Investigating Pulse Wave Velocity and Phase Difference as Novel Biomarkers in Neonatal Haemodynamics

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Abstract

Introduction:

Despite its prevalence and associated morbidity and mortality, there are still significant drawbacks to the diagnosis and management of patent ductus arteriosus (PDA). There is a lack of consensus about when a PDA is haemodynamically significant, the nature and timing of intervention and even whether or not it is pathological or a mere innocent bystander of prematurity. Furthermore, drawbacks in PDA research such as a lack of core data sets, lack of consistency in applying treatments during clinical trials and lack of robust long-term outcome measures have hindered advancement of clinical management. Utilisation of novel biomarkers has improved the research quality and clinical care of a number of paediatric conditions. Despite a number of studies assessing possible novel biomarkers in the management of PDA, none have been deemed suitable for clinical application. Pulse Wave Velocity (PWV) is haemodynamic biomarker used in adult populations as a marker of cardiovascular morbidity that may be relevant to PDA. Evidence of its use in paediatric populations is minimal and a process of validation is yet to be undertaken in neonatal populations.

Aim:

The aims of this study are to highlight the current benefits and drawbacks in the diagnosis and monitoring of PDA, begin a process of validation for PWV and its expression in terms of phase difference as novel biomarkers in neonatal populations, and to assess the relationship between these biomarkers and echocardiographic markers of PDA to evaluate its potential future role in clinical practice.

Methods:

A systematic review was conducted to assess current practices in defining haemodynamically significant PDA (hs-PDA). A subsequent prospective observational study was conducted to assess the measurement of PWV/phase difference in neonatal populations, the effects of various demographic

and clinical parameters on their measurement and their relationship with echocardiographic parameters used in the diagnosis of PDA.

Results:

Our systematic review highlighted significant variation in what clinical and echocardiographic parameters were used to define hs-PDA in randomised controlled trials. The prospective observational study found weak associations between the measurement of PWV/phase difference and most demographic/clinical variables. The study also highlighted poor correlations between their measurement and the presence of PDA. However, the small sample size means future research is required before firm conclusions can be drawn about the relationship between PWV/phase difference and the demographic, clinical and echocardiographic variables encountered in neonatal care.

Conclusion:

Current research about PDA in neonates is suboptimal and could benefit from the formation of a core data set to improve meta-analysis. The identification of novel biomarkers to aid echocardiography as a diagnostic tool could also be beneficial. PWV/phase difference have shown potential as useful novel biomarkers in neonatal haemodynamics, however further research is required to fully evaluate their relationship with PDA.

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List of Abbreviations

AG	Anna Glaser
ANP	Atrial Natriuretic Peptide
ANS	Autonomic Nervous System
BNP	Brain Natriuretic Peptide
BPD	Bronchopulmonary Dysplasia
BW	Birthweight
CBF	Cerebral Blood Flow
CO	Cardiac Output
DA	Ductus Arteriosus
DH	Dr Daniel Hawcutt
DV	Ductus Venosus
ECG	Electrocardiogram
FB	Fariba Bannerman
GA	Gestational Age
GJ	Graham Jeffers
HFOV	High-Frequency Oscillatory Ventilation
HIE	Hypoxic-Ischaemic Encephalopathy
hs-PDA	Haemodynamically Significant Patent Ductus Arteriosus
IRAS	Integrated Research Application System
IVC	Inferior Vena Cava
IVH	Intraventricular Haemorrhage
LA	Left Atrium
LA:Ao	Left Atrium:Aortic Root Ratio
MAS	Merconium Aspiration Syndrome
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
NO	Nitric Oxide
NPV	Negative Predictive Value
NSAID	Non-Steroidal Anti-Inflammatory Drug
NT-proBNP	Amino-terminal pro-B-type Natriuretic Peptide
PD	Paraskevi Dimou
PDA	Patent Ductus Arteriosus

PFC	Persistent Fetal Circulation
PGHS	Prostaglandin Synthetase
PNS	Parasympathetic Nervous System
POX	Peroxidase Region
PPD	Pulse Phase Difference
PPV	Positive Predictive Value
PWV	Pulse Wave Velocity
RA	Right Atrium
RAAS	Renin Angiotensin Aldosterone System
RCT	Randomised Controlled Trial
RDS	Respiratory Distress Syndrome
ROC	Receiver Operating Characteristics
SNS	Sympathetic Nervous System
s-PDA	Symptomatic PDA
T3	Triiodothyronine
T4	Thyroxine
TTN	Transient Tachypnoea of the New-born
UAC	Umbilical Arterial Catheter

Chapter 1: Introduction

1.1 Background

Preterm birth, defined as birth at less than 37 weeks gestational age (1), occurs in 5-10% of births in resource-rich countries such as the UK and USA (2). Although evidence is limited, the prevalence of preterm birth in developing countries has been estimated at approximately 25% (3). Preterm neonates are a population vulnerable to a unique set of pathologies that are associated with high rates of mortality and long-term morbidity. It is encouraging that from the development of antepartum steroid and surfactant therapy for neonatal respiratory distress syndrome (RDS) (4, 5), to the use of therapeutic hypothermia in the management of hypoxic ischaemic encephalopathy (HIE) (6), survival rates in both preterm and term neonates have improved drastically across the globe (7, 8). However, in 2014 there were still 2.9 million neonatal deaths worldwide (9) and the Office for National Statistics reported that between 2014 and 2015, immaturity-related conditions were responsible for between 49.3-50.3% of neonatal deaths in England and Wales (10). This demonstrates that there remains considerable scope to improve outcomes for these children.

Holistic care in neonatology involves much more than just the treatment of illness. Patients are often subjected to constant monitoring and repeated investigations which can have a number of adverse effects on the neonate. Not only are procedures and investigations distressing to both the patient and their parents, disruption of the baby and their environment whilst being treated in neonatal intensive care units (NICU) can also hinder their development (11-13). There is therefore a need for research into the development of non-invasive methods of monitoring to help provide optimal care to patients and give an already vulnerable population the best chance to thrive and survive.

One of the commonest conditions to affect preterm infants is the patent ductus arteriosus (PDA). This condition affects between 30-50% of all preterm neonates depending on gestation (14). Although necessary for survival of the fetus in utero and normal for the first 24-48 hours of life, persistence of the ductus arteriosus after birth is associated with a number of serious adverse outcomes. These include the development of intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD) and necrotizing enterocolitis (NEC) (15-18). The current gold standard investigation for the diagnosis of PDA is the echocardiogram. Although this can accurately measure the size of the PDA as well as a number of other haemodynamic characteristics of the neonate, it has a number of drawbacks. Echocardiograms are disruptive to the neonate and only provide a one-off reading at a particular point in time. This means that in the process of investigating patients and monitoring their response to treatment, neonates are often subjected to multiple echocardiograms. This can cause disruption to their sleep cycle, increased noise around the incubator and temperature changes, all of which can be detrimental to the patient (11-13). This chapter aims to review the fetal and neonatal cardiovascular systems, the pathophysiology, diagnosis and management of PDA, the use of novel biomarkers in neonatology and finally, the current and potential uses of pulse wave velocity (PWV) and phase difference as novel biomarkers in neonatal care.

1.2 Fetal and Transitional Circulation

1.2.1 Vascular Physiology

With each beat of the heart, blood passes from the ventricles through the arterial system and around the body. The flow of blood around the body is achieved through a combination of mass flow of blood (i.e. blood that is ejected from the ventricles

pushing along any residual blood in the arteries) supplemented by recoil of the blood vessel walls that relates to the vascular tone of the arteries. Vascular tone refers to a vessels ability to constrict relative to its maximally dilated state. A vessels ability to constrict or dilate contributes to vascular resistance, and can therefore increase or decrease blood flow to the target organs based on metabolic demand. Tone is modulated through contraction or relaxation of the vascular smooth muscle cells under the influence of a number of intrinsic and extrinsic factors. Intrinsic factors (e.g. nitric oxide (NO), prostacyclin, thromboxane A_2 etc) (19) derive from within the vascular endothelium, whereas extrinsic factors (e.g. angiotensin II, atrial natriuretic peptide (ANP)) derive from a source outside of the vessel or the organ or tissue where the vessel is located. Another important extrinsic factor which plays a significant role in the control of haemodynamics within the body is input from the autonomic nervous system (ANS). The ANS is part of the peripheral nervous system which controls multiple physiological processes such as maintenance of blood pressure, regulation of heart rate, pupillary dilatation and constriction and control of secretions such as sweat and saliva (20). There are two branches of the ANS, the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). Activation of the SNS causes an increase in heart rate, cardiac contractility and peripheral resistance via post-ganglionic nerve fibres (20-22). Activation of the PNS causes a decrease in these haemodynamic properties of the cardiovascular system, predominantly via the action of the vagus nerve (20-22). Any alteration to the development or functioning of the ANS can therefore have significant consequences to the haemodynamic control within the neonate.

1.2.2 Fetal Circulation

There are a number of differences between the circulatory system of a fetus (Figure 1) (23) and that of a newborn. The circulatory system in utero is different to that after birth as the right ventricle is involved in the ejection of oxygenated blood. Whilst in utero, the fetus relies on the delivery of oxygenated blood from the mother via the umbilical vein (24). Blood from the umbilical vein either travels through the liver microcirculation or bypasses it entirely by entering the inferior vena cava (IVC) via the ductus venosus (DV). The IVC then allows the oxygen-rich blood to enter the right side of the heart. The Eustachian valve helps direct the flow of blood from the right atrium (RA) to the left atrium (LA) via an intra-cardiac shunt known as the foramen ovale (24). The patency of the foramen ovale is maintained in utero by a combination of factors. The collapsed and fluid-filled fetal lungs cause increased resistance to the right outflow tract due to high pulmonary vascular resistance (PVR). This results in decreased venous return to the LA via the pulmonary veins. The combination of raised PVR and reduced venous return to the LA causes a pressure gradient across the atria, allowing blood to shunt from right to left. An additional embryological adaptation that aids the distribution of maternal blood around the fetus is the ductus arteriosus (DA). The DA is an extra-cardiac shunt that allows communication between the left pulmonary artery and the descending aorta. High pulmonary vascular resistance causes a right-to-left shunt, allowing between 75-90% of right ventricular output to bypass the pulmonary circulation and enter the systemic circulation (24). During fetal life, the DA's patency is maintained by a combination of low partial pressure of oxygen in the non-functioning fetal lungs, vasodilatory prostaglandins (e.g. PGE2) secreted by the placenta and the secretion of NO by ductal endothelial cells (25). Together, this system of shunts allows optimal distribution of oxygenated maternal blood around the fetal body in an attempt to ensure favourable growth and development.

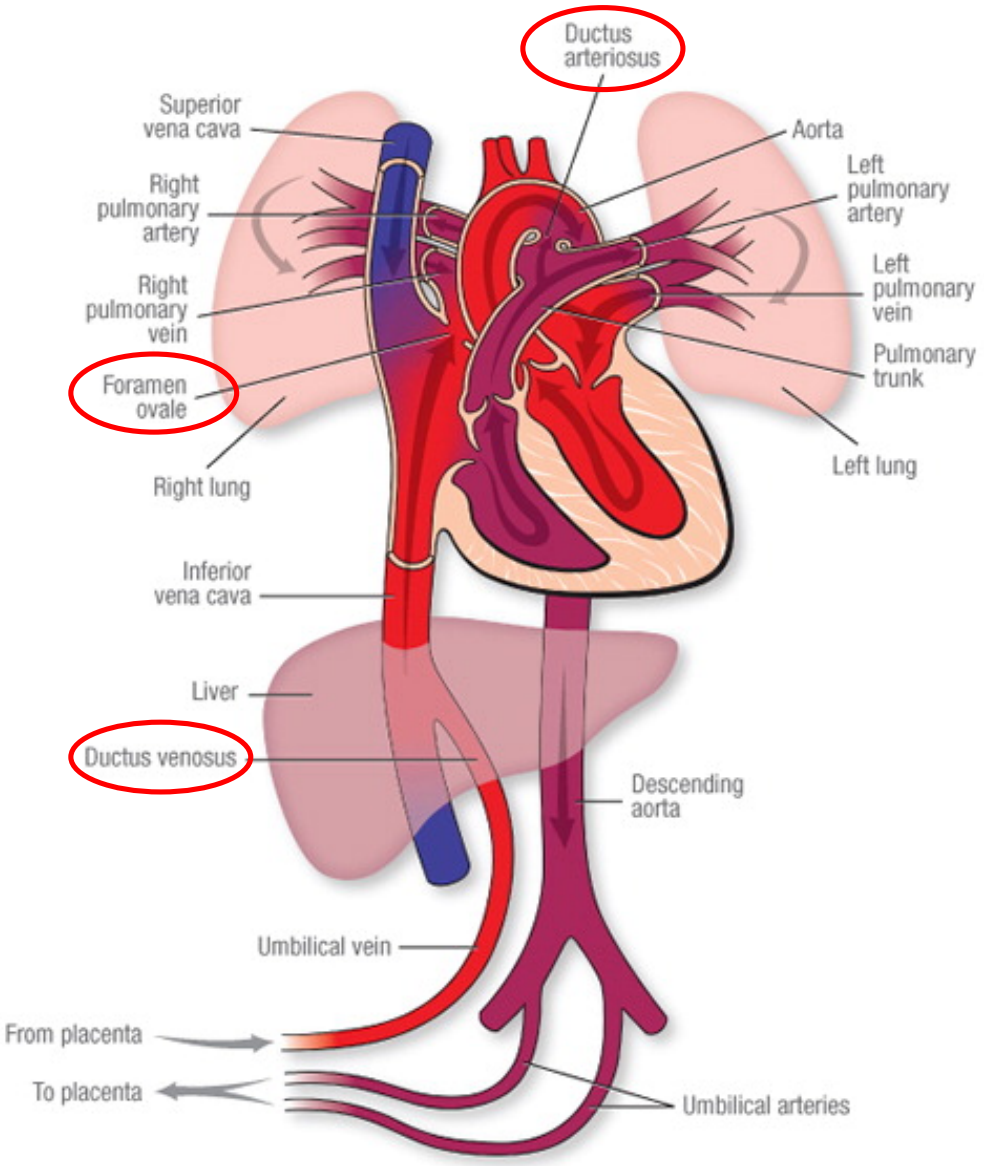


Figure 1 The fetal circulation. Adapted from the American Heart Association (23)

1.2.3 Transitional Circulation of the Newborn

Prior to birth, the fetal circulation is maintained by two main factors. The placenta acts as a low pressure vascular bed which allows deoxygenated blood from the fetus to return to the mother via the placenta through the umbilical arteries (24). Secondly, the high vascular resistance of the pulmonary circulation caused by the non-functioning fetal lungs maintains a right-to-left cardiac shunt, thus ensuring the distribution of oxygenated maternal blood throughout the systemic circulation. Although these factors are key to the survival of the fetus in utero, a number of changes must occur for the fetus to successfully transition to life outside the womb. The term "transitional circulation" refers to cardiopulmonary changes that occur as the baby is born that allows them to adapt to post-natal life. During labour, pressure from the birth canal aids the removal of some of the fluid that occupies the fetal lungs. This in combination with a number of thermal, chemical and tactile stimuli at birth initiates breathing (26). As the baby takes their first breath, inflation of the lungs causes a significant reduction in pulmonary vascular resistance. The mechanism of this reduction is thought to be due to a combination of factors. The stimulation of pulmonary stretch receptors stimulates vasodilatation of the pulmonary vasculature and the improved oxygenation of blood reduces vasoconstriction secondary to hypoxia (27). The reduction in pulmonary vascular resistance results in increased pulmonary blood flow and therefore increased venous return to the LA. This causes a subsequent rise in left atrial pressure. In combination with this, the removal of the placental circulation through clamping of the umbilical cord causes an increase in systemic vascular resistance and a significant reduction in the venous return to the right side of the heart via the DV and IVC (27). The resulting increased left atrial pressure and decreased right atrial pressure culminate in the closure of the foramen ovale, causing a cessation of shunting between the atria. The DA is closed by a combination of decreased pulmonary vascular

resistance along with a reduction in vasodilatory prostaglandins. This is achieved through cessation of placental blood flow and pulmonary metabolism of PGE2. “Functional” closure of the DA, whereby there is complete cessation of left-to-right shunting, typically occurs within 24 hours; however, there is still the possibility of the DA becoming patent again (25). “Anatomical” closure of the DA, characterised by fibrous proliferation of the intima and thus an inability to reopen, typically occurs within 2-3 weeks after birth (25). Anatomical closure of the DA results in the formation of the ligamentum arteriosum, allowing the infant to develop a normal dual circulatory system.

1.2.4 Factors affecting the Transitional Circulation in Term Infants

Although almost all term neonates adapt well to post-natal life, the persistence of the fetal circulation can be extremely detrimental to their health. The presence of abnormal physiological states such as hypoxia, hypercarbia and hypothermia caused by birth complications can result in vasoconstriction of sensitive neonatal pulmonary arterioles (27). This results in an increase in pulmonary vascular resistance, causing a persistence of right-to-left shunting which inhibits the natural closure of the DA and foramen ovale (Figure 2) (27).

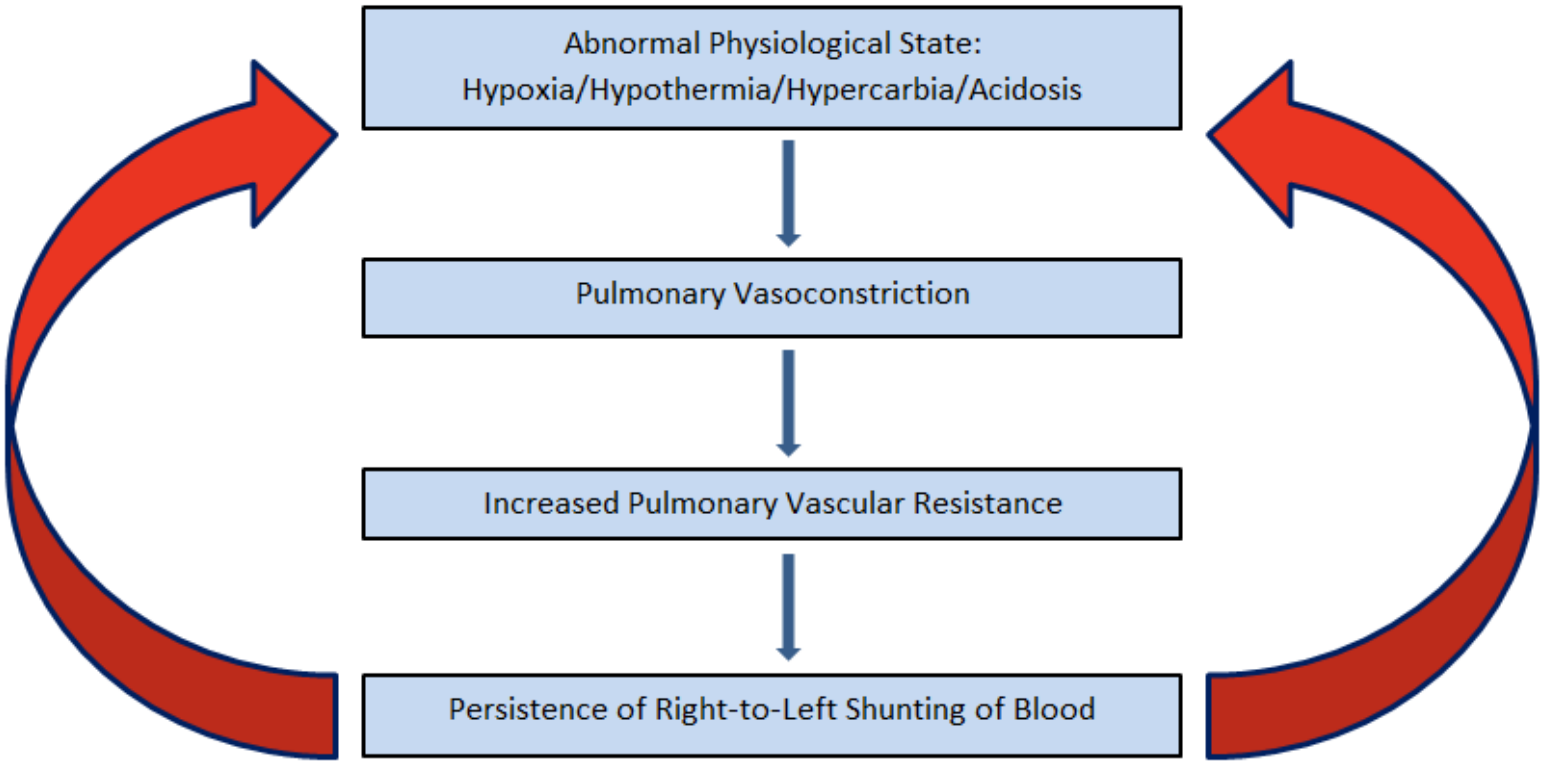


Figure 2 The "Vicious Cycle" of Persistent Fetal Circulation (PFC) - Adapted from Fetal Circulation by *Murphy* (27)

1.2.5 Factors affecting the Transitional Circulation in Preterm Infants

Preterm infants are particularly susceptible to disruptions to the development of a functional post-natal circulatory system. Although they too are susceptible to the effects of the "vicious cycle" of PFC, the incomplete development of key organs can lead to abnormal physiological functioning.

Cardiovascular System:

Preterm infants are particularly susceptible to the persistence of a PDA, which will be discussed in detail later in the chapter. Immature cardiac myocytes present in preterm infants may result in reduced cardiac contractility (24). Their increased heart rate also impairs diastolic filling, which in combination with reduced contractility, significantly reduces their cardiac output (CO) (24).

Central Nervous System:

Preterm infants with reduced CO may be at increased risk of cerebral hypoperfusion due to altered cerebral autoregulation. Although its mechanism is poorly understood, cerebral autoregulation refers to the process by which the vasculature within the brain rapidly responds to alterations in blood pressure in order to maintain cerebral perfusion and prevent hypoxic injury (28, 29). The "autoregulatory plateau" shows the maintenance of stable cerebral blood flow (CBF) at varying mean arterial blood pressures (Figure 3) (30). It has been suggested that extremely premature neonates have a much narrower autoregulatory plateau (31), resulting in increased fluctuations in CBF (32). Failure of cerebral autoregulation (commonly referred to as "pressure passivity") whereby the infant is unable to maintain their CBF even with minor falls in arterial blood pressure, is associated with cerebral ischaemia (33).

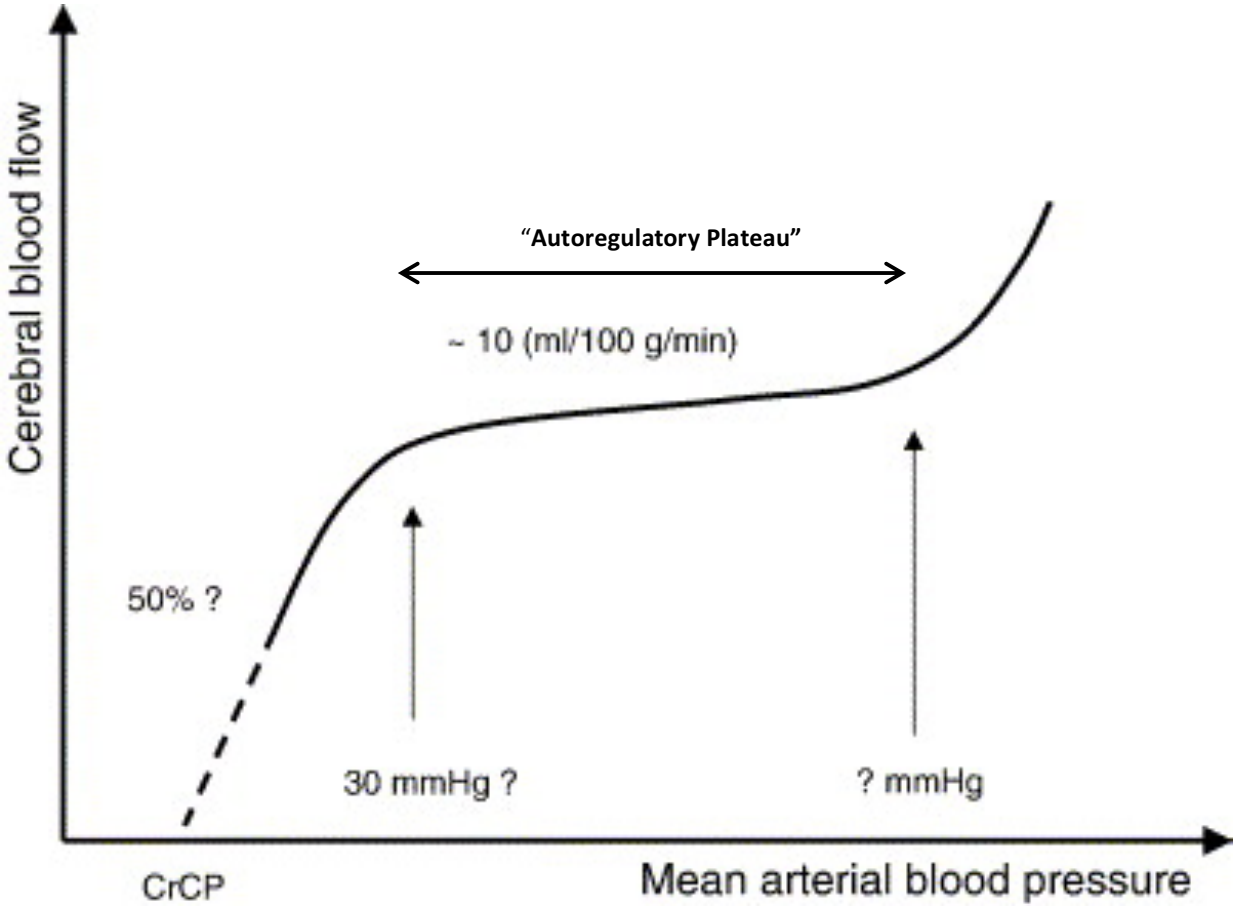


Figure 3 The "Autoregulatory Plateau" of cerebral autoregulation – adapted from Autoregulation of cerebral blood flow in newborn babies by *Greisen* (30). CrCp = Critical Closing Pressure

It is thought that this may be associated with the absence of muscle in premature penetrating cerebral arterioles (34). Neurological insults such as cerebral ischaemia may affect the transitional circulation through their effect on the development of the ANS. As previously mentioned, the ANS plays a key role in the regulation of vascular tone and thus haemodynamic control of the newborn. It has been suggested that perinatal neurological damage can cause alterations to the development of the ANS (35). A recent study outlined the effects of hypoxic-ischaemic encephalopathy (HIE) on the development of the ANS in the new-born. HIE is a perinatal neurological injury associated with hypoxia and reduced CBF. It was found that although injury to different parts of the cerebral cortex and cerebellum had differing effects on the development of the ANS, hypoxic-ischaemic injury of the brain causes alterations to the sympatho-vagal balance of the ANS (22). This alteration in the functioning of the neonatal ANS may affect their ability to adjust their pulmonary and systemic vascular resistance which are key aspects of the transitional circulation.

Endocrine System:

Preterm infants may be susceptible to endocrine dysfunction which can affect their ability to adapt to life outside the womb. A cortisol "surge" occurs after the placental circulation is disrupted. Cortisol stimulates a number of key physiological processes in the neonate. It is involved in the increase in beta-adrenergic receptor density in the heart and lungs, the induction of the maturation of the surfactant system and stimulating the release of catecholamines which are responsible for thermogenesis and increasing blood pressure after birth (36, 37). Cortisol also aids the conversion of the thyroid hormone thyroxine (T4) to its active metabolite triiodothyronine (T3). T3 and cortisol are associated with the activation of the $Na^{+}K^{+}$ATPase responsible for aiding

the clearance of fluid from the fetal lungs (37). Preterm infants may be susceptible to a reduction in cortisol due to immature adrenal glands at the time of birth (37).

1.3 The Patent Ductus Arteriosus

1.3.1 The Pathophysiology of the Patent Ductus Arteriosus

A PDA is a common condition within neonatology. It occurs in approximately 1 in 2000 term neonates (38) and is inversely proportional to gestational age in premature neonates, with the incidence ranging from 30-50% (14). Combined data from five studies showed that spontaneous closure of a PDA after 7 days of life occurred in only 36% of patients born at 27-28 weeks gestational age (GA), in 32% of those born at 25-26 weeks GA, and in just 13% of those born at 24 weeks GA (15). There are a number of factors that contribute to the persistence of a PDA in preterm neonates. It is thought that as term babies approach birth, there is a decrease in the sensitivity of ductal cells to the vasodilatory prostaglandins that help maintain the patency of the DA (39). Ductal cells in the preterm infant are therefore thought to be more sensitive to vasodilatory prostaglandins. This in combination with the immature fetal lungs' reduced ability to metabolise prostaglandins contribute to the failure of the DA to close (40, 41).

PDA is associated with a number of life-threatening conditions such as IVH, NEC and BPD (15-18). Adverse outcomes secondary to PDA, particularly BPD, can be attributed to the so called "steal phenomenon". A left-to-right shunt caused by the lower vascular resistance of the pulmonary vessels results in pulmonary hyperperfusion and systemic hypoperfusion (42). This has a particularly significant effect on the cerebral, renal and mesenteric systems (15, 43). It is hypothesised that this may be exacerbated by the

effect of a PDA on the ANS. One study showed that preterm infants with a PDA had an association with predominance of the PNS (44). It was hypothesised that a PDA caused an increased end diastolic pressure (secondary to the persistent shunting of blood) which subsequently caused a release of atrial natriuretic peptide (ANP), resulting in widespread hypotension and systemic hypoperfusion. It was suggested that the systemic hypoperfusion results in decreased cerebral oxygenation which further disrupts the normal development of the ANS, impairing the sympatho-vagal balance.

1.3.2 Investigating the Patent Ductus Arteriosus

Although investigations such as electrocardiogram (ECG) and chest radiographs can provide additional information when assessing an infant with a PDA, the gold standard investigation is the echocardiogram (43). Echocardiograms in the investigation of a PDA are transthoracic ultrasound scans of the cardiovascular anatomy. Ultrasound composes of high frequency sound waves beyond the human auditory range travelling through tissues of various densities. A transducer emits the waves through a conductive medium which eliminates power loss at the air-tissue interface. Tissues of different densities then reflect different quantities of the ultrasound wave which are collected by the transducer before being converted into an image on the ultrasound screen. The resistance of a tissue to the passing of ultrasound waves is known as the "acoustic impedance" (45). Fluid allows greater transmission of ultrasound waves and in doing so reflect little of the ultrasound waves back to the transducer (45). This produces a "hypoechoic" or dark image (45). Denser tissues such as bone reflect much more of the ultrasound waves and in doing so produce a "hyperechoic" or light image (45). This allows the practitioner to differentiate between different structures based on the density of tissue. Doppler ultrasonography provides additional information on the velocity and direction of the flow of fluid.

PDA is diagnosed by using echocardiography to demonstrate evidence of blood flow of abnormal origin along the pulmonary artery. Once visualised, the diameter of the PDA can be measured, along with a number of other parameters to assess its haemodynamic significance. Echocardiography is considered the gold-standard investigation of PDA as it provides the clinician with direct visualisation of the duct, as well as measurement of its severity from a haemodynamic standpoint.

Despite its widespread use in the diagnosis of PDA, there are a number of potential drawbacks to the use of echocardiography in neonatal care. Concerns surrounding its repeatability and accessibility have been raised in literature, whilst the need for repeated measurements may make it inappropriate for monitoring a dynamic condition such as a PDA. The current uses, benefits and drawbacks of echocardiography in neonatal care will be discussed in more detail in chapter 2.

1.3.3 Management of Patent Ductus Arteriosus

Management of PDA remains controversial. Not only are there multiple treatment options, but there are also conflicting opinions on whether or not a PDA requires intervention due to the lack of evidence for improvement in long term outcomes associated with treatment (46, 47). Current established treatment options typically include medical management with indomethacin or ibuprofen, or surgical ligation. Indomethacin and ibuprofen are non-steroidal anti-inflammatory drugs (NSAIDs) which act by inhibiting vasodilatory prostaglandins such as PGE2 which maintain the patency of the DA. Although treatment with NSAIDs is successful in treating PDA in 70-80% of cases, they are associated with a number of adverse side effects including decreased renal function and gastrointestinal bleeding (48, 49). Surgical ligation is typically reserved for PDA that is refractory to medical treatment or when NSAIDs are

contraindicated (50). Although it has been shown to reduce mortality, management with surgical ligation has been associated with a number of complications including hypotension, adverse neurodevelopmental outcomes and increased rates of pneumothorax and infection (48, 49). Paracetamol has recently been studied as a possible alternative to medical management with NSAIDs that is associated with lower rates of toxicity. It is thought that this is due to paracetamol's different mechanism of action. Like traditional NSAIDs, it reduces synthesis of prostaglandins through the inhibition of prostaglandin synthetase (PGHS), however, it acts on a different enzyme binding site known as the peroxidase region (POX) (51). A recent review found it was equally as efficacious, but that further evidence was required to assess long-term effects and potential adverse reactions before it should be considered a first-choice drug in the management of PDA (52).

1.4 Novel Biomarkers in Neonatology

1.4.1 Background

Biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention" (53). Neonatology has seen an increase in research in biomarkers to help improve the diagnosis and management of life-threatening conditions. For example, conventional biomarkers such as interleukins and cytokines involved in the immune cascade have been extensively researched in relation to neonatal sepsis (54-56). However, these biomarkers are not specific to a particular disease or organ system. There has thus been a call for further investigation into "novel" biomarkers to help improve diagnostic capabilities and drug development in neonatology and paediatrics (57, 58). Disease-specific biomarkers have

been used to great effect in paediatric populations. Examples of established biomarkers include haemoglobin A1C in the diagnosis and monitoring of diabetes mellitus, and the MYCN oncogene in the assessment of risk of developing neuroblastoma (58). The use of established biomarkers such as these in everyday paediatric practice highlights their clinical potential.

1.4.2 Desirable Traits of a Biomarker

The desirable traits of a biomarker have been well documented (59). Ultimately, a biomarker should consist of a sample that is acceptable and accessible combined with an appropriate and accurate assay. However, when investigating a novel biomarker for its association with a specific clinical outcome, the importance of considering the following has been outlined:

I. Assay performance – is the assay accurate, reliable and responsive to the condition under investigation?
II. Qualification – does the biomarker have a robust relationship with the current gold standard investigation for the proposed clinical outcome?
III. Utility – can the biomarker improve care beyond what is already available? (59)

The importance of considering the effects of normal growth and development before a biomarker is implemented in clinical practice has also been outlined (58). In order for biomarkers to be considered useful, there therefore needs to be a rigorous process of validation before they can be considered for application in clinical practice (60-62).

1.4.3 Drawbacks in Biomarker Research

Researching novel biomarkers in paediatric populations is subject to a number of unique drawbacks (59). The first of which is the incidence and prevalence rates of morbidity in children. In general, there are lower incidence and prevalence rates of disease in children. This often results in a significantly smaller sample size for analysis, affecting the validity of results. Even in conditions that typically predominantly affect children such as congenital conditions and chromosomal abnormalities, the sample sizes are still significantly smaller than other conditions that are well researched in adults. As well as this, due to reluctance to perform unnecessary investigations/interventions on healthy children, it is often more difficult to obtain control groups to use as comparison when investigating biomarkers. Another important aspect to consider with research in paediatrics is the changes in physiology and pathophysiology between differing age groups. Considerable changes to normal physiology occur as children grow, with different organ systems developing at different rates. This further hinders researchers' ability to gather large, reliable sample sizes. Furthermore, when considering conditions that are common in adults *and* children (e.g. diabetes mellitus), comparisons cannot be made between the two groups due to significant differences in pathophysiology. Although novel biomarkers have the potential to make a significant impact to patient care in neonatology, performing quality research into their clinical application can be challenging.

1.4.4 Biomarker Method Validation

Before implementation in clinical practice, biomarkers should be subject to a rigorous process of method validation. Method validation can be defined as "the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled" (62). When seeking to validate a

novel biomarker, it has been suggested that first the purpose, target values and acceptance limits of the method should be agreed upon before assessing the biomarkers performance by experimentation (62).

1.4.5 Novel Biomarkers in the Investigation of Patent Ductus Arteriosus

The use of alternative biomarkers in the monitoring of PDA has been investigated, most notably the use of brain natriuretic peptide (BNP) and amino-terminal pro-B-type natriuretic peptide (NT-proBNP). BNP and NT-proBNP are released in response to cardiac wall stress and act by inducing naturesis/diuresis and vasodilatation, whilst concurrently inhibiting the renin-angiotensin-aldosterone system (RAAS) and SNS (63). Although some studies showed promising results for the use of BNP and NT-proBNP in the diagnosis and monitoring of haemodynamically significant PDA (hs-PDA) (64-66), a recent systematic review reported that results varied widely depending on assay and patient characteristics (67). This outlines the need for further research into specific biomarkers for PDA. Novel biomarkers in PDA can help avoid unnecessary investigation and treatment, whilst also monitoring progress and response to treatment, however, taking a consistent approach to testing each biomarker is imperative.

A recent unpublished pilot study performed at the Liverpool Women's Hospital assessed the use of phase difference and pulse wave velocity (PWV) as potential novel biomarkers in the haemodynamic assessment of extremely preterm neonates. Although it was a small pilot study consisting of just 14 patients, it showed promising results with phase difference showing significant correlation to the PDA diameter ($P = <0.001$; $r = 0.820$). Another recent study also assessed the measurement of PWV expressed as phase difference as a non-invasive method of detecting PDA. Data was

collected on those aged <32 weeks GA and using arterial photoplethysmography collected from a pulse oximeter, they found that phase difference was strongly associated with PDA (optimal pulse phase difference (PPD) cut-off of ≥1.65 deg/cm, with an area under the receiver operating characteristics (ROC) curve of 0.98 (95% confidence interval, 0.96-1), sensitivity = 94.2%; specificity = 98.3%) and should be considered as a non-invasive method of detection (68). *Oishi et al* reviewed the same technique as a method of evaluating neonatal peripheral circulation. Within their study they included a case study of a patient who was diagnosed with symptomatic PDA. They noted a decrease in PPD whilst the PDA was symptomatic and an increase in PPD following treatment with anti-prostaglandin therapy (69). The results of these studies highlight the potential uses of PWV and phase difference as novel biomarkers in the diagnosis and management of PDA.

1.5 Pulse Wave Velocity and Phase Difference

1.5.1 Background

The speed at which blood is propagated around the body can be used as a method of analysing vascular compliance. PWV is a method of measuring arterial compliance that is calculated from measurements of the time it takes the impulse arising from a contraction of the heart's ventricles to travel between two recording sites – the so-called "pulse transit time" (70). The measurement of arterial stiffness, which is inversely proportional to PWV, helps us understand the mechanics of large vessels and thus circulatory physiology and disease (70). The factors affecting PWV can be shown by the Moens-Korteweg equation (Figure 4):

$$PWV = \sqrt{\frac{E \cdot h}{\rho \cdot 2R}},$$

Figure 4 Moens Korteweg equation – E = incremental elastic modulus of the vessel wall; h = wall thickness; r = vessel radius; p = blood density.

PWV can also be expressed in terms of phase difference. Phase difference refers to the difference between two waves of the same frequency that are referenced to the same point (71). The speed of pulse wave propagation can be measured as the difference in time between the R wave of an electrocardiogram (ECG) wave (corresponding to ventricular contraction) and the peak of the systolic blood pressure wave measured at a known point. The phase difference can be expressed in time or as a proportion of a total wave (in degrees).

1.5.2 Practical considerations of measuring Pulse Wave Velocity

There are a number of different methods of measuring PWV including intra-arterial transducers, arterial tonometry, Doppler ultrasound and oscillometry (72, 73). The waves need to be detected at two set points in order to calculate a value for PWV. The most common is the aortic PWV, whereby measuring points used are either at the aortic arch or the common carotid artery and the femoral artery (72). Once the two measurement points have been set, the distance between them in calculated. This then allows the PWV to be calculated using the equation:

PWV = d (metres) / t (seconds)

Measurements of this value in neonates are met with a number of potential drawbacks. Methods such as oscillometry require cuffs to be applied over the carotid artery in the neck and the femoral artery in the leg (73). Applying a cuff to the neck of a

neonate has the potential to be not only practically difficult but also distressing for the patient and their family. Other methods such as Doppler ultrasound require accurate measurement of the pulse wave at two determined points which may be difficult to achieve and maintain in a moving neonate. We postulate that for the measurement of this value in patients admitted to the NICU, the most reliable and minimally disruptive method is through measurement via an intra-arterial line; more specifically, through the use of an umbilical arterial catheter (UAC).

UAC insertion is common practice in neonates who require invasive haemodynamic monitoring or frequent arterial blood tests (74). The UAC is connected to a transducer which provides real-time information to the patients monitor, allowing the arterial waveform to be observed. In order to use the data from the UAC to calculate the PWV, a number of steps must be taken. First, as per common practice, all patients fitted with a UAC must have an appropriate x-ray to ensure the line is adequately placed. Based on this radiograph, a calculation of the distance of the tip of the catheter from the aortic valve can be calculated to provide the distance the pulse wave is travelling. If compared to the patient's ECG data, we can calculate the PWV through measuring the time difference between the R wave of the ECG (corresponding to ventricular contraction and the ejection of blood from the heart) and the peak of the systolic waveform as measured by the UAC. This provides a time measurement to how fast the pulse wave is travelling between the two set measurement points. This value may also be expressed in the form of "phase difference". The proportion of the cardiac cycle whereby the pulse wave is being transmitted can therefore be represented as a phase difference in degrees (°) whereby the total cardiac cycle represents 360°.

There are benefits and drawbacks of expressing haemodynamics in terms of PWV or phase difference which are outlined in Table 1.

	Pulse Wave Velocity (PWV)	Phase Difference
Benefits	Calculation is based on a more scientifically familiar concept of calculating speed from distance and time.	Calculation is based purely on ECG and arterial waveforms, eliminating the measurement error associated with expression of PWV.
Drawbacks	Relies on the measurement of distance from Aortic Valve to UAC tip – subject to potentially significant error of measurement.	Expression of phase difference is uncommon practice in medicine and therefore is a more abstract concept than measurement of PWV.

Table 1 Benefits and Drawbacks of PWV and Phase Difference

1.5.3 Current and Potential Uses of Pulse Wave Velocity and Phase Difference in the measurement of haemodynamic function

PWV is a well-established biomarker used in the investigation of cardiovascular disease in adults. It is considered to be a very accurate method of investigating arterial stiffness (75), but it has also been shown to be associated with variation in left ventricular systolic function as well (76). Although it is typically used to measure arterial stiffening in a clinical setting, it has been suggested that the concurrent analysis of heart rate variability and PWV can be used to assess peripheral vascular sympathetic functions as well (77). Due to its accuracy and repeatability as a cardiovascular biomarker in adults, PWV has been investigated for its potential uses in paediatric populations. It has been shown that PWV can be accurately measured and easily reproduced when assessing the vasculature of the neonate (78) and although predominantly an illness of older populations, arterial stiffening has been noted in paediatric populations with conditions associated with cardiovascular disease such as diabetes mellitus and chronic kidney disease (79, 80). Phase difference is not currently used as a method of expressing variations in haemodynamic function. We hypothesise that based on the factors affecting the Moens-Korteweg equation (see Figure 4 above) there are a number of other potential conditions which may cause variations in the measurement

of PWV. These include conditions which may cause variation to the radius of blood vessels, namely patent ductus arteriosus (PDA) and hypotension. Based on the existing evidence regarding the accuracy and repeatability of measuring PWV in paediatric populations, we believe the research into the use of PWV and phase difference as potential biomarkers in neonatal haemodynamics is warranted.

1.6 Aims and Objectives

This study aims to challenge current practices in the field of neonatal haemodynamics. Current practices regarding the investigation and management of PDA will be reviewed. We will also seek to improve the current understanding of the factors affecting the measurement of PWV in neonatal populations. It is hoped that this will stimulate a process of validation for the use of PWV/phase difference as a novel biomarker in the field of neonatal haemodynamics. With the vascular tone of arteries being controlled by a careful balance between the SNS and PNS, and PDA being associated with PNS predominance and hypotension, we postulate that the association between PDA and PWV/phase difference may be related to increased radius of blood vessels, thus altering the Moens-Korteweg equation.

Chapter two will review the use of echocardiography as an investigation in neonatal care, specifically reviewing its current uses in the haemodynamic assessment of the neonate, its accuracy, reliability and accessibility, as well as potential alternatives to its use.

Chapter three will consist of a systematic review of the clinical and echocardiographic markers of haemodynamic significance used in the assessment of the neonate with suspected hs-PDA.

Chapter four describes a prospective observational study analysing the factors affecting the measurement of PWV and phase difference in the first three days of life. The impact of numerous continuous and dichotomous variables that frequently occur in neonatal care will be reviewed.

Chapter five describes the relationship between pulse wave velocity, phase difference and echocardiographic markers of PDA.

Chapter six discusses the findings from the previous chapters and outlines their relevance to clinical practice. This chapter will critically evaluate the potential use of PWV/phase difference as continuous non-invasive biomarkers in neonatal haemodynamics.

Chapter 2: Echocardiography – A Review

2.1 Introduction

Echocardiography has been considered the "gold standard" investigation for the diagnosis of PDA for decades. Hailed for its ease of use and accuracy, it has become a mainstay of investigation in neonatal units across the UK. However, there are a number of potential drawbacks which reduce the value of echocardiography. Furthermore, there are a number of areas where the use of echocardiography could be supplemented by additional investigations. This chapter aims to critically evaluate the pros and cons of the use of echocardiography as part of the management of PDA in patients that are treated in NICU.

2.2 Current Uses of Echocardiography in Neonatal Haemodynamics

Echocardiography allows the clinician to directly view and measure the size of the PDA. This allows the investigator to make a bedside diagnosis as well as a prediction of the severity of disease based on the PDA diameter. The development of Doppler ultrasound has since provided the clinician with a means of assessing a number of additional parameters which aid in the diagnosis of an hs-PDA. The ability to assess direction and velocity of blood flow adds further value to the echocardiographic evaluation of a patient as it allows the clinician to assess the direction of the shunting as well as the rate of flow through the ductus. Although the assessment of numerous haemodynamic parameters is useful in theory, their application to a clinical scenario is somewhat limited by the lack of a universally accepted grading criteria to confidently declare haemodynamic significance. *McNamara et al* stated that "the lack of a standardised approach in assigning echocardiographic significance is a major barrier

towards better understanding the clinical impact of the ductus arteriosus" (81). Although they subsequently proposed a system for determining haemodynamic significance, the authors of more recent literature still note that there is no clear clinical definition (82, 83). This lack of clinical clarity has the potential to result in significant variation in the use of echocardiography as a means of investigating the haemodynamic status of the neonate.

2.3 The Accuracy of Echocardiography in the Haemodynamic Assessment of the Neonate

In the hands of an experienced clinician, echocardiography is deemed to be an accurate method of investigating cardiovascular anatomy. A number of studies conducted in the 1980s showed echocardiography's potential as a useful bedside investigation in the detection of PDA. *Smallhorn et al* assessed 94 patients ranging from 28 weeks' gestation to 8 years of age using suprasternal cross-sectional echocardiography and compared them to a group of 37 patients without a diagnosis of PDA. In all patients, angiographic, surgical or necropsy confirmation was available. Of the 94 patients with PDA, 87 were correctly identified with 7 false negatives where the PDA was <2mm (84). This highlighted the reliability of echocardiography to identify moderate-to-large PDAs whilst also exposing its inaccuracy when assessing small PDAs. The authors felt, however, that this was not a major drawback as a PDA of diameter <2mm was unlikely to have any haemodynamic significance (84). *Rigby et al* investigated 35 preterm infants ranging from 26 to 30 weeks' gestational age using cross-sectional echocardiography. Although they did not specifically measure PDA diameter as part of their study, they were able to identify the presence of a PDA in 100% of patients in their study population (85). The authors agreed with the findings of *Smallhorn et al,* deducing that it was possible for small ductuses <2mm to be missed on

echocardiography (85). *Vick et al* used a combination of 2D echocardiography and Doppler ultrasound to assess preterm infants who were suspected of having PDA based on clinical findings (auscultation of a murmur, ventilator dependence, and chest x-ray findings) (86). They found satisfactory detection of PDA in 33 of 36 examinations (92%) (86). These early studies showed a high degree of accuracy in the detection of PDA using echocardiography.

Whilst drawbacks in the detection of small PDAs were identified, modern technology has improved the accuracy of echocardiography in the 30 years since these studies. *Hiraishi et al* compared two-dimensional and Doppler echocardiography data to angiographic, surgical and post-mortem data of 213 patients ranging from birth to 4 years old with a diagnosis of congenital heart disease. Of the 79 patients observed to have PDA via invasive methods, 76 (96.2%) were correctly identified by echocardiography (sensitivity = 96%; specificity = 100%) (87). *Lee et al* looked at the ability of both a neonatologist and cardiologist to diagnose PDA using a portable ultrasound machine. Of the 24 examinations, 15 were ultimately deemed to be positive for PDA. Neonatologist examinations had a sensitivity of 69% and a specificity of 88%, compared to the cardiologist's examinations which had a sensitivity of 87% and specificity of 71% (88). On 5 occasions, a neonatologist believed there to be no PDA present when a cardiologist evaluation was positive for PDA, however, in 3 of these cases, the PDA was considered "small" or "tiny" based on pre-set criteria (88). This study highlighted the ability of both cardiologists and neonatologists (who had limited training in the diagnosis of PDA using echocardiography) to detect patency with a moderate degree of accuracy and certainty. Furthermore, this study was conducted using a portable ultrasound machine. It was suggested that rates of detection would therefore likely improve if more sophisticated equipment was used. *Sohn et al* compared preoperative echocardiographic findings to surgical findings in patients with

congenital heart disease. 26 of the patients reviewed as part of their study were found to have PDA, 22 of whom were <1 year-old. 100% of the children with PDA in this study were correctly diagnosed with pre-operative echocardiography (89).

Literature spanning the last 30 years is in agreement that echocardiography is an accurate method of diagnosing PDA in infants. *Galante et al* reported that all ultrasound measurements have an error range of 10-20% (90), however, this value seems to be greatly overestimated in the case of diagnosing PDA in new-borns. Nonetheless, the studies presented highlight the fact that there is still a small degree of error in echocardiography's diagnostic capabilities.

One particular area where the diagnostic capabilities of echocardiography may be limited further is in patients suffering from lung disease. *Hiraishi et al* reported that respiratory problems such as hyperinflation and pneumothorax resulted in a "poor parasternal window", causing suboptimal imaging of the PDA (87). Furthermore, in 5 cases in the *Smallhorn et al* study, an inadequate suprasternal window was observed due to secondary hyperinflation of the chest (84). Hyperinflation can occur in a number of conditions likely to coexist with PDA such as BPD, transient tachypnoea of the new-born (TTN) and meconium aspiration syndrome (MAS). BPD affects at least a quarter of those born with a birth weight of less than 1500g, with its incidence increasing with reduced gestational age and birth weight (91). TTN is the most common respiratory disease of new-borns occurring in approximately 5.7 per 100 term births in the UK (92, 93). Although predominantly a condition affecting babies with increasing gestational age, MAS is still a highly prevalent condition in neonatology, occurring in 0.43 per 1000 births in a recent study conducted in Australia and New Zealand (94). Often patients with severe lung disease will require high-frequency oscillatory ventilation (HFOV) which may further limit the diagnostic abilities of echocardiography due to increased

thoracic movement. The high rates of incidence of these conditions with the potential to affect the accuracy of PDA diagnosis highlight a potential pitfall in the use of echocardiography in NICU.

2.4 Repeatability of Echocardiography

In order for an investigation to be considered useful, there must be a high degree of repeatability in its measurement to ensure consistency of diagnosis. Echocardiography is highly dependent on both the practical and interpretive skills of the user, resulting in the potential for misdiagnosis in inexperienced hands. The repeatability of echocardiographic parameters has been described as “far from optimal” (83), with another review claiming left-atrium-to-aortic-root ratio (LA:Ao) is “subject to considerable operator variability” (81). An overview of the literature reviewing repeatability of echocardiographic parameters is presented in Table 2. This lack of repeatability may result in misdiagnosis, over- or underestimation of disease severity and conflicting clinical pictures produced by different examiners.

Author (Year):	Echocardiographic Parameters Assessed:	Results:							
		Within-Observer Repeatability:				Between-User Repeatability			
		RepC	95% CI	RepI	95% CI	Rep C	95% CI	RepI	95% CI
Schwarz et al (2016)(83)	RI_CA					0.09	0.07-0.13	11	9-17*
	RI_ACA					0.11	0.08-0.16	14	11-20*
	LA:Ao					0.23	0.17-0.33	16	12-23
	LVPEP/LVET					0.08	0.06-0.11	23	18-32
	VTI_Ao/VTI_Pa					0.28	0.21-0.40	26	20-38
	PDA Diameter					0.28	0.15-1.47	21	12-112
Groves et al (2008)(95)	SVC Flow	30**	17-43**			85**	35-136**		
	DAo Flow	60**	34-86**			80**	33-127**		
Skinner et al (1996)(96)	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
Moorthy et al (1990)(97)	Cerebral Artery Pulsatility Index			17				20	

Table 2 Repeatability of Echocardiographic Parameters; RepC = Repeatability Coefficient; 95%CI = 95% Confidence Interval; RepI = Repeatability Index; RI_CA/ACA = Resistance Index in Coeliac Artery/Anterior Cerebral Artery; LA:Ao = Left Atrium:Aoric Root Ratio; LVPEP/LVET = Left-ventricular-preejection-period-to-ejection-time-ratio; VTI_Ao/VTI_Pa = ratio of the velocity time integrals in the large vessels; SVC = Superior Vena Cava; Dao = Descending Aorta; *indicates statistically significant result; **values stated in ml/kg/min.

2.5 Accessibility of Neonatologist-performed Echocardiography

In order to ensure optimal accuracy and repeatability of diagnosis whilst using echocardiography, users require thorough training and sophisticated technology (98). Echocardiograms are often performed by paediatric cardiologists who are attending the NICU, with many neonatologists lacking the expertise to perform these examinations themselves. Although in some large centres there is no shortage of paediatric cardiologists, this is not the case for many smaller centres and there have therefore been calls for more neonatologists to be trained in echocardiography (88, 90). Despite increasing prevalence of neonatologist-performed echocardiography, the number of neonatal physicians with adequate training is still limited. *Schachinger et al* conducted a survey in 2014 covering 95% of neonatal-perinatal training programs in the US. They found that just 9% of neonatal units had a functional echocardiography-trained neonatologist, whilst only 8% of centres offered echocardiography training as part of their neonatal-perinatal training pathways (99). In the UK, only 56% of neonatal training programs offer training in echocardiography (100). Additionally, 78% of neonatal consultants reportedly expressed an interest in achieving formal accreditation in neonatal echocardiography (100). These statistics highlight the lack of neonatologists adequately trained in the use of echocardiography in countries which are deemed to be at the forefront of medical care. In the wake of these studies, the need for structured echocardiography training for neonatologists has been highlighted in a number of expert consensuses from the UK, US and Europe (100-102).

Some potential barriers remain to the widespread use of echocardiography in neonatal units. Aside from the lack of established training programs highlighted by numerous expert consensuses, they suggested other pitfalls included the difficulty in recruiting experienced cardiologists to teach the technique to neonatologists and a lack of equipment (90). It has been

suggested that technological solutions, such as telemedicine, may help alleviate some of these barriers for those practicing in rural areas with limited access to expertly trained physicians or substandard equipment. A 2010 study performed between a tertiary care hospital in Lisbon and three rural hospitals in Portugal and the Azores reviewed the used of teleconsultations for the assessment of echocardiograms performed on foetuses, newborns and children (103). They concluded that it was not only diagnostically beneficial, but also aided the training of medical staff and provided socio-economic benefit to centres operating in remote conditions (103). The risk of misdiagnosis and unnecessary intervention has also been reported to affect the decision of neonatologists to train in echocardiography. This suggestion is somewhat validated by *Lee et al* finding a number of neonatologists performing echocardiography missed PDAs that paediatric cardiologists were able to detect. Furthermore, one study investigated the rates of treatment between disclosure and non-disclosure groups who received echocardiographic assessment of suspected PDA. Although the statistical differences between their two groups were deemed insignificant, it was suggested that avoiding routine echocardiography could reduce the use of drug therapy in very preterm neonates (104).

Overall, it is evident that there are inadequacies in the current training of neonatologists in echocardiography. Current expert consensuses have somewhat addressed the issue by providing guidance on prospective training programs, however, until widespread application occurs, these inadequacies are likely to continue. Furthermore, the economic impact of the introduction of a training program such as this cannot be ignored. Although the use of technologies such as telemedicine can help improve care in rural areas or low-income countries, this has the potential to add to the already significant workload of those trained in neonatal echocardiography elsewhere. We believe that improving echocardiography training for neonatologists would help improve accessibility to this valuable investigation.

2.6 Echocardiography as a non-continuous method of Investigating PDA

Despite its accuracy and non-invasiveness, one of the major drawbacks of echocardiography is the need for repeated measurements. Echocardiography shows a snapshot of the cardiovascular anatomy and haemodynamic status of the patient at a particular point in time. Therefore patients with persistent PDA are often subject to multiple echocardiograms. Although it has been suggested serial echocardiography can help improve diagnostic accuracy (83), this can be disruptive to the neonate. There have thus been calls for a continuous method of monitoring (84) which would not only be beneficial in identifying those suitable for diagnostic scanning, but would also aid in the monitoring of response to pharmacological or surgical management. Further research into non-invasive continuous methods of monitoring PDA is believed to be beneficial for a number of reasons. Firstly, many units employ routine echocardiography for preterm neonates, many of whom will not have pathological PDAs. This subjects newborns to unnecessary scanning which, as previously mentioned, can be distressing to the patient. The ability to identify a continuous monitoring system could potentially highlight those requiring further investigation with echocardiography, thus reducing the number of unnecessary investigations. This would not only be beneficial for patients, but could also reduce the workload of those trained in neonatal echocardiography. This could be beneficial for those working in smaller health care centres. As well as this, a continuous monitoring method could be utilised to monitor response to treatment, to identify those with PDAs refractory to medical management and who may benefit from surgical treatment.

2.7 Conclusion

Despite its reported accuracy in the measurement of PDA diameter and certain haemodynamic parameters, there are a number of inadequacies in echocardiography as a method of

investigating PDA. These include issues surrounding repeatability and the lack of structured training programs for neonatologists affecting accessibility to echocardiography on certain neonatal units. Furthermore, we believe the non-continuous nature of echocardiography is a major drawback in the measurement of haemodynamic and PDA status as they are highly variable states that change frequently and rapidly. Research into the development of a continuous, non-invasive method of monitoring PDA in neonates is therefore deemed to be appropriate.

Chapter 3: Clinical and Echocardiographic Markers of Haemodynamically Significant PDA in Neonatology: A Systematic Review

3.1 Introduction

Despite its prevalence in neonatology, there are currently no widely accepted criteria for the diagnosis of a haemodynamically significant PDA (hs-PDA) (82, 83). Although suggestions as to what defines a hs-PDA have been made in the literature (101), different centres will use different parameters and cut-offs resulting in greatly varying thresholds for treatment across the globe. This variation is just one of a number of drawbacks prevalent in PDA research, which also includes high contamination rates between treatments during clinical trials, and the lack of validated long-term outcome measures. These factors have limited the implementation of evidence based treatment strategies for the management of PDA (81).

The decision to initiate or withhold treatment is often based on the results of clinical trials and meta-analysis. Evidence-based medicine relies on the production of robust evidence that is free from significant bias. Core data sets refer to an agreed standardised collection of measurements and outcomes which should be reported on when conducting research on a clinical topic (105). These help facilitate meta-analysis by reducing heterogeneity, reducing the risk of bias, and ensuring clinically relevant outcomes are identified and reported on (106). It is important to ensure that meta-analyses of studies of PDA therapy are not based on different definitions of a hs-PDA. Development of consensus is required before any core data sets for PDA can be determined. Throughout this review, when discussing the formation of a "core data set", the authors refer to the process of standardising the clinical and/or echocardiographic biomarkers of haemodynamic significance used in PDA research.

The aim of this study was to conduct a systematic review of the randomised controlled trials (RCTs) of the medical management of PDA in neonates. The primary outcome was the clinical and echocardiographic parameters used for determining haemodynamic significance to assess

the extent of variation in clinical practice. This represents the first step towards development of a core data set for PDA disease.

3.2 Methods

A literature search of relevant studies was performed using the PubMed, Scopus and Web of Science databases. Boolean operators and MeSH terms were used to help eliminate irrelevant studies. Two investigators (BR and DH) independently performed the initial screening of titles and abstracts, before BR reviewed full text reports for appropriateness. All papers deemed suitable by either BR or DH were subject to full text review. Any uncertainty regarding the appropriateness of including a study was discussed between researchers BR and DH before a consensus was reached. A full search strategy is shown in Appendix 1. The terms "haemodynamically significant PDA" and "symptomatic PDA (s-PDA)" were deemed interchangeable, and so studies using either term were included for review.

Inclusion Criteria:

Publications evaluating medical management of PDA in neonates of all gestations were searched for using appropriate search terms (see Appendix 1). Only RCTs were included, as the authors were aware that there are a considerable number in this area. Only studies which specifically outlined their criteria for determining haemodynamic significance via clinical and/or echocardiographic means were included for review.

Exclusion Criteria:

Studies on animal models; surgical trials; non-RCT; no definition of haemodynamic significance.

Primary Outcomes:

Specific clinical or echocardiographic parameters used as part of a study's definition of hs-PDA.

Secondary Outcomes:

- To review literature regarding the repeatability of echocardiographic parameters to evaluate echocardiography's usefulness as a means of measuring markers of haemodynamic significance.

Data Extraction:

Data extraction was performed by researcher BR with the help of authors DH, AG, FB, GJ and PD for studies published in languages other than English. Trials were studied for their inclusion of criteria for defining a hs-PDA. Studies that specifically stated their criteria for determining haemodynamic significance were included for review. The clinical/echocardiographic parameters used as part of their definition were extracted, as well as interventions studied and the number of study participants.

Data Presentation:

Data was presented in tables and diagrams intended to highlight the variation and relationship between the clinical and echocardiographic parameters used to define hs-PDA.

Quality Assessment:

A critique of the methodological quality of the included studies was deemed unnecessary, as this research did not involve the synthesis of outcome data.

3.3 Results

A total of 40 studies were found to meet the inclusion and exclusion criteria. The review flowchart is shown in Figure 5, with the reasons individual studies were excluded summarised in Appendix 2. Table 3 contains details of the studies included for review. A total of 3,723 participants were included across the 40 studies.

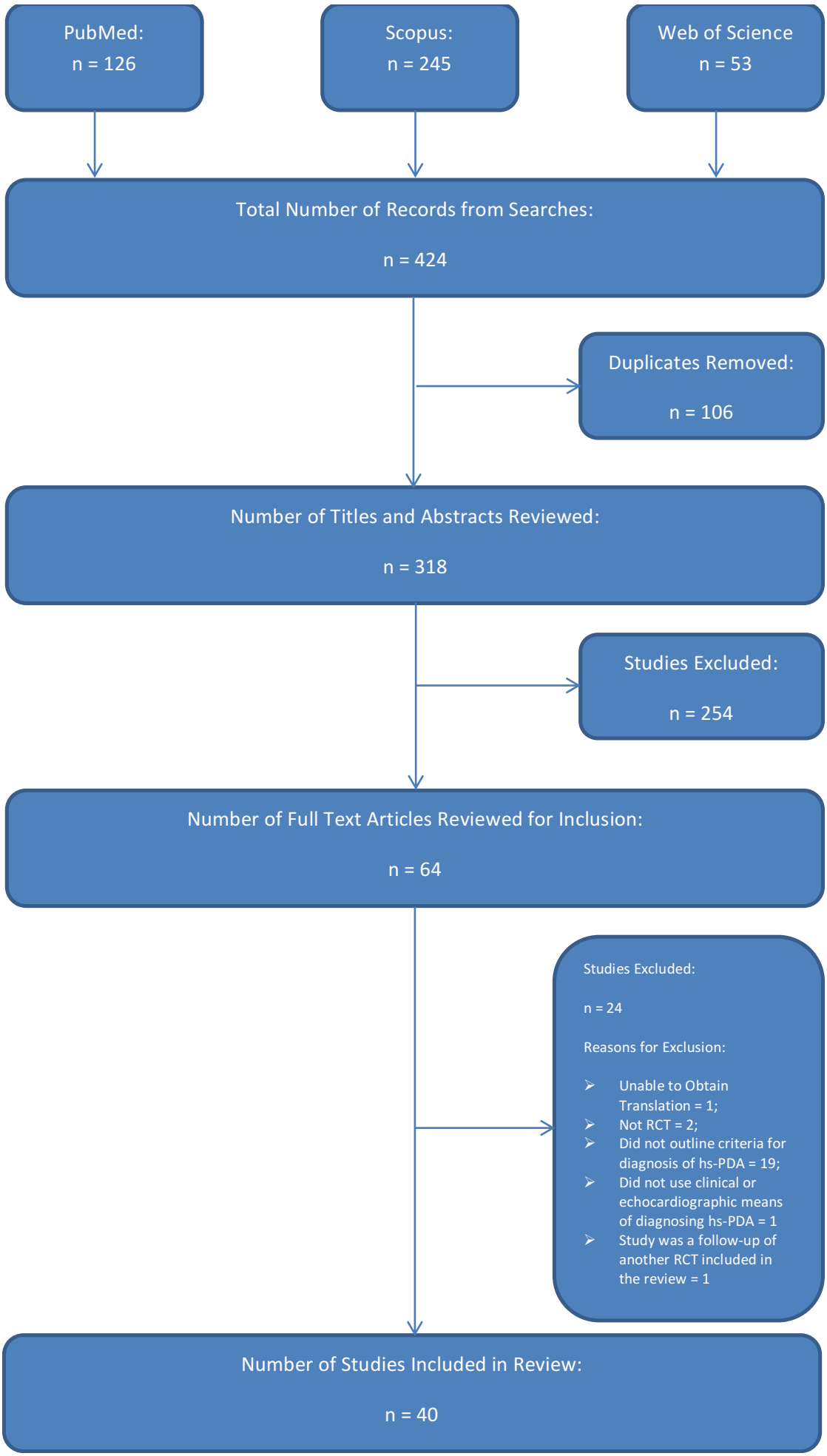


Figure 5 Review Flowchart

Author (Year):	n:	Interventions:	Clinical Markers of Haemodynamic Significance Used:	Echocardiographic Markers of Haemodynamic Significance Used:
El-Mashad et al (2017)(107)	300	IV PARA vs IV IBU vs IV INDO	N/A	• LA:Ao >1.6:1 • Pulmonary artery backflow • Duct Diameter >1.5mm • Reverse end-diastolic flow in the descending aorta/mesenteric artery
Lin et al (2017) (108)	144	IV INDO vs IV IBU	• Cardiovascular Dysfunction Score >3 (includes heart rate, quality of peripheral pulsation, degree of precordial pulsation, duration of murmur and cardiothoracic ratio)	• LA:Ao >1.3:1
Sadeghi-Moghaddam et al (2017) (109)	80	Oral IBU vs Oral INDO	N/A	• LA:Ao >1.4:1 • Duct Diameter >1.5mm
Demir et al (2017) (110)	72	Oral IBU vs Rectal IBU	• Tachycardia • Apnoea • Thrill • Active, heaving precodium • Bounding peripheral pulses • Wide pulse pressure • Murmur • Increasing oxygen dependence	• Duct Diameter >1.5mm • LA:Ao >1.5:1 • Evidence of left-to-right shunting • Evidence of reverse end-diastolic flow
Harkin et al (2016) (111)	48	IV PARA vs Placebo	• Increased need for respiratory support • Decreased systolic/ mean BP • Increased pulse pressure • Pulmonary congestion • Cardiomegaly • Hepatomegaly • Murmur	• LA:Ao >1.4:1 • PDA Diameter >50% wider than LPA • Flow pattern evidence of left-to-right shunting

			• Hyperdynamic precordium • Bounding pulses	
Dani et al (2016) (112)	110	IV PARA vs IV IBU	N/A	• Evidence of left-to-right shunting • LA:Ao >1.3:1 • Duct Diameter >1.5mm
Yang et al (2016) (113)	87	Oral PARA vs Oral IBU	• Systolic or consecutive murmur in the left border of sternum; • Strengthened beat of anterior thorax; • Locomotive pulse; • Tachycardia in quiet state; • Unexplainable deterioration of respiratory condition; • Increased pulmonary vasculature shadows and enlarged heart or signs of pulmonary oedema under CXR examination	• LA:Ao >1.4:1 • Pulmonary Artery Diastolic Backflow; • PDA Diameter >1.4mm
Dash et al (2015) (114)	77	Oral PARA vs IV INDO	N/A	• Duct Diameter >1.5mm • Evidence of left-to-right shunting • LA:Ao >1.5:1
Yadav et al (2014) (115)	83	Oral IBU vs Oral INDO	N/A	• Duct Diameter >1.5mm • Systolic and diastolic pressure gradients • LA:Ao >1.4:1 • Evidence of PDA flow (hypertensive, growing or pulsatile pattern)
Oncel et al (2014) (116)	90	Oral PARA vs Oral IBU	• Clinical signs of PDA not outlined.	• Duct Diameter >1.5mm • LA:Ao >1.5:1mm • End diastolic reversal of blood flow in the aorta • “Poor cardiac function in

				additional to clinical signs of PDA"
Kluckow et al (2014) (117)	92	Early IV INDO vs Placebo	N/A	• Duct Diameter >1.5mm
Lago et al (2014) (118)	112	Bolus IV IBU vs Continuous IV IBU	N/A	• Duct Diameter >1.4mm/kg • Unrestrictive pulsatile transductal flow (PDA maximum velocity <2.0m/s) • LA:Ao >1.4:1 • Mean and end diastolic flow velocity in the LPA >0.42 and >0.20 m/s respectively • LVO/SVC flow ratio >4
Bagnoli et al (2013) (119)	134	IV IBU vs Placebo	N/A	• LA:Ao >1.4:1 • LV:Ao >2.1:1 • Duct Diameter >1.5mm • Evidence of left-to-right shunting • Diastolic reversal of flow in the aorta
Sosenko et al (2012) (120)	105	Early IV IBU vs Placebo	• Signs of PDA (not specified) plus evidence of pulmonary haemorrhage (bloody secretions from ETT) • Signs of PDA (not specified) plus cardiomegaly and pulmonary oedema on CXR plus hypotension requiring inotropes or respiratory failure (defined as at least two of: FIO_2 >0.5, intermittent mandatory ventilation >40 breaths/min, peak inspiratory pressure >20cm H_2O, high frequency ventilation	N/A

			with Paw >13 and FIO_2 >0.5 [for >8h])	
Erdeve et al (2012) (121)	80	Oral IBU vs IV IBU	• Clinical signs of PDA not outlined.	• Duct Diameter >1.5mm • LA:Ao >1.5:1 • Left-to-right Shunting • End-diastolic reversal of aortic blood flow • "Poor cardiac function in addition to the signs of PDA"
Dani et al (2012) (122)	70	High-dose IV IBU vs Standard-dose IV IBU	N/A	• Evidence of left-to-right shunting • LA:Ao >1.3:1 • Duct Diameter >1.5mm
Cheng et al (2012) (123)	30	IV INDO vs Oral IBU	N/A	• LA:Ao >1.4:1 • Duct Diameter >1.5mm
Gokmen et al (2011) (124)	102	IV IBU vs Oral IBU	• Clinical signs of PDA not outlined.	• Duct Diameter >1.5mm • Evidence of left-to-right shunting • LA:Ao >1.5:1 • End-Diastolic reversal of aortic blood flow • "Poor cardiac function in addition to signs of PDA"
Aranda et al (2009) (125)	136	IV IBU vs Placebo	N/A	• LA:Ao >1.4:1 • LV:Ao >2.1:1 • Duct Diameter >1.5mm
Sangtawesin et al (2008) (126)	62	Oral IBU vs Placebo	• Bounding pulses • Pulse pressure >35mmHg • Hyperactive precordium • Tachycardia (HR >170bpm) • Hepatomegaly • Cardiomegaly • Increased pulmonary vasculature on CXR	• Left-to-right/bidirectional shunting of blood • LA:Ao >1.4:1 • Duct Diameter >1.5mm

Cherif et al (2008) (127)	64	Oral IBU vs IV IBU	N/A	• Disturbed diastolic flow in PA/PT • Diastolic backflow in aorta • Diastolic backflow in medium cerebral artery/superior mesenteric artery/renal arteries • LA:Ao >1.6:1
Su et al (2008) (128)	119	IV IBU vs IV INDO	N/A	• Evidence of left-to-right shunting (pulsatile/growing pattern only)
Salama et al (2008) (129)	41	IV INDO vs Oral IBU	N/A	• Duct Diameter >1.5mm
Jegatheesan et al (2008)(130)	105	Low dose INDO vs High dose INDO	N/A	• Evidence of Left-to-Right flow
Fakhraee et al (2007) (131)	36	Oral INDO vs Oral IBU	N/A	• Evidence of left-to-right shunting • Duct Diameter >1.5mm • LA:Ao >1.6:1 • Severe diastolic backflow in PT/Aorta
Sangtawesin et al (2006) (132)	42	Oral IBU vs Placebo	N/A	• LA:Ao >1.4:1 • Duct Diameter >1.5mm
Gimeno Navarro et al (2005) (133)	47	IV INDO vs IV IBU	N/A	• Presence of a dominant aortopulmonary shunt • Pulmonary Trunk/DAP ratio >0.3 • Inversion of the Diastolic Flow in the Abdominal Aorta
Gournay et al (2004) (134)	131	IV IBU vs Placebo	N/A	• LA:Ao >1.48:1 • Retrograde/Absent diastolic flow in the cerebral anterior artery/descending thoracic aorta • Pulsatile flow in the DA • Diastolic flow velocity in PA >20m/s
Su et al (2003) (135)	63	IV IBU vs IV INDO	N/A	• Evidence of left-to-right shunting • LA:Ao >1.3:1

				• Duct Diameter >1.5mm
Lee et al (2003) (136)	140	Conventional-dose IV INDO vs Prolonged low-dose IV INDO	• Systolic Murmur • Hyperactive precordium • Wide Pulse pressure (diastolic pressure less than half systolic) • Hypotension • Apnoea • Rising CO_2	• Duct Diameter >1.5mm
Lago et al (2002) (137)	175	IV INDO vs IV IBU	N/A	• Disturbed diastolic flow in main PA with diastolic backflow in the aorta immediately below the DA and a forward flow above the ductal insertion
Supapannachart et al (2002)(138)	18	Oral IBU vs Oral/IV INDO	• Systolic murmur at left upper parasternal border; • Continuous murmur at left upper parasternal border; • Active precordium; • Bounding pulse; • Wide Pulse Pressure (>35mmHg); • Tachycardia (>170bpm); • Hepatomegaly; • CXR showing cardiomegaly (CT ratio >0.6) or increased pulmonary vasculature	N/A
Dani et al (2000) (139)	80	Prophylactic IV IBU vs Post-Echocardiography IV IBU	N/A	• LA:Ao >1.3:1 • Evidence of left-to-right shunting • Duct Diameter >1.5mm
Tammela et al (1999) (140)	61	Short-course IV INDO vs Long-course IV INDO	• Systolic or continuous murmur at LSE • Increased precordial impulse	• Continuous left-to-right shunting

			• Bounding peripheral pulses • Resting tachycardia • Unexplained deterioration of respiratory status • Increased pulmonary markings/cardiomegaly/pulmonary oedema on CXR	
Lai et al (1990)(141)	32	Oral INDO vs Placebo	• Systolic or continuous cardiac murmur; • Hyperactive precordium; • Bounding pulses; • Tachycardia; • Tachypnoea; • Hepatomegaly; • Cardiomegaly with pulmonary plethora on CXR	• Used echocardiography, but did not outline their specific criteria.
Vogtmann et al (1988)(142)	41	Oral INDO vs Non-Treatment	• Murmur; • Active Precordium; • Bounding Pulses; • Hypotension; • Tachycardia; • Evidence of Respiratory Decompensation;	• Systolic Time Intervals (PEP/LVET <0.3)
Gersony et al (1983) (143)	421	UMT + IV INDO vs UMT + IV INDO as Rescue Therapy vs UMT + Rescue Surgery	• Continuous murmur • Need for ventilator support at at least 48h • Hyperactive precordium • Increased pulse pressure • Bounding pulses • Tachycardia (HR >170bpm) • Tachypnoea (RR >70) • Hepatomegaly	• LA:Ao >1.15:1

Rudd et al (1983)(144)	30	Oral INDO vs Placebo	• Full pulses in the absence of anaemia; • Active cardiac impulse; • Loud Pulmonary component of 2nd Heart Sound; • Cardiomegaly on CXR; • Pleonaemia	• LA:Ao >1.2:1
Monset-Couchard et al (1983) (145)	24	IV INDO vs Non-Treatment	• Precordial Shock; • Increased Peripheral Pulsatility; • Systolic Murmur; • Evaluation of Heart Rate; • Assessment of hepatic volume; • Assessment of pulmonary vascularisation; • Cardiac Volume; • Electrocardiogram (evidence of pressure overload);	• LA:Ao (significant value not stated)
Yanagi et al (1981)(146)	39	Oral INDO vs Placebo	• Continuous Ventilator Support	• LA:Ao >1.3:1

Table 3 Summary of Trials included for Review: IV = intravenous; PARA = Paracetamol; IBU = Ibuprofen; INDO = Indomethacin; LA:Ao = Left Atrium:Aortic Root Ratio; LPA = Left Pulmonary Artery; BP = Blood Pressure; PDA = Patent Ductus Arteriosus; LVO/SVC = Left Ventricular Output/Superior Vena Cava; LV:Ao = Left Ventricle:Aortic Root Ratio; ETT = Endotracheal Tube; CXR = Chest X-ray; FiO_2 = Fraction of Inspired Oxygen; HR = Heart Rate; PA = Pulmonary Artery; PT = Pulmonary Trunk; DAP = Diameter of Pulmonary Trunk; DA = Ductus Arteriosus; CT ratio = cardiothoracic ratio; LSE = Left Sternal Edge; PEP = pre-ejection period; LVET = Left Ventricular Ejection Time; UMT = Usual Medical Therapy (in the case of *Gersony et al(143),* UMT = fluid restriction, diuretics and digoxin); RR = Respiratory Rate

Clinical Markers of Haemodynamic Significance:

Of the 40 trials reviewed, eighteen (45.0%) used clinical signs as part of their definition for hs-PDA (number of participants = 1596 [42.9%]). Sixteen trials used a combination of clinical and echocardiographic markers to define haemodynamic significance (number of participants = 1473 [39.6%]) and two used clinical features alone (number of participants = 123 [3.3%]). The three most common clinical markers were hyperdynamic precordium, bounding peripheral pulses and heart murmur.

Four of the trials that used clinical markers as part of their definition did not provide specific details of the clinical signs used for diagnosis of hs-PDA. Three of the four studies (116, 121, 124) used unspecified clinical features of PDA in conjunction with "poor cardiac function" as part of their definition for haemodynamic significance. Another (120) used unspecified clinical signs of PDA along with a number of clinical findings indicative of haemodynamic compromise and pulmonary haemorrhage to define hs-PDA.

The most commonly used clinical markers of haemodynamic significance used by the remaining 14 studies, plus the signs of haemodynamic compromise used by (120), are summarised in Table 4 (total number of participants across 15 trials specifically outlining clinical features of haemodynamic significance = 1324). One study (108) used a previously described cardiovascular dysfunction score as clinical markers of haemodynamic significance. This scoring system includes evaluation of heart rate, quality of peripheral pulsation, degree of precordial pulsation, duration of murmur and cardiothoracic ratio (147). These values will thus be considered individually as part of Table 4.

Echocardiographic Markers of Haemodynamic Significance:

Of the 40 trials reviewed, 38 (95.0%) used echocardiographic markers as part of their definition for hs-PDA (number of participants = 3600 [96.7%]). Sixteen trials used a combination of

echocardiographic and clinical biomarkers to define haemodynamic significance (number of participants = 1473 [39.2%]), whereas 22 used echocardiographic markers alone (number of participants = 2127 [57.5%]). The echocardiographic parameters used in the assessment of haemodynamic significance can be seen in Table 4. The echocardiographic parameters of "poor cardiac function" (116, 121, 124) or "evidence of reverse diastolic flow" (110) are not included due to lack of specificity. *Lai TH et al* (141) used echocardiography to confirm the presence of symptomatic PDA but did not specify the individual parameters and hence is not included in the echocardiographic markers section of Table 4 (number of studies included = 37; number of participants = 3568).

Left Atrium:Aortic Root Ratio as an Echocardiographic Marker of Haemodynamic Significance:

LA:Ao was the single most commonly used marker of haemodynamic significance (Table 3), with studies including 2723 neonates utilising it. There was wide variation in the threshold used to define a hs-PDA (>1.15:1 - >1.6:1). Table 5 outlines the frequency of each LA:Ao threshold value used, and the proportion of neonates from the entire study population this would have captured.

Relationship between Echocardiographic Markers of Haemodynamic Significance:

LA:Ao, duct diameter, and evidence of shunting were the three most commonly used echocardiographic parameters. At least one of the three was used in 35 out of 37 [94.6%; number of participants = 3352 (93.9%)] studies which used echocardiography for the definition of hs-PDA (Figure 6). Despite almost 95% of all patients having one of these parameters used in the determination of hs-PDA, only 1129/3352 (33.7%) had all of them measured simultaneously.

Repeatability of Echocardiographic Markers of Haemodynamic Significance:

An overview of the literature reviewing repeatability of echocardiographic parameters is presented in Table 2.

Clinical Marker:	Number of Studies (%):	Number of Participants (%)	Echocardiographic Marker:	Number of Studies (%):	Number of Participants (%):
Hyperdynamic Precordium	11 (73.3%)	1115 (84.2%)	LA:Ao	28 (75.7%)	2747 (77.0%)
Bounding Peripheral Pulses	10 (66.7%)	975 (73.6%)	Duct Diameter	24 (64.9%)	2167 (60.7%)
Murmur	9 (60.0%)	1023 (77.2%)	Evidence of Left-to-Right/Bidirectional Shunting**	19 (51.4%)	1592 (44.6%)
Cardiomegaly	9 (60.0%)	587 (44.3%)	Reverse End-Diastolic Flow in the Aorta	10 (27.0%)	1159 (32.5%)
Deterioration of Respiratory Status*	8 (53.3%)	973 (73.5%)	Disturbed PA/PT Flow ***	7 (18.9%)	905 (25.4%)
Tachycardia	8 (53.3%)	897 (67.7%)	Reverse End-Diastolic Flow in the Mesenteric Artery	2 (5.4%)	364 (10.2%)
Wide Pulse Pressure	6 (40.0%)	761 (57.5%)	LV:Ao	2 (5.4%)	270 (7.6%)
Increased Pulmonary Vasculature on Chest Radiograph	6 (40.0%)	290 (21.9%)	Diastolic Backflow in Cerebral Arteries	2 (5.4%)	195 (5.5%)
Hepatomegaly	5 (33.3%)	581 (43.9%)	LVO/SVC Flow Ratio	1 (2.7%)	112 (3.1%)
Pulmonary Oedema/Congestion	4 (26.7%)	301 (22.7%)	Systolic and Diastolic Pressure Gradients	1 (2.7%)	83 (2.3%)
Tachypnoea	2 (13.3%)	453 (34.2%)	Diastolic Backflow in Renal Arteries	1 (2.7%)	64 (1.8%)
Hypotension	2 (13.3%)	245 (18.5%)	Pulmonary Trunk/DAP Ratio	1 (2.7%)	47 (1.3%)
Apnoea	2 (13.3%)	212 (16.0%)	PEP/LVET	1 (2.7%)	41 (1.1%)
Evidence of Pulmonary Haemorrhage	1 (6.7%)	105 (7.9%)			
Thrill	1 (6.7%)	72 (5.4%)			
Decreased Systolic/Mean Blood Pressure	1 (6.7%)	48 (3.6%)			
Loud Pulmonary Component of 2nd Heart Sound	1 (6.7%)	30 (2.3%)			

Table 4 Summary of clinical and echocardiographic markers of haemodynamic significance. *Deterioration of Respiratory Status includes rising CO2, increased ventilator/oxygen dependence and respiratory failure. LA:Ao = Left Atrium:Aortic Root Ratio; PA = Pulmonary artery; PT = Pulmonary Trunk; LV:Ao = Left Ventricle:Aortic Root Ratio; LVO/SVC = Left Ventricular Output/Superior Vena Cava; DAP = Diameter of Pulmonary Trunk; PEP/LVET = pre-ejection period/left ventricular ejection time. **includes flow patterns (e.g. hypertensive, pulsatile, growing. ***includes PA backflow, mean/end diastolic flow velocity in LPA and disturbed diastolic backflow in PA

LA:Ao Value:	Frequency:	Studies:	Number deemed to have hs-PDA (%):
>1.6:1	3	(107, 127, 131)	400/2723 (14.7%)
>1.5:1	5	(110, 114, 116, 121, 124)	821/2723 (30.2%)
>1.48:1	1	(148)	952/2723 (35.0%)
>1.4:1	10	(109, 111, 113, 115, 118, 119, 123, 125, 126, 132)	1766/2723 (64.9%)
>1.3:1	6	(108, 112, 122, 135, 139, 146)	2272/2723 (83.4%)
>1.2:1	1	(144)	2302/2723 (84.5%)
>1.15:1	1	(143)	2723/2723 (100%)

Table 5 LA:Ao as an echocardigraphic marker; one study (145) used LA:Ao as a marker of hs-PDA without defining a threshold and therefore is excluded from table 4.

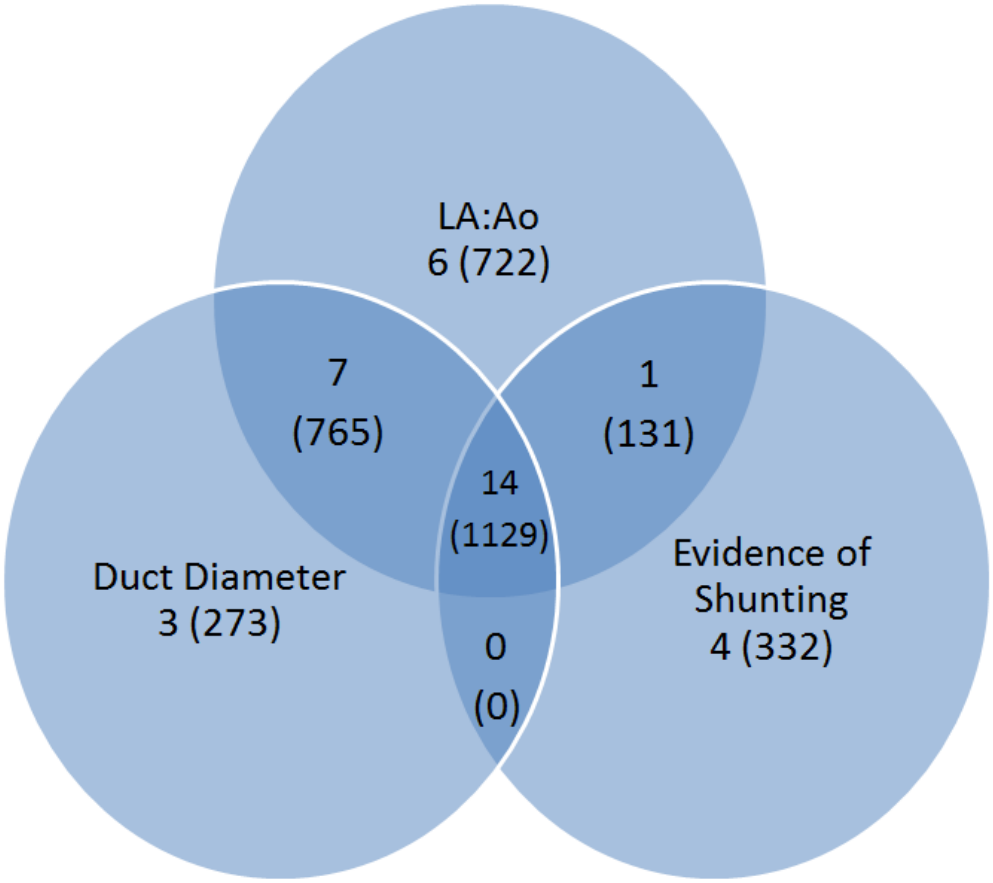


Figure 6 The relationship between the three most common echocardiographic markers of haemodynamic significance; evidence of shunting incorporates evidence of left to right shunting, flow/growing/pulsatile/hypertensive patterns and presence of a dominant aortopulmonary shunt.

3.4 Discussion

Ideally the decision to intervene in a neonate with a PDA should be based on the echocardiographic documentation of a significant left-to-right transductal shunt, with measurable haemodynamic effects, leading to clinical instability (81). This approach ensures those with haemodynamic compromise are treated, without exposing those in whom intervention may be unnecessary to the potentially harmful side effects of treatment. However, it is clear from this systematic review that RCTs tend to use inclusion criteria based on echocardiographic biomarkers to define hs-PDA. Less than half of studies used any clinical markers as part of their definition for a haemodynamically significant PDA, and only 5% used clinical markers alone. The use of clinical markers was particularly infrequent in studies published from 2003-2015 (73.9% [17/23] used echocardiography alone). However, more recent studies (2016-date) have re-incorporated clinical markers (42.9% [3/7] used echocardiography alone).

There are advantages to using echocardiography, including the non-invasive nature of the investigation as well as its reported accuracy in diagnosing PDA compared to clinical signs (87, 89). However, issues surrounding repeatability remain a potential shortcoming for echocardiography. Our review can only identify assessment of inter-observer variability for two of the most commonly-used echocardiographic parameters (LA:Ao and duct diameter –Table 2). Moreover, the use of LA:Ao as an inclusion criterion in RCTs and its ideal threshold depends on the timing of the echocardiography as PDA shunting causes several neuro-hormonal adaptations which result in progressively increased preload and stretching of the LA during postnatal maturation (37, 38). It is thought the development of a standardised and structured neonatal echocardiography training curriculum might help reduce inter- and intra-observer variation in echocardiographic measurements.

The range of criteria used and cut off points in PDA RCTs has previously been noted in 2011 (149). The current review contains additional details about the specific criteria used, the

combinations chosen, and repeatability of echocardiographic makers. In addition, since the 2011 review an additional 18 RCTs have been published containing 1816 babies, which are included in our systematic review. We believe there is an ethical requirement to determine the most clinically-useful markers of hsPDA to ensure neonates in these studies are optimally managed.

While there is no consensus for either the definition of hs-PDA or the strength of the association between hs-PDA and the associated adverse outcomes (IVH, NEC, BPD) (150, 151), improvements could be made through the formation of a core data set for PDA research. The formation of a core data set would reduce the wide variation of parameters reported in literature. Ensuring research reports on the same parameters would have significant benefits for future meta-analyses, allowing more rigorous comparisons between markers of haemodynamic significance. If agreement on how to utilise the current selection of clinical and echocardiographic criteria cannot be reached, it may be that novel biomarkers need to be developed and validated.

A limitation of this review is that several studies conducted by the same authors used the same or similar definitions of haemodynamic significance. Individual parameters may therefore be over-represented in our review due to the volume of published material from individual groups. Furthermore, when comparing the number of participants who were subject to measurement of each parameter, the presence of large studies may have skewed our results.

4.4 Conclusion

There is wide variation in clinical and echocardiographic parameters used to define haemodynamic significance in published literature. Development of a core data set for PDA would help improve the quality of future PDA research.

Chapter 4: Factors affecting the measurement of Pulse Wave Velocity and Phase Difference in the first three days of Neonatal Life

4.1 Introduction

The early postnatal period is a particularly challenging time for any newborn. In both term and preterm neonates, a number of changes occur to help them adapt to life outside the womb. These include cardiorespiratory adaptations as well as alterations to energy metabolism and temperature control. Those born prematurely may struggle to adapt to life outside of the womb due to underdevelopment of organ systems, traumatic birth conditions or illnesses associated with prematurity such as PDA, sepsis and RDS. Furthermore, neonates born prematurely are more likely to be treated with medications such as inotropes or vasopressors in early life to help them survive. It is therefore believed that there are a number of variables which should be assessed when investigating a potential haemodynamic biomarker to be used in the early neonatal period.

PWV has long been validated as a useful method of measuring haemodynamic function in adults, however, investigations into its use in paediatric populations is limited and research into its use in neonatal populations is even more uncommon. There is also limited literature reviewing the expression of PWV in terms of phase difference. As a result, in order for PWV or phase difference to be considered as potential novel biomarkers, it is crucial that descriptive data is collected to help identify what can be considered as a "normal" value, as well as any potential variables which may affect its measurement.

Since PWV and phase difference are measurements of haemodynamic function, we believe it is important to assess the impact of factors which may affect vascular tone or cardiac output on their measurement. Furthermore, due to the rapid development of the neonate in early life, it is also important to consider factors such as age (days), postnatal age (hours), birth weight (BW),

GA and multiple pregnancies on their measurement. It has already been shown that there are significant differences in the measurements of PWV in different paediatric age groups ranging from 6 to 18 years (152), so we hypothesise that their measurement in neonates may also be affected by age. Obtaining baseline information on values measured in this population and the variables causing alterations in their measurement is a key first step in their validation as clinical biomarkers to be used in neonatology. As outlined by *Kearns et al,* it is crucial to assess the impact of the growth and development of the child before a biomarker can be considered for implementation in clinical practice (58). The aim of this chapter is therefore to improve our understanding of the demographic and post-natal factors affecting the measurement of PWV and phase difference in the first three days of life, in the hope of beginning a process of validation for these biomarkers.

4.2 Methods

4.2.1 Study Design

A prospective observational study was conducted at Liverpool Women's Hospital. All neonates who had a UAC fitted within the first three days of life were eligible for recruitment in the study. A demographic profile (consisting of routinely collected data only) was collected from existing clinical records. This included maternal data as well as neonatal data relating to age (days), postnatal age (hours), GA, BW and whether the participant was a twin or higher order multiple pregnancy. Data was also collected on vital signs and arterial waveforms (allowing calculation of PWV and phase difference), dose and rate of infusion of inotropes, and additional comorbidities/medications that were deemed to affect the patient's haemodynamics (sepsis, IVH and NSAID, caffeine or surfactant administration).

All clinical data collected as part of this study was in line with standard care at the Liverpool Women's Hospital. No investigations/procedures were performed in addition to the standard care patients receive whilst placed in the NICU.

4.2.2 Inclusion and Exclusion Criteria

Inclusion Criteria:

- Neonate admitted to Liverpool Women's Hospital Neonatal Unit;
- Age 1-3 days;
- UAC placed in situ for clinical care within 3 days of birth;
- Transducer capable of being fitted to UAC without additional intervention/disruption to the patient.

Exclusion criteria:

- Age >3 days;
- UAC not sited or not capable of being fitted to transducer;
- Survival of baby expected to be <24 hours;
- Additional congenital heart disease or conditions expected to alter cardiovascular function (ventricular septal defect, atrioventricular septal defect, tetralogy of Fallot, transposition of great vessels, truncus arteriosus, coarctation of the aorta, congenital valvular disease, and congenital hydrops).
- Chromosomal abnormalities.
- Considered unsuitable for recruitment by nursing staff and Consultant Neonatologist at Liverpool Women's Hospital.

4.2.3 Study Outcomes

The primary outcome of this study was to collect descriptive data about PWV and phase difference in the neonatal period.

The secondary outcome of this study was to examine relationships between PWV and phase difference in relation to demographic data (age, postnatal age, GA, BW, multiple pregnancies) and other post-natal events deemed likely to have an impact on neonatal haemodynamics (sepsis, intraventricular haemorrhage and inotrope, NSAID, caffeine or surfactant administration).

4.2.4 Study Procedures

Ethical Approval:

Proportionate review was sought for this research project due to the non-interventional and pseudo-anonymised nature of data collection. HRA approval and a favourable opinion from the North-East Tyne and Wear Research Ethics Committee was obtained before initiation of the study (REC Reference: 18/NE/0058).

Recruitment:

All patients admitted to the unit who had a UAC in situ within the first three days of life were included in this study provided no exclusion criteria were met. Clinical data that is collected in accordance to the standard care protocol was anonymised and analysed in accordance with the aims of the study. Based on the typical patient population that is observed at the Liverpool Women's Hospital, we believed it was reasonable to recruit approximately 30 patients in this study. Posters were placed in the neonatal unit to make staff aware of eligible participants in the hope this would improve participant recruitment. Parents were able to opt out of the study.

Consent:

All clinical data used as part of this prospective observational study is data that was already collected as part of each patient's routine clinical care at the Liverpool Women's Hospital. Additional information on PWV and phase difference was synthesised without further intervention to the patient. These values were calculated based on the routine clinical data collected. Each participant was assigned a study number and no identifiable information was linked to the data that is collected. Based on the fact this study was performed using pseudo-anonymised data that requires no alteration to patient care, parental consent was not sought for this study.

If parents who became aware of the study (e.g. seeing equipment being added to the patient monitors or seeing study posters erected on the ward) wanted more information on the details of the study, an information leaflet would be made available on request. The leaflet, along with the aforementioned ward posters and verbal explanations provided by clinical staff and the research team, would give parents the ability to opt out of the study.

Data Collection:

Demographic data on maternal and neonatal characteristics, as well as data on management whilst in the NICU was collected from patient records via the Liverpool Women's Hospital MediTech and Badger systems.

PWV data collection would occur on days 1-3 of life where an infant has a UAC in situ as part of the routine pathway of clinical care. Days of life were calculated according to the ward policy on the beginning of day 1 of life. Neonates are considered day 1 of life for the first 24 hours, day 2 of life from 24-48 hours etc. If a baby had a UAC fitted on day 2 (or 3), a reduced number of

day's data was collected. Also, if a UAC was removed or stopped functioning prior to day 3, a reduced number of day's data was collected.

The data was collected from the routine monitoring undertaken on the baby and will be stored on the Liverpool Women's Trust system. Access to raw data on this system was limited to the named study team. The collection and storage of this information was password protected to ensure security of identifiable information. Prior to statistical analysis, all data was anonymised and transferred to a University of Liverpool computer based shared drive to allow all members of the research team to have access to the relevant information without compromising patient confidentiality.

Invasive Blood Pressure Monitoring:

Continuous invasive BP was monitored via a UAC (3.5 Fr) that was inserted soon after birth. The catheters are typically positioned between the sixth and ninth thoracic vertebra. The catheter is connected to the electronic transducer via a 38 cm extension made from rigid plastic.

Catheter position is confirmed with radiography. 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 (153). There are no data for preterm babies and for this reason we reviewed CT scans from term infants with cardiac conditions and found that the relation of the aortic valve to the vertebral level varied greatly, but the average was found to be around the 6th thoracic vertebra. The distance of the catheter tip from the aortic valve was estimated using PACS software. The location of the aortic valve was assumed to be at the level of T6. Anatomical landmarks such as the carina were used where possible to account for the arch of the aorta.

All possible steps were taken to ensure the collection of quality data. The UACs and the data being displayed on the monitor were assessed by a member of the research team prior to data collection. Ultimately, the decision to collect data was at the discretion of the member of the clinical care/research team who was collecting it. The assessment of the data was qualitative and included assessment of the following:

- Pulse pressure: normal pulse pressure for preterm infants is approximately 15-25mmHg (154). A narrow pulse pressure may be seen if the UAC is blocked;
- Arterial waveform: the arterial trace was assessed for the presence of a normal waveform and the absence of dampening (the absorption of energy within the oscillating system resulting in artificial reduction in the amplitude of the arterial waveform) (155). Although the presence of a dichrotic notch is deemed normal, the absence of a clearly defined single peak in such waveforms eliminates the possibility for PWV/phase difference analysis. Arterial waveforms were also therefore assessed for the presence of a dichrotic notch;
- Appearance of UAC: visual assessment of the UAC (e.g. checking for air bubbles in the tubing between the UAC and the blood pressure transducer);
- Transducer location: ensuring the blood pressure transducer is situated at the level of the patient's heart.

The data collection software allowed review of the waveform data that had been collected. This acted as an aid in the identification of "dampened" signals which occur when the UAC becomes blocked. This was used to ensure the collection of quality data. Based on the data collected we can therefore classify the quality and usefulness of the arterial waveforms as "adequate", "suboptimal" (inadequate for analysis but adequate for patient monitoring, e.g. presence of a significant dichrotic notch), or "inadequate" (e.g. significantly dampened signal).

Pulse Wave Velocity and Phase Difference:

The PWV and phase difference were calculated by combining the ECG and peak systolic blood pressure waveform collected by the IxTrend software® (Ixellence GmbH, Wildau, Germany). The time difference between the R wave of the ECG waveform and the peak of the systolic blood pressure waveform was calculated using the MATLAB software and expressed as PWV and phase, i.e. the proportion of one cardiac cycle (Figure 7). This data was collected by connecting the patient's monitor (which is already receiving information via the transducer attached to the patient's UAC) to a computer with the appropriate software installed. This was achieved by the insertion of a Bluetooth device into the back of the patient's monitor and the insertion of a paired dongle into the researcher's computer. Data was therefore collected without any additional disruption to the infant. We aimed to collect data for a minimum of 30 minutes a day for the first three days of life for those included in the study. This provided baseline information on the variation of PWV/phase difference in early life. This is the data that would be compared to the variables stated above to better understand the characteristics of these biomarkers in the neonate.

4.3 Results

A total of 32 UACs were inserted during the data collection period with 14 patients being recruited for inclusion in the study. A total of 3 patients were subsequently excluded due to data being deemed unsuitable for analysis. A total of 16 days' worth of data collection across 11 participants was therefore used for analysis. The reasons for exclusion for the other 18 patients are outlined in Figure 8.

4.3.1 Analysis of Arterial Waveform Morphology

A total of seven days' worth of data was deemed unsuitable for PWV/phase difference calculation due to suboptimal arterial blood pressure waveforms. In order for the appropriate

analysis to be carried out, a single clear peak of the arterial waveform must be identified. In the excluded cases, the arterial waveform was considered either too “damp”, or the presence of a significant dichrotic notch meant that analysis could not be conducted. This was because a single clear peak could not be identified. Examples of both are illustrated in Figure 9.

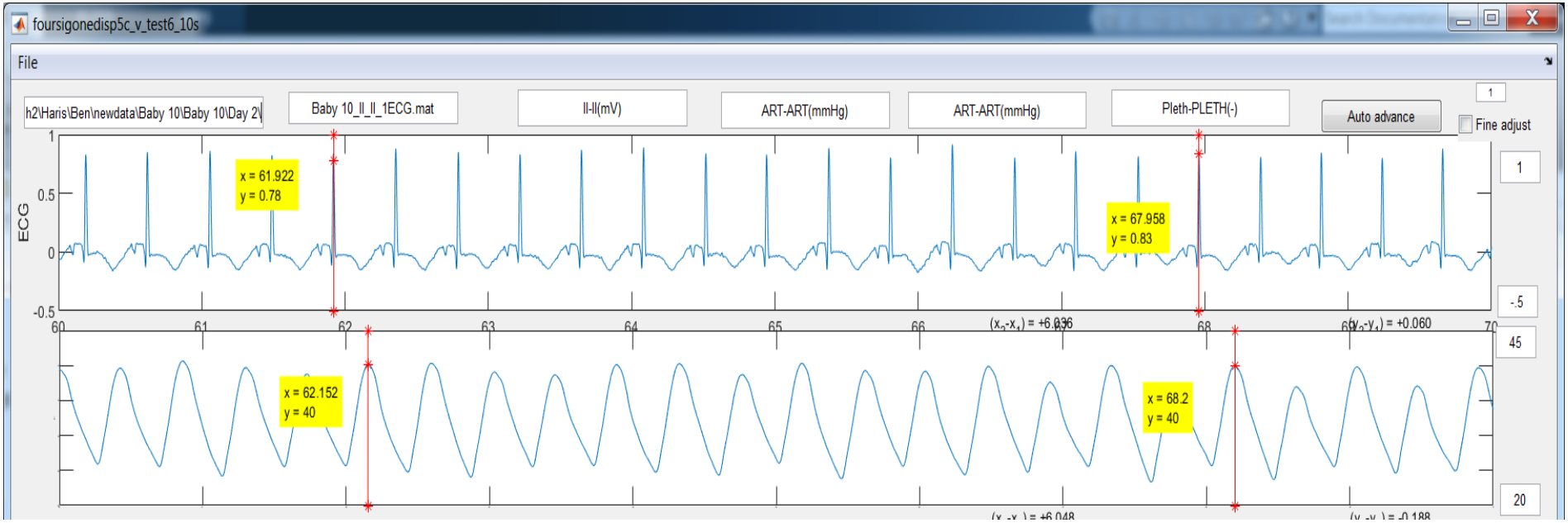


Figure 7 ECG and Arterial Waveform Analysis for PWV calculation

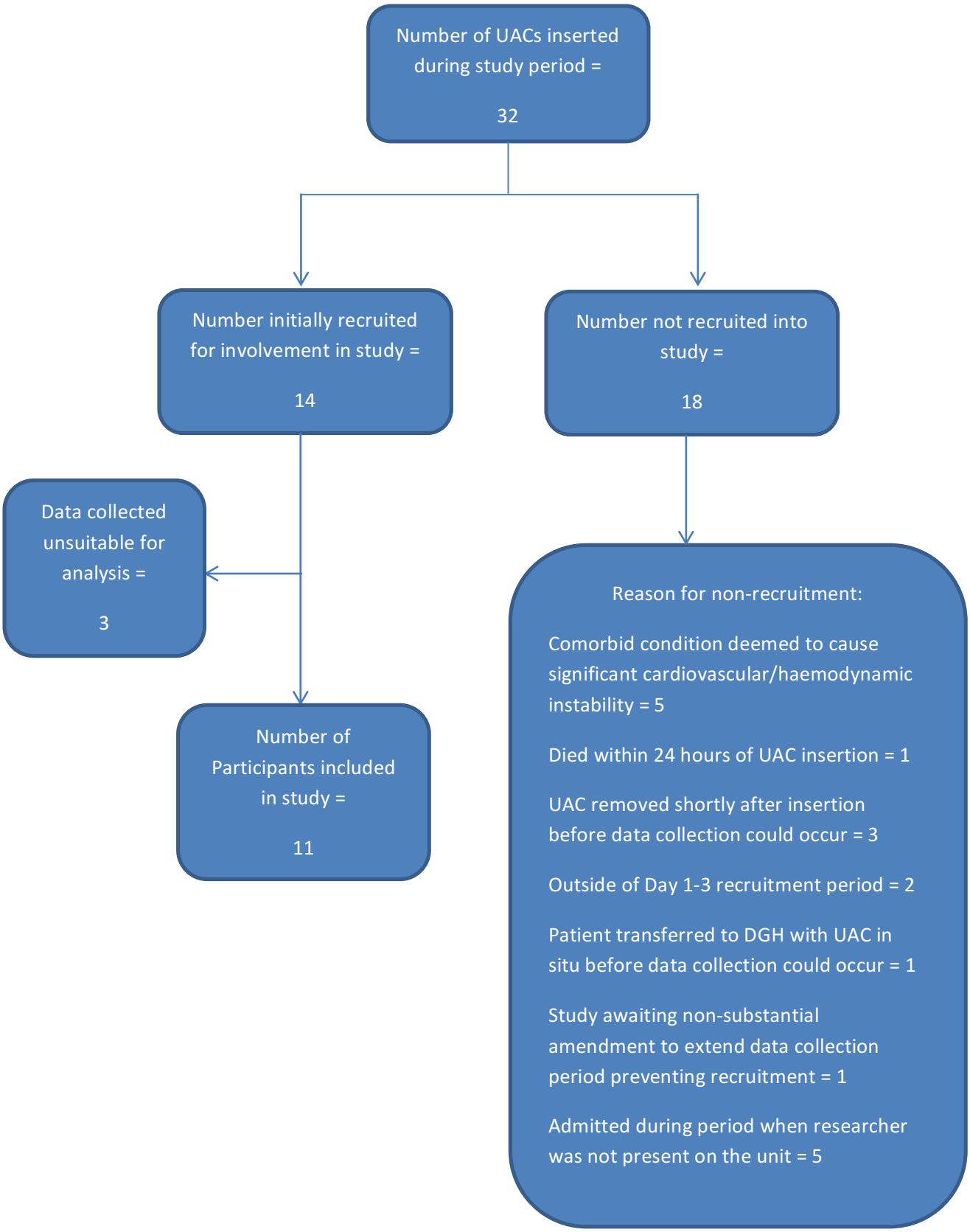


Figure 8 Recruitment Flowchart; UAC = Umbilical Arterial Catheter; DGH = District General Hospital.

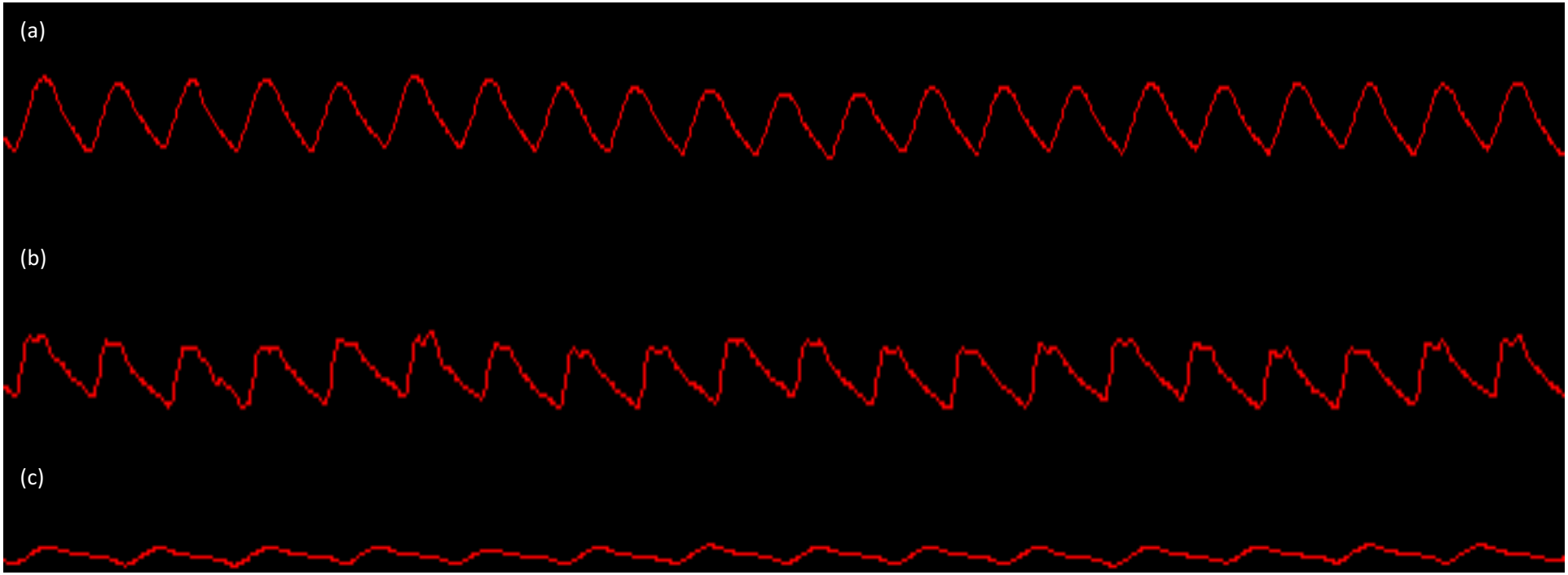


Figure 9 Examples of Arterial Waveform Morphology: (a) - an example of an adequate waveform extracted from the data collected on Baby 10 Day 2; (b) – an example of a suboptimal waveform exhibiting a dominant dichrotic notch resulting in a "double-peak" phenomenon extracted from the data collected on Baby 11 Day 1; (c) – an example of an unusable "dampened" waveform extracted from the data collected on Baby 12 Day 1.

4.3.2 Results – Preterm Neonates

Of the 11 participants recruited as part of the study, 9 were preterm (GA <37 weeks). The demographic data of the preterm neonates included in this study is shown in Table 6. The initial measurement data noted in this population is illustrated in Table 7.

Relationship between Pulse Wave Velocity/Phase Difference and Demographic Variables:

The following demographic variables were assessed for their impact upon the measurement of PWV and Phase Difference:

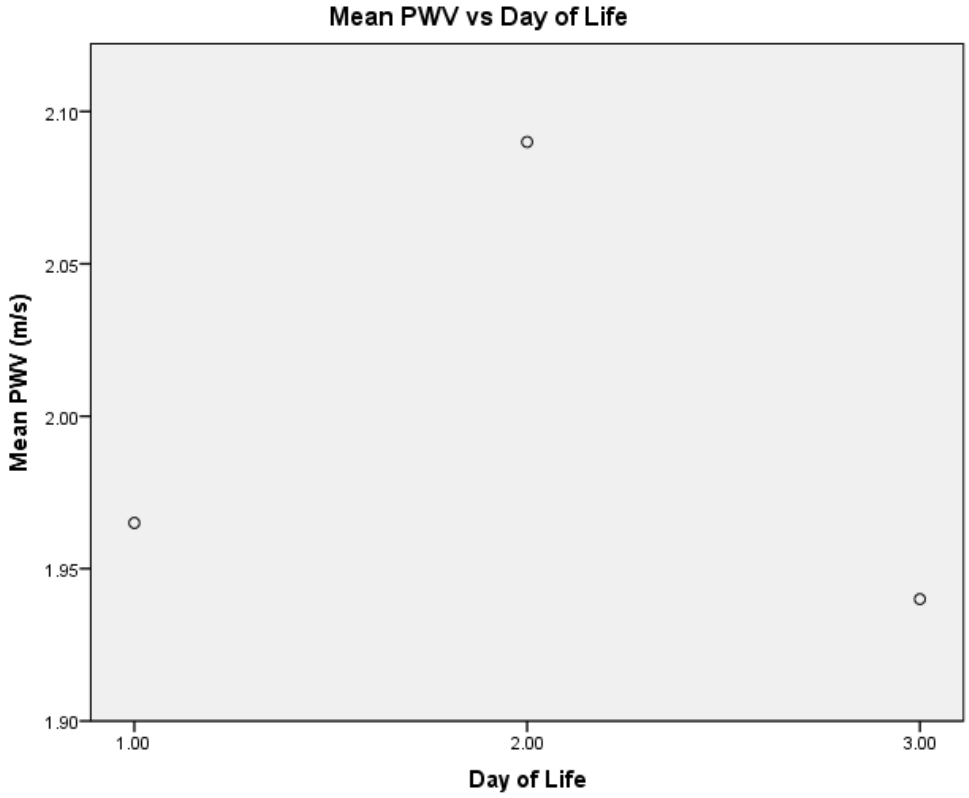
- Day of life (Figure 10);
- Sex (Figure 11).
- Birthweight (Figure 12);
- Gestational Age at Birth (Figure 13);
- Corrected Gestational Age (Figure 14);
- Post-Natal Age (Figure 15);

Total Participants	**9**
Days of Data Collection: **Total** **Day 1** **Day 2** **Day 3**	 16 4 7 5
Sex: **Male** **Female**	 6 3
Birthweight (g)*	930 (820 – 1020)
Gestational Age at Birth (weeks)*	26.29 (25.43 – 27.00)
Corrected Gestational Age (weeks)*	26.50 (24.86 – 26.78)
Post-Natal Age (hours)*	34.5 (22.75 – 49.75)
Maternal Age (years)*	25 (22 – 31)
Antenatal Steroid Administration (complete or incomplete)	5
Maternal Smoking	4 (Data not recorded for Babies 3, 5 and 6)
Surfactant Administration	6
5-Minute APGAR Score*	4 (2 – 6; Data not recorded for Babies 2, 6 and 10)
Mean Arterial Pressure (mmHg)*	32 (27.33 – 34.33)
HeRO Score*	2.62 (0.96 – 3.39)
Lactate (mmol/L)*	1.95 (1.37 – 2.17)
Haemoglobin (g/L)*	139 (111.75 – 158.25)
CRP (mg/L)*	7.6 (3.15 – 15.73)
Fluid Replacement (ml/kg/day)*	113.25 (89.05 – 144.13)
SIMV: **Days** **Mean Airway Pressure*** **Peak Inspiratory Pressure (PIP)*** **Peak Expiratory End Pressure (PEEP)***	 5 8 (6.5 – 12.5) 18 (16 – 20) 5 (4 – 5.5)
CPAP: **Days** **Peak Expiratory End Pressure (PEEP)***	 5 4 (4 – 6)
Caffeine Administration	5
Inotrope Administration	**1**

Table 6 Demographic Data of Study Participants; *expressed as median with interquartile range (IQR); CRP = C-Reactive Protein; SIMV = Synchronised Intermittent Mechanical Ventilation; CPAP = Continuous Positive Airway Pressure

	Pulse Wave Velocity	Phase Difference
Median (IQR)	1.94 (1.74 – 2.27)	180.66 (176.96 – 194.84)
Range	1.63 – 2.63	158.66 – 203.08

Table 7 Baselines values of PWV and Phase Difference observed in the preterm population



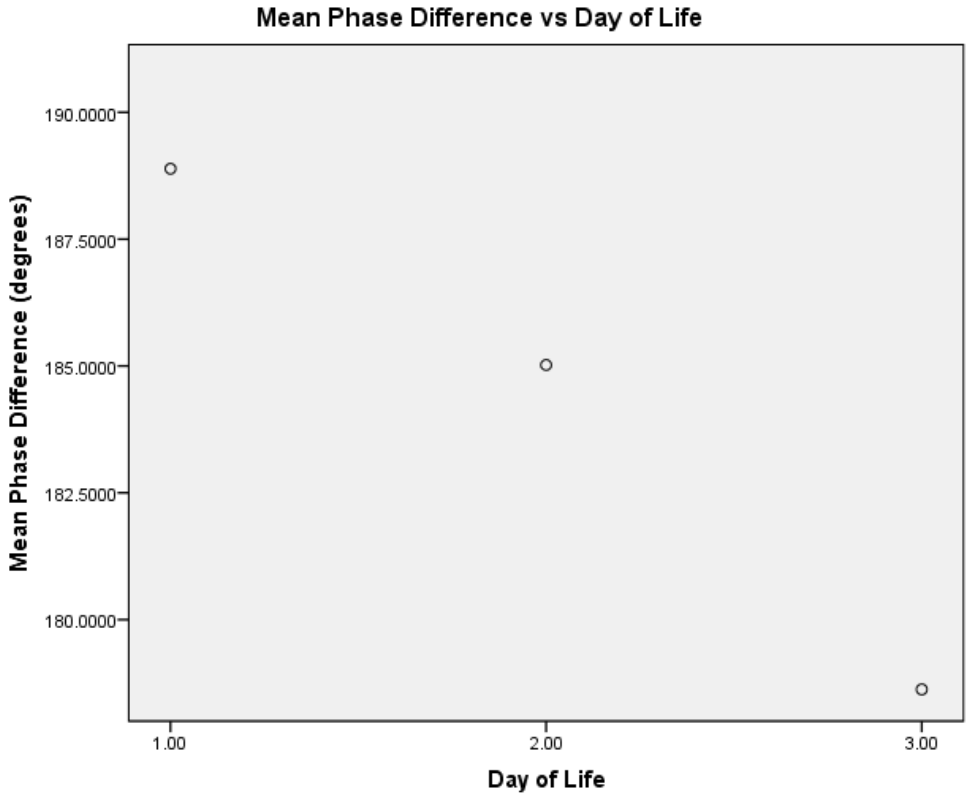
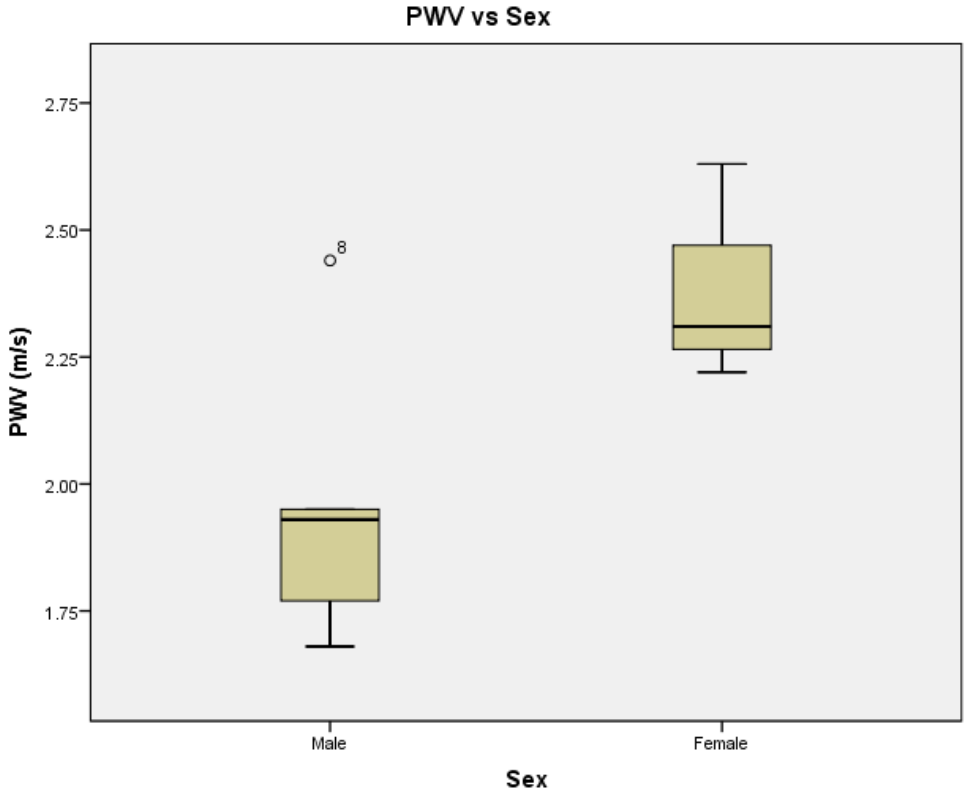


Figure 10 The Relationship between Mean PWV/Phase Difference and Day of Life



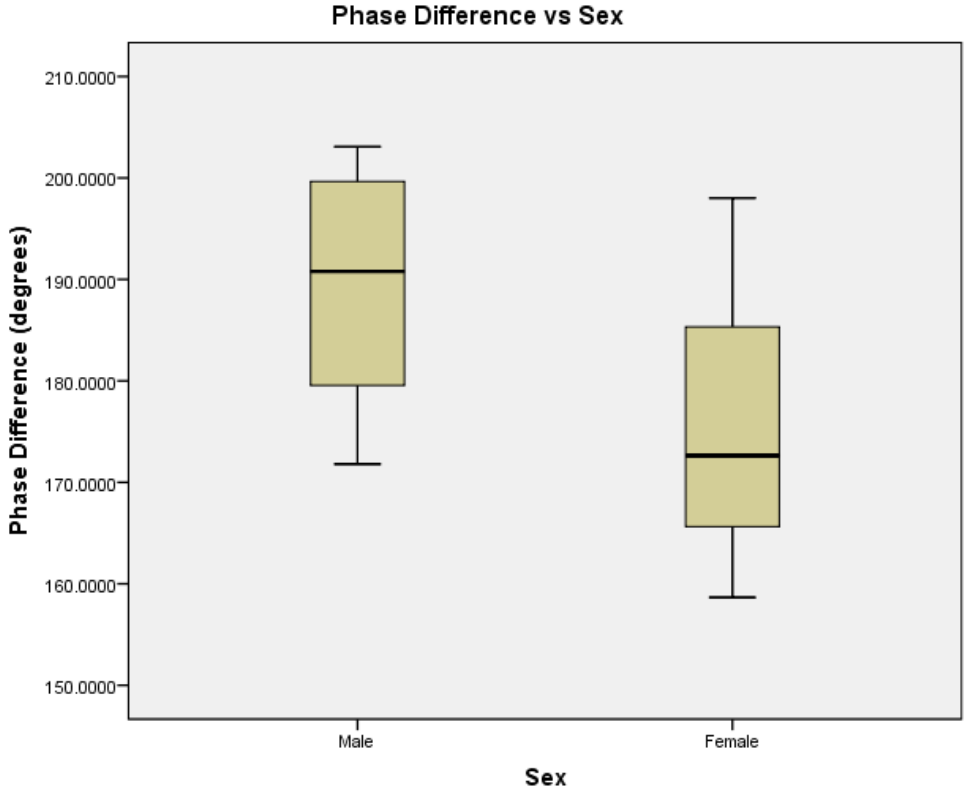
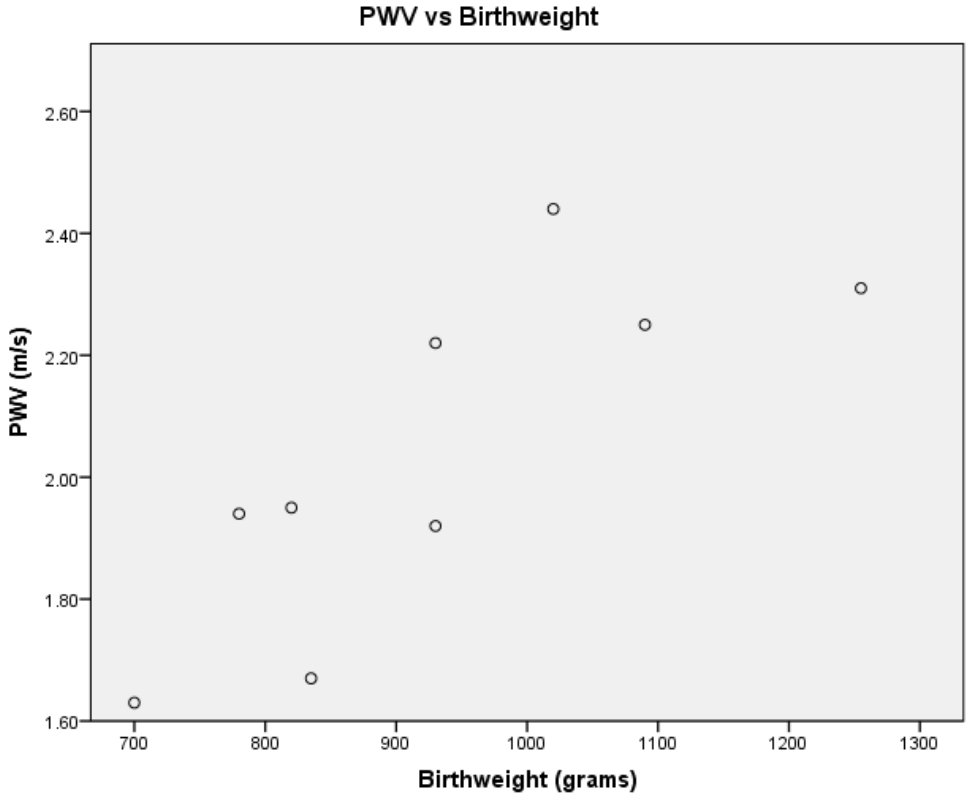


Figure 11 The Relationship between PWV/Phase Difference and Sex



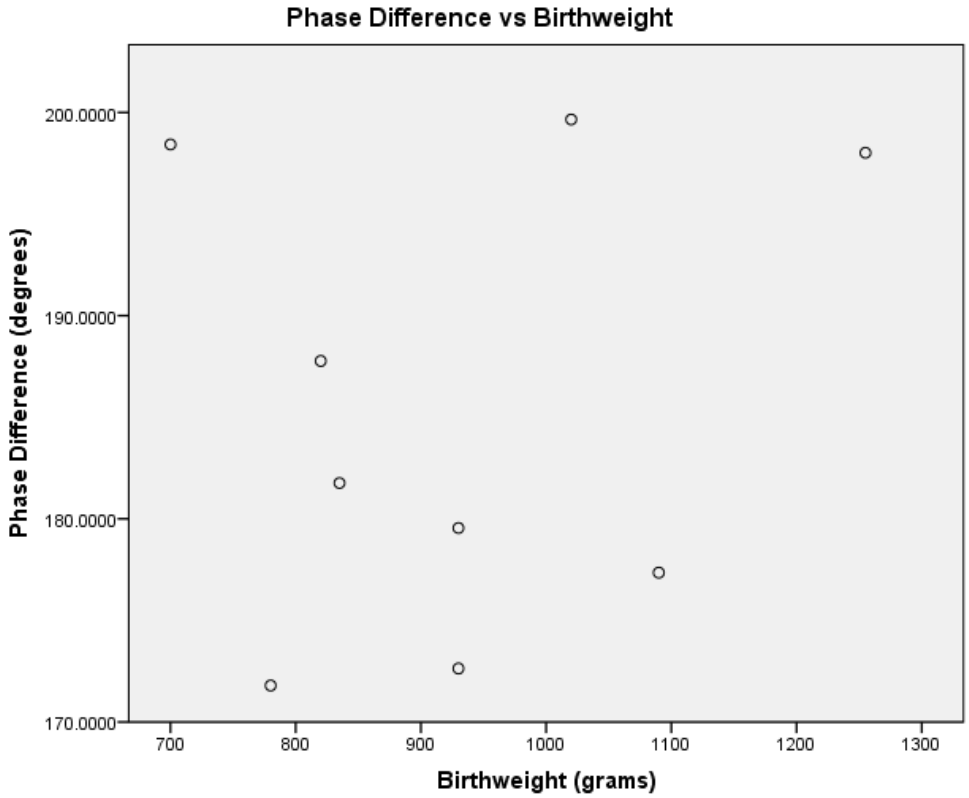


Figure 12 The Relationship between PWV/Phase Difference and Birthweight

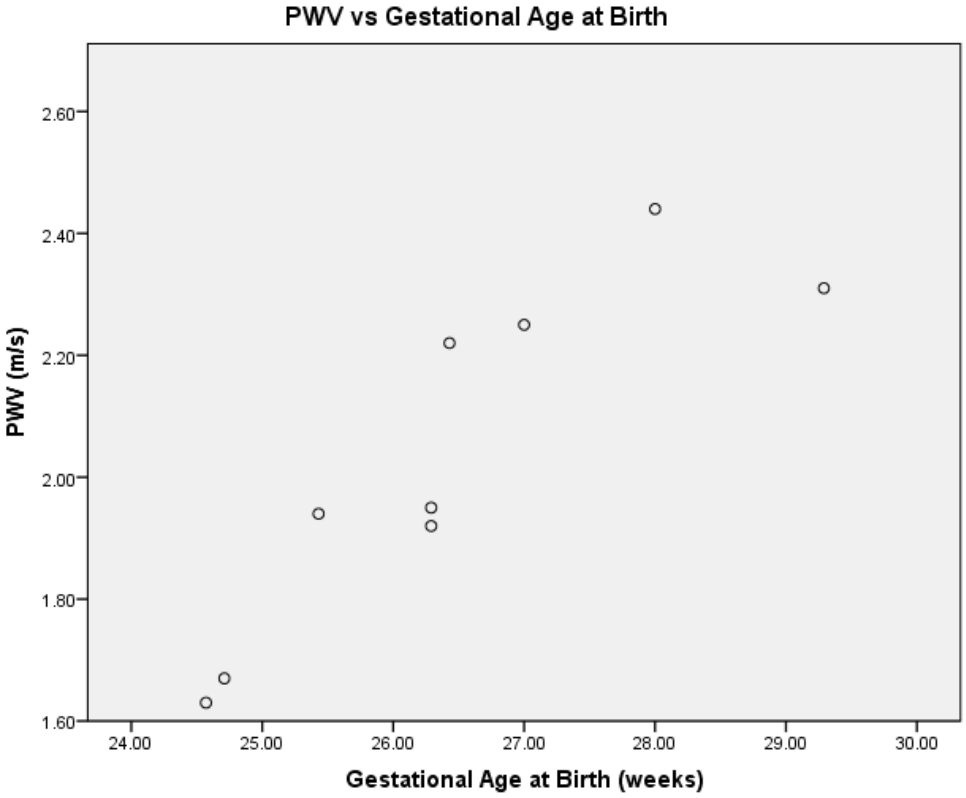
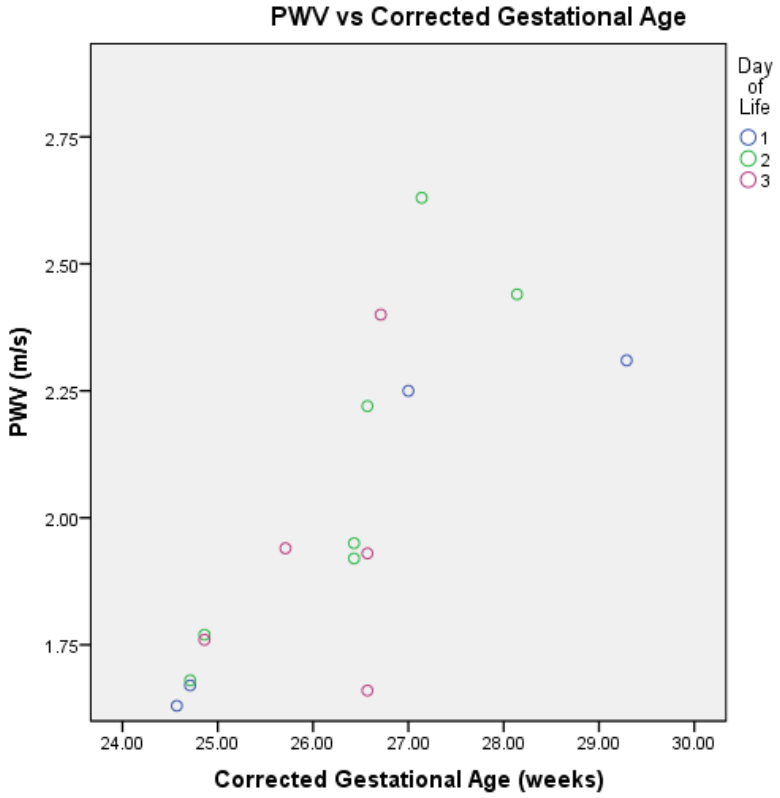


Figure 13 The Relationship between PWV/Phase Difference and Gestational Age at Birth



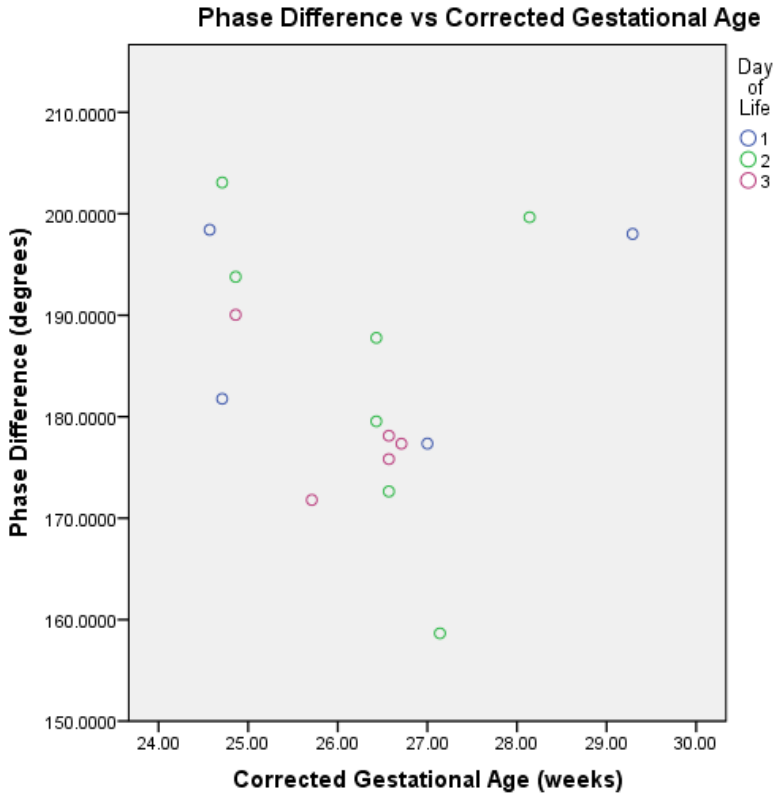
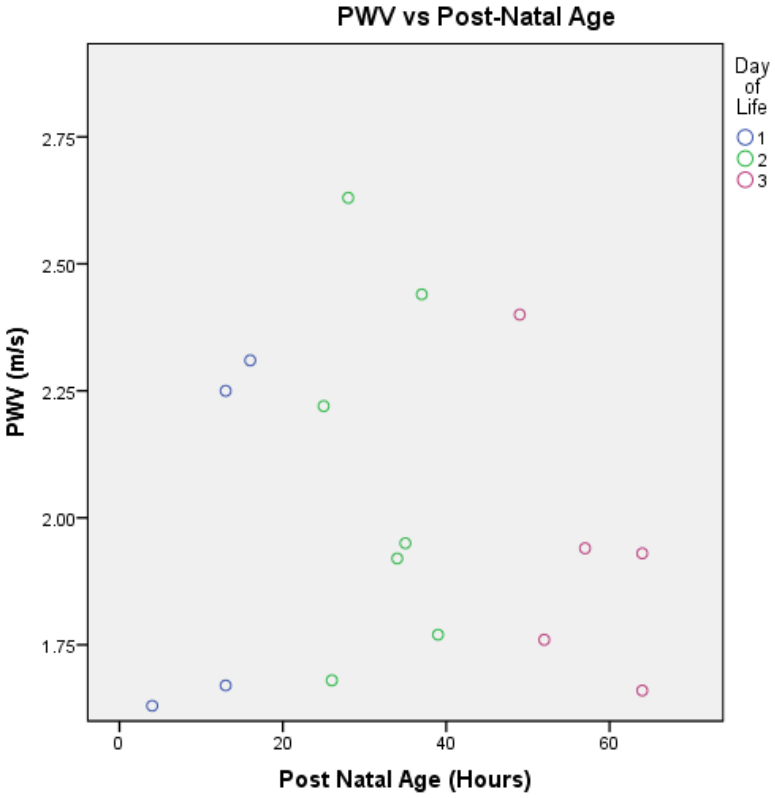


Figure 14 The Relationship between PWV/Phase Difference and Corrected Gestational Age



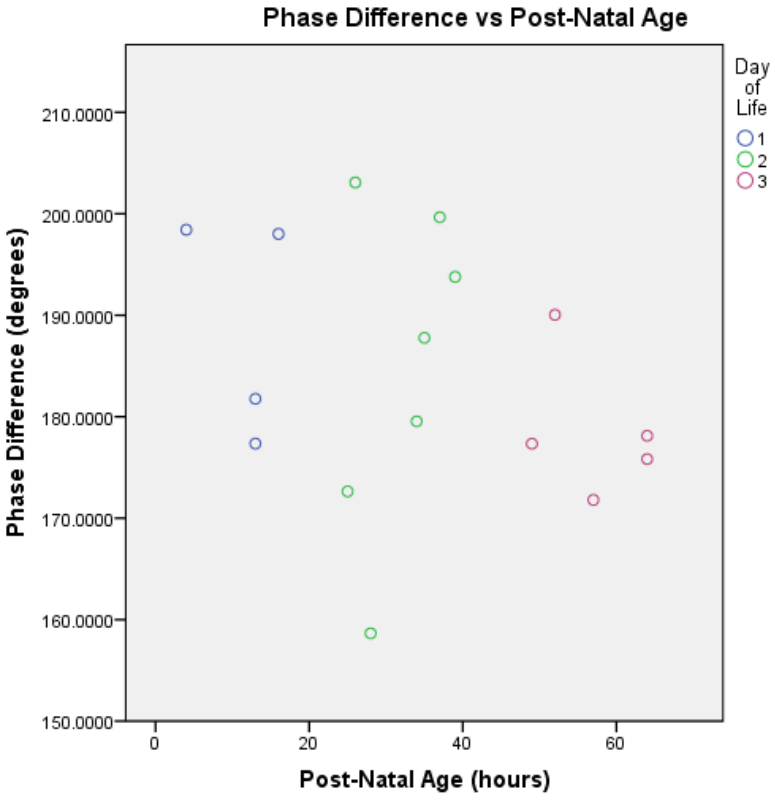


Figure 15 The Relationship between PWV/Phase Difference and Post-Natal Age

An overview of the relationships between PWV/Phase Difference and the above demographic variables, as well as the strength of their associations, is exhibited in Table 8.

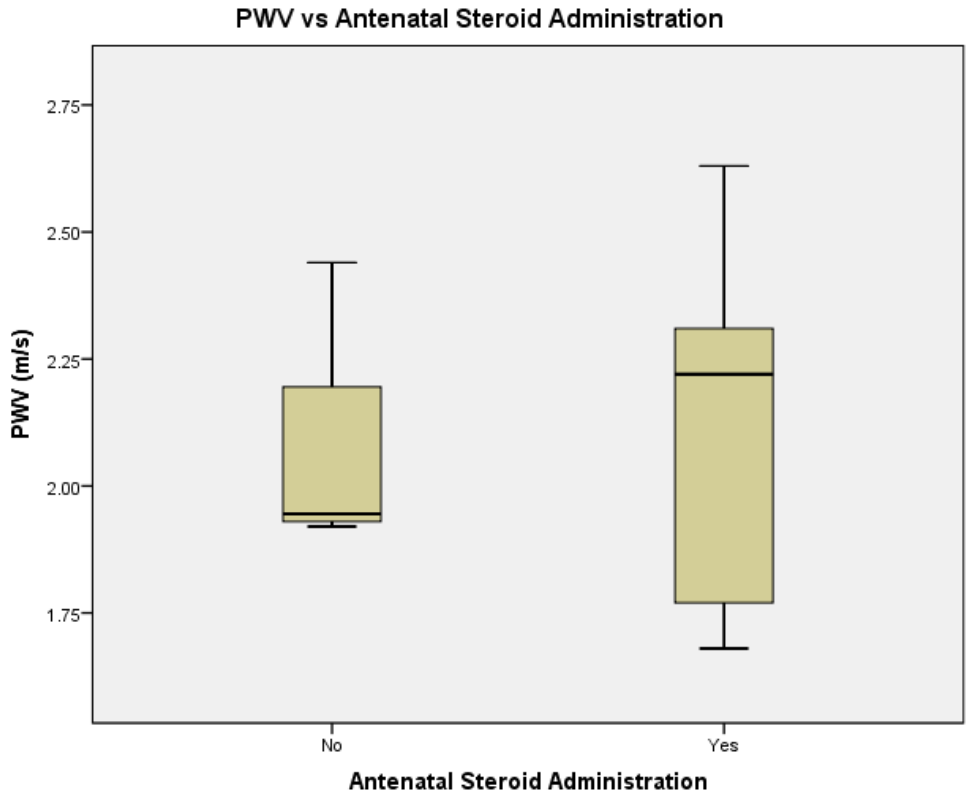
PWV:			
Variable:	**Rho (2 Significant Figures):**	**Strength of Correlation:**	**P value:**
Day of Life	0.00	Weak	0.667
Birthweight	0.80	Strong	0.01*
Gestational Age at Birth	0.95	Strong	0.01*
Corrected Gestational Age	0.90	Strong	0.001*
Post-Natal Age	0.04	Weak	0.987
Phase Difference:			
Day of Life	-1.00	Strong	0.01*
Birthweight	0.10	Weak	0.797
Gestational Age at Birth	0.15	Weak	0.699
Corrected Gestational Age	-0.09	Weak	0.814
Post-Natal Age	-0.29	Weak	0.283

Table 8 Overview of the relationship between PWV/Phase Difference and Demographic variables; *indicates significance at the 0.05 level (2-tailed).

Relationship between Pulse Wave Velocity/Phase Difference and Clinical Variables:

The following clinical variables were assessed for their impact upon the measurement of PWV and Phase Difference:

- Antenatal Steroid Administration (Figure 16);
- Surfactant Administration (Figure 17);
- Mean Arterial Blood Pressure (Figure 18);
- HeRO Score (Figure 19);
- Lactate (Figure 20);
- Haemoglobin (Figure 21);
- CRP (Figure 22);
- Fluid Replacement (Figure 23);
- Mean Airway Pressure (Figure 24);
- Caffeine Administration (Figure 25);
- IVH (Figure 26).



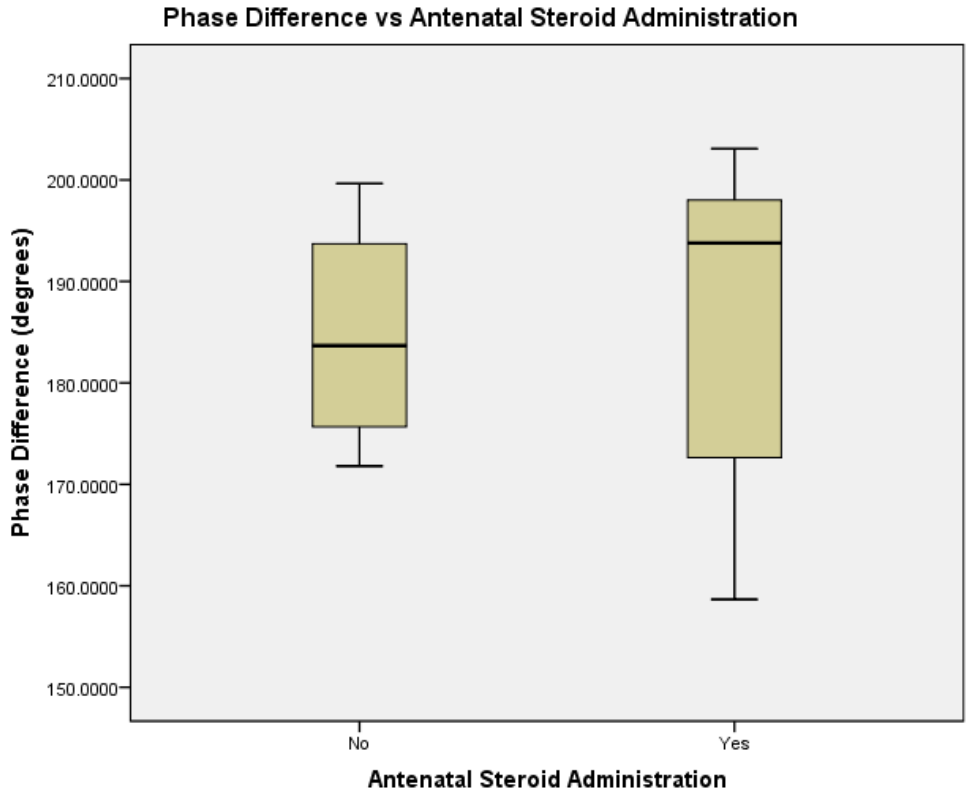
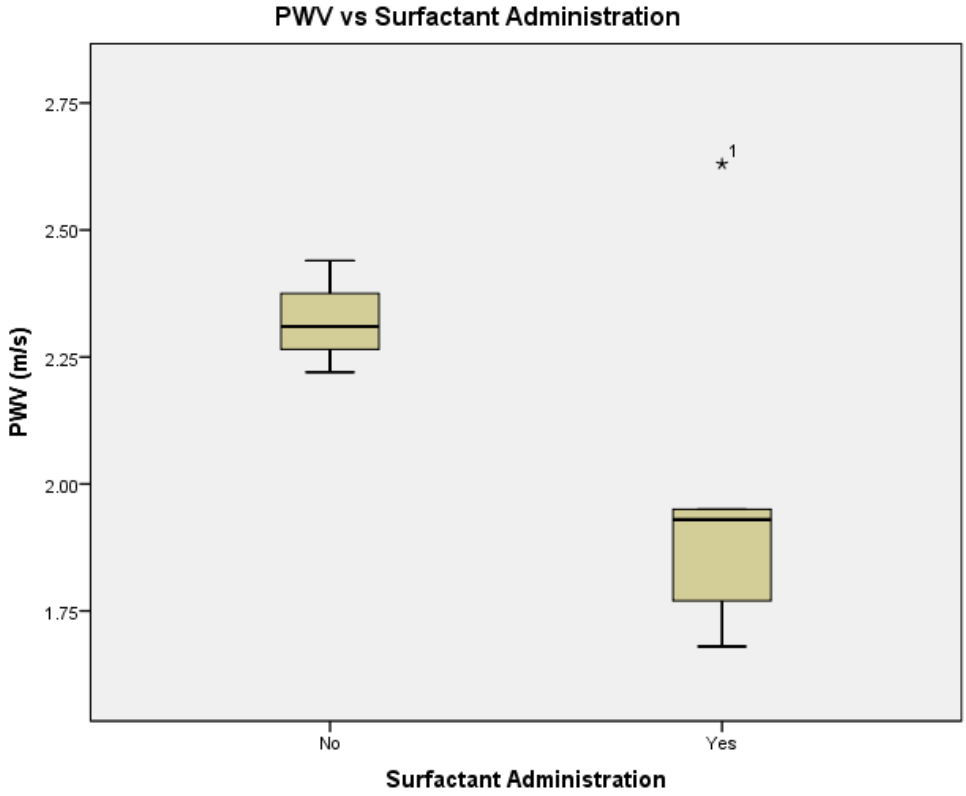


Figure 16 The Relationship between PWV/Phase Difference and Antenatal Steroid Administration



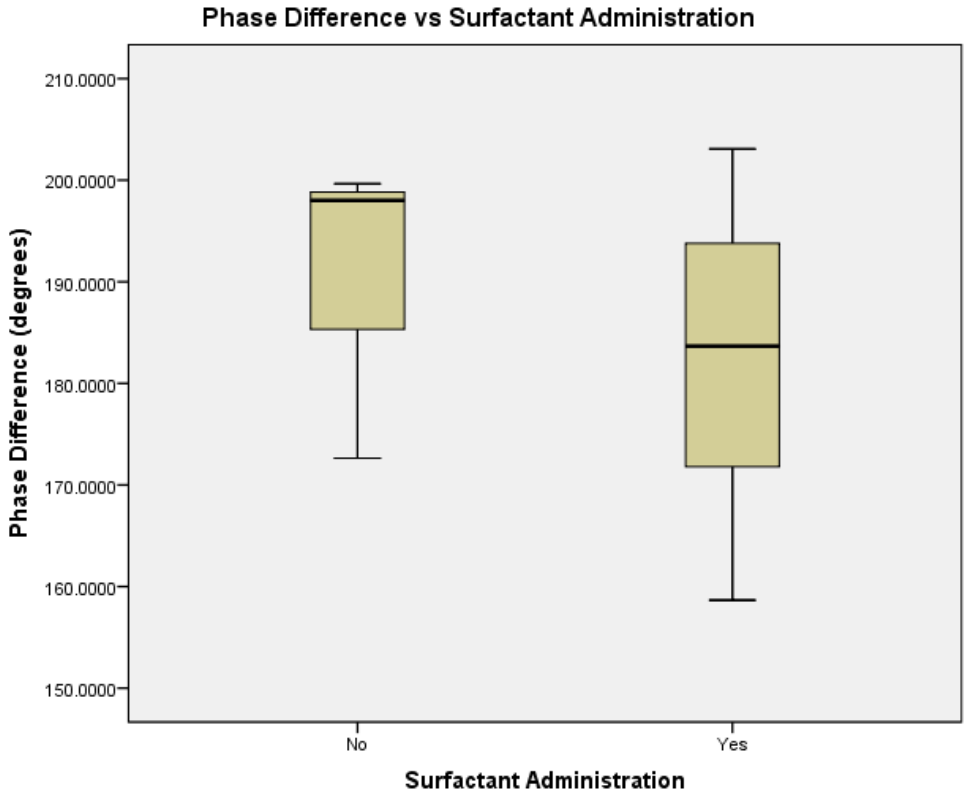
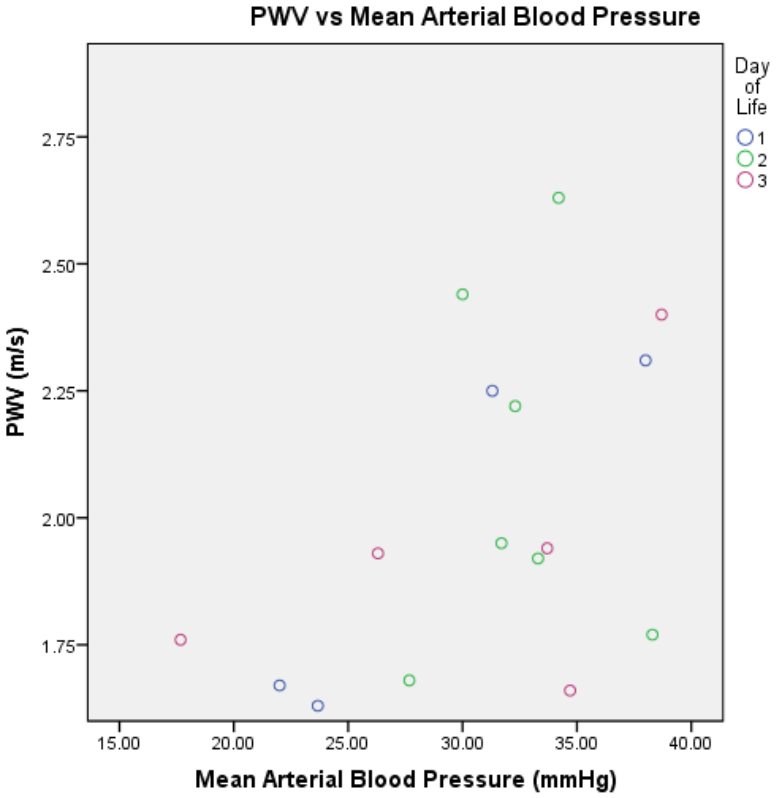


Figure 17 The Relationship between PWV/Phase Difference and Surfactant Administration



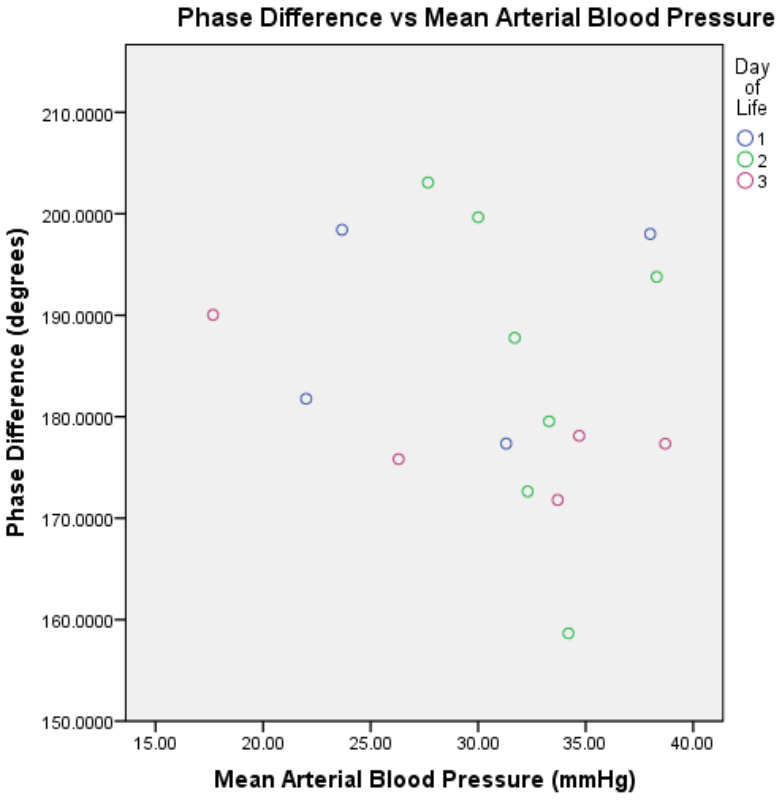
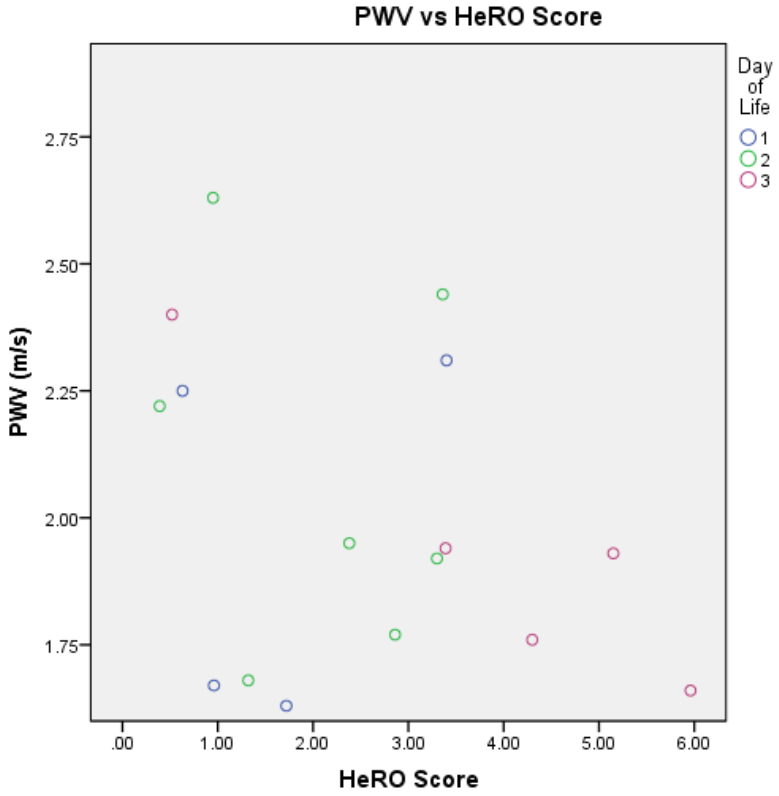


Figure 18 The Relationship between PWV/Phase Difference and Mean Arterial Blood Pressure



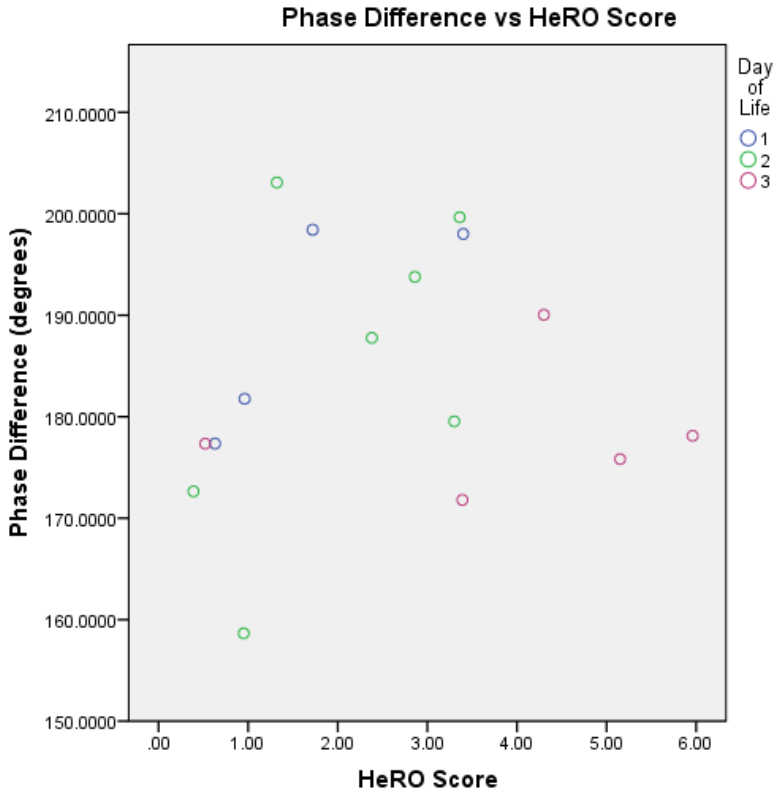
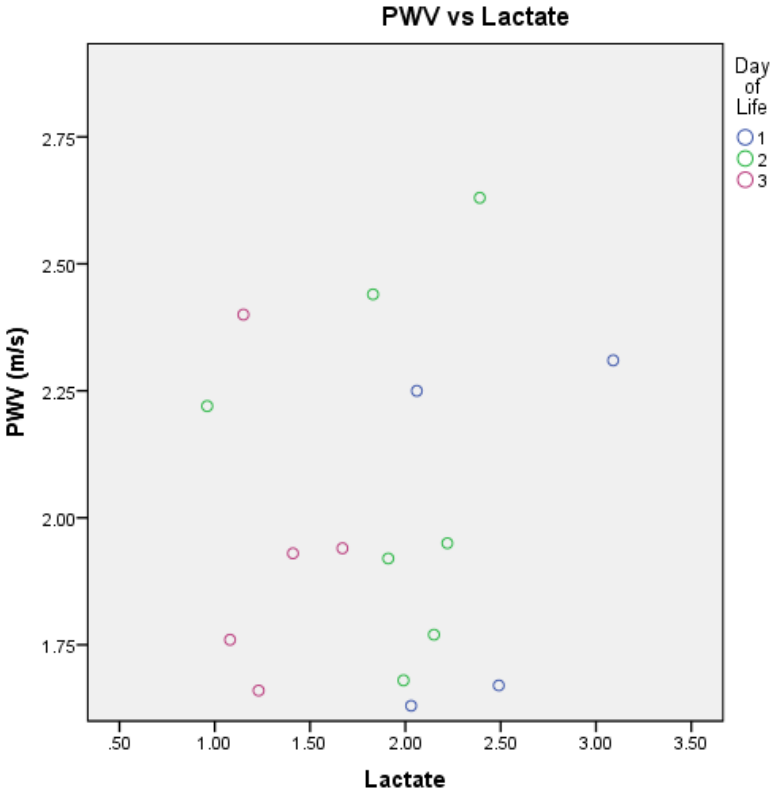


Figure 19 The Relationship between PWV/Phase Difference and HeRO Score



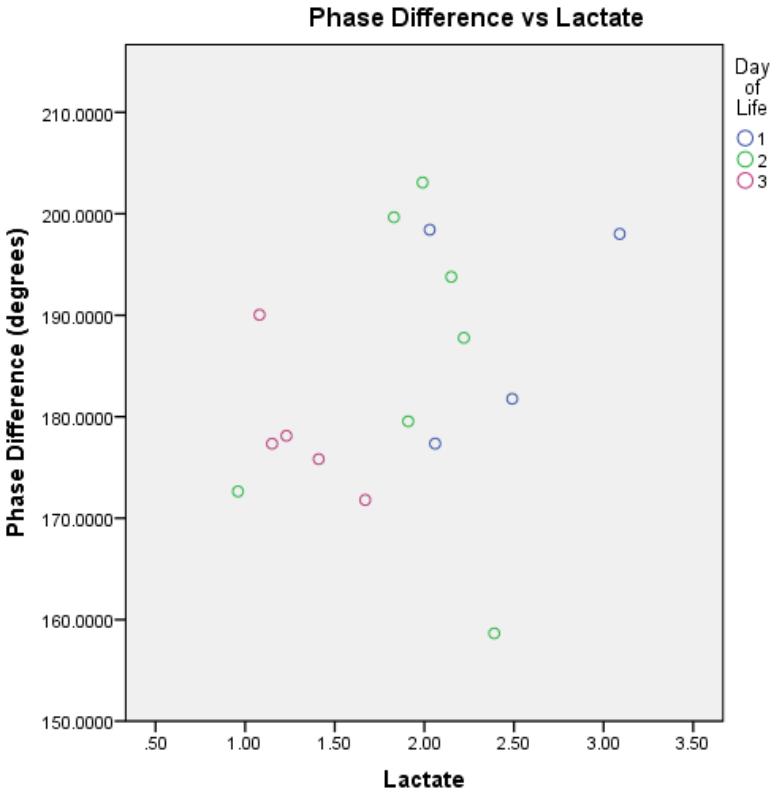
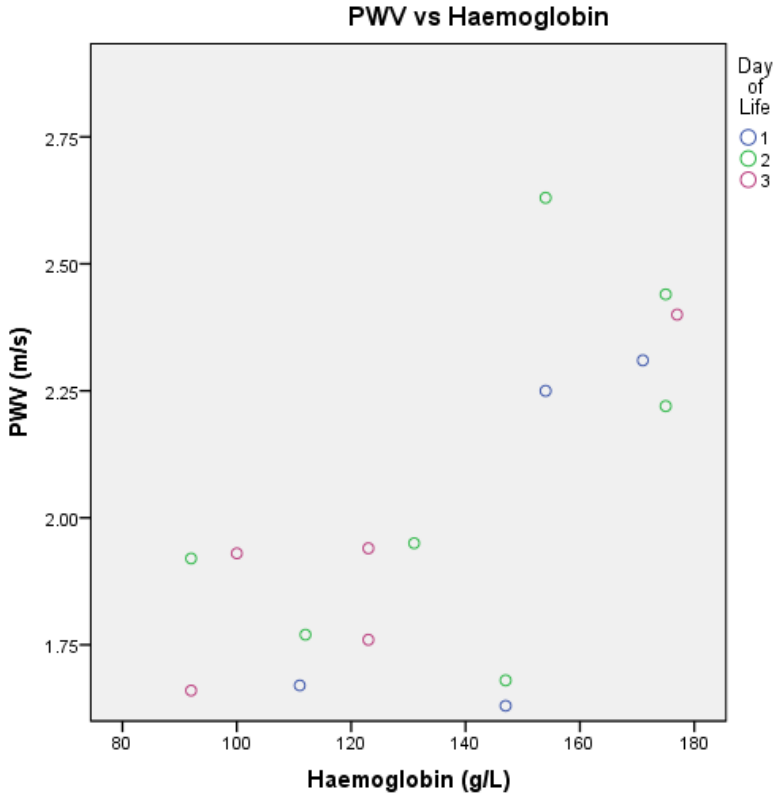


Figure 20 The relationship between PWV/Phase Difference and Lactate



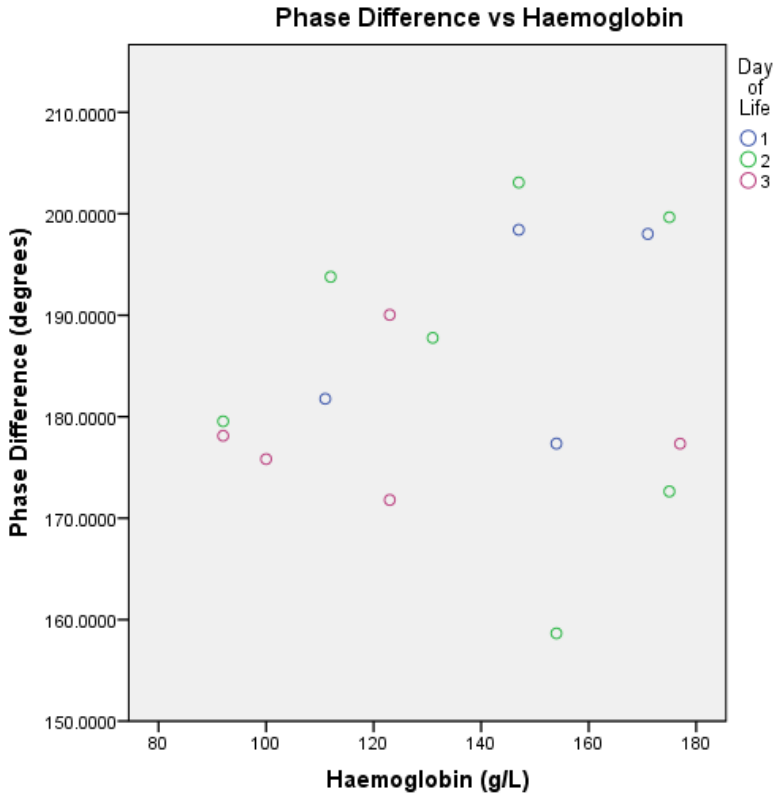


Figure 21 The Relationship between PWV/Phase Difference and Haemoglobin

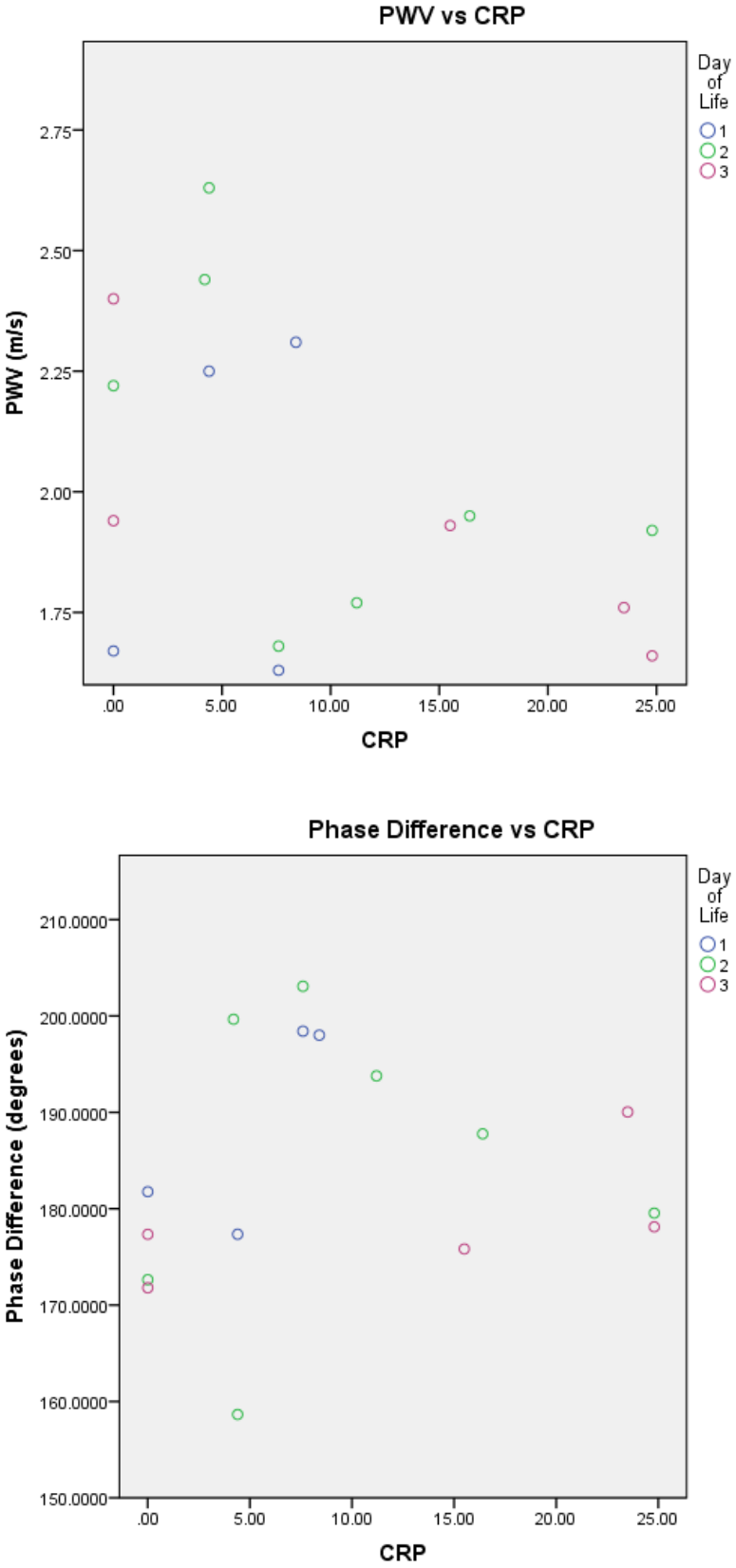
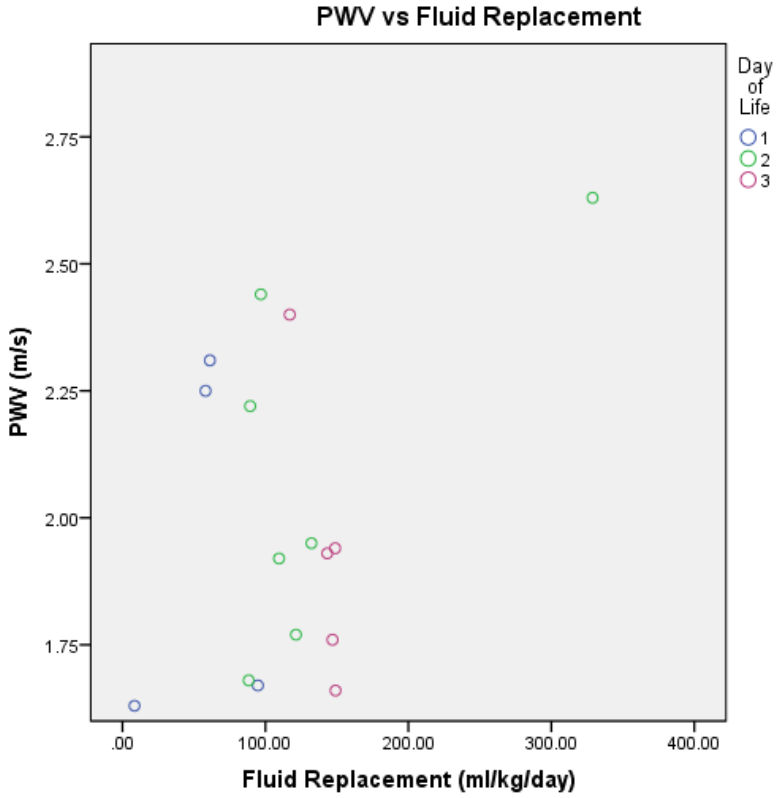


Figure 22 The Relationship between PWV/Phase Difference and CRP



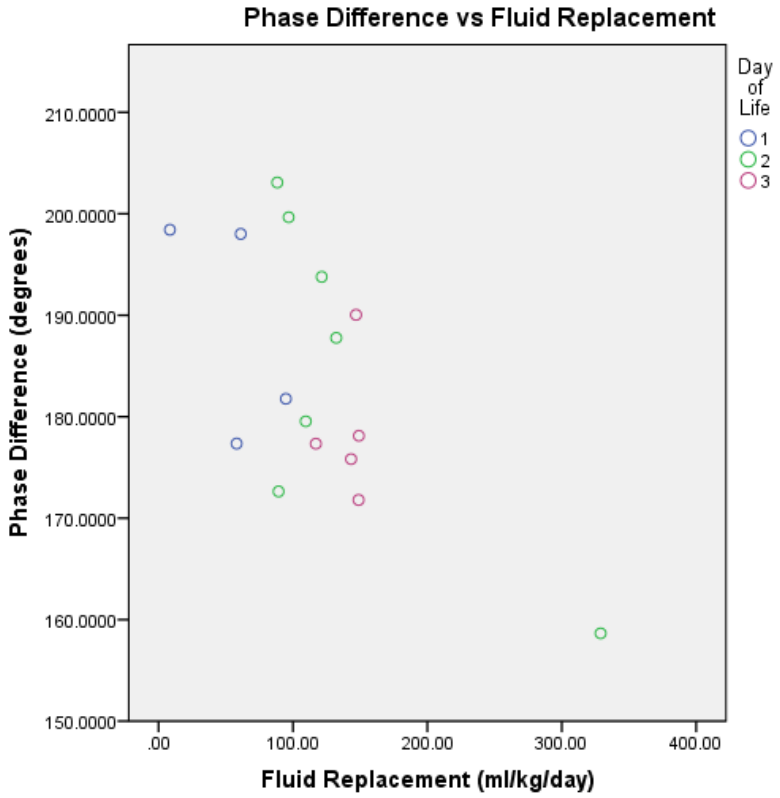
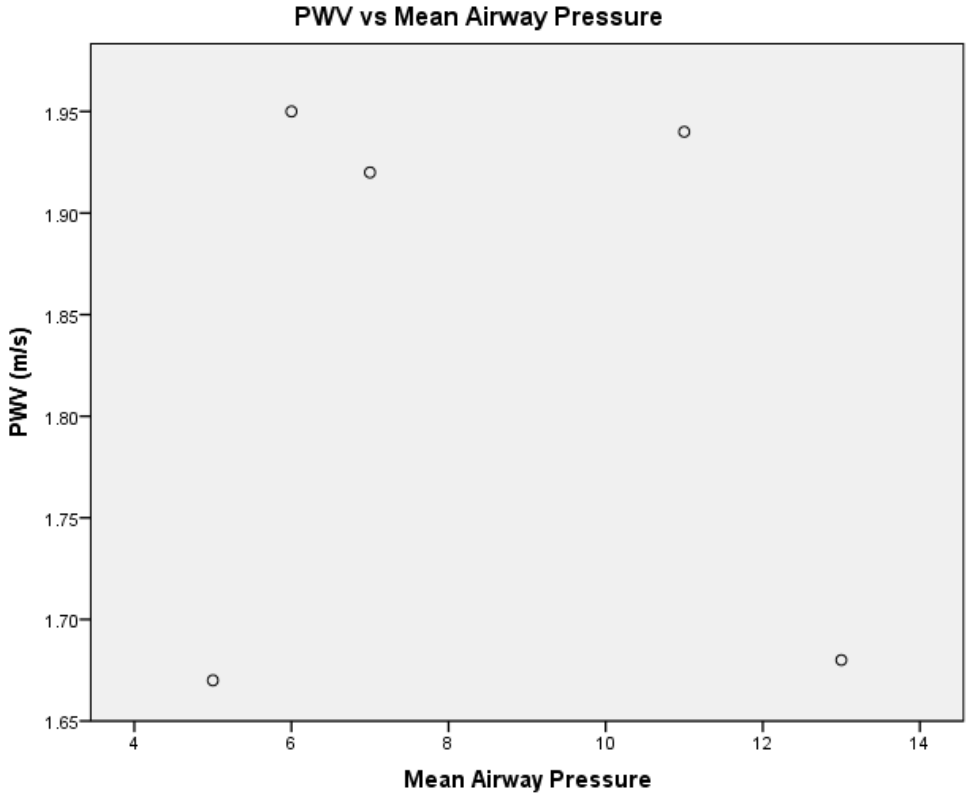


Figure 23 The Relationship between PWV/Phase Difference and Fluid Replacement



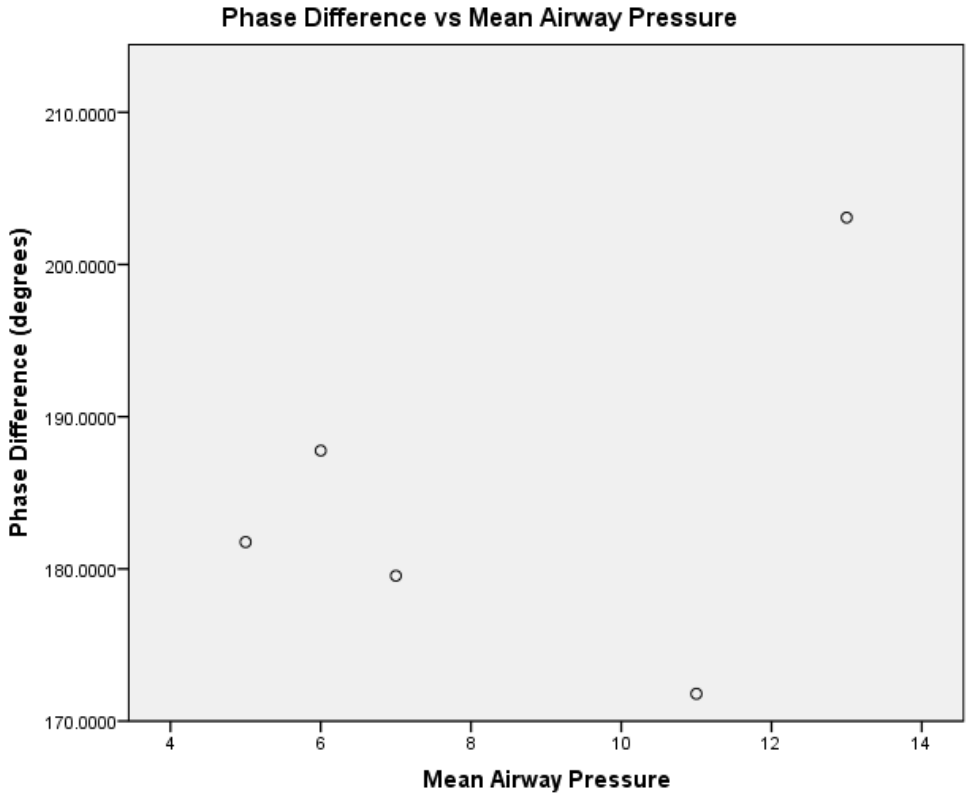


Figure 24 The Relationship between PWV/Phase Difference and Mean Airway Pressure

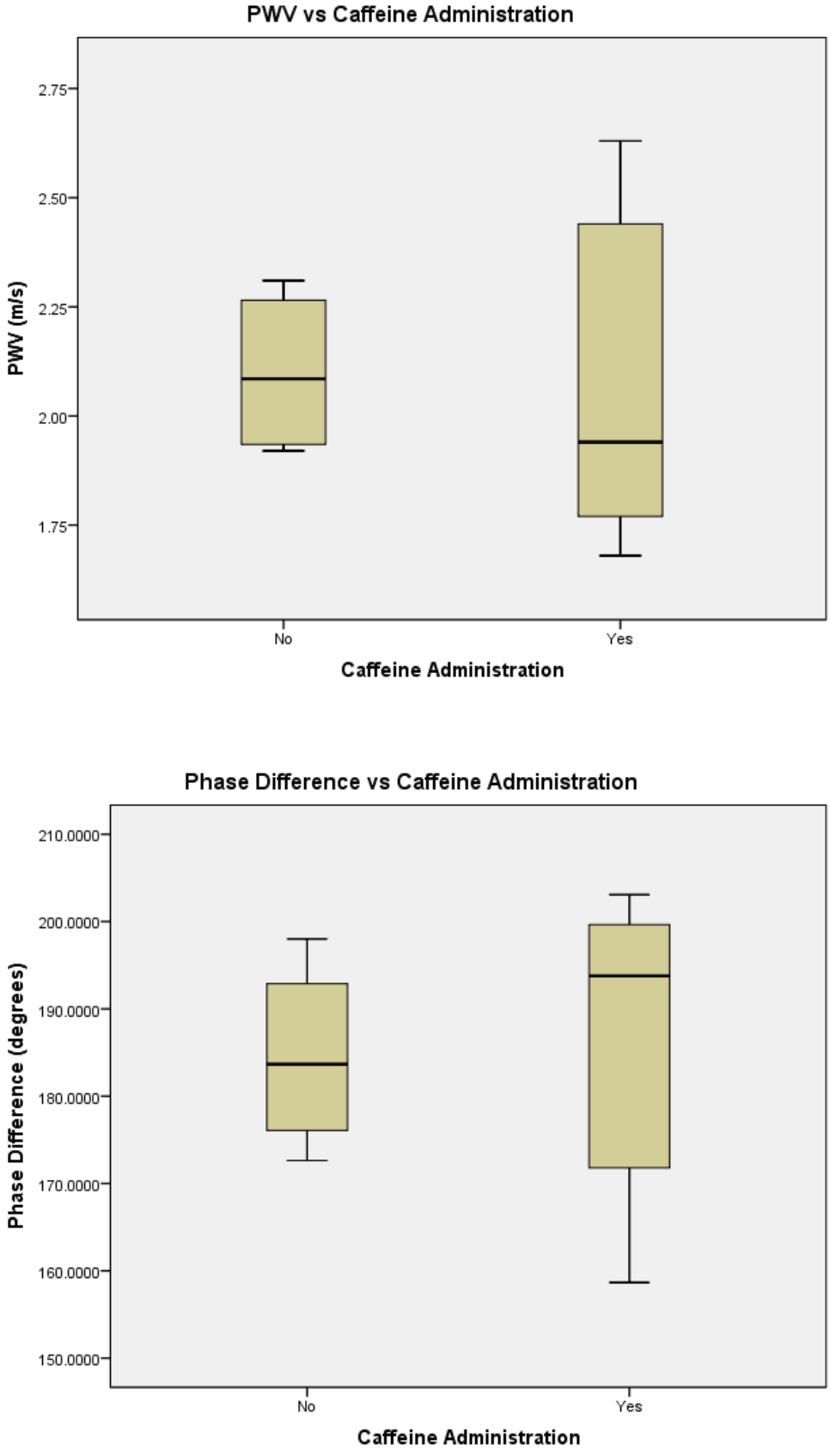
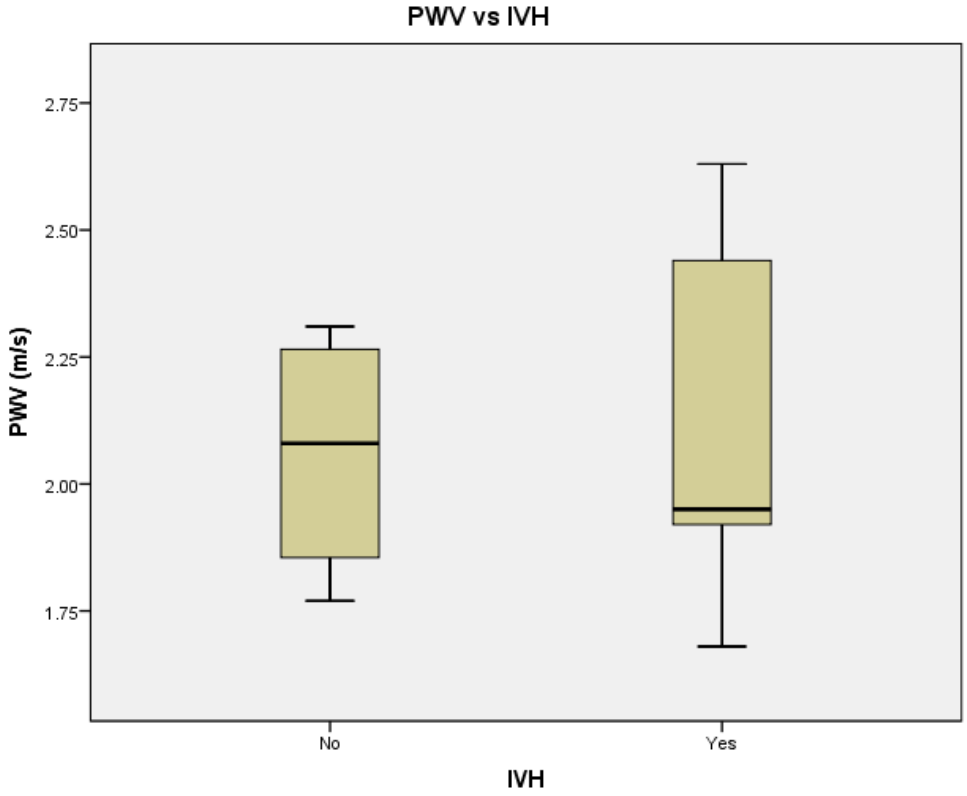


Figure 25 The Relationship between PWV/Phase Difference and Caffeine Administration



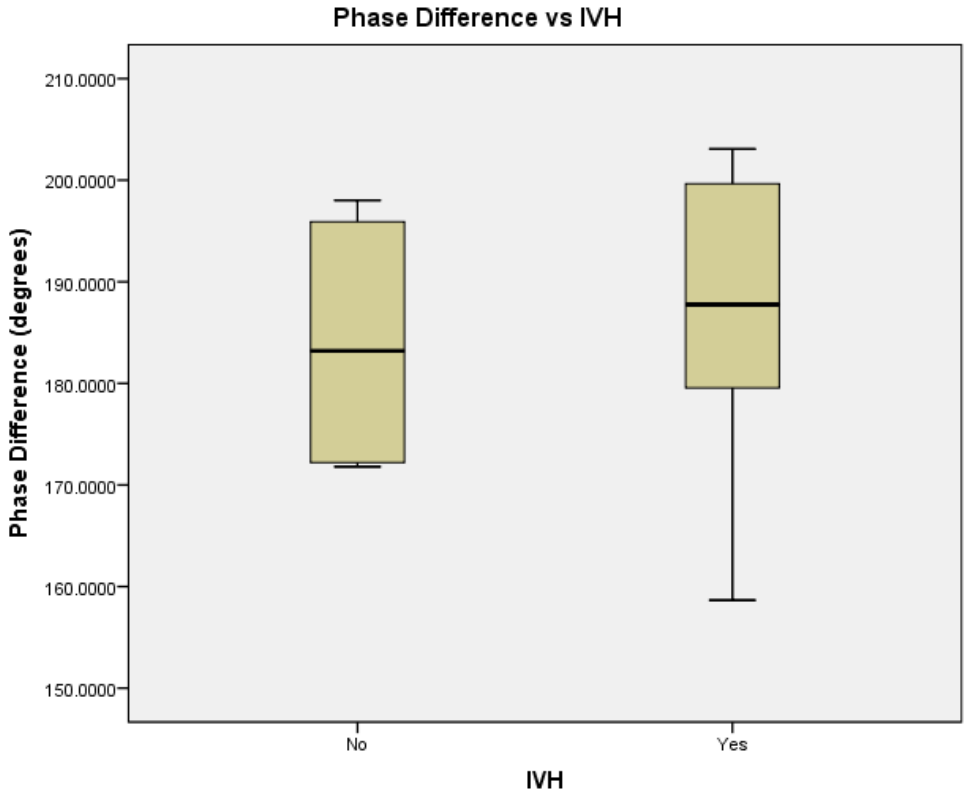


Figure 26 The Relationship between PWV/Phase Difference and IVH

An overview of the relationships between PWV/Phase Difference and the above clinical variables, as well as the strength of their associations, is exhibited in Table 9.

PWV:			
Variable:	**Rho (2 Significant Figures):**	**Strength of Correlation:**	**P value:**
Mean Arterial Blood Pressure	0.12	Weak	0.765
HeRO Score	0.02	Weak	0.966
Lactate	0.20	Weak	0.606
Haemoglobin	0.69	Moderate	0.041*
CRP	-0.37	Weak	0.330
Fluid Replacement	0.15	Weak	0.700
Mean Airway Pressure	-0.20	Weak	0.800
Phase Difference:			
Mean Arterial Blood Pressure	-0.40	Weak	0.286
HeRO Score	0.27	Weak	0.488
Lactate	0.15	Weak	0.700
Haemoglobin	0.18	Weak	0.651
CRP	0.26	Weak	0.500
Rate of Fluid Replacement	-0.72	Moderate	0.030*
Mean Airway Pressure	0.60	Moderate	0.400

Table 9 Overview of the relationship between PWV/Phase Difference and Clinical Variables; *indicates significant value at the 0.05 level (2-tailed).

4.3.3 Results – Term Neonates

Of the 11 participants recruited as part of this study 2 were born at term. Both neonates however, suffered hypoxic-ischaemic injury at birth and were therefore cooled during the period of data collection. As well as this, they required significant haemodynamic support with administration of inotropes and vasopressors. It was therefore deemed inappropriate to conduct analysis on their data due to the number of potential confounders and very small sample size.

It is hoped that this data could be used in the future if additional data was collected on term infants through subsequent studies.

Chapter 5: Investigating the Relationship between Patent Ductus Arteriosus and the Measurement of Pulse Wave Velocity and Phase Difference

5.1 Introduction

Collection and collation of normative data is an important first step in the validation of novel biomarkers. However, in order to justify further research, it is important to identify the potential clinical impact of a proposed biomarker. As highlighted by *Savage and Everett*, in order for a biomarker to be considered for inclusion in clinical practice, it is crucial to highlight the relationship between it and the current gold standard investigation, as well as highlighting areas where its use could improve care beyond what is already available (59).

As demonstrated in previous chapters, the use of PWV and Phase Difference has the potential to improve current management of PDA. Due to the nature of its measurement, the formation of an algorithm providing an hourly PWV/Phase Difference "score" predictive of PDA diameter could act as a useful screening method for those suffering from large, potentially haemodynamically significant PDAs. Furthermore, calculation of such a score could help improve monitoring capabilities of those who are currently undergoing PDA treatment. The ability to review trends of such a score would allow clinicians to identify a worsening or improving PDA. This could improve neonatal care through the reduction of unnecessary echocardiograms being performed. Such scoring systems have already been used to great effect in specialist neonatal units. The HeRO system provides an hourly score for patients using an algorithm based on heart rate variability. It is thought that alterations in heart rate variability are linked to the onset of sepsis, and so those with a rising HeRO score can be screened for infection and promptly treated with antibiotics (156). Implementation of a similar system using PWV/Phase Difference could be a valuable supplement to echocardiography in the investigation and management of PDA.

A PDA has the potential ability to affect the measurement of PWV/Phase Difference in a number of ways. As illustrated in Figure 4, the Moens-Korteweg equation exhibits the variables that affect the measurement of PWV. We hypothesise that the presence of a PDA will have a two-fold effect on the measurement of PWV. Firstly, the presence of a PDA will increase the vessel radius (*r*) aspect of the equation. An increasingly large PDA diameter will therefore cause a larger *r* value, resulting in a hypothetically reduced pulse wave velocity. This effect may also be exacerbated by the effect of the PDA on the functioning of the ANS. As mentioned previously, a recent study by *Goudjil et al* highlighted an increase in parasympathetic activity in those suffering from PDA (44). This parasympathetic dominance may further contribute to the effect on vessel radius through vasodilatation.

The aim of this chapter is to investigate the relationship between measurements of PDA severity (PDA diameter, LA:Ao) and PWV/phase difference.

5.2 Methods

A prospective observational study was conducted on the neonatal intensive care unit of the Liverpool Women's Hospital following confirmation of ethical approval as outlined in section 4.2.4. All neonates with a UAC in situ within the first three days of life were eligible for recruitment provided they did not fulfil any of the exclusion criteria. Routine clinical data (ECG and arterial waveform) allowing the calculation of PWV/Phase difference was to be collected, as well as echocardiographic parameters related to PDA severity (PDA Diameter, LA:Ao, Transductal velocity).

All inclusion and exclusion criteria and data collection procedures were the same as mentioned in Chapter 4 with one exception. During days 3-10, additional data was collected when participants were undergoing echocardiographic evaluation. Therefore, PWV/ Phase Difference data was compared to echocardiographic data within the first 10 days of life for those recruited

in the study. This data was collected within a 4 hour window (between 2 hours before to 2 hours after) of the echocardiogram.

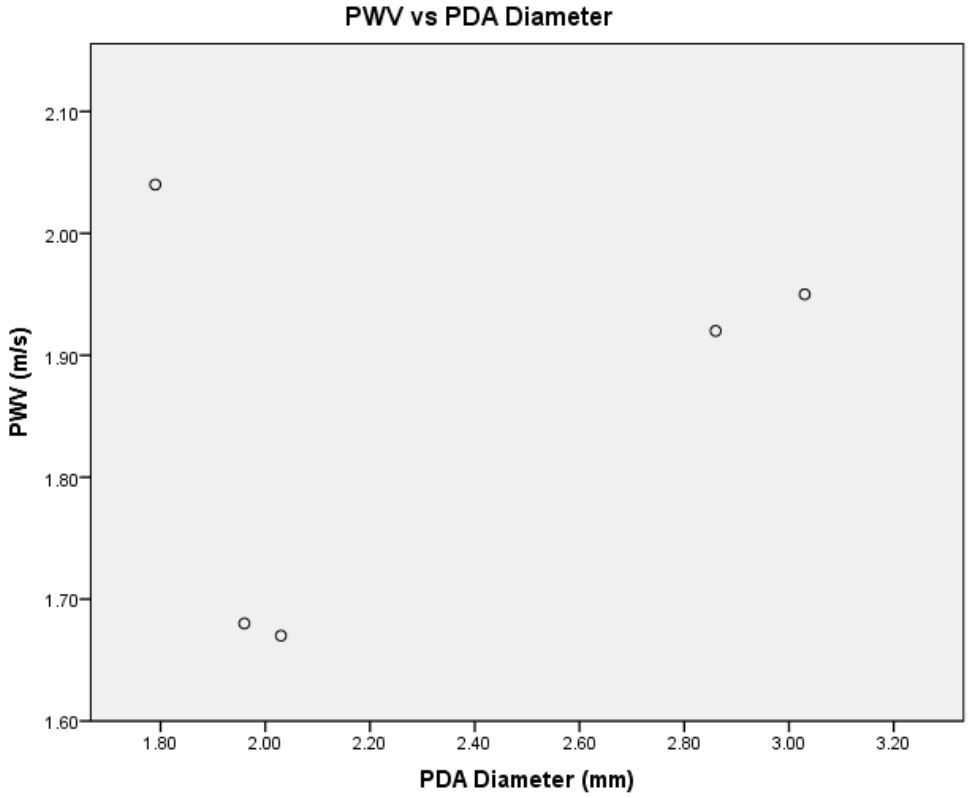
Graphical and non-parametric correlational analysis to assess the relationship between PWV, Phase Difference and markers of PDA severity will be performed in accordance with the methods outlined in Chapter 4.

5.3 Results

Of the 11 participants recruited as part of the study, a total of 6 neonates had an echocardiogram performed whilst their UAC remained in situ. One participant was found to have bi-directional blood flow through their PDA and thus was excluded from the analysis. This was to avoid the potentially confounding effects of the differing physiology between a bi-directional and a purely left-to-right shunt. The data from 5 echocardiograms was therefore used.

The following echocardiographic parameters were compared to PWV and Phase Difference to assess their relationship:

- PDA Diameter (Figure 27);
- Left Atrial:Aortic Ratio (Figure 28).



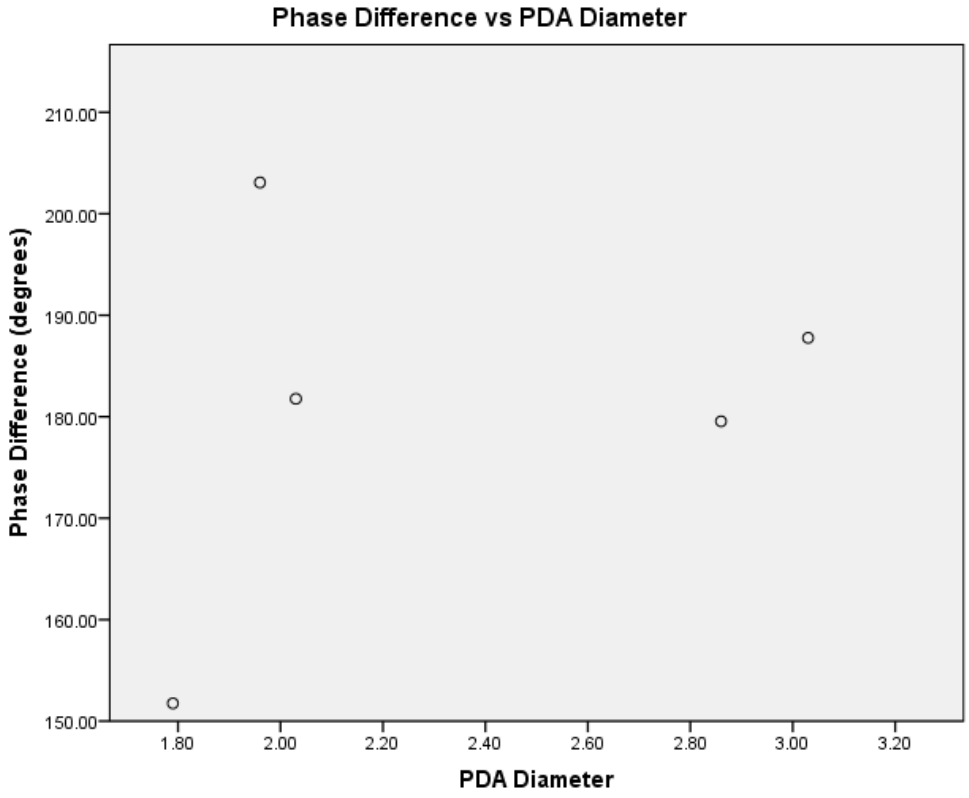
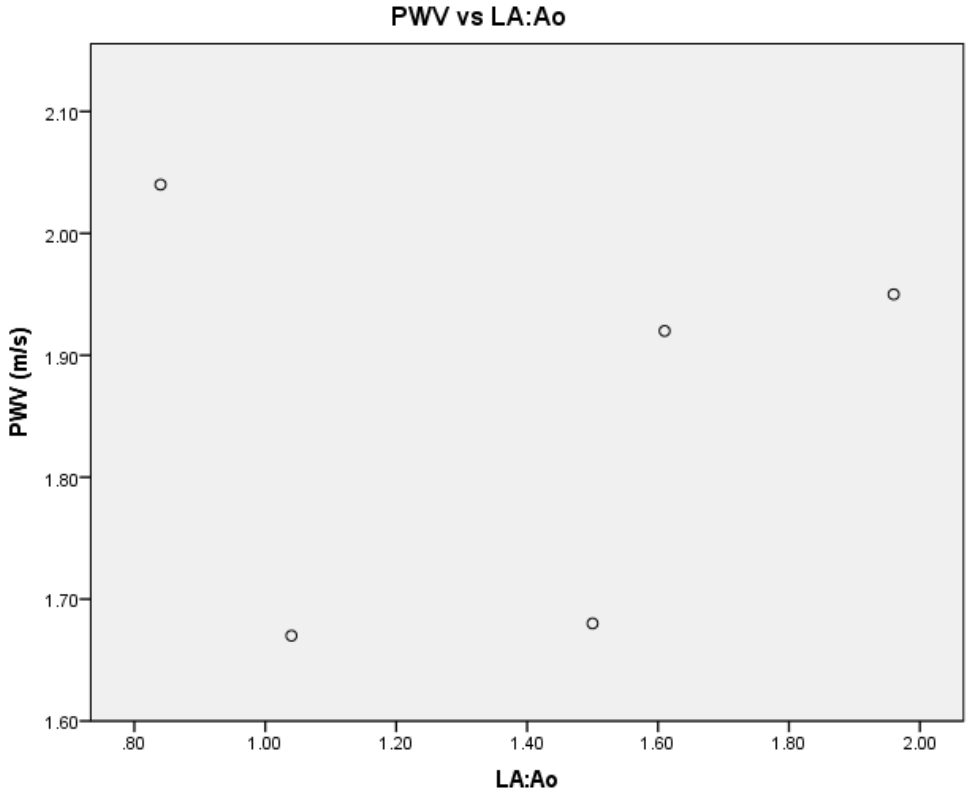


Figure 27 The Relationship between PWV/Phase Difference and PDA Diameter



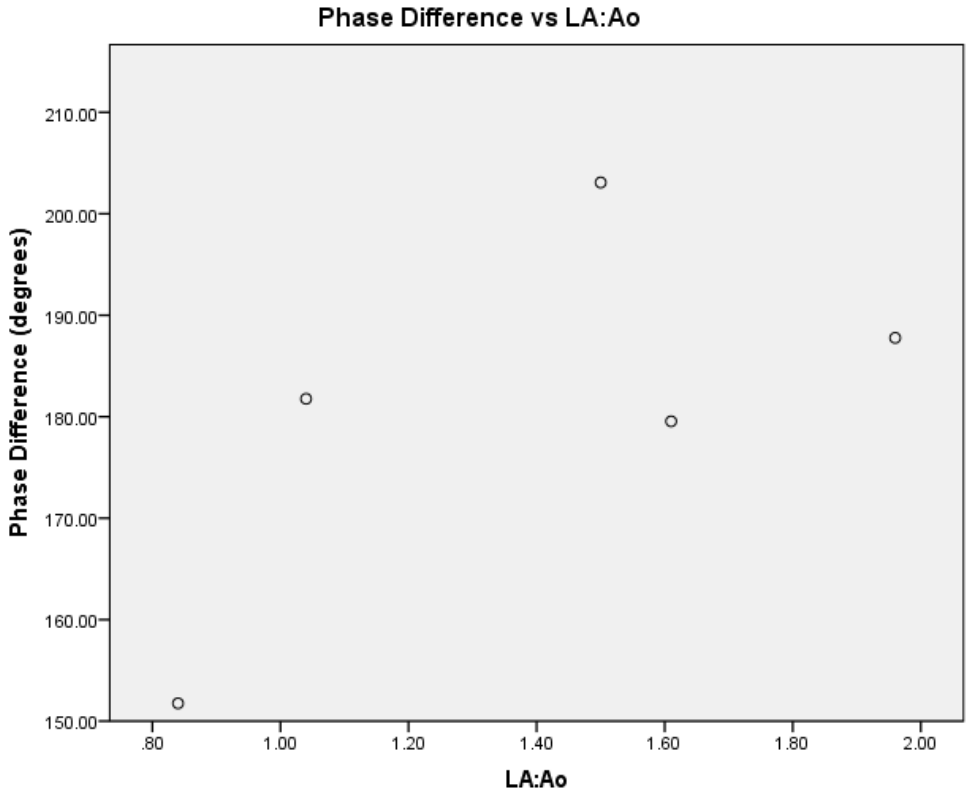


Figure 28 The Relationship between PWV/Phase Difference and LA:Ao

An overview of the relationships between PWV/Phase Difference and the above echocardiographic variables, as well as the strength of their associations, is exhibited in Table 10.

PWV:			
Variable:	**Rho:**	**Strength:**	**P value:**
PDA Diameter	-0.10	Weak	0.873
LA:Ao	0.00	Weak	1.000
Phase Difference:			
PDA Diameter	0.30	Weak	0.624
LA:Ao	0.50	Weak	0.391

Table 10 Overview of the Relationship between PWV/Phase Difference and Echocardiographic Variables

Chapter 6: Investigating Novel Biomarkers in Neonatal Haemodynamics – A Discussion

The analysis of the variation of measurement and the association between PWV/Phase difference and demographic variables is an important first step in their validation as potential biomarkers in neonatology. We demonstrated median values for PWV and phase difference in our study population. We also outlined the variability of distribution and range of values observed. Although this is an important initial step in the estimation of "normal" values, conclusions cannot be made due to the small sample size of this study. It is hoped that future research can build on this data to help identify a normal range of values for this population.

There was a strong association between PWV and birthweight/gestational age at birth (r = 0.8, p = 0.01 and r = 0.95, p = <0.01 respectively, Table 8). Furthermore, a strong association was observed between PWV and corrected GA (r = 0.90, p = 0.001, Table 8). Phase difference showed weak, non-significant associations with all demographic variables except for the comparison between mean phase difference and day of life (r = -1.00, p = 0.01, Table 8). This strong relationship exhibited an overall decrease in phase difference across the first three days of life. This relationship differed from the comparison between phase difference and postnatal age. Based on the relationship between mean phase difference and day of life, we would expect the relationship between phase difference and postnatal age to follow a similar trend; however, no association was exhibited. The different associations between PWV and phase difference suggest that these parameters are capturing different aspects of haemodynamic physiology. We can speculate that a biomarker that is strongly associated with maturity (indicated by GA) may be less strongly associated with the state of a PDA than a biomarker that is associated with day-to-day changes.

We note a potential drawback of monitoring systems that calculate algorithmic "scores" based on the patient's condition at different time intervals. Although every effort was made to collect

data on patients after cares had been performed, they often still required investigations during periods of data collection. The process of disrupting the patient – for arterial blood gas sampling for example – may cause changes to their physiological state or the functioning of the UAC. This may temporarily alter measurements during that period of time. The patient may also undergo weaning of oxygen or medication causing a temporary alteration to their physiological state whilst they adapt to new levels of intervention. Furthermore, data may be collected across a period during which the patient suffers a significant apnoea or bradycardia. All of these factors could cause substantial hour-to-hour variation, possibly explaining the lack of correlation noted when comparing phase difference to smaller time intervals. It may be useful for future studies to compare PWV/phase values before and after alterations in care or changes in clinical state to assess their impact on the measurement of these biomarkers.

Analysis of clinical variables showed some potential associations with PWV and phase difference. A notable difference was observed between those who had and had not received treatment with surfactant. It has been suggested that sustained high pressure ventilation following the administration of surfactant can cause increased intrathoracic pressure (157). This subsequent raised intrathoracic pressure can impede venous return, thus reducing cardiac output and systolic blood pressure. Based on this observation, the reduction in PWV noted in patients administered with surfactant may be explained by the effect of a reduced blood vessel radius on the Moens-Korteweg equation. However, increased intrathoracic pressure may also affect the elasticity of arterial vessels. Based on the Moens-Korteweg equation, this would cause an increase in PWV. Although there was no evidence of an association between mean airway pressure and PWV, future research should aim to further analyse the effect of raised intrathoracic pressure on these biomarkers. One aspect of our results that was not in keeping with what we would expect based on the Moens-Korteweg equation was the relationship between PWV and haemoglobin level. An increase in haemoglobin causes an increase in blood viscosity (158), which we would expect to cause a decrease in PWV. This was not the case as a

moderate and significant association was noted with an increase in haemoglobin being associated with a higher PWV value ($r = 0.69$, $p = 0.04$, Table 9). Due to the small sample size associated with this study, multivariate analysis to assess potential confounding effects on this relationship could not be performed. Although weak, non-significant correlational analysis was noted ($r = 0.27$, $p = 0.488$ and $r = 0.26$, $p = 0.500$ respectively, Table 9), the trend on visual inspection suggested a possible positive relationship between phase difference and both HeRO score and CRP on day 1 of life. HeRO score and CRP are used as infection markers. HeRO score is used as an early warning scoring system on the Liverpool Women's Hospital NICU. It works by detecting alterations to heart rate variability, thought to be caused by the release of inflammatory cytokines during a period of infection. An algorithm subsequently produces a score for the patient every hour. Identification of an increasing HeRO score is suggestive of a progressing infection; a septic screen is therefore performed and the patient will be started on a course of empirical antibiotic therapy whilst awaiting results from the screen. We would therefore expect to see a negative correlation between them and PWV due to the vasodilatory effects of infection. However, no participant recruited as part of this study was diagnosed with blood culture confirmed sepsis and no significant correlation was noted between PWV and markers of infection. Although the mechanism is unclear, the association between phase difference and HeRO/CRP may be explained by other factors affecting the normal physiological functioning of the neonate. It has been noted that preterm neonates have reduced heart rate variability compared to term neonates (159). It is thought that this may be due to an immature autonomic nervous system. One explanation for this could be a potential association between autonomic function/heart rate variability and phase difference, exhibiting as a positive trend in relation to HeRO score in this study. Future research could benefit from assessing this relationship. Neonates can exhibit an inflammatory response for a number of reasons apart from infection, including hypoxia or poor tissue perfusion (160). The trend observed between CRP and phase difference may therefore be due to an unrelated inflammatory response that happened to

coexist at the time of data collection. A moderate and statistically significant negative correlation was observed between phase difference and fluid replacement ($r = -0.72$, $p = 0.03$, Table 9). A possible explanation for this phenomenon may be the effect of circulating volume on the mass flow of blood in the aorta. For example, a reduced rate of fluid replacement may result in lower circulating volume which will minimise the effect of mass flow of blood thus prolonging the pulse transit time. A moderate but non-significant positive association was noted between phase difference and mean airway pressure. This is in keeping with the negative association between PWV and mean airway pressure, as it would be expected for phase difference to increase as PWV decreases.

The comparison of PWV in male and female populations showed a considerable difference between sexes, however, the overlap in error bars suggests that this difference is not statistically significant. One possible reason for this possible discrepancy is the differences in male and female populations included in this study (6 males, 3 females). Another possible reason for the observed difference is the difference in average gestational age of the two populations. The mean gestational age at birth in the male population was 25.88 weeks compared to a mean of 27.57 weeks in the female population. PWV is dependent on the measurement of the approximate distance for blood to travel between the aortic valve and the tip of the UAC catheter. We speculate that infants with increased gestation age in the absence of intrauterine growth restriction will have increased length and weight, thus increasing the distance aspect of the PWV calculation leading to a greater overall value.

There were a number of demographic and clinical variables that the authors wanted to compare to the proposed biomarkers but could not due to their absence in the study population. There was only one set of twins included as part of this study. It was therefore not possible to investigate the effects of multiple pregnancies on the measurement of PWV and phase difference. Inotropes were administered for one day in one participant and so the impact of

their use could not be assessed. Furthermore, at no point was any neonate treated with ibuprofen or diagnosed with blood culture confirmed sepsis. Future research should endeavour to analyse the effects of these variables on PWV and phase difference as they have the potential to cause significant effects on their measurement.

Comparison of PWV and phase difference with echocardiographic variables showed no strong or statistically significant correlations. This has somewhat disproved the authors' hypothesis of the presence of a PDA causing a measurable effect on the measurement of PWV and phase difference. However, the trend on visual analysis suggestion suggests a possible positive trend between phase difference and both PDA diameter and LA:Ao. Notably, the correlation coefficient for phase difference and LA:Ao was 0.5. Furthermore, there was a potential outlier in the comparison of PDA diameter and PWV. This participant exhibited a high PWV (2.03 m/s) despite a relatively small PDA diameter (1.79mm). This did not appear to be in keeping with the trend exhibited by the other four readings. On review of the patient's history and management, no identifiable cause for this discrepancy could be identified. Since this data is based on only 5 echocardiographic readings, a possible association cannot be excluded. It is therefore believed that further research is needed into the association between echocardiographic markers of PDA and PWV/phase difference in order to make more conclusive comments regarding their relationship.

This study was informed by the evidence outlined in chapters 2 and 3. The continuing lack of consensus regarding the clinical importance, investigation and management of PDA in neonatal care highlights the need for continued research. We identified the potential benefit of novel biomarkers to supplement the use of echocardiography in the diagnosis and prognostication of PDA disease. The non-invasive nature of the measurement of PWV in those with UACs in situ was also thought to be clinically beneficial by providing an alternative method of investigation that would help reduce the number of procedures performed on patients in NICU.

The process of validation of a novel biomarker requires a rigorous process of validation. "Normal" distribution of its measurement across the proposed population of benefit must first be conducted. Potential confounders and variables thought to significantly alter its measurement must also be sought. This provides additional data in relation to those in whom using the biomarker is not indicated. Without this, comparison of a biomarker with its proposed diagnosis is irrelevant. Throughout biomarker validation the appropriateness, acceptability and accessibility of its use in clinical practice should be reviewed. We believe that the aims of this study were appropriate for beginning the process of validation for a novel biomarker. Chapter 4 reviewed the variation in measurement of PWV in a population where there is limited evidence. It also assessed the association between a number of potential clinical confounders with the proposed biomarker. Chapter 5 then illustrated its potential impact on current practice by comparing its measurement to a clinical condition.

Our study sought to add additional information to what had already been published in literature. Previous studies reviewing pulse phase difference as a method of evaluating PDA (*Goudjil et al, Kotidis et al*) conducted their studies exclusively in preterm neonates (<32 weeks and <29 weeks, respectively). Our study aimed to include babies of all gestations provided they had a UAC in situ. This would have allowed us to compare PWV and phase difference measurements in both term and preterm populations. Although our sample size for term neonates was deemed too small to assess as a separate population, we hope this data can be used in future research to assess the variation in measurements in term neonates. We also sought to investigate additional clinical parameters to evaluate their effect on PWV/Phase Difference measurement. These include variables such as mean airway pressure that were not assessed in previous literature. This study was also conducted using a UAC and arterial waveform rather than a pulse oximeter and arterial photoplethysmography. This was in the hope of identifying a new and more accurate method of measuring the proposed biomarkers.

We believe that our methodology was appropriate to answer our proposed questions. The non-interventional nature of such a study was favourable with regards to study uptake. No parents opted-out of study participation. Every effort was made to provide parents with information about the study. This was via ward posters and discussions at the bedside with researcher BR. At no point was additional information in the form of information leaflets required. It is thought that this was due to the non-interventional nature of the study with zero physical patient contact. Furthermore, the 0% opt-out rate noted in this study will have eliminated the risk of volunteer bias. Our inclusion criteria ensured that there was a clearly outlined study population that would be studied within a set period of time in early neonatal life. Furthermore, our exclusion criteria helped minimise the effect of potential clinical confounders which may have skewed our results. Removal of patients with conditions such as significant congenital cardiac disease or hydrops fetalis helped reduce the risk of anomalous results due to abnormal physiology. Although future studies reviewing the use of PWV in neonatal populations may benefit from reviewing its measurement in patients with physiological or anatomical anomalies causing alterations in haemodynamic function, this was deemed inappropriate for the early stages of biomarker validation. For those recruited as part of the study, all appropriate routinely collected clinical data was documented. This allowed the comparison of a number of factors thought to potentially effect the measurement of PWV. Examples include ventilation settings (effects of intrathoracic pressure), fluid replacement (effects of circulating volume) and haemoglobin level (viscosity of blood). The use of arterial waveforms provided by the presence of a UAC helped reduce variation in readings. UACs are placed between T6-T9 and confirmed by radiography, providing consistent placement between participants. As well as this, the close proximity of the catheter tip to the site of the PDA improves the accuracy of the measurement of its effect on PWV.

Every attempt was made to ensure consistency and validity of data collected. Researchers ensured data collection occurred while the patient was not being disturbed to avoid any external

influence on the results recorded. Data collection was almost always conducted between cares and after any scans had been performed. The only exception to this was when patients were having echocardiograms performed as this allowed us to collect data relative to the patient's disease state at that particular time. Prior to data collection, arterial and ECG waveforms were subject to quality assessment by researcher BR. Arterial waveforms were optimally scaled and the ECG lead (I, II or III) that was producing the largest and most consistent R waves was selected. This ensured clarity of the peaks of both the R wave and arterial wave, minimising the potential for error in data analysis. All echocardiograms were reviewed by one member of the research team (CK – paediatric cardiology registrar). Measurements of PDA diameter and LA:Ao were determined by CK using pre-recorded images.

There were a number of limitations associated with our study design. Although the aim of one aspect of the study was to collect normative data to evaluate the biomarker in early neonatal life, the ability to only recruit participants for the study within the first 3 days of life impacted echocardiogram data collection. In a number of instances, a patient may have been >3 days old with a UAC in situ and having an echocardiogram performed, however, if they had not been recruited in the first 3 days of life, their data could not be included in the study. Although a functional UAC was deemed to be an accurate method of measuring the arterial waveform, in many cases they proved problematic. Often the UAC was left in situ despite not tracing as it still provided access for arterial blood sampling. This was the case for one study participant on day 10 of life, meaning that no data collection could occur during an echocardiogram. There were times where, despite UAC quality assurance checks and optimisation of scale, the trace was still considered poor. This inconsistency of readings highlights a potential drawback in the use of UACs for determining PWV. Furthermore, the distance aspect of the PWV equation was determined by a researcher measuring the approximate route of the aorta based on the most recent chest radiograph performed at the time of data collection. Although anatomical landmarks such as the carina were used to maintain consistency in the tracing of the

approximate route of the aorta, this measurement was subject to a number of potential limiting factors. A chest radiograph does not take into account posterior-to-anterior orientation of the aorta; the aortic valve is radiolucent and thus its approximate location was used; clarity of the catheter tip and location of the carina were occasionally difficult to precisely measure due to suboptimal radiograph exposure; and finally, radiographs performed on NICU patients are often rotated. Despite the same method being used by the same researcher for all measurements, all of these factors may have resulted in inaccuracies in the calculation of PWV. For this reason it is believed that phase difference may be a more appropriate method of expressing pulse transit time as it is not dependent on aortic measurements. Certain aspects of data collection were also dependent on other members of the clinical care team. Collection of demographic, perinatal and clinical data was dependent on proper documentation on the Trust's "Badger" system. Although documentation was usually of a high standard, there were a number of areas where data was often missing. Maternal details such as smoking history or steroid exposure was often missing. This was possibly due to the fact many participants in the trial were born preterm and this data had not been recorded, or there was not time to provide steroids. This may have potentially skewed our analysis of the study demographic, as well as under- or overestimated the impact of surfactant administration on the measurement of PWV in early neonatal life. Furthermore, echocardiograms were performed by whichever neonatologist was working on NICU that day. This may have introduced some degree of between-user variability within the echocardiographic data recorded.

The Baby OSCAR early intervention in PDA trial was running concurrently with this research. This is a multi-centre randomised placebo controlled trial of ibuprofen for extremely preterm babies who had been diagnosed with a large PDA. This was both beneficial and detrimental to data collection. Those who were potentially eligible were screened by Doppler echocardiogram in the first 72 hours of life. This allowed researchers to collect time-specific data that could be accurately compared to the current PDA status of a study participant. However, those deemed

eligible for participation in the OSCAR trial do not have another echocardiogram for 3 weeks unless there is a decline in clinical condition. This may have reduced the number of babies having echocardiograms in our 3-10 day window as it may have affected the threshold at which a clinician deemed echocardiography was appropriate in study participants. Furthermore, babies recruited into the Baby OSCAR trial are treated with either ibuprofen or a placebo. Although there was no open-label use of ibuprofen in our study population, we cannot exclude the possibility that participants may have been potentially administered ibuprofen as part of their inclusion in the Baby OSCAR trial. The blinded nature of this concurrent study meant we could not assess whether there was not any association between PWV/phase difference and ibuprofen administration in those recruited in our study that were also part of the treatment arm of the Baby OSCAR trial. The results of our study are also likely to be subject to an element of sample size bias due to the small number of participants recruited as part of the study. UAC insertion rates in the three months prior to protocol submission were reviewed in an attempt to provide an estimate of the number of participants we expected to recruit to this study. This review projected that we could expect to recruit approximately 30 participants over a period of three months. During this sample size estimate, we failed to take into account patients in this period that would not have been suitable for recruitment. UACs are often inserted to critically ill neonates who require inotropes or regular arterial blood gas monitoring. Often these patients are suffering from serious conditions that would have excluded them from our study. Our inability to take this into account during our sample size estimate may have led to an over-ambitious prospective number of recruits. As highlighted by Figure 8, 32 UACs were inserted during a 3 month data collection period but 6 were unable to be recruited due to significant comorbidity or death within 24 hours. Exposure to potential participants was also hindered by issues surrounding ethical approval for the study. Initial delay in beginning data collection due to incomplete IRAS documentation meant data collection was started almost seven weeks later than anticipated. Between the proposed study start date (02/01/18) and the actual study start

date (18/02/18) there were 24 UACs inserted on the unit, 16 of whom would have been deemed suitable for recruitment in this study. In addition to this, a non-substantial amendment form had to be submitted to extend the period of data collection initially suggested in the protocol. Delay in the issue of extension resulted in an additional potential participant not being included in the study. There is, however, potential for the data of this study to be combined with that collected by *Kotidis et al* as part of the pilot study that informed this research project. Of the nine participants in this study, 8 were below the gestational age cut-off point (29 weeks) for inclusion in the pilot study. This would provide a larger sample size from which more reliable data could be extracted in the hope of improving the likelihood of future publication.

Our study exemplifies a number of the drawbacks of biomarker research in paediatric populations highlighted by *Savage and Everett* (59). Our study was subject to a small sample size meaning that firm conclusions cannot be made from our data set alone. As well as this, our study lacked a true control group. Although we were able to demonstrate baseline values of PWV and phase difference in our study population, there were no truly "healthy" participants in the study as they were all premature. Furthermore, a control group to compare to the term infants included in our study in future studies would not be plausible as UAC insertion in healthy term neonates would be unethical. Therefore assessment of "normal" PWV and phase difference values cannot be truly estimated using this method. Use of a method of calculating PWV/phase difference without the insertion of an arterial line may therefore be indicated in the estimation of "normal" thresholds. For example, further research regarding the use of peripheral saturation probes and photoplethysmography as exhibited in a recent study (68) may be beneficial.

Although a number of the drawbacks of this study were outside of the control of the research team, there are a number of ways the study could be improved if it was repeated. Expanding the inclusion criteria to allow recruitment outside of the first three days of life may have helped increase the amount of data collected between days 3-10 of life. Furthermore, conducting the

study in a centre that was not part of the Baby OSCAR trial may have increased the number of echocardiograms performed during this period. All data collection was performed by researcher BR. Although this ensured consistency in data collection methods, the lack of a larger research team contributed to reduced participant recruitment. A number of potential candidates were missed during periods when BR was not on the unit. It would be unfeasible for one researcher to be present on the unit at all times to ensure all potential candidates were recruited. If this study was to be repeated on a larger scale, it would be beneficial to have a number of researchers trained in the method of data collection. This would ensure that neonates admitted to the unit overnight or during weekends had a better rate of recruitment. This would also improve the power of the study, and allow additional statistical analysis to be performed on a larger cohort. Conducting the research across a number of centres and across a greater period of time would further improve the sample size of subsequent research. This would remove any potential sample size bias that this study may have been subject to.

6.1 Conclusion

This study aimed to review the use of PWV and phase difference as potential biomarkers for implementation in clinical practice. Based on our comparisons between demographic and clinical variables, it is thought that there are few variables that have significant impact on their measurement. All variables which were shown to have a strong correlation with the biomarkers (birthweight, gestational age at birth, corrected gestational age and day of life) could be easily accounted for if these biomarkers were to be introduced into clinical practice. Although no firm associations were noted between these biomarkers and echocardiographic markers of PDA, the overall trend exhibited by visual analysis suggested a potential positive association between phase difference and PDA diameter/LA:Ao.

Overall, the sample size for this study is too small to make conclusions about the effect of demographic, clinical and echocardiographic variables on the measurement of PWV and phase

difference; however, we believe this study warrants further research in the field of PWV/phase difference validation and the use of novel biomarkers in the management of PDA.

Appendix 1 – Systematic Review Literature Search Results

PubMed Literature Search Results:

Search Term(s):	Results:
"Patent Ductus Arteriosus"	11,365
"Patent Ductus Arteriosus" OR "PDA"	18,747
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus"	19,088
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate"	5461
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal"	5754
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn"	5895
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm"	5987
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature"	6098
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature" OR "Prematurity"	6104
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature" OR "Prematurity" AND "Ibuprofen"	378
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature" OR "Prematurity" AND "Ibuprofen" OR "Indomethacin"	1211
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature" OR "Prematurity" AND "Ibuprofen" OR "Indomethacin" OR "Indometacin"	1214
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature" OR "Prematurity" AND "Ibuprofen" OR "Indomethacin" OR "Indometacin" OR "Paracetamol"	1238
Limited to Randomized Controlled Trials	126

Scopus Literature Results:

Search Term(s):	**Results:**
"Patent Ductus Arteriosus"	16,386
"Patent Ductus Arteriosus" OR "PDA"	37,179
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus"	37,330
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate"	2069
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal"	3877
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn"	8221
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm"	8399
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature"	8645
"Patent Ductus Arteriosus" OR "PDA" OR "Persistent Ductus Arteriosus" AND "Neonate" OR "Neonatal" OR "Newborn" OR "Preterm" OR "Premature" OR "Prematurity"	8741
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OR “Premature” OR “Prematurity” AND “Ibuprofen” OR “Indomethacin” OR “Indometacin” OR “Paracetamol” AND “randomi*ed controlled trial”	
Minus duplications from PubMed Search	171

Web of Science Literature Search Results:

Search Term(s):	**Results:**
“Patent Ductus Arteriosus”	6951
“Patent Ductus Arteriosus” OR “PDA”	21,527
“Patent Ductus Arteriosus” OR “PDA” OR “Persistent Ductus Arteriosus”	21, 739
“Patent Ductus Arteriosus” OR “PDA” OR “Persistent Ductus Arteriosus” AND “Neonate”	1132
“Patent Ductus Arteriosus” OR “PDA” OR “Persistent Ductus Arteriosus” AND “Neonate” OR “Neonatal”	1908
“Patent Ductus Arteriosus” OR “PDA” OR “Persistent Ductus Arteriosus” AND “Neonate” OR “Neonatal” OR “Newborn”	2393
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OR “Neonatal” OR “Newborn” OR “Preterm” OR “Premature” OR “Prematurity” AND “Ibuprofen” OR “Indomethacin” OR “Indometacin” OR “Paracetamol”	
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Appendix 2 – Systematic Review: Excluded Papers

Author (Year):	Reason for Exclusion:
Adamska et al (2005) (161)	Unable to attain translation.
Aly et al (2007) (162)	Did not outline criteria for haemodynamic significance.
Asbagh et al (2015) (163)	Did not outline criteria for haemodynamic significance.
Bagheri et al (2016) (164)	Did not outline criteria for haemodynamic significance.
Bravo et al (2014) (165)	Did not outline criteria for haemodynamic significance.
Chotigeat et al (2003) (166)	Did not outline criteria for haemodynamic significance.
Couser et al (1996) (167)	Did not outline criteria for haemodynamic significance.
Dang et al (2013) (168)	Did not outline criteria for haemodynamic significance.
DeMauro et al (2013) (169)	Did not outline criteria for haemodynamic significance.
Hammerman et al (2005) (170)	Not an RCT.
Hammerman et al (2008) (171)	Did not outline criteria for haemodynamic significance.
Kaapa et al (1982) (172)	Used aortograms, not echocardiograms.
Krueger et al (1987) (173)	Did not outline criteria for haemodynamic significance.
Lin et al (2012) (174)	Did not outline criteria for haemodynamic significance.
Merritt et al (1981) (175)	Did not outline criteria for haemodynamic significance.
Nestrud et al (1980) (176)	Did not outline criteria for haemodynamic significance.
Peckham et al (1984) (177)	One-year follow-up of another paper included in study.
Pourarian et al (2008) (178)	Did not outline criteria for haemodynamic significance.
Pourarian et al (2015) (179)	Did not outline criteria for haemodynamic significance.
Rhodes et al (1988) (180)	Did not outline criteria for haemodynamic significance.
Van Overmeire et al (2000) (181)	Did not outline criteria for haemodynamic significance.
Van Overmeire et al (2001) (182)	Did not outline criteria for haemodynamic significance.
Van Overmeire et al (2003) (183)	Not an RCT.
Van Overmeire et al (2004) (184)	Did not outline criteria for haemodynamic significance.

Appendix 3 – Research Protocol

Investigating Pulse Wave Velocity as a Novel Biomarker in Neonatal Haemodynamics

Version 2.0

06/11/18

Main Sponsor: University of Liverpool

Funders: Student project and Liverpool Women's NHS Foundation Trust

Study Coordination Centre: Liverpool Women's Hospital NHS Trust

NRES Reference: 18/NE/0058

Study Team:

Chief Investigator: Dr Mark Turner

Co- Investigators: Dr Charalampos Kotidis, Dr Daniel Hawcutt, Ben Rodgers

Statistician: Antonio Eleuteri.

Study Manager: Not applicable.

Study Coordination Centre:

For general queries, supply of study documentation, and collection of data, please contact Ben Rodgers who will discuss with the appropriate clinician:

Tel: 07714002504

Email: hlbrodge@student.liverpool.ac.uk

Clinical Queries:

Clinical queries should be directed at Ben Rodgers (see details above).

Sponsorship:

The University of Liverpool is the main sponsor for this Study. For further information regarding the sponsorship conditions, please contact:

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Study Summary:

This protocol describes the "Pulse Wave Velocity and Phase Difference as Novel Biomarkers in Neonatal Haemodynamics" study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the primary investigator (Ben Rodgers).

This study will adhere to the principles outlines in the NHS Research Governance Framework for Health and Social Care (2nd Edition). It will be conducted in compliance with the protocol, Data Protection Act and other regulatory requirements as appropriate.

Glossary of Abbreviations:

ANP	Atrial Natriuretic Peptide
ANS	Autonomic Nervous System
BNP	Brain Natriuretic Peptide
BPD	Bronchopulmonary Dysplasia
BW	Birth Weight
DA	Ductus Arteriosus
ECG	Electrocardiogram
GA	Gestational Age
GI	Gastrointestinal
HRV	Heart Rate Variability
hsPDA	Haemodynamically significant Patent Ductus Arteriosus
IVH	Intraventricular Haemorrhage
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
NO	Nitric Oxide
NPV	Negative Predictive Value
NSAID	Non-Steroidal Anti-Inflammatory Drug
NT-proBNP	Amino-terminal pro-B-type Natriuretic Peptide
PDA	Patent Ductus Arteriosus
PNS	Parasympathetic Nervous System
PPV	Positive Predictive Value

PWV	Pulse Wave Velocity
RAAS	Renin-Angiotensin-Aldosterone System
RDS	Respiratory Distress Syndrome
REC	Research Ethics Committee
SNS	Sympathetic Nervous System
SSA	Site Specific Assessment
UAC	Umbilical Arterial Catheter

Keywords:

Pulse Wave Velocity, Patent Ductus Arteriosus (PDA), Neonatology

Title:

Investigating Pulse Wave Velocity as a Novel Biomarker in Neonatal Haemodynamics.

Design:

Prospective Observational Study

Aims:

To better understand the effects of a number of continuous and dichotomous variables on the measurement of pulse wave velocity in neonates.

Outcome Measures:

The relationship between pulse wave velocity and a number of variables affecting neonatal haemodynamics in the first three days of life.

Population Eligibility:

Inclusion Criteria:

- Neonate admitted to Liverpool Women's Hospital Neonatal Unit;
- Age 0-3 days;
- Umbilical Arterial Catheter (UAC) placed in situ for clinical care within 3 days of birth;
- Transducer capable of being fitted to UAC without additional intervention/disruption to the patient.

Exclusion Criteria:

- Age >3 days;
- UAC not sited or not capable of being fitted to transducer;
- Survival of baby expected to be <72 hours;
- Additional congenital heart disease or conditions expected to alter cardiovascular function (ventricular septal defect, atrioventricular septal defect, tetralogy of Fallot, transposition of great vessels, truncus arteriosus, coarctation of the aorta, congenital valvular disease, and congenital hydrops).
- Chromosomal abnormalities;
- Considered unsuitable for recruitment by nursing staff and Consultant Neonatologist at Liverpool Women's Hospital.

Duration:

Data collection will occur on site by the primary investigator (Ben Rodgers) from February 18th to 1st June 2018. Statistical analysis and write up will be performed in June 2018.

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1. Introduction:

1.1. Background:

Preterm birth, defined as birth at less than 37 weeks gestational age(1), occurs in 5-10% of births in resource-rich countries such as the UK and USA(2). Preterm neonates are a population with a unique set of potential pathologies associated with high rates of mortality and long-term morbidity. From the development of antepartum steroid and surfactant therapy for neonatal respiratory distress syndrome (RDS) (4, 5), to the use of probiotics in the prevention of necrotising enterocolitis (NEC) (185), survival rates in both term and preterm neonates have improved drastically across the globe(7, 8). However, *Lawn et al* reported in 2014 that there were still 2.9 million neonatal deaths worldwide(9) and the Office for National Statistics reported that between 2014 and 2015, immaturity-related conditions were responsible for between 49.3-50.3% of neonatal deaths in England and Wales(10). This demonstrates that there is still a clear need for improved care within the field of neonatology to help improve outcomes.

Biomarkers in Neonatology:

Biomarkers are defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological response to a therapeutic intervention"(53). Neonatology has seen an increase in biomarker research to help improve the diagnosis and management of life-threatening conditions. For example, conventional biomarkers such as interleukins and cytokines involved in the immune cascade have been extensively researched in relation to neonatal sepsis (54-56). However, these biomarkers are not specific to a particular disease or organ system. There has thus been a call for further investigation into "novel" biomarkers to help improve diagnostic capabilities and drug development in neonatology and paediatrics (57, 58). *Savage and Everett* outlined the desirable traits of biomarkers (59). Ultimately, a biomarker should consist of a sample that is acceptable and accessible combined with an appropriate and accurate assay. However, when investigating a novel biomarker for its association with a specific clinical outcome, they suggest it is important to consider the following:

IV. Assay performance – is the assay accurate, reliable and responsive to the condition they are investigating?
V. Qualification – does the biomarker have a robust relationship with the current gold standard investigation for the proposed clinical outcome?

VI. Utility – can the biomarker improve care beyond what is already available? (59)

Kearns has also outlined the importance of considering the effects of normal growth and development before a biomarker is implemented in clinical practice (58). In order for biomarkers to be considered useful, there therefore needs to be a rigorous process of validation before they can be considered for implication in clinical practice (60-62).

Pulse Wave Velocity and Phase Difference: Novel Biomarkers in the Assessment of Neonatal Haemodynamics?

Pulse wave velocity (PWV) is a method of measuring arterial stiffness that is calculated from measurements of the time it takes the impulse arising from a contraction of the heart's ventricles to travel between two recording sites – the so-called "pulse transit time" (70). The measurement of arterial stiffness, which is inversely proportional to PWV, helps us understand the mechanics of large vessels and thus circulatory physiology and disease (70). It has been suggested that the concurrent analysis of heart rate variability and PWV can also be used to assess peripheral vascular sympathetic functions in adults which reflect a number of pathologies (77). Furthermore, although it has typically been used in adult populations in the past, it has been shown that PWV can be accurately measured and easily reproduced when assessing the vasculature of the neonate (78). The factors affecting PWV can be shown by the Moens-Korteweg equation (figure 1):

$$PWV = \sqrt{\frac{E \cdot h}{\rho \cdot 2R}},$$

Figure 4 Moens-Korteweg equation; E = incremental modulus of stiffness of the vessel; h = vessel wall thickness; p = density of blood; R = radius of blood vessel

Any factors that affect the components of this equation can cause variations to the measurement of PWV. Although predominantly a disease of older populations, arterial stiffening has been noted in paediatric populations with conditions associated with cardiovascular disease such as diabetes mellitus and chronic kidney disease (79, 80). Hypotension is a common problem for patients admitted to NICU and the resulting increased diameter of blood vessels will result in a lower pulse wave velocity.

Further insight into the stiffness of blood vessels can be gained by measuring the difference in time between the R wave of an electrocardiogram (ECG) wave (corresponding to ventricular contraction) and the peak of the systolic blood pressure wave measured at a known point. This value is known as the phase difference. This helps measure the differences in mass flow of blood compared to that of the transmission of the pulse waves within the vessels.

In neonates, the recording of the systolic blood pressure wave requires the insertion of an umbilical arterial catheter (UAC). UAC's are standard practice in neonates who require invasive haemodynamic monitoring or frequent blood tests in the days after birth (74). The UAC is connected to a transducer which provides real-time information to the patients monitor, allowing the arterial waveform to be observed. The use of appropriate software allows the information on the arterial waveform of the neonate to be collected and compared to the ECG R waves to calculate the phase difference.

PWV is an established biomarker in adult populations and has shown potential with regards to its use in paediatrics (152). However, the transitional circulation is extremely complex, with a number of variables affecting the haemodynamics of the neonate. These include gestational age, weight, the open fetal channels (patent foramen ovale (PFO) and PDA), inotropic support, or comorbidities such as IVH and lung disease. Further research into the effects of these potential variables is necessary to further validate PWV and phase difference as novel biomarkers in neonatology before their use in clinical practice is further assessed.

The Patent Ductus Arteriosus:

Although essential for fetal life whilst in utero, persistence of the DA into infancy can have significant haemodynamic consequences, notably disruption to cerebral and systemic circulation.

A persistent patent ductus arteriosus (PDA) is a common condition within neonatology. It occurs in approximately 1 in 2000 neonates born at term (38) and is inversely proportional to birthweight in premature neonates, with the incidence ranging from 30-50% (14). *Clyman et al* reported that combined data from five other studies showed that spontaneous closure of a PDA after 7 days of life occurred in only 36% of patients born at 27-28 weeks gestational age (GA), in 32% of those born at 25-26 weeks GA, and in just 13% of those born at 24 weeks GA (15). There are a number of factors that contribute to the persistence of a PDA in preterm neonates. It is thought that as term babies approach birth, there is a decrease in the sensitivity of ductal cells to the vasodilatory prostaglandins that help keep the DA patent (39). Ductal cells in the preterm

infant are thought to be more sensitive to vasodilatory prostaglandins, and this in combination with the immature fetal lungs' reduced ability to metabolise prostaglandins contribute to the failure of the DA to close (40, 41).

PDA is associated with a number of adverse outcomes in infancy. It has been associated with life-threatening conditions such as IVH, NEC and bronchopulmonary dysplasia (BPD) (15-18). Adverse outcomes secondary to PDA, particularly BPD, can be attributed to the so called "steal phenomenon". A left-to-right shunt caused by the lower vascular resistance of the pulmonary vessels results in pulmonary hyperperfusion and systemic hypoperfusion (42). This has a particularly significant effect on the cerebral, renal and mesenteric systems (15, 43).

Current Practices in the Detection and Monitoring of the Patent Ductus Arteriosus:

Although investigations such as ECG and chest radiographs can provide additional information when assessing an infant with a PDA, the gold standard investigation is the echocardiogram (43). This allows direct visualisation and measurement of the severity of the PDA. However, accurate use of echocardiography requires specialist training and exhibits approximately a 10% intra-observer variability and a 15-20% inter-observer variability (186). Furthermore, investigations in neonatal intensive care (NICU) can be detrimental to the health and the development of the preterm infant. Disruption of sleep, increased noise around the incubator and temperature changes associated with interventions can all have adverse effects on the neonate (11-13). Echocardiography provides only a one-off measurement and therefore requires multiple readings to monitor PDA closure, or to assess patients with possible PDA reopening. It is therefore believed that a reduction in unnecessary echocardiography in neonates suspected of suffering from PDA would be beneficial to the overall care of neonates. We postulate that novel biomarkers that allow continuous non-invasive monitoring of PDA in preterm neonates will help improve diagnostic and monitoring practices in NICU.

A recent pilot study performed at the Liverpool Women's Hospital assessed the use of phase difference and pulse wave velocity as potential novel biomarkers in the haemodynamic assessment of extremely preterm neonates. Although it was a small pilot study consisting of just 14 patients, it showed promising results with phase difference showing significant correlation to the PDA diameter ($P = <0.001$, $r = 0.820$). The pilot study also suggested that PWV was related to PDA size in preterm neonates as it alters the parameters of the Moens-Korteweg equation. We postulate that changes to the ANS affecting vascular tone will alter the measurement of PWV in

newborn babies due to changes in vessel diameter. Although PWV and phase difference require further research into the effect of common variables found in neonatology on their validity, it is believed that the results of this pilot study warrant further research.

1.2. Rationale for Study:

Novel biomarkers used in the monitoring of neonatal haemodynamics could significantly improve neonatal outcomes. PWV is a useful biomarker in the investigation of haemodynamics in adult and paediatric populations; however, there is limited evidence in the neonatal population. The continuously changing nature of neonatal haemodynamics during the first days of life possess a significant challenge for the validation of haemodynamic biomarkers due to the multiple simultaneously changing parameters (cardiac, vascular, pulmonary and autonomic adaptation to extrauterine life). Disease and prolonged patency of the fetal channels can further complicate the above interactions. Assessing the impact of potential variables affecting the measurement of PWV and phase difference is crucial for their validation as potential biomarkers in neonatology.

PDA is extremely prevalent in preterm neonates. It often requires serial monitoring in order to both diagnose the condition and assess its response to treatment. It is believed that further research into other non-invasive methods of investigation is warranted to improve its overall management in secondary care. Reducing the number of potentially unnecessary investigation may also help reduce the distress experienced by the patient and their family. Based on the results of the recent pilot study performed at the Liverpool Women's Hospital, we hypothesise that a PDA is associated with alterations in the PWV and phase difference. We postulate that this is likely to be due to the effect of vasodilatation associated with PDA on the Moens-Korteweg equation. We anticipate that the larger the PDA diameter, the lower the PWV and the larger the phase difference.

2. Study Objectives:

The aim of the study is to improve our understanding of the factors affecting the measurement of PWV and phase difference in neonates. A prospective cohort study of neonates admitted to the NICU with a UAC in situ will be performed in the Liverpool Women's Hospital.

The primary objective of this study is to investigate the associations of a number of demographic variables with PWV and phase difference. These are:

I. Gestational Age;
II. Birth Weight;
III. Postnatal Age.
IV. Twin or higher multiple pregnancies.

The rationale for the investigation of the link between PWV, phase difference and these variables is to better understand patient factors that may affect the measurement of these novel biomarkers. In order to validate their measurement as potential biomarkers, it is crucial to identify any features that may cause anomalous results or that may need to be taken into account when calculating their value and applying it to a clinical picture. Features such as GA, weight, age and HRV are greatly variable in neonatal populations and have the potential to affect the overall haemodynamics of the patient. Twin or higher multiple pregnancies can result in disturbances to fetoplacental haemodynamics.

The secondary objective is to investigate the associations of variables that vary after birth on the PWV and phase difference. These include:

I. Inotropic support;
II. Infection – suspected or blood culture confirmed;
III. Intraventricular Haemorrhage (IVH) – presence yes/no +/- grade (1-4);
IV. Autonomic Function (as measured by heart rate variability (HRV));
V. Oxygen saturation;
VI. The tertiary objective is to assess the relationship between the measurement of PWV, phase difference and PDA diameter.

The rationale for investigating these dichotomous variables is their effect on patient haemodynamics. Inotropic support has a direct effect on the blood flow due to their effect on heart contractility. Sepsis causes widespread vasodilatation with a reduction in systemic vascular resistance and changes to cardiac output. Intraventricular haemorrhage is linked to pressure passive cerebral circulation and changes to cerebral autoregulation which are particularly common in preterm infants(187). As previously mentioned, it has been hypothesised that the presence of a PDA leads to a predominance of the PNS, resulting in vasodilatation and thus alterations to the PWV. However, the development of the autonomic nervous system has also been shown to be altered in those born prematurely who do not necessarily suffer from PDA (188). Data on HRV is to be collected to better understand the relationship between the ANS, PDA and PWV. The pilot study previously conducted at Liverpool Women's Hospital assessing

the correlation between PDA and phase difference also found a potential association between the arterial waveform produced as part of the measurement of oxygen saturation and phase difference. A recent study by *Goudjil et al* assessed the measurement of PWV expressed as phase difference as a non-invasive method for the detection of a PDA (68). They collected data on those aged <32 weeks GA and using arterial photoplethysmography collected from a pulse oximeter, they found that phase difference was strongly associated with PDA (optimal pulse phase difference (PPD) cut-off of ≥1.65 deg/cm, with an area under the ROC curve of 0.98 (95% confidence interval, 0.96-1), sensitivity = 94.2%; specificity = 98.3%) and should be considered as a non-invasive method of detection (68). Since oxygen saturation is routinely collected on all babies on the unit, we hope to gather some additional data to further research into this relationship, whilst also comparing it to the data collected from the arterial waveform of the UAC. This will enable us to further assess the validity of PWV/phase difference as a novel biomarker in the investigation of PDA whilst also identifying the optimal method of measurement.

3. Study Design:

This prospective observational study will be conducted at Liverpool Women's Hospital. All neonates admitted who have a UAC within the first three days of life will be eligible for recruitment in the study.

A demographic profile (consisting of routinely collected data only) will be collected from existing clinical records. Data will also be collected on PWV, phase difference, haemodynamic biomarkers of PDA via echocardiogram, dose and rate of infusion of inotropes, and additional comorbidities that are deemed to affect the patient's haemodynamics (appendix 1).

All clinical data to be collected as part of this study will be in line with the standard of care protocol at the Liverpool Women's Hospital. No investigations/procedures will be performed in addition to the standard care patients receive whilst placed in the NICU.

Inclusion and Exclusion Criteria:

Inclusion Criteria:

- Neonate admitted to Liverpool Women's Hospital Neonatal Unit;
- Age 0-3 days;
- UAC placed in situ for clinical care within 3 days of birth;

- Transducer capable of being fitted to UAC without additional intervention/disruption to the patient.

Exclusion criteria:

- Age >3 days;
- UAC not sited or not capable of being fitted to transducer;
- Survival of baby expected to be <24 hours;
- Additional congenital heart disease or conditions expected to alter cardiovascular function (ventricular septal defect, atrioventricular septal defect, tetralogy of Fallot, transposition of great vessels, truncus arteriosus, coarctation of the aorta, congenital valvular disease, and congenital hydrops).
- Chromosomal abnormalities.
- Considered unsuitable for recruitment by nursing staff and Consultant Neonatologist at Liverpool Women's Hospital.

Study Outcomes:

1. Descriptive data about PWV and phase difference in the neonatal period;
2. Information about relationships between PWV and phase difference and:

    A. Demographic data

    B. Variables influences by postnatal events and PDA diameter.

Study Procedures:

Recruitment:

All patients admitted to the unit who have a UAC in situ within the first three days of life will be included in this study. Clinical data that is collected in accordance to the standard of care protocol will be anonymised and analysed in accordance with the aims of the study. Based on the typical patient population that is observed at the Liverpool Women's Hospital, we believe it is reasonable to recruit a minimum of 30 patients in this study. Posters will be erected in the neonatal unit to make staff aware of eligible participants in the hope this will improve participant recruitment (appendix 2). Parents will be able to opt out of the study.

Consent:

All clinical data used as part of this prospective observational trial is data that will already be collected as part of each patient's routine clinical care at the Liverpool Women's Hospital. Additional information on PWV and phase difference will be collected without further intervention to the patient. These values will be calculated based on the routine clinical data collected (see "Pulse Wave Velocity and Phase Difference" section below). Each participant will be assigned a study number and no identifiable information will be linked to the data that is collected. Based on the fact this study will be performed using anonymised data that requires no alteration to patient care, we will not be seeking parental consent for this study.

The parents may become aware of the study procedures (e.g. adding the monitoring equipment to the monitoring procedures, even though the study monitoring equipment has no impact on the baby). A study information leaflet will be made available for families (appendix 3). The leaflet, along with the aforementioned ward posters and verbal explanations provided by clinical staff and the research team, will give parents the ability to opt out of the study.

Data Collection:

Data collection will occur on days 0-3 of life where an infant has a UAC in situ as part of the routine pathway of clinical care. Days of life will be calculated according to the ward policy on the beginning of day 1 of life. If a baby has a UAC fitted on day 2 (or 3) a reduced number of days data will be collected. Also, if a UAC is removed or stops functioning prior to day 3, a reduced number of days data will be collected.

The data will be collected from the routine monitoring undertaken on the baby (see below) and will be stored on the Liverpool Women's Hospital Trust system. Access to raw data on this system will be limited to the named study team. The collection and storage of this information will be password protected to ensure security of identifiable information. Prior to statistical analysis, all data will be anonymised and transferred to a University of Liverpool computer based shared drive to allow all members of the research team to have access to the relevant information without compromising patient confidentiality.

Invasive Blood Pressure Monitoring:

Continuous invasive BP will be monitored via an umbilical artery catheter (3.5 Fr) that was inserted soon after birth. The catheters are typically positioned between the sixth and tenth

thoracic vertebra. The catheter will be connected to the electronic transducer via a 38 cm extension made from rigid plastic. The distance from the blood pressure transducer will be calculated based on UAC and extension length.

Catheter position is confirmed with radiography. 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 a lot and the average position, according to Eycleshymer and Schoemaker, is at the level of the middle third of the seventh thoracic vertebra(153). There are no data for preterm babies and for this reason we reviewed CT scans from term infants with cardiac conditions and found that the relation of the aortic valve to the vertebral level varied greatly, but the average was found to be around the 6th thoracic vertebra. The distance of the catheter tip from the aortic valve will be traced using PACS software.

All possible steps will be taken to ensure the collection of quality data. The UACs and the data being displayed on the monitor will be assessed by a suitably experienced member of staff prior to data collection. Ultimately, the decision to collect data will be at the discretion of the member of the clinical care/research team who is collecting it. The assessment of the data will be qualitative and will include assessment of the following:

- Pulse pressure: normal pulse pressure for preterm infants is approximately 15-25mmHg(154). A narrow pulse pressure may be seen if the UAC is blocked;
- Arterial waveform: the arterial waveform will be assessed for the presence of normal features such as the percussion wave, the tidal wave and the dicrotic notch and wave;
- Appearance of UAC: visual assessment of the UAC (e.g. checking for air bubbles in the tubing between the UAC and the blood pressure transducer);
- Transducer location: ensuring the blood pressure transducer is situated at the level of the patient's heart.

Photographs will be taken with the absence of identifiable information to aid in the identification of "dampened" signals which occur when the UAC becomes blocked. This will be used to ensure the quality of data collection. We will also endeavour to grade the images depending on the amount of dampening (absent, mild, moderate, severe) to assess the effect of dampening on other variables.

Pulse Wave Velocity and Phase Difference:

The PWV and phase difference will be calculated by combining the ECG and peak systolic blood pressure waveform collected by the IxTrend software® (Ixellence GmbH, Wildau, Germany). The time difference between the R wave of the ECG waveform and the peak of the systolic blood pressure waveform will be calculated and expressed as phase, i.e. the proportion of one cardiac cycle. This data is collected by connecting the patient's monitor (which is already receiving information via the transducer attached to the patient's UAC) to a computer with the appropriate software installed (figure 2). Data is therefore collected without any additional disruption to the infant. We aim to collect data for between a minimum of 30 minutes to a maximum of 2 hours a day for the first three days of life for those included in the study. This will provide baseline information on the variation of PWV/phase difference in early life. This is the data that will be compared to the variables stated above to better understand the characteristics of these biomarkers in the developing neonate. As much data will be collected as possible for the first three days of life. For example, if a neonate is fitted with a UAC on day 2, they will still be eligible for inclusion in the study but only 2 days' worth of data will be collected.

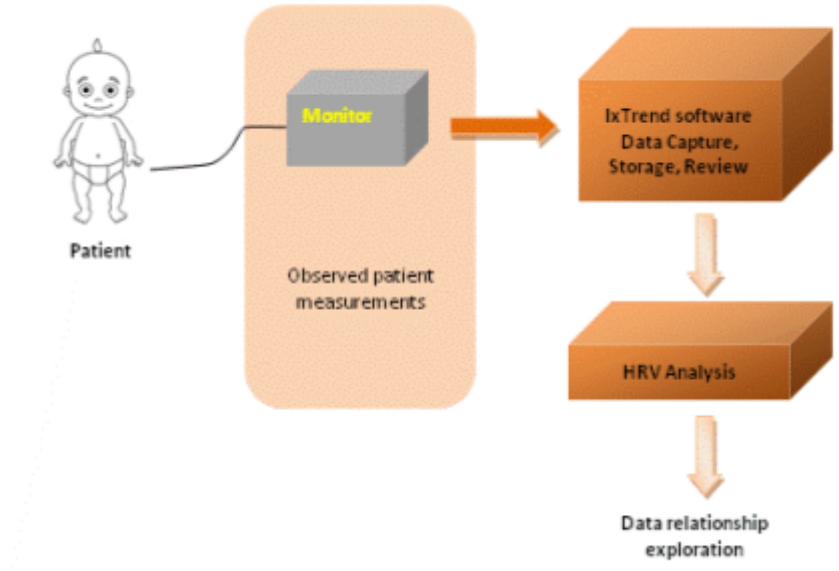


Figure 2 the proposed method of collection of data for PWV and Phase Difference

Additional data collection will be performed for between a minimum of 30 minutes and a maximum of 2 hours before each echocardiogram. We aim to complete this data collection within a 4 hour window of the echocardiogram (i.e. from 2

hours before to 2 hours after the procedure). This will provide more time-specific data that can be more accurately compared to PDA diameter.

Echocardiography:

All echocardiography studies will be performed using a Vivid E9 GE machine and 12 or 6-Hz probe. Echocardiography will be conducted according to the standard operating procedures of the Liverpool Women's Hospital depending on clinical need for each individual patient. The PDA diameter will be recorded during each echocardiogram that is performed. No additional echocardiograms will be performed than is clinically necessary in order to minimise distress to the patient and their family. We anticipate that due to the patient's admission to the neonatal unit and their need for the insertion of a UAC, each patient will receive at least one echocardiogram during their admission.

Autonomic Function:

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. IxTrend software® (Ixellence GmbH, Wildau, Germany) will be used to capture ECG raw data. The signal will be sampled at 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. Sleep state will be justified according to criteria set by Sheldon (189). Of note, this procedure does not cause any disturbance to the participant and so can be conducted at the same time as other assessments. Methods for HRV are described in the Task Force of the European Society of Cardiology report and the North American Society of Pacing and Electrophysiology (1996) (190). Spectral analysis is commonly applied to a series of cardiac cycle RR intervals derived from an ECG signal over a 3- to 15-minutes period (190, 191). The spectral analysis of the RR series will be performed by using an algorithm of Fourier Transform (MATLAB®) (192). The goal of spectral analysis was to determine the repartition of power (190) across low frequencies (LFs, 0.04–0.15 Hz) reflecting both the sympathetic and parasympathetic stimulation and high frequencies (HFs, 0.15–1.5 Hz) reflecting parasympathetic activity in the RR series.

4. Statistical Analysis:

All data collected will be pseudoanonymised. Data without personal identifiers will be passed to shared areas in the University of Liverpool file system. Graphical analysis will be performed to assess gross association between PWV, phase difference and days of life. This will be combined with data related to the variables anticipated to affect the measurement of PWV and phase difference to assess their association. Additional data collected on PWV and phase difference in correlation with echocardiography and measurement of the PDA as mentioned above will also be included. Correlation coefficient will also be calculated. Intergroup comparisons will be conducted on demographic variables collected. The statistical significance will be set depending on the sample size collected. Linear regression analysis will be performed to identify the correlation between multiple variables. Non-parametric statistical analysis will be conducted to assess the distribution of variables measured as part of the study. The number of variables to be included in multivariate analysis will rely on the number of participants recruited as part of the study (see "Sample Size" below). Any additional statistical analysis performed will be according to recommendation by the statistician for this study.

Sample Size:

We aim to recruit as many patients as possible to the study within the time available. Based on the intake of patients at the Liverpool Women's Hospital and the percentage of those requiring the insertion of UACs, we aim to complete the data collection for this study over approximately 4 months and believe it is reasonable to collect data on a minimum of 40 patients. In order to conduct multivariate analysis, we aim to recruit 10 patients per variable we aim to compare to PWV/phase difference. Since we cannot accurately predict the number of participants that will be recruited to the study, we acknowledge the possibility that the number of variable compared to PWV may need to be reduced accordingly. The decision based on what variable(s) to exclude in this instance would be based on the clinical judgement of the research team.

5. Regulatory Issues:

This Study will be conducted according to the National Institute of Health Research's Good Clinical Practice guidelines. The Chief Investigator aims to gain ethical approval from the relevant Research Ethics Committee (REC). The study will be submitted for Site Specific Assessment (SSA)

at each participating NHS Trust. The Chief Investigator will require a copy of the Trust research and development approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

6. End of Study:

The end of study will be defined as database lock.

7. Archiving:

Data and all appropriate information will be stored for a minimum of 5 years after the completion of the study.

8. Publication Policy:

We hope that this study will produce results that will be eligible for publication in peer-reviewed journals and for presentation at scientific conferences.

9. Intellectual Property:

We will be in contact with the University of Liverpool regarding gaining intellectual property for the potential use of this biomarker as a detection/monitoring tool in the management of neonates with PDA in the future.

Appendix 4: Sample Data Collection Form

Patient ID Number: Day of Life:

Maternal Demographics:

Age:	
Antenatal Steroid Use (Completed Course):	Yes / No
Maternal Hypertension:	Yes / No
Type of Delivery:	Vaginal / Caesarean / Instrumental
Past Medical History:	
Medication History:	
Family History:	
Social History:	

Perinatal Data:

GA:	
BW (kg):	
Small for Gestational Age:	
Multiple (Order):	
Sex:	Male / Female
Need for Advanced Resuscitation at Birth (intubation +/- assisted circulation):	
Placental Transfusion Procedures (Delayed Cord Clamping (DCC)/Intact Umbilical Cord Milking (IUCM)/Cut Umbilical Cord Milking (CUCM):	None / DCC / IUCM / CUCM
5-minute APGAR Score:	/10
Working Weight (kg):	

Vital Signs: Date: Time:

Heart Rate (bpm):	
Blood Pressure:	mmHg/ mmHg
SpO_2:	%
Respiratory Rate:	/min
HeRO Score:	

Biochemical Data: Date: Time:

pH:	
PaCO2:	
Lactate:	
Base Excess:	

Hb:	
White Cell Count:	
Blood Culture:	Positive / Negative
CRP:	

Treatment Data: Date: Time:

Ventilator Status:	
Fluid Replacement:	
Inotrope Dose:	
Inotrope Rate:	
Other:	

Co-Morbidity Data: Date: Time:

Intraventricular Haemorrhage (Grade):	N / 1 / 2 / 3 / 4
Sepsis (Blood Culture Positive):	Y / N
Other:	

PWV/Phase Difference/PDA Data: Date: Time:

Pulse Wave Velocity (m/s):	
Phase Difference (°):	
PDA Diameter (mm):	

Appendix 5 – Sample Ward Posters

PDA BIOMARKER STUDY

1. What is a PDA?

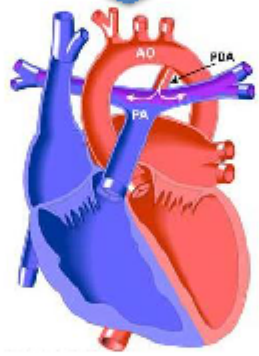
A patent ductus arteriosus (PDA) is the persistence of one of the cardiac shunts that keep fetus' alive in utero. It helps send oxygenated maternal blood around the baby's body before it begins to use its lungs after birth.

2. Why is a PDA bad?

A PDA causes alterations to the blood flow around the baby's body. This can leave the baby susceptible to conditions such as brain haemorrhages and chronic lung disease.

3. How are PDA's currently detected?

Diagnosis of a PDA requires an ultrasound of the baby's heart, also known as an echocardiogram. One of these must be performed every time a clinician wants to assess disease progression or response to treatment.



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4. What are we researching?

We are researching a new biomarker which may help aid the diagnosis and monitoring of PDAs in the future. It is known as "Pulse Wave Velocity". It is a value that can be calculated by downloading information from the baby's monitor.

5. Who is eligible?

Babies admitted to the unit who are <3 days old and who has an umbilical arterial catheter (UAC) in situ is eligible for recruitment in the study.

Those with chromosomal or other congenital cardiac abnormalities are not eligible for participation.

Parents may decide to opt out of the study. Parent information leaflets can be made available on request.

6. Who to contact?

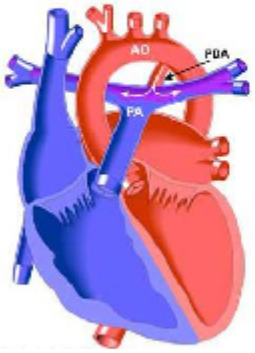
If you have identified a patient who may be eligible for participation in this trial, please contact:

Ben Rodgers

Mobile – 07714002504

Email – hlbrodge@student.liverpool.ac.uk

PDA BIOMARKER STUDY

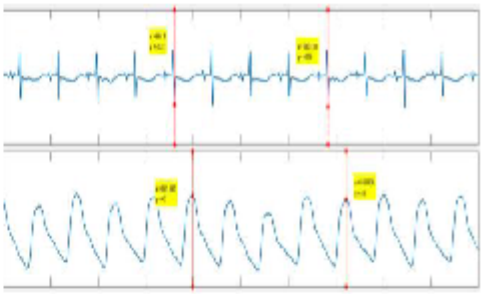


AIM AND RATIONALE:

- PDA is a form of congenital heart defect that can contribute to the development of conditions such as intraventricular haemorrhage and bronchopulmonary dysplasia.
- It is present in 30-50% of preterm neonates.
- The current gold-standard investigation is the echocardiogram – this investigation requires multiple readings on different days to monitor disease progress and can be very disruptive to patients and their families.
- We hope to investigate new, non-invasive methods of monitoring this condition in patients admitted to neonatal intensive care (NICU).

METHODS:

- Pulse Wave Velocity is a value that can be calculated from ECG and Arterial pulse wave data.
- This value will be calculated and compared to routine clinical data collected on each patient.
- We postulate that this value is affected by a number of variables including PDA due to their effects on the neonates haemodynamics



PARTICIPATION:

- Patients aged <3 days who have an umbilical arterial catheter (UAC) in situ are eligible for participation in this trial.
- If you identify a possible participant, please contact:
  Ben Rodgers
  Mobile – 07714002504
  Email – hlbrodge@student.liverpool.ac.uk
- Parents may decide to opt out of this study; parent information leaflets can be made available upon request.

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PDA BIOMARKER STUDY

Background and Rationale:

The Patent Ductus Arteriosus (PDA) is a common congenital heart defect that is particularly common in preterm babies. It is associated with a number of severe conditions such as intraventricular haemorrhage (IVH) and chronic lung disease. Diagnosis of this condition currently relies on performing echocardiograms. These only provide a one-off reading and therefore repeating the procedure multiple times is often necessary to monitor the progression of disease. Constantly disrupting the baby in this way can be distressing for parents and can also be detrimental to the development of the neonate due to effects on its temperature and sleep cycle.

Study and Methods:

We hope to investigate a new biomarker which could potentially be used to identify and monitor PDA in the future. Pulse Wave Velocity (PWV) can be calculated using patient's ECG and arterial waveform data to provide an assessment of the patient's haemodynamics. We are therefore looking to recruit any neonates within the first 3 days of life who have an umbilical arterial catheter (UAC) in situ. We are also hoping to collect data on any patients <10 days old who still have a UAC in situ and are being investigated with an echocardiogram.

WHO TO RECRUIT:

- ✓ Babies with a UAC in situ
- ✓ Babies aged 0-3 days

WHO NOT TO RECRUIT:

- × Age >3 days
- × No UAC in situ
- × Chromosomal abnormalities
- × Other cardiac malformations
- × Not deemed clinically fit for participation

WHO TO CONTACT:

If you believe there are patients appropriate for participation in this trial please contact:

Ben Rodgers

Mobile Number – 07714002504

Email Address – hlbrodge@student.liverpool.ac.uk

Parents can decide to opt out of the study; parent information leaflets can be made available on request.

Appendix 6 – Parent/Caregiver Information Leaflet

What will happen to the data that is collected?

No data collected from your child will be identifiable, i.e. the identity of your child will not be linked to the information collected and so no one will know they were a participant in the study. The data will be analyzed to see if there are any associations between PWV and the information collected from participants in the hope of furthering research into the use of PWV in newborn babies. We hope for the results of this study to be published in scientific journals and for their results to be presented at scientific conferences.

Are there any risks/benefits of my child taking part?

There is no added risk to your child participating in this study as no additional interventions will be performed other than downloading and analyzing the information already collected on their monitor. Your child will not directly benefit from participation but we hope their involvement in this study will help benefit other babies in a similar situation in the future.

Why my Child?

In order for the appropriate information to be collected, a number of criteria must be met. They include:

1. The baby is currently admitted to the NICU at Liverpool Women's Hospital;
2. The baby is less than 3 days old at the time of recruitment in the trial;
3. The baby has an umbilical arterial catheter (a tube going through their bellybutton into one of their blood vessels) fitted.

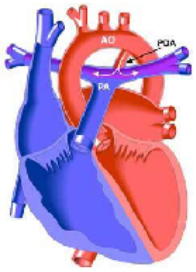
If your baby fulfills these criteria, they will be recruited as part of the study. If you do not wish for your child to participate in the study, you may opt out of the study at any time. If you have any further questions about the study that have not been addressed by this leaflet please contact:

Ben Rodgers

Mobile – 07714002504

Email – hlbrodge@student.liverpool.ac.uk

Investigating Pulse Wave Velocity (PWV) as a Novel Biomarker in Neonatal Haemodynamics



Information leaflet for parents and care givers

Liverpool Women's NHS Foundation Trust

University of Liverpool

What is this about?

This study is researching the characteristics of a new way of investigating disease in newborn babies. We are researching a value called "Pulse Wave Velocity" (PWV) which is a measurement of how blood flows around the body. This is an investigation that is already used in adults, but we are researching its use in newborn babies in the hope of improving the standard of care in our neonatal unit.

What is it used to investigate?

In adults, PWV is used to assess how stiff the arteries that carry blood around the body are. Certain conditions that can affect newborn babies can also alter how stiff or loose their arteries are. The condition we are investigating its use in is known as "Patent Ductus Arteriosus" (PDA). This is a very common heart condition that affects 1 in 2000 term babies and between 30-50% of preterm infants. It is currently diagnosed by taking an ultrasound of the baby's heart (known as an "echocardiogram"). Although this is a very accurate method of diagnosing the condition, it has a number of drawbacks. These include the need to do multiple scans to monitor the disease, and the risk of disrupting the sleep of the baby and the temperature within the incubator. We hope to investigate PWV as a potential non-invasive, continuous method of monitoring this condition.

How is the data collected?

All the data required to calculate a value of PWV is collected by downloading the information that is already being displayed on your baby's monitor. This information is then converted into a number using an equation. Data will also be collected on any blood tests, medications or diagnoses that your baby has received since they were admitted to the neonatal intensive care unit (NICU). The collection of data for this study will in no way cause any change to the care they are already receiving. All the data collected as part of this study has received the appropriate ethical approval.

Does participation in this study mean my baby has a PDA?

Not necessarily. If your baby has a PDA, a member of the clinical care team will have informed you. This study is looking to collect as much data as possible for this new investigation so all children who are eligible will be included in the study.

Bibliography

1. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bulletin of the World Health Organization. 2010;88:31-8.
2. Haas DM. Preterm birth. BMJ clinical evidence. 2011;2011.
3. Steer P. The epidemiology of preterm labour. BJOG : an international journal of obstetrics and gynaecology. 2005;112 Suppl 1:1-3.
4. Fujiwara T, Maeta H, Chida S, Morita T, Watabe Y, Abe T. Artificial surfactant therapy in hyaline-membrane disease. Lancet (London, England). 1980;1(8159):55-9.
5. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics. 1972;50(4):515-25.
6. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. The Cochrane database of systematic reviews. 2013(1):Cd003311.
7. Battin MR, Knight DB, Kuschel CA, Howie RN. Improvement in mortality of very low birthweight infants and the changing pattern of neonatal mortality: the 50-year experience of one perinatal centre. Journal of paediatrics and child health. 2012;48(7):596-9.
8. Thompson K, Conlon J, Magowan BA, Bain M. Neonatal mortality amongst Scottish preterm singleton births (2001-2010): record linkage of maternity data and neonatal mortality data. Public health. 2015;129(12):1597-601.
9. Lawn JE, Blencowe H, Oza S, You D, Lee AC, Waiswa P, et al. Every Newborn: progress, priorities, and potential beyond survival. Lancet (London, England). 2014;384(9938):189-205.
10. Office for National Statistics. Childhood mortality in England and Wales: 2015 2017 [Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/birthsdeathsandmarriages/deaths/bulletins/childhoodinfantandperinatalmortalityinenglandandwales/2015#infant-mortality-rates-increase-for-the-first-time-since-2006.
11. Allen KA. Promoting and Protecting Infant Sleep. Advances in neonatal care : official journal of the National Association of Neonatal Nurses. 2012;12(5):288-91.
12. Lai TT, Bearer CF. Iatrogenic Environmental Hazards in the Neonatal Intensive Care Unit. Clinics in perinatology. 2008;35(1):163-ix.
13. Deguines C, Degrugilliers L, Ghyselen L, Chardon K, Bach V, Tourneux P. Impact of nursing care on temperature environment in preterm newborns nursed in closed convective incubators. Acta paediatrica (Oslo, Norway : 1992). 2013;102(3):e96-e101.
14. Mezu-Ndubuisi OJ, Agarwal G, Raghavan A, Pham JT, Ohler KH, Maheshwari A. Patent Ductus Arteriosus in Premature Neonates. Drugs. 2012;72(7):907-16.
15. Clyman RI, Couto J, Murphy GM. Patent ductus arteriosus: are current neonatal treatment options better or worse than no treatment at all? Seminars in perinatology. 2012;36(2):123-9.
16. Noori S, McCoy M, Friedlich P, Bright B, Gottipati V, Seri I, et al. Failure of ductus arteriosus closure is associated with increased mortality in preterm infants. Pediatrics. 2009;123(1):e138-44.
17. Evans N, Kluckow M. Early ductal shunting and intraventricular haemorrhage in ventilated preterm infants. Archives of disease in childhood Fetal and neonatal edition. 1996;75(3):F183-6.
18. Rojas MA, Gonzalez A, Bancalari E, Claure N, Poole C, Silva-Neto G. Changing trends in the epidemiology and pathogenesis of neonatal chronic lung disease. The Journal of pediatrics. 1995;126(4):605-10.
19. Sandoo A, van Zanten JJCSV, Metsios GS, Carroll D, Kitas GD. The Endothelium and Its Role in Regulating Vascular Tone. The Open Cardiovascular Medicine Journal. 2010;4:302-12.

20. Jänig W. Autonomic Nervous System. In: Schmidt RF, Thews G, editors. Human Physiology. Berlin, Heidelberg: Springer Berlin Heidelberg; 1989. p. 333-70.
21. Florea VG, Cohn JN. The autonomic nervous system and heart failure. Circulation research. 2014;114(11):1815-26.
22. Schneebaum Sender N, Govindan RB, Sulemanji M, Al-Shargabi T, Lenin RB, Eksioglu YZ, et al. Effects of regional brain injury on the newborn autonomic nervous system. Early human development. 2014;90(12):893-6.
23. Association AH. Fetal Circulation 2017 [Available from: http://www.heart.org/HEARTORG/Conditions/CongenitalHeartDefects/SymptomsDiagnosisofCongenitalHeartDefects/Fetal-Circulation_UCM_315674_Article.jsp#.WowUE65l-Uk.
24. Finnemore A, Groves A. Physiology of the fetal and transitional circulation. Seminars in fetal & neonatal medicine. 2015;20(4):210-6.
25. Gournay V. The ductus arteriosus: physiology, regulation, and functional and congenital anomalies. Archives of cardiovascular diseases. 2011;104(11):578-85.
26. Lissauer T, Fanaroff AA. Neonatology at a Glance. Hoboken, UNKNOWN: John Wiley & Sons, Incorporated; 2011.
27. Murphy PJ. The fetal circulation. Continuing Education in Anaesthesia Critical Care & Pain. 2005;5(4):107-12.
28. Cipolla MJ. Integrated Systems Physiology: From Molecule to Function. The Cerebral Circulation. San Rafael (CA): Morgan & Claypool Life Sciences

29. Hahn GH. Testing impact of perinatal inflammation on cerebral autoregulation in preterm neonates: evaluation of a noninvasive method. Danish medical journal. 2013;60(4):B4628.
30. Greisen G. Autoregulation of cerebral blood flow in newborn babies. Early human development. 2005;81(5):423-8.
31. Wu T-W, Azhibekov T, Seri I. Transitional Hemodynamics in Preterm Neonates: Clinical Relevance. Pediatrics & Neonatology.57(1):7-18.
32. Soul JS, Hammer PE, Tsuji M, Saul JP, Bassan H, Limperopoulos C, et al. Fluctuating pressure-passivity is common in the cerebral circulation of sick premature infants. Pediatric research. 2007;61(4):467-73.
33. Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Archives of disease in childhood Fetal and neonatal edition. 2008;93(2):F153-61.
34. Kuban KC, Gilles FH. Human telencephalic angiogenesis. Annals of neurology. 1985;17(6):539-48.
35. Thiriez G, Mougey C, Vermeylen D, Wermenbol V, Lanquart JP, Lin JS, et al. Altered autonomic control in preterm newborns with impaired neurological outcomes. Clinical autonomic research : official journal of the Clinical Autonomic Research Society. 2015;25(4):233-42.
36. Padbury JF, Ervin MG, Polk DH. Extrapulmonary effects of antenatally administered steroids. The Journal of pediatrics. 1996;128(2):167-72.
37. Hillman N, Kallapur SG, Jobe A. Physiology of Transition from intrauterine to Extrauterine Life. Clinics in perinatology. 2012;39(4):769-83.
38. Schneider DJ, Moore JW. Patent ductus arteriosus. Circulation. 2006;114(17):1873-82.
39. Clyman RI, Mauray F, Rudolph AM, Heymann MA. Age-dependent sensitivity of the lamb ductus arteriosus to indomethacin and prostaglandins. The Journal of pediatrics. 1980;96(1):94-8.
40. Clyman RI, Campbell D, Heymann MA, Mauray F. Persistent responsiveness of the neonatal ductus arteriosus in immature lambs: a possible cause for reopening of patent ductus arteriosus after indomethacin-induced closure. Circulation. 1985;71(1):141-5.

41. Thebaud B, Michelakis ED, Wu XC, Moudgil R, Kuzyk M, Dyck JR, et al. Oxygen-sensitive Kv channel gene transfer confers oxygen responsiveness to preterm rabbit and remodeled human ductus arteriosus: implications for infants with patent ductus arteriosus. Circulation. 2004;110(11):1372-9.
42. Lemmers PM, Toet MC, van Bel F. Impact of patent ductus arteriosus and subsequent therapy with indomethacin on cerebral oxygenation in preterm infants. Pediatrics. 2008;121(1):142-7.
43. Dice JE, Bhatia J. Patent Ductus Arteriosus: An Overview. The Journal of Pediatric Pharmacology and Therapeutics : JPPT. 2007;12(3):138-46.
44. Goudjil S, Imestouren F, Chazal C, Ghostine G, Wallois F, Leke A, et al. Patent ductus arteriosus in preterm infants is associated with cardiac autonomic alteration and predominant parasympathetic stimulation. Early human development. 2013;89(9):631-4.
45. Abu-Zidan FM, Hefny AF, Corr P. Clinical ultrasound physics. Journal of Emergencies, Trauma and Shock. 2011;4(4):501-3.
46. Fowlie PW, Davis PG, McGuire W. Prophylactic intravenous indomethacin for preventing mortality and morbidity in preterm infants. The Cochrane database of systematic reviews. 2010(7):Cd000174.
47. Ohlsson A, Walia R, Shah SS. Ibuprofen for the treatment of patent ductus arteriosus in preterm or low birth weight (or both) infants. The Cochrane database of systematic reviews. 2015(2):Cd003481.
48. Bhat R, Das UG. Management of patent ductus arteriosus in premature infants. Indian journal of pediatrics. 2015;82(1):53-60.
49. Perez KM, Laughon MM. What is new for patent ductus arteriosus management in premature infants in 2015? Current opinion in pediatrics. 2015;27(2):158-64.
50. Malviya MN, Ohlsson A, Shah SS. Surgical versus medical treatment with cyclooxygenase inhibitors for symptomatic patent ductus arteriosus in preterm infants. The Cochrane database of systematic reviews. 2013(3):Cd003951.
51. Bardanzellu F, Neroni P, Dessì A, Fanos V. Paracetamol in Patent Ductus Arteriosus Treatment: Efficacious and Safe? BioMed research international. 2017;2017:1438038.
52. Bardanzellu F, Neroni P, Dessi A, Fanos V. Paracetamol in Patent Ductus Arteriosus Treatment: Efficacious and Safe? BioMed research international. 2017;2017:1438038.
53. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology and therapeutics. 2001;69(3):89-95.
54. Ng PC. Diagnostic markers of infection in neonates. Archives of disease in childhood Fetal and neonatal edition. 2004;89(3):F229-35.
55. Ng PC, Li K, Leung TF, Wong RP, Li G, Chui KM, et al. Early prediction of sepsis-induced disseminated intravascular coagulation with interleukin-10, interleukin-6, and RANTES in preterm infants. Clinical chemistry. 2006;52(6):1181-9.
56. Ng PC, Li K, Chui KM, Leung TF, Wong RP, Chu WC, et al. IP-10 is an early diagnostic marker for identification of late-onset bacterial infection in preterm infants. Pediatric research. 2007;61(1):93-8.
57. Ng PC, Lam HS. Biomarkers in neonatology: the next generation of tests. Neonatology. 2012;102(2):145-51.
58. Kearns GL, Artman M. Functional Biomarkers: an Approach to Bridge Pharmacokinetics and Pharmacodynamics in Pediatric Clinical Trials. Current pharmaceutical design. 2015;21(39):5636-42.
59. Savage WJ, Everett AD. Biomarkers in pediatrics: children as biomarker orphans. Proteomics Clinical applications. 2010;4(12):915-21.
60. Wu AH. Analytical validation of novel cardiac biomarkers used in clinical trials. American heart journal. 2015;169(5):674-83.

61. Ensor JE. Biomarker validation: common data analysis concerns. The oncologist. 2014;19(8):886-91.
62. Cummings J, Raynaud F, Jones L, Sugar R, Dive C. Fit-for-purpose biomarker method validation for application in clinical trials of anticancer drugs. British Journal of Cancer. 2010;103(9):1313-7.
63. Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. Heart. 2006;92(6):843-9.
64. Kim JS, Shim EJ. B-type natriuretic Peptide assay for the diagnosis and prognosis of patent ductus arteriosus in preterm infants. Korean circulation journal. 2012;42(3):192-6.
65. Lee JH, Shin JH, Park KH, Rhie YJ, Park MS, Choi BM. Can early B-type natriuretic peptide assays predict symptomatic patent ductus arteriosus in extremely low birth weight infants? Neonatology. 2013;103(2):118-22.
66. Mine K, Ohashi A, Tsuji S, Nakashima J, Hirabayashi M, Kaneko K. B-type natriuretic peptide for assessment of haemodynamically significant patent ductus arteriosus in premature infants. Acta paediatrica (Oslo, Norway : 1992). 2013;102(8):e347-52.
67. Kulkarni M, Gokulakrishnan G, Price J, Fernandes CJ, Leeflang M, Pammi M. Diagnosing significant PDA using natriuretic peptides in preterm neonates: a systematic review. Pediatrics. 2015;135(2):e510-25.
68. Goudjil S, Imestouren F, Armougon A, Razafimanantsoa L, Mahmoudzadeh M, Wallois F, et al. Noninvasive technique for the diagnosis of patent ductus arteriosus in premature infants by analyzing pulse wave phases on photoplethysmography signals measured in the right hand and the left foot. PloS one. 2014;9(6):e98763.
69. Oishi M, Nishida H, Kabe K, Hoshi J. Monitoring neonatal peripheral circulation by electrocardiogram-to-oximeter pulse velocity. Pediatric research. 1993;33(6):653-7.
70. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac A-M, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Hypertension. 1995;26(3):485-90.
71. Ballou G. Handbook for Sound Engineers: Focal; 2005.
72. Calabia J, Torguet P, Garcia M, Garcia I, Martin N, Guasch B, et al. Doppler ultrasound in the measurement of pulse wave velocity: agreement with the Complior method. Cardiovascular Ultrasound. 2011;9(1):13.
73. Kracht D, Shroff R, Baig S, Doyon A, Jacobi C, Zeller R, et al. Validating a New Oscillometric Device for Aortic Pulse Wave Velocity Measurements in Children and Adolescents. American journal of hypertension. 2011;24(12):1294-9.
74. Wallenstein MB, Stevenson DK. New technique for umbilical artery catheter placement in the neonate. The Journal of pediatrics. 2015;166(2):501.
75. Najjar SS, Scuteri A, Shetty V, Wright JG, Muller DC, Fleg JL, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. Journal of the American College of Cardiology. 2008;51(14):1377-83.
76. Salvi P, Palombo C, Salvi GM, Labat C, Parati G, Benetos A. Left ventricular ejection time, not heart rate, is an independent correlate of aortic pulse wave velocity. Journal of applied physiology (Bethesda, Md : 1985). 2013;115(11):1610-7.
77. Okada M, Matsuto T, Satoh S, Igarashi S, Baba M, Sugita O, et al. Role of pulse wave velocity for assessing autonomic nervous system activities in reference to heart rate variability. Medical informatics = Medecine et informatique. 1996;21(1):81-90.
78. Koudsi A, Oldroyd J, McElduff P, Banerjee M, Vyas A, Cruickshank JK. Maternal and neonatal influences on, and reproducibility of, neonatal aortic pulse wave velocity. Hypertension. 2007;49(1):225-31.

79. Shah AS, Gao Z, Urbina EM, Kimball TR, Dolan LM. Prediabetes: the effects on arterial thickness and stiffness in obese youth. The Journal of clinical endocrinology and metabolism. 2014;99(3):1037-43.
80. Shroff R, Degi A, Kerti A, Kis E, Cseprekal O, Tory K, et al. Cardiovascular risk assessment in children with chronic kidney disease. Pediatric nephrology (Berlin, Germany). 2013;28(6):875-84.
81. McNamara PJ, Sehgal A. Towards rational management of the patent ductus arteriosus: the need for disease staging. Archives of disease in childhood Fetal and neonatal edition. 2007;92(6):F424-7.
82. Arlettaz R. echocardiographic evaluation of Patent Ductus Arteriosus in Preterm infants. Frontiers in pediatrics. 2017;5:147.
83. Schwarz CE, Preusche A, Baden W, Poets CF, Franz AR. Repeatability of echocardiographic parameters to evaluate the hemodynamic relevance of patent ductus arteriosus in preterm infants: a prospective observational study. BMC pediatrics. 2016;16:18.
84. Smallhorn JF, Huhta JC, Anderson RH, Macartney FJ. Suprasternal cross-sectional echocardiography in assessment of patient ducts arteriosus. British heart journal. 1982;48(4):321-30.
85. Rigby ML, Pickering D, Wilkinson A. Cross sectional echocardiography in determining persistent patency of the ductus arteriosus in preterm infants. Archives of disease in childhood. 1984;59(4):341-5.
86. Vick GW, 3rd, Huhta JC, Gutgesell HP. Assessment of the ductus arteriosus in preterm infants utilizing suprasternal two-dimensional/Doppler echocardiography. Journal of the American College of Cardiology. 1985;5(4):973-7.
87. Hiraishi S, Horiguchi Y, Fujino N, Agata Y, Kawai H, Ohe M, et al. Two-dimensional and Doppler echocardiographic assessment of variably shaped ductus arteriosus by the parasternal approach. Pediatric cardiology. 1991;12(1):6-12.
88. Lee HC, Silverman N, Hintz SR. Diagnosis of patent ductus arteriosus by a neonatologist with a compact, portable ultrasound machine. Journal of perinatology : official journal of the California Perinatal Association. 2007;27(5):291-6.
89. Sohn S, Kim HS, Han JJ. Pediatric cardiac surgery with echocardiographic diagnosis alone. Journal of Korean medical science. 2002;17(4):463-7.
90. Galante D. Echocardiography as a reliable tool in neonates with ductal shunting. Minerva anestesiologica. 2010;76(3):178-80.
91. Jensen EA, Schmidt B. Epidemiology of bronchopulmonary dysplasia. Birth defects research Part A, Clinical and molecular teratology. 2014;100(3):145-57.
92. Kumar A, Bhat BV. Epidemiology of respiratory distress of newborns. Indian journal of pediatrics. 1996;63(1):93-8.
93. Morrison JJ, Rennie JM, Milton PJ. Neonatal respiratory morbidity and mode of delivery at term: influence of timing of elective caesarean section. British journal of obstetrics and gynaecology. 1995;102(2):101-6.
94. Dargaville PA, Copnell B. The epidemiology of meconium aspiration syndrome: incidence, risk factors, therapies, and outcome. Pediatrics. 2006;117(5):1712-21.
95. Groves AM, Kuschel CA, Knight DB, Skinner JR. Echocardiographic assessment of blood flow volume in the superior vena cava and descending aorta in the newborn infant. Archives of Disease in Childhood - Fetal and Neonatal Edition. 2008;93(1):F24.
96. Skinner JR, Boys RJ, Heads A, Hey EN, Hunter S. Estimation of pulmonary arterial pressure in the newborn: study of the repeatability of four Doppler echocardiographic techniques. Pediatric cardiology. 1996;17(6):360-9.
97. Moorthy B, Colditz PR, Ives KN, Rees DG, van't Hoff WG, Hope PL. Reproducibility of cerebral artery Doppler measurements. Archives of disease in childhood. 1990;65(7 Spec No):700-1.

98. Iyer P, Evans N. Re-evaluation of the left atrial to aortic root ratio as a marker of patent ductus arteriosus. Archives of disease in childhood Fetal and neonatal edition. 1994;70(2):F112-7.
99. Schachinger S, Stansfield RB, Ensing G, Schumacher R. The prevalence of and attitudes toward neonatal functional echocardiography use and training in the United States: a survey of neonatal intensive care unit medical directors. Journal of neonatal-perinatal medicine. 2014;7(2):125-30.
100. Singh Y, Gupta S, Groves AM, Gandhi A, Thomson J, Qureshi S, et al. Expert consensus statement 'Neonatologist-performed Echocardiography (NoPE)'-training and accreditation in UK. European journal of pediatrics. 2016;175(2):281-7.
101. Mertens L, Seri I, Marek J, Arlettaz R, Barker P, McNamara P, et al. Targeted Neonatal Echocardiography in the Neonatal Intensive Care Unit: practice guidelines and recommendations for training. Writing Group of the American Society of Echocardiography (ASE) in collaboration with the European Association of Echocardiography (EAE) and the Association for European Pediatric Cardiologists (AEPC). Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography. 2011;24(10):1057-78.
102. de Boode WP, Singh Y, Gupta S, Austin T, Bohlin K, Dempsey E, et al. Recommendations for neonatologist performed echocardiography in Europe: Consensus Statement endorsed by European Society for Paediatric Research (ESPR) and European Society for Neonatology (ESN). Pediatric research. 2016;80(4):465-71.
103. Gomes R, Rossi R, Lima S, Carmo P, Ferreira R, Menezes I, et al. Pediatric cardiology and telemedicine: seven years' experience of cooperation with remote hospitals. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2010;29(2):181-91.
104. DeMauro SB, Cohen MS, Ratcliffe SJ, Abbasi S, Schmidt B. Serial echocardiography in very preterm infants: a pilot randomized trial. Acta paediatrica (Oslo, Norway : 1992). 2013;102(11):1048-53.
105. Williamson PR, Altman DG, Blazeby JM, Clarke M, Devane D, Gargon E, et al. Developing core outcome sets for clinical trials: issues to consider. Trials. 2012;13(1):132.
106. Webbe J, Sinha I, Gale C. Core Outcome Sets. Archives of disease in childhood - Education & practice edition. 2017.
107. El-Mashad AE, El-Mahdy H, El Amrousy D, Elgendy M. Comparative study of the efficacy and safety of paracetamol, ibuprofen, and indomethacin in closure of patent ductus arteriosus in preterm neonates. European journal of pediatrics. 2017;176(2):233-40.
108. Lin YJ, Chen CM, Rehan VK, Florens A, Wu SY, Tsai ML, et al. Randomized Trial to Compare Renal Function and Ductal Response between Indomethacin and Ibuprofen Treatment in Extremely Low Birth Weight Infants. Neonatology. 2017;111(3):195-202.
109. Sadeghi-Moghaddam P, Arjmandnia MH, Heidari A, Mohagheghi-Kamal SM, Aghaali M. Comparison of therapeutic effects and side effects of oral ibuprofen and indomethacin on the closure of patent ductus arteriosus in premature infants. Journal of Babol University of Medical Sciences. 2017;19(9):7-12.
110. Demir N, Peker E, Ece I, Balahoroglu R, Tuncer O. Efficacy and safety of rectal ibuprofen for patent ductus arteriosus closure in very low birth weight preterm infants. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2017;30(17):2119-25.
111. Harkin P, Harma A, Aikio O, Valkama M, Leskinen M, Saarela T, et al. Paracetamol Accelerates Closure of the Ductus Arteriosus after Premature Birth: A Randomized Trial. The Journal of pediatrics. 2016;177:72-7.e2.

112. Dani C, Poggi C, Mosca F, Schena F, Lista G, Ramenghi L, et al. Efficacy and safety of intravenous paracetamol in comparison to ibuprofen for the treatment of patent ductus arteriosus in preterm infants: study protocol for a randomized control trial. Trials. 2016;17:182.
113. Yang B, Gao X, Ren Y, Wang Y, Zhang Q. Oral paracetamol vs. oral ibuprofen in the treatment of symptomatic patent ductus arteriosus in premature infants: A randomized controlled trial. Experimental and therapeutic medicine. 2016;12(4):2531-6.
114. Dash SK, Kabra NS, Avasthi BS, Sharma SR, Padhi P, Ahmed J. Enteral paracetamol or Intravenous Indomethacin for Closure of Patent Ductus Arteriosus in Preterm Neonates: A Randomized Controlled Trial. Indian pediatrics. 2015;52(7):573-8.
115. Yadav S, Agarwal S, Maria A, Dudeja A, Dubey NK, Anand P, et al. Comparison of oral ibuprofen with oral indomethacin for PDA closure in Indian preterm neonates: a randomized controlled trial. Pediatric cardiology. 2014;35(5):824-30.
116. Oncel MY, Yurttutan S, Erdeve O, Uras N, Altug N, Oguz SS, et al. Oral paracetamol versus oral ibuprofen in the management of patent ductus arteriosus in preterm infants: a randomized controlled trial. The Journal of pediatrics. 2014;164(3):510-4.e1.
117. Kluckow M, Jeffery M, Gill A, Evans N. A randomised placebo-controlled trial of early treatment of the patent ductus arteriosus. Archives of disease in childhood Fetal and neonatal edition. 2014;99(2):F99-f104.
118. Lago P, Salvadori S, Opocher F, Ricato S, Chiandetti L, Frigo AC. Continuous infusion of ibuprofen for treatment of patent ductus arteriosus in very low birth weight infants. Neonatology. 2014;105(1):46-54.
119. Bagnoli F, Rossetti A, Messina G, Mori A, Casucci M, Tomasini B. Treatment of patent ductus arteriosus (PDA) using ibuprofen: renal side-effects in VLBW and ELBW newborns. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2013;26(4):423-9.
120. Sosenko IR, Fajardo MF, Claure N, Bancalari E. Timing of patent ductus arteriosus treatment and respiratory outcome in premature infants: a double-blind randomized controlled trial. The Journal of pediatrics. 2012;160(6):929-35.e1.
121. Erdeve O, Yurttutan S, Altug N, Ozdemir R, Gokmen T, Dilmen U, et al. Oral versus intravenous ibuprofen for patent ductus arteriosus closure: a randomised controlled trial in extremely low birthweight infants. Archives of disease in childhood Fetal and neonatal edition. 2012;97(4):F279-83.
122. Dani C, Vangi V, Bertini G, Pratesi S, Lori I, Favelli F, et al. High-dose ibuprofen for patent ductus arteriosus in extremely preterm infants: a randomized controlled study. Clinical pharmacology and therapeutics. 2012;91(4):590-6.
123. Cheng DD, Ortiz EE, Angtuaco JL. A single-blind randomized controlled trial comparing the efficacy of two doses of oral ibuprofen with intravenous indomethacin in terms of ductus arteriosus closure among premature infants with patent ductus arteriosus: A phase 2A clinical trial. Acta Medica Philippina. 2012;46-47(4-1):51-5.
124. Gokmen T, Erdeve O, Altug N, Oguz SS, Uras N, Dilmen U. Efficacy and safety of oral versus intravenous ibuprofen in very low birth weight preterm infants with patent ductus arteriosus. The Journal of pediatrics. 2011;158(4):549-54.e1.
125. Aranda JV, Clyman R, Cox B, Van Overmeire B, Wozniak P, Sosenko I, et al. A randomized, double-blind, placebo-controlled trial on intravenous ibuprofen L-lysine for the early closure of nonsymptomatic patent ductus arteriosus within 72 hours of birth in extremely low-birth-weight infants. American journal of perinatology. 2009;26(3):235-45.
126. Sangtawesin C, Sangtawesin V, Lertsutthiwong W, Kanjanapattanakul W, Khorana M, Ayudhaya JK. Prophylaxis of symptomatic patent ductus arteriosus with oral ibuprofen in very low birth weight infants. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2008;91 Suppl 3:S28-34.

127. Cherif A, Khrouf N, Jabnoun S, Mokrani C, Amara MB, Guellouze N, et al. Randomized pilot study comparing oral ibuprofen with intravenous ibuprofen in very low birth weight infants with patent ductus arteriosus. Pediatrics. 2008;122(6):e1256-61.
128. Su BH, Lin HC, Chiu HY, Hsieh HY, Chen HH, Tsai YC. Comparison of ibuprofen and indometacin for early-targeted treatment of patent ductus arteriosus in extremely premature infants: a randomised controlled trial. Archives of disease in childhood Fetal and neonatal edition. 2008;93(2):F94-9.
129. Salama H, Alsisi A, Al-Rifai H, Shaddad A, Samawal L, Habboub L, et al. A randomized controlled trial on the use of oral ibuprofen to close patent ductus arteriosus in premature infants. Journal of neonatal-perinatal medicine. 2008;1(3):153-8.
130. Jegatheesan P, Ianus V, Buchh B, Yoon G, Chorne N, Ewig A, et al. Increased indomethacin dosing for persistent patent ductus arteriosus in preterm infants: a multicenter, randomized, controlled trial. The Journal of pediatrics. 2008;153(2):183-9.
131. Fakhraee SH, Badiee Z, Mojtahedzadeh S, Kazemian M, Kelishadi R. Comparison of oral ibuprofen and indomethacin therapy for patent ductus arteriosus in preterm infants. Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics. 2007;9(5):399-403.
132. Sangtawesin V, Sangtawesin C, Raksasinborisut C, Sathirakul K, Kanjanapattanakul W, Khorana M, et al. Oral ibuprofen prophylaxis for symptomatic patent ductus arteriosus of prematurity. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2006;89(3):314-21.
133. Gimeno Navarro A, Cano Sanchez A, Fernandez Gilino C, Carrasco Moreno JI, Izquierdo Macian I, Gutierrez Laso A, et al. [Ibuprofen versus indomethacin in the treatment of patent ductus arteriosus in preterm infants]. Anales de pediatria (Barcelona, Spain : 2003). 2005;63(3):212-8.
134. Gournay V, Roze JC, Kuster A, Daoud P, Cambonie G, Hascoet JM, et al. Prophylactic ibuprofen versus placebo in very premature infants: a randomised, double-blind, placebo-controlled trial. The Lancet.364(9449):1939-44.
135. Su PH, Chen JY, Su CM, Huang TC, Lee HS. Comparison of ibuprofen and indomethacin therapy for patent ductus arteriosus in preterm infants. Pediatrics international : official journal of the Japan Pediatric Society. 2003;45(6):665-70.
136. Lee J, Rajadurai VS, Tan KW, Wong KY, Wong EH, Leong JY. Randomized trial of prolonged low-dose versus conventional-dose indomethacin for treating patent ductus arteriosus in very low birth weight infants. Pediatrics. 2003;112(2):345-50.
137. Lago P, Bettiol T, Salvadori S, Pitassi I, Vianello A, Chiandetti L, et al. Safety and efficacy of ibuprofen versus indomethacin in preterm infants treated for patent ductus arteriosus: a randomised controlled trial. European journal of pediatrics. 2002;161(4):202-7.
138. Supapannachart S, Limrungsikul A, Khowsathit P. Oral ibuprofen and indomethacin for treatment of patent ductus arteriosus in premature infants: a randomized trial at Ramathibodi Hospital. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2002;85 Suppl 4:S1252-8.
139. Dani C, Bertini G, Reali MF, Murru P, Fabris C, Vangi V, et al. Prophylaxis of patent ductus arteriosus with ibuprofen in preterm infants. Acta paediatrica (Oslo, Norway : 1992). 2000;89(11):1369-74.
140. Tammela O, Ojala R, Iivainen T, Lautamatti V, Pokela ML, Janas M, et al. Short versus prolonged indomethacin therapy for patent ductus arteriosus in preterm infants. The Journal of pediatrics. 1999;134(5):552-7.
141. Lai TH, Soong WJ, Hwang B. Indomethacin for the prevention of symptomatic patent ductus arteriosus in very low birth weight infants. Zhonghua Minguo xiao er ke yi xue hui za zhi [Journal] Zhonghua Minguo xiao er ke yi xue hui. 1990;31(1):17-23.
142. Vogtmann C, Grubbe G, Ruckhaberle KE, Bottcher H, Ockert C. [Effects of early therapy with indomethacin on the manifestation of a persistent ductus arteriosus in extremely

underweight premature infants]. Monatsschrift Kinderheilkunde : Organ der Deutschen Gesellschaft fur Kinderheilkunde. 1988;136(9):636-9.
143. Gersony WM, Peckham GJ, Ellison RC, Miettinen OS, Nadas AS. Effects of indomethacin in premature infants with patent ductus arteriosus: results of a national collaborative study. The Journal of pediatrics. 1983;102(6):895-906.
144. Rudd P, Montanez P, Hallidie-Smith K, Silverman M. Indomethacin treatment for patent ductus arteriosus in very low birthweight infants: double blind trial. Archives of disease in childhood. 1983;58(4):267-70.
145. Monset-Couchard M, Dias-Mancano D, Murat I, Relier JP. [Controlled trial of intravenous lyophilized indomethacin in the treatment of persistent ductus arteriosus in premature infants]. Pediatrie. 1983;38(6):365-77.
146. Yanagi RM, Wilson A, Newfeld EA, Aziz KU, Hunt CE. Indomethacin treatment for symptomatic patent ductus arteriosus: a double-blind control study. Pediatrics. 1981;67(5):647-52.
147. Yeh TF, Raval D, Luken J, Thalji A, Lilien L, Pildes RS. Clinical evaluation of premature infants with patent ductus arteriosus: a scoring system with echocardiogram, acid-base, and blood gas correlations. Critical care medicine. 1981;9(9):655-7.
148. Gournay V, Roze JC, Kuster A, Daoud P, Cambonie G, Hascoet JM, et al. Prophylactic ibuprofen versus placebo in very premature infants: a randomised, double-blind, placebo-controlled trial. Lancet (London, England). 2004;364(9449):1939-44.
149. Zonnenberg I, de Waal K. The definition of a haemodynamic significant duct in randomized controlled trials: a systematic literature review. Acta paediatrica (Oslo, Norway : 1992). 2012;101(3):247-51.
150. Laughon MM, Simmons MA, Bose CL. Patency of the ductus arteriosus in the premature infant: is it pathologic? Should it be treated? Current opinion in pediatrics. 2004;16(2):146-51.
151. Benitz WE. Treatment of persistent patent ductus arteriosus in preterm infants: time to accept the null hypothesis? Journal Of Perinatology. 2010;30:241.
152. Thurn D, Doyon A, Sozeri B, Bayazit AK, Canpolat N, Duzova A, et al. Aortic Pulse Wave Velocity in Healthy Children and Adolescents: Reference Values for the Vicorder Device and Modifying Factors. American journal of hypertension. 2015;28(12):1480-8.
153. Eycleshymer A, Schoemaker D. Cross-Section Anatomy Atlas. New York: D. Appleton and Company; 1911.
154. Vega-Barrera C, Muraskas J, Guo R, Ray B. The pulse pressure in a premature infant less than 37 weeks gestational age with a patent ductus arteriosus. Open Journal of Pediatrics. 2013;Vol.03No.02:6.
155. Rook WH, Turner JD, Clutton-Brock TH. Analysis of damping characteristics of arterial catheter blood pressure monitoring in a large intensive care unit. Southern African Journal of Critical Care (Online). 2017;33:8-10.
156. Fairchild KD. Predictive monitoring for early detection of sepsis in neonatal ICU patients. Current opinion in pediatrics. 2013;25(2):172-9.
157. Skinner J. The effects of surfactant on haemodynamics in hyaline membrane disease. Archives of Disease in Childhood Fetal and Neonatal Edition. 1997;76(2):F67-F9.
158. Atsma F, Veldhuizen I, de Kort W, van Kraaij M, Pasker-de Jong P, Deinum J. Hemoglobin level is positively associated with blood pressure in a large cohort of healthy individuals. Hypertension. 2012;60(4):936-41.
159. Selig FA, Tonolli ER, Silva EV, Godoy MF. Heart rate variability in preterm and term neonates. Arquivos brasileiros de cardiologia. 2011;96(6):443-9.
160. Newnam KM, McGrath JM. Understanding the Inflammatory Response of the Neonate: Clinical Implications for Caregivers in the Neonatal Intensive Care Unit. Newborn and Infant Nursing Reviews. 2010;10(4):165-76.

161. Adamska E, Helwich E, Rutkowska M, Zacharska E, Piotrowska A. [Comparison of the efficacy of ibuprofen and indomethacin in the treatment of patent ductus arteriosus in prematurely born infants]. Medycyna wieku rozwojowego. 2005;9(3 Pt 1):335-54.
162. Aly H, Lotfy W, Badrawi N, Ghawas M, Abdel-Meguid IE, Hammad TA. Oral Ibuprofen and ductus arteriosus in premature infants: a randomized pilot study. American journal of perinatology. 2007;24(5):267-70.
163. Akbari Asbagh P, Zarkesh MR, Nili F, Nayeri FS, Tofighi Naeem A. Prophylactic teatment with oral paracetamol for patent ductus arteriosus in preterm infants: a randomized clinical trial. Tehran University Medical Journal. 2015;73(2):86-92.
164. Bagheri MM, Niknafs P, Sabsevari F, Torabi MH, Bahman Bijari B, Noroozi E, et al. Comparison of Oral Acetaminophen Versus Ibuprofen in Premature Infants With Patent Ductus Arteriosus. Iranian Journal of Pediatrics. 2016;26(4):e3975.
165. Bravo MC, Cabanas F, Riera J, Perez-Fernandez E, Quero J, Perez-Rodriguez J, et al. Randomised controlled clinical trial of standard versus echocardiographically guided ibuprofen treatment for patent ductus arteriosus in preterm infants: a pilot study. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2014;27(9):904-9.
166. Chotigeat U, Jirapapa K, Layangkool T. A comparison of oral ibuprofen and intravenous indomethacin for closure of patent ductus arteriosus in preterm infants. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2003;86 Suppl 3:S563-9.
167. Couser RJ, Ferrara TB, Wright GB, Cabalka AK, Schilling CG, Hoekstra RE, et al. Prophylactic indomethacin therapy in the first twenty-four hours of life for the prevention of patent ductus arteriosus in preterm infants treated prophylactically with surfactant in the delivery room. The Journal of pediatrics. 1996;128(5 Pt 1):631-7.
168. Dang D, Wang D, Zhang C, Zhou W, Zhou Q, Wu H. Comparison of oral paracetamol versus ibuprofen in premature infants with patent ductus arteriosus: a randomized controlled trial. PloS one. 2013;8(11):e77888.
169. DeMauro SB, Cohen MS, Ratcliffe SJ, Abbasi S, Schmidt B. Serial Echocardiography in Very Preterm Infants: A Pilot Randomized Trial. Acta paediatrica (Oslo, Norway : 1992). 2013;102(11):10.1111/apa.12389.
170. Hammerman C, Kaplan M. Prophylactic ibuprofen in premature infants: a multicentre, randomised, double-blind, placebo-controlled trial. The Journal of pediatrics. 2005;146(5):709-10.
171. Hammerman C, Shchors I, Jacobson S, Schimmel MS, Bromiker R, Kaplan M, et al. Ibuprofen versus continuous indomethacin in premature neonates with patent ductus arteriosus: is the difference in the mode of administration? Pediatric research. 2008;64(3):291-7.
172. Kaapa P, Lanning P, Koivisto M. Early closure of patent ductus arteriosus with indomethacin in preterm infants with idiopathic respiratory distress syndrome. Acta paediatrica Scandinavica. 1983;72(2):179-84.
173. Krueger E, Mellander M, Bratton D, Cotton R. Prevention of symptomatic patent ductus arteriosus with a single dose of indomethacin. The Journal of pediatrics. 1987;111(5):749-54.
174. Lin XZ, Chen HQ, Zheng Z, Li YD, Lai JD, Huang LH. [Therapeutic effect of early administration of oral ibuprofen in very low birth weight infants with patent ductus arteriosus]. Zhongguo dang dai er ke za zhi = Chinese journal of contemporary pediatrics. 2012;14(7):502-5.
175. Merritt TA, Harris JP, Roghmann K, Wood B, Campanella V, Alexson C, et al. Early closure of the patent ductus arteriosus in very low-birth-weight infants: a controlled trial. The Journal of pediatrics. 1981;99(2):281-6.
176. Nestrud RM, Hill DE, Arrington RW, Beard AG, Dungan WT, Lau PY, et al. Indomethacin treatment in patent ductus arteriosus. A double-blind study utilizing indomethacin plasma levels. Developmental pharmacology and therapeutics. 1980;1(2-3):125-36.

177. Peckham GJ, Miettinen OS, Ellison RC, Kraybill EN, Gersony WM, Zierler S, et al. Clinical course to 1 year of age in premature infants with patent ductus arteriosus: results of a multicenter randomized trial of indomethacin. The Journal of pediatrics. 1984;105(2):285-91.
178. Pourarian S, Pishva N, Madani A, Rastegari M. Comparison of oral ibuprofen and indomethacin on closure of patent ductus arteriosus in preterm infants. Eastern Mediterranean health journal = La revue de sante de la Mediterranee orientale = al-Majallah al-sihhiyah li-sharq al-mutawassit. 2008;14(2):360-5.
179. Pourarian S, Takmil F, Cheriki S, Amoozgar H. The Effect of Oral High-dose Ibuprofen on Patent Ductus Arteriosus Closure in Preterm Infants. American journal of perinatology. 2015;32(12):1158-63.
180. Rhodes PG, Ferguson MG, Reddy NS, Joransen JA, Gibson J. Effects of prolonged versus acute indomethacin therapy in very low birth-weight infants with patent ductus arteriosus. European journal of pediatrics. 1988;147(5):481-4.
181. Van Overmeire B, Smets K, Lecoutere D, Van de Broek H, Weyler J, De Groote K, et al. A Comparison of Ibuprofen and Indomethacin for Closure of Patent Ductus Arteriosus. New England Journal of Medicine. 2000;343(10):674-81.
182. Van Overmeire B, Van de Broek H, Van Laer P, Weyler J, Vanhaesebrouck P. Early versus late indomethacin treatment for patent ductus arteriosus in premature infants with respiratory distress syndrome. The Journal of pediatrics. 2001;138(2):205-11.
183. Van Overmeire B. The use of ibuprofen in neonates in the treatment of patent ductus arteriosus. International journal of clinical practice Supplement. 2003(135):23-7.
184. Van Overmeire B, Allegaert K, Casaer A, Debauche C, Decaluwe W, Jespers A, et al. Prophylactic ibuprofen in premature infants: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet (London, England). 2004;364(9449):1945-9.
185. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. The Cochrane database of systematic reviews. 2014(4):Cd005496.
186. Wyllie J. Neonatal echocardiography. Seminars in fetal & neonatal medicine. 2015;20(3):173-80.
187. Perlman JM. The relationship between systemic hemodynamic perturbations and periventricular-intraventricular hemorrhage--a historical perspective. Seminars in pediatric neurology. 2009;16(4):191-9.
188. Yiallourou SR, Witcombe NB, Sands SA, Walker AM, Horne RS. The development of autonomic cardiovascular control is altered by preterm birth. Early human development. 2013;89(3):145-52.
189. Sheldon SH. Sleep in Infants and Children. Sleep: A Comprehensive Handbook: John Wiley & Sons, Inc.; 2005. p. 507-10.
190. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation. 1996;93(5):1043-65.
191. Longin E, Schaible T, Lenz T, König S. Short term heart rate variability in healthy neonates: Normative data and physiological observations. Early human development. 2005;81(8):663-71.
192. Valimaki I, Rantonen T. Spectral analysis of heart rate and blood pressure variability. Clin Perinatol. 1999;26(4):967-80, x.