expected. There is large variation of the observed values. cTOI decreases with maturation (Figure 10). Low values (cTOI<55) were observed up to the eighth week. RI initially increased with maturation, but following the sixth week it started decreasing (Figure 11). Only one infant in our cohort had reversed end diastolic flow in the ACA. Burdjalov score increased exponentially during the first two weeks after birth and there was slowing of the increase rate on subsequent weeks up to week ten when a plateau was reached (Figure 12).

Generally, there was large variation of all the aforementioned biomarkers. Participants with IVH had no difference from participants without IVH except with respect to the Burdjalov score.

All cases of IVH occurred during the first three days after birth. However, I was unable to determine the exact timing due to the methodological limitations and the fact that some patients were recruited on third day after birth. To assess further whether patients who developed severe IVH had different biomarker trajectory during the transitional period and the subsequently weeks, separate smoothed local polynomial regression fits were created for patients with and without severe IVH (Figures 13-17). Infants with severe IVH had higher PDA score from birth to around fourth week with confidence intervals of the two populations not overlapping (Figure 13). Following the fourth week there was a regression towards the mean with confidence intervals overlapping each other. Infants with severe IVH had overall larger PDA diameter and this became more apparent following the second postnatal week, when there was no overlap of the confidence intervals (Figure 14). Infants with severe IVH had lower Burdjalov score from birth until the third postnatal week with regression towards the mean the following weeks (Figure 15). Infants with severe IVH had overall higher RI, but confidence intervals of the two groups were overlapping apart from postnatal weeks 4 and 5 (Figure 16). Infants with severe IVH had lower cTOI from birth until the sixth postnatal week with regression towards the mean and overlapping confidence intervals the following weeks (Figure 17). The confidence intervals of the severe IVH group were wide due to number of patients contributing data (N=5).

Subsequently, I assessed whether there was a statistically significant difference of the relevant biomarkers between patients with and without severe IVH during the transitional

period by performing Mann Whitney test for each of the first three days after birth. Table 10 summarises the results. Differences became more prominent on Day 3 after birth. Babies with severe IVH had higher PDA score (median (IQR), P-value: 17 (4.5) vs. 11 (8.5), 0.044), lower cTOI (58.9 (10) vs. 68.7 (9.9), <0.001) and Burdjalov score (2 (3) vs. 6 (4), 0.010). However, following Bonferroni correction only the difference of cTOI between patients with and without severe IVH remained statistically significant. There were only three babies with severe IVH in the first day after birth which restricted statistical analysis.

In summary, patients with severe IVH had different course of the studied biomarkers from the first days after birth indicating possibly different underlying pathophysiology. PDA score had slightly better ability compared to PDA diameter to separate patients with severe vs. non severe IVH. The time point analysis during the transitional period demonstrated that only cTOI was statistically different in patients with severe IVH on the third day after birth.

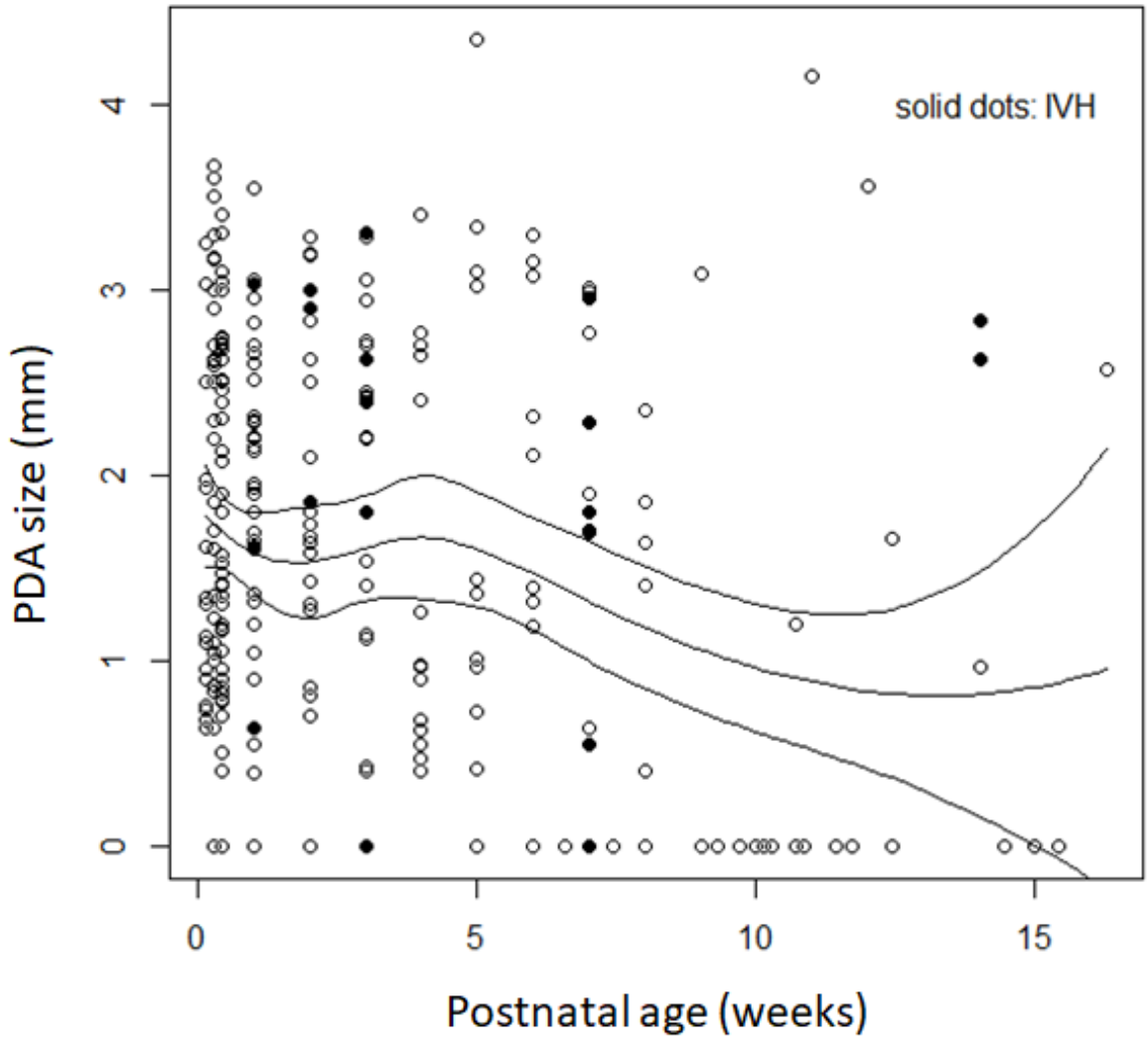

Figure 9: Smoothed local polynomial regression fits (with 95% confidence intervals of the PDA size changes from birth to discharge.

Solid dots represent patients with severe IVH (Grade 3 and 4). PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

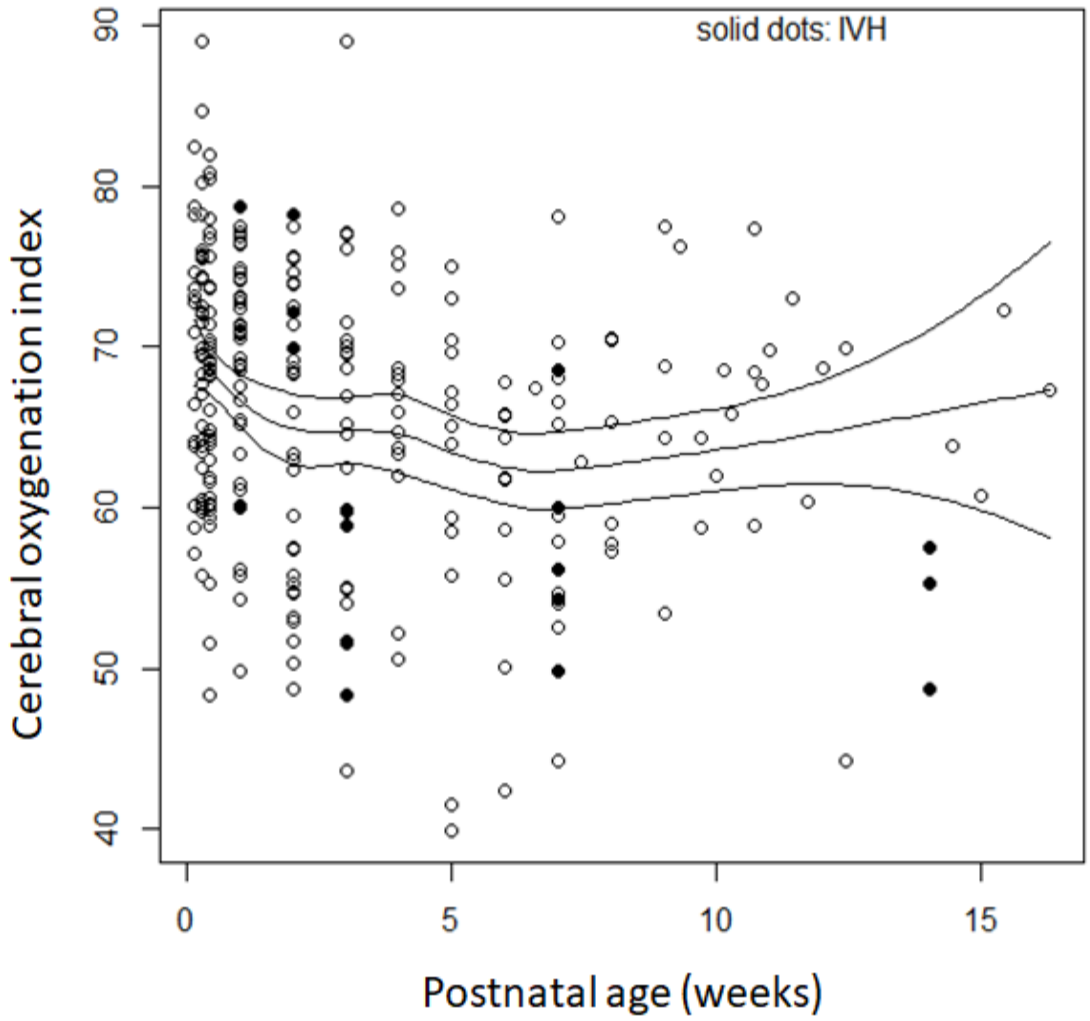

Figure 10: Smoothed local polynomial regression fits (with 95% confidence intervals) of the cerebral tissue oxygenation index changes from birth to discharge.

Solid dots represent patients with severe IVH (Grade 3 and 4). IVH: intraventricular haemorrhage.

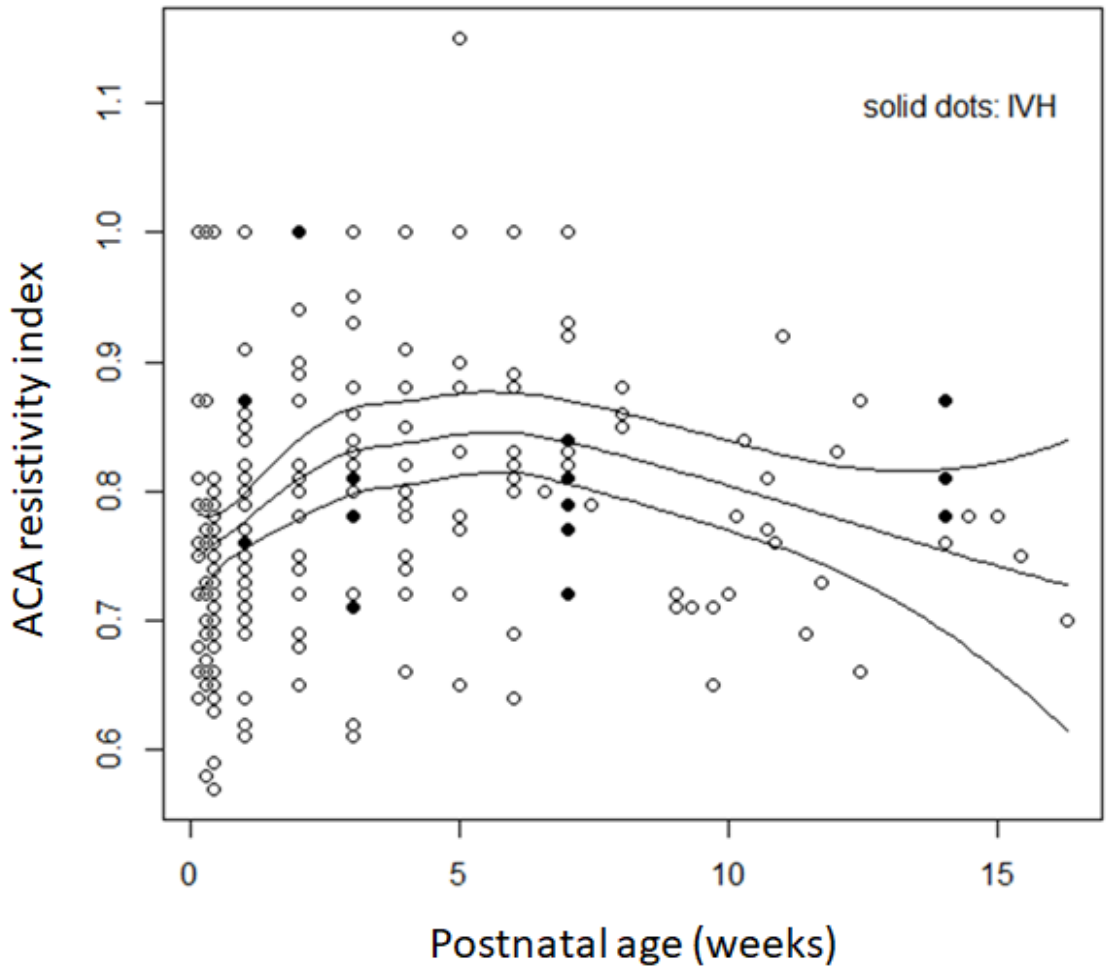

Figure 11: Smoothed local polynomial regression fits (with 95% confidence intervals) of the anterior cerebral artery resistivity index changes from birth to discharge.

Solid dots represent patients with severe IVH (Grade 3 and 4). ACA: anterior cerebral artery, IVH: intraventricular haemorrhage.

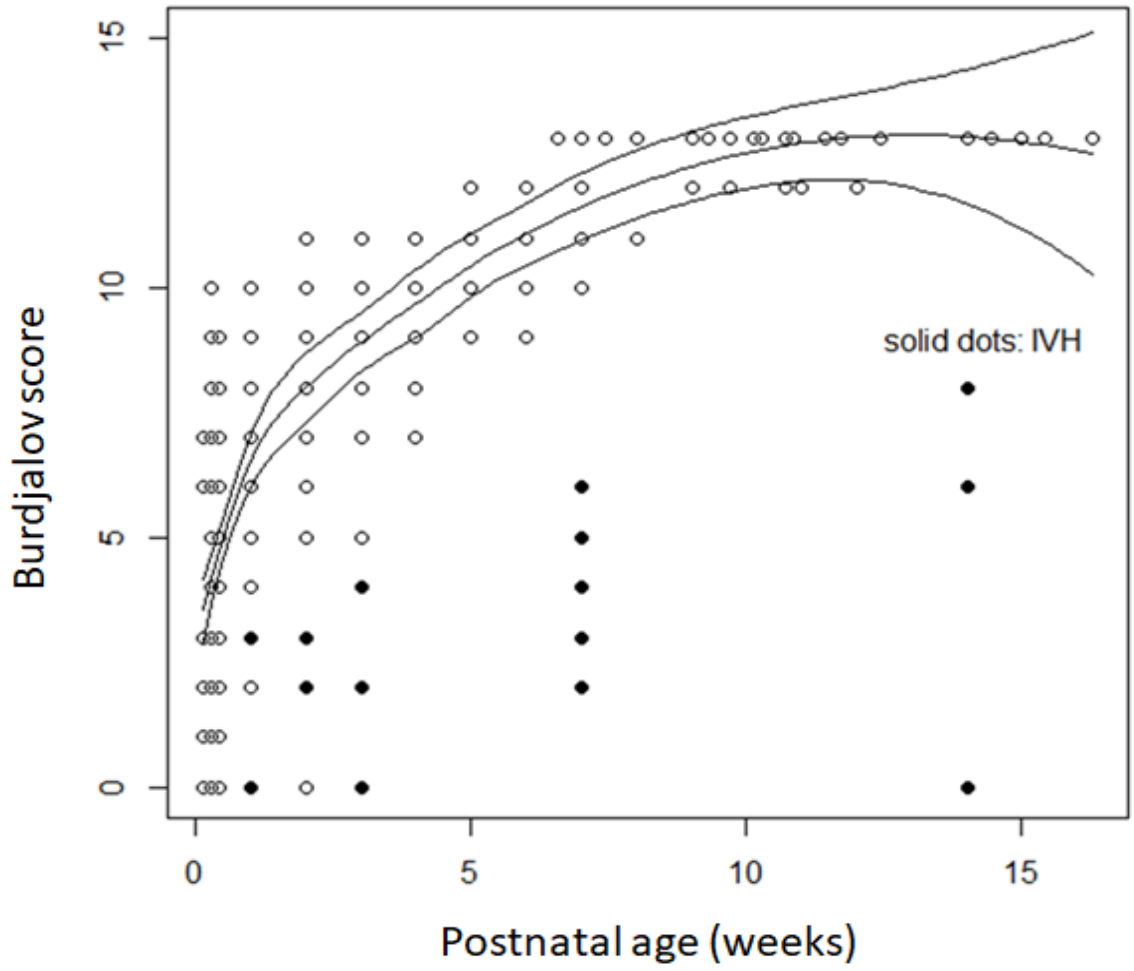

Figure 12: Smoothed local polynomial regression fits (with 95% confidence intervals) of the Burdjalov score changes from birth to discharge.

Solid dots represent patients with severe IVH (Grade 3 and 4). IVH: intraventricular haemorrhage.

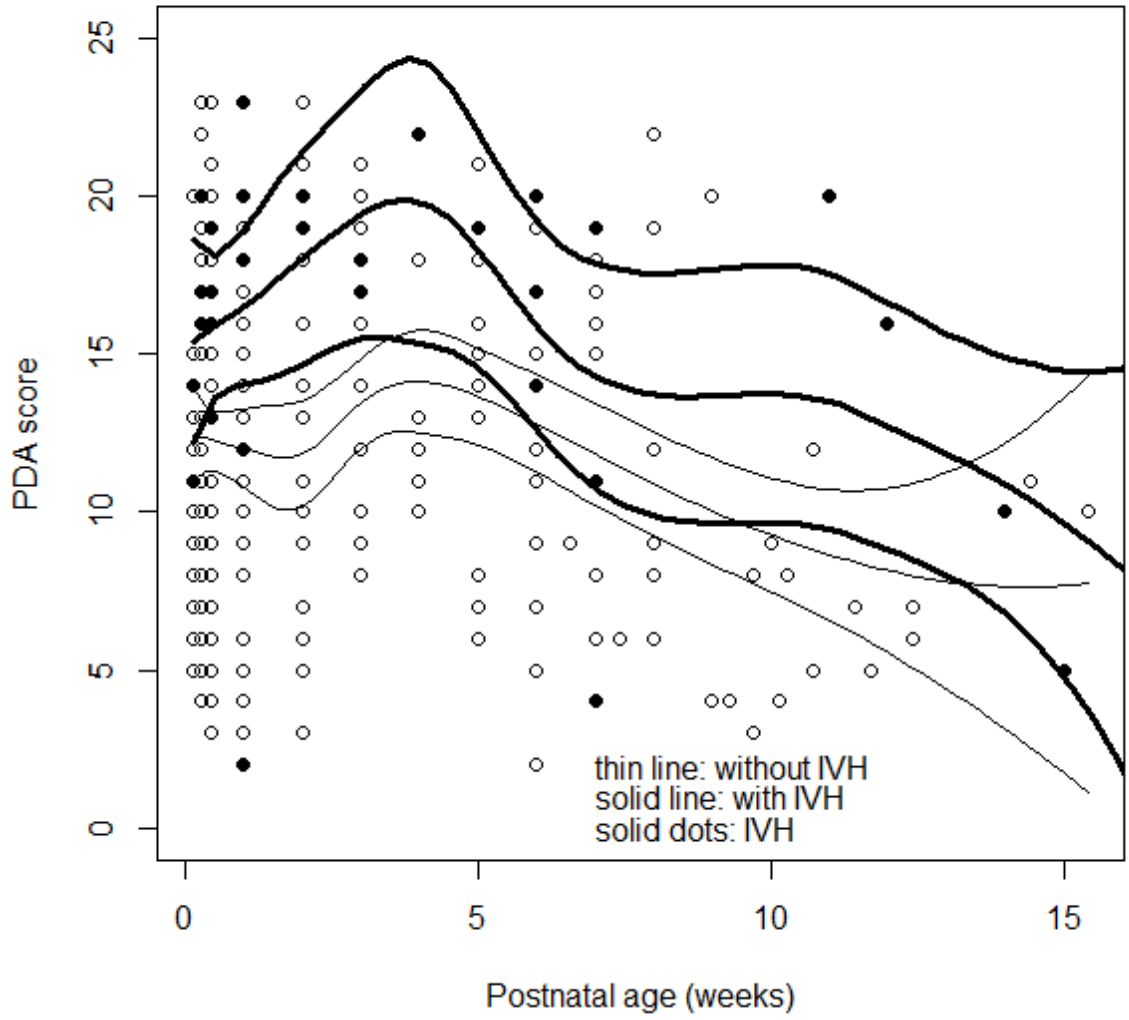

Figure 13: Smoothed local polynomial regression fits (with 95% confidence intervals) of postnatal natural evolution of PDA score in patients with and without severe IVH.

Solid dots and lines represent patients with severe IVH. PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

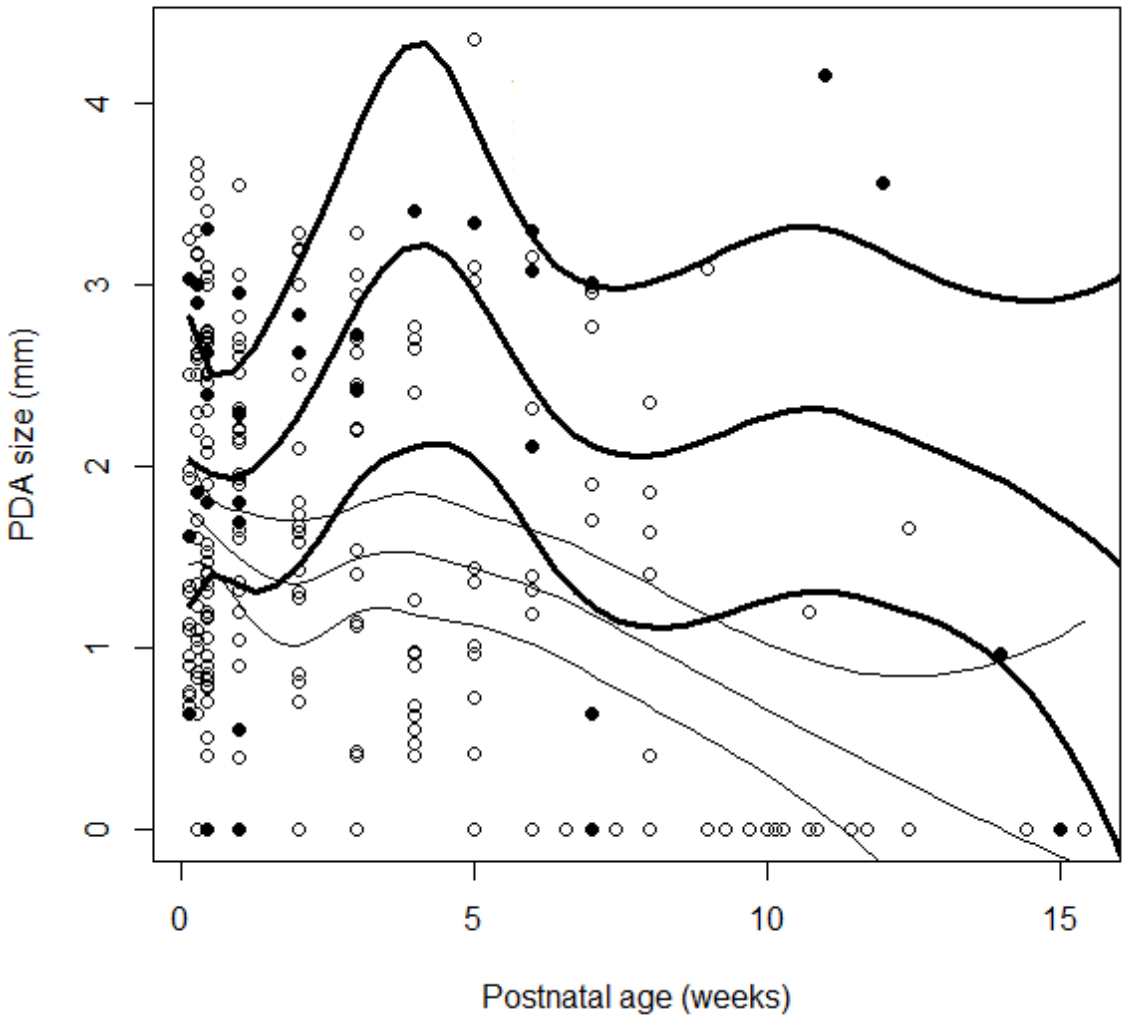

Figure 14: Smoothed local polynomial regression fits (with 95% confidence intervals) of postnatal natural evolution of PDA diameter in patients with and without severe IVH.

Solid dots and lines represent patients with severe IVH. PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

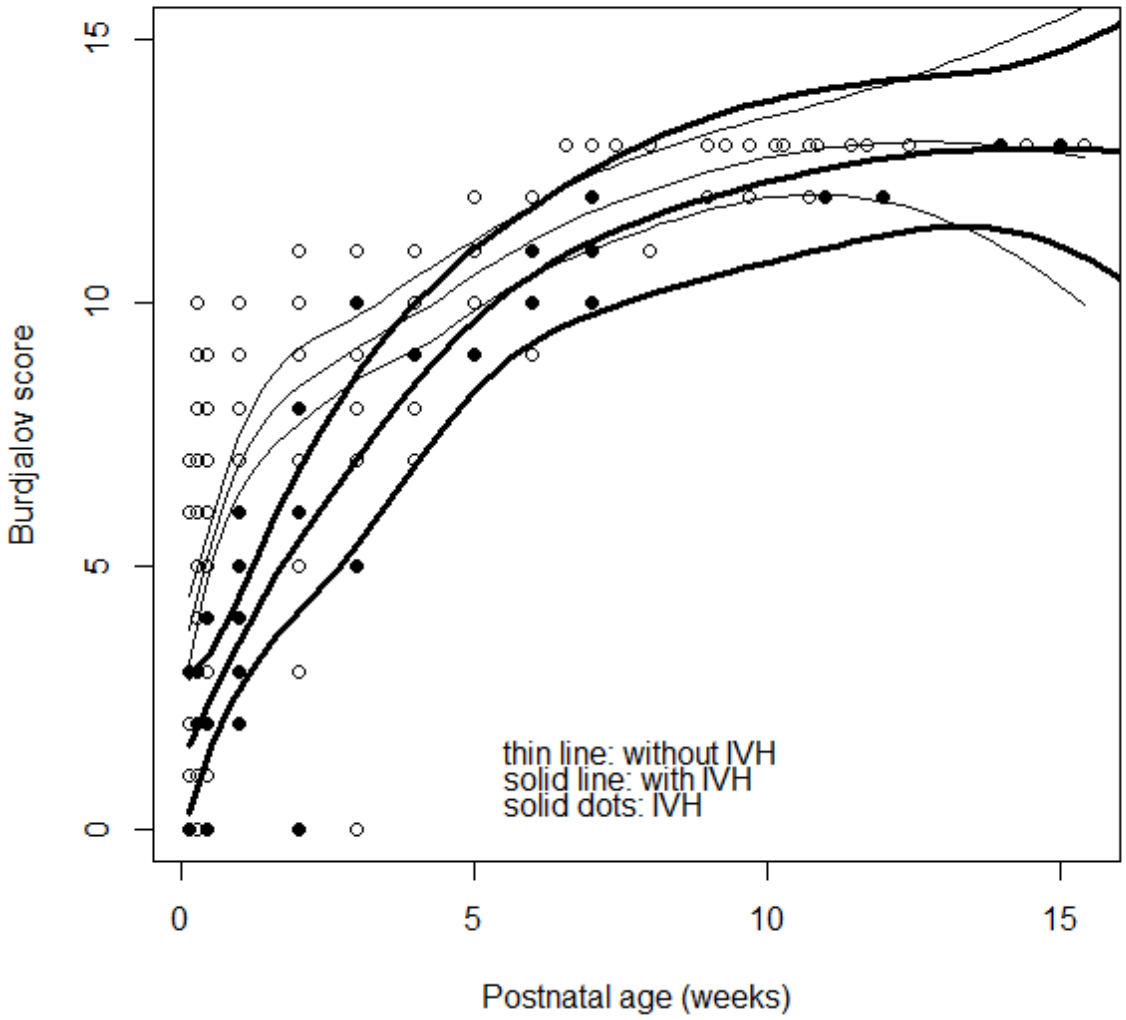

Figure 15: Smoothed local polynomial regression fits (with 95% confidence intervals) of postnatal natural evolution of Burdjalov score in patients with and without severe IVH.

Solid dots and lines represent patients with severe IVH. IVH: intraventricular haemorrhage.

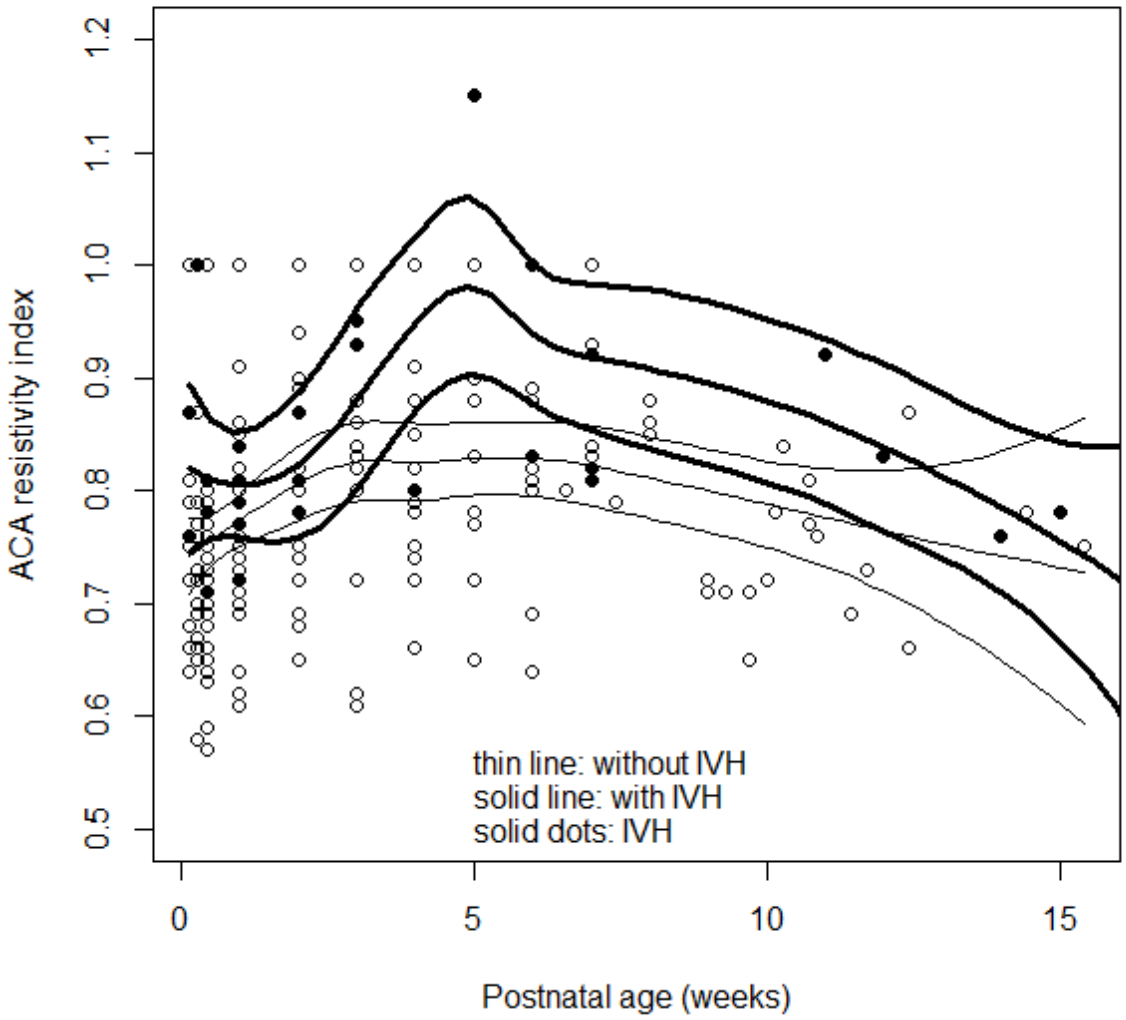

Figure 16: Smoothed local polynomial regression fits (with 95% confidence intervals) of postnatal natural evolution of anterior cerebral artery resistivity index in patients with and without severe IVH.

Solid dots and lines represent patients with severe IVH. ACA: anterior cerebral artery, IVH: intraventricular haemorrhage.

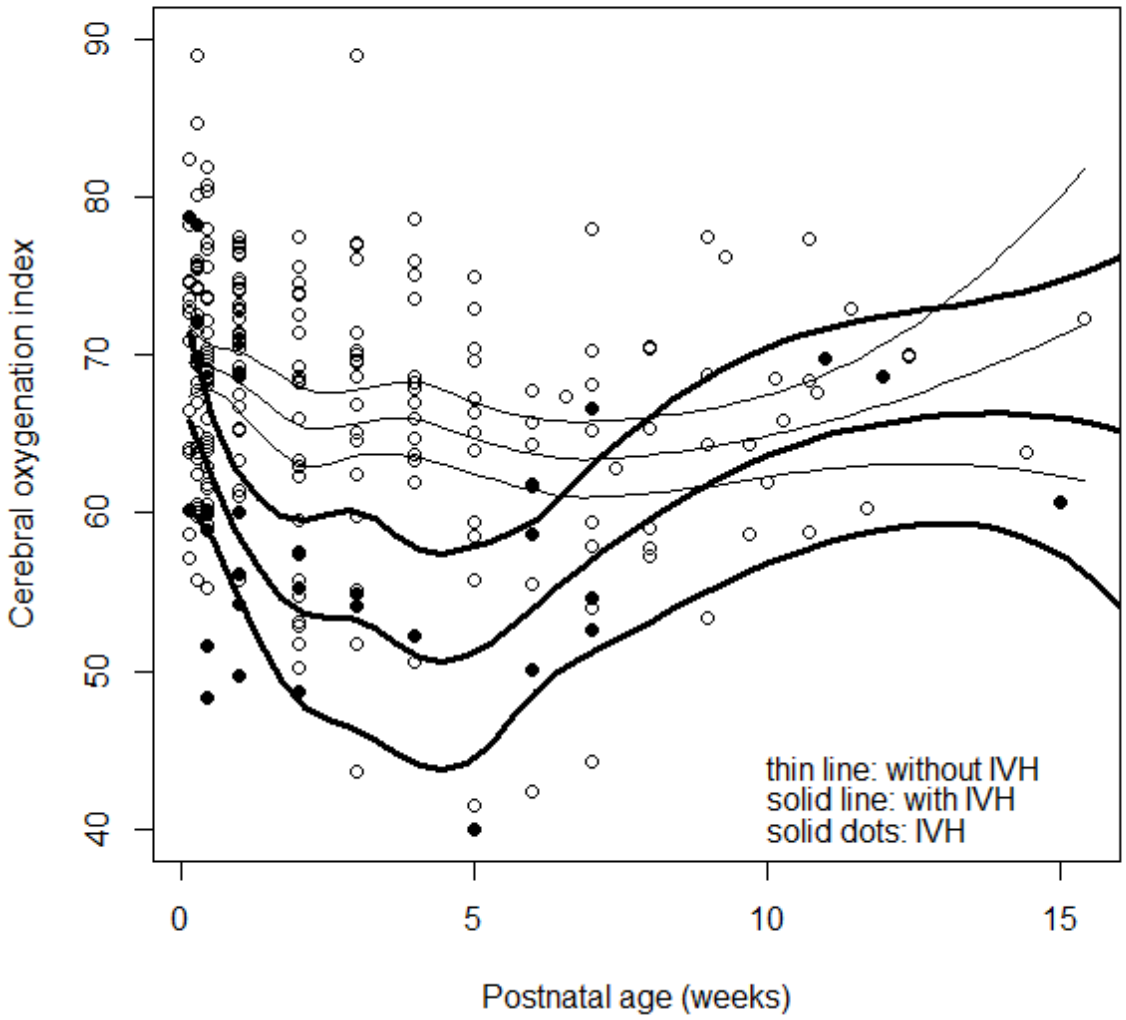

Figure 17: Smoothed local polynomial regression fits (with 95% confidence intervals) of postnatal natural evolution of cerebral oxygenation index in patients with and without severe IVH.

Solid dots and lines represent patients with severe IVH. IVH: intraventricular haemorrhage.

Infants with severe IVH had lower cTOI from birth until the sixth postnatal week.

			PDA diameter (mm)		PDA score		cTOI		Burdjalov score		ACA RI	
Day 1 (N=17)	Severe	No	1.1 (1.2)	0.90	11 (6)	0.16	70 (10.7)	0.95	3 (5)	0.15	0.73 (0.2)	0.39
	IVH	Yes	-		-		-		-		-	
Day 2 (N=31)	Severe	No	1.95 (1.7)	0.29	12 (11)	0.24	68.8 (11.6)	0.26	4.5 (5)	0.32	0.73 (0.19)	0.18
	IVH	Yes	2.9 (2.1)		17 (4)		72.2 (8.3)		2 (1)		-	
Day 3 (N=46)	Severe	No	1.42 (1.7)	0.28	11 (8.5)	0.044	**68.7 (9.9)**	**<0.001**	6 (4)	0.010	0.71 (0.05)	0.18
	IVH	Yes	2.39 (2.5)		17 (4.5)		**58.9 (10)**		2 (3)		0.71 (0.09)	

Table 10 Assessment of the studied biomarkers in patients with severe vs. non severe IVH during the transitional period. There were only three babies with severe IVH in the first day after birth which restricted statistical analysis. Data are presented as median and interquartile ranges and the corresponding P values (Mann Whitney test).

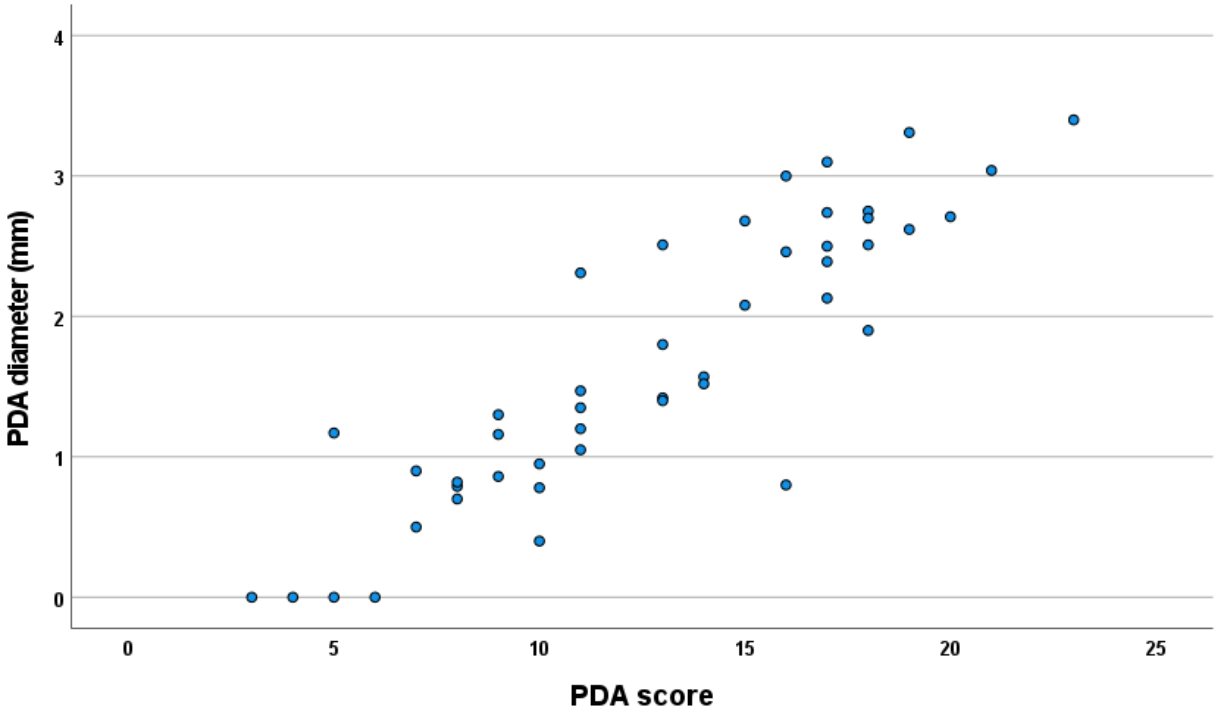

Figure 18: Correlation between PDA diameter and PDA score in preterm infants on third day after birth (N: 46, Spearman's ρ: 0.888, P <0.001).

Association between PDA and cTOI, Burdjalov score and RI

Figures 19-22 show scatter graphs with smoothed local polynomial regression fits (with 95% confidence intervals) of PDA vs. cTOI, Burdjalov score and RI. Figure 19 demonstrates that many patients with PDA larger than 2 mm have cerebral oxygenation less than 55 which is considered the lower end of normal range (145, 228). Many of these observations were from patients with severe IVH. Figure 20 indicates that as PDA diameter increases Burdjalov score decreases, but beyond 2.5mm this trend is skewed towards higher Burdjalov scores and the confidence intervals also widen as fewer participants contribute data. Looking into the individual data, these observations came from more mature babies (higher postnatal age) and persistent PDA, who had larger PDA size as their body size was bigger and had higher Burdjalov score as their brain was more mature. An attempt to adjust for postnatal age resulted in Figure 21. The estimation method (smoothing by local polynomials) does not allow adjustments by continuous variables, so two categories were created based on median postnatal age (two weeks). The skewness of the trend on Figure 20 was reduced and there was consistent trend of larger PDA size correlating with lower Burdjalov score for both groups (younger and older than two weeks). Figure 22 shows that only after PDA diameter becomes larger than 1mm there is an effect on cerebral RI with positive correlation between PDA diameter and anterior cerebral artery RI.

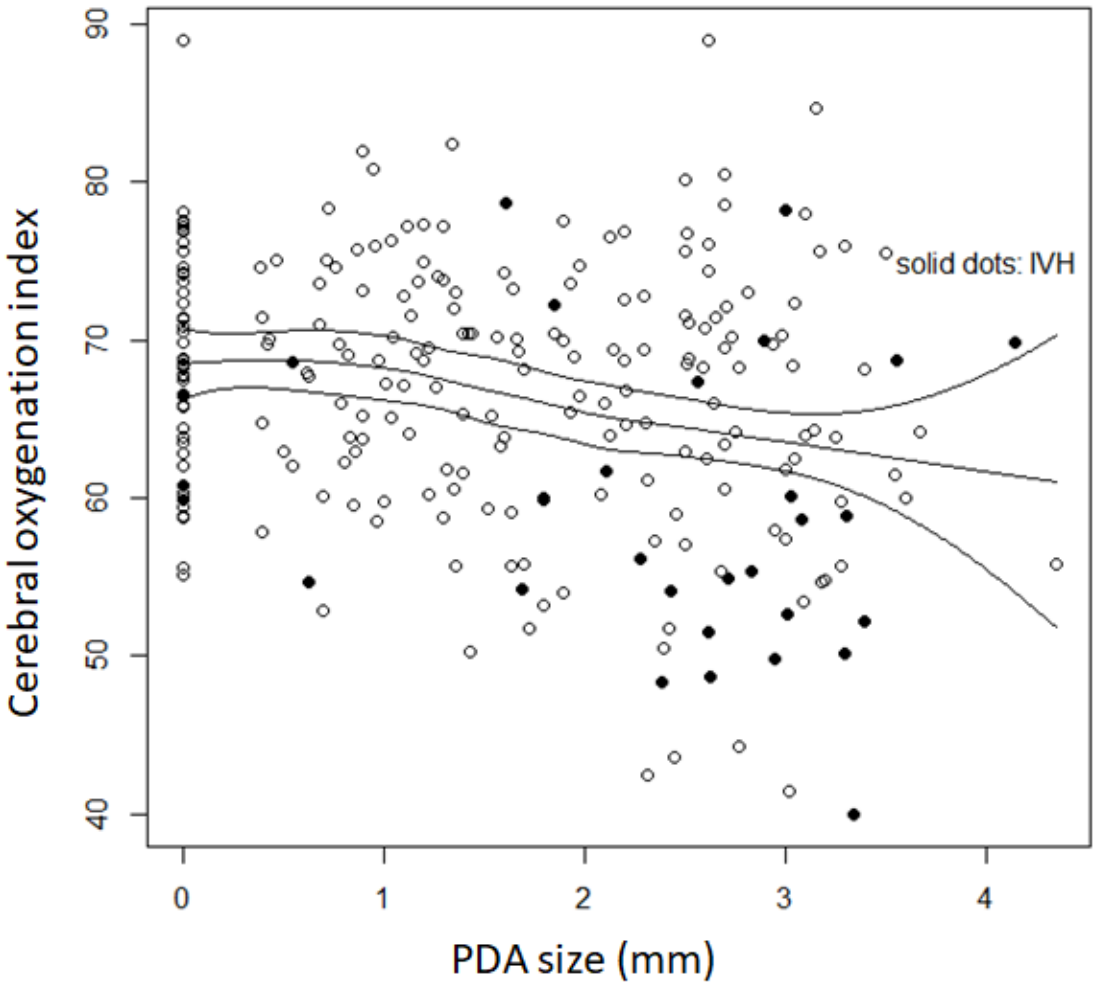

Figure 19: Smoothed local polynomial regression fits (with 95% confidence intervals) of PDA size vs. cerebral oxygenation index.

Patients with severe IVH are represented by solid dots. PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

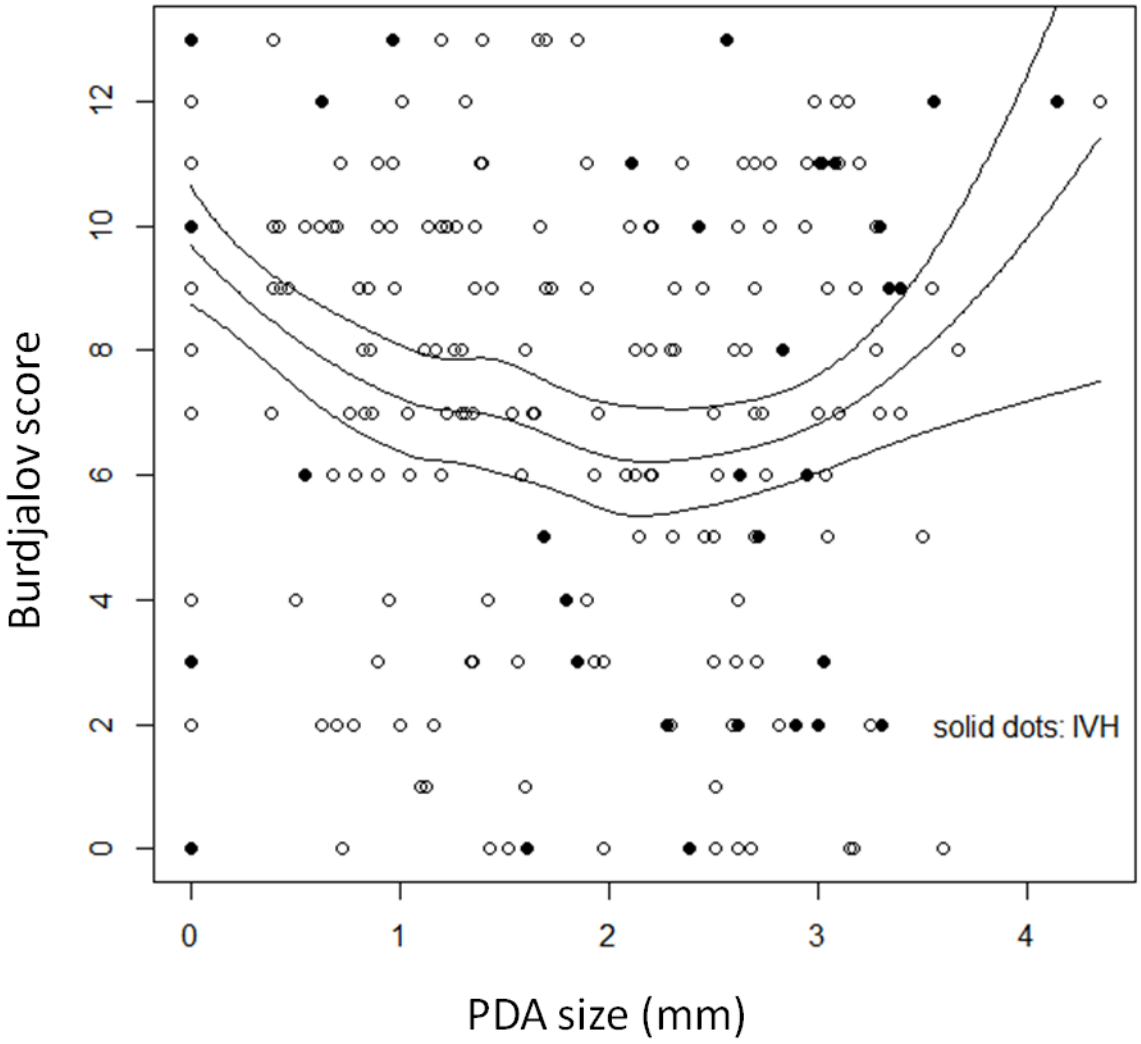

Figure 20: Smoothed local polynomial regression fits (with 95% confidence intervals) of PDA size vs. Burdjalov score.

Patients with severe IVH are represented with solid dots. PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

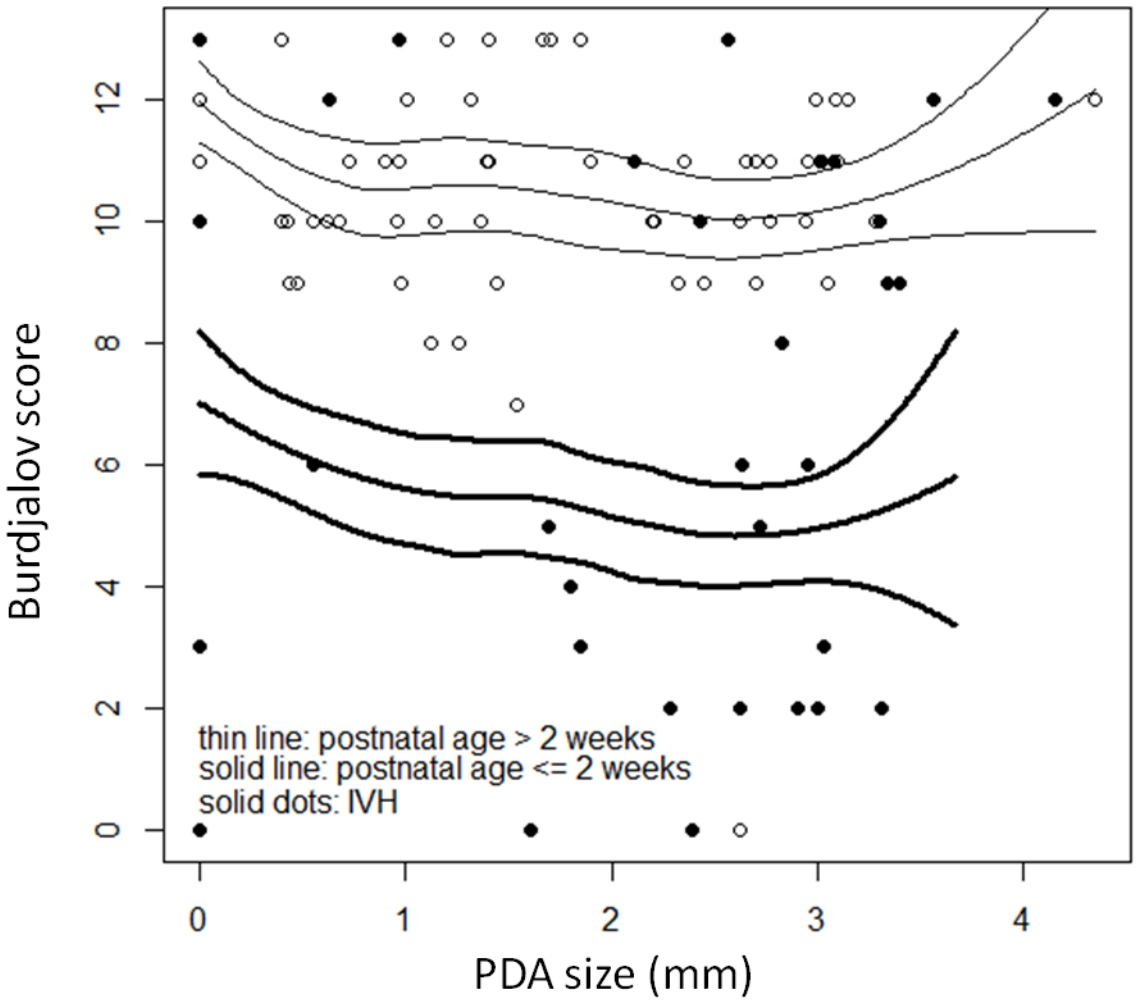

Figure 21: Smoothed local polynomial regression fits (with 95% confidence intervals) of PDA size vs. Burdjalov score adjusted for postnatal age.

Solid lines represent patients younger than two weeks postnatal age. Patients with severe IVH are represented with solid dots. PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

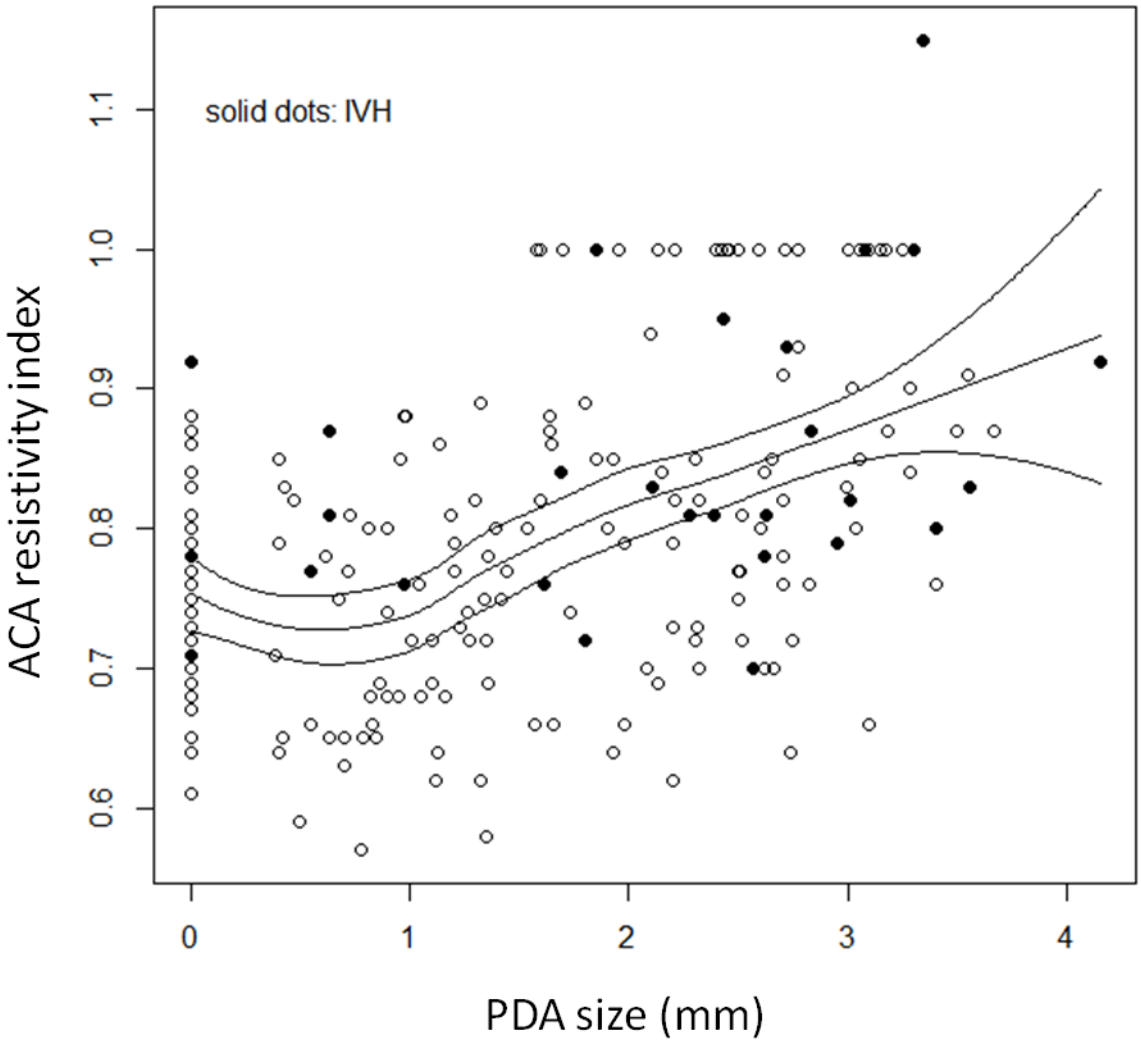

Figure 22: Smoothed local polynomial regression fits (with 95% confidence intervals) of PDA size vs. anterior cerebral artery resistivity index.

Patients with severe IVH are represented with solid dots. ACA: anterior cerebral artery, PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

Model specification

The associations between PDA diameter and cTOI, Burdjalov score and RI shown in the previous figures were further explored by developing parametric regression models using the imputed data sets. The models specify whether PDA diameter has any significant impact on the cerebral biomarkers studied. Table 11 shows the F statistics and corresponding P-values. Interaction of PDA with IVH severity (severe IVH: IVH> Grade 2) was also included in the regressions. The model specifies how the observed factors impact the separation of the classes non severe IVH vs. severe IVH.

The parametric regression model showed an association between PDA diameter and RI ($F=19.78$, $P<0.0001$), cTOI ($F=-4.16$, $P=0.017$) and Burdjalov score ($F=-12.55$, $P<0.0001$). The coefficients mean that as PDA became larger the RI increases (reduced diastolic velocity in the cerebral artery) and Burdjalov score and cerebral oxygenation decreased. However, when the interaction of IVH severity was included in the previous regression analysis, the association became statistically non-significant (Table 11). The implications of the interaction are that despite PDA having a significant effect on cerebral blood flow velocities, oxygenation and electrical activity, there is not enough evidence that this is associated with severe IVH.

PDA diameter		
Association	**F**	**P-value**
Cerebral oxygenation index	4.16	0.017
IVH interaction	0.19	0.67
Burdjalov score (nonlinear)	12.55	<0.0001
IVH interaction	0.37	0.54
ACA resistivity index	19.78	<0.0001
IVH interaction	0.75	0.39

Table 11: PDA diameter association with the cerebral biomarkers including the interaction with the severe IVH.

Associations stratified according to IVH severity

To investigate further whether patients with severe IVH have a specific pattern of observations which is lost during the generalised statistical analysis, smoothed local polynomial regression fits (with 95% confidence intervals) between those with and without severe IVH were made. These did not show any significant effects (data not shown). Due to the small number of patients with severe IVH the confidence intervals in this group were wide. There was a difference between those with and without severe IVH, with confidence intervals not overlapping, only for the cerebral oxygenation with a range of PDA sizes and ACA RI. Patients with severe IVH who had larger PDA had also lower cTOI compared to patients with similar size PDA, but without severe IVH (Figure 23). Patients with severe IVH had lower cTOI when PDA size was greater than 2mm (Figure 23) and had lower cTOI even when RI was within normal limits (Figure 24).

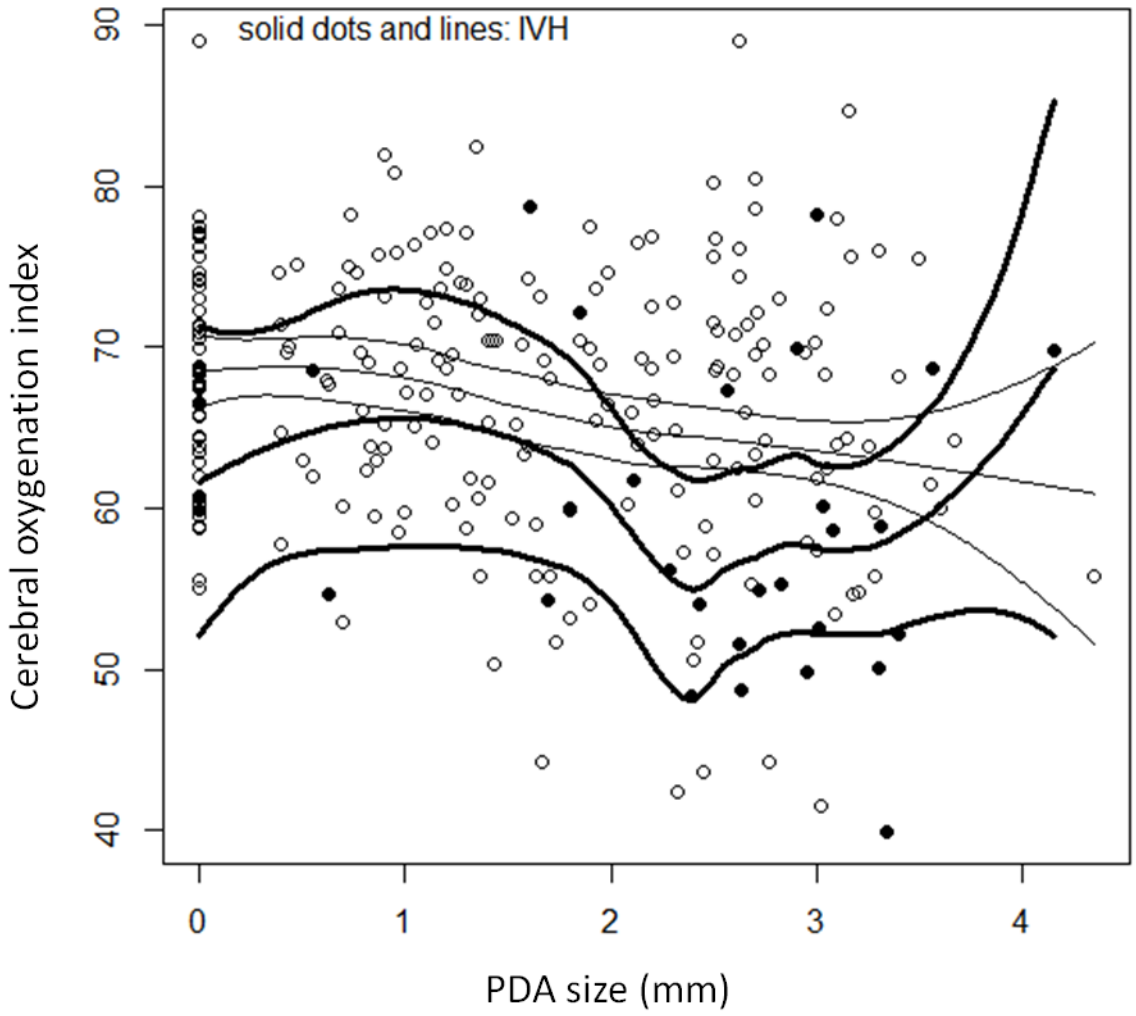

Figure 23: Smoothed local polynomial regression fits (with 95% confidence intervals) of cerebral oxygenation index and PDA size dichotomised according to the presence of severe IVH.

Solid dots and lines represent patients with severe IVH. PDA: patent ductus arteriosus, IVH: intraventricular haemorrhage.

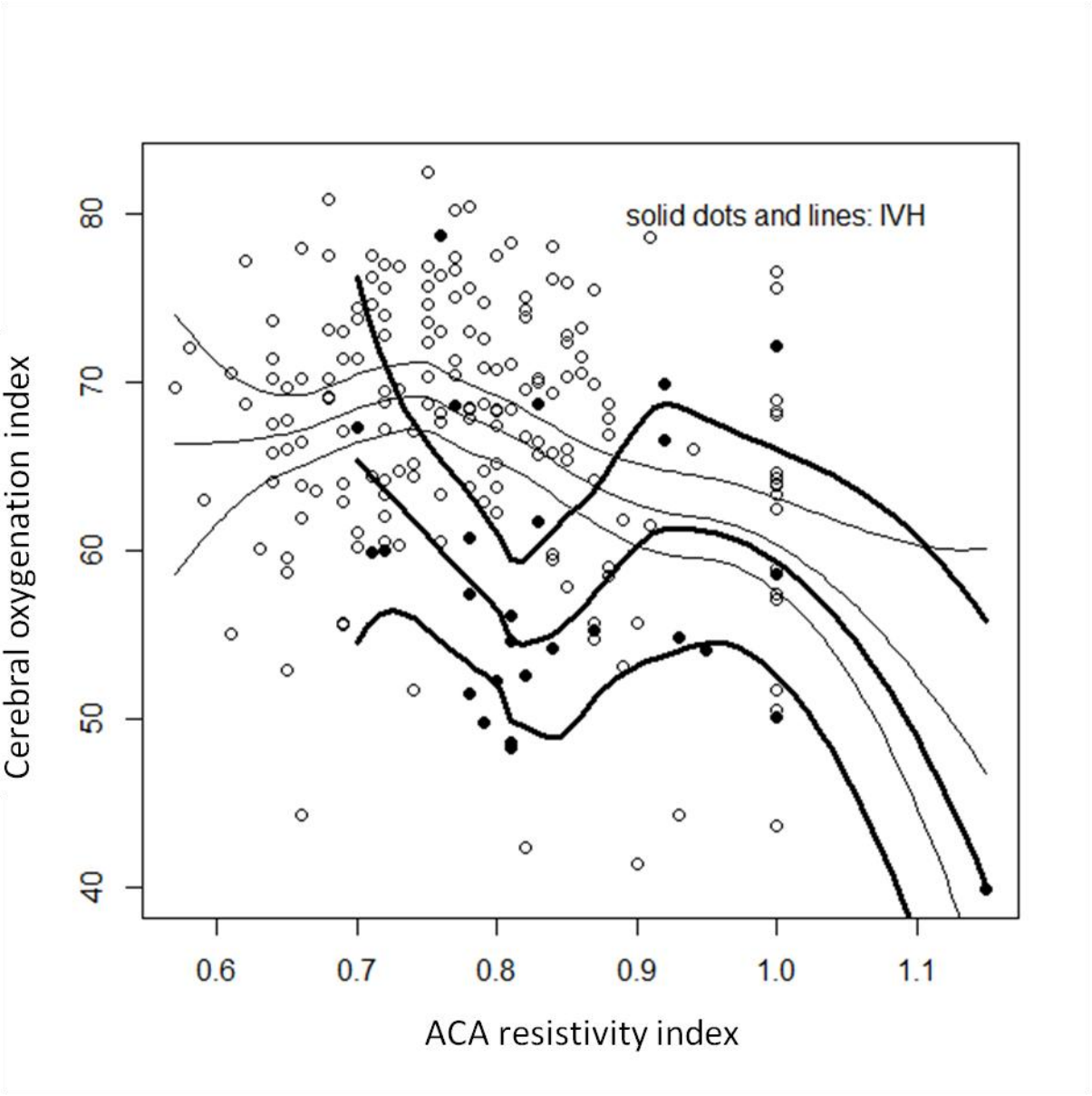

Figure 24: Smoothed local polynomial regression fits (with 95% confidence intervals) of anterior cerebral artery resistivity index and cerebral oxygenation index dichotomised according to the presence of severe IVH.

Solid dots and lines represent patients with severe IVH. ACA: anterior cerebral artery, IVH: intraventricular haemorrhage

Association between RI vs. cTOI and Burdjalov score

The interrelationship between the cerebral biomarkers was subsequently assessed. Figures 25-27 show smoothed local polynomial regression fits (with 95% confidence intervals) of RI vs. cTOI and Burdjalov score.

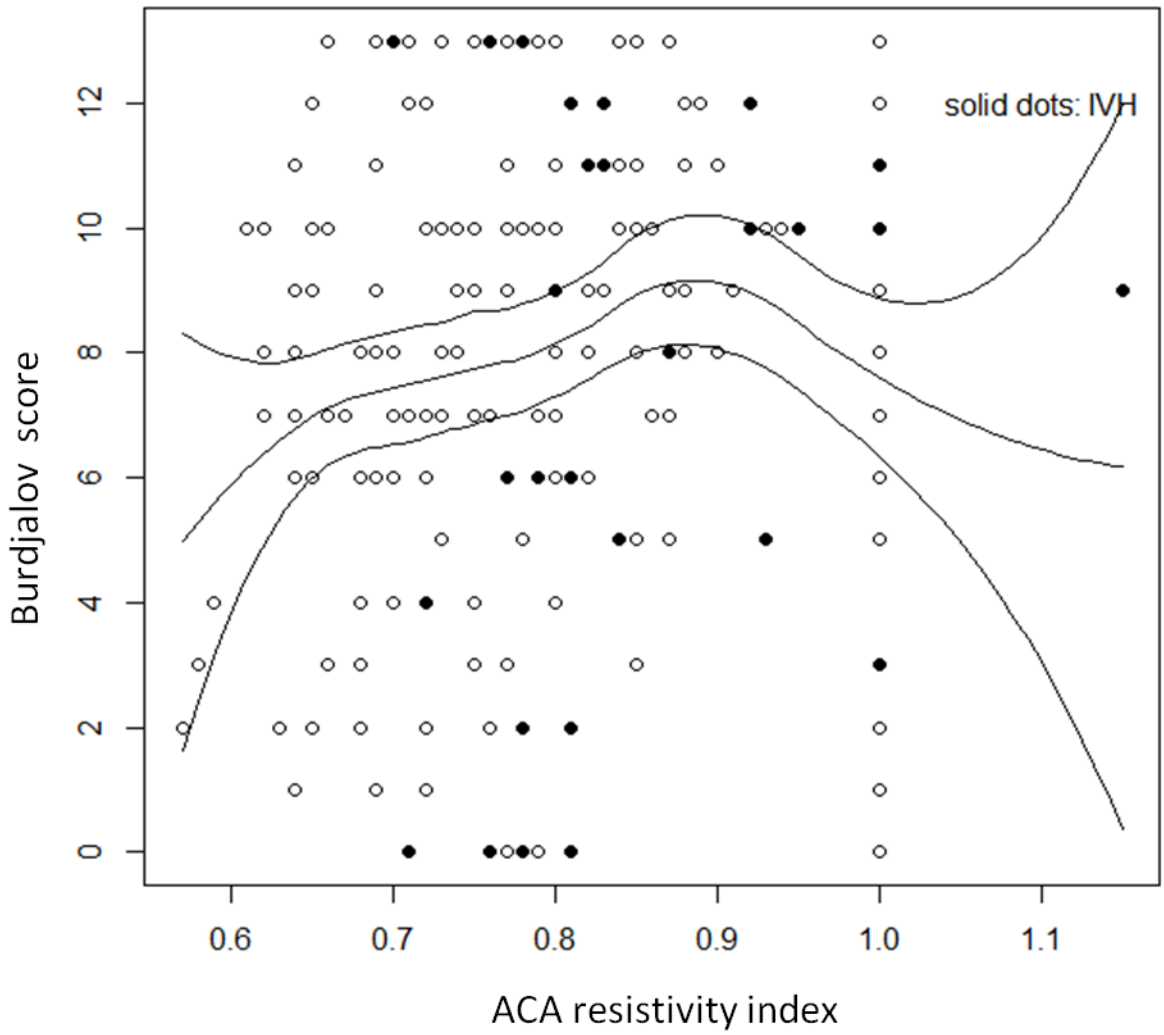

Figure 25: Smoothed local polynomial regression fits (with 95% confidence intervals) of Burdjalov scrore vs. anterior cerebral artery resistive index.

Patients with severe IVH are represented with solid dots. ACA: anterior cerebral artery, IVH: intraventricular haemorrhage

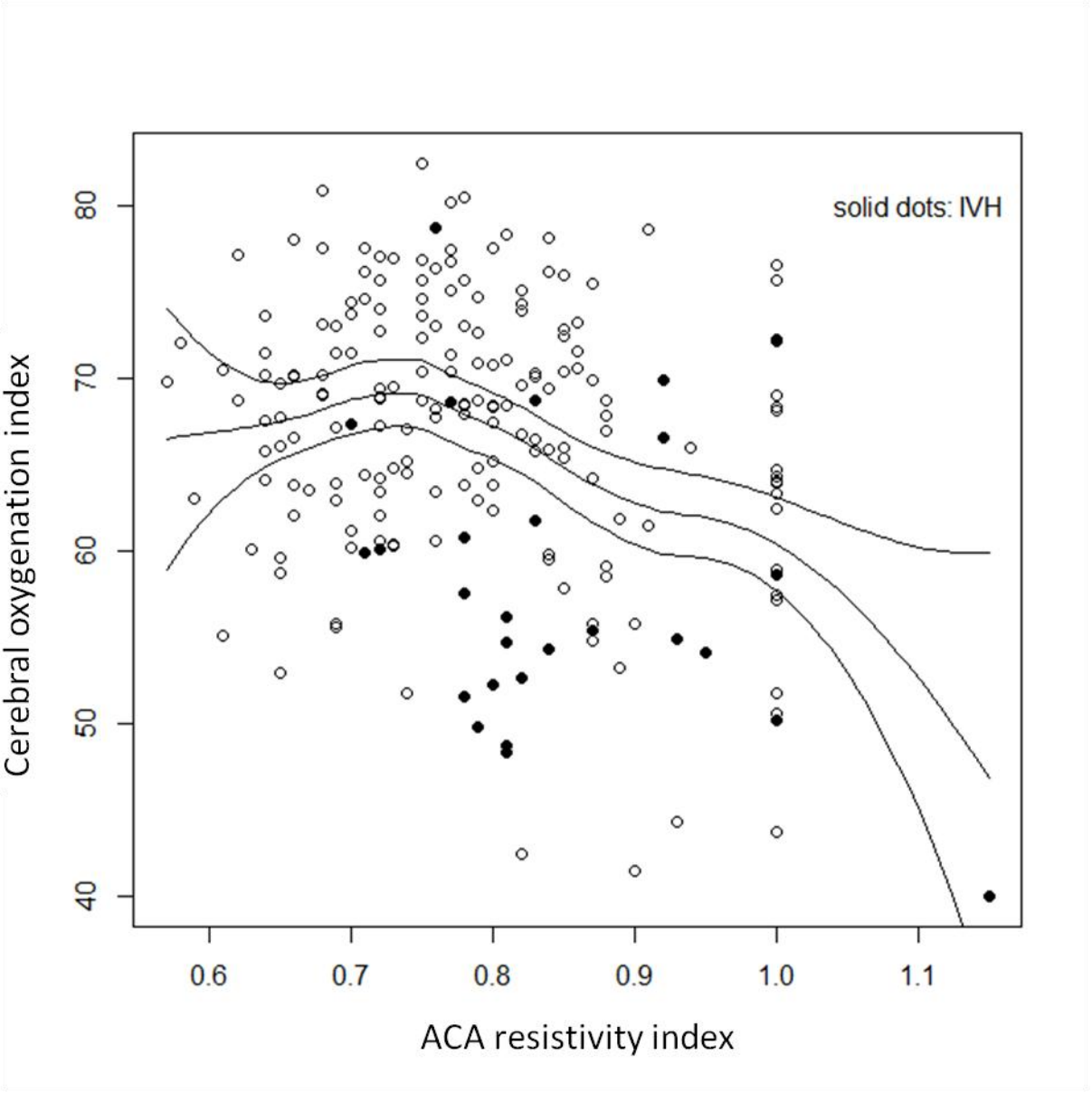

Figure 26: Smoothed local polynomial regression fits (with 95% confidence intervals) of anterior cerebral artery resistive index vs. cerebral oxygenation index.

Patients with severe IVH are represented with solid dots. ACA: anterior cerebral artery, IVH: intraventricular haemorrhage.

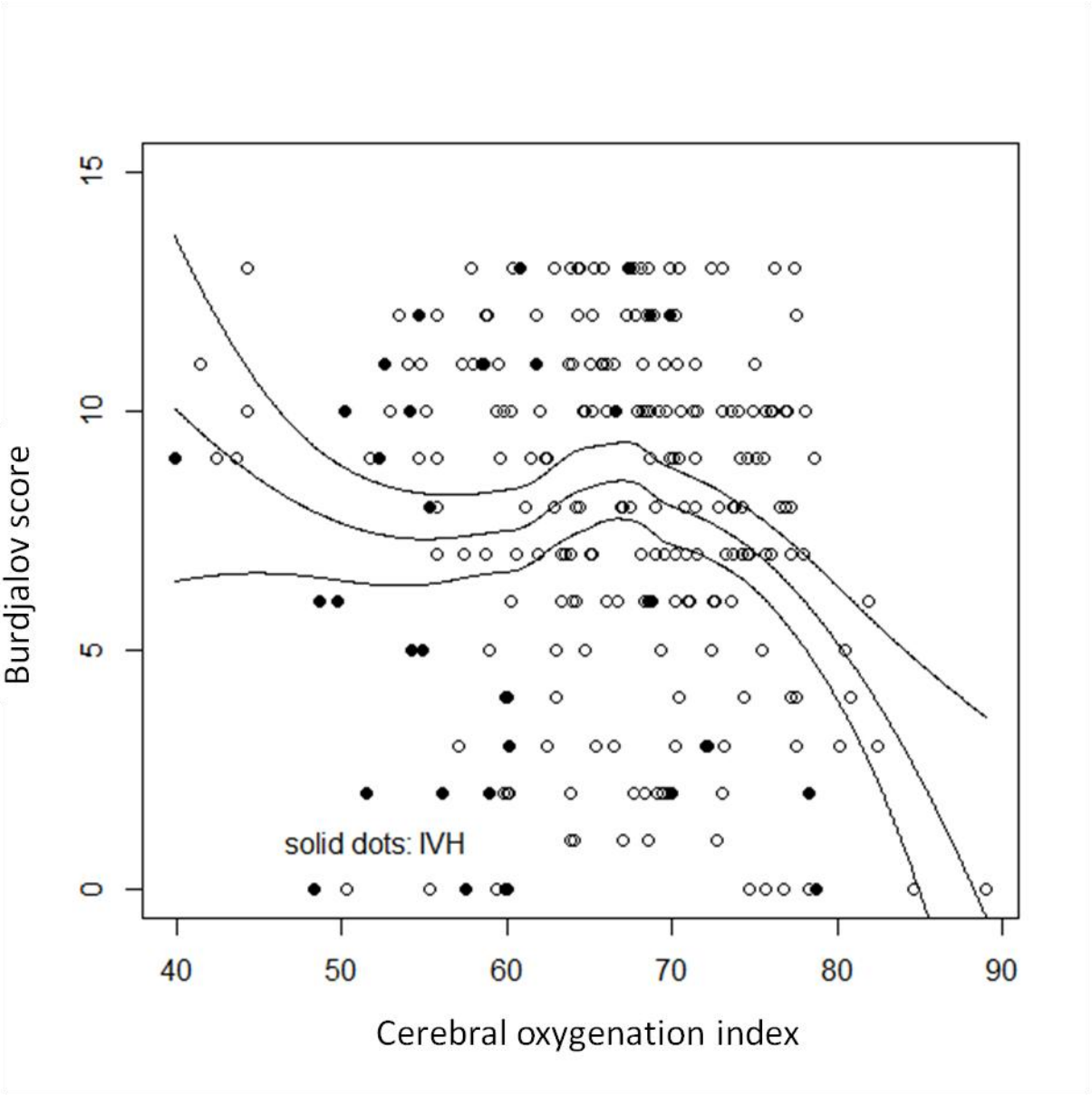

Figure 27: Smoothed local polynomial regression fits (with 95% confidence intervals) showing the relationship between cerebral oxygenation index and Burdjalov score.

Patients with severe IVH are represented with black dots. IVH: intraventricular haemorrhage.

Figure 27 shows an apparent increase in Burdjalov score as cerebral oxygenation decreased. This was unexpected. Normal cTOI in the range of 55-80 should be correlating with a higher Burdjalov score and an expected relationship should be a bell shape relationship as abnormal cerebral oxygenation (outside the considered normal range of 55-80) should be associated with abnormal/lower cerebral electrical activity (lower Burdjalov score). The relationship was further explored by considering separately the data for younger and older babies (less and above two weeks postnatal age). This is shown in Figure 28, where there is bell shaped curve relationship in both groups.

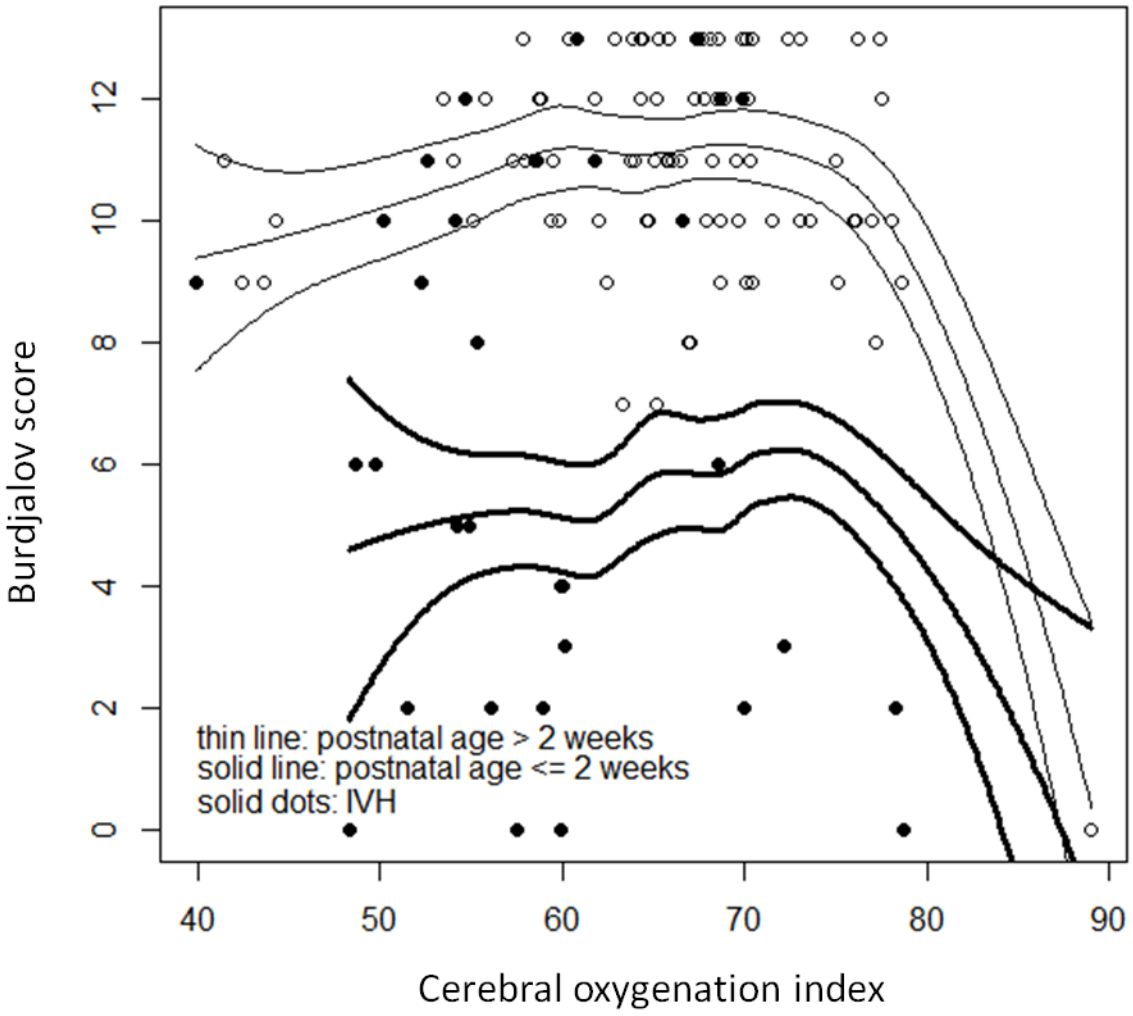

Figure 28: Smoothed local polynomial regression fits (with 95% confidence intervals) of cerebral oxygenation index vs. Burdjalov score adjusted for postnatal age.

Solid lines represent patients younger than two weeks postnatal age. Patients with severe IVH are represented with solid dots. cTOI: cerebral tissue oxygenation index, IVH: intraventricular haemorrhage.

The associations between RI, cTOI and Burdjalov score were further assessed by using the imputed data sets and fitting parametric regression models. Table 12 shows the F statistics and corresponding P-values. Interaction of RI with IVH severity (severe IVH) was also included in the regressions. cTOI was strongly related with RI and Burdjalov score (F=4.03, P=0.0035 and F=5.78, P=0.0002 respectively). As cTOI decreases, RI increases. Burdjalov score and cTOI have a more complex relationship as shown in Figures 27 and 28 and already described. There was no significant association between RI and Burdjalov score.

Anterior cerebral artery resistive index		
Association	**F**	**P-value**
cTOI (non-linear)	4.03	0.0035
IVH interaction	0.47	0.62
Burdjalov score (non-linear)	1.44	0.10
IVH interaction	1.10	0.34
Burdjalov score		
cTOI (non-linear)	5.46	0.0003
IVH interaction	1.35	0.26
IVH	2.49	0.061

Table 12: Parametric regression model assessing the relationship between RI, cTOI and Burdjalov score.

cTOI: cerebral oxygenation index, PDA: PDA diameter, RI: anterior cerebral artery resistive index.

Association between IVH outcome and PDA, cTOI, Burdjalov score and RI

A logistic regression model was designed to estimate the association between the outcome (severe IVH) and PDA diameter and the cerebral biomarkers (including interactions of PDA with cTOI, Burdjalov score and RI; and interaction of cTOI with RI). The model specified how the observed factors impacted the separation of babies with non-severe IVH vs. severe IVH. Table 13 shows the Chi-square statistics and P-values of the factors in the model.

A backward variable selection procedure, using Akaike's information criterion (AIC) to select the factors, was then applied to obtain a parsimonious description of the relationship between IVH and the predictive factors. Table 14 shows the model coefficients which are found by the selection procedure, the Z statistic and related P-value. Note that under this procedure, factors which were not statistically significant in the original model may become significant; this is because the AIC does not use P-values for selection, but instead measures a trade-off between predictive power and model complexity (penalising complex models that can lead to over-fitting the data).

The coefficients of the model in Table 14 can be interpreted as follows: as PDA increases, there is an increase of the probability that a subject will belong to the severe IVH class. Moreover, as cTOI increases, there is a decrease of the probability that the subject will belong to the severe IVH class.

Severe IVH		
Factor	**Chi-square**	**P-value**
PDA	4.50	0.34
cTOI	14.56	0.0022
Burdjalov score	2.27	0.32
RI	2.85	0.41
Interactions of PDA with other factors	2.61	0.46
Interaction of cTOI with RI	0.30	0.59

Table 13: Logistic regression model describing the association between severe IVH and PDA, cTOI, Burdjalov score and RI (including the interaction of PDA with other factors and cTOI with RI).

IVH: intraventricular haemorrhage, cTOI: cerebral oxygenation index, PDA: PDA diameter, RI: anterior cerebral artery resistive index.

Severe IVH			
Factor	**Coef**	**Z**	**P-value**
PDA	0.532	3.105	0.00190
cTOI	-0.0882	-3.394	0.00690

Table 14: Backward variable selection procedure to describe the relationship between IVH and the predictive factors identified PDA and cTOI as statistically significant factors.

It is important to estimate the prediction bias, as testing the performance of the model on the data used to fit it may lead to optimistic conclusions. The model was assessed both in terms of discrimination and calibration accuracy using bootstrapping techniques (C-index of 0.70, shrinkage coefficient 0.74). Calibration is measured both by the shrinkage coefficient (which is an estimate of overfitting; in our analysis, we can expect 1-0.74=0.26, i.e. 26% over fitting) and graphically by comparing predicted and observed risks (expressed as probabilities). Ideally, the predicted and observed risks should be equal (represented as a dashed diagonal calibration relationship line in Figure 29). Figure 29 showed an estimate of the calibration performance of the model. In this case, from the bias-corrected performance graph it can be seen that the model predicted higher risks of IVH than were actually observed for risks higher than 40%. However, this is not always the case due to the limited sample size and possible model inadequacies. Hence, this results in the calibration relationship between observed and predicted risks (represented by the dotted line in Figure 29). Moreover, the estimation of the shrinkage coefficient provides information whether the model may overfit the data, so the apparent calibration may be optimistic and biased. Therefore, it is useful to estimate a bias-corrected calibration relationship (the continuous line in the graph), where the bias is estimated by bootstrapping. Figure 29 demonstrated that the bias-corrected calibration relationship was less optimistic than the apparent calibration relationship, as it was farther from the theoretical ideal calibration than the latter.

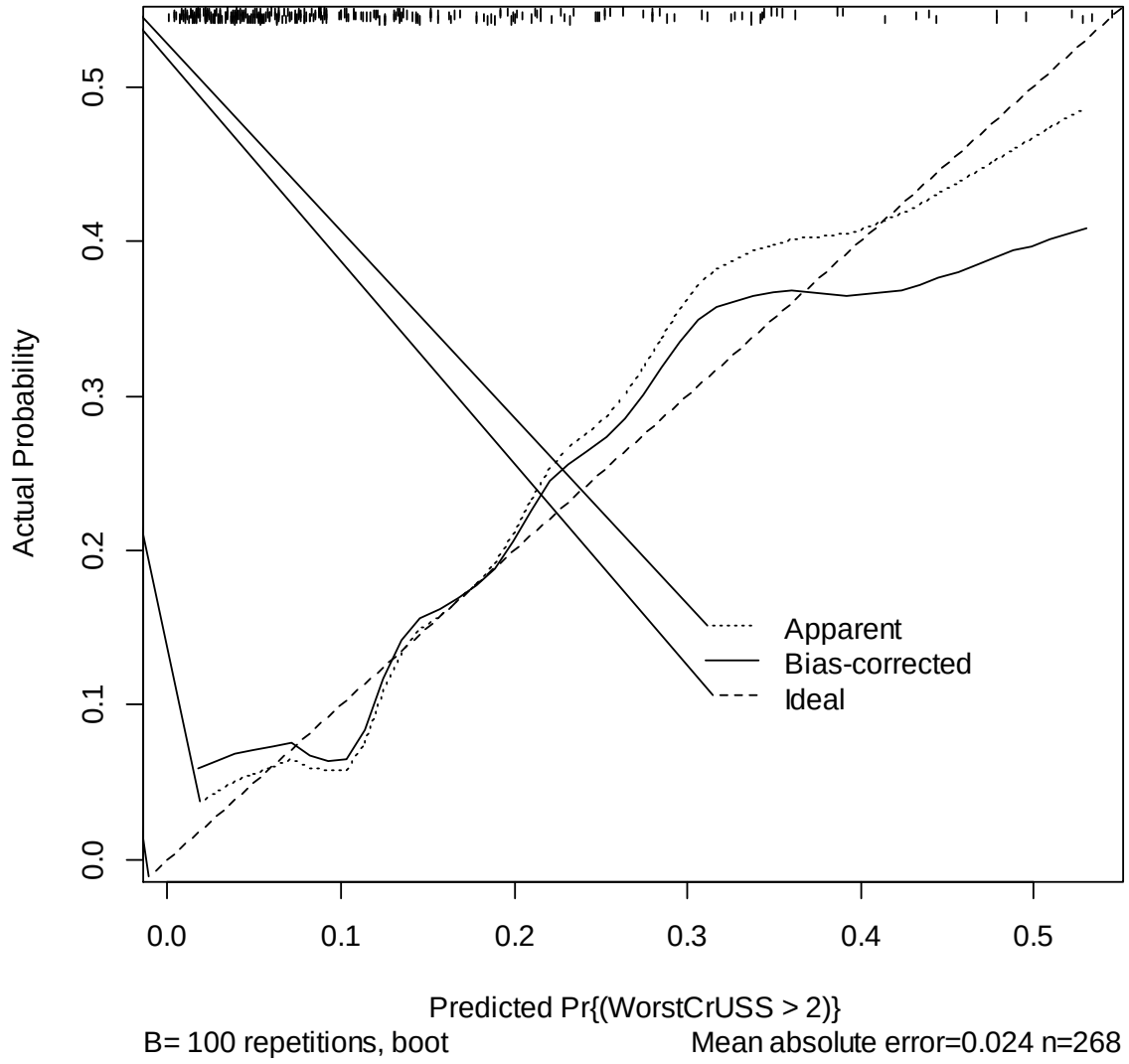

Figure 29: Calibration performance of the classification model.

The apparent line denotes the predictive performance of the model on the data used to fit the model. The bias-corrected line denotes the expected out-of-sample performance of the model, estimated by bootstrap resampling (100 repetitions of the sampling process). The rug plot at the top of the graph denotes the observed data.

Association between PDA, cTOI and death

Finally, an interaction of the PDA and cTOI with death was investigated (Figure 30). A parametric regression model was fitted using the imputed data to assess the associations between PDA and cTOI evidenced in the previous Figures. No association was found between PDA and death including the interaction of two biomarkers with death.

Backward logistic regression model in predicting severe IVH including inotropic support and sepsis on Day 3 after birth

The cohort had a high incidence of sepsis and inotropic support. Two backward logistic regression models were applied to account for these variables, assess the interaction of the biomarkers of the original model (cTOI, Burdjalov score, RI) with the evolution of severe IVH and whether the use of PDA score or PDA diameter had a different predictive value. There were 46 babies contributing data for both backward logistic regression models using PDA diameter or PDA score as marker of PDA significance. Both models resulted in identical outcomes with only cTOI being associated with severe IVH (N= 46, B= -0.467, S.E: 0.22, Wald: 4.52, P= 0.033). There was a very strong correlation between PDA diameter and PDA score on Day 3 after birth (N: 46, Spearman's ρ: 0.888, P value<0.001) (Figure 18).

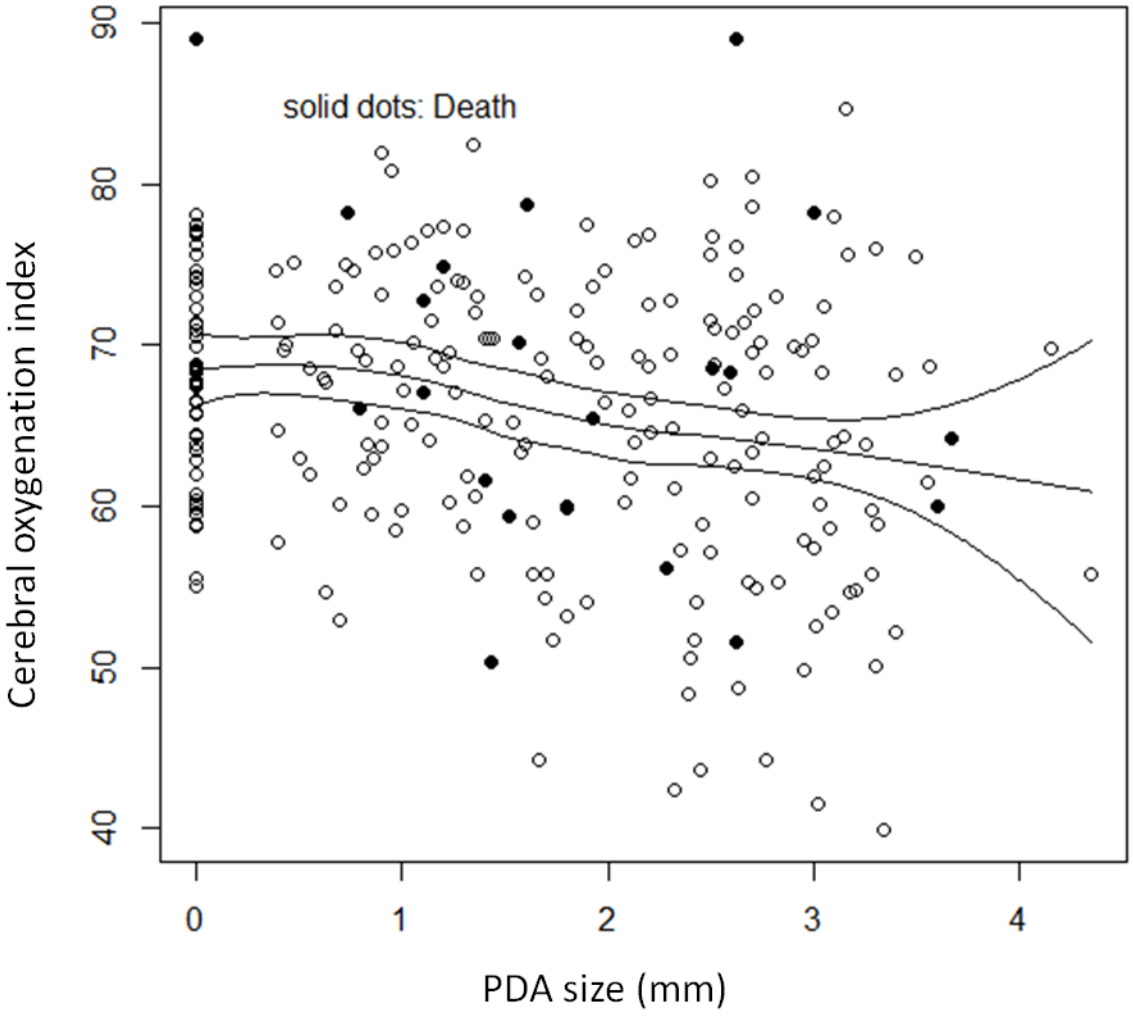

Figure 30: Smoothed local polynomial regression fits (with 95% confidence intervals) of PDA size vs. cerebral oxygenation index.

The black dots represent patients who died. PDA: patent ductus arteriosus.

Summary

The statistical associations reported in this chapter are summarised in Figure 31. In conclusion, there was evidence of an association between IVH outcome and PDA and cTOI. Furthermore, there was evidence of association between cTOI, Burdjalov score and RI with PDA diameter, but not in interaction with IVH severity. Despite the individual statistically important associations the two-step model was not valid. These data suggested that PDA had a significant effect on the incidence of severe IVH, but that effect was not mediated by cTOI, RI or Burdjalov score. Time point analysis and logistic regression analysis on third day after birth when all the cases of IVH had occurred demonstrated that only cTOI was statistically different in patients with severe IVH. These results did not change when inotropic support and incidence of sepsis were taken into account. Two backward logistic regression models using PDA score and PDA diameter alternatively as markers of PDA severity resulted in identical model characteristics possibly due to collinearity of the two biomarkers.

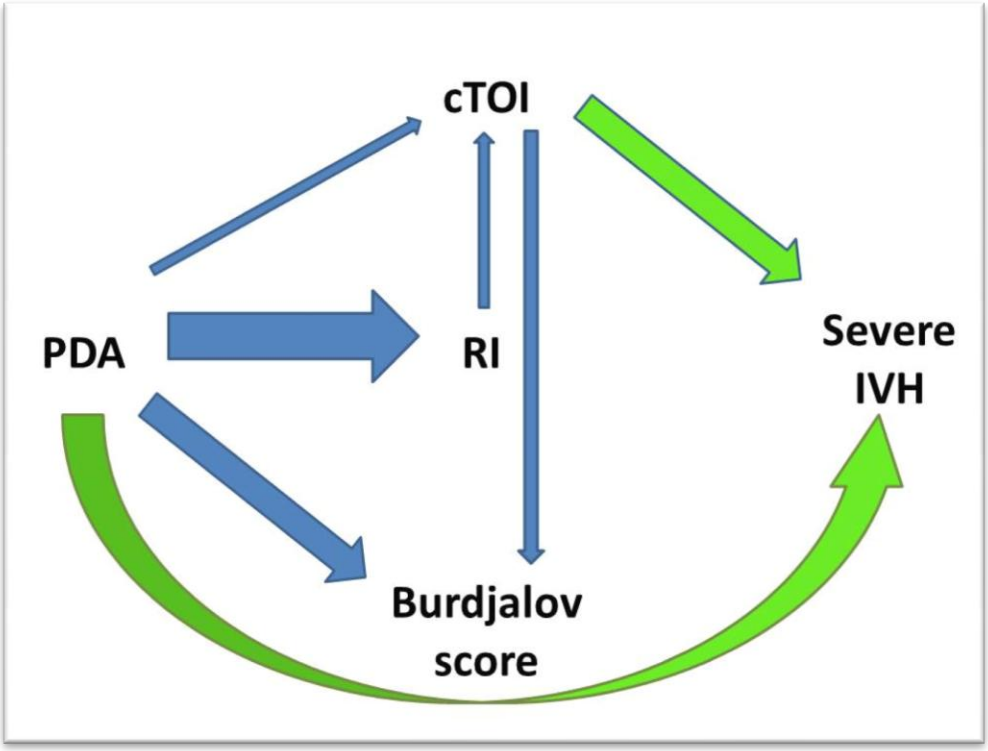

Figure 31: Summary of the significant interactions between the factors of the two-step biological model.

The blue arrows represent statistically significant interactions revealed by the parametric logistic regression analysis. The green arrows represent the statistically significant associations found after the backward variable selection procedure. The arrow width is proportional to the F statistics value.

Discussion

This is the first study to my knowledge that assessed the impact of PDA size on cerebral function and incidence of IVH using simultaneous echocardiography, cerebral ultrasonography, cerebral NIRS and aEEG during the natural evolution of PDA disease. There is evidence of an association between severe IVH with PDA and cTOI. Furthermore, there is evidence of an association between cTOI, Burdjalov score and RI with PDA, but not in interaction with IVH severity. This means that isolated steps of two step model (Figure 3) specified ante hoc are valid, but not the entire two-step model. Alternatively, the effects of cerebral oxygenation, blood flow and/or electrical activity are not captured adequately with these measurements. Single elements of the model were more influential than the combined effects that were postulated before the study was done.

The novelty of my approach was the simultaneous assessment of cardiac and cerebral function using multiple biomarkers for a long period and their combination in the construction of a statistical model based on a two-step biological model that was identified ante hoc. PDA-related cerebral effects were observed well beyond the period of transitional circulation. The cohort included infants who became unwell, needed inotropic support and developed severe IVH. It was demonstrated that it is feasible to perform this complex monitoring in critically ill patients without significant adverse events. Factors that affected the consent process, recruitment and data collection are discussed in Appendix 11. Moreover, clinicians in the unit had a relatively

conservative approach to PDA treatment and this allowed me to observe the natural history of PDA closure and study the PDA and cerebral haemodynamics without significant contamination from PDA treatment. None of these infants was a participant in the Baby OSCAR trial. Our treatment rate with ibuprofen was 12% and 2% with ligation which are very low compared to other units nationally and internationally (Australia up to 74% and 12%, Canada 51% and 7% respectively) (229, 230).

Demographics, clinical outcomes and assessment of selection bias

I assessed whether there is selection bias due to the other competing studies by comparing the thesis population with the LWH population, the UK population and the VON population during the same period. This analysis demonstrated that overall the thesis cohort did not have significant selection bias within the centre. GA at birth and birth weight, which are the strongest predictors of neonatal complications and clinical outcomes (7), were almost identical to the local population during the same period. The median maternal age at birth (31 years) was similar to the national average age of all mothers of babies born in England and Wales in 2017 (30.5 years) (231). The maternal booking BMI was 26.2 and slightly above the ideal range 18.5-25 and similar to the national average for this age, but this may was influenced by the weight gain during the first trimester (232). The reported smoking (12%) and alcohol (6%) consumption was low compared to the local high smoking rates in Merseyside (22%) (233) and the national high alcohol consumption rate in second trimester (34%) (234). Offensive liquor and/or chorioamnionitis incidence was similar to the LWH population

and the international population. Our population had high prevalence of chorioamnionitis, PROM and use of antibiotics consistent with published data (235). Indomethacin was used for tocolysis in 10% of the cases. There is controversy regarding its postnatal effects with some studies proposing antenatal indomethacin is associated with increased risk for severe intraventricular haemorrhage, necrotizing enterocolitis, and periventricular leukomalacia (236) and others not (237). A recent report proposed that antenatal indomethacin may be related to early PDA closure in preterm infants in contrast to previous studies (238). However, other reports suggested the opposite with infants receiving antenatal indomethacin being resistant to postnatal indomethacin and requiring PDA ligation (239, 240). In our cohort, only one out of six infants who received antenatal indomethacin had severe IVH (Grade III). Two-thirds of the infants received antenatal $MgSO_4$ for neuroprotection with the majority of them being delivered in LWH. The haemodynamics associated with the antenatal administration of $MgSO_4$ is discussed in detail in Chapter 5. Almost all infants were exposed to at least one course of steroids and 75% had a complete course. Half of the infants were born via a spontaneous vaginal delivery.

My cohort had significant higher incidence of BPD compared to national and international populations, but was in line with the local population incidence. The reasons for this local difference from benchmarks are not known. The incidence of "advanced resuscitation" was higher in the study cohort than among the LWH population. This may be a terminological difference. In the LWH records, "advanced resuscitation" means use of inotropes and/or cardiac massage. In this study, "advanced

resuscitation" meant additionally not straightforward and/or prolonged attempts for intubation that resulted in compromised peripheral perfusion and tone. This was thought necessary as it is known that increased duration of cerebral hypoxia is associated with poorer neurodevelopmental outcomes in preterm babies or death (241). In addition, there may have been a selection bias towards a sicker population in this study for two reasons. Firstly, it is possible that sicker babies were not recruited to other studies. Secondly, because sicker babies were transferred into the unit making it difficult to recruit them to other studies that recruited in the first days after birth. The higher incidence in the use of inotropes in my cohort is notable because of the focus on haemodynamics in this study and may also reflect a selection bias towards sicker babies. There was a higher incidence of sepsis in the study cohort, 46% vs 32% in the local cohort, but this cohort was in line with the international population. This may be due to the variable thresholds used to define sepsis and chorioamnionitis in the different databases. Overall, apart from advanced resuscitation and inotrope use, there was no clinically significant selection bias related to the recruitment process and the other competing clinical trials.

The presence of sicker babies in this cohort is likely to mean that the associations observed have been modulated, in some babies, by pathophysiological processes seen following difficult birth and leading to the use of inotropes. There is no reason to think that the pathophysiology seen in the study cohort is different from that seen among other babies from this center, or from other centers. However, as with all studies of neonatal critical care, interpretation of the results needs to be coloured by the

realisation that the reported associations may have a different pattern in babies with a different mixture of severe and non-severe clinical courses in the days after birth.

Similarly, the generalisability of this study – and any study that recruits exclusively from a single centre – needs careful attention. The events and processes that lead to BPD are overrepresented in this sample compared to other populations. It is possible that some of these events and processes influence the processes studied in this thesis. If the processes that lead to BPD in Liverpool are qualitatively different from processes that lead to in other centres then any Liverpool-specific processes could modulate the associations described in this thesis. There is no evidence that Liverpool has different pathophysiology from other units. If the same processes are relevant in Liverpool as in other centres then the centre-specific effects relate to the mixture of pathophysiologies. Future studies should be multicentred to allow the effects of centre specificities to be diluted.

Limitations of this comparison

The cohort size was small (52 patients) and one patient corresponds to 2% difference. This should be considered to assess the comparisons between the thesis cohort and the other neonatal populations. High percentage of missing data on the local database and questionable quality of the presented data are some of the main limitations. The analysis of the VON database raised also some concerns for the quality of the data and possible impact of missing data as the reference population was variable for the different assessed parameters despite assessing the same GA range. Varying

definitions of the different demographic and outcome parameters can give rise to variable measurements.

Evolution of biomarkers with postnatal maturation

PDA diameter decreased over time as expected (Figure 9). Monitoring infants with closed PDA after the second week was discontinued and this is the reason for the observed increase of PDA diameter after the second week because infants with a closed PDA did not contribute data. Many infants were discharged to Level II units. The PDA may have reopened in some infants. However, it is not appropriate to reconstruct reliably the graph assuming the PDA diameter is zero following its closure. There was large variation of the observed values. 54% of our patients had PDA diameter >1.5mm at some point during the transitional period, which has been the threshold for PDA treatment for many clinical trials, but only 15% received medical therapy. There was a patient who did not receive treatment and PDA persisted up to the discharge at 16 weeks postnatal age. These findings agree with a recent report which found the median time to ductal closure to be 71 days in infants <26+0 weeks gestation (117).

cTOI decreases with postnatal maturation (Figure 10). This is consistent with previous reports (160). The drop of cerebral oxygenation over time occurred in conjunction with decreasing PDA size as shown in Figures 9 and 10. Supranormal values (cTOI >80) mainly occurred during the first three days after birth when intensive care and treatment with oxygen are more likely. This is consistent with data from SafeBooC clinical trial, which found considerable burden of hypoxia and hyperoxia during the period of transitional circulation (156). Low values (cTOI<55) were observed up to the

eighth week. It is interesting that patients with severe IVH had cTOI values concentrating on the extremes of the considered normal range indicating possible cerebral hyperoxia or hypoxia.

RI initially increased with maturation, but following the sixth week it started decreasing. This is consistent with the trend of reduced PDA diameter following the fifth week. Similar patient selection limitations apply for the interpretation of this biomarker trend. The increasing of RI with postnatal age is consistent with a previous study (207). Only one infant in our cohort had reversed end diastolic flow in the ACA and had also severe IVH.

The Burdjalov score increased exponentially during the first two weeks after birth. After slowing off during the subsequent weeks a plateau was reached at week ten. This is consistent with observations by Burdjalov et al. (174). More detailed description of the evolution of Burdjalov score and the parameters which affect it can be found in Chapter 4.

Summary

There are significant changes with all studied biomarkers with advancing postnatal age representing a continuous change of interplay between postnatal maturation, PDA haemodynamics and cerebral biomarkers. Generally, there is large variation of all the aforementioned biomarkers on each time point. Our data has the limitation that after the second week only patients with persistent PDA were studied.

Temporal changes of the biomarkers in patients with and without severe IVH

Babies with severe IVH had different temporal trajectories of the studied biomarkers (PDA diameter, PDA score, cTOI, Burdjalov score and RI) during the first days after birth and this persisted for weeks after birth (Figures 13-17). During the first days after birth, PDA score was shown to have better ability compared to PDA diameter to separate cases with severe IVH from cases with non-severe IVH. This observation is important as it points towards the PDA induced haemodynamic disturbances possibly related to the IVH pathophysiology. Moreover, it means that the PDA score can potentially serve as a screening tool for inclusion in clinical trials. However, as described in the following section (Biomarkers during the transitional period) this observation was not statistically significant. All assessments of PDA will benefit from larger studies that incorporate more advanced technologies to assess the timing of brain injury insult and the evolution of IVH.

PDA diameter was previously found to be the single most important parameter that determines the magnitude of the PDA shunt (378) and it is easily understood by clinicians with no cardiac expertise. However, any single parameter cannot describe and assess comprehensively the complex haemodynamics related to PDA shunting. Therefore, there is currently a shift away from using single echocardiographic parameters to assess PDA haemodynamic significance. Different PDA scores have been devised in order to assess better the magnitude of the PDA shunt and the multiple

effects PDA has on cardiac and respiratory status of the preterm babies (133, 137, 242). However, there is lack of robust data from large observational studies and clinical trials including heterogenous populations of preterm babies to validate the use of PDA scores in predicting certain clinical outcomes.

The window of opportunity to target PDA treatment for IVH prevention is very narrow (maximum 72 hours). IVH usually occurs in babies who become critically unwell, haemodynamically unstable and usually have relative or absolute contraindications for medical PDA treatment. PDA ligation is now resorted only for the cases that medical treatment has failed so that this window of opportunity further narrows. Moreover, the precise identification of when IVH starts is challenging even in research settings. It is very possible that previous clinical trials for primary prevention of IVH and early targeted PDA treatment have treated babies in whom the IVH disease process had already started (cerebral ischemia, disturbed cerebral autoregulation), but was not macroscopically evident by CrUSS or even MRI. Hence, treating this population at risk of developing IVH with agents inhibiting platelet function can have possible iatrogenic side effects and worsen the severity of IVH. This can be one the reasons behind the observed lack of long-term clinical efficacy of NSAIDs in treating PDA. There is a need for new biomarkers in screening extremely preterm babies in risk for IVH, better understanding of the IVH pathophysiology and its underlying molecular pathways and a need for new PDA treatment agents targeting molecular pathways which do not interfere with the initiation and progression of IVH lesions.

A recent study has demonstrated that babies with IVH and large PDA had lower cerebral perfusion measured by MRI. However, the assessments were performed after IVH occurred and hence these relationships reflect more the effects of the IVH on cerebral perfusion and the PDA the epiphenomenon of IVH (243). In the present thesis, an attempt was made to do serial measurements to follow the evolution of IVH and other findings. As noted, comprehensive serial measurements were not possible. The finding of reduced cTOI on day 3 is likely to reflect events after IVH which may or may not be relevant to the pathophysiology of IVH. Definitive studies of the time course of these complex physiological interactions will need to be done in multiple centres that do not allow recruitment to other studies to reduce the participation of babies in a cohort that deploys multiple assessments starting on the day of birth.

Moreover, there is collinearity between PDA diameter and PDA score and it is uncertain whether PDA score can provide additional information when used as screening tool for clinical trials. This is needs to be assessed in a large multicenter feasibility study.

Biomarkers during the transitional period

On the third day after birth babies with severe IVH had lower cTOI [58.9 (10) vs. 68.7 (9.9), <0.001]. This is consistent with the logistic regression analysis when all data points were used for the biological model (Table 13). Further studies with larger populations in the first days after birth are needed to increase the power and reach definitive conclusions and assess which assessments of PDA can serve as screening

tools to assess babies in risk to develop IVH. In conclusion, babies with severe IVH had significantly lower cerebral oxygenation only on Day 3.

Backward regression model on Day 3

The model combining all datasets from birth to discharge demonstrated that severe IVH was associated with the presence of large PDA and lower cerebral oxygenation. Furthermore, there was a significant association between lower cerebral oxygenation, lower cerebral electrical activity and higher resistivity index on anterior cerebral artery with larger PDA size, but not in interaction of these variables with IVH severity. This means that separate steps of a proposed two step model were valid, but not the entire two-step model. However, IVH happens early during extrauterine life or even during pregnancy and during the study there were more observations reflecting the aftermath following the IVH. Hence, it is uncertain whether the observed relationships are the cause or the epiphenomenon of brain injury. Moreover, the study population had high incidence of sepsis and use of inotropic support that are both known to be associated with IVH and predominantly occurred during the transitional period (23, 29). This led to the design of a new logistic regression model to investigate further the interaction of all these parameters at the third day after birth which was the closest time point during the transitional circulation period when all IVH cases had already occurred, and the maximum number of individual observations were available. A backward regression model was designed to assess whether PDA diameter, Burdjalov score, cTOI, RI, sepsis and/or use of inotropes on Day 3 after birth could predict the occurrence of

severe IVH. An additional backward regression model was designed substituting PDA diameter with PDA score to assess whether PDA score had better predictive value. Both models resulted in absolute the same model outcomes with only cTOI being associated with severe IVH (N= 46, B= -0.467, S.E: 0.22, Wald: 4.52, P= 0.033). This confirms the association between reduced cerebral oxygenation and the presence of IVH in this cohort. There was a very strong correlation between PDA score and PDA diameter (N: 46, Spearman's ρ: 0.888, P value<0.001) which can be considered collinear. Combined with relatively small sample size this may explain the identical performance of the backward model irrespective of using PDA diameter or PDA score.

PDA size and severe IVH

A significant association between PDA diameter and severe IVH was found, but not in interaction with the cerebral biomarkers. There was an increased incidence of severe IVH in infants with a larger PDA. Patients with IVH had the same number of observations (median of 5) compared to control group. Hence, the two groups (severe vs. non severe IVH) are equally represented. Moreover, as can be assessed in Figures 23 and 24, patients with severe IVH and larger PDA had lower cTOI and higher RI. The underlying pathophysiology is believed to be related to the PDA induced hypoxia and hypoperfusion because of shunting. PDA increased pulmonary flow and the consequent pulmonary plethora may interfere with gas exchange and causing systemic hypoxia (244).

There are conflicting data in the literature regarding the impact of PDA on IVH. Many studies have demonstrated an association between PDA and IVH (245, 246). However,

many others did not (247). This can be possibly attributed to the differences of power and sample size of the studies, the biological variability, the imprecision of measurements, the background demographics (IUGR incidence, severity of prematurity, use of $MgSO_4$ and antenatal steroids), which all alter the background risk of developing IVH. Our cohort had significantly higher rate of severe IVH (12%) compared to national (~6%) (248) and international average (~3.5%) (249). It is unlike clinical practice in LWH to differ significantly to national and international standards; hence the underlying causes may be related to the background socioeconomic and antenatal characteristics of our population (250). Nevertheless, the higher incidence of severe IVH in our population allowed us to reach statistically significant conclusions with a relatively small sample size.

Despite the observed association between PDA and IVH, a causal relationship has not been established, despite decades of intensive research and many clinical trials involving PDA treatment. There is evidence that prophylactic indomethacin and ibuprofen decrease the risk of severe IVH, but there is no evidence of a difference in mortality, chronic lung disease, neurodevelopmental outcome and other major neonatal outcomes (122, 199, 251). However, NSAID treatment is often associated with side effects (gastrointestinal bleeding and perforation, renal impairment). This is the reason why a recent Cochrane review recommended that no more studies of prophylactic PDA treatment are needed until evidence from ongoing trials with early targeted treatment become available (122).

The uncertainty regarding the causal relationship between PDA and IVH is also partially due to the complex pathophysiology of IVH. There are no validated biomarkers to assess the onset of IVH at a cellular level as there is a time delay between the initial cerebral insult and the appearance of ultrasonographic evidence of IVH. IVH is believed to occur predominantly during the first three days after birth (252, 253). However, there is latency between the initial cerebral injury and the appearance of the IVH on CrUSS. There is not yet a valid biochemical biomarker to assist IVH diagnosis in preterm infants. However, there are several biomarkers under investigation with the most promising being S100β (a calcium sensor protein) and activin (254). It is interesting that S100β measured in the maternal blood of pregnancies complicated by IUGR had very high predictive value for the development of IVH (sensitivity 100%, specificity 99.3% and 0.999 area under the ROC curve) (32). Activin was found to be in high concentrations in blood of preterm infants in the first hour after birth that subsequently developed IVH (sensitivity 100%, specificity 93% and positive predictive value of 79%) (255). If the antecedents of IVH can be traced back to pregnancy, it is uncertain whether PDA has a significant effect on IVH pathophysiology or the opposite, as the existence of an association does not imply the direction of this association. This finding also raises the issue of the antenatal events and sequelae leading to IVH before the delivery and the importance of working together with obstetric researchers to develop appropriate biomarkers to phenotype each pregnancy appropriately.

It is possible that IVH and/or cerebral dysfunction can actually disrupt normal PDA closure by altered autonomic modulation of vascular control. There is evidence that

altered autonomic function is detected before the appearance of IVH (42, 256) and IVH is subsequently associated with altered autonomic function (257). It is known that DA receives rich supply by adrenergic nerves which end in the ductal smooth muscle layer (73). The parasympathetic nerves are practically absent (258). However, parasympathetic activity increases quickly with maturation (259, 260). There is evidence that parasympathetic dominance or reduced sympathetic activity is associated with the presence of PDA (76). It is known from animal studies that adrenaline or noradrenaline can constrict the foetal DA (259). It is also suggested that PDA causes parasympathetic stimulation through its effect on left atrial enlargement and stimulation of atrial natriuretic peptide secretion (261). However, there are no studies investigating specifically the interplay between IVH, autonomic function and PDA.

Summary

PDA is associated with severe IVH (N=52, Coef=0.532, Z=3.105, P=0.00190) and this is supported by previous published data. PDA steal phenomenon is possibly related to this interaction. However, there is uncertainty regarding any causal association and the direction of the association.

PDA size and cerebral oxygenation

A negative association between cerebral oxygenation and PDA diameter was found. As PDA diameter increased cTOI slowly decreased in a linear fashion. Overall, there is no evidence of IVH interaction with this association and there is large variability of the observed values. Many patients with PDA larger than 2 mm had cerebral oxygenation

less than 55 which is considered the lower end of the normal range. Many of these observations were from patients with severe IVH, which led to the variation in cTOI according to the size of the PDA observed in Figure 23 with patients with severe IVH and larger PDA having lower cTOI. As PDA diameter increases the blood volume stolen from the systemic circulation also increases and the brain perfusion may become compromised. However, the increased preload leads to higher stroke volume and higher left ventricular output, which becomes hyperdynamic and compensates the volume run off. The threshold when the steal phenomenon overcomes the compensatory ability of the premature myocardium which has reduced systolic and diastolic reserves is still unknown (262). There is also the component of the cerebral autoregulation which is impaired in the premature infant, but previous studies have demonstrated premature brain to be able to regulate cerebral blood flow in a considerably wide range of BP (21). These thresholds are possibly shifting depending on multiple other parameters affecting cerebral and cardiac function (GA, medication, background disease severity, infection, carbon dioxide etc).

There is conflicting evidence in the literature regarding the interaction of PDA with cerebral oxygenation. A study involving 38 extremely preterm infants found that there was no difference on cerebral oxygenation between infants with large (diameter >3mm) and moderate PDA size (diameter between 1.5-3mm) (159). However, Lemmers et al. found that there was a significant difference between infants with a PDA (PDA diameter > 1.4mm and LA:Ao ratio >1.4) and equally matched infants without clinical signs of PDA (however only 50% had echocardiographic confirmation)

(263). This study excluded infants with IVH > Grade 2. A more recent study from the same group claimed that “Cerebral oxygenation is related to the ductal diameter, as measured by echocardiographic examination, and cerebral oxygenation takes a different course over time, depending on the status of the duct”. However, these claims were not supported well by the presented data as there was no statistically and clinically significant difference between the compared groups (264).

Summary

There was a negative linear correlation between PDA diameter and cTOI (N=52, F=4.16, P=0.017). Many patients with PDA larger than 2 mm had cerebral oxygenation less than 55 which is considered the lower end of normal range. The PDA steal phenomenon is possibly involved in this interaction.

Cerebral oxygenation and severe IVH

There was a significant association between cTOI and severe IVH. cTOI was used as a marker of cerebral oxygenation and cerebral blood flow in our study. IVH occurred mainly in the first days after birth. All data before and after IVH appearance were included and, since there were more observation time points beyond the transitional period, the observed association could be a consequence of the altered cerebral haemodynamics imposed by the severe cerebral injury. However, it was previously demonstrated patients with severe IVH having simultaneously lower cTOI and larger PDA and higher RI. These complex interactions make the discussion regarding causation difficult. Nevertheless, cerebral hypoxia and hypoperfusion, whether a cause or aftermath, are implicated in IVH pathophysiology as previously discussed.

These results are consistent with the published literature. It has been previously shown that cTOI was higher and cerebral fractional oxygen extraction (cFOE) lower before the development of severe IVH in preterm infants <32 weeks (164). In the same study significantly more infants with severe IVH needed PDA treatment. These results were also replicated by Noori et al. who found cardiac function and cerebral blood flow to be stable in preterm infants who do not develop IVH during the transitional period. Those developing IVH during this period had lower systemic perfusion and cerebral blood flow followed by an increase in these variables preceding the development of IVH (265). Alderliesten et al. recently published a study demonstrating that cerebral hypoxia was associated with lower composite cognitive outcome at 24 months. However, the odds ratio was very low 1.019 (95% CI 1.003-1.035) (266). The same study found that a cTOI of <55% was significantly associated with severe IVH (OR 1.017; CI 1.007-1.026). This group did not find an association between hyperoxia and adverse neurodevelopmental outcome. However, another group found not only cerebral hypoxia, but also cerebral hyperoxia to be associated with poorer cognitive outcome. They found also that high cFOE is associated with poorer motor outcome (155).

Summary

Lower cTOI was associated with severe IVH (N=52, Coef=-0.0882, Z=-3.394, P=0.00690), higher RI and larger PDA. Reduced cerebral oxygenation may have been a reflection of the altered cerebral haemodynamics induced by the cerebral injury.

PDA size and anterior cerebral artery resistivity index

A positive correlation was found between PDA diameter and RI during a long period of observation from birth to discharge. RI is considered a marker of arterial resistance and it has been used as surrogate marker of blood flow for trend analysis (267). Resistance in systemic circulation is mainly affected by the peripheral vasculature (small arteries and arterioles). However, this applies more in adult studies where the cardiac anatomy and function, preload and afterload remain considerably stable over the study period. However, this is not applicable in studies in preterm infants where the PDA and PFO shunting change continuously with postnatal maturation and affect preload and afterload. This is also supported by animal studies which found that PI is not a direct measure of cerebral arterial resistance (268).

PDA connects the high resistance systemic circulation to the low resistance pulmonary circulation. DA diameter is equal to the descending aorta diameter at birth and subsequently decreases (269). This means that PDA can divert considerable blood volume leading to the PDA-induced steal phenomenon. The PDA steal phenomenon decreases diastolic blood pressure and the end-diastolic velocity in the cerebral arteries. Current ultrasound technology does not allow accurate measurement of the ACA diameter in extremely preterm infants and hence absolute blood flow cannot be calculated. RI was found to correlate linearly with cerebral perfusion pressure in an animal study (270). However, as an index its absolute value may remain stable if both systolic and diastolic velocities are equally affected and hence it may be unaffected in low blood flow situations which affect both the numerator and denominator of the

ratio. It is well known that PDA increases preload which leads to left atrial and left ventricular dilatation and subsequently increase in stroke volume (271). Increased stroke volume is associated with increased peak systolic velocity (272). PDA leads to increased peak systolic velocity induced by the increased preload and decreased diastolic velocity induced by the drop in vascular resistance which finally results in higher RI. Hence, RI can be also considered a surrogate marker of “steal phenomenon”.

Previous studies had mixed results regarding the effect of PDA on cerebral RI. Perlman et al. investigated the possible association between PDA and IVH (273). In a small population of more mature infants (mean GA 30 weeks) in the pre-steroids and pre-surfactant era, when PDA diagnosis was made from clinical signs and indirect echocardiographic biomarkers, they found that PDA increased the ACA pulsatility index (PI). They speculated, since fluctuations of blood flow velocity in the ACA may cause rupture of the capillaries of the germinal matrix, PDA maybe involved in the IVH pathophysiology (273). Subsequent studies demonstrated similar results (274). Shimada et al. found that ACA PI was higher in infants with haemodynamically significant PDA, but other biomarkers (ACA temporal mean blood flow velocities and vascular resistance) were not (97). These findings led them to the conclusion that compensatory increase of cardiac output can maintain cerebral perfusion despite significant left to right shunt across the PDA.

However, a recent small study of 25 extremely preterm infants with mean GA 27 weeks did not find a correlation between PDA diameter and middle cerebral artery RI in the

first week after birth (275). This was in contrast with the observed impaired blood flow patterns in renal and celiac arteries and attributed to the pre-ductal position of the neck vessels and the intact cerebral autoregulation of the study population. However, this may be due to the study being underpowered to show any significant difference.

Another large study which followed up 235 extremely preterm infants up to discharge found that RI indices in carotid arteries, basilar artery, anterior cerebral artery, and pial and striatal arteries differed with larger arteries showing significantly higher RI (276). RI in ACA was significantly higher in infants with hsPDA, but the difference was not large (0.75 vs. 0.82). However, this difference was not present in the rest of the arteries. Moreover, in contrast with previous studies (274, 277), they found no significant difference of RI pre- and post-PDA ligation. However, the sample size for this comparison was again small for this subgroup analysis. The same study emphasised the need for future studies to develop a methodology for simultaneous assessment of multiple biomarkers similar to the current study.

Summary

Increasing PDA size was associated with an increased RI (N=52, F=19.78, P<0.0001) and this could be attributed to the steal phenomenon.

Anterior cerebral artery resistivity index and cerebral oxygenation

There was a negative association between RI and cTOI during a long period of monitoring from birth to discharge. cTOI was used in the present study as a surrogate marker of cerebral oxygenation (228) and RI as a surrogate of steal phenomenon and

cerebral blood flow. It was previously demonstrated that PDA correlated with both cTOI and RI (263, 278). The fact that RI was correlated to cTOI means that PDA effects are not restricted locally in the descending aorta, but have further haemodynamic effects on cerebral circulation and affect cTOI. cTOI remained stable until a RI value of 0.75 and following this point started decreasing (Figure 26). This may represent a cut off point for the transition to loss of cerebral autoregulation (279). It is interesting that all patients with RI=1 had PDA diameter >1.5mm which is the cut off point for the diagnosis of hsPDA for many clinical trials for PDA treatment (Figure 22). Moreover, many patients with severe IVH had reduced cerebral oxygenation even when cerebral arterial resistance remained within normal limits (Figure 24). This maybe a reflection of the disturbed cerebral vascular control following a severe cerebral injury. However, there were many observations from patients without severe IVH who had very low cTOI and high RI. This, combined with the wide confidence intervals observed in the IVH group, suggested that the interaction of the aforementioned biomarkers with severe IVH was not statistically significant.

There are limited studies investigating the interaction of RI with cTOI in patients with PDA. A study involving 31 extremely preterm infants who were treated for hsPDA with ibuprofen during the transitional period found that the MCA RI and mean blood flow velocity had a positive correlation with cFOE before and after PDA treatment. Moreover, the mean blood flow velocity correlated positively with cTOI. However, the study did not find a significant correlation between PDA diameter and cTOI (280). Hoodbhoy et al. studied preterm infants who underwent PDA ligation at one month

after birth with a matched control group without PDA and found that infants in the PDA group had higher MCA RI, which normalized following ligation (281). Both MCA RI and PI decreased in the PDA group with concomitant decrease of peak systolic and increase in diastolic velocity.

Summary

There was a positive correlation between RI and cTOI (N=52, F=4.03, P=0.0035). This finding in combination with the other associations found in current thesis suggests that PDA has a potentially significant effect on the cerebral circulation resulting in diminished blood flow. Moreover, patients with severe IVH had reduced cerebral oxygenation even when cerebral arterial resistance remained within normal limits.

PDA size and Burdjalov score

A significant negative non-linear correlation was found between PDA diameter and Burdjalov score. However, there was no association between Burdjalov score and IVH severity. Infants with larger PDA had lower Burdjalov score, but this relationship was not linear (Figure 20). Burdjalov score increased with increasing maturity and the analysis included data over a long maturation period from birth to discharge from hospital. Moreover, beyond two weeks postnatal age only infants with persistent PDA were studied. This skewed the observed relation between the biomarkers as some infants in later postnatal age had both large PDA (as PDA size was increasing according to their body growth) and higher Burdjalov score as their brain was becoming more mature. Adjustment for postnatal age (Figure 21) abolished the skewness of the trend on Figure 20 and demonstrated a consistent trend of larger PDA size correlating with

lower Burdjalov score for both groups (infants younger and older than two weeks). These findings suggest that PDA may have a significant effect on cerebral electrical activity.

There are only few studies using EEG/aEEG to study PDA pathophysiology. A study with a similar population to the present study found that PDA has an independent negative effect on Burdjalov score and mainly affects aEEG continuity (184). Griemaier et al. found that Burdjalov score was reduced in preterm infants (GA 28–31 weeks) who received sedation and had a PDA as compared to preterm infants who received only sedation (205). However, the group of patients who received only sedation had significantly higher GA (30.1 vs. 29.5 weeks) and GA, as also shown in our study, is one of the main factors affecting Burdjalov score.

In the present study, the Burdjalov score increased rapidly during the first days after birth. This is consistent with a previous study demonstrating that rapid changes of EEG and aEEG traces take place during the first three days after birth and these are distinct to the slow changes associated with maturation (282, 283). During the transitional period from the intra- to extra-uterine life, evolution of EEG traces over one day is equivalent to 1-2.5 weeks of maturation of lateral postnatal ages (282). There are also other contributing factors affecting Burdjalov score. PNA and GA had the strongest influence on the development of the aEEG tracing as well as the administration of drugs such as morphine and caffeine (283).

Summary

There was a negative non-linear correlation between Burdjalov score and PDA diameter (N=52, F=12.55, P<0.0001). This is possibly due to lower cerebral blood perfusion induced by the PDA related steal phenomenon. However, this relationship is complex as postnatal maturation has a significant effect on evolution of Burdjalov score and will be further analysed in Chapter 4.

Burdjalov score and cerebral oxygenation

A significant association was found between the Burdjalov score and cTOI (nonlinear association). The ideal association according to physiology should be a bell shape curve as normal cTOI values (55-80) should correspond to higher Burdjalov score and abnormal values on each side of this range with lower Burdjalov score. However, as can be assessed in Figure 27 this relationship was skewed by low cTOI values which were related to higher Burdjalov score. These datasets came from more mature infants at a later postnatal age when infants with persistent large PDA had higher Burdjalov score values due to the maturation process. Adjustment for postnatal age (Figure 28) abolished the skewness of the trend on Figure 27 and demonstrated a bell shape relationship between cTOI and Burdjalov score for both groups (younger and older than two weeks) with cTOI values outside the normal range correlating with lower Burdjalov scores. This also signifies the importance of considering postnatal maturation when assessing the biomarker relationship during a long observation period.

It should be also noted that cerebral oxygenation was measured from the frontal lobes and aEEG was measured on the parietal lobes. Despite this spatial difference, a significant association was found, which means that the reduced cerebral oxygenation

in frontal lobe reflecting cerebral hypoperfusion may be more generalised and affect cerebral electrical activity in other brain areas. This is very possible as all cerebral arteries are connected and receive blood supply from the circle of Willis. However, a previous study demonstrated regional and hemispheric asymmetries in newborn cerebral blood flow using non-invasive optical techniques (158).

To the best of my knowledge this is the first time these two cerebral biomarkers have been used in the assessment of preterm infants with PDA. This methodology has previously mainly been used in infants with hypoxia-induced encephalopathy (284). A study which utilised this methodology during resuscitation found that preterm infants who developed severe IVH or died had significantly lower cTOI from 8 to 10 min of life (285). In that study aEEG was not predictive of death or IVH. There is evidence that changes in cTOI are likely to precede changes in EEG activity as indicated by a study in infants undergoing sedation with propofol. However, the underlying pathophysiology may be different in PDA physiology (286). A recent report emphasised the need for studies which utilise similar study methodology and pointed out that this may become the future of neonatal neuromonitoring (287).

Summary

There was a non-linear association between Burdjalov score and cerebral oxygenation in infants with PDA (N=52, F=5.78, P=0.0002). However, this association was skewed as the monitoring period was long with postnatal maturation also having a significant impact on Burdjalov score. Adjustment for postnatal maturation revealed a bell shape

relationship between Burdjalov score and cerebral oxygenation demonstrating that biological phenomena should not only be studied using linear models. Simultaneous monitoring of cerebral oxygenation and electrical function may provide additional information on the impact of PDA on cerebral haemodynamics.

Burdjalov score and anterior cerebral artery resistivity index

There was no association between Burdjalov score and ACA RI. To the best of my knowledge there are no studies addressing this relationship in infants with PDA. A negative correlation between Burdjalov score and RI would be expected on physiologic grounds; however, the overall trend pointed towards a positive correlation. The interpretation of the data was difficult as there was large variability of the observed biomarkers and maturation may have interfered with any possible relationship. Studies from different populations have shown conflicting outcomes. A study in extremely preterm infants showed that common carotid artery blood flow was not associated with EEG discontinuity. However, mean arterial BP had a negative relationship with EEG discontinuity (288). A study in very preterm IUGR infants found Burdjalov score to be lower and middle cerebral artery velocity to be higher compared to matched normal weight infants (289). It is interesting that in infants with complex congenital heart disease lower fetal cerebral Doppler indices showed significant association with decreased postnatal EEG indices and both were predictive of lower 18-month cognitive development scores (290). Victor et al. found in a population of preterm infants without a significant/persistent IVH no correlation between cardiac output and cerebral electrical activity (291). This study demonstrated that even the premature

brain has the ability for cerebral autoregulation and cerebral indices remain stable over a wide range of BP and cerebral flow.

A recent study in infants <32 weeks GA found that the Burdjalov score on day one was associated with trans-cerebellar diameter and biparietal width. On day three and in the first 72 hours, the Burdjalov score correlated with deep grey matter volumes. In the same study, Burdjalov score could also predict psychomotor developmental index (Bayley Scales of Infant Development II) at two years of age (292). There is evidence that early aEEG, recorded during the first hours after birth, correlates with long term outcomes in extremely preterm infants (293).

Summary

There was no significant association between Burdjalov score and ACA RI.

Burdjalov score and severe IVH

There was no association between Burdjalov score and severe IVH. This may be true or a consequence of reduced power of study in combination with our analysis methodology as data obtained over a long period of observations was aggregated into a single analysis. As it is demonstrated in Chapter 4 the stronger factors affecting Burdjalov score are postnatal age, morphine administration and gestation at birth. Hence, any useful predictive aEEG information detected over a short period may have been diluted by the noise created by the other parameters over a long observation period. However, results from studies focussed on the transitional period have shown that identifiable aEEG/EEG patterns are associated with IVH. EEG monitoring has a

predictive value on the grade of IVH and abnormal EEG patterns often precede ultrasound evidence of IVH (294). There are multiple studies which relate abnormal aEEG patterns with abnormal neurological and clinical outcomes (40, 177, 295). There is also emerging evidence that aEEG parameters observed during the first 72 hours of life were associated with altered deep grey matter volumes, biparietal width and transcerebellar diameter at term equivalent age, as measured by MRI, and they also correlated with neurodevelopmental outcome (292). However, there are also negative reports which did not detect any association between aEEG and severe IVH. In one study, this outcome may have been due to the many artefacts generated during the resuscitation process (285).

Summary

There was no demonstrable association between the Burdjalov score and severe IVH.

Death

There was no association between PDA and death. Almost all the deaths occurred well after the first week of life. Only one-third of patients died from respiratory failure and the rest died from late onset sepsis. No patient died or care withdrawn due to IVH. Researchers are encouraged to use death as clinical outcome as it is an irreversible outcome and has important impact on the wider family and society. However, this is not always appropriate when assessing causal associations as the link between the disease and death maybe indirect and/or weak. Based on pathophysiology, PDA can contribute to respiratory failure due to the pulmonary overperfusion and increased pulmonary pressures and finally to death. However, the link between PDA and sepsis

may not be direct and intuitive. Extremely preterm infants have a higher background risk of death and many predisposing risk factors (immature anatomy and physiology, unknown response to medication and multiple simultaneous medical interventions). Sepsis in preterm infants is primarily related to the immature immune system and protective mechanisms, the presence of multi-resistant microbial species in NICU and the multiple foreign body/equipment introduced to support their function and improve outcomes (intravascular lines, endotracheal tubes, gastroesophageal tubes etc). Hence, aggregating all causes of death in a composite death rate may not be informative of causation process or treatment efficacy.

Limitations

The study has several limitations. The sample size did not allow me to utilise more cardiac and cerebral biomarkers to build a more complex model as the study would have become underpowered. Hence, the analysis was limited to five biomarkers (one for every 10 patients). Consent issues made it challenging to recruit patients during the first three days of transitional circulation, when the most striking changes of cardiac and cerebral haemodynamics are observed. Many patients came from Level II neonatal units and enrolment was only possible on the second or third day of life. The same patients also had to be discharged back earlier, when they did not require intensive care. This led to variable number of different patients contributing data on each time point (Figure 8).

Technical issues and patients' critical clinical condition led to small percentage of missing data. Missing data on different observation time points necessitated the

application of multiple imputation procedure. However, it is always statistically preferable to impute rather than discard data. I discontinued monitoring patients with closed PDA after the second week, hence there were more data coming from patients with persistent PDA which skewed the observed chronological trends. The analysis utilised all the available data from birth to discharge and hence it is challenging to disentangle causation from aftermath relationship. RI and cTOI were obtained from the frontal lobe area, but aEEG from parietal area. Moreover, PDA and RI were instantaneous observations in comparison to cTOI and aEEG, which were the average of four hours monitoring and potentially could be influenced by other parameters.

Conclusion

I recruited 52 infants, monitored the PDA size, cerebral oxygenation, cerebral blood flow and electrical activity from birth to discharge and related these biomarkers to the occurrence of severe IVH. The results should be interpreted in view of the limitations described in pages 180-183. Babies with severe IVH had different temporal trajectories of the studied biomarkers (PDA diameter, PDA score, cTOI, Burdjalov score and RI) during the transitional period and this persisted for weeks after birth indicating significant interaction between cardiac and cerebral haemodynamics pre and post IVH occurrence. Time point analysis during the transitional period demonstrated that babies with severe IVH had lower cTOI on Day 3 after birth and this persisted after accounting for sepsis and inotropic support on logistic regression analysis. There was a very strong correlation between PDA score and PDA diameter, which can be considered collinear.

When all data were assessed simultaneously an association between severe IVH and PDA and cTOI was demonstrated. Furthermore, there was evidence of association between cTOI, Burdjalov score and RI with PDA, but not in interaction with IVH severity. This means that isolated steps of the two-step model are valid, but not the entire two-step model. This may be a true observation, a result of the study methodology as data were aggregated over a long period into a single analysis or related to the small sample size of the study. It seems that IVH is associated with the presence of large PDA and lower cerebral oxygenation. However, taking into consideration that IVH happens early during the extrauterine life or even during the

pregnancy and there were more observations reflecting the aftermath following the IVH, it is uncertain whether the observed relationships are the cause or the aftermath of IVH. There was also a strong association between PDA size with all the cerebral biomarkers, which implied that PDA may have important effects on global cerebral function. Patients with severe IVH had reduced cerebral oxygenation even when cerebral arterial resistance remained within normal limits. I speculate that this may be the reflection of disturbed cerebral vascular control following a severe cerebral injury.

We are all born with a DA and this may allow the left ventricle to adapt to the acutely increased afterload after birth. However, DA in some preterm infants has a different natural course and is linked to cerebral injury. Whether and at which point the PDA becomes a disease is still uncertain and it is very possible this goalpost to be shifting depending on the rest of the neonatal comorbidities. Future studies can investigate possible interactions between cerebral injury, altered autonomic function and altered natural course of DA closure leading to a viscous circle of hsPDA, steal phenomenon, cerebral hypoperfusion, autonomic dysfunction and prolonged PDA patency.

Chapter 4: Linear mixed-effects analysis of longitudinal measurements of Burdjalov score with assessment of the impact of morphine and gestational age

Purpose of the section

Chapter 3 shows that there are multiple inter-relationships between parameters related to cerebral haemodynamics. Of these, the parameter that is closest to "brain function" is the Burdjalov score. As a summary of electrical activity within the skull, the Burdjalov score reflects brain activity and may serve as a real-time marker of the impact of prematurity, and subsequent treatments. The Burdjalov score is a potential pharmacodynamic endpoint that could be used to monitor the effects and safety of treatments. There are other influences on the Burdjalov score, and it is important to understand these influences so that the effects of treatments can be recognised.

The purpose of this chapter is to characterise some associations with the Burdjalov score and assess whether any effects of a medicine such as morphine are expected to affect Burdjalov score. These findings will contribute to decisions about the use of Burdjalov score as a pharmacodynamic outcome. GA and postnatal age are known to affect Burdjalov score (174, 184). Morphine is widely used as an analgesic in neonatal practice and is known to have effects on the Burdjalov score (296, 297). The associations between PDA (the focus of this thesis) and Burdjalov score taking account of age have not been described. Accordingly, the objective of this chapter is to build a linear mixed effect model to assess the impact of gestational age at birth, postnatal age/maturation, morphine administration and PDA diameter on Burdjalov score.

Methods

Population

The population studied was the same as in Chapter 3.

Statistics

Statistical analysis was performed using the R statistical package and the rms library in consultation with Dr Antonio Eleuteri. Demographic factors were assessed using descriptive statistics. Descriptive data are reported as median and interquartile range for continuous parameters and as proportion (%) of the whole population for categorical outcomes. Groups compared using Mann-Whitney. A P value < 0.05 was considered significant. Detailed description of the statistical methods and model building is provided in the Results section.

Results

The data

The analysis included 52 infants with 268 complete observations. The observed values for time after birth ranged from 1 day to 114 days. Due to the extreme dispersion of observations (extreme values only appear once in the data, which can cause stability problems in the analysis) natural logarithm of time was considered. Figure 32 shows the Burdjalov scores vs. postnatal age for all patients. The time points at which a patient was treated with morphine are marked with red colour. Inspection of the Burdjalov score data suggested a general increase with postnatal maturation and that morphine administration corresponded to lower scores.

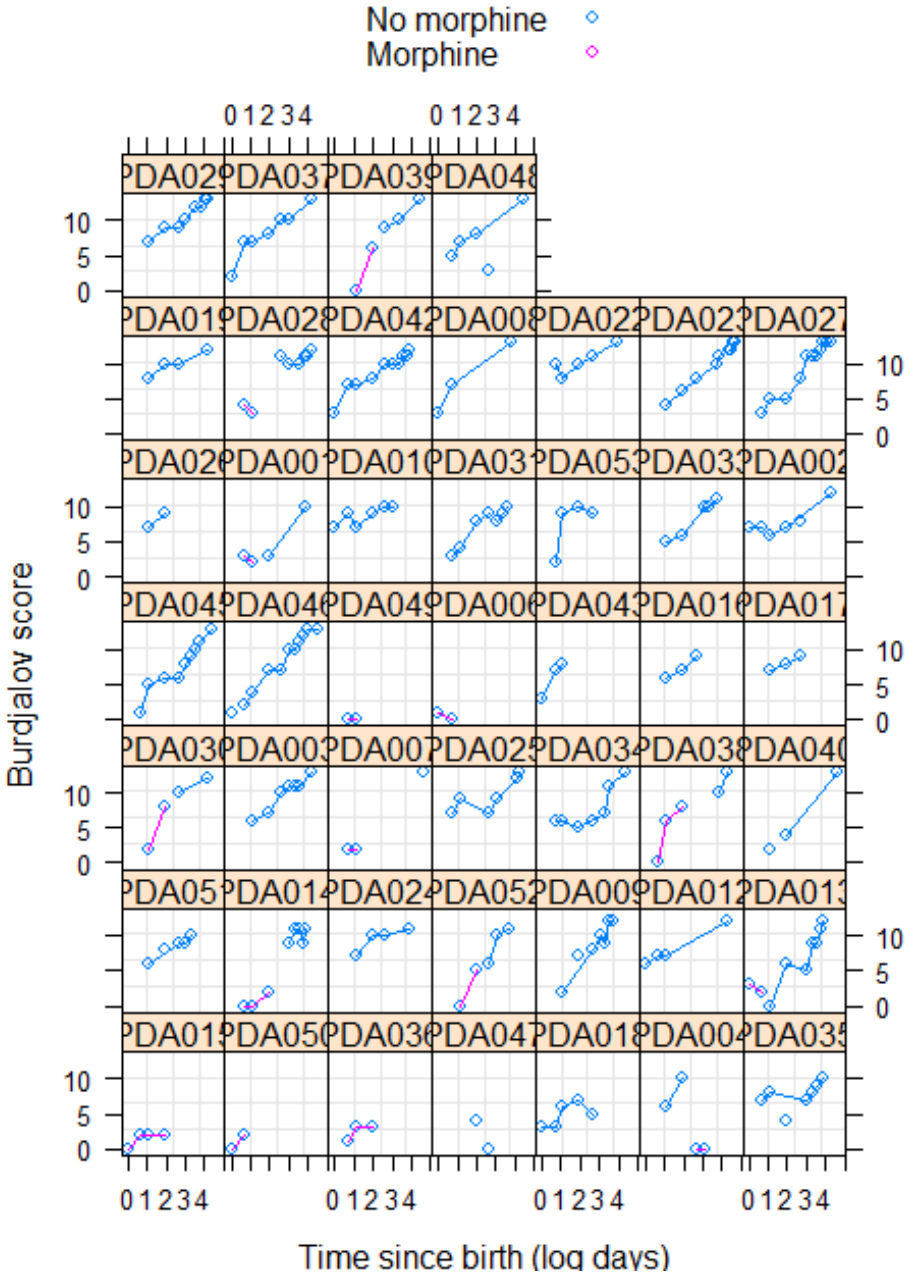

Figure 32: Observed Burdjalov scores vs. postnatal age (log scale) for each subject. Time points of subjects treated with morphine are shown in red.

Preliminary analysis

A simple linear regression of Burdjalov score vs. (log) postnatal age shows that it is important to explicitly model the longitudinal nature of the data (repeated measures on the same subject at different time points) (Figure 33). The boxplots of the regression residuals by subject demonstrate the importance of consideration for a subject effect in the model, as the average residuals (represented by dots) are not all centred around zero, and their variabilities (denoted by the blue boxes) are of different magnitude. If the data did not exhibit subject variability, the residuals would all have been centred around zero and their magnitudes would have been comparable. Thus, the same model cannot describe the whole population.

Model

Following consultation with Dr Antonio Eleuteri we subsequently fitted a series of linear regressions of Burdjalov score vs. (log) postnatal age for each subject (only 46 subjects could be used in this analysis since five patients had only one observation, and we required at least two observations per patient to assess the trend). Figure 33 shows the extreme inter-subject variability both in the intercept and slope of the model. Hence, it became necessary to build a more complex model in which both the intercept and model coefficients were random rather than fixed. Neither morphine nor gestational age information were used in these simple models, since at this stage we were only interested in assessing the appropriateness of a longitudinal model.

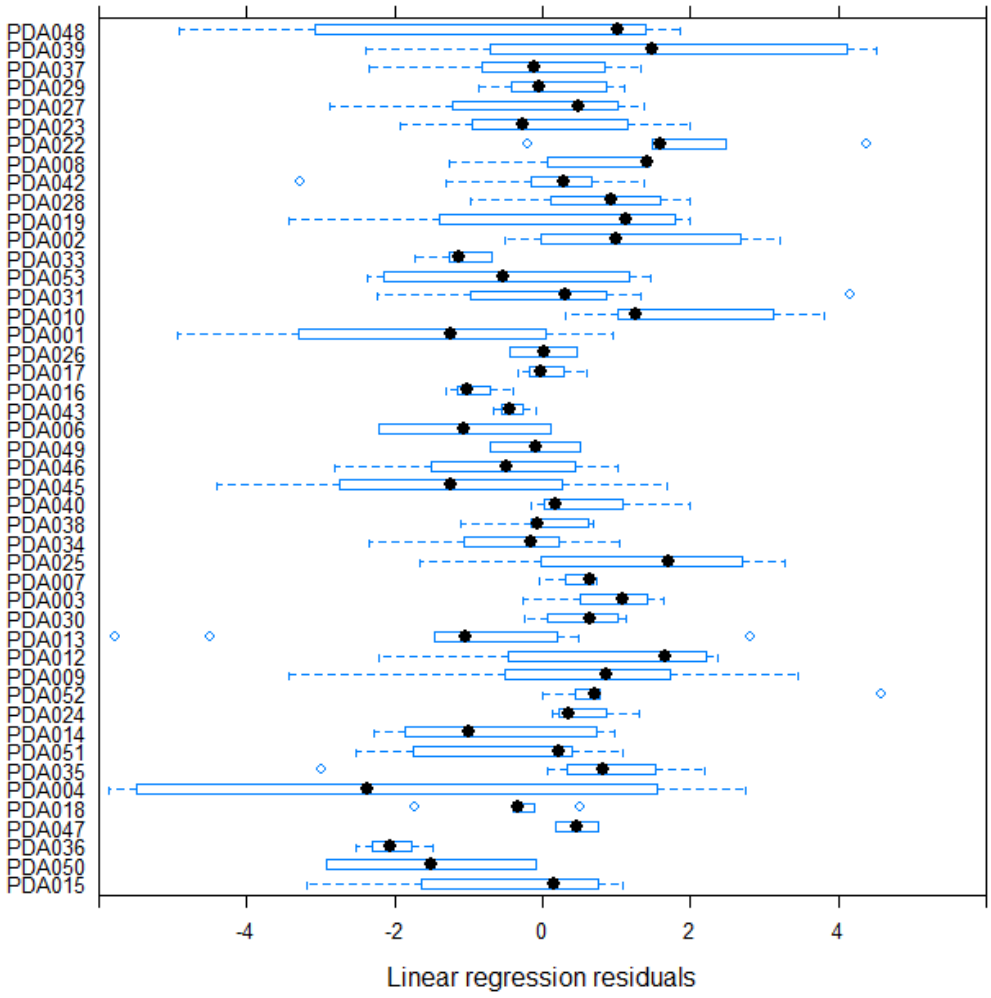

Figure 33: Linear regression residual plots of all individuals of the study for the assessment of model fit.

Demographics

The background demographics were similar to Chapter 3 and are presented divided into two groups: morphine (24 infants) vs. no morphine administration (22 infants) (Table 15). Morphine was given in 46 occasions out of 262 observations (17.5%). Morphine was given as an infusion and at a variable dose as per patients' condition and preference of the attending medical team. Morphine was mainly administered during the transitional circulation (30 out of 46 occasions) and subsequently on occasions when the infants became unwell with sepsis and needed intubation (up to the third week after birth). Infants treated with morphine had a significantly higher incidence of PROM (14 vs. 6, P-value=0.036), received antenatal antibiotics more frequently (20 vs. 12, P-value=0.036), were also significantly less mature (median (IQR), 25.9 (1.7) vs. 27.4 (2.2), P-value=0.002) and had lower BW (0.78 (1.7) vs. 0.95 (0.27), P-value=0.002) (Table 15). These infants had also lower APGAR scores at 5 min (5 (4) vs. 5 (5), P-value=0.013) and lower cord pH (P-value=0.019). All infants who died received morphine and this resulted in a statistically significant difference (7 vs. 0, P-value=0.007). Almost all the severe IVHs occurred also in the morphine group (five out of the six cases), however the difference was not statistically significant.

Maternal demographics		
	Morphine (N=24)	No Morphine (N=22)
Maternal BMI	27.3 (10.1)	24.8 (8.4)
Maternal age (years)	31 (5.5)	31 (7.5)
Twin pregnancy	4 (17%)	4 (19%)
Alcohol	1 (4%)	1 (5%)
Smoking	3 (13%)	1 (9%)
Hypertension	3 (13%)	4 (18%)
Chorioamnionitis	9 (38%)	6 (27%)
PROM	14 (58%) *	6 (27%) *
Perinatal antibiotics	20 (83%) *	12 (55%) *
Indomethacin	2 (8%)	2 (9%)
$MgSO_4$	15 (63%)	15 (68%)
IUGR	3 (13%)	0
Steroids		
Complete course	20 (83%)	15 (68%)
Any dose	4 (17%)	5 (23%)
Perinatal demographics		
GA (weeks)	25.9 (1.7) **	27.4 (2.2) **
BW (kg)	0.78 (0.23) **	0.95 (0.27)**
Male	18 (75%)	12 (55%)
Mode of delivery		
SVD	12 (50%)	12 (55%)
Emergency CS	12 (50%)	8 (35%)
Elective CS	0	1 (5%)
Instrumental	0	1 (5%)
Advanced resuscitation	12 (50%)	5 (23%)
APGAR 5	7 (4) *	8 (5) *
Cord pH	7.28 (0.19) *	7.34 (1.95) *

BMI: body mass index, PROM: prolonged rupture of membranes, SVD: spontaneous vaginal delivery, IUGR: intrauterine growth restriction. Data presented as number of patients or median and IQR or percentage as appropriate.
* P-value < 0.05, ** P-value= 0.002

Table 15: Summary of the background maternal and perinatal demographics.

.

Linear mixed-effects models

We next considered a longitudinal model where both the intercept and time coefficient were normal random variables. We adjusted also for gestational age and morphine. We also assessed whether gestational age and/or morphine enter the model as fixed effects or random effects. Three models were fitted, corresponding to different hypotheses as shown in Table 16. The best model was the one with morphine as an additional random effect as it had the lower AIC. This model is described in Table 17. All four parameters (postnatal maturation, use of morphine, GA and PDA size) had statistically significant impact on Burdjalov score variability with the postnatal maturation and use of morphine having the highest P-values. Postnatal maturation and use of morphine were the parameters with the stronger contribution to the Burdjalov score variability in our population, which was roughly ten times stronger in comparison to the PDA size and baseline GA at birth.

Random effect	df	AIC
Intercept + Time after birth	8	1012.05
Intercept + Time after birth + Morphine	11	967.47
Intercept + Time after birth + Gestational age	11	1018.05

Table 16: Assessment of best longitudinal model fit of the association with Burdjalov score.

df: number of parameters (degrees of freedom), AIC: Akaike Information Criterion, an information-theoretic measure of the trade-off between model complexity and model fit (the lower the AIC, the better the model).

Parameter	Estimated coefficient	Standard deviation of random effect	F	P-value
Postnatal age	2.047	0.671	268.12	<0.0001
Morphine	-2.91	2.91	473.99	<0.0001
Gestational age	0.279	-	7.72	0.0077
PDA	-0.194	-	4.45	0.036

Table 17: Random effects model of the interaction of Burdjalov score with the rest of the model parameters.

We next assessed whether the model assumptions were satisfied. The two fundamental assumptions were:

1- The within-group errors are normally and independently distributed with zero mean and constant variance
2- The random effects are normally distributed with mean zero and are independent for different groups

Figure 34 shows the residual boxplots from each subject. This was compared with the previous residual graph of the naive linear model (Figure 33). The boxplots in Figure 34 cluster around zero and they are much shorter, denoting a smaller variation. This suggests that the new model describes better the observation variation in the data. Figure 35 shows the quantile-quantile plot of the residuals vs. the standard normal. The assumption of normality seems reasonable although there is some asymmetry. Figure 36 shows the quantile plot of the estimated random effects in the model. The plots in Figure 36 are approximately linear, which means the model provides an accurate description of the observed variability in the data. In all cases, no strong deviation from normality can be detected, with the exception of the lower tail.

Interpretation

Our results can be interpreted as follows: for each week increase of postnatal and gestation age the Burdjalov score increases by 2.047 points and 0.279 respectively, whereas for each mm of PDA diameter increase Burdjalov score decreases by 0.194 and morphine administration results in Burdjalov score decrease by an average of 2.91

points. These results should be considered in conjunction with the fact that Burdjalov score ranges from zero to maximum fourteen points.

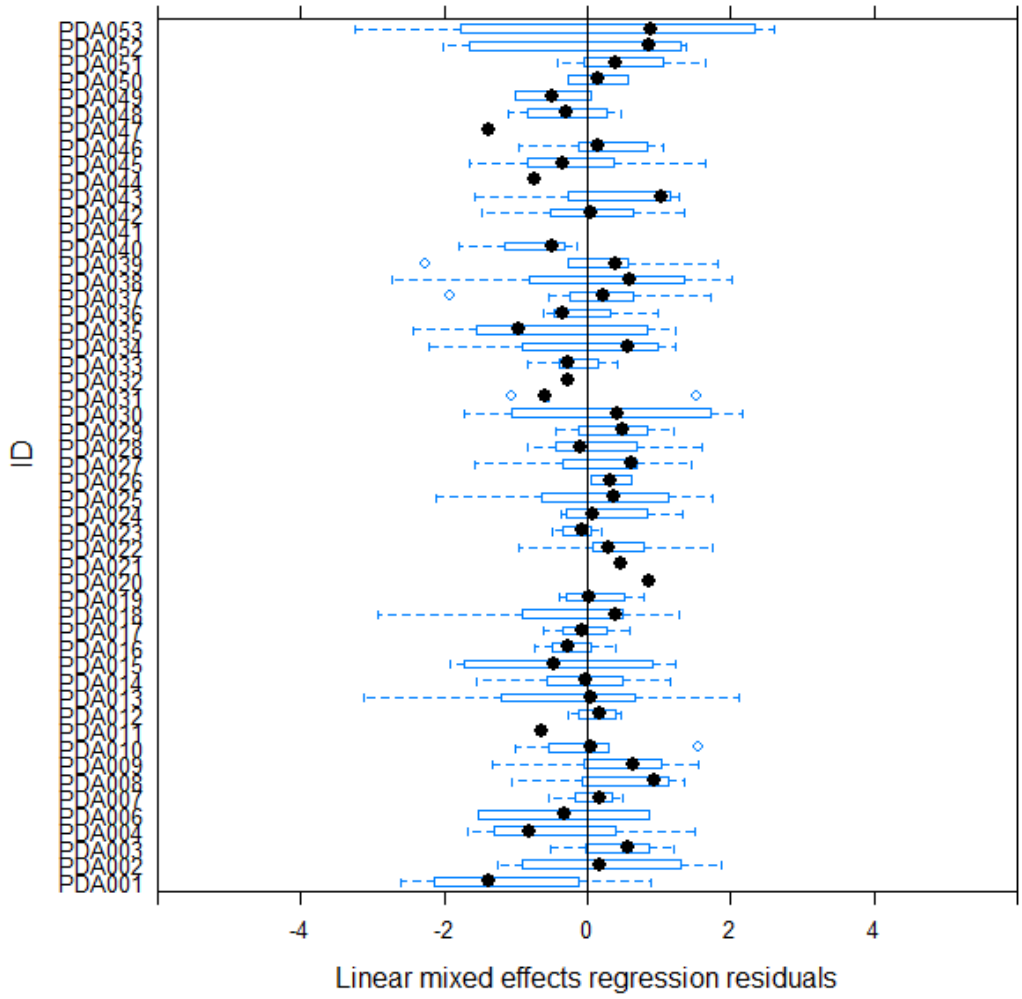

Figure 34: Linear mixed effects regression residuals for the model described in Table 17 assessing residual boxplots from each subject.

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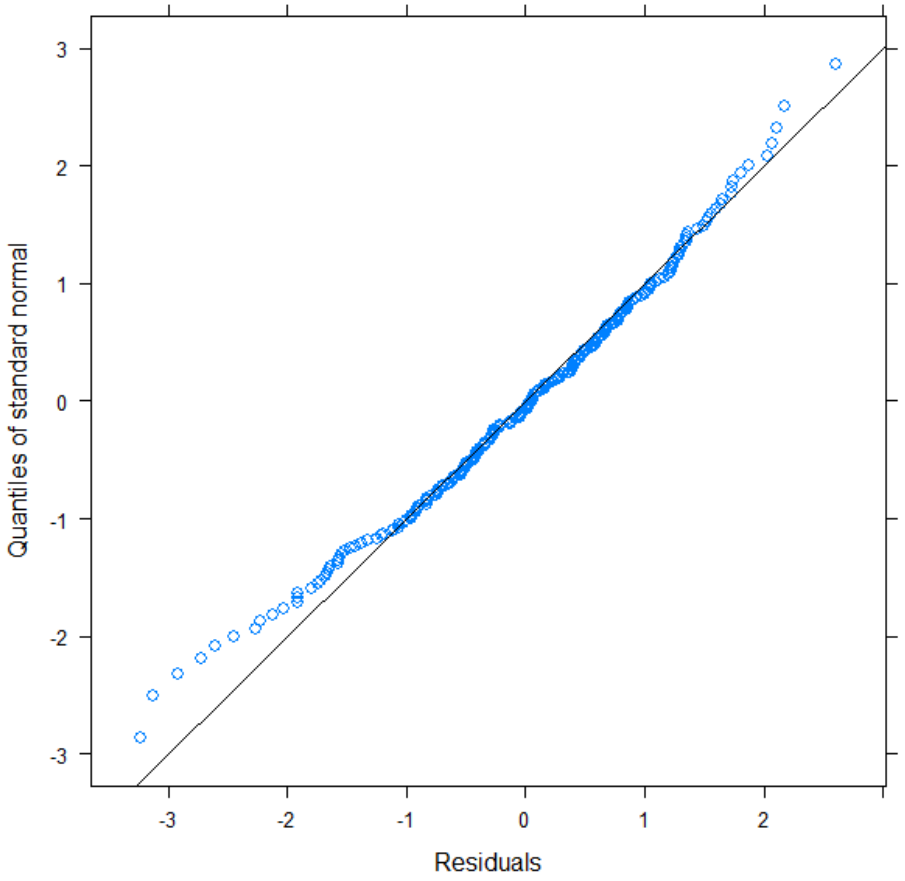

Figure 35: Quantile plot of the random effects in the model described in Table 17.

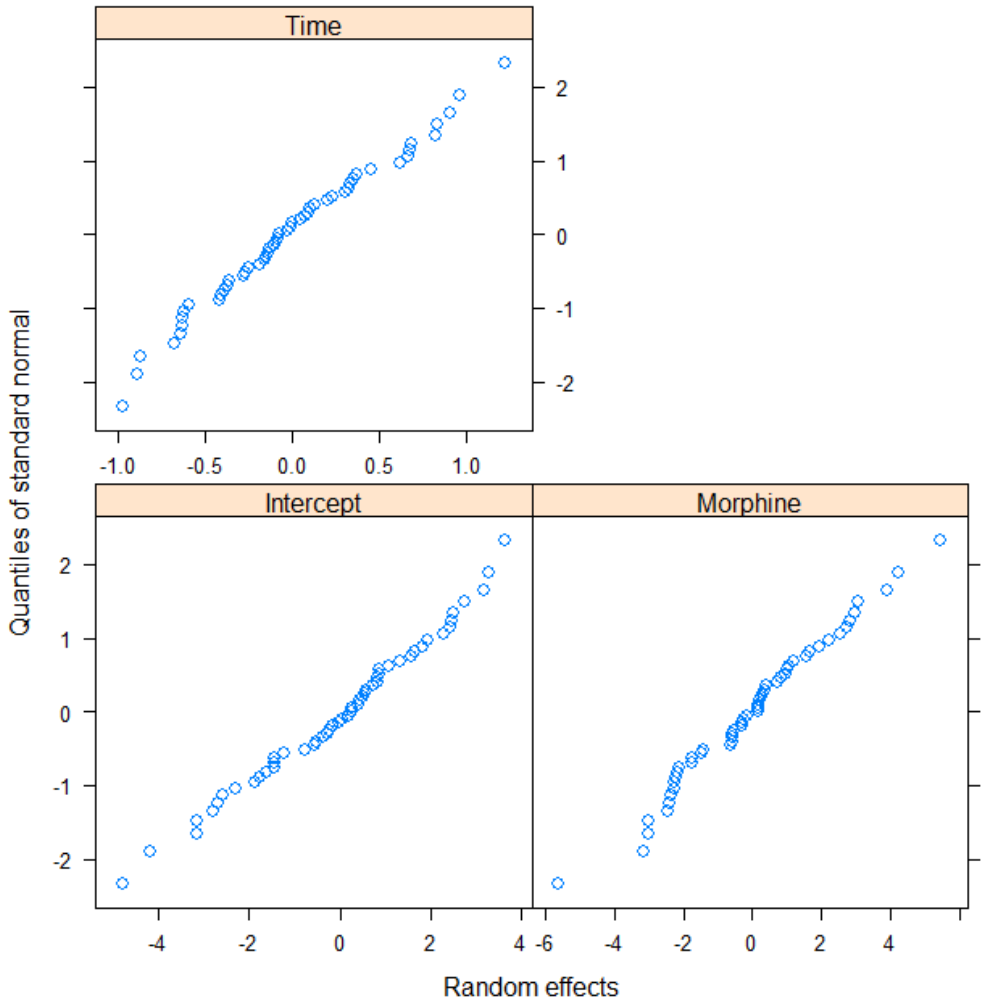

Figure 36: Quantile plots of random effects for intercept, time and morphine for the model shown in Table 17.

Summary

In conclusion, we modelled the longitudinal measurement of the Burdjalov score and its dependence on gestation and postnatal age, the PDA diameter and administration of morphine. The model suggests heterogeneity amongst subjects of time evolution of the Burdjalov score, and the effect of morphine on subjects. On average, Burdjalov score increases with postnatal age and with baseline gestational age; but decreases with morphine administration and the presence of a larger PDA.

Discussion

This is the first study which investigated the interaction between Burdjalov score, GA at birth, postnatal maturation, neonatal morphine administration and PDA diameter over a long period from birth to discharge in extremely preterm infants with all the common neonatal comorbidities. There are previous studies assessing the PDA effect on Burdjalov score, but PDA was considered as categorical variable (205). I found a significant relationship of all the studied parameters with Burdjalov score progression, but morphine administration had the strongest association with Burdjalov score followed by postnatal maturation, GA and PDA.

Morphine

It is well known that morphine administration has significant effects on aEEG activity (296, 297) and would therefore be expected to result in a lower total Burdjalov score. However, we monitored our patients from birth to discharge and morphine administration coincided with the first period of transitional circulation when infants were haemodynamically unstable and subsequently when they became unwell usually due to sepsis and required reintubation. Hence, morphine administration in our study coincided with periods of increased disease severity. Morphine also was given to more immature infants with lower BW who had higher prevalence of PROM and received more often antenatal antibiotics. Our model has accounted for GA and postnatal age, but any residual effects could not be excluded as it is known that all these parameters are related to worsen neonatal outcomes and higher prevalence of cerebral injury (298, 299). The higher prevalence of death in the infants receiving morphine should be

assessed in conjunction with the previous observations and considering that morphine administration is part of treatment protocol in critically unwell infants.

Our results are consistent with previous studies, which demonstrated that morphine depresses aEEG/EEG activity (283, 300, 301). Morphine is a strong opioid that activates four different opioid receptors and causes sedation and analgesia through its actions in the central nervous system (302). Morphine activates a G-protein coupled receptor. When the receptor is activated, calcium influx decreases resulting in a lower pre-synaptic neurotransmitter release. Moreover, morphine acts on potassium channels and promotes potassium efflux, which hyperpolarise post-synaptic neurons and results to a decrease of neurotransmitters excretion. These actions inhibit ascending neuronal pain pathways and the perception and response to pain is changed (303). The inhibitory neuronal effect is associated with the overall reduced cerebral activity observed by aEEG (304).

Moreover, there are possible interactions of morphine with the rest of the model parameters:

i. Morphine has vasodilatory properties, but its impact on PDA in preterm infants is unknown. Animal studies in rats have shown that morphine has cardioprotective effects and its underlying mechanism is possibly modulation of endothelial function and vasodilatation (305). Moreover, a study in humans demonstrated that morphine administration induces peripheral venous and arteriolar dilation by a reflex reduction in

sympathetic alpha adrenergic tone (306). There is lack of similar studies in preterm infants, but it is possible morphine to have vasodilatory effect on DA as we previously discussed the presence of sympathetic innervation in the DA and it is known from animal studies the in-utero preterm DA to be more sensitive to nitric oxide signalling to maintain its patency compared to term DA (84). However, a RCT has shown no difference in the incidence of PDA in infants treated with morphine, but the study was possibly underpowered to detect any difference (307).

ii. Morphine has also potential neuroprotective effects. Opioid receptors are involved on the neuroprotective effects during remote limb ischemic postconditioning demonstrated in neonatal rat pups and direct morphine administration after hypoxia was found to decrease infarct size (308). There is lack of clinical studies, but the δ-opioid receptor is considered a potential therapeutic target for stroke (309).

iii. There are many studies demonstrating the adverse effects of pain on cerebral growth and neurodevelopmental outcome of preterm infants (310). Two large RCTs have demonstrated beneficial effects of morphine administration in ventilated infants with reduction of IVH incidence in the treated group (311, 312).

iv. On the other hand, there are concerns that morphine may have toxic effects on rapidly growing and developing brain of extremely preterm infants. There is inconsistent evidence regarding its long-term effects. In

vitro and animal studies raised concerns regarding a possible apoptotic effect after neonatal administration of morphine with long term consequences (313, 314). Concerns were raised by some clinical studies that its inhibitory action may alter cerebral anatomy or structure (315-317). However, existing studies have not demonstrated any strong evidence of adverse neurodevelpmental outcomes (316, 318, 319).

v. GA and postnatal maturation can also have a role in the morphine-brain function interaction as there is evidence of developmental and maturation processes in the presence and function of opioid receptors in the preterm brain (320). However, there are no data from human studies.

Gestation age and postnatal maturation

GA and postnatal age have significant impact on Burdjalov score progression and these results are consistent with previous studies (170, 171, 174, 205, 321-324). A study in preterm infants without IVH found that the effect of postnatal age on aEEG parameters was 6.4 to 11.3 times higher than GA (283). In univariate analysis, indomethacin and morphine administration were associated with aEEG measures. However, on multivariate regression only GA and PNA were associated with increased qualitative and quantitative aEEG measures. It is possible that the significance of GA in our study would have been stronger, if the inclusion criteria allowed recruitment of infants with wider GA range.

The biological background of these observations lies on the progressive maturation of fetal and neonatal brain with advanced GA and postnatal maturation (325, 326). The underlying mechanisms between neuronal activity and connectivity in relation to the observed EEG/aEEG patterns are poorly understood in preterm infants and are beyond the scope of the current analysis (327). Neuronal migration is almost completed by 23 weeks gestation age, but the cerebral cortex is still unfolded and undergoes rapid folding and maturation in the subsequent weeks (328). A small study of very preterm infants without IVH showed the significant correlation between the inter-burst interval in the EEG and cortical folding on magnetic resonance imaging with postnatal age (329). Moreover, the burst interval duration is a marker of the overall EEG background activity and was found to be correlated to the overall cortical folding. Finally, a previous study demonstrated Burdjalov score and MRI to be predictive of neurodevelopmental outcome at 2 years of corrected age in very preterm infants (292).

In Chapter 3 an association between IVH and Burdjalov score was demonstrated. However, IVH was not included in the current model due to the limited power of the study (one factor per ten patients). It is known that severe IVH has significant and prolonged effects on aEEG as all patterns assessed in Burdjalov score were found to be lower in infants with IVH and some of them persisted up to 36 weeks' corrected age (206). Clinical studies demonstrated that early aEEG pattern can predict both short- and long-term outcome in preterm infants (172, 293).

Patent Ductus Arteriosus

PDA has significant effects on cerebral electrical activity. Chapter 3 discussed the interaction of PDA with the rest of the cerebral biomarkers and more specifically with Burdjalov score. On the basis of the current analysis the strength of each individual parameter in the progression of Burdjalov score was evaluated. Considering PDA as a reference for comparison of the strength of the other variables on the Burdjalov score, each mm increase of PDA diameter was equivalent to 1 week of gestation age at birth, morphine administration had an effect that was 15 times stronger and one week of postnatal age had 10.5 times stronger effect. These results provide a novel perspective on the assessment of the significance of PDA in relation to the risk of cerebral injury as it is very important to take into account the GA. If we assume a causative role for PDA, in an infant born at 24 weeks a 2mm PDA will have a different impact on cerebral function with different added risk for cerebral injury than the same size PDA in an infant born at 27 weeks. The results of the present analysis are consistent with other studies (184) and the results in Chapter 3, however the present study is the first to evaluate and quantify the impact of the PDA diameter on Burjdalov score during a period from birth to discharge.

Limitations

The study size did not allow inclusion of more variables in the model. This was the main reason for including the Burdjalov score which is a composite score. Sample size considerations were also the reason for not including IVH severity in the model. Morphine administration in our study coincided with periods of greater disease severity and was associated with lower gestation and BW, which are all associated with

impaired cerebral function and worse clinical outcomes. The statistical model has accounted for GA and postnatal age, but any residual effects could not be excluded as it is known that all these parameters are related to worsen neonatal outcomes and higher prevalence of cerebral injury.

Future studies

These studies need to be replicated in order to establish whether the results are generalisable. Comparisons between studies, sites and populations will be facilitated by the establishment of technical standards for the conduct of each assessment. If the results are consistent across sites and populations, it will be important to identify the relationship between the Burdjalov score and clinically meaningful outcomes. Outcomes that are meaningful to clinicians could include neurodevelopmental assessments and educational attainment. It is important to consider outcomes that are important to survivors of birth at extreme prematurity and their parents. The Burdjalov score will only be a useful pharmacodynamic outcome if changes in Burdjalov score predict changes in clinically meaningful outcomes. Once these essential studies to underpin the use of Burdjalov score have been conducted, it could be used in early phase studies of interventions to prevent or treat cerebral complications of birth at extreme prematurity.

Conclusion

This was a prospective observational study which followed up extremely preterm infants with a PDA from birth to discharge. It demonstrated that cerebral electrical

activity increased with postnatal age and with baseline gestational age, but decreased with morphine administration and the presence of a larger PDA. The findings confirmed:

- the significant effects of morphine administration on cerebral function and the potential short- and long-term effects
- the maturation processes that take place following preterm birth
- that GA at birth is an independent significant parameter when aEEG patterns are assessed
- PDA has significant effects on cerebral electrical activity.

The study showed that the Burdjalov score can be standardised for known influences and used to assess the effects of a treatment.

Chapter 5: Neonatal cardiovascular and cerebral function following antenatal maternal $MgSO_4$ administration

Purpose

This Chapter addresses Objective III in Chapter 1 in order to assess the extent to which an intrapartum administered vasodilator ($MgSO_4$) is associated with PDA function and cerebral physiology. The null hypothesis was that antenatal $MgSO_4$ administration has no effect on neonatal serum Mg^{2+}, PDA score and cerebral biomarkers.

Methods

This was a prospective convenience observational study recruiting infants 24-28+6 weeks' gestation and postnatal age ≤72 hours. The same cohort of patients as described in Chapter 3 was investigated and methods were previously described in Chapter 2 (page 71).

Results

Data

Fifty-one infants were recruited with median GA 26.6 weeks [Interquartile range (IQR) 25.7-28.0] and median BW 900 grams (IQR 760-1,080). Other demographic variables are shown in Table 18. Thirty-three mothers (65%) received antenatal $MgSO_4$ for neuroprotection (including seven who had preeclampsia) and eighteen did not receive $MgSO_4$. Half of the infants, who were not exposed to maternally administered $MgSO_4$, were transferred from a hospital where $MgSO_4$ administration for neuroprotection was not standard practice; the rest were not exposed to $MgSO_4$ due to emergency situations [home birth (one), cord prolapse (two), placental abruption (two), not enough time (two) and $MgSO_4$ not being considered as a therapeutic option (three)]. The median duration of $MgSO_4$ infusion was 7.5 hours (IQR 3.0-12.0). Background

demographics were similar between the two groups without a statistically significant difference.

	No antenatal $MgSO_4$	Antenatal $MgSO_4$
Patients, n (%)	18 (35)	33 (65)
Maternal age (years)	32 (31-35)	31 (26-34)
Maternal booking BMI (kg/m^2)	24.7 (23.6-37.1)	26.2 (22.0-32.4)
SGA, n (%)	1 (2)	4 (8)
Chorioamnionitis, n (%)	4 (8)	11 (21)
Onset of labour		
Spontaneous preterm labour, n (%)	17 (33)	26 (51)
Iatrogenic: Pre-eclampsia	1 (2)	7 (14)
Antenatal steroids, n (%)		
No	2 (4)	0
1 dose	5 (10)	6 (12)
2 doses	11 (21)	27 (53)
Mode of delivery, n (%)		
Vaginal	12 (24)	17 (33)
Caesarean	6 (12)	16 (31)
$MgSO_4$ infusion duration (hours)	0	7.5 (3-12)
Male infants, n (%)	11 (21)	21 (41)
Gestation (weeks)	26.5 (25.7-27.7)	26.7 (25.4-28.2)
Birth weight (Kg)	0.92 (0.718-1.17)	0.89 (0.76-0.975)
Cord BE (mmol/L)	-6.4 (-0.6-(-16.3))	-3.4 (-2-(-6.1))
APGAR 5 minutes	8 (3-9)	7 (6-8)
Data presented as median and IQR. BMI: Body mass index, PROM: prolong rupture of membranes, PIH: Pregnancy induced hypertension, BE: base excess, SGA: small for gestational age.		

Table 18: Maternal and neonatal demographics among those with and without antenatal $MgSO_4$ administration.

Preliminary analysis

Regression analysis showed a significant association between $MgSO_4$ exposure and neonatal Mg^{2+} during the first three days after birth (coefficient=0.21, F= 5.31, P=0.0024, Figure 37). Figure 37 suggests that the relationship is nonlinear, so it cannot be described by a single parameter and the model coefficient provides a simple estimate of the "trend" demonstrated by the graph.

Model

BW and maternal BMI are the background demographic parameters which may have a significant effect on Mg^{2+} and hence on cerebral and cardiovascular biomarkers and severity of cerebral injury. There was no significant association between Mg^{2+} and neonatal BW, PDA score, cerebral oxygenation index, Burdjalov score or IVH (Table 19). Maternal BMI was inversely strongly associated with Mg^{2+} (coefficient=-0.0029, F=6.86, P=0.0018). There was also evidence of a moderate interaction between BMI and $MgSO_4$ exposure (coefficient=-0.0078, F=5.01, P=0.028). Figure 38 shows a graphical representation of these associations and interactions.

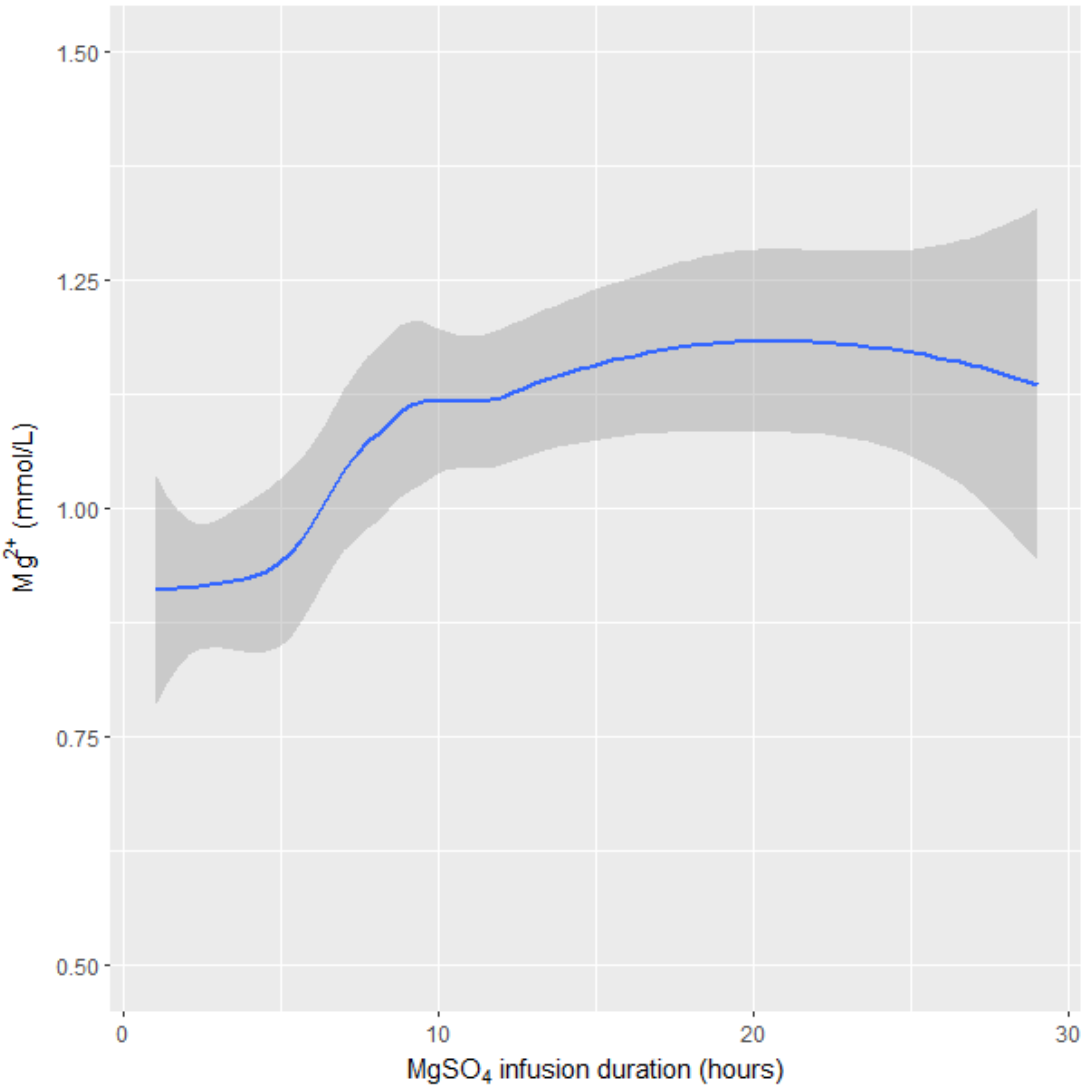

Figure 37: Smoothed local polynomial regression fits (with 95% confidence intervals) of the relationship between duration of antenatal $MgSO_4$ infusion and the longitudinal measurements (over a 72 hours period) of neonatal Mg^{2+}.

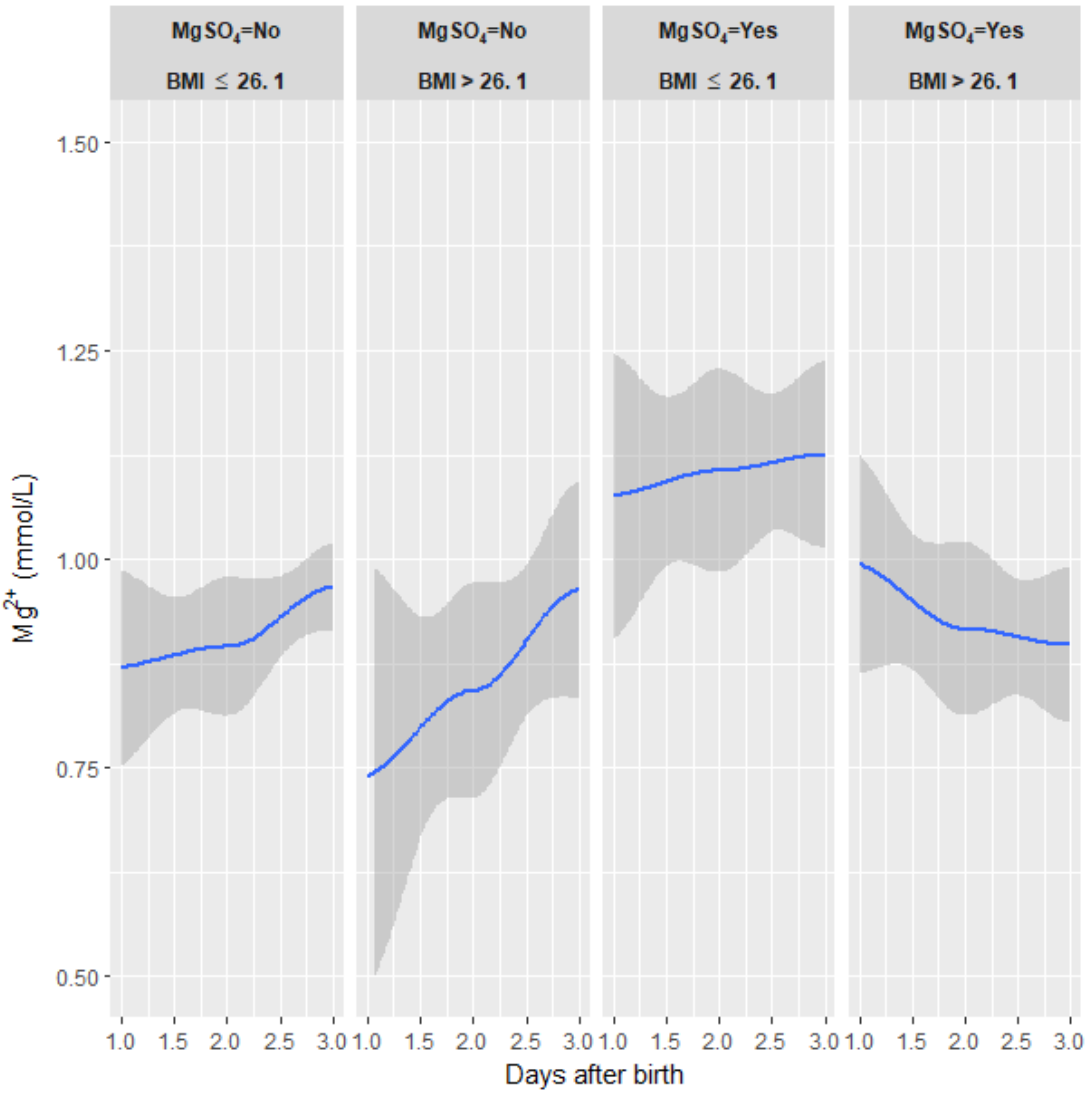

Figure 38: Impact of BMI and antenatal $MgSO_4$ administration on neonatal serum magnesium concentrations.

Factor	Coefficient	F	P-value
Antenatal $MgSO_4$	0.21	5.31	0.0024
BMI	-0.0029	6.86	0.0018
BMI * Antenatal $MgSO_4$	-0.0078	5.01	0.028
BW	0.0022	0.51	0.60
BW * Antenatal $MgSO_4$	0.15	0.61	0.44
PDA score	0.0015	0.48	0.62
PDA score * Antenatal $MgSO_4$	0.0039	0.22	0.64
Burdjalov score	0.0002	1.82	0.17
Burdjalov score * Antenatal $MgSO_4$	0.017	1.82	0.18
cTOI	-0.000036	0.13	0.88
cTOI * Antenatal $MgSO_4$	0.0052	0.20	0.65
IVH severity	-0.066	1.66	0.20
IVH severity * Antenatal $MgSO_4$	-0.014	0.03	0.86
BMI: Body mass index, BW: birth weight, PDA: patent ductus arteriosus, cTOI: cerebral tissue oxygenation index, IVH: intraventricular haemorrhage. The asterisks (*) denote interaction (modelled as multiplication).			

Table 19: Linear regression model of neonatal Mg^{2+} and associated variables and biomarkers.

Statistics interpretation

A maternal BMI increase from 20 to 40 kg/m^2 was associated with a decrease in neonatal Mg^{2+} by an average of 0.06mmol/L and 0.16mmol/L, if $MgSO_4$ is not given. $MgSO_4$ administration results on average in a 0.21mmol/L increase of neonatal Mg^{2+}. Neonatal Mg^{2+} does not have any significant effect on PDA score and cerebral biomarkers.

Protocol of power analyses for cerebral oxygenation and Burdjalov score

Purpose

To inform a future study which will assess the relationship between antenatal $MgSO_4$ and perinatal pharmacokinetics and pharmacodynamics to indicate the optimal dose and infusion duration of $MgSO_4$, the sample size required to detect differences in the two parameters' means (cTOI and Burdjalov score) between subjects who have received or not $MgSO_4$ were calculated. To perform power calculations an estimate of the effect size was required. To calculate the effect size, assuming equal sample sizes in both groups, estimates of the standard deviations of the measurements in the two groups were also required. Since these are not known a priori, estimates from the available data were used (measurements were taken at day 3 after birth).

Cerebral oxygenation

Table 20 shows the sample standard deviation with 95% confidence intervals for the measurements of cerebral oxygenation.

Number of measurements	Sample standard deviation	95% Confidence interval
17 (no $MgSO_4$)	8.65	[6.45, 13.17]
28 ($MgSO_4$)	6.99	[5.53, 9.51]

Table 20: Cerebral oxygenation: sample standard deviation with 95% confidence intervals of the measurements.

The previous analysis in Chapter 3 (Figure 10) as well as previous studies have demonstrated that the mean standard deviation of the cTOI values in preterm infants are in the range of five units (330). Moreover, the normal cTOI range is considered between 55-80 (25 units difference) which results in a calculated standard deviation approximately 5 units. Hence, it was assumed that a clinically relevant difference is 5 units. Table 21 shows the estimated sample sizes in two different scenarios: the first one using the sample standard deviation estimated from the data, the second one using the upper confidence limit on the sample standard deviation (for a conservative estimate of sample size). Both one tail and two tail options were considered, and three power levels. The significance level was set to 0.05.

	One tail	Two tails
Sample standard deviation (effect size: 0.64)	Power 0.95: 55 per group (total 110) Power 0.9: 43 per group (total 86) Power 0.8: 31 per group (total 62)	Power 0.95: 66 per group (total 132) Power 0.9: 53 per group (total 106) Power 0.8: 40 per group (total 80)
Upper confidence limit of sample standard deviation (effect size: 0.44)	Power 0.95: 115 per group (total 230) Power 0.9: 90 per group (total 180) Power 0.8: 65 per group (total 130)	Power 0.95: 139 per group (total 278) Power 0.9: 110 per group (total 220) Power 0.8: 83 per group (total 166)

Table 21: Summary of estimated sample sizes in two different scenarios using cerebral oxygenation index as surrogate biomarker to demonstrate clinical efficacy: 1. the sample standard deviation estimated from the data, 2. using the upper confidence limit on the sample standard deviation (for a conservative estimate of sample size). Both one tail and two tails options were considered, and three power levels. The significance level was set to 0.05.

Burdjalov score

Table 22 shows the sample standard deviation with 95% confidence intervals for the measurements of Burdjalov score. It was assumed that a clinically relevant difference is two units. Rasler et al. studied 232 babies and found that the difference in median Burdjalov scores between infants who had a severe outcome at one year corrected gestational age and those with a normal outcome was two points (331). Table 23 shows the estimated sample sizes in two different scenarios: the first one using the sample standard deviation estimated from the data, the second one using the upper confidence limit on the sample standard deviation (for a conservative estimate of sample size). Both one tail and two tails options were considered, and three power levels. The significance level was set at 0.05.

Number of measurements	Sample standard deviation	95% Confidence interval
17 (no $MgSO_4$)	2.00	[1.49, 3.05]
27 ($MgSO_4$)	3.11	[2.45, 4.26]

Table 22: Burdjalov score: sample standard deviation with 95% confidence intervals of the measurements.

	One tail	Two tails
Sample standard deviation (effect size: 0.76)	Power 0.95: 38 per group (total 76) Power 0.9: 31 per group (total 62) Power 0.8: 23 per group (total 46)	Power 0.95: 46 per group (total 92) Power 0.9: 38 per group (76 total) Power 0.8: 29 per group (58 total)
Upper confidence limit of sample standard deviation (effect size: 0.54)	Power 0.95: 75 per group (total 150) Power 0.9: 60 per group (total 120) Power 0.8: 44 per group (total 88)	Power 0.95: 91 per group (total 182) Power 0.9: 74 per group (total 148) Power 0.8: 55 per group (total 110)

Table 23: Summary of estimated sample sizes in two different scenarios using Burdjalov score as surrogate biomarker to demonstrate clinical efficacy: 1. the sample standard deviation estimated from the data, 2. using the upper confidence limit on the sample standard deviation (for a conservative estimate of sample size). Both one tail and two tails options were considered, and three power levels. The significance level was set to 0.05.

Interpretation of statistics

If a future two-armed clinical trial uses cTOI as surrogate biomarker for demonstrating the clinical efficacy of antenatal $MgSO_4$, it will need to recruit a total number of patients ranging from 80 to 132 depending on the desirable power (0.8 to 0.95 respectively, effect size: 0.64) (Table 21). If the probability to detect a positive result needs to be higher, 278 patients should be recruited (considering the upper confidence limit of sample standard deviation with 0.95 power and P-value 0.05; effect size 0.44). Respectively, Table 23 demonstrates that if Burdjalov score is used instead of cerebral oxygenation index, the number of patients needed to be recruited is lower; 58, 92 and 182 respectively.

Discussion

This is the first study to examine the complex interaction of antenatal $MgSO_4$ administration with neonatal Mg^{2+} and neonatal haemodynamics using echocardiography, cTOI and aEEG during the first three days after birth. We found that $MgSO_4$ administration, infusion duration and maternal BMI had a significant influence on neonatal Mg^{2+}. $MgSO_4$ administration and neonatal Mg^{2+} did not have a significant effect on cardiovascular and cerebral biomarkers following multivariate analysis.

There is limited evidence regarding $MgSO_4$ PK in pregnancy (332) and whether an one- or two- compartment model is appropriate appears to depend on the clinical situation. Thus, Lu and colleagues described the $MgSO_4$ PK profile after intravenous administration as a 2-compartment model with a rapid distribution alpha phase followed by a relative slow beta phase of elimination (57), but Brookfield et al. suggested that preeclampsia may alter the Mg^{2+} pharmacokinetics and described an one-compartment model (332). $MgSO_4$ half-life in eclamptic mothers is four hours and appears to increase with renal failure (333). Animal studies found that $MgSO_4$ administration in pregnant rats crosses the placenta within two hours, can be detected in the fetal cerebrospinal fluid and preferentially concentrates into the forebrain (334, 335). Moreover, Mg^{2+} crosses the placental barrier actively (336) and there is positive correlation between maternal serum and umbilical cord Mg^{2+} (337, 338).

I found that neonatal Mg^{2+} increased during the first three days after birth in infants not treated with $MgSO_4$ (Figure 38) as previously reported (339), but remained stable in infants exposed to $MgSO_4$ and Mg^{2+} levels were higher than those considered

"normal". Figure 38 shows that the infants with the highest Mg^{2+} were those of mothers with the lowest BMI scores. Those infants with supranormal Mg^{2+} warrant further investigation as the biological actions of magnesium have been shown to be beneficial (44). However, a retrospective cohort study demonstrated that in a subset of preterm human infants with serum magnesium levels >2.3 mmol/L, there was increased mortality independent of BW and GA (340, 341). The definition of normality in a preterm population is difficult, but future studies should be designed in view of the present findings to assess an "ideal" or "normal" range.

The duration of $MgSO_4$ infusion is an important factor affecting Mg^{2+} as it determines the total amount of $MgSO_4$ administered to mothers and infants and is consistent with previous studies (342). Infants in the present study needed a maternal $MgSO_4$ infusion for more than eight hours to achieve Mg^{2+} levels above 1 mmol/L (Figure 37). This may indicate the need of at least 8 hours of infusion prior to birth for neuroprotective effects to be evident or that the loading dose was inadequate. However, another study did not find an association between infusion duration and clinical effect (neonatal death, cerebral palsy and multiple other neonatal morbidities) (52).

The effect of maternal BMI on Mg^{2+} was more apparent on days 2 and 3 (Figure 38). The clinical significance of BMI on $Mg2^{+}$ was mild as increasing BMI from 20 to 40 kg/m2 decreased Mg^{2+} by around 0.06mmol/L without $MgSO_4$ infusion, and by 0.2mmol/L with $MgSO_4$ infusion. A recent study found that women with higher BMI needed a longer duration of $MgSO_4$ infusion to reach a steady state, suggesting that

they may benefit from a tailored loading dose (343). Furthermore, in a study for eclampsia prophylaxis half of women who received 6 g $MgSO_4$ and then 2 g/h had subtherapeutic Mg^{2+} (aimed therapeutic range of 2 to 3.45 mmol/L) and BMI>30 was associated with subtherapeutic levels (344). Pregnancy may be associated with altered distribution of maternal Mg^{2+} due to weight gain that includes oedema and increased fat (58). These observations, supported by the results of our study, highlight the importance of achieving a better understanding of the handling of drugs during pregnancy and their effects on the fetus, and more precise individualised prescribing.

The multivariate model did not find a significant association between neonatal Mg^{2+} and PDA score (including interactions of the latter with $MgSO_4$). This is consistent with previous reports that found that antenatal $MgSO_4$ produced no consistent cardiovascular effects in the infant in the first 24 hours (345). However, other investigators found that higher Mg^{2+} at birth, but within normal limits, were associated with a delayed closure of the ductus arteriosus, and less severe IVH (346).

There is limited evidence regarding the effect of Mg^{2+} on cerebral haemodynamics and function. The multivariate model did not find any significant association between Mg^{2+} and cTOI or cerebral electrical activity. Verhagen et al. found that treating pregnant women with labetalol and/or $MgSO_4$ may influence cerebral oxygen extraction in their offspring shortly after birth as this intervention decreased cFOE on the first day after birth (347). Infants exposed to $MgSO_4$ compared to non-exposed babies had similar systemic and cerebral hemodynamics, but lower cFOE. These findings suggest reduced

cerebral metabolism may be a component of the neuroprotective actions of antenatal $MgSO_4$.

Study limitations

This study was based on a cohort with a small number of participants. The study was not specifically designed to assess the effect of antenatal $MgSO_4$ on neonatal haemodynamics and was underpowered to detect significant neonatal outcomes. Due to consent issues and other concurrent studies, a small number of infants were recruited from the first day after birth (Figure 8), when the haemodynamic changes are expected to be more significant and the difference in neonatal Mg^{2+} higher. Sample size limitations restricted the number of maternal covariates that could be studied. Moreover, the group of babies not received $MgSO_4$ were more likely to receive incomplete course of steroids, be born in a district hospital with less expertise in resuscitation and initial management of extreme prematurity, and underwent the stress of neonatal transfer.

Implications for future work

There is an urgent need for a well-designed study of the relationship between antenatal $MgSO_4$ and perinatal pharmacokinetics and pharmacodynamics to indicate the optimal dose and infusion duration of $MgSO_4$ to maximize neonatal Mg^{2+} (348). In the light of our results, points to consider in the planning (design and sample size) of perinatal PK-PD studies are the needs: 1) to resolve the interactions between BW,

maternal BMI, GA and pre-eclampsia; 2) to explore whether the loading dose for mothers is sufficient to attain steady state for the fetus and whether personalising the loading dose makes a difference; 3) for sufficient participants to explore biomarkers of neonatal brain and haemodynamic function, in this case their relationships to Mg^{2+}; 4) the potential for different relationships between the variables on different days after birth; 5) to pull data, when possible, from other available studies, but it will be important to capture the data consistently so that new studies maybe needed.

To inform these future studies we performed power analyses using the cerebral biomarkers as surrogate biomarkers to assess whether neuroprotection therapy had a significant effect on postnatal cerebral function. The analysis demonstrated that Burdjalov score may be a useful biomarker or surrogate outcome for the effect of Mg^{2+} in the brain and requires the recruitment of fewer patients. Rasler et al. studied 232 babies and found that the difference in median Burdjalov scores between babies who had a severe outcome at one year corrected gestational age and those with a normal outcome was two points (331). Accordingly, a two-armed trial that aimed to find the same difference in Burdjalov scores would need to recruit 74 babies in each arm (total 148 babies, P-value 0.05, power 90%, effect size of 0.54 when the clinically significant effect size was standardized by the upper limit of the 95% confidence intervals for standard deviation in Burdjalov scores found in this study). The same study to detect 5 units difference in cerebral oxygenation between the two arms would need to recruit more patients (110 on each arm, 220 in total). However, cerebral oxygenation is more easily measured and does not require a lot of offline signal processing and analysis

(349). The validity of both biomarkers to predict later neurodevelopmental outcome in preterm infants has been previously demonstrated (155, 349).

Conclusion

Maternal BMI and duration of $MgSO_4$ infusion (and their interaction) appear to have significant impact on neonatal Mg^{2+} that may merit changes to the dosing regimen, for example individualising the loading dose. This may also have important implications for other drugs used during pregnancy. No associations were demonstrated of Mg^{2+} with PDA score, cerebral oxygenation and cerebral electrical activity, although the number of patients studied was small.

Chapter 6: Pulse Transit Time, Pulse Wave Velocity and a novel biomarker in Preterm Infants with Patent Ductus Arteriosus

Introduction

PTT and PWV are validated biomarkers in adult vascular physiology (350-353). PTT is the time it takes the pulse pressure wave to travel through a specific segment of the arterial tree. PWV can be calculated from the physical distance between the heart and the signal detector divided by PTT. PWV is considered a surrogate of systemic vascular resistance. There has been little reported use of PWV or PTT in preterm infants probably in part due to the much higher HR and smaller physical distances, making the precision of the measurements having a greater influence.

PDA can have significant effects on cardiac and aortic haemodynamics. The presence and severity of PDA are difficult to assess continuously in preterm neonates. Although echocardiography is the gold standard for PDA assessment, it is a single time-point assessment which requires expertise that is not always readily available; it is subject of considerable variation and sometimes is not well tolerated by critically ill preterm infants. PTT and PWV may detect altered haemodynamics due to the PDA steal phenomenon. Moreover, combined information from PWV and BP can possibly provide information for the cardiac output as it is known that cardiac output is proportional to BP and the systemic vascular resistance.

PTT and PWV are affected by HR (190) and for this reason we devised the novel variable (PD) to take the variable HR into account. PD is a marker of the difference in time between the ECG R wave and the following systolic BP wave peak divided by the time of the cardiac cycle and expressed as phase, i.e. the proportion of one cardiac

cycle expressed in degrees where one cycle is 360^{O}. Our hypothesis was that PD could be related to the size of the PDA.

Aim

The aims of this study were:

1. To investigate whether measurements of PTT and PWV in extremely preterm infants are affected by PDA. The null hypothesis was that PDA diameter does not affect measures of PTT aortic PWV in the first days after birth in extremely preterm infants.
2. To explore the associations between PTT and PWV with other echocardiographic and cerebral biomarkers.
3. To explore the relationship between PD and the size of the PDA and test the null hypothesis that there is no relationship between these variables.

Methodology

Population

Patients from the same cohort as in Chapter 3 were included in the present study, if they had an umbilical artery catheter inserted for clinical reasons and their continuous physiological data were captured with IxTrend software (Ixellence, Wildau Germany).

The methodology applied was previously described in Chapter 2.

Results

The data

Fourteen infants were studied either on Day 2 or Day 3 after birth and a single measurement was analysed for each neonate (ten observations on Day 2). The demographic details of the study population and a summary of measurements are shown in Table 24. The main demographics were: BW [Median (IQR)] 0.89 kg (0.76-1.15), gestation 26.3 weeks (24.9-28.1), PDA size 1.6 mm (1.2-2.5) and BP 32 mmHg (29 - 35). The analysis included four neonates who received inotropes, one who subsequently developed severe IVH and three who later died. One infant (born at 24 weeks) was hypotensive in one of the recordings with systolic BP 23mmHg and diastolic 13 mmHg.

Analysis

There were no significant associations between the background demographics and PTT, PWV or PD (Mann Whitney test). There was a positive correlation between PTT and GA that was statistically non-significant after Bonferroni correction (Spearman's rho, P-value) (0.631, 0.015).

Demographics		
Patients (N)	14	Percentage or IQR
Birth weight (kg)	0.89	0.76 - 1.17
Male (N)	10	67%
Hypertension (N)	3	20%
Chorioamnionitis (N)	7	47%
Steroids (N)	13	87%
APGAR 5'	6	5-8
Inotropes (N)	4	27%
Gestation (weeks)	26.6	24.8 - 28.2
PTT (sec)	0.20	0.18 - 0.21
PWV (m/sec)	2.10	1.75 - 2.63
Phase difference (°)	175	166 – 189
PDA (mm)	1.6	1.1 - 2.7
UAC distance from AoV (mm)	43	36-48
Heart rate (beats/min)	151	138-159
Mean BP (mmHg)	32	29 - 35
Haemoglobin	147	123-163
Cerebral tissue oxygenation	69	63-73
Burdjalov score	2	0-7
ACA resistive index	0.72	0.65-0.84
Data expressed as number of patients (N) or median and IQR or percentage as appropriate. PTT: pulse transient time, PWV: pulse wave velocity, UAC: umbilical artery catheter, AoV: aortic valve, BP: blood pressure, ACA: anterior cerebral artery.		

Table 24: Summary of patient demographics.

Aortic biomarkers			
	PTT	**PWV**	**PD**
Gestation	0.631 (0.015)	NS	NS
BW	NS	NS	NS
HR	-0.660 (0.014)	NS	NS
MBP	NS	NS	NS
cTOI	0.680 (0.007)	-0.666 (0.009)	0.534 (0.049)
Burdjalov score	NS	NS	NS
ACA RI	NS	NS	NS
PDA size	0.535 (0.049)	NS	0.820 (<0.001)
LVEDD:Ao ratio	0.914 (<0.001)	-0.613 (0.034)	0.732 (0.007)
E/A wave ratio	0.876 (<0.001)	-0.595 (0.041)	NS
IVRT	NS	NS	NS
BW: birth weight, HR: heart rate, MBP: mean blood pressure, cTOI: cerebral tissue oxygen extraction, PTT: pulse transient time, PD: phase difference, PWV: pulse wave velocity, PDA: patent ductus arterious, LVEDD/Ao: left ventricular end diastolic diameter to aortic root ratio, IVRT: isovolumetric relaxation time, ACA RI: anterior cerebral artery resistivity index, NS: no significance. In each box first value represents Spearman's correlation coefficient and second P-value.			

Table 25: Summary of the relations between the aortic biomarkers, background demographics, echocardiographic and cerebral biomarkers.

Spearman's rho and P-values are presented only for correlations with initial P-values <0.05. Statistical significance P-value <0.0042 (after Bonferroni correction).

Pulse transient time and pulse wave velocity

Positive correlations (Spearman's rho, P value) were found between PTT and cTOI (0.680, 0.007), PDA diameter (0.540, 0.046), LVEDD:Ao ratio (0.914, 0.001), E/A wave ratio (0.876, <0.001) and negative correlations with HR (-0.791, 0.015) and PWV (-0.659, 0.010). There was a positive correlation between PTT and the distance of UAC tip (0.807, <0.001). There were negative correlations between PWV and PTT (-0.659, 0.010), LVEDD:Ao ratio (-0.613, 0.034), E/A wave ratio (-0.595 and 0.041) and cTOI (-0.666, 0.009). However, after Bonferroni correction only PTT vs. E/A wave ratio, PTT vs. distance of UAC tip and PTT vs. LVEDD:Ao ratio remained statistically significant. The relationship between PTT and E/A wave ratio became non-significant when corrected for HR. There was no statistically significant association of the aortic biomarkers with haemoglobin levels, Burdjalov score or cerebral Doppler indices (Table 25).

The assessment of the scatter plots on Figure 39 identified a common outlier. The patient on Figure 39 (PDA vs. PWV) was one of the most mature in our cohort (born at 27 weeks GA and BW 1.26kg), but was critically unwell on second day after birth with septic shock (maternal chorioamnionitis and PROM) and lactate of 9 mEq/L, mean BP 32mmHg, very tachycardic (HR 191), on high dose inotropic support and intravenous morphine. PDA diameter was 3.6mm (the largest in the cohort), cTOI was 60 and there was no diastolic flow on the ACA Doppler ultrasonography (RI=1). The patient died from late onset sepsis at day 43 after birth. If data from this patient is excluded due to the presence of septic shock while being on high dose inotropic support, the

correlation between PDA vs. PTT and PWV vs. PDA became stronger [(0.709, 0.007) and (0.632, 0.021) respectively].

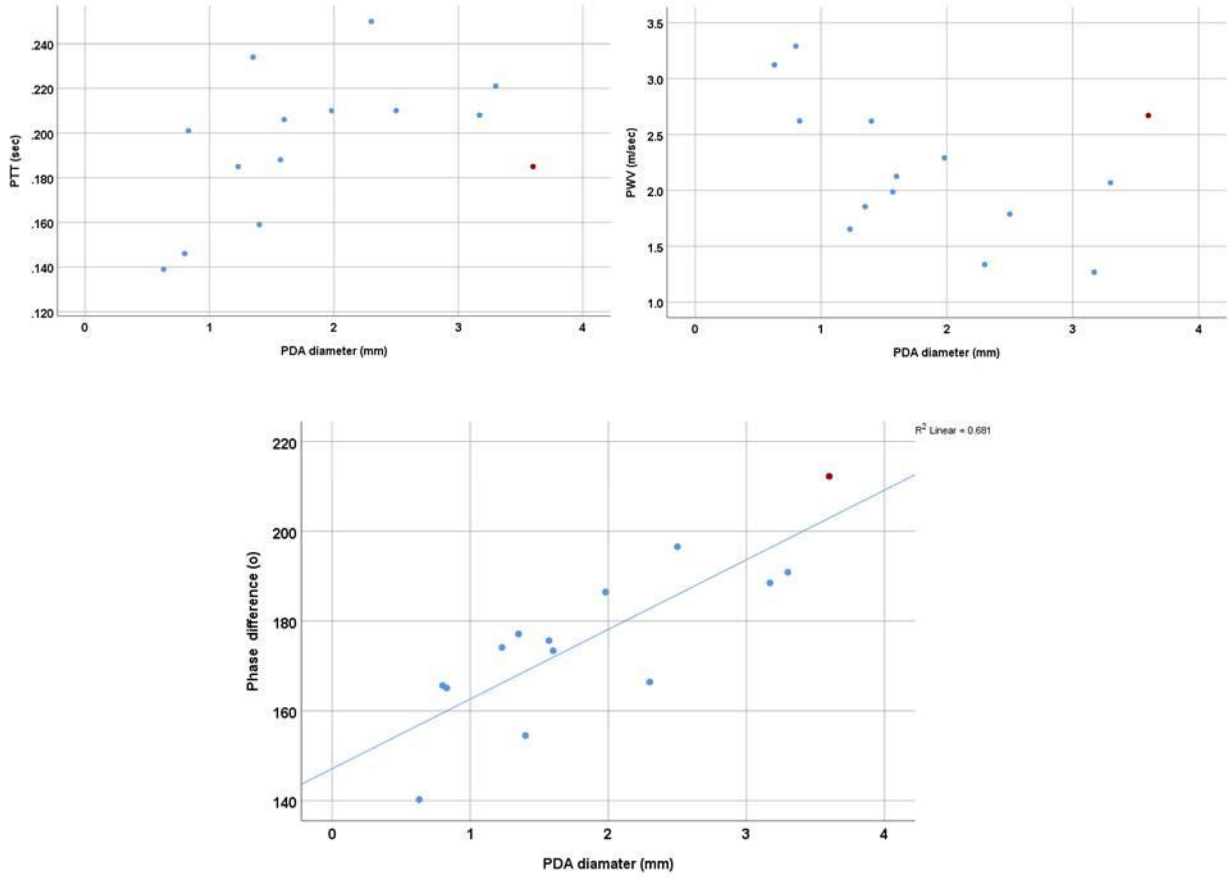

Figure 39: PDA size versus PTT (left), PWV (right) and PD (bottom).

PDA size versus PTT (left), PWV (right) and PD (bottom) demonstrating a stronger correlation for PDA size against PD. An outlier was identified on the top scatter plots and highlighted with red colour in all scatter plots. Large PDA: PDA diameter >2mm.

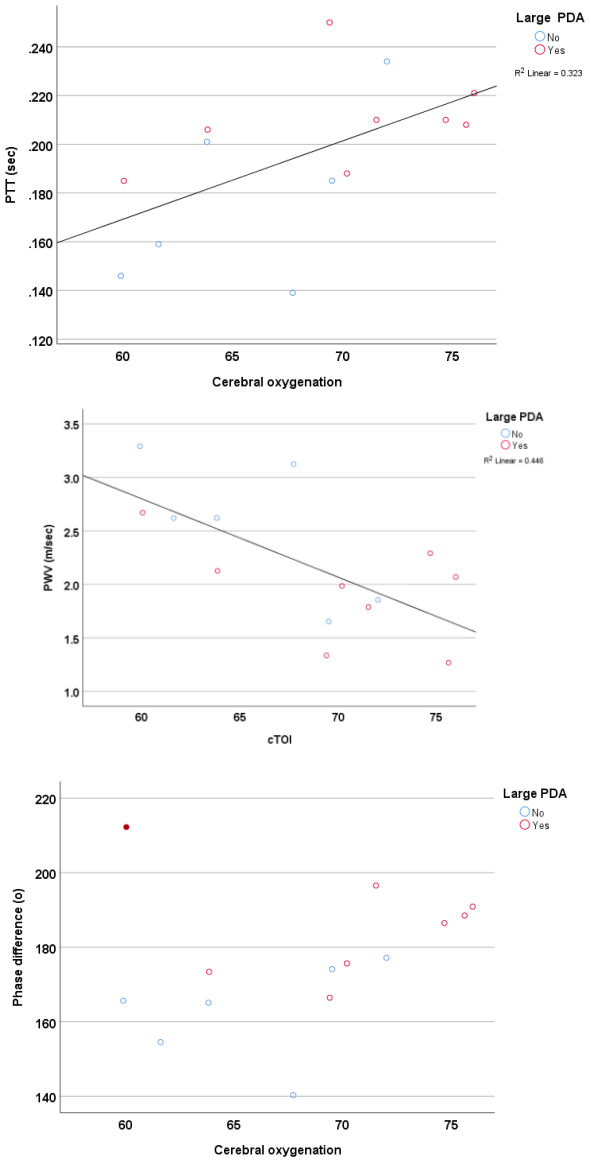

Figure 40: Cerebral oxygenation index versus PTT (top panel), PWV (middle panel) and PD (bottom panel). Cerebral oxygenation index versus PTT (top panel), PWV (middle panel) and PD (bottom panel) demonstrating a relationship between cerebral oxygenation and the aortic biomarkers that is opposite to the expected in the light of data from Chapter 3. The same patient appears as outlier in the last figure with the larger PDA diameter and low cerebral oxygenation. Large PDA: PDA diameter >2mm.

A second outlier was identified on scatter plot in Figure 41. This patient was septic with CRP 104 mg/L (normal range 0-10) following birth at 24 weeks (BW 480gr) and needed advanced resuscitation at birth with cardiac massage and drugs. Despite inotropic support the patient was severely hypotensive with systolic BP 23mmHg (mean BP 16mmHg) on a morphine infusion, but maintained a high left ventricular output (223mls/kg/min) with lactate of 2.2 mEq/L. PDA diameter was 3.17mm, cTOI was 76 and there was no diastolic flow on the anterior cerebral artery Doppler ultrasonography (ACA RI=1). The removal of this patient rendered the correlation between PWV and systolic BP (-0.645, 0.017), diastolic BP (-0.651, 0.016) and mean BP (-0.635, 0.020) stronger.

In adult and paediatric studies there is a positive correlation between PWV and BP (355). However, the presence of a negative correlation between PWV and BP in the present study led to further analysis of the echocardiographic data and their interaction with PWV to identify the underlying pathophysiological processes which may explain this specific interaction in the neonatal population. PWV is considered a surrogate of systemic vascular resistance. It is known that vascular resistance is the ratio of BP and left ventricular output. These data were available for the individual patient from the invasive BP monitoring and the echocardiographically measured left ventricular output and hence, a substitute of vascular resistance (VR) was calculated by dividing BP with left ventricular output. To confirm whether our observation of negative correlation between PWV and BP was true, the possibility of a relation between PWV and VR was assessed. There were eleven patients contributing data for

this analysis and although the correlation was not statistically significant, there was a moderate correlation between PWV and the estimated VR (0.536, 0.089) (Figure 42).

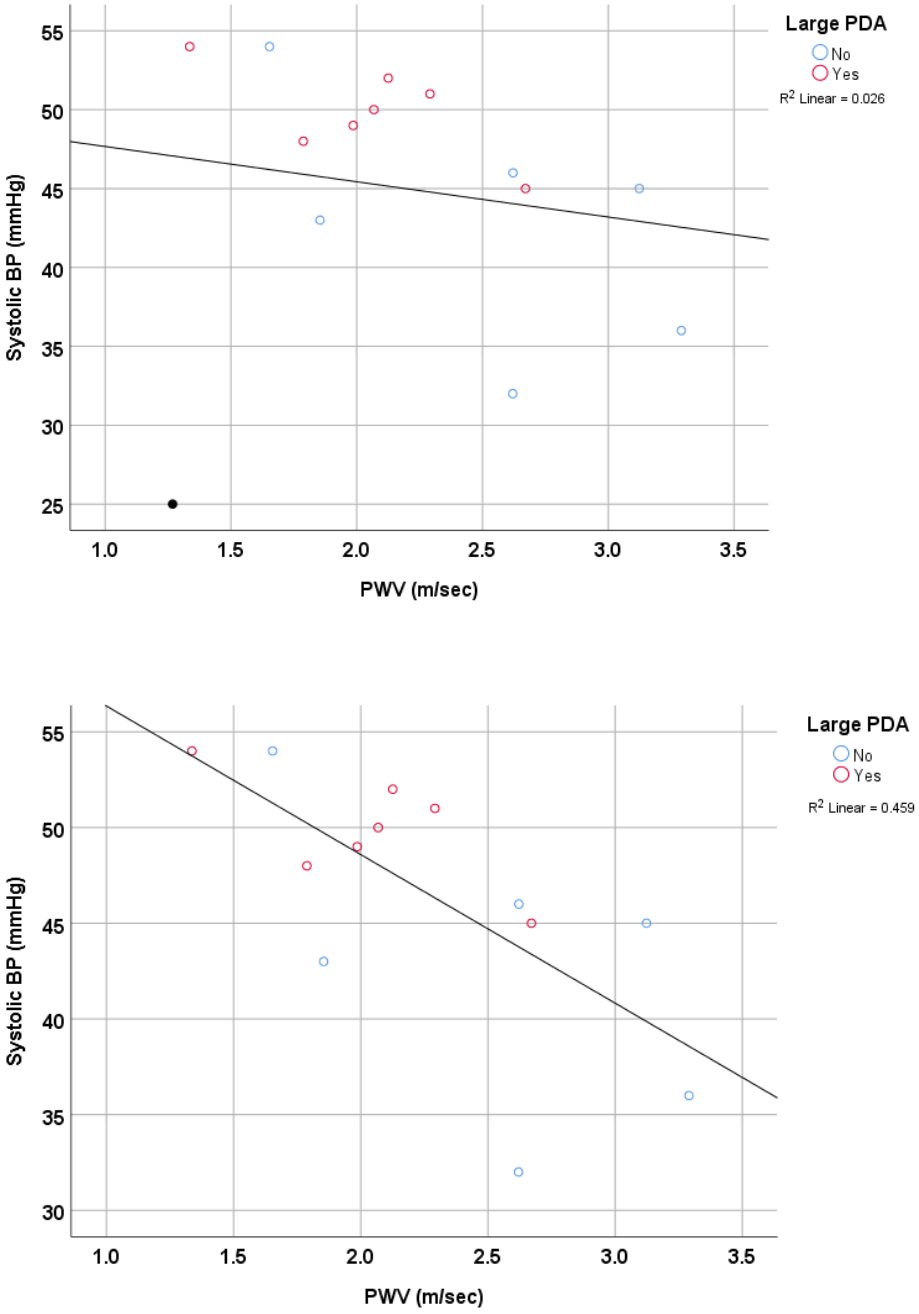

Figure 41: The relationship between PWV and systolic BP.

In the top graph, an outlier (black colour) is identified with very low systolic BP 25mmHg (mean BP 16mmHg) while born at 24 weeks. In the bottom graph, the removal of this outlier significantly improves the correlation [Spearman's rho, P-value (-0.363, 0.223) vs (-0.645, 0.017)]. Large PDA: PDA diameter >2mm.

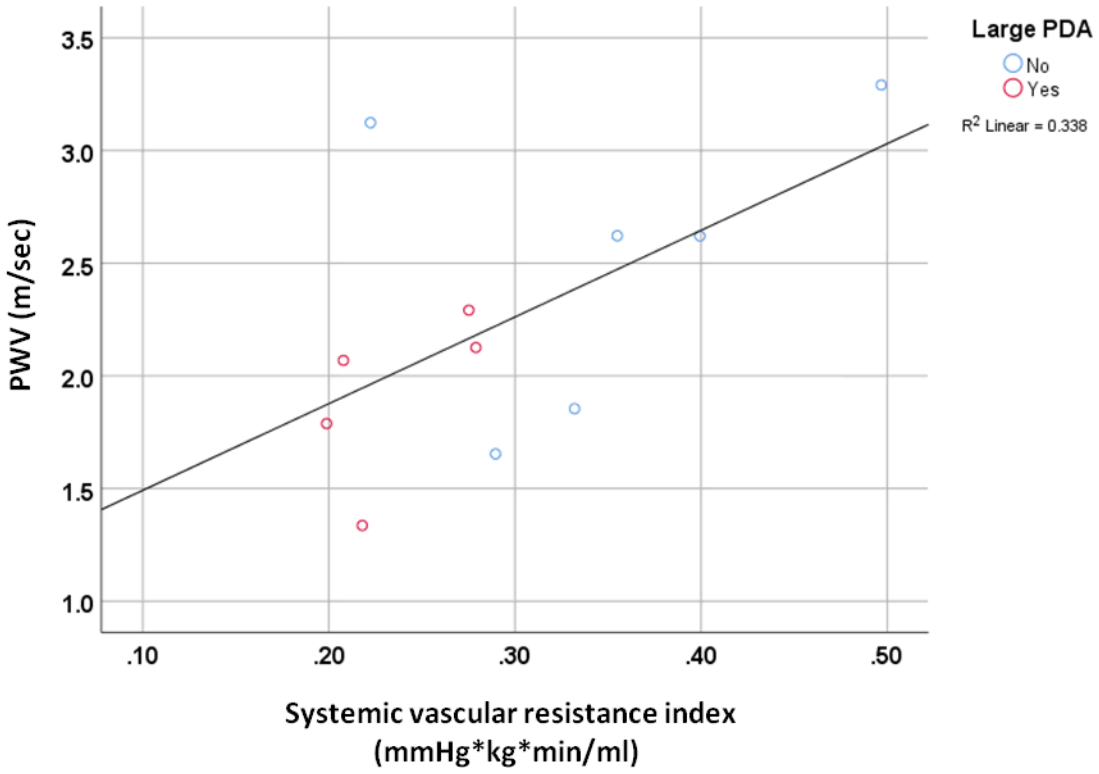

Figure 42: The relationship between PWV and the calculated systemic vascular resistance index (VR) based on echocardiographically derived cardiac output and the aortic BP.

The relationship between PWV and the calculated systemic vascular resistance index (VR) based on echocardiographically derived cardiac output and the aortic BP. There is a positive correlation, however not statistically significant [Spearman's rho, P-value (0.536, 0.089)]. Large PDA: PDA diameter >2mm.

Further assessment of the echocardiographic parameters demonstrated a positive correlation between PDA and LVEDD:Ao (0.907, 0.001), LVEDD:Ao and stroke volume (0.770, 0.006), left ventricular stroke volume and left ventricular output (0.818, 0.004) and left ventricular output and BP (0.560, 0.058) (Figures 36-41). These analyses demonstrated the larger the PDA diameter the higher is the preload (LVEDD:Ao), stroke volume and cardiac output and lower the aortic resistance. However, it is possible the increase in cardiac output to outpace the decrease in PWV (representing systemic vascular resistance). This finally results in relatively increased blood pressure (Blood pressure = Resistance x Cardiac output) observed in our cohort. A summary of the interactions and the corresponded correlations are displayed in Figure 49.

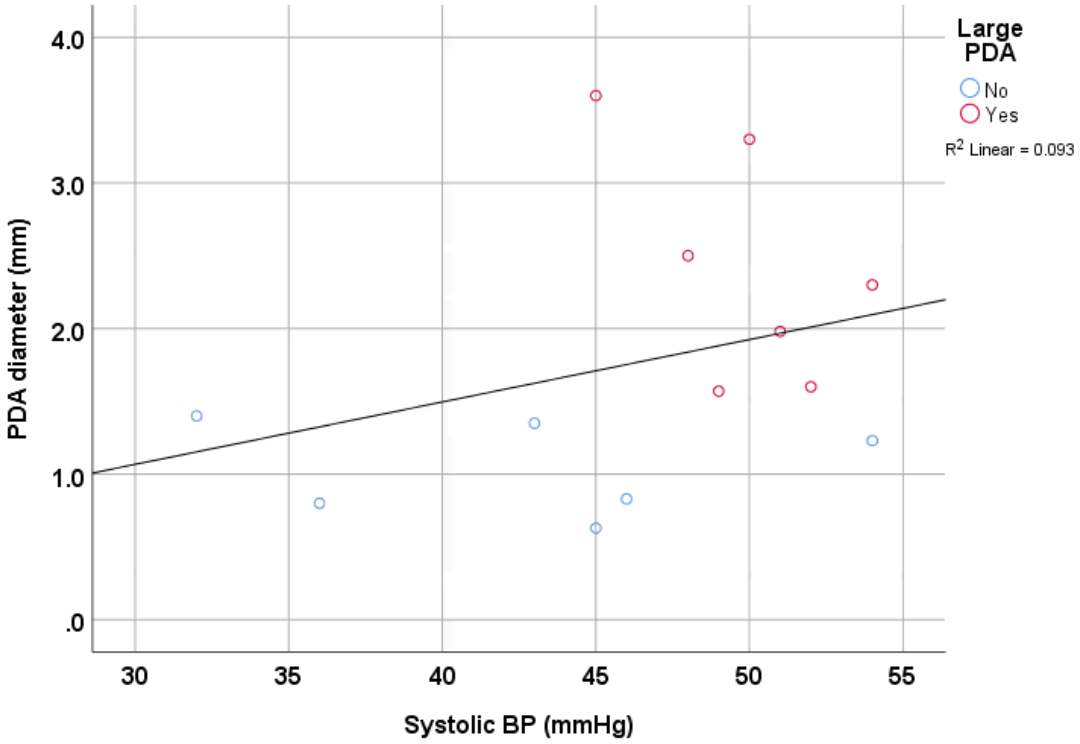

Figure 43: The relationship between PDA size and systolic BP.

There is a weak statistically non-significant positive correlation. Large PDA: PDA diameter >2mm.

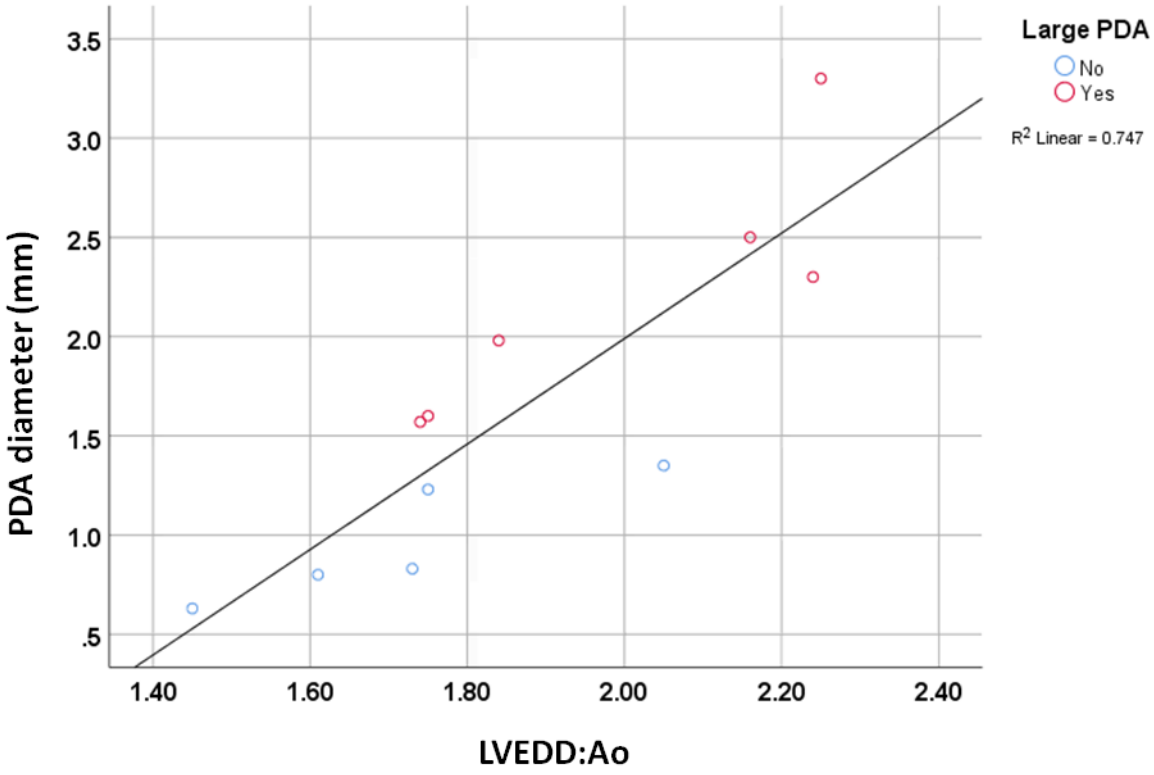

Figure 44: The relationship between PDA size and LVEDD:Ao representing preload.

There is a strong statistically significant positive correlation [Spearman's rho, P-value (0.907, 0.001)]. Large PDA: PDA diameter >2mm, LVEDD:Ao: left ventricular end diastolic diameter to aortic root ratio.

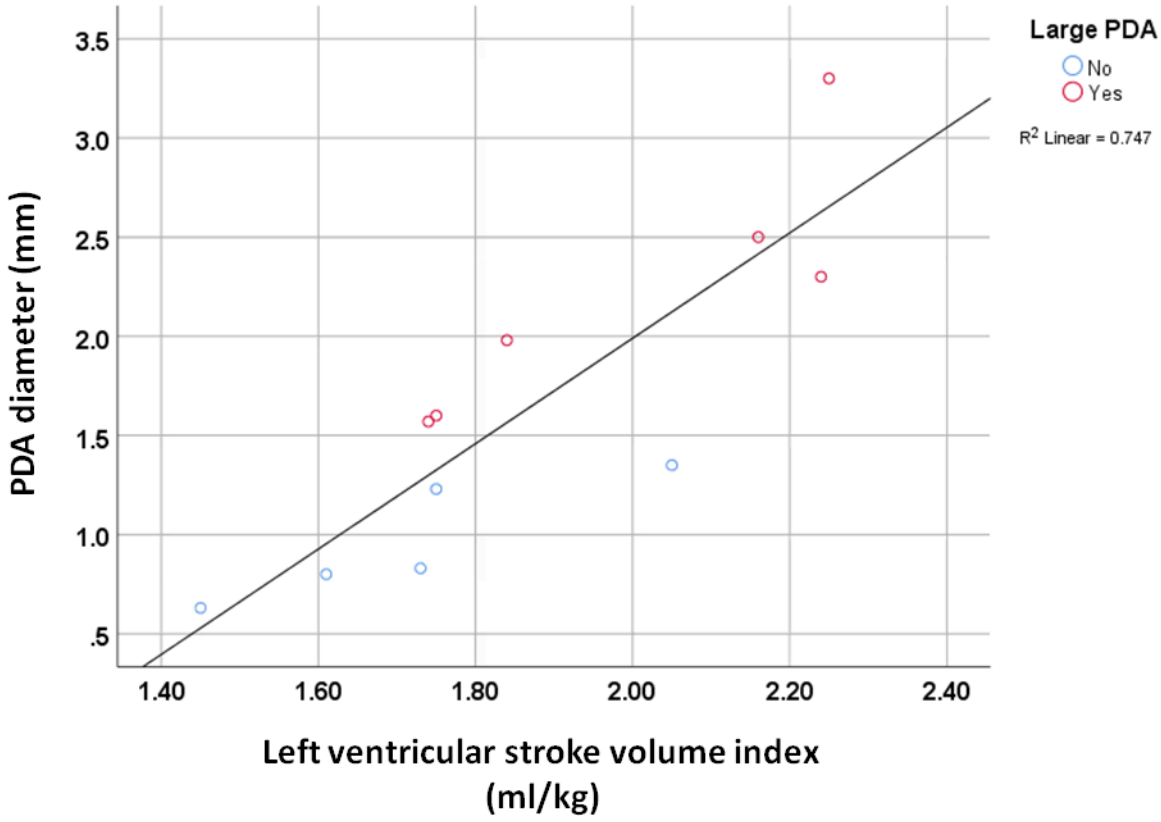

Figure 45: The relationship between PDA size and left ventricular stroke volume index.

There is a strong statistically significant positive correlation [Spearman's rho, P-value (0.748, 0.005)]. Large PDA: PDA diameter >2mm.

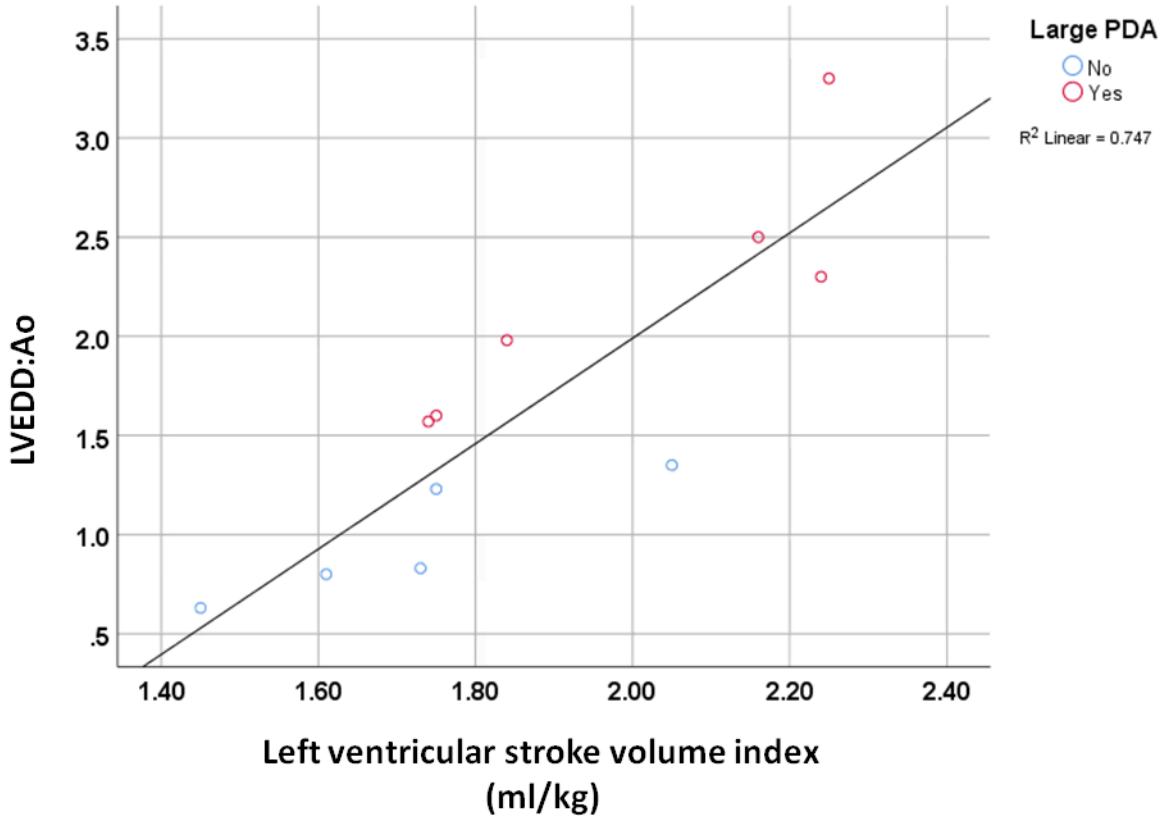

Figure 46: The relationship between LVEDD:Ao and left ventricular stroke volume index.

There is a statistically significant strong positive correlation [Spearman's rho, P-value (0.770, 0.006)]. Large PDA: PDA diameter >2mm.

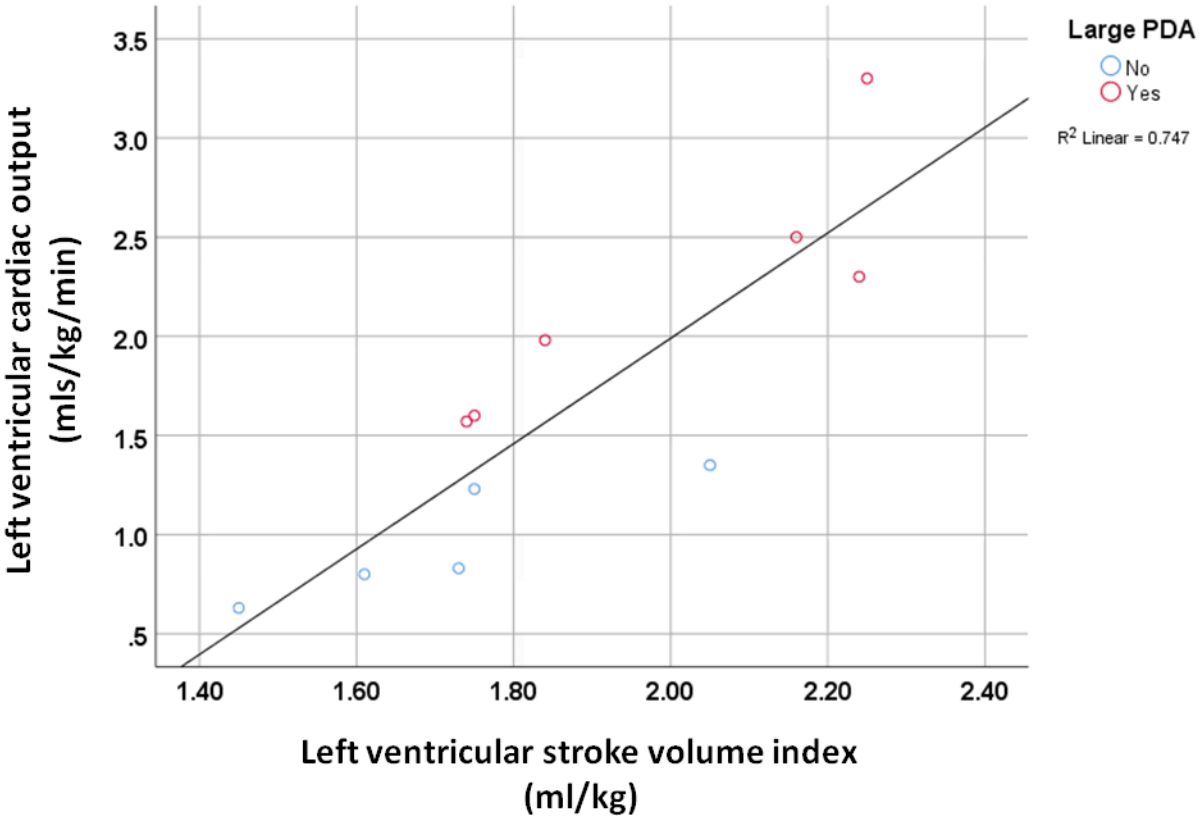

Figure 47: The relationship between left ventricular stroke volume index and cardiac output.

There is a strong statistically significant positive correlation [Spearman's rho, P-value (0.818, 0.004)]. Large PDA: PDA diameter >2mm.

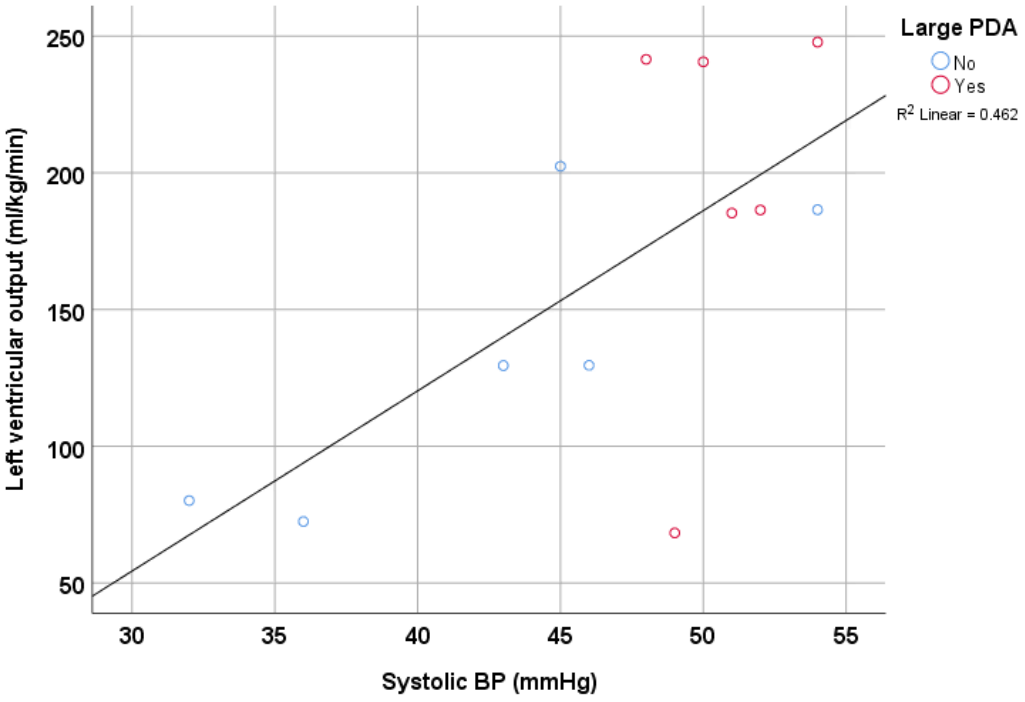

Figure 48: The relationship between left ventricular cardiac output and systolic BP.

There is a statistically not significant positive correlation [Spearman's rho, P-value (0.560, 0.058)]. Large PDA: PDA diameter >2mm.

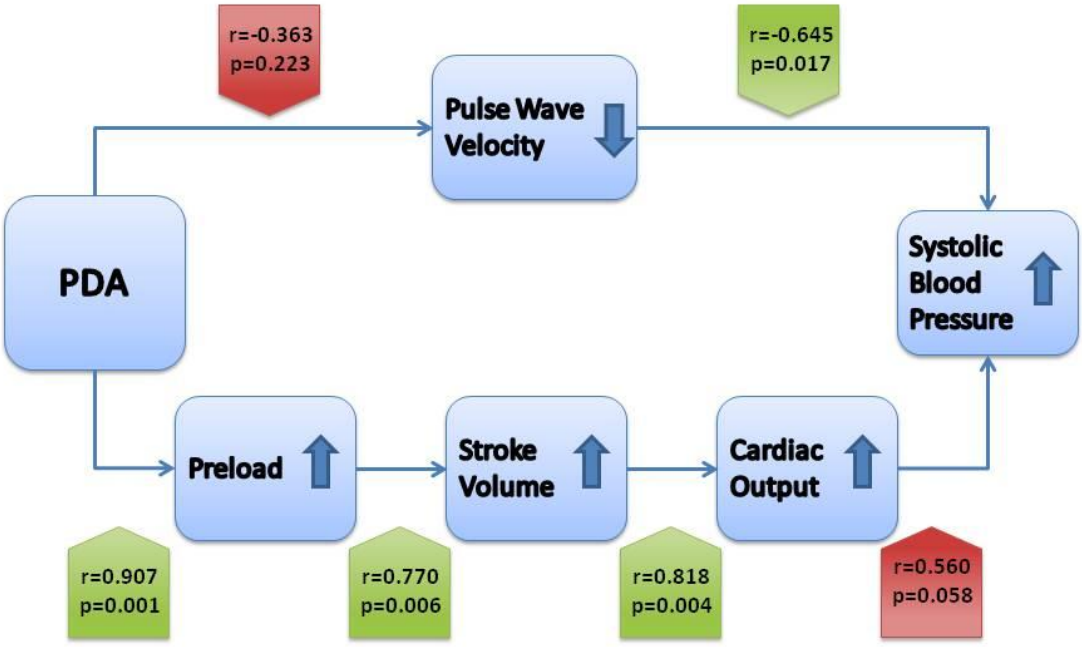

Figure 49: Summary of proposed mechanisms of interactions between PDA, systemic vascular resistance (PWV) and echocardiographic biomarkers.

PDA has multiple effects on haemodynamic and echocardiographic biomarkers. The large blue arrows in the boxes represent the increase or decrease of the relevant parameter, the green arrow boxes point out interactions with P-value <0.05 and the red arrow boxes point out interactions with P-value >0.05. PDA: patent ductus arteriosus, r: Spearman's correlation coefficient, p: P-value.

Phase difference

The median and IQR PD values are shown in Table 24. The maximum observed difference in the cohort was 74°. A positive correlation was found between PD and cTOI (0.534, 0.049), PDA diameter (0.820, <0.001) and LV/Ao ratio (0.732, 0.007). Only PD vs. PDA met the threshold for statistical significance after Bonferroni correction.

Repeatability

The intra-patient repeatability of PD was good with mean difference of -0.21° and two standard deviations of the differences being 15.6° (Figure 50).

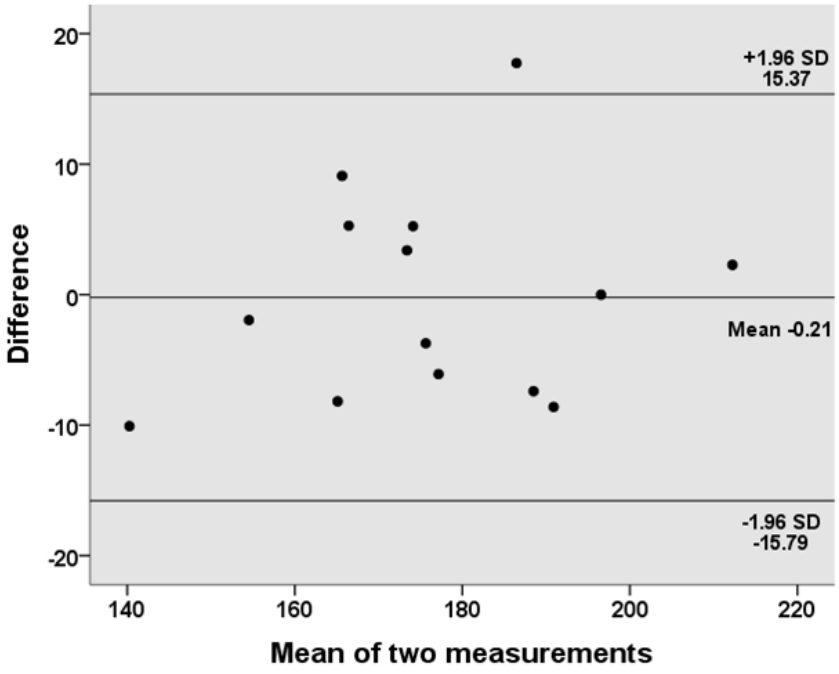

Figure 50: Bland–Altman plot assessing the repeatability of phase difference.

Discussion

This is the first study to measure PWV and PTT using ECG and BP waveforms in extremely preterm infants and assess their relation to echocardiographic and cerebral biomarkers. A new biomarker, phase difference, was devised to account for the variable HR in the neonatal population. PD correlates well with PDA size and merits further investigation as a continuous biomarker for neonatal monitoring. The method is highly reproducible similar to previous studies (354), however it has the advantage of allowing continuous measurement. Moreover, combined PWV and BP data can help provide information of cardiac output which can be used in clinical and research settings for aiding making rational decisions when treating unstable patients and expand our knowledge of haemodynamics in preterm inants.

I found PTT to be correlated with GA, HR and PDA size. However, after Bonferroni correction these correlations became non-significant. It was previously demonstrated that pulse wave conduction time, as measured by pulse wave plethysmography peripherally, is strongly correlated with BW (189). Goudjil et al. previously demonstrated by using pulse wave photoplethysmography from pre- and post-ductal sites that PDA is correlated with pulse phase difference changes (355). However, the methodology used in the current thesis has the benefit of not needing extra probes placed to the baby, is straightforward and independent of the frequent motion artefacts observed with pulse oximetry as well as heavy signal processing involved in

the generation of the signal and does not involve advanced techniques and algorithms including decomposition of the signals.

A positive correlation between PTT vs. LVEDD:Ao and PTT vs. E/A wave ratio was also demonstrated. Both associations were significant even after Boneferroni correction, but PTT vs. E/A wave ratio became not significant when corrected for HR. E/A wave ratio and diastolic function are known to be affected by tachycardia (356) and preterm infants can have highly variable HR. LVEDD:Ao is marker of PDA volume shunting and indicates increased preload. As observations from the second and third day after birth were used for this analysis, this may have allowed able time for the left ventricle to dilate due to the PDA shunting. This is also supported by the observed correlation trend between PTT and PDA, which however was not statistically significant due to the Bonferroni correction.

Amirtharaj et al. found that PDA treatment in ex-preterm infants with ligation or ibuprofen resulted in prolongation of PTT measured by photoplethysmography on hand and foot (357). This is opposite to our findings. However, the study had different methodology and several limitations, which render the results hard to interpret. PDA treatment occurred at variable postnatal age and infant size (post-natal age: 10 to 79 days and weight: 605 to 2000gr). The study did not specify the population characteristics and time of the initial measurements before the PDA ligation and post ligation assessment was performed 24 hours following the procedure. Hence, if there was considerable time delay between the two assessments, it is possible maturation

processes to alter the haemodynamics. Moreover, they did not provide HR data during the recordings that is known to significantly affect PTT. Finally, PTT was derived peripherally from high resistant vessels in the limbs. In contrast, recordings in the current study were from the descending aorta which is central and elastic with lower arterial stiffness compared to peripheral (muscular) arteries.

There was no statistically significant association between PDA and PWV. The absence of statistical significant correlation can be partially explained by the variable HR in our population, the imprecise method to assess the distance of the UAC from the aortic valve or can be a type 2 error. The natural variation of heart anatomical position, mode of ventilation, heart size, respiratory cycle phase when the X-ray was taken, and the angle of the X-ray can all contribute to the imprecise distance measurement.

The values of PWV (median 2.1 m/s, IQR 1.75-2.63) are consistent with previous studies on older infants and children (358, 359), albeit being slightly lower. Broadhouse et al. studied infants with a median GA of 32 weeks and reported a mean PWV of 3.2 m/s (range 2.3-5.1). Our population consisted of more premature infants and PWV was on the lower range of this cohort. This is expected as there is a positive correlation between GA and arterial stiffness and Moens-Kortewer equation indicates that PWV is proportional to vascular stiffness (360). It is known that preterm infants in the first days after birth have reduced sympathetic activity compared to term and reduced capacity to up-regulate their sympathetic activity (361). It has been suggested that autonomic immaturity makes them more susceptible to hypotension due to reduced

vascular tone which can lead to cardiovascular instability (21). Additionally, BP in the current thesis was measured in the aorta (central vessel) instead of peripheral vessels and, in order to improve precision, a modified measurement of PTT was adopted using the peak of the systolic BP trace.

There was a negative correlation although not significant between PWV and BP opposite to the adult and paediatric studies (362, 363). This observation highlights the differences between adult and neonatal vascular haemodynamics and emphasises the importance of PDA on aortic haemodynamics as patients with low PWV in our population had also large PDA and elevated systolic BP. PWV is a surrogate biomarker for systemic vascular resistance and is directly proportional to BP and inversely proportional to left ventricular output. In neonates the presence of a negative correlation is the result of the combined effect of PDA on the overall vascular resistance in the descending aorta and the increased left to right shunting leading to increased left ventricular cardiac output. The observation that low resistance with high BP can only occur if left ventricular output increases out of proportion and overcompensates the reduced systemic vascular resistance is important. Calculation of the vascular resistance from the echocardiographic data (systolic BP divided by the left ventricular output) demonstrated a positive correlation between VR and PWV (although not statistically significant) confirming the previous speculation. Further analysis revealed that increased stroke volume was the main drive for the cardiac output increase as HR was not correlated with PDA size. The increased stroke volume was generated by the increased preload due to the PDA as there was a significant

correlation between LVEDD:Ao with both stroke volume and PDA size. This is supported by previous studies (91). The correlation between cardiac output and BP was not statistically significant (red arrow box), but this is expected as previous studies have demonstrated a weak correlation between BP and cardiac output in preterm infants (364). Moreover, there is evidence that many infants with reduced systemic perfusion have normal or high blood pressure in the first hours of life, suggesting that a high systemic vascular resistance may lead to reduced blood flow (365). The summary of the correlations and the interactions are shown on Figure 49. This analysis demonstrates the clinical potential and usefulness of PWV in the neonatal unit as it can be continuously measured and displayed real time on the patient's monitor. Knowing the vascular resistance and BP can be informative of the left ventricular output without the need for repeated echocardiographic assessment. Both these parameters can be continuously available to the clinical team and in combination with other biomarkers (lactate, urine output, NIRS data) can potentially help to make rational clinical management decisions based on pathophysiological grounds and expand our knowledge of haemodynamics in preterm infants.

Based on Moens-Korteweg equation (Figure 51) we expect PWV to decrease when there is a PDA present. Moens-Korteweg equation indicates that PWV is inversely related to the radius of the vessel. The right panel in Figure 51 shows a diagrammatic illustration when there is a PDA attached to the descending aorta. The insertion of PDA increases the total cross-sectional area at the level of the descending aorta and hence

the radius in the Moens-Kortewer equation. The corresponding new PWVb in the descending aorta (which was detected by the UAC) is expected to be lower than PWVa.

PWV can be affected by multiple parameters. PWV was shown to increase by 1 m/sec in normal fetuses from 20 weeks to term (366). PWV is proportional to the mean BP inside the artery, the properties of the artery wall and wall thickness. The mean BP increases with gestation, and so does the thickness of the aortic wall relative to the lumen and the supporting connective tissue. The presence of elastin in the aortic wall is one the main determinants of the incremental elastic modulus (Einc on Figure 51). The deposition of elastin on aortic wall starts early during the fetal life and its production peaks in the perinatal period (367). Previous studies in a paediatric population found that gender, age, height, weight, mean BP and HR are independent predictors of PWV (363). Neonatal PWV correlates with BW (354, 368) and IUGR infants tend to have lower PWV in early infancy (368). Maternal anaemia prior to 20 weeks gestation is associated with an increased infant PWV (369) and infants of diabetic mothers with higher haemoglobin A1c have a higher PWV (370). There is inconsistent evidence whether maternal BP during pregnancy has any impact on neonatal PWV. Koudsi et al. found an inverse relationship between maternal systolic BP at 28 weeks gestation and neonatal aortic PWV (354). However, another study failed to reproduce the same findings as showed no evidence of association between infant PWV at 2–6 weeks of age and maternal BP in early or late pregnancy (371).

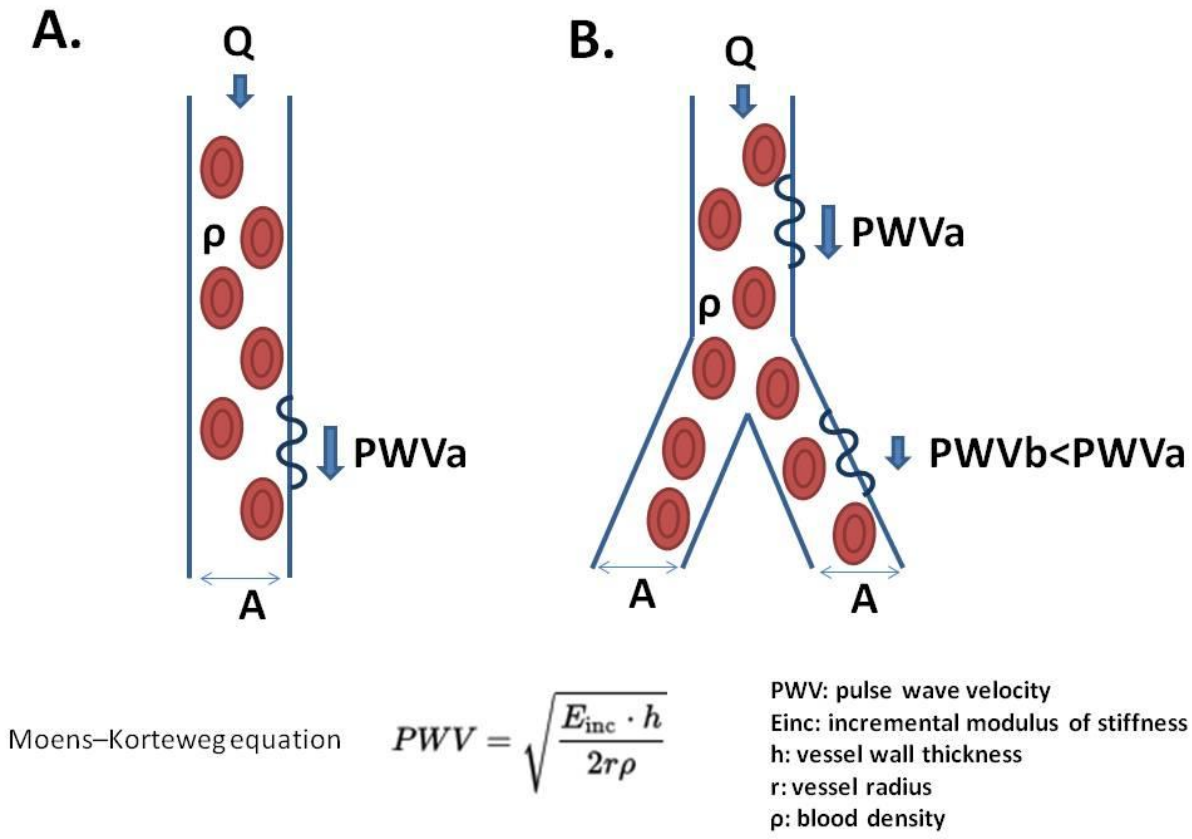

Figure 51: Diagrammatic illustration of the descending aorta and the correspondent haemodynamics without (A) and with the presence of PDA (B).

The insertion of PDA increases the total effective cross-sectional area at the level of the descending aorta and hence the radius in the Moens-Kortewer equation. The corresponding new PWVb in the descending aorta will be lower than PWVa.

It was found also that PDA shunting leads to increased stroke volume. Previous studies from animal and human studies claim that the preterm myocardium is stiff, and preterm infants have diastolic dysfunction and cannot increase stroke volume (372, 373). Hence, they rely on tachycardia to increase cardiac output. However, other studies have demonstrated that preterm infants with large PDA are able to increase stroke volume and their HR remains stable (91, 94, 374). These studies speculated on the underlying mechanism and assumed this was due to decreased afterload. The current study demonstrated that the underlying mechanism of this adaptation is likely to be the decreased afterload with reduced systemic vascular resistance due to the PDA shunt and this is associated with increased systolic BP due to increased stroke volume secondary to increased preload. Moreover, it seems likely that homeostatic mechanisms compensate for the haemodynamic consequences of PDA and maintain adequate cerebral perfusion in a wide range of PDA size. It is possible that the loss of vascular control due to a severe underlying clinical condition (sepsis in our cases) to weaken the homeostatic mechanism and lead to decompensation.

The present study demonstrated a significant positive correlation between PD and PDA size. It was hypothesised that higher PD (lower PWV) could be an indicator of PDA presence. It was found that a PD above 180^{o} was indicative of a PDA diameter larger than 2mm, which is considered large and haemodynamically significant by many clinical trials investigating PDA treatment (135, 375).

PTT, PWV and PD are all interrelated as PWV is derived from PTT accounting for the distance of UAC catheter from the aortic valve and PD is derived from PTT accounting for HR. Substantial HR variation was observed in our population (maximum range 111 to 191), which can have a major impact on PTT measurement. PTT was subsequently indexed accounting for the variable HR and this resulted in the development of PD, which correlated better with PDA size (Figure 39). It is known that PTT and subsequently PWV can be affected by HR (371, 376). There was also substantial variability of BW (maximum range 0.48-1.3 kg), GA (24 to 28+6 weeks) and severity of the underlying clinical condition with critically ill patients who needed inotropic support. Medication can possibly have an impact on the aortic haemodynamics and measured biomarkers (377).

PDA diameter was used as a biomarker for PDA shunting as, according to Hagen-Poiseuille's law, the blood flow through a vessel is proportional to the fourth power of the radius of the vessel and the pressure gradient across the vessel and inversely related to the length of the tube and the blood viscosity. Hence, the vessel diameter is the most important parameter. However, preterm DA is often elongated and its length can be much larger and out of proportion even to the fourth power of radius. Nevertheless, the impact of PDA length has not been yet studied as it is very difficult to measure accurately with echocardiography. Moreover, preterm DA is often tortuous with multiple points of stenosis (71). In these situations Hagen-Poiseuille's law cannot describe the underlying haemodynamics. Previous studies have demonstrated that PDA size is the single most useful parameter for determining the haemodynamic

significance (378). Moreover, if PD is used only for trend monitoring of PDA in an individual patient, then the rest of the Hagen-Poiseuille's law parameters become irrelevant as they remain relatively stable and only PDA diameter changes in short term.

Cerebral oxygenation and aortic biomarkers

This is the first study to demonstrate a correlation between PTT, PWV, PD and cTOI which became non-significant when Bonferroni correction was applied. This merits further investigation and confirmation in future studies. In Chapter 3 and in the previously described literature, it has been shown that PDA size is inversely correlated with cerebral oxygenation. However, in the small population studied here the sign of the relation is opposite to that expected as there was a positive correlation between PD and cTOI. Cerebral oxygenation in the subpopulation of the present analysis was within the considered “normal” range (55 to 85) (160). This may have affected the relationship between the aortic biomarkers and the cerebral oxygenation index which is opposite to the expected. The BP measurements were obtained from the descending aorta distal to the neck arterial branches. Hence, it is unknown whether cerebral arteries react in a different way to compensate for the PDA induced steal phenomenon. Voges et al. have previously demonstrated that different sections of the aortic arch have different PWV values (379). Cerebral circulation has autoregulatory abilities, despite its impairment due to immaturity (380). Finally, there is evidence that PWV in pregnant women with gestational diabetes is negatively correlated with

cerebral oxygenation despite the fact that the background pathophysiology is different (381). In conclusion, the observed correlation between the aortic biomarkers and cTOI are opposite to expected and require further investigation in future studies with larger population size and individuals with abnormal cerebral oxygenation.

Limitations

The natural variation of heart anatomical position, mode of ventilation, heart size, respiratory cycle phase, when the X-ray was taken, and the angle of the X-ray can all contribute to the imprecise measurement of the distance between the UAC tip and the aortic valve and the measurement of the aortic biomarkers (PTT, PWV and PD). Sampling frequency and filtering resulted in more smoothed BP waveforms, which may have impacted on the accurate detection of the BP waveform peak and the calculation of PTT. The small population size with multiple comparisons may have resulted in spurious correlations. However, Bonferroni correction was used to account for the multiple comparisons. The small population did not allow us to perform regression analysis to assess any possible confounders.

Conclusions

PDA has significant effects on cardiac and aortic haemodynamics. In a small population of preterm infants a significant positive correlation was found between PDA and PD which renders further investigation with validation studies. If proved valid it can serve as a non-invasive continuous biomarker of monitoring PDA size and may be useful in clinical trials. Moreover, it is possible that combined PWV and BP data from the UAC will be predictive of cardiac output in the descending aorta which is useful clinical

information for the management of this fragile population. This methodology can provide continuous non-invasive data which combined with the rest of the cerebral biomarkers can advance the knowledge of haemodynamics in preterm infants.

Chapter 7: Overall conclusions

Aims addressed

The primary aims addressed by my thesis were a multimodal, integrated assessment of influences of PDA haemodynamics on cerebral haemodynamics using echocardiography, NIRS, aEEG and cerebral ultrasonography, and the evaluation of a pre-defined two-step model for the causation of brain injury by PDA shunt (Chapter 3). The dataset was rich and provided an opportunity to characterise components of the multimodal assessment including: influences on the aEEG score used in the multimodal assessment (Chapter 4) and antenatal influences on elements of the multimodal assessment (Chapter 5). Moreover, I investigated further existing biomarkers (PTT and PWV) of aortic haemodynamics and related them to PDA and cerebral biomarkers and devised a novel biomarker (PD), which was found to be strongly correlated with PDA size (Chapter 6).

Key findings

Chapter 3

This is the first study to assess the impact of PDA size on cerebral function and incidence of IVH using simultaneously echocardiography, cerebral ultrasonography, cerebral NIRS and aEEG during the natural evolution of PDA disease. The novelty of this approach was the integrated assessment of cardiac and cerebral function using multiple biomarkers and their combination in the construction of a two-step biological model linking PDA size to markers of cerebral dysfunction and these markers to the presence of severe IVH. The PDA related cerebral effects were monitored from birth to discharge. The study cohort included infants who became unwell, needed inotropic support and developed severe IVH. It was demonstrated that it is feasible to perform

this complex monitoring in critically ill patients without significant adverse events and achieve high recruitment rate. As RCTs increasingly adopt similar methodology, the experience collected in the current thesis can inform future RCTs designs.

Babies with severe IVH had different temporal trajectories of the studied biomarkers (PDA diameter, PDA score, cTOI, Burdjalov score and RI) during the transitional period and this persisted for weeks after birth indicating significant interaction between cardiac and cerebral haemodynamics pre- and post-IVH occurrence. The analysis demonstrated that severe IVH was associated with the presence of large PDA and lower cerebral oxygenation. Furthermore, there was a significant association between lower cerebral oxygenation, lower Burdjalov score, and higher RI with larger PDA size, but not in interaction of these variables with IVH severity. However, IVH happens early during extrauterine life or even during pregnancy and there were more observations reflecting the aftermath following the IVH. Accordingly, it is uncertain whether the observed relationships are the cause or the epiphenomenon of brain injury. The strong association between PDA size and all the cerebral biomarkers implies that PDA has important and simultaneous effects on global cerebral function. Patients with severe IVH had reduced cerebral oxygenation even when cerebral arterial resistance remained within normal limits. This is likely to be a reflection of the disturbed cerebral vascular control following a severe cerebral injury. In conclusion, the study demonstrated that separate steps of a proposed two step model (Figure 3) were valid, but not the entire two-step model. This maybe a true observation, a consequence of the study

methodology as data aggregated over a long period were combined into a single analysis or related to the small sample size of the study.

The study demonstrated that PDA and its impact on cerebral biomarkers can be considered a continuum. There was a clear association between PDA and severe IVH. However, many infants with large PDAs did not develop severe IVH and had normal CrUSS at discharge. This supports the ongoing controversy regarding the definition of haemodynamically significant PDA and its impact on cerebral haemodynamics in extremely preterm infants. Severe IVH has devastating implications for individuals, their families and society. If severe IVH is used as clinical outcome to assess PDA disease, it is clear that there are two distinct periods of PDA disease. One during the transitional circulation when in a subset of preterm infants PDA can be an additional strain and offset cardiovascular reserves of the premature heart (these reserves are labile and incomplete) and finally contribute to the multifactorial causal pathway of IVH. The second period during the subsequent weeks is not associated with IVH, but with other disease processes (NEC, BPD, periventricular leucomalacia) when near optimal systemic perfusion maybe maintained due to the high left ventricular output in expense of pulmonary hyperperfusion, higher Qp:Qs and evolving heart failure. Most of the studies consider “PDA disease” as a uniform entity that is either present or absent. However, it is more likely that PDA should be considered as a spectrum from biological normality to a pathological disease state with clinical instability and varying effects on organs. The richness of this dataset suggests that personalised medicine may be difficult in the absence of machine learning techniques. Machine learning

techniques with utilisation of big data analysis have promising applications in other medical specialties (e.g. cancer, neurology, psychiatry) (382, 383). On the other hand, this move towards a personalised medicine approach in neonates could be difficult to achieve as there are many unknown parameters in a multifactorial disease process and these parameters have variable effect size.

A universal cut-off point for PDA size across a wide range of GA may was a pragmatic approach in the past, but this has proved counterproductive and moving forward in the era of precision medicine and research, future clinical trials need to take account of body size, the physiological adaptations, the postnatal maturation and quantify the haemodynamic impact on target organs. In the future additional data from genomic, proteomic and pharmacogenomic studies may allow a more integrated and individualised approach using big data analysis (87). However, there is a need for precise and accurate biomarkers to reduce the signal to noise ratio especially when integrated analysis requires multiple comparisons of many biomarkers over a long observation period.

The study population had a high mortality rate (17%) with considerable morbidity as half of the infants required inotropic support and 12% developed severe IVH. It is paramount to recruit critically unwell patients as this is the group with disturbed haemodynamics and the worse clinical outcomes. Hence, it is more possible to demonstrate significant trends, when performing correlation analysis, and to find significant differences even with relatively small populations. However, the

recruitment of unwell patients is often very difficult, as nurses and clinical staff consider these infants should be protected from extra stress caused by the study procedures. Moreover, there is a culture of nursing and clinical staff being suspicious regarding research recruitment and not considering research as one of main priorities in the clinical practice (384). This is despite the fact that there is little evidence behind the majority of the neonatal interventions and the majority of neonatal drugs are used off-label (385). Hence, it is important to collect data in well designed studies to understand better the underlying physiology of a continually expanding population and inform evidence based interventions and treatments.

Clinicians in the neonatal unit had a relatively conservative approach to PDA treatment and this allowed me to observe the natural history of PDA closure and study the PDA and cerebral haemodynamics without significant contamination from PDA treatment. Moreover, the study managed to recruit a cohort of critically unwell patients and this had significant impact on results. Many studies in neonatal medicine are not sufficiently powered to demonstrate a significant difference/effect between the comparing groups. This is either because the comparing populations are not sufficiently different or the biomarkers used are not precise, accurate or do not have sufficient dynamic range to detect the differences. Hence, there are often conflicting data in the literature as was shown in this thesis.

Limitations

Some limitations of the thesis were mentioned in Chapter 3 (pages 207-8). This single centre study, done in parallel with other studies, needs careful interpretation when

considering generalisability. There is some suggestion of selection bias, most likely due to factors external to the study. Completely generalisable results will need multicentre studies. While feasible in one centre, using similar methods in multiple centres will need careful attention to consistency in using investigative techniques. Some of the sources of potential selection bias recognised in this study will also be present in other centres, e.g. postnatal transfer. The sample size did not allow me to utilise more cardiac and cerebral biomarkers to build a more complex model as the study would have become underpowered. Hence, we limited the biomarkers to five (one for every 10 patients). Consent issues rendered the recruitment of patients during the transitional circulation challenging. However, it is known that the most significant changes of cardiac and cerebral haemodynamics are observed during the first three days after birth. Additionally, many patients came from Level II neonatal units and enrolment was only possible on the second or third day of life. The same patients also had to be discharged back earlier when they did not require intensive care. This led to variable number of different patients contributing data on each time point.

Technical issues and patients' critical clinical condition led to small percentage of missing data. Missing data on different observation time points necessitated the application of multiple imputation procedure. However, it is always statistically preferable to impute rather than discard data. I discontinued monitoring patients with closed PDA after the second week, hence from that time point there were more data coming from patients with persistent PDA which skewed the observed chronological trends. The analysis utilised all the available data from birth to discharge and hence it is

challenging to disentangle causation from aftermath relationship. RI and cTOI were obtained from the frontal lobe area, but aEEG from parietal area. Moreover, PDA and RI were instantaneous observations in comparison to cTOI and aEEG, which were the average of four hours monitoring and potentially could be influenced by other physiological processes.

Future studies

We plan to investigate further a possible causal relationship between PDA, autonomic nervous system, markers of cerebral function and cerebral injury (severe IVH) using Direct Acyclic Graph methodology restricting the analysis only during the transitional circulation when most of the IVH episodes occur (386, 387). Antenatal IVH can be studied by working together with obstetric researchers for better obstetric phenotypic characterisation during premature labour, monitoring of obstetric interventions, and improvement of antenatal consent rates. This collaboration can improve the phenotypic characterisation of the population in risk for IVH and identify cases of antenatal brain injury with the use of antenatal biomarkers. The integration of neurodevelopmental data (collected but not yet analysed) will provide evidence of the long term consequences of the above interactions. Future studies with a combination of MRI studies with blood or urine derived biomarkers of cerebral injury to assess accurately the onset of IVH and relate them to PDA and cerebral biomarkers will possibly facilitate better understanding of the interaction between PDA and IVH.

Chapter 4

The analysis in Chapter 4 demonstrated that cerebral electrical activity increases with postnatal age and with baseline gestational age, but decreases with morphine administration and the presence of a larger PDA. These findings emphasise the significant effects of morphine administration on cerebral function and the potential short- and long-term effects; point to the quick maturation processes which take place following preterm birth; demonstrate that GA at birth is an independent significant parameter when aEEG patterns are assessed, and PDA has significant effects on cerebral electrical activity. Moreover, it became evident that Burdjalov score can be standardised for known influences and used to assess the effects of a treatment.

Limitations and future studies

Limitations and future studies were discussed in Chapter 4.

Chapter 5

Chapter 5 investigated the neonatal cardiovascular and cerebral function following antenatal maternal $MgSO_4$ administration. Maternal BMI and duration of $MgSO_4$ infusion (and their interaction) appeared to have significant effect on neonatal Mg^{2+} that may merit changes to the dosing regimen, for example individualising the loading dose. No associations were demonstrated between neonatal Mg^{2+} with PDA score, cerebral oxygenation and cerebral electrical activity, although the number of patients studied was small. Future dose finding studies should be based on multicompartmental population PK studies that include maternal and neonatal pharmacodynamic measures. To inform these future studies, a sample size calculation was performed

considering different methods of cerebral monitoring as surrogate end point biomarkers.

Limitations and future studies

Limitations and future studies were discussed in Chapter 5.

Chapter 6

Chapter 6 demonstrated that PDA has significant effects on aortic haemodynamics as measured by PTT, PWV and PD. In a small population of extremely preterm infants a strong positive correlation was found between PDA and PD which renders further investigation with validation studies. A continuous predictive biomarker for PDA size can be helpful screening tool for infants needing echocardiography in the neonatal unit in an effort to use human resources more effectively and reduce the disturbance of the vulnerable preterm infants by repeated echocardiograms. Moreover, it can possibly serve as an alarm for those infants who have already received PDA medical treatment as it is known that the PDA reopening rate is moderate (388). Compared to previously used methods which measure the peripheral plethysmographic signals from the limbs, the methodology used in the current thesis has the advantage that the UAC tip lies in close proximity to the PDA in the descending aorta and hence it is believed to detect better the local PDA induced aortic haemodynamics. If proved valid, it can serve as a bedside non-invasive continuous biomarker for monitoring PDA size and can be useful in clinical trials.

Moreover, the analysis demonstrated the clinical potentials of combining PWV and BP data from the UAC to predict left ventricular cardiac output in the descending aorta without the need for echocardiographic assessment, which can be useful clinical information for the management of the extremely preterm infants. There is a clinical need for new biomarkers to measure the effective cardiac output when treating unwell preterm infants as the previous clinical practice to rely on BP has been proved counterproductive. If further validated, the methodology used in the current thesis can provide continuous non-invasive data which can be displayed real time on the patient's monitor and combined with other biomarkers (lactate, urine output, NIRS data) can potentially help to make rational clinical management decisions based on pathophysiological grounds and expand our knowledge of haemodynamics in preterm infants.

Limitations

Limitations were discussed in Chapter 6.

Future studies and potential clinical applications

The PD between ECG and BP is straightforward to measure and hence the results of this pilot study suggest that this approach merits further investigation as a possible biomarker in trend monitoring of PDA size and function. Further studies with more patients are needed to confirm our findings and investigate other covariates related to the PWV and PD in extremely preterm infants with PDA and their relation to long term clinical outcomes. Moreover, if PD is used only for trend monitoring of PDA in an individual patient, then the rest of the Hagen-Poiseuille's law parameters become irrelevant as they remain relatively stable and only PDA diameter changes in short

term. Future studies can also confirm the relationship between PDA, PWV, cardiac output and BP in preterm infants. These studies will provide more information whether it is possible combined PWV and BP data from the UAC to predict cardiac output in the descending aorta. If these biomarkers are proved valid to assess haemodynamics in preterm infants, it is possible to use them as surrogate markers in pharmacodynamic studies as they can be measured continuously during drug administration without interfering with the underlying haemodynamics and without disturbing infants' wellbeing.

Overall conclusions

The current thesis has proved that multimodal assessment of cardiac and cerebral biomarkers over a long period is well tolerated by extremely preterm infants and useful to elucidate the complex interactions between PDA and cerebral function. It was found that PDA is associated with severe IVH and altered cerebral function, however a causal relationship could not be proved and it is uncertain whether the observed relationships are the cause or the epiphenomenon of brain injury. The study also demonstrated a strong association between PDA size and the studied cerebral biomarkers, which implies that PDA has important and simultaneous effects on global cerebral function. I found that although separate steps of the proposed two step model were valid (Figure 3), the entire two-step model was not. This may be a true observation or the consequence of the study methodology as data aggregated over a long period were combined into a single analysis and might be related to the small sample size of the study. I speculate that PDA should be considered as a spectrum from

biological normality to a pathological disease state with varying effects on target organs. Cutoffs may be needed for trial eligibility criteria but, ideally, the cut off should be evaluated by relating the characteristics of the PDA to the risk of clinically important outcomes. Moreover, biological models are a good basis for research given that there is sufficient understanding of the biology to develop these models. The data analysis made evident that conventional statistics may be difficult to use and alternatives are DAGs or machine learning techniques.

In Chapters 4 and 5 it was demonstrated that pharmacodynamic end point biomarkers can be characterised during the study of neonatal cerebral and cardiac haemodynamics. In Chapter 4 it was found that cerebral electrical activity (Burdjalov score) increases with postnatal age and with baseline gestational age, but decreases with morphine administration and the presence of a larger PDA. Moreover, it became evident that Burdjalov score can be standardised for known influences and used to assess the effects of a treatment. Chapter 5 investigated the neonatal cardiovascular and cerebral function following antenatal maternal $MgSO_4$ administration and found maternal BMI and duration of $MgSO_4$ infusion (and their interaction) to have significant effect on neonatal Mg^{2+}. Despite the absence of associations between neonatal Mg^{2+} with PDA score, cerebral oxygenation and cerebral electrical activity, a sample size calculation was performed to inform future studies considering different methods of cerebral monitoring as pharmacodynamic end point biomarkers.

Finally, in Chapter 6 it was found that new PDA biomarkers can be developed, but should be characterised and validated before being evaluated in clinical studies. I found a strong positive correlation between PDA and PD, which has the potentials to be a bedside non-invasive continuous biomarker for monitoring PDA size and possibly an alarm for PDA reopening in infants already received PDA medical treatment. Moreover, the analysis demonstrated the clinical potentials of combining PWV and BP data to predict left ventricular cardiac output in the descending aorta without the need for echocardiographic assessment, which can be useful clinical information for the management of the extremely preterm infants.

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Appendices

Appendix 1: Study protocol

Haemodynamic biomarkers in preterm infants with Patent Ductus Arteriosus (PDA) (HAPI-PDA study): a pilot cohort study of the relationships between blood flow through a PDA and surrogate outcomes for brain injury

REVISION HISTORY:			
Version	Authors	Effective Date	Changes Made
1.0	C.Kotidis, M. Weindling, M. Turner	27.05.14	Minor changes
1.2	Louise Hardman	30.05.14	Minor changes
1.3	C. Kotidis, M. Turner	24.09.14	Minor changes in Data Protection section according to ethics committee recommendations
1.4	C. Kotidis	12.01.15	Major changes on inclusion criteria

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Contents

PROTOCOL SYNOPSIS

Title of Study	**Haemodynamic biomarkers in preterm infants with Patent Ductus Arteriosus PDA (HAPI-PDA study): a pilot cohort study of the relationships between blood flow through a PDA and surrogate outcomes for brain injury**
Part of an FP7 Collaborative Project titled	'Dobutamine for NEOnatal CIRCulatory failure defined by novel biomarkers.' Grant agreement: FP7; HEALTH-2011.4.2-1 [Investigator-driven clinical trials on off-patent medicines for children]. Grant Agreement Number: 282533
Sponsor	University of Liverpool
Study site	Liverpool Women's Hospital
Background	Babies born at extreme prematurity have a distinct pattern of blood flow as they adapt to extrauterine life. One feature of this transition is a patent ductus arteriosus (PDA) that is found commonly in babies born prematurely. PDA is associated with poor outcomes of preterm birth such as brain injury. Excess blood flow through a PDA may be a causative factor in brain injury affecting babies born at extreme prematurity. However, there is little published work about how the extent of blood flow through a PDA is associated with brain injury. Surrogate outcomes are needed to study this association in clinical settings.
Principal objective	To validate study procedures, biomarkers of the severity of PDA and surrogate outcomes of brain injury in order to develop a preliminary estimate of the extent to which biomarkers of PDA flow correlate with surrogate outcomes of brain injury. Specifically, a core dataset will be collected in all participants to examine the association between a biomarker of PDA flow (PDA severity score) and the surrogate outcome of cerebral blood flow (cerebral tissue oxygenation) measured using Near Infra Red Spectroscopy (NIRS).
Study design	Cohort study of babies born at extreme prematurity. Run-in phase before full cohort opens. When possible participants in the cohort will contribute to substudies to validate other biomarkers of PDA and surrogate outcomes.
Inclusion criteria	• neonates 24 to 28^{+6} weeks´ gestation

	• postnatal age ≤72 hours • PDA confirmed by echocardiography • parental informed consent
Exclusion criteria	• non-viability (unlikely to survive more than 48 hours in the opinion of the attending physician) • congenital hydrops, congenital heart disease or other malformations likely to affect cardiovascular adaptation • surgery planned within 72 hours of birth • chromosomal anomalies • IVH> grade 2 in the initial cranial ultrasound scan • informed consent form (ICF) not signed.
Treatment duration / duration of observation	To be defined by the clinical team. The study will have no influence on clinical management.
Assessment of exposure	Using echocardiography, the magnitude of blood flow through the PDA will be assessed by measuring a set of variables that will generate a severity score. The feasibility of of this score will be assessed during the run-in phase
Primary Outcome measure	Cerebral tissue oxygenation index measured (cTOI) by NIRS To be confirmed during the run-in phase.
Secondary Outcome measures	Other echocardiography measurements (including Superior Vena Cava (SVC) flow) Other NIRS variables EEG Beat-to-beat variability
Sample size	Time-limited pilot study for 15 months: run-in phase of 3 months and full study for 12 months. We estimate that 15 babies will contribute to the run-in study and 60 to the full study
Substudies	During the full study groups of babies will contribute to validation studies of other biomarkers and surrogate outcomes depending on how long babies can be studied for.
Statistics	<u>Cohort study</u> Correlation between PDA severity score and cTOI measured on NIRS as a marker of cerebral blood flow. Descriptive analyses of other variables.

	Sub studies Descriptive analyses of variables.

Background

Preterm labour and its accompanying morbidity and mortality in preterm babies has long term consequences on the life of the individual, their family and the community [1]. The shift from in utero life to life in air is a big challenge that the preterm infant has to overcome with immature organs and poor reserves. A key element of this transition is closure of the ductus arteriosus. Delayed closure of ductus arteriosus in premature neonates is associated with significant morbidity. Closure of the duct involves multiple complex mechanisms that are compromised in preterm infants. Patent ductus arteriosus (PDA) is a common finding in the first weeks of life occurring in about 1/3 infants <30 weeks' gestational and up to 60% of infants <28 weeks. The incidence increases linearly with decreasing gestational age (GA) [2]. Several studies have identified genetic and environmental risk factors contributing in patency of ductus arteriosus in term and preterm infants [3]. PDA is associated with increased mortality [4], bronchopulmonary dysplasia (BPD) [5], necrotizing enterocolitis (NEC) [5], peri-intraventricular haemorrhages (PIVH) and subsequent neurodisability [6, 7] and renal impairment [8]. Multi-organ failure is the usual cause of death in infants with persistent PDA despite therapy.

PDA is considered to be a shunt that diverts blood flow from the systemic to the pulmonary circulation. This "steal phenomenon" and the accompanying pulmonary plethora are considered as the main pathophysiologic mechanisms that contribute to the observed outcomes in preterm infants: higher incidence of BPD, lower peripheral tissue oxygen delivery and subsequently higher incidence of NEC and renal failure, lower cerebral oxygenation and subsequently higher incidence of PIVH, that all contribute to higher rate of death [9]. However, although PDA is associated with a range of problems it is not clear whether PDA is the cause of effect of its associated morbidities.

PDA management is very controversial because of the diversity of PDA physical history, lack of widely accepted definition and criteria for PDA assessment, medication side effects and limited access to echocardiography in neonatal units. There is no clear answer in the literature regarding optimal therapy [10-12]. Several clinical trials and systematic reviews have failed to demonstrate a meaningful long term beneficial effect of PDA treatment [13, 14]. PDA is closed spontaneously in the majority (94%) of infants with birth weight >1000gr before discharge [15]. PDA might be just a biomarker of disease severity associated with prematurity. There are no randomized placebo-controlled trials of ductal treatment with a real no treatment arm focusing on the long term outcomes. There is a growing discussion among neonatologists regarding the need for a placebo-controlled trial where the PDA will be left untreated.

Most of the studies in the literature consider PDA as a uniform entity that is either present or absent. However, there are accumulating data that PDA can be considered as a spectrum from biological normality to a pathological disease state with clinical instability and varying effects on organs [16, 17]. The lack of precise characterisation of the PDA might partially explain the controversial results from the studies that deal with causality and treatment. Recent studies underlined the need for classification of PDA disease and presented a correlation between disease staging and outcome (BPD) [18, 19]. Serial echocardiography can identify haemodynamically significant PDA before clinical signs present and reduce severe IVH and days on ventilator [20]. It is important to stratify disease in order to prevent harm from known drug side effects (ibuprofen, indomethacin) [14] and intervention related morbidity (PDA ligation) [21, 22]. Moreover, patients need treatment as individuals and clinicians have to apply the knowledge obtained by research in a personalised manner. It would be very useful if we could predict which infants are susceptible to PDA related co-morbidities.

A number of techniques can be used to assess the extent and consequences of PDA.

Echocardiography

Echocardiography is considered as the gold standard in diagnosis and management of PDA. Precise measurement of ductal size and its hemodynamic impact on the pulmonary and systemic circulation is feasible. Most of the studies in the past focused on PDA size as severity biomarker. Sehgal et al have described a scoring system in order to evaluate PDA severity [19]. There is a correlation between a high composite PDA severity score with a higher incidence of subsequent chronic lung disease in preterm infants. The proposed assessment of the haemodynamic impact of ductal flow might allow the determination of responders and non-responders to the medical or surgical treatment of PDA. SVC flow is considered a surrogate marker of systemic blood flow especially in the early postnatal period, when shunts confound the classic echocardiographic measurement of left and right ventricular output [23]. Low SVC flow has been shown to be associated with surrogate markers of adverse long-term outcomes [24, 25], particularly late intraventricular haemorrhage (IVH) occurring more than 6 hours after birth [24]. There is a positive association between SVC flow and cTOI in very low birth weight infants [26].

Autonomic dysfunction

The autonomic nervous system regulates visceral homeostasis by functioning below the level of consciousness. Autonomic dysfunction is a well-studied entity in adult medicine and has been associated with multiple outcomes in cardiovascular disease. Heart rate variability (HRV) is a well established marker of autonomic dysfunction which was first described in perinatal medicine in 1965 [27]. However, there is limited evidence of the impact of PDA on autonomic dysfunction especially during the critical period of transitional circulation and/or vice versa. A recent study revealed that PDA alters the equilibrium of autonomic function by stimulating the parasympathetic system. The authors suggested that fluid overload may stimulate receptors placed in the pulmonary artery, ventricles and atria. Atrial natriuretic peptide (ANP) released by atria in response to fluid overload may imply a second pathophysiologic mechanism as

ANP decreases HR and has vasodilator properties [28]. Another study found that infants with a PDA had a higher heart rate and short-term variability, but reduced long-term variability when compared with matched controls. PDA treatment with indomethacin did not have any impact on these observations [29]. However another study did not replicate these results. PDA closure with indomethacin reduced the mean heart rate and increased the long-term variability. The authors attributed the observations to improved brain stem oxygenation [30].

Near infrared spectroscopy

Near infrared spectroscopy (NIRS) provides a non invasive method of assessing cerebral and systemic haemodynamics by measuring tissue oxygenation [31]. Human tissues are relatively transparent to near infrared light. Haemoglobin absorbs near infrared light of specific wavelength and through spectroscopy, it is possible to monitor tissue oxygenation. New generation NIRS can measure absolute values. In contrast to pulse oximetry, that measures arterial oxygen saturation, NIRS measures oxygenation in the tissue as a whole. As the venous blood volume accounts for 75% of the total, we can assume that NIRS measures mainly venous saturation. By comparing arterial and venous oxygenation it is possible to calculate cerebral blood flow (CBF) and fractional oxygen extraction (FOE). TOI is the ratio of oxygenated haemoglobin to total haemoglobin in the tissue, has a good correlation with the cerebral venous saturation in animal studies and can be used as a non invasive parameter for monitoring of cerebral oxygenation [32].

Inadequate haemodynamic adaptation during the first 72 hours after birth can affect cerebral perfusion [33]. NIRS may be an effective tool for screening infants at risk for adverse outcomes due to PDA [34]. Cerebral hyperperfusion has been associated with severe PIVH [35]. Preterm infants with haemodynamically significant PDA have impaired regional cerebral oxygenation [9] and higher risk of IVH [7]. NIRS is a reliable method of assessing superior vena cava saturations and superior to arterial saturation

in monitoring systemic perfusion in infants with single ventricle heart defects [36]. There are conflicting data in the literature regarding the effect of PDA treatment, both pharmacological and ligation, in cerebral oxygenation [9, 34, 37-39]. The impact of NIRS monitoring on long term outcomes in neonates with PDA is unknown.

Electroencephalogram

Electroencephalogram (EEG) is a useful tool in assessing brain activity, especially in sedated and ventilated preterm infants. The EEG pattern of infants between 26 and 30 weeks gestation has been well characterized [40]. Reduced cerebral perfusion is associated with decreased amplitude of EEG [41]. There are limited numbers of studies assessing EEG in neonates with PDA. In an old and small study assessing PDA severity using carotid Doppler, no difference was found in spectral analysis of EEG. This study concluded with the assumption that the degree of decreased cerebral blood flow in infants with a significant PDA is not sufficient to cause measurable alteration in electrocortical activity [42]. In another study, where only 1/4 of the study population had a small PDA, infants with low cardiac output and normal blood pressure were able to maintain a normal electrocortical activity. They presumed that cerebral autoregulation preserves blood flow even in very preterm babies, but the limits of autoregulation are uncertain [43]. Indomethacin treatment for PDA does not seem to impair cerebral function as measured by quantitative EEG [44]. PDA ligation has been associated with compromised cerebral oxygenation and decreased aEEG measured electrical brain activity [22].

Rationale for the study

Neonatology is one of the most rapidly changing medical specialties due to technological and medical progress. Surfactant administration, antenatal steroids, nitric oxide and sophisticated ventilators rendered old studies not applicable to the

new era. Pooling results from these studies in order to conduct systematic reviews or formulating clinical protocols doesn't really apply on current clinical practice. There is a need for new studies that will address the pathophysiology of PDA in the growing population of extremely low birth infants.

Consequently we postulate that PDA disease severity will affect cerebral oxygenation and possibly major neonatal clinical diagnoses in a continuum. This leads to the hypothesis that magnitude of blood flow through the PDA as assessed by flow through the PDA measured using echocardiography is linearly proportional to cerebral oxygenation (measured by NIRS). Secondary hypotheses are that the magnitude of PDA is linearly proportional to other surrogate outcomes representing brain injury: cerebral blood flow (measured by SVC flow), cerebral electrical activity (measured by EEG), beat-to-beat variability (as a marker of autonomic dysfunction), and is associated with major neonatal diagnoses.

One important aspect of this therapeutic area is the use of haemodynamic assessments as biomarkers during clinical drug development and clinical practice. In general, biomarkers can be used for diagnosis, indication for treatment and monitoring treatment. Biomarkers need to be validated and qualified. Validation refers to assessing the extent to which a measurement is reliable and includes assessments of repeatability, inter- and intra-user variability. Qualification refers to assessing the extent to which a measurement reflects clinically important outcomes.

Despite the widespread use of these assessments in clinical practice there has never been a systematic evaluation of validation and qualification. This study will contribute to the evaluation of haemodynamic assessments in neonates through sub studies relating to specific biomarkers and surrogate outcomes. The lack of prior data means that this study needs to identify the extent to which the assessments are validated and also identify sources of uncertainty that need to be addressed in future studies. With respect to qualification some relevant data about outcomes at hospital discharge will be gathered from data that is collected in routine clinical practice. This provides the

opportunity to conduct preliminary studies to qualify haemodynamic biomarkers of PDA and qualify surrogate outcomes of cerebral perfusion.

An important influence on haemodynamic assessments is the impact of medicines administered to neonates. A number of studies of medicines are underway on the Neonatal Unit and these provide the opportunity to examine the impact of different medicines.

This study will be conducted in parallel to other studies on the Neonatal Unit:

1. OSCAR: Randomised control trial of ibuprofen vs placebo among neonates with significant PDA within 72 hours of birth.

2. NeoCirculation: observational study of haemodynamics and dobutamine PK.

These studies are both multi-centred and so will provide the opportunities for comparison of the findings between sites. In particular, NeoCirculation has a particular emphasis on developing haemodynamic biomarkers in this population.

Subject to the relevant consents from parents, this study will take baseline measurements and follow-up for four groups of babies:

a) babies enrolled in OSCAR and allocated to placebo

b) babies enrolled in OSCAR and allocated to ibuprofen

c) babies enrolled in NeoCirculation

d) babies not enrolled in OSCAR or NeoCirculation

In the past parents have not objected to being approached about multiple studies and have been comfortable agreeing to participation in no, one or two parallel studies. The burdens imposed by these studies are not large and are consistent between studies. At all times parents will be able to change their minds and withdraw their baby from any or all of these studies without any impact on the care their baby receives.

Objectives

The primary objective of this project is to make a preliminary estimate of the magnitude of the association between the haemodynamic impact of PDA (as measured by the PDA severity score) and cTOI (as measured by NIRS).

Secondary objectives of this project are:

1. To validate components of a PDA severity score within 72 hours of birth among babies born at less than 29 weeks gestation
2. To validate measurement of surrogate outcomes for brain injury: haemodynamic parameters within 72 hours of birth among babies born at less than 29 weeks gestation, echocardiographic assessment of haemodynamic status (such as SVC flow), EEG and autonomic dysfunction.

Methods

This prospective observational study will be performed in Liverpool Women's Hospital. All infants born <29 weeks' gestation are eligible for recruitment in the study.

A demographic dataset will be compiled from the clinical record. This will be supplemented by a haemodynamic dataset including biomarkers of PDA and surrogate outcomes relating to cerebral oxygenation. The core haemodynamic dataset will include the PDA severity score as a marker of PDA evaluation and cTOI on NIRS. Sub studies will contribute data about other potential surrogate outcomes.

Inclusion and exclusion criteria

Inclusion criteria are:

- neonates 24 to 28^{+6} weeks´ gestation

- postnatal age ≤72 hours
- parental informed consent

Exclusion criteria are:

- non-viability (unlikely to survive more than 48 hours in the opinion of the attending physician)
- congenital hydrops, congenital heart disease or other malformations likely to affect cardiovascular adaptation
- surgery planned within 72 hours of birth
- chromosomal anomalies
- IVH> grade 2 in the initial cranial ultrasound scan
- informed consent form (ICF) not signed.

Primary Outcome

The primary outcome will be the relation of PDA severity score to cTOI.

Secondary outcomes

a) cerebral electrical activity

b) cerebral tissue oxygenation index

c) cerebral blood flow

d) beat-to-beat variability

f) arterial blood pressure (measured invasively)

g) capillary refill time (CRT)

h) urine output

i) blood lactate concentration

j) base excess.

Tertiary outcomes will be part of the core dataset and will contribute to the qualification of surrogate outcomes measured earlier in the clinical course.

- Clinical outcomes at term equivalent:
    - death
    - composite outcome of IVH grade 2-4
    - major clinical neonatal diagnoses at 40 weeks' gestation: prevalence of hypotension, days to reach total enteral nutrition, necrotizing enterocolitis (± surgery); treatment for PDA; age at PDA treatment; retinopathy of prematurity (ROP) (worst grade); ROP requiring treatment, renal insufficiency, oxygen dependency at 36 weeks' gestation (chronic lung disease); oxygen dependency at discharge; nosocomial infection.
    - If funds are secured in the future, we will proceed to assess the neurodevelopmental outcome at 2 years corrected age and compare it with the background risk of PDA severity.

Study procedures

Recruitment

Families will be approached before birth if possible or in the first day after birth. An information leaflet has been specifically designed and will be provided to parents before asking consent. Consent will be required for all study procedures. Consent will be obtained when convenient after admission before a baby becomes eligible. Parents will have ample time to think and ask any possible questions regarding the study. Parents can withdraw consent at any point of the study without an explanation. The investigator's contact information will be provided.

All neonates born at less than 29 weeks' gestation will have a screening echocardiogram done as part of routine clinical care. Babies will be recruited once they meet the study inclusion criteria and consent is given.

Echocardiography

All echocardiography studies will be performed using a Vivid E9 GE machine and 12 or 6-Hz probe. Two dimensional, M-mode, pulse and colour flow Doppler imaging will be performed. Echocardiography will be done every 24 hours with at least 12 hours between assessment in the first 3 days of life, once weekly until the PDA closes and then before discharge. Infants without PDA or with a PDA closed in the first 3 days of life will be used as a comparison group to assess "normality" and echocardiography will be still performed until 2 weeks of age at the aforementioned time intervals. If the clinical condition of the babies allows, PDA will be classified using previously described methods (Table 1) [19]. Individual parameters are graded from 1 to 3 based on the magnitude with a composite score range 0-30.

Feature quantified	Modality/position of sample gate	Score 0	Score 1	Score 2	Score 3
Ductal features					
Transductal diameter (mm)	Two-dimensional, short axis view	0	<1.5	1.5–3	>3
Ductal velocity Vmax(m/s)	PWD at pulmonary end of duct	0	>2	1.5–2	<1.5
Magnitude of ductal shunt					
PDA:LPA diameter			<0.5	≥0.5–1	≥ 1
Antegrade PA diastolic flow (cm/s)	PWD within main pulmonary artery	0	0–20	>20	
Antegrade LPA diastolic flow (cm/s)	PWD within left pulmonary artery	0	<30	30–50	>50
Left atrial: aortic ratio	M-mode, long axis view	1.13 ± 0.23*	<1.4:1	1.4–1.6:1	>1.6:1
Left ventricular: aortic ratio	M-mode, long axis view	1.86 ± 0.29*	–	2.15 ± 0.39*	≥ 2.5
Features of myocardial performance					
LVO/SVC flow ratio	PWD of flow in superior vena cava	2.4 ± 0.3*	–	–	≥ 4
E wave/A wave ratio	Transmitral Doppler	<1		1–1.5	>1.5
IVRT (ms)	Between mitral and aortic valves	>55	46–54	36–45	<35

Table 1. Scoring system of hemodynamic significant DA. *Mean ± SD, Vmax: maximum velocity, LPA: left pulmonary artery, PA: pulmonary artery, PWD: pulse wave doppler, isovolumetric relaxation time (IVRT).

NIRS measurements

The cTOI will be measured as described elsewhere [45] using NIRO-200NX; Hamamatsu Photonics KK, Shizuoka, Japan. The combined use of NIRS and pulse oximetry allows the continuous monitoring of the cerebral fractional oxygen extraction (FOE) [32]. Recordings will start as soon as possible after enrolment; 4 hours every 12 hours in the first 3 days, 4 hours daily in the rest of the first week, 4 hours on alternate days until the PDA closes and before discharge. If PDA closes earlier than 2 weeks, NIRS will be still applied until 2 weeks of age in order to collect "normal"data. NIRS monitoring will always precede heart scans. NIRS application procedure and monitoring are specified in details in the relevant Appendix and Standard Operating Procedure.

Other assessments will be conducted in sub studies.

Autonomic dysfunction

The ECG data will be recorded as part of the routine neonatal care, from three lead electrodes forming the Einthoven triangle - two electrodes are placed onto either side of the chest and a third on the outer aspect of one thigh. The signal will be sampled at 256 or 512 Hz and stored on trust shared drive. Recordings will be performed whilst the infants are asleep and where they will not be disturbed throughout the duration of the recording. Recordings will precede echocardiography and will be performed simultaneously with EEG and NIRS, if possible. Otherwise recordings will be done as closer to echocardiography as possible. Sleep state will be justified according to criteria set by Sheldon [46].

Of note, this procedure does not cause any disturbance to the participant and so can be conducted at the same time as other assessments.

Electroencephalography

Digital EEG recordings will be performed using a Nicolet 16-channel system (Optima Medical Ltd., UK). Six electrodes will be placed on the frontopolar (Fp1, Fp2), central (C3, C4), and occipital (O1, O2) positions bilaterally according to the International 10-20 System [47]. A reference electrode will be placed at the vertex (Cz). Skin impedance of 2 k will be maintained for all recordings. A sampling rate of 256 Hz will be used for digitization. The EEG will be displayed on a computer screen as four bipolar channels (Fp1 – C3, C3 – O1, Fp2 – C4, and C4 – O2) using a high-pass filter of 0.3 Hz, a low-pass filter of 70 Hz, a notch filter of 50 Hz, a base time of 10 s, and a gain of 100 V. The EEG recordings will be analysed for degree of discontinuity, amplitude, presence of abnormal transients, and synchrony. Quantitative analysis of EEG will be done by a) spectral analysis and b) manual calculation of the interburst interval. To calculate the interburst intervals, gross artefacts (activity with no identifiable normal EEG activity) will be identified by eye and removed. The interburst interval will be defined as a period between electrical bursts during which activities are lower than 30 V in all leads and calculated manually [48]. The 90th centile (P90) for interburst interval will be then calculated for the first 60 min of artefact-free recording for each baby. The normal range (10th to 90th centile) of P90 interburst interval in infants between 24 and 30 wk gestation is 7–25 s during the first 2 days after birth [49]. Spectral analysis using Fast Fourier transformation will be performed using the manufacturer's software (Nicolet) and is described in detail elsewhere [49]. The 75 min of EEG recorded from six monopolar channels (Fp1, Fp2, C3, C4, O1, and O2) will be subjected to spectral analysis. The spectrum will be subdivided into delta (0.5–3.5 Hz), theta (4 –7.5 Hz), alpha (8 –12.5 Hz), and beta (13–30 Hz) frequency bands. The absolute power of a frequency band is defined as the integral of the power values within the frequency range and expressed as V2. The relative power of a frequency band is defined as the

ratio of the absolute power of that frequency band to the total power of the EEG signal and expressed as a percentage. The absolute and relative powers of each frequency band will be calculated for every 10-s epoch. Gross artefacts (activity with no identifiable normal EEG activity) will be identified by eye and removed manually in 10-s epochs. The first 60 min of artefact-free EEG will be then used to calculate the median absolute and the median relative powers of each frequency band. For very low birth weight infants, the relative power of the delta frequency band is the most discriminatory and repeatable (coefficient of repeatability of 8%) spectral measurement [49]. The normal range (10th to 90th centile) of the relative power of the delta frequency band in infants between 24 and 30 w gestation is 62–82% during the first 2 d after birth [49].

Cranial ultrasound

Cranial ultrasound scan (CUS) will be done as soon as possible after enrolment, preferably within the first 6 hours of birth. Then between 48 to 72 hours, at postnatal day 7 (±1), 14 (±1), and at 36 (± 2) week's gestation. CUS is part of the routine clinical care of premature infants. Eleven pre-specified views will be recorded and stored.

Priority of measurements

There will be a number of assessments for each participant.

Given the clinical state of the participants some assessments will not be possible in some participants. The measurements will be done in a set order so that consistent results are obtained for the core dataset and substudies are done only if core studies have been completed.

The order of assessment will be:

1. Set-up NIRS
2. Assess cTOI on NIRS
3. Assess other NIRS variables

4. Set-up EEG
5. Conduct EEG
6. Conduct echocardiogram

Assessment of autonomic dysfunction has no impact on the participant and so will be done on all participants who have an echocardiogram.

Clinical data

Obstetric and intrapartum data will be collected from hospital records. Neonatal data will be collected prospectively. All clinical data of patients (vital signs, ventilator settings, laboratory blood results, medication and possible interventions) will be recorded electronically in our central database and analysed retrospectively. Description of specific data to be entered in the case report form are as follows:

- Maternal history:
  - Age.
  - Social history: job, education, income (will be used to calculate socioeconomic index)
  - Antenatal corticosteroid use (complete course).
  - Maternal hypertension and treatment.
  - Chorioamnionitis: fever (>38ºC), foul smelling amniotic fluid, tenderness of the uterus, maternal/fetal tachycardia, maternal leukocytosis, pathological diagnosis.
  - Prolonged rupture of membranes (>24 hours to delivery).
  - Type of delivery.
  - Previous medical history, medication and family history.
  - Others (window for description).

- Perinatal data:
  - Gestational age.
  - Birth weight.
  - Small for gestational age (defined by weight <3rd percentile).
  - Multiple (order).
  - Gender.
  - Need for advanced resuscitation at birth (intubation ± assisted circulation).
  - Placental transfusion procedures (e.g. delayed cord clamping, milking of the cord).
  - 5 min Apgar score.
  - Working weight and head circumference.

- Neonatal outcomes:
  - Invasive BP (mean, max, min) while the infant is on inotropes.
  - Capillary Refill Time (CRT) while the infant is on inotropes.
  - Biochemical data: plasma lactate, base excess, pH, blood gases, central haemoglobin, white cell count, neutrophil count, C-reactive protein, blood cultures, creatinine and glucose serum levels. A blood gas will be done after the end of NIRS and EEG recording and before the start of Echo-D, as EEG parameters are also closely associated with pCO2 and ventilatory status.
  - Physiological data: heart rate, pulse oximetry
  - Urine output: mean diuresis, expressed as ml/kg/h
  - Volume replacement: total amount of i.v. bolus administered as volume expansion (crystalloids or blood products) and time

- Cranial ultrasound scan findings

- Major neonatal diagnoses/outcomes (at 40 weeks' gestation ± two weeks, or earlier if died or discharged)

- Survival free of severe brain injury as defined by CUS
- Overall mortality
- Major CUS diagnoses: IVH 2-4; cystic and non-cystic PVL, porencephalic cyst, ventriculomegaly, cerebellar haemorrhage; widening of interhemispheric fissure; others (window for description)
- Hypotension
- Days to reach full enteral nutrition
- Necrotizing enterocolitis or bowel perforation
- Oxygen dependence at 36 weeks´ gestation
- Oxygen dependence at discharge
- Nosocomial infection
- Ductus arteriosus patency duration and treatment (age)
- Retinopathy of prematurity (grade) and treatment

Measures to minimize pain and distress

All study related procedures will be undertaken in line with local procedures to minimise pain and optimise welfare. All study-related procedures will be conducted by professionals experienced in neonatal care who have received study-specific training.

Clinical management

All infants will receive standard routine clinical treatment according to the local neonatal guidelines in Liverpool Women's Hospital. The attending physician, who will not be a member of the research group, will coordinate the management of the infants. The unit has adopted a symptomatic way of PDA treatment, but practice varies among clinicians. In 2012 among children with echocardiographically confirmed PDA, 20% received ibuprofen and 3% had duct ligation. It is the policy of the neonatal unit to keep infant’s mean BP (mmHg) above the infant’s gestation (weeks) and instituting treatment when mean BP is that limit plus one or more of the following clinical/laboratory features: urine output < 0.5 mL/kg/hour, poor peripheral perfusion (CRT > 3s) or lactate > 4 mmol/L. This will be achieved by volume expansion using

blood transfusion or normal saline and/or inotropes such as dopamine, dobutamine, adrenaline or hydrocortisone either singly or in combination. Equipment used in the study is checked by the Medical Engineers of Liverpool Women's Hospital and will be used under the local Infection Control Guidelines.

Follow-up measurements

If funds are secured in the future, we will proceed to assess neurodevelopmental outcome at the age of 24 months (corrected for prematurity) and correlate it with PDA severity.

Sample size considerations

As this is a pilot study, power calculation is not applicable. We will study the existence and nature of possible associations between PDA score and different clinical outcomes. In a sample of 75 participants contributing to the core dataset associations of interest would be defined as those with moderate correlation of 0.4 ($p<0.05$).

Study duration

In Liverpool Women's Hospital we admit annually 110 preterm infants born less than 29 weeks GA with 85 of them having echocardiographically confirmed PDA. We expect a high consent rate as the study is observational with non-invasive monitoring methods. Thus we consider that 1.5 years is a reasonable time period to recruit 75 patients.

Statistical analysis

We will produce summary statistics for each time point of the study. The PDA severity score will be the predictor of outcomes and cTOI measured using NIRS will be the primary outcome. Correlation coefficients will be the main form of analysis. Autonomic dysfunction, cerebral electrical activity and cerebral blood flow will be the main secondary outcomes. Simple graphical analysis will be used to examine the distribution of the variables and their interrelationship. We will assess also if PDA severity score has a predictive value in evaluating future death, composite outcome of IVH grade 2-4 and

major clinical neonatal diagnoses at 40 (± 2) weeks' gestation: prevalence of hypotension, days to reach total enteral nutrition, necrotizing enterocolitis (± surgery); treatment for PDA; age at PDA treatment; retinopathy of prematurity (ROP) (worst grade); ROP requiring treatment, renal insufficiency, oxygen dependency at 36 weeks' gestation (chronic lung disease); oxygen dependency at discharge; nosocomial infection. The Mann–Whitney U-test (or Student's t-test) will be used to investigate population characteristics, and linear regression analysis will be performed to identify the correlation between the multiple variables.

Ethical considerations

Ethical approval will be asked from a Research Ethics Committee. Parental informed consent will be obtained prior to any study procedures. The trial will be conducted in accordance with the principles of Good Clinical Practice. Procedures will be established to prevent and/or minimise risk of complication for participants, such as complications related to the device and the treatment guideline includes only interventions that are commonly used during intensive care in this population.

Data Protection

The study staff involved with this study will comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants. Only designated university personnel has access to the department through a secure swipe card system. Extra encoded door protects the room where the paper files will be stored. Paper and other manual files will be filed and stored securely in a locked cabinet. Computers used to collate the data will have limited access measures via user names and passwords. Encrypted external hard drives will be used to transfer data from Echo, NIRS and EEG machines to the central database. Published results will not contain any personal data that could allow identification of individual participants.

This study is done as part of a project involving six countries in the European Union, Turkey and the USA. Some of the data may be shared with partners in these other countries who are doing similar work on babies in their countries. All data will be shared following linked anonymisation. Consent will be sought for the transfer of data outside the EU. If parents decide to withdraw from this study, all anonymised information collected about the mother and baby will be retained for the purpose of completing the research, unless parents tell us otherwise. If parents withdraw their baby from the study, we will ask them whether we can keep the data that relates to their baby.

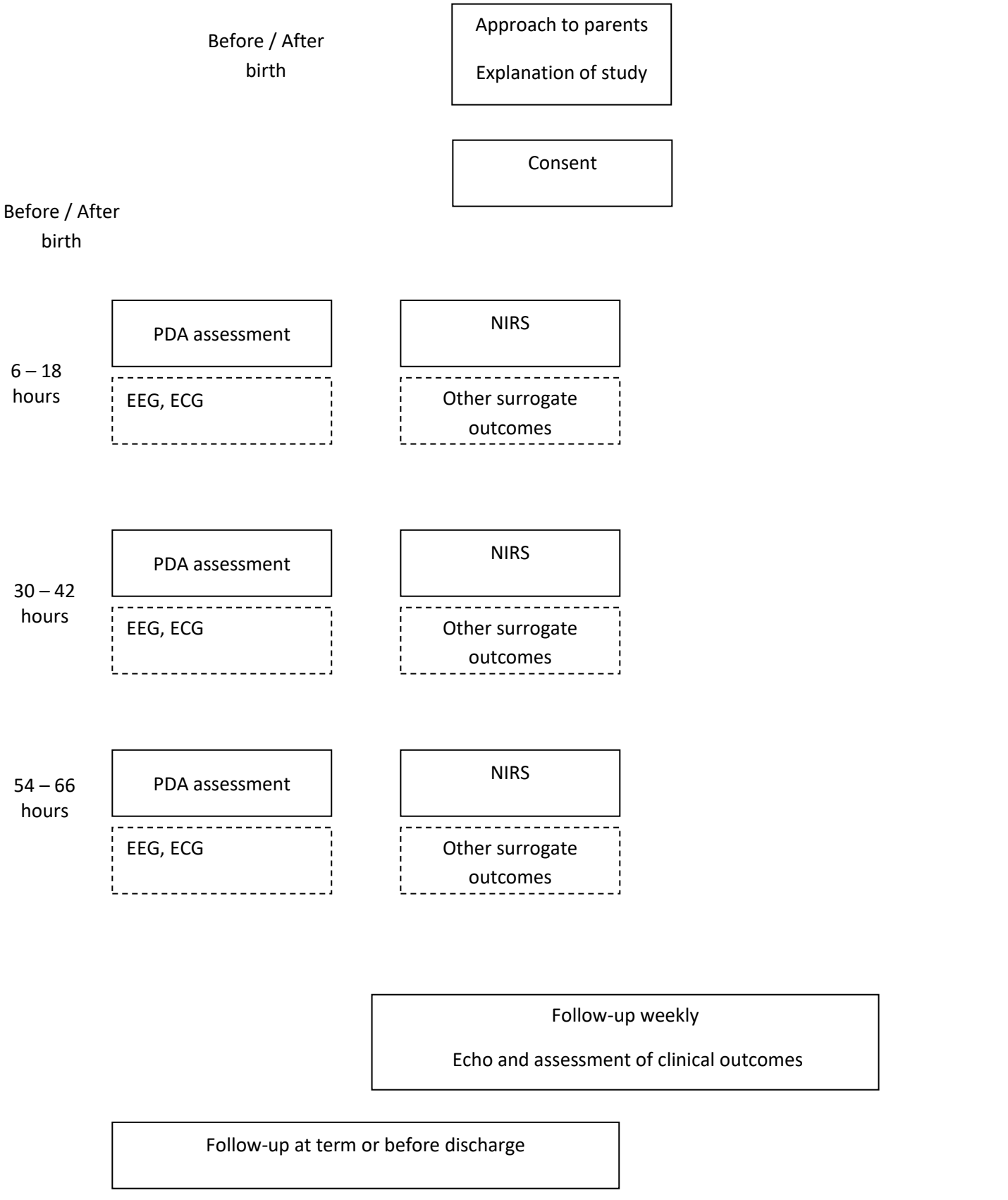
Publication

The clinical study report will be used for publication and presentation at scientific meetings. Families will be informed for the study results by a letter.

Resources

The study is funded by European Commission grant Neocirculation.

Patient Flow



Boxes with dashed borders indicate supplementary measurements made during substudy if baby is stable.

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Appendix 2: Procedures for echocardiographic assessment

The infant has to be in a comfortable environment. Handling should be minimised. Gel should be warmed before it is applied. The infant should be supine with the chest and abdomen exposed. The welfare of the neonate will take priority at all times.

Features for the interpretation of abnormal structure or haemodynamics are specific to the ultrasound machine. The ultrasound machine will be configured appropriately for the neonate, providing cross-sectional, M-mode, colour Doppler, pulsed and continuous wave Doppler images.

Outcome measures:

SVC flow and RVO are derived from the vessel cross-sectional area and the velocity measurements [velocity time integral (VTI)], according to the equations (Stroke volume: VTI x Pi x mean diameter2/4):

SVC flow (ml/kg/min): SVC stroke volume x heart rate /body weight (kg)

RVO (ml/kg/min): RVO stroke volume x heart rate/body weight (kg)

LVO (ml/kg/min): LVO stroke volume x heart rate/body weight (kg)

Corrected IVRT=IVRT/ √RR

Method of assessment:

SVC flow

SVC diameter is measured using left-mid parasternal view in a true sagittal plane, by positioning the transducer over mid sternum, a bit towards left side, the notch pointing upwards. The SVC usually funnels out as it enters the right atrium. The diameter is measured at the base of the funnel by M-mode. As SVC diameter varies during the cardiac cycle, the maximum and minimum diameter are averaged. Additional 2D images will be recorded and frozen. SVC velocity will be measured using the subcostal

window. The transducer will be positioned in the midline midway between the xiphisternum and umbilicus. The beam is aligned to transect the baby in the short axis with the notch pointing to the left. Angling the beam anteriorly the SVC appears as a diverticulum in the right atrium next to the aorta. The transducer will be as close to the umbilicus as possible to decrease the angle of insonation. The pulsed-Doppler range gate will be placed at the junction of the SVC and right atrium. SVC flow trace has a first peak corresponding to filling in ventricular systole, and a second peak representing positive ventricular filling in early diastole. Additionally, atrial systole is sometimes marked by a reduction in flow velocity that becomes negative. The Doppler velocity envelope will be traced using the calculation package on the Echo-D machine. If there is negative flow (atrial systole), this should be also incorporated, which is subtracted from the positive forward flow. Measure for 5-10 cardiac cycles.

Right ventricular Output

Right ventricular Output (RVO) is derived by measuring blood flow in the main pulmonary artery, which is imaged in the long axis. The pulmonary artery diameter is measured in the high parasternal long axis view by tilting slightly down the transducer. The diameter is measured from the 2-D image at the insertion of the pulmonary valve leaflets, advancing frame by frame until mid- to end-systole, when the leaflets are in parallel position. Doppler is performed from the low parasternal long axis view, tilting upwards the transducer. The range gate is positioned just beyond the valve leaflets to minimize any disturbance to the flow pattern from ductal shunting. The pulmonary artery is particularly good for Doppler studies because its posterior direction takes the blood directly away from the transducer at a minimal angle of insonation from the low parasternal window.

Left ventricular Output

Left ventricular Output (LVO) is derived by measuring blood flow in the aorta. The aortic diameter is measured from the parasternal long axis view on the level of aortic valve. The diameter is measured from the 2-D image at the insertion of the aortic valve

leaflets, advancing frame by frame until end-systole, just before valve closes. Doppler is performed from the 4 chambers apical view modified in order to visualise the ascending aorta. The range gate is positioned just beyond the valve leaflets. The main problem is the ankle of insonation that should be close to 10-20^{o}. Try to avoid angle correction.

E wave/A wave ratio

E wave/A wave ratio is a marker of diastolic function. Cardiac diastole is divided into four phases: 1) isovolumic relaxation time, 2) early filling, 3) diastasis and 4) atrial contraction. We can assess this phases by studying the mitral flow pattern with PWD. Doppler is performed from the 4 chambers apical view. The PWD cursor is placed between the tips of the open mitral valve leaflets. We usually derive a dicrotic flow pattern, consisting of E and A wave. E wave corresponds to early filling and A wave to atrial contraction.

Isovolumic relaxation time

Isovolumic relaxation time (IVRT) is a marker of diastolic function. Prolongation of IVRT indicates poor myocardial relaxation. IVRT is measured from an apical 4-chamber view, positioning the pulsed wave Doppler sample volume in the left ventricle as much deviated from mitral inflow to left-ventricular outflow so as to visualize, sequentially, both the cessation of aortic outflow and the offspring of mitral flow from the Doppler shift 0 line. We can normalize the absolute value of IVRT for heart rate by dividing the absolute value by the square root of cardiac cycle length.

Timetable for echocardiographic assessment

As described in the protocol.

Several interventions/treatments could be relevant to the echo derived parameters or are mandatory per protocol, therefore should be recorded at the time of each Echo-D scan:

Type of medication

Total i.v. volume infusion rate (ml/h)

Ventilation: type of ventilation, mean airway pressure, fractional inspired oxygen fraction

Sedation type

MBP

CRT

Acknowledgement

My PhD was funded by NEOCRULATION consortium (Grant agreement ID: 282533) and I was co-leading the development of the biomarkers used in the clinical trial (Dobutamine for NEOnatal CIRCulatory failure defined by novel biomarkers) and co-designed the standard operating procedures. The same standard operating procedures were used also in the present study.

Appendix 3: Procedures for cranial ultrasound and Doppler studies

Cranial ultrasound (CrUSS) scan will be done as soon as possible at enrolment, preferably within the first 6 hours of birth. Then between 48 to 72 hours, at postnatal day 7 (±1), 14 (±1), and at 36 (± 2) week's gestation. Eleven pre-specified views will be recorded and stored. Cerebral blood flow velocity measurements at the anterior (ACA) and middle (MCA) cerebral arteries will be recommended but not mandated by protocol.

Outcome Measures

Outcome measure 1: Parenchymal/periventricular haemorrhagic infarction / inhomogeneous flaring

Outcome measure 2: IVH Grade III

Outcome measure 3: Cerebellar haemorrhage

Outcome measure 4: Posthaemorrhagic hydrocephalus

Outcome measure 5: cPVL

Outcome measure 6: Cerebral atrophy

Technique

Each cranial ultrasound scan will comprise 11 standard views through the anterior fontanel (6 coronal, 5 sagittal), 1 optional coronal view through mastoid and 2 optional (coronal and parasagittal) through the posterior fontanel. Additional coronal view through the temporal squama for Doppler flow measurements at the MCA.

Anterior fontanel

Coronal:

C1 frontal lobes

C2 anterior frontal horns of the lateral ventricles

C3 third ventricle, connections between the lateral and third ventricle (foramen of Monro); measurements: lateral ventricles with largest size of ventricular index (VI); anterior horn width; width of third ventricle; interhemispheric fissure

C4 cerebellum largest diameter left-right

C5 level of the trigone of the lateral ventricles, posterior horns with plexus (glomus)

C6 occipital lobes beyond the posterior horns

Sagittal:

S1 midline; measurement: depth of fourth ventricle ; Doppler flow measurements at the ACA

S2 left lateral ventricle;

S3 left periventricular through the Sylvian fissure

S4 right lateral ventricle; measurement: right TOD

S5 right periventricular through the Sylvian fissure

Temporal squama

Axial: Doppler flow measurements at the MCA

Timing of Scan

Early scans (day 1, 2, 3)

Late scans (day 7, 14, 35)

Near-term/ Term scan: at 36 (± 2) weeks' postmenstrual age

Method of Classification

1. Normal brain scan

No cysts

No ventricular dilatation

No enlargement of extra-cerebral spaces

Normal cortical grey matter

2. Mild brain injury:

Grade 1-2 IVH

Persistent pathological non-decreasing flaring at days 7 and 14

Thinning of the corpus callosum

Ventriculomegaly at term, ventricular index < P97

3. Severe brain injury :

IVH grade 3 (VI>P97 during the acute phase)

Posthaemorrhagic ventricular dilatation with the ventricular index (VI) exceeding the 97th percentile (VI>p97)

Parenchymal/periventricular haemorrhagic infarction

Local cystic lesions (unilateral)

Cystic periventricular leukomalacia (bilateral)

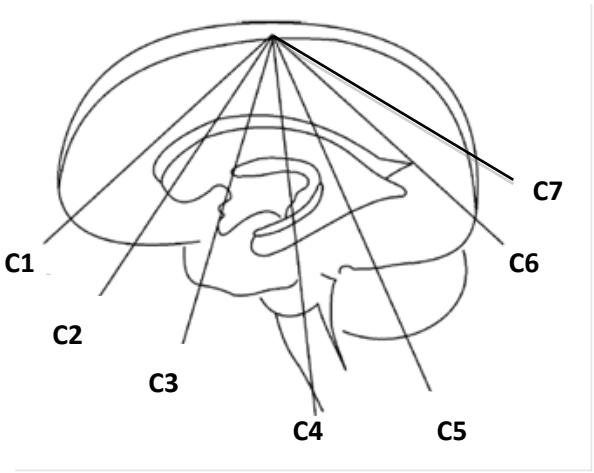
Cerebellar haemorrhage

Cerebral atrophy at term age (ventricular dilation and/or increased extra-cerebral spaces)

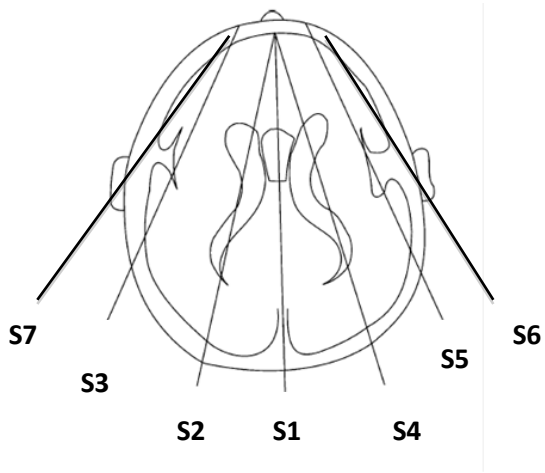
CrUSS-Doppler derived measurements

1. Peak systolic flow velocity (PSFV), end-diastolic flow velocity (EDFV), and temporal mean flow velocity (TMFV)

2. Resistance index: (PSFV-EDFV)/ PSFV



Standard six coronal views



Standard 3 sagittal

Timing of Scan

1st CUS	Within 12h of enrolment
2nd CUS	Between 49-72 h of treatment start
3rd CUS	Day 7 from birth (6-8)
4th CUS	Day 14 from birth (13-15)
5th CUS	Day 35 from birth (34-36)
6th CUS	Near-term/ Term scan: at 36 (35-37) weeks' gestation
7th CUS	Optional: additional, if clinically indicated

Appendix 4: Procedures for near-infrared spectroscopy studies

NIRS provides continuous non-invasive monitoring of the cerebral tissue oxygenation index (cTOI), which is a proxy of the cerebral venous saturation. The combined used of NIRS and pulse oximetry allows the continuous monitoring of the cerebral fractional oxygen extraction (cFOE).

Outcome measure: cTOI

Technique

NIRO-200NX, a spectrophotometer using spatially resolved spectroscopy, will be used. Quality of NIRS signal will be checked on the forearm before placing the sensor on the infant. The device should give readings between 50 and 90%. The sensor will be applied on a clean surface. Aim for a site with as little hair as possible. Do not place the sensor over sinus cavities, superior sagittal sinus, hematomas or other anomalies. Ensure intact skin surface. For prolonged monitoring periods, the sensor will be moved every 3-4 hours, if possible as part of routine handling to avoid additional disturbances to the infant.

Timing

Recordings will start as soon as possible after enrolment. 4 hours every 12 hours in the first 3 days, 4 hours daily in the rest of the first week and 4 hours on alternative day until PDA closes. NIRS monitoring will always precede echocardiography.

Method of assessment

Data will be recorded and stored. For analysis purposes, the averaged value for all samples of the continuous NIRS and pulse oximeter data recordings obtained during 10 min intervals every 60 min will be calculated. SaO2 variation will not exceed 1% within the measurement time point. cFOE will be calculated as (SaO2-CTOI)/SaO2 (expressed as a percentage). NIRS data sampling time at 5 sec.

SOP: Near infra-red spectroscopy recording

1. Introduction

Before a procedure is initiated please read carefully the instructions for every step of the procedure.

2. How to begin patient NIRS monitoring in NeoCirc-001 clinical trial

NIRS monitoring will precede echocardiography and will be combined simultaneously with EEG monitoring for 75 min. If infant's head size or ventilation mode do not allow it, separate recordings will be performed.

Procedure

- Turn on the NIRS device and make sure the correct sensor is connected (Adult SomaSensor for INVOS 5100 C; Probe Holder S type with NIRO 200 NX)
- Place probe on your own forearm . The device should give readings between 50% and 90%. If not
- Check correct placement of sensor
- Change sensor
- Position the sensor on the infants head as described below
- Press 'START'

3. How to position the NIRS sensor on the infant's head

Procedure

- To ensure good contact, clean/degrease the skin using water. Ensure patient's skin is completely dry with a gauze pad.

- Check that the sensor is not defect. If it is, discard it and take a new.
- Select sensor site on the head. Aim for a site with as little hair as possible, as this can introduce inaccurate readings. Do not place the sensor over sinus cavities, the superior sagittal sinus, subdural or epidural haematomas or other anomalies such as arterio-venous malformations, as this may cause readings that are not reflective of brain tissue or no readings at all.

Emitter – Detector)(Detector – Emitter

- Ensure intact skin surface.
- Apply sensor to the head so that the light source is facing towards the skin. The sensor may be fixed with a self-adhesive single use bandage.
- Secure the cable to a fixed object to avoid strain on the sensor to skin interface.
- CAUTION: In order to avoid pressure sores, keep the external pressure on sensor to a minimum, while maintaining sufficient sensor-skin contact.
- CAUTION: The sensor is not MRI compatible.

4. How to change the position of the NIRS sensor

For extended monitoring the sensor should be repositioned at a different location. For infants with scalp oedema or poor perfusion this may be as often as every 4 hours to

avoid damage from heat and/or pressure. If possible, do it as part of the routine handling of the infant to disturb the infant as little as possible.

Procedure

- Press 'NIRS resiting' on the lower right of the screen
- Gently remove the sensor from the skin.
- Carefully inspect the skin for any sensor related marks, if any, please fill in the relevant information in the adverse events form (to be formulated) after the sensor has been repositioned.
- Apply the sensor on a different site according to the instructions above.
- Press 'NIRS resiting' on the lower right of the screen again
- CAUTION: If the sensor is difficult to remove, the local protocol for protection of the integrity of the skin should be followed.

Appendix 5: Procedures for aEEG/EEG assessment

The aEEG/EEG provides non-invasive continuous monitoring of cerebral electrical activity. Decreased cerebral perfusion is associated with recognizable changes in the aEEG pattern.

Outcome measures

Outcome measure 1: Burdjalov score

Technique

Digital aEEG/EEG recordings will be performed. Electrode positioning must always include P3-P4 from where the IBI will be estimated. A frontal reference electrode is also applied. Additional electrodes may be used depending on the ventilation mode, head size and application of NIRS.

Raw aEEG/EEG data will be transferred to central data manager.

Timing

As described in the protocol.

SOP: EEG/aEEG

1 Introduction

1.1 The following is based on the protocol developed for the SafeBoosC study (http://www.rigshospitalet.dk/menu/AFDELINGER/Juliane+Marie+Centret/Klinikker/Neonatalklinikken/Forskningihovedmenu/SafeboosC/SOPs.htm).

1.2 EEG monitoring will precede echocardiography and will be combined simultaneously with NIRS monitoring for 75 min. If infant's head size or ventilation mode do not allow it, separate recordings will be performed.

1.3 Infants' well being should be the first priority.

1.4 All precautions should be taken in order to minimize the risk for infection (hand wash with soap and water, single use electrodes, EEG device sanitisation before and after use according to local protocols).

2 Recording the aEEG/EEG. The procedure

2.1 Fill in the aEEG/EEG recording protocol in the eCRF.

2.2 Explain the procedure to parents and the staff caring for the baby.

2.3 Prepare the equipment.

- Check that the date and time in the monitor is correct.
- Enter patient identification as usual

3. Prepare the infant, if necessary remove the NIRS sensor

4. Place the electrodes:

- Avoid abrased skin, obvious cephalohematoma, severe oedema or other abnormality
- Place the P3 and P4 electrodes according to the 10-20-system (figure 1). Check that the electrode impedance is less than 20 kOhm

5. Check that electrode signals have low noise (identified by a thin line, with minimal artefacts from electrocardiogram, respiratory movements, hiccups or other muscle artefact). The electrodes should be checked for correct connection and labelling by touching them slightly and noting the signal on the screen.

6. Start recording.

7. Note any disturbance or pain or sedative medication using the event marking system.

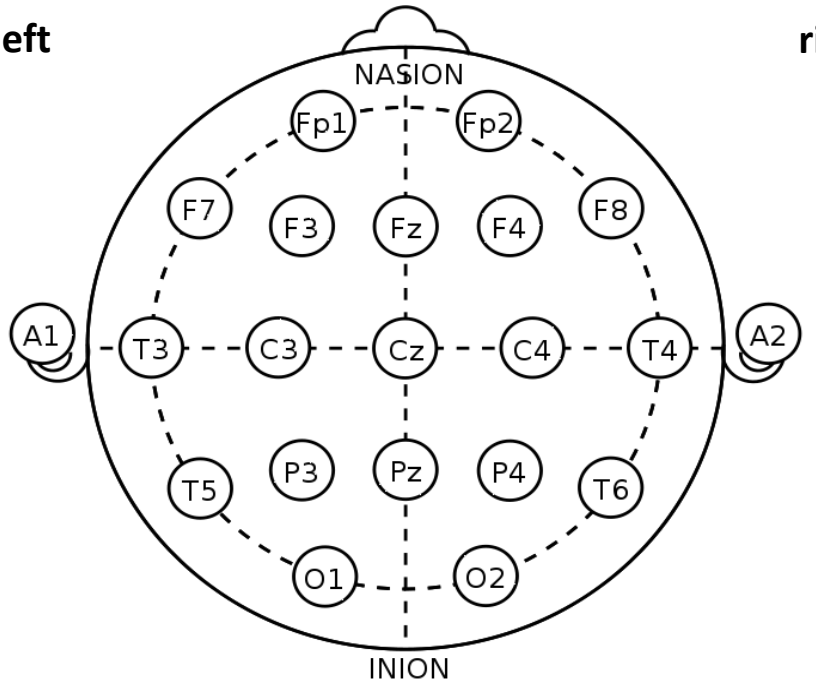


Figure 1. 10-20- electrode placement scheme.

SOP: Amplitude-integrated encephalography (aEEG) interpretation and scoring system

1. PURPOSE

The purpose of this document is to define the technique and timing of aEEG assessment based on previous literature (1).

2. SCOPE

This standard operating procedure (SOP) applies to all staff involved in the interpretation of aEEG data for the HAPI-PDA study.

3. ABBREVIATIONS/DEFINITIONS

aEEG - Amplitude-integrated electroencephalography
SOP - Standard Operating Procedure

4. RESPONSIBILITIES

Not Applicable

5. ASSOCIATED DOCUMENTS

- Procedures for aEEG assessment
- PDA table of assessments

6. MATERIALS

OBM viewer software-Natus Olumpic Brainz Monitor

7. PROCEDURE

(i) Timing

aEEG will be scored at the defined time points according to PDA table of assessments.

(ii) Definitions

a. **Continuity**

- ***Continuous Normal Voltage*** – A narrow band with minimum voltage above 5 microvolts. Maximum voltage above 10 microvolts.

- ***Discontinuous Normal Voltage*** – A moderately wide band. Minimum voltage below 5 microvolts, but variable. Maximum voltage above 10 microvolts.

- ***Burst Suppression*** – An extremely wide band with maximum and minimum voltages both very low and very high, and without variability to the lower margin.

- ***Continuous Low Voltage*** – A narrow band with the maximum and minimum voltages below 10 microvolts (not shown below).

- ***Inactive*** – A very narrow band with all activity below 5 microvolts

b. **Sleep wake cycling (SWC).** SWC refers to the emergence and progression of periods, where the peak-to-trough width of the recording would expand and subsequently contract. Cycling was observed as variations in both amplitude and continuity of electrical activity on aEEG tracings.

c. **Amplitude (in _V) of the lower border:** The magnitude of the CFM tracing's lower border (voltage troughs) is estimated as the average lower microvolt level during the recording epoch. A line drawn though the lower margin of the aEEG band should be with half of the microvolt troughs below the line and half above.

d. **Bandwidth of the aEEG:** Bandwidth reflects a combination of the voltage span (peak-to-trough) of the tracing and the magnitude of the aEEG depression (amplitude of the lower border). The span is calculated as the difference between the upper and lower voltage margins of the tracing's narrowest part.

(iii) Procedure

The aEEG trend, displayed at 6 cm/h, will be visually rated in 6- hour blocks by investigators blinded for the patients' identity and clinical data. 6y hours blocks more close to echocardiogram will be selected. The assessment of the aEEG background pattern will be repeatedly aided by inspection of original single-channel EEG at 15 mm/s and 100 µV/cm sensitivity. aEEG trend data will be evaluated according to two methods:

a) The aEEG background pattern of every epoch will be interpreted as burst-suppression, discontinuous or continuous according to Hellstrom-Westas et al {Hellström-Westas L, 2006 #45}, and thereafter the activity dominating the 4-h epoch will be further classified into 6 categories: 0 = Inactive/flat, 1 = Sparse BS, 2 = Dense BS, 3 =Mix of both BS and discontinuous (DC), 4 =Discontinuous (DC), 5 =Continuous (C). Cyclicity will be rated in 4 categories: 0 = Absent, 1 = Imminent, 2 = Established but immature, 3 = Developed and mature. The CFM monitor generates automatically a report with the time spent on each background activity. Examples of different patterns of background activity are displayed in pictures 1 and 2.

b) Background scoring according to Burdjalov et al [1] including four single parameters, continuity, cyclicity, amplitude of lower band, bandwith span and amplitude of lower band together with the their sum as displayed in Table 1.

Score	Continuity	Cycling	Amplitude of Lower Border	Bandwidth Span and Amplitude of Lower Border
0	Discontinuous	None	Severely depressed (<3 μV)	Very depressed: low span (≤15 μV) and low voltage (5 μV)
1	Somewhat continuous	Waves first appear	Somewhat depressed (3–5 μV)	Very immature: high span (>20 μV) or moderate span (15–20 μV) and low voltage (5 μV)
2	Continuous	Not definite, some cycling	Elevated (>5 μV)	Immature: high span (>20 μV) and high voltage (>5 μV)
3		Definite cycling, but interrupted		Maturing: moderate span (15–20 μV) and high voltage (>5 μV)
4		Definite cycling, noninterrupted		Mature: low span (<15 μV) and high voltage (>5 μV)
5		Regular and mature cycling		

Table 1: The Burdjalov CFM score (1).

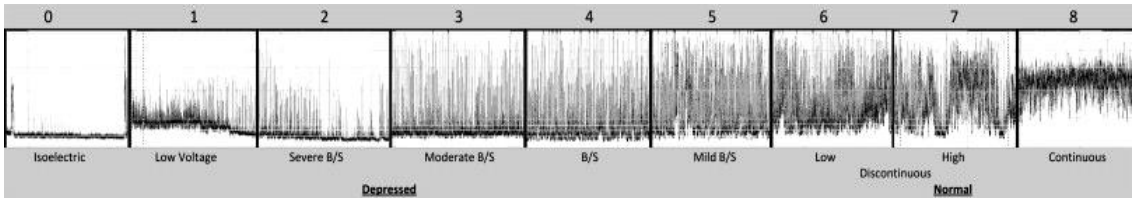


Figure 1: Different aEEG background patterns

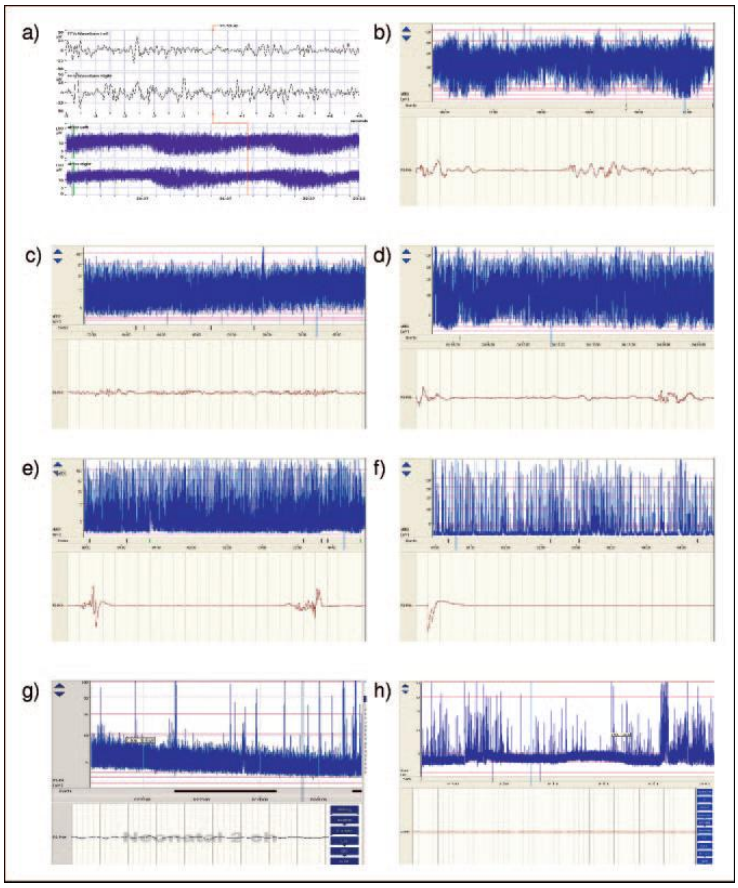


Figure 2. The classification of primary aEEG background patterns, as well as the three degrees of sleep-wake cycling (SWC) .

a) Continuous background with **SWC** in healthy term infant.

b) Continuous and discontinuous aEEG background with immature SWC in an infant who has Dandy Walker malformation at 35 weeks' gestation.

c) Discontinuous background that gradually becomes more continuous, as seen by the rise in the minimum amplitude.

d) DC background in normal very preterm infant

e) Burst-suppression with >100 bursts/h

f) Burst-suppression with <100 bursts/h

g) Low voltage (LV)

h) Flat (FT) aEEG.

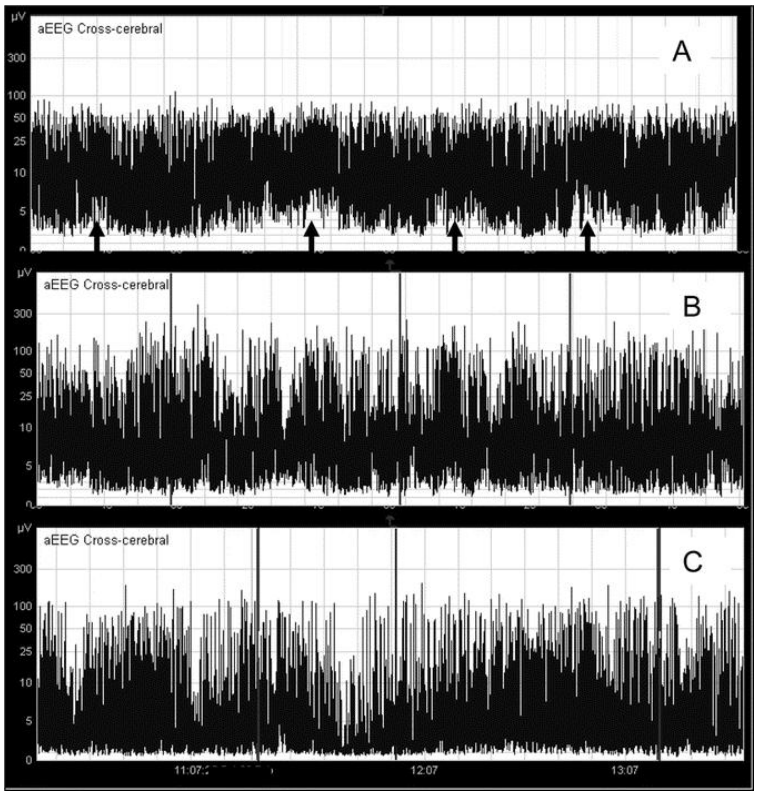
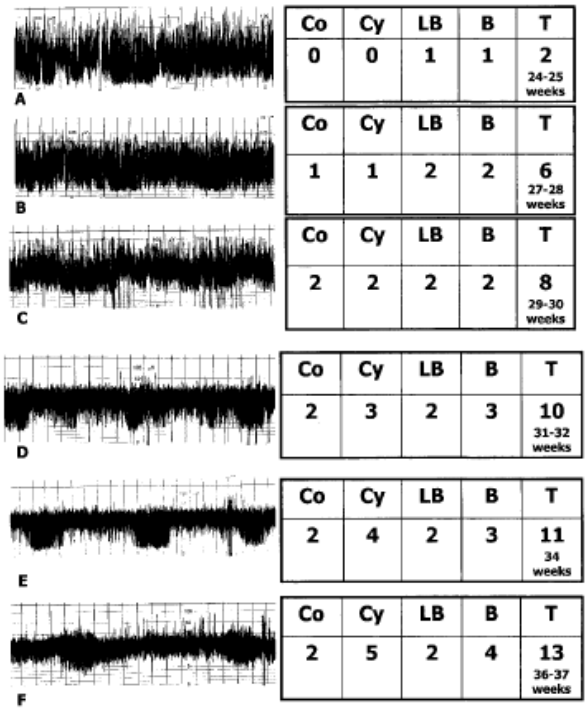


Figure 3. **Cyclicity Assessment**

A, Periods of continuity with baseline >5 µV (identified with arrows). **Developed sleep-wake cycles with sinusoidal variation** >20-min duration.

B, Discontinuous baseline 2–5 µV. **Immature sleep-wake cycling**: baseline variability >2 µV.

C, Suppressed trace: baseline <2 µV. **No sleep-wake cycling**: baseline variability <2 µV



Co	Cy	LB	B	T
0	0	1	1	2 24-25 weeks

Co	Cy	LB	B	T
1	1	2	2	6 27-28 weeks

Co	Cy	LB	B	T
2	2	2	2	8 29-30 weeks

Co	Cy	LB	B	T
2	3	2	3	10 31-32 weeks

Co	Cy	LB	B	T
2	4	2	3	11 34 weeks

Co	Cy	LB	B	T
2	5	2	4	13 36-37 weeks

Figure 4. Burdjalov aEEG scoring system.

Co, continuity of the recording; Cy, presence of cycling; LB, lower border amplitude score; B, bandwidth; T, total score (1).

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Information proforma for EEG/aEEG recording

Patient number:_____

Date of aEEG/EEG recording:_____

Start time:_____

Stop time:_____

Equipment

Nicolet □
BRM (BrainZ) □
Micromed □
g.tec □
Other (name):_____

Electrodes

Disc □ Hydrogel □ Needle □

Electrode positions

Standard P3/P4 □
Abnormal P3/P4 □
Additional electrodes. Describe:_____

Medications baby received within 24 h prior to recording:

Caffeine □ YES □ NO

Last dose before aEEG/EEG: Date:_____ Time:_____

Comment:_____

Theophylline □ YES □ NO

Last dose before aEEG/EEG: Date_____ Time:_____

Comment:_____

Opioid □ YES □ NO

Please specify opioid (e.g. morphine, fentanyl):_____

Last dose before aEEG/EEG: Date:_____ Time:_____

Comment:_____

Sedative □ YES □ NO

Please specify sedative_____

Last dose before aEEG/EEG: Date:_____ Time:_____

Comment:_____

Anticonvulsant □ YES □ NO

Please specify_____

Last dose before aEEG/EEG: Date:_____ Time:_____

Comment:_____

Mode of ventilation

SIMV □ **HFOV** □ **CPAP** □

Appendix 6: ixTrend data collection

- Insert the Blue ixTrend dongle into the USB port on the laptop.

  This must be done to allow data to be recorded for longer than 60 seconds.

- Connect the Bluetooth dongle (labelled client) to the P.C. via the USB adaptor.

  This will collect the data from the Bluetooth dongle and transfer it into the computer.

- Connect the Bluetooth dongle (labelled server) to the MIB/Serial port on the back of the monitor, using the RJ45 Ethernet adaptor.

  This will transmit the data from the monitor to the other Bluetooth dongle. The Bluetooth connection has a range of 10m. Moving the dongles further apart than this, will break the connection or cause data to be lost during transfer.

- Restart the HeRO computer.

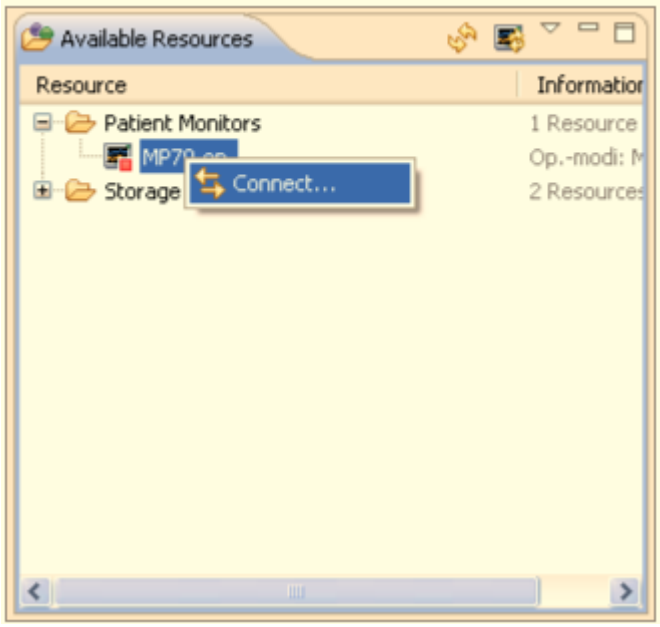
  To allow the software to capture waveform data.

- Start the ixTrend software.

  This must be done whilst the HeRO computer is restarting.

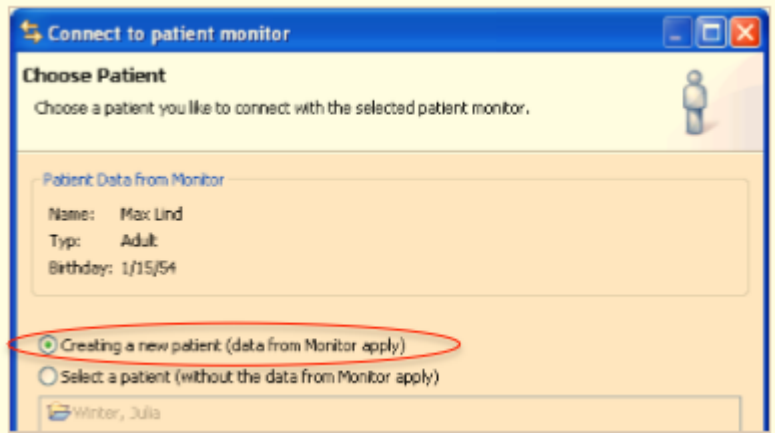
- Click x on welcome screen.
- Look for the monitor connection (bottom Left).

  This will appear as a “Resource” in the available resources tab, expand the “Patient Monitors” section.



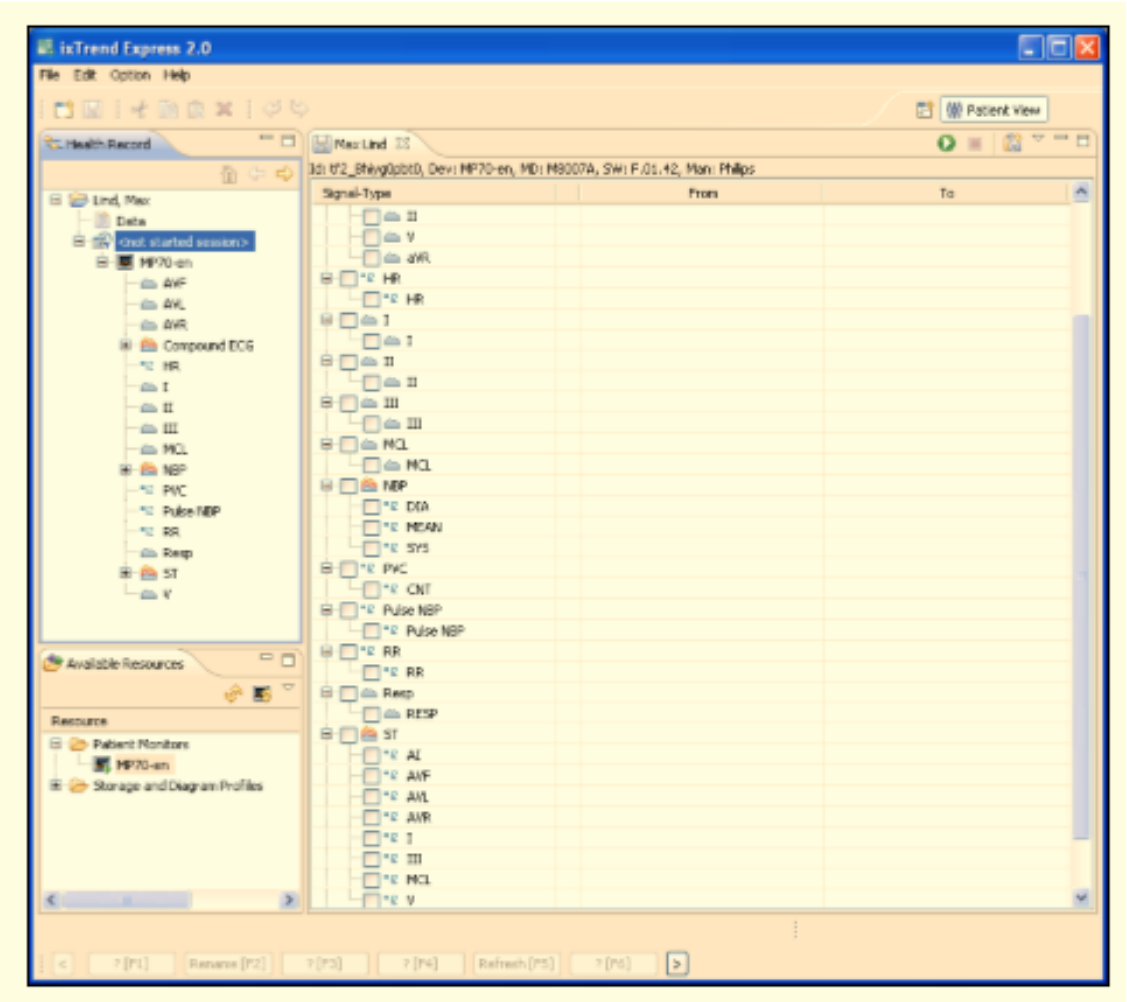
- Right click the monitor and click connect.

- Create a new patient using data from the monitor/ select a patient from the



list of current patients.

- Double click the “<not started session>” heading, in the “Health Record”



tab.

- Select the measurements you want to record.

The waveform measurements have a cloud next to the measurement name. For single value recordings the icon is: “^{0}i2”.

If no waveform measurement options are visible, the HeRO computer system must be restarted and the monitor reloaded into the ixTrend software. This can be done by right clicking on the monitor resource and clicking disconnect. Then right clicking again and selecting connect. This must be done before the HERO computer completes its restart.

- Click the green arrow (top right) to begin recording the selected measurements.

- To stop recording click the red square (top right, next to the green arrow).

  The measurements that you have recorded will appear on the left under the heading of the session date and time. To view the captured data you can double click on the data you want to view. This will display the data for the recording session, you can scroll throughout the time of the recording. This data will be stored in the ixTrend software for as long as you choose to keep it.

- To export the data right click the measurement you want and select "export".

  You will be prompted to select export settings. Select the top format option: .csv file. The next options will be to select "List Separator": comma, and "Decimal Separator": point.

- Choose the destination folder appropriate for the measurement.

  These can be found in your documents folder. There are three folders: ECG data, Pleth data and resp data. You can create more folders for other types of measurement. Keep this data named or dated in some order that lets you keep track of which recording is which.

- Save the data to the specified folder.

  You can change the prefix of the file so that you know what the name of the file will start with.

- Transfer this data to your desktop computer using a USB stick.
- Save this patient data on the networked server.
- If you only want to view the start of the data you can open the file in excel.

Appendix 7: Case reporting form

PDA haemodynamics: observational study

Infant's details

Name: _

Hosp number: _ _ _ _ _ _ _ _ _ _ _ _ _ Study number:_ _ _ _ _ _ _ _ _ _ _ _ _ _

Maternal details

Name: _

Address: _

_ _

Tel: _

Marital status: Single Married Cohabiting
Divorced

Ethnic background: British/Irish Other white White and Black Caribbean

White and Black African White and Asian
Indian

Pakistani Chinese
Asian other

African Carribean
Arabic

Other: _ _ _ _ _ _ _ _ _ _ _ _ _

Educational background: Primary Secondary
College

University Master PhD

Job:

Income: Less than 10,000 10-20,000 20-30,000 30-40,000

40-50,000 50-60,000 60-80,000 >80,000

Vaccinations:

Medication:

PMH: _

FH:

Antenatal history

Pre and post pregnancy Weight:

Height:

Scans:

Previous pregnancies/miscarriages:

Haemorrhages No ☐ Yes ☐ _ _ _ _ _ _ _ _ _ _ _ _

Infections No ☐ Yes ☐ _ _ _ _ _ _ _ _ _ _ _ _

Smoking No ☐ Yes ☐ _ _ _ _ _ _ _ _ _ _ _ _

Diabetes or GD No ☐ Yes ☐ _ _ _ _ _ _ _ _ _ _ _ _ _

Hypertension No ☐ Yes ☐ _ _ _ _ _ _ _ _ _ _ _ _

Alcohol No ☐ Yes ☐ _ _ _ _ _ _ _ _ _ _ _ _

Chorioamnionitis: Fever (>38ºC), Foul smelling amniotic fluid

Tenderness of the uterus Maternal/fetal tachycardia

Maternal leukocytosis

Prolonged rupture of membranes (>24 hours to delivery)

Other: _
_ _
_ _
_ _

<u>Baby's demographics</u>

Name: _
_ _ _ _ _ _ _ _ _ _

Gender: Male ☐ Female ☐

BW: ☐ kgr SGA? _ _ _ _ _ _ HC: ☐

Steroids: _ _ _ _ _ _courses

GA: _ _ _ _ _ _weeks _ _ _ _ _ _days

Onset of labour: _
_ _ _ _ _ _ _ _ _ _

Mode of delivery SVD Instrumental ElLSCS EmLSCS

Complications _
_ _ _ _ _ _ _ _ _ _

APGAR score 1min 5 min

Resuscitation notes: _ _ _ _ _ _ _ _ _ _ _ _ _

Condition at birth: _
_ _ _ _ _ _ _ _ _ _

Cord gases: Arterial pH Venous pH

Admission temp: _
_ _ _ _ _ _ _ _ _ _

Courses of surfactant: _
_ _ _ _ _ _ _ _ _ _

Days on ventilation: Intubated CPAP

Days on O2: _
_ _ _ _ _ _ _ _ _ _ _

Inotropic support: _

Courses of Ibuprofen: _ Postanatal age: _ _ _ _ _ _ _ _ _ _ _ _ _ _

PDA ligation: No ☐ Yes ☐ Age: _

Major neonatal diagnoses :

REVISION HISTORY:			
Version	Authors	Effective Date	Changes Made
1.1	C.Kotidis, M. Weindling, M. Turner	27.05.14	Minor changes
1.2	C.Kotidis, M. Turner	22.09.14	Minor changes according to ethics committee

Table of assessments

Study phase	Screening	Follow up						
Day or week	D1	D2	D3	W1	W2	Wn	Discharge	
Screening log	1							
Informed consent	1							
Maternal medical history	1							
Perinatal data	1							
BW and HC	1							
Echo	1	1	1	1	1	1	1	
NIRS	1	1	1	1	1	1	1	
aEEG	1	1	1	1	1	1	1	
CrUSS	1	1	1	1	1	1	1	
HERO score	2	2	2	1	1	1	1	
Gas profile *	2	2	2	1	1	1	1	
Lactate*	2	2	2	1	1	1	1	
Base excess*	2	2	2	1	1	1	1	
Biochemical profile*	1	1	1	1	1	1	1	
Haematology*	1	1	1	1	1	1	1	
CRT	2	2	2	1	1	1	1	
BP, HR, SaO2	2	2	2	1	1	1	1	
FiO2	2	2	2	1	1	1	1	
Ventilation settings	2	2	2	1	1	1	1	
Medication	1	1	1	1	1	1	1	

***if clinically indicated**

Appendix 8: Parents' Information Sheet

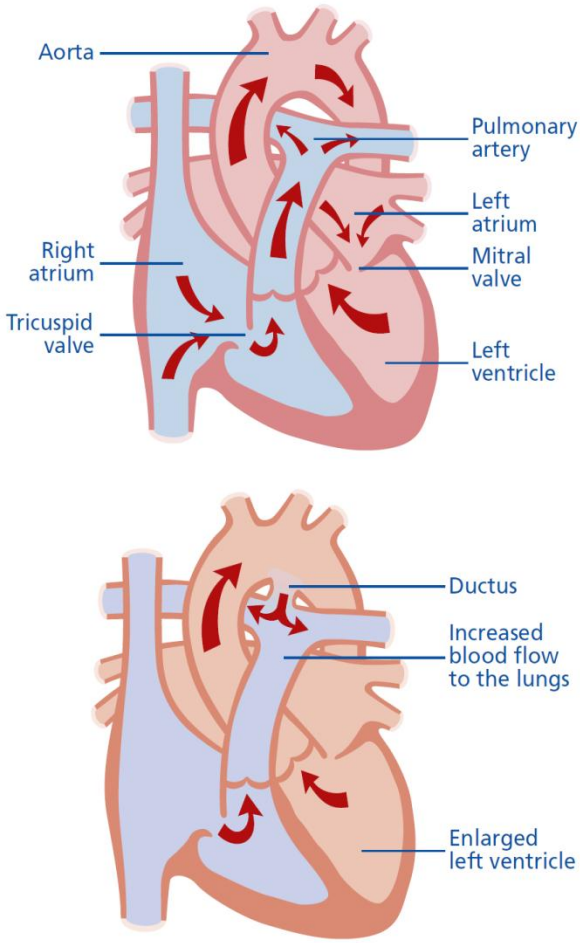
Hemodynamic Effect of Patent Ductus Arteriosus in Preterm Infants: an observational study

Your baby is being invited to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it will involve. Please ask us if there is anything that you do not understand or if you would like more information about the research study.

Background

The ductus arteriosus is a blood vessel that connects the pulmonary artery (main vessel supplying the blood to the lungs) to the aorta (main vessel supplying the blood to the body). By this way blood bypasses the lungs that are not in use during life inside the womb. This connection is present in all newborn babies, but should close shortly after birth. In some babies, especially those born prematurely, this vessel may be open. This is called a patent ductus arterious (PDA).

The size of the PDA may vary, but, if it is large, the amount of the blood that goes to lungs is increased, which can cause problems in some babies. The brain, gut and kidneys may also not work properly or be damaged.



Picture 1: Normal heart and patent ductus arteriosus. Blue represents blood without oxygen that comes from the body and goes to the lungs in order to have oxygen added. Red represents the blood with oxygen that comes from the lungs and goes to rest of the body.

How do we detect PDAs?

A doctor will do a special ultrasound scan called an echocardiogram to make precise measurements of the size of the PDA.

What is the purpose of the Study?

This study will identify babies that are at higher risk for developing a large PDA and problems linked to this. Data from this study will be used to improve the care of babies like yours in future but will not directly affect the care of your baby.

Why has my baby been chosen?

You are being invited to take part because your baby is premature and we know that all premature babies are potentially at risk of PDA. By comparing the results from all babies in the study we hope to identify patients who are at higher risk of developing more problems.

Do I have to participate?

No, you are free to refuse to take part in this study on behalf of your child. If you decide not to take part it will not alter your baby's treatment in any way. If you decide to take part, you are still free to withdraw at any time. If you decide to withdraw from this study, information collected about you and your baby will be retained for the purpose of completing the research unless you tell us otherwise. If you withdraw your baby from the study we will ask you whether we can keep the data that relates to your baby.

If you decide to take part in the study your baby's care will remain the same.

What will happen if I agree to participate on behalf of my baby?

If you decide to take part we will ask you to sign a consent form on behalf of your baby and give you a copy of this information sheet and the consent form for you to keep. In this study we do NOT test new drugs or new treatments. Your baby's treatment will be unaffected. If you agree to take part, a doctor will monitor your baby's heart function with ultrasound once daily for the first 3 days, once weekly until 2 weeks of age, subsequently once weekly until the PDA closes and then before discharge. Ultrasound does NOT contain radiation and does NOT harm your baby. Six probes will be attached to special monitors in order to monitor brain function.

What are the possible advantages of taking part?

The study will not be of any direct benefit to your child, but the results from the study may help us to help children in the future.

What are the possible side effects of taking part?

There might be a slight irritation of forehead skin due to pressure of the bandage that holds the NIRS probe in place. For this reason, we will change the position of the probe every 4 hours.

What if something goes wrong?

If you have a concern about any aspect of the study, you can speak to one of the researchers who will do their best to answer your questions. If you are unhappy with your participation, and wish to complain formally, you can do this through the NHS complaints procedure, details of which can be obtained from the Patient Quality Manager Liverpool Women's NHS Foundation Trust.

Will my baby's participation in the study remain confidential?

All information (for example date of birth, sex, race, height, weight, physical health) gathered about your baby by health professionals will be stored securely by the Doctor at the Neonatal Intensive Care Unit and will remain confidential. The data collected will be kept for the maximum period permitted by the hospital in accordance with their local requirements. Anonymous information about your baby will be made available to the hospital's ethics committee or study personnel who may be evaluating the study. The data

collected may be made available to authorized representatives of the national and foreign government agencies. This is in compliance with European data protection laws, which are designed to protect your baby's privacy.

This study is done as part of a project involving six countries in the European Union, Turkey and the USA. Some of the data, but not personal data, may be shared with partners in these other countries who are doing similar work on babies in their countries.

What will happen to the results of the study?

The results of the study will not be known until the data is analysed. The results will be reported in professional publications or meetings but babies will not be individually identified. We will make our results widely available so that doctors and nurses can use the information to help babies in the future.

Expenses and payments

We are not able to offer any payments for your child's involvement in this study.

Who has reviewed the study?

This study has been reviewed by the NRES Committee North West – Lancaster Research Committee and by the Research and Development Department at Liverpool Women's Hospital.

What do I do now?

Thank you for considering taking part in this research. If you have any further questions about the study please do not hesitate to contact a member of our team.

Complaints

If you have any concerns or complaints during the study please let the team know. You may also wish to contact the PALS team who will help you use the Hospital complaints procedure.

Dr Haris Kotidis - Tel. 0151795 9561

PALS (Patient Advice & Liaison Service) Liverpool Women's Hospital - Tel. 01517024297

REVISION HISTORY:			
Version	Authors	Effective Date	Changes Made
1.1	C.Kotidis, M. Weindling, M. Turner	27.05.14	Minor changes
1.2	Louise Hardman	30.05.14	Minor changes
1.3	C.Kotidis, M. Turner	24.09.14	Minor changes according to ethics committee recommendations
1.4	C.Kotidis, M. Turner	04.11.14	Minor changes approved by R&D
1.5	C.Kotidis, M. Turner	16.02.15	Changes of time points of assessment according to the substantial protocol amendment

Appendix 9: Consent form

Direct dial: 0151 795 9561

Email: c.kotidis@liv.ac.uk

Consent form

Study title: **Haemodynamic biomarkers in preterm infants with Patent Ductus Arteriosus (PDA) (HAPI-PDA study): a pilot cohort study of the relationships between blood flow through a PDA and surrogate outcomes for brain injury**

Patient Details:.....

Please Initial Boxes

1. I confirm that I have read and understand the information sheet PIS Version 1.5 dated 10th February 2015 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered to my satisfaction. ☐

2. I understand that my baby's participation is voluntary and that I am free to withdraw at any time without my baby's medical care or legal rights being affected. ☐

3. If I withdraw from the study I consent that data gathered by study team members for the study relating to me and my baby up to the time at which I withdraw consent will be retained by the study team unless I withdraw that consent. ☐

4. I understand that information about my baby needed for the study (including personally identifiable information) may be collected from their medical records and looked at by the research team during the ☐

study. It may also be looked at by regulatory authorities supervising the trial, the NHS Trust and other investigators in countries in the European Union (EU) and other countries outside EU (USA and Turkey), where it is relevant to my taking part in this research. I give permission for these individuals to have access to these records.

5. I agree that my baby can take part in the above study ☐

6. I confirm that I am willing to be approached in the future about further contributions to the study. I am willing to be approached in 24 months time (if my infant/ward is under routine neonatal out-patient follow-up) to give further consent for a more detailed assessment of my child's developmental progress. ☐

Name of Parent/guardian	Signature	Date

Name of Person Taking Consent	Signature	Date

Appendix 10: Ethical approvals

Health Research Authority
National Research Ethics Service

NRES Committee North West - Lancaster
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Tel: 0161 625 7818
Fax: 0161 625 7299

26 March 2015

Dr Mark Turner
Senior Lecturer, Consultant Neonatologist
University of Liverpool
Liverpool Women's Hospital
Department of Women's and Children's Health
Crown Street, Liverpool
L8 7SS

Dear Dr Turner

Study title:	**Haemodynamic biomarkers in preterm infants with patent ductus arteriosus (PDA) (HAPI-PDA study): a pilot cohort study of the relationships between blood flow through a PDA and surrogate outcomes for brain injury**
REC reference:	**14/NW/1274**
Amendment number:	**1.4**
Amendment date:	**16 February 2015**
IRAS project ID:	**127099**

Change to inclusion criteria and protocol.

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The members had no issues with this application.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering letter on headed paper		
Notice of Substantial Amendment (non-CTIMP)	Amendment 1.4	16 February 2015
Participant consent form	1.4	16 February 2015
Participant information sheet (PIS)	1.5	16 February 2015
Research protocol or project proposal	1.4	16 February 2015

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

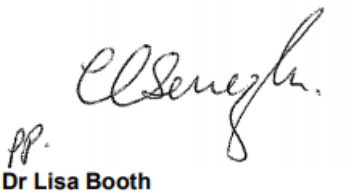
Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

14/NW/1274:	**Please quote this number on all correspondence**

Yours sincerely



Dr Lisa Booth
Chair

E-mail: nrescommittee.northwest-lancaster@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Gillian Vernon, Liverpool Women's NHS Foundation Trust*
Mr Alex Astor

Appendix 11: Qualitative assessment of the factors affected recruitment and data collection

There was a high recruitment rate (66%) and sufficient number of patients was recruited despite other competing studies at the same time. This was attributed to a number of reasons:

i. A presentation was prepared to inform clinical and nursing staff regarding the study rationale and methodology before the study opening. The main message conveyed was that there is a lot of uncertainty regarding PDA and its impact on cerebral function and the study may provide a better insight of PDA pathophysiology and allow designing better studies in the future. It is the "unwell" preterm infant who needs treatment and currently we do not know the appropriate treatment and hence this is the infant we have to study and not only the "well" premature infant. This allowed better understanding of the study and resulted for the majority of the staff to embrace the study.

   I believe that the inclusion and good relationship with the nursing staff had major impact on recruitment rates and on study population demographics. Nursing staff build a more close relationship with the parents and parents always ask their opinion. Negative non-verbal language by the nursing staff is enough to put-off a family especially after the stressful experience of preterm labour and birth and deny consent.

ii. LWH has a long standing portfolio of delivering high quality research and this has created a culture of encouraging research among the staff. Most of

the clinicians have also higher academic qualifications. These were reflected in parent-staff interactions and the way the study was introduced to the parents at a very stressful period of their life.

iii. The study did not have a therapeutic intervention. These led to less ethical dilemmas and easier agreement for participation.

iv. Families were identified in the obstetric ward and informed for the study in advance before the traumatic experience of preterm labour and its physiological and psychological impact on informed-consent process.

v. Pointing to parent's altruism and the possible benefit for future infants was also proved efficient as pointed also by a previous study (Morley CJ, Lau R, Davis PG, et al. What do parents think about enrolling their premature babies in several research studies?)

The majority of missing observations was attributed to the following reasons:

i. Approach to seek informed-consent after the birth was difficult as parents were distressed and difficult to comprehend the new situation. Many observations on first day after birth were missed due to this reason as there was a time delay between birth and signing the consent form

ii. At least 12 hours were allowed after information was provided to the parents to discuss and decide whether they would agree to participate.

iii. Many patients came from Level II neonatal units and enrolment was only possible on the second or third day of life.

iv. Many patients were also discharged back to the Level II neonatal units once they did not require intensive care.

v. There was only one machine for aEEG and NIRS available and there were weeks with many patients in the study with prolonged PDA patency and newborns which needed to be recruited. Hence observations were missed from the more mature infants.

vi. Data were lost due to technical issues with the operating machines.

vii. There were periods of annual leave that led to missing observations.

viii. There were occasions that the critical clinical condition of the patient did not allow us to make an observation

There were no major complications from the study procedures. Due to the bulky size of NIRS probes in comparison to the size of preterm infants' head, temporary minor skin irritation and markings were observed, which lasted for couple of hours. The repeated echocardiograms demonstrated on many occasions malpositioned umbilical venous catheters which initially were appropriately positioned and subsequently needed adjustment.

Appendix 12: Publications

2021

- Kotidis C, Wertheim D, Weindling M, Rabe H, Turner MA. Assessing patent ductus arteriosus in preterm infants from standard neonatal intensive care monitoring. Eur J Pediatr. 2021.

2017

- JENS 2017, 30/10-03/11/17, Venice Italy. C. Kotidis, A. Eleuteri, A. M. Weindling, M. Turner "Cerebral Biomarkers In Extremely Preterm Infants With A Patent Ductus Arteriosus". Oral presentation
- XXV Biennial Meeting of the International Perinatal Collegium, Edinburgh, Scotland, 2–6th July 2017, "Cerebral Biomarkers In Extremely Preterm Infants With A Patent Ductus Arteriosus". Oral presentation
- 6th European Society for Developmental Perinatal and Paediatric Congress, 20-23rd June 2017, Leuven, Belgium. C. Kotidis, A. Sharp, Z. Alfirevic, A.M. Weindling, M.Turner "Neonatal cardiovascular and cerebral function after antenatal maternal exposure to magnesium sulfate". Oral presentation
- Neonatal Society 2017 Summer meeting, Brighton, UK. C. Kotidis, D. Wertheim, A. M. Weindling, H. Rabe , M. Turner. "Phase Difference, a Novel Tool for the Assessment of PDA and Haemodynamics in Extremely Preterm Neonates". Oral presentation

2016

- ESPR 2016, 21-25/10/16, Geneva, Switzerland.
    - C. Kotidis, M. Weindling, N. Subhedar, M. Turner. “Validation Of PDA Severity Score In Predicting Chronic Lung Disease (CLD) In Extremely Preterm Infants With Patent Ductus Arteriosus” Oral presentation
    - Lucas, C., Rodgers, B., Patel, A., Turner, M., Weindling, M., & Kotidis, C. (2016). Non-Infective Influences On A Continuous Predictor Of Infection (HeRO Score). In EUROPEAN JOURNAL OF PEDIATRICS Vol. 175 (pp. 1775-1776). e-Poster
    - C. Kotidis, M. Weindling, M. Turner. Neonatal Haemodynamics: An Empirical Evaluation Of One Causal Pathway. e-Poster
- Neonatal Society 2016 Autumn Meeting, London UK. C. Kotidis, M. Weindling, M. Turner. “Biomarkers associated with haemodynamically significant patent ductus arteriosus”. Oral presentation

2015

- Neonatal Society Autumn Meeting 2015, Royal Society of Medicine, London. Thursday, 5th November 2015. C Kotidis,N. Subhedar, M Weindling, M Turner. Haemodynamically Significant Patent Ductus Arteriosus Impairs Cerebral Oxygenation and May Be Linked With Intraventricular Haemorrhage. Oral presentation
- ITM Research Day, Eleanor Rathbone Theatre, Institute of Translational Medicine, University of Liverpool, 6th July 2015. C Kotidis, M Weindling, M

Turner. Cerebral Haemodynamics In Preterm Infants With Patent Ductus Arteriosus (PDA): A Pilot Study. e-Poster

- The 24th Biennial Meeting of the International Perinatal Collegium, Silverado Resort & Spa Napa, California July 12-16, 2015. C. Kotidis, N. Subhedar, M. Weindling, M. Turner. Haemodynamics In Preterm Infants With Patent Ductus Arteriosus (PDA). Oral presentation
- 1st Congress of joint European Neonatal Societies (jENS 2015); Budapest (Hungary); September 16-20, 2015. C. Kotidis, M. Turner. A study to validate the duration of assessment of baseline NIRS values in preterm babies with echocardiographically-confirmed PDA. e-Poster