

**B-Type Natriuretic Peptide and
Novel Cardiac Ultrasound
in Very Preterm Neonates:
Potential Markers for the Detection of Pulmonary
Hypertension and for Risk Stratification**

Sarah Harris

MBChB, DCH, FRACP

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Abstract

Very premature birth causes an interruption of cardiorespiratory development. Successful pulmonary adaptation requires synchronous development of alveoli and pulmonary capillary networks. Very premature birth threatens this process. Exposure to excess oxygen, combined with the modifying effects of growth and inflammation, can cause bronchopulmonary dysplasia characterised by maldevelopment of alveoli and pulmonary vasculature. This may be complicated by pulmonary hypertension causing right heart dysfunction which may persist to adulthood.

As pulmonary hypertension develops insidiously, and is associated with significant morbidity and mortality, screening is recommended. However, there are no consensus diagnostic criteria and a paucity of validated heart ultrasound parameters in the very preterm population. N-Terminal proB-type Natriuretic Peptide (NTproBNP), a cardiac hormone, is a promising biomarker as it is a sensitive marker of ventricular wall pressure and volume stress and predicts pulmonary hypertension in other settings. There is limited data in very preterm infants. Premature infants have unstable oxygen saturations due to multifactorial causes which may be exacerbated by pulmonary hypertension. Oxygen saturation targeting attempts to reduce hypoxia and hyperoxia. Modern oximeters allow detailed analysis of oxygen saturation patterns but optimal measures of instability have not been established.

We investigated temporal changes in NTproBNP and factors influencing levels, in a cohort of very preterm infants. We evaluated NTproBNP as a biomarker for pulmonary hypertension by pairing NTproBNP with heart ultrasound and pulse oximetry data in the neonatal period and report clinical and neurodevelopmental outcomes at two years. Quantitative measures of right heart function and compliance with oxygen saturation alarm limits in our unit were evaluated. Current screening recommendations were reviewed.

We demonstrated high NTproBNP on days 3 and 10 then decreasing NTproBNP beyond the period of cardiac transition. NTproBNP was a highly sensitive and specific marker of haemodynamically significant patent ductus arteriosus and a modest predictor of severe bronchopulmonary dysplasia. None of our cohort had evidence of pulmonary hypertension at 36 weeks post menstrual age by predefined conventional heart ultrasound criteria. However, we demonstrated it is feasible for more advanced quantitative measures of right heart function to be incorporated into clinician performed heart ultrasound that may be of value to screening

programmes. We showed changes in these parameters over time, reviewed their reliability, and investigated differences in infants with and without bronchopulmonary dysplasia. Oxygen saturation instability increased in our cohort over time peaking at day 28 and was greater in infants with bronchopulmonary dysplasia. We identified oxygen saturation coefficient of variation and percentage time less than 88% as useful measures of instability. Compliance with oxygen saturation alarm limits in our unit was low despite high levels of nursing experience potentially exposing extremely premature infants to iatrogenic hyperoxia which may contribute to pulmonary hypertension.

In summary, we found a lower than expected rate pulmonary hypertension in our very preterm cohort. There is insufficient evidence for NTproBNP as a standalone biomarker for pulmonary hypertension but it may be useful as an adjunct to comprehensive heart ultrasound evaluation. To reduce pulmonary hypertension, surveillance of at risk infants and oxygen saturation stewardship is needed.

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My role in this study

Supported by all of those mentioned above, I conceived the study question, conducted the literature review, designed the study, wrote the grant applications to secure funding and submitted the ethics application. I was also responsible for the day to day running of the study including recruitment, data collection and data entry and ongoing communication with families. I conducted 90% and reported all of the heart ultrasounds and was responsible for analysis of NTproBNP results, heart ultrasound and oxygen saturation data as well as health and neurodevelopmental outcome data. I also observed the BNP isoform testing and designed the respiratory health questionnaire. I have submitted manuscripts for publication and presented results from this study at conferences. Finally I have written and formatted this thesis.

"My journey took me somewhat further down the rabbit-hole than I'd intended and, though I dirtied my fluffy white tail, I've emerged enlightened."

Guy Ritchie's Sherlock Holmes

PREFACE

Ethical Approval

This study complied with the Declaration of Helsinki regarding the ethical principles for medical research involving human participants. Ethical approval was granted by the University of Otago Human Ethics Committee (12/298). Local study approval was granted after consultation with Māori through Te Komiti Whakarite and the study was conducted in accordance with the principals of the Treaty of Waitangi.¹

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Disclosures

No conflicts of interests to declare or financial interests to disclose

Publications and Proceedings

This work has been published presented in part in the following publications and meetings:

- Harris SL, More K, Dixon B, Troughton, R Pemberton C, Horwood J, Ellis N, Austin N. Factors affecting N-terminal pro-B-type natriuretic peptide levels in preterm infants and use in determination of haemodynamic significance of patent ductus arteriosus. January 2018 European Journal of Pediatrics DOI10.1007/s00431-018-3089-y
- Harris S, Austin N, Horwood J, Pemberton C, Troughton R. Factors influencing NTproBNP levels over time in preterm infants. Poster at the Pediatric Academic Societies Meeting, San Francisco, 2017
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Abbreviations

ANOVA	Analysis of variance
ANZNN	Australia and New Zealand Neonatal Network
AT	acceleration time
BP	blood pressure
BPD	bronchopulmonary dysplasia
BNP	B-type natriuretic peptide
CIH	chronic intermittent hypoxia
CPAP	continuous positive airway pressure
CRP	C- reactive protein
CV	coefficient of variation
cGMP	cyclic guanosine monophosphate (cGMP)
DOHAD	developmental origins of health and disease
EDTA	ethylene diamine tetra acetic acid
FS	fractional shortening
HbA1c	haemoglobin A1c
HIF	hypoxic- inducible factor
HPLC	high performance liquid chromatography
HsPDA	hemodynamically significant patent ductus arteriosus
IAAV	intrapulmonary arteriovenous anastamotic vessels
ICC	intraclass correlation coefficient
IUGR	intrauterine growth restriction

LV	left ventricle or left ventricular
LVEI	left ventricular eccentricity index
MPA	main pulmonary artery
MHC	myosin heavy chain
NICHHD	National Institute of Child Health and Human Development
NICU	Neonatal Intensive Care Unit
NTproBNP	N-terminal pro B-type natriuretic peptide
PASP	pulmonary artery systolic pressure
PDA	patent ductus arteriosus
PH	pulmonary hypertension
PMA	post-menstrual age
PPHN	persistent pulmonary hypertension of the newborn
PVD	pulmonary vascular disease
RA	right atrial
RDS	respiratory distress syndrome
RIMP	right index of myocardial performance (also known as right Tei index)
ROC	receiver operating curve
ROP	retinopathy of prematurity
RV	right ventricle or right ventricular
RVET	right ventricular ejection time
RVSP	right ventricular systolic pressure
SGA	small for gestational age
SpO ₂	peripheral capillary oxygen saturation

TAPSE	tricuspid annular plane of systolic excursion
TAPSV	tricuspid annular peak systolic velocity
TDI	tissue Doppler imaging
TnT	troponin T
TR	tricuspid regurgitation
USS	ultrasound scan
VEGF	vascular endothelial growth factor

BACKGROUND

The Impact of Premature Birth on Cardiopulmonary Development

Importance of premature birth

Globally one in ten babies are born prematurely.² Premature birth is defined by the World Health Organisation as birth before 37 completed weeks gestation.^{3,4} Rates of premature birth are rising and there is considerable geographic variation (Fig.1).^{2,3} Common causes of preterm birth are maternal preeclampsia (a hypertensive disorder of pregnancy), chorioamnionitis (uterine infection), uteroplacental haemorrhage, premature rupture of membranes and spontaneous preterm labour of unclear aetiology.⁵

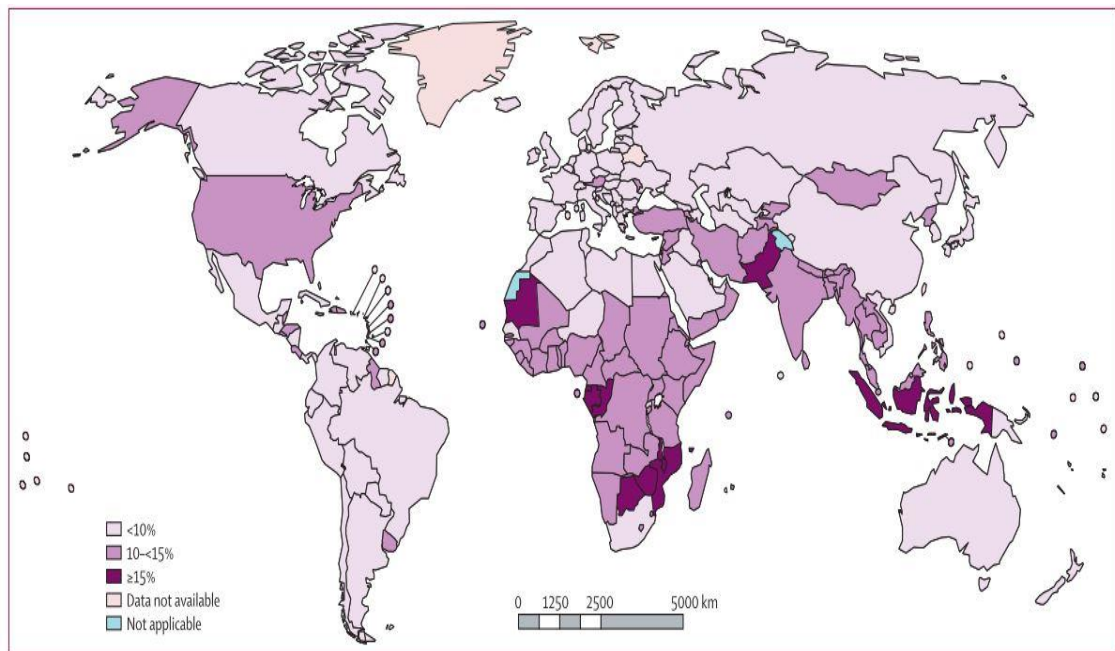


Figure 1: Estimated preterm birth rates by country for the year of 2010

Reproduced with permission from: Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet*. 2012;379(9832):2162-72. Copyright Elsevier

In New Zealand 7.4% of live-born infants are born prematurely and 1.3% are born at < 32 weeks gestation.⁶ When intensive care is available survival rates are high. The Australia and New Zealand Neonatal Network (ANZNN), has prospectively collected data on all high risk infants, admitted to the 27 regional neonatal intensive care units (NICU) since 1995 including New Zealand level 2 units from 1999. ANZNN survival rates for infants admitted to a neonatal unit in 2013 rose from 66% for babies born at 24 weeks to 98% for those born at 29 weeks gestation.⁷

Several major advancements have contributed to the rise in survival rates. In 1972 New Zealanders Sir Graham Liggins and Dr Ross Howie made the serendipitous discovery that antenatal corticosteroids accelerate fetal lung maturity. This intervention reduced mortality from premature lung disease to one third of that in the untreated group.⁸ Subsequent advances, such as lung surfactant replacement therapy which prevents collapse of alveolar lung units and improves ventilation and the shift away from invasive ventilation to gentler modes of non-invasive ventilation have contributed to the now very high survival rates seen in the developed world.^{9 10} There remains, however, significant global inequity in healthcare provision and premature birth remains the number one cause of newborn deaths globally and the second leading cause of death in children under five.³ It is estimated that >75% of deaths secondary to premature birth are preventable without intensive care.³

As survival has increased, long term sequelae of premature birth have become apparent. Premature-born children at risk of poor neurodevelopmental and learning outcomes, poor respiratory health and impaired growth.^{11, 12} There is also significant evidence for the developmental origins of health and disease (DOHAD) in which the in-utero and early life environment influence the risk of disease in adulthood.¹³ Three of the most significant non-communicable disease challenges facing society, heart disease, diabetes and obesity, are thought to have their origin in the in-utero and perinatal milieu of experiences.¹³ The fetus has the capacity to adapt to a sub-optimal intrauterine environment to ensure survival. However, when there is a mismatch of in-utero and post-natal conditions, such as a period of growth restriction followed by liberal nutrition, this adaptive programming can predispose an individual to obesity, cardiovascular disease, dyslipidaemia and diabetes.^{14 15, 16} Premature infants are at particularly high risk of this developmental plasticity having long term health consequences.¹⁷⁻²¹

The societal cost of preterm birth is considerable.²² In 2005 the United States annual minimum cost of preterm birth was estimated to be \$26.2 billion, while in the United Kingdom costs to the public sector were estimated to be £2.946 billion.^{23 24} These are underestimates as these studies only focus on the immediate costs of neonatal care and the long term costs of major

childhood morbidities. They do not include the cost to caregivers or the late effects on adult health.

Thus prematurity is common, is a significant contributor to global child mortality, has lasting health effects and consumes a large portion of health budgets. However, tremendous improvements have been made in outcomes after prematurity through high quality research and evidenced based practice. Preventing premature birth or the complications of premature birth is of great importance as it can have far reaching effects on individuals, families and society.



Figure 2: Premature baby in incubator

Permission to use image for educational and teaching purposes granted by family.

The impact of premature birth on lung development

Lung development remains the single greatest challenge for infants born too early, contributing to long hospital stays, respiratory morbidity in early childhood and late deaths beyond neonatal discharge.^{25, 26} Effective respiration requires complex synchrony between the neural drive of the respiratory control centre in the brain, the mechanics of airway, diaphragm and intercostal musculature and gas exchange at the level of alveoli and pulmonary vasculature.²⁷ Premature birth disrupts the normal embryological development of this system.

Animal and human studies have demonstrated that the detrimental effects of premature birth on lung architecture are influenced by antenatal factors, such as placental insufficiency, chorioamnionitis, antenatal steroids and postnatal factors including surfactant deficiency, oxygen exposure, mechanical ventilation and sepsis (Fig. 3).²⁸

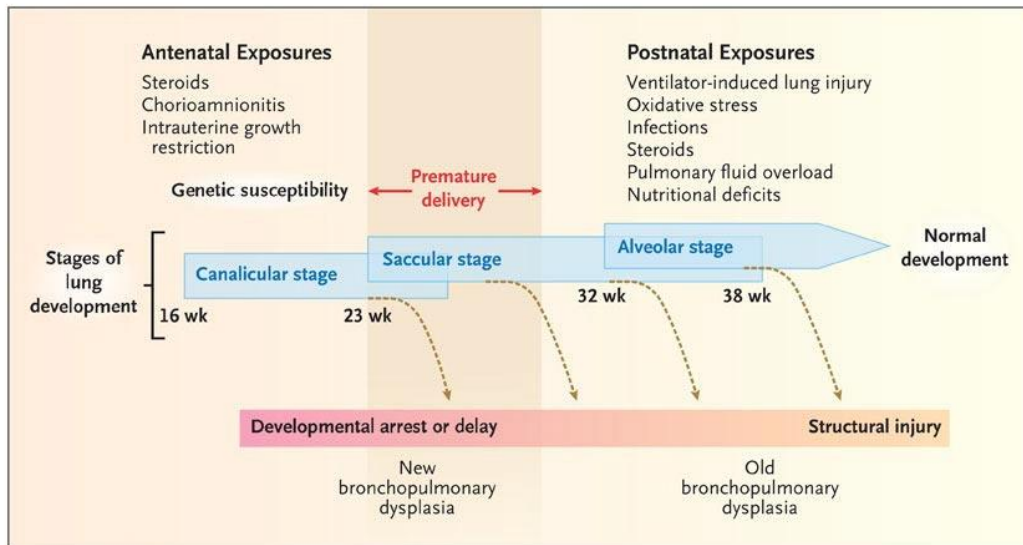
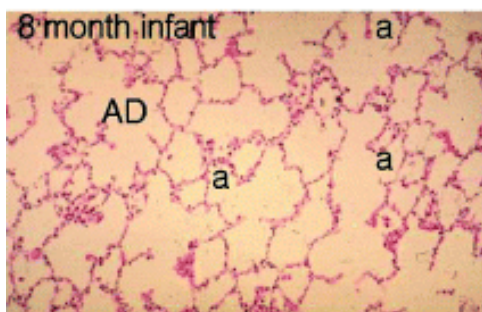
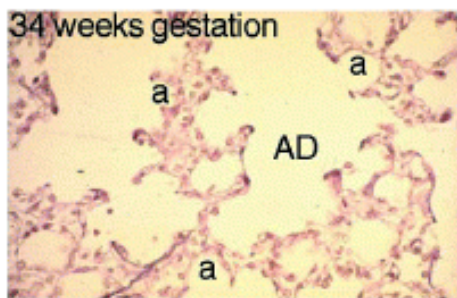
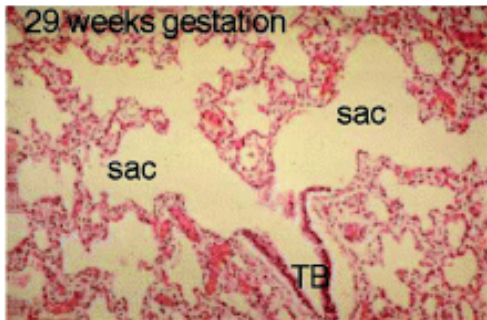
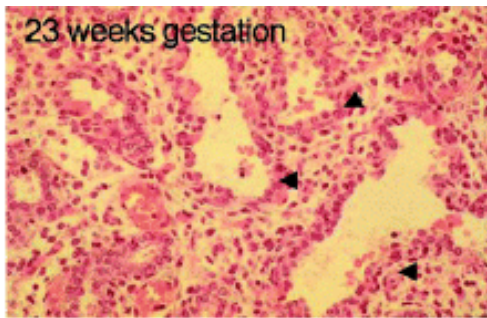


Figure 3: The impact of premature birth on lung development

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An infant born at < 28 weeks gestation has lungs that are transitioning between the canalicular and saccular stages of development. At this stage air sacs and the vascular network surrounding them are primitive with reduced surface area for gas exchange (Fig. 4). The interstitial gap is wide, reducing efficiency of gas exchange. Surfactant-producing cells are only just beginning to be differentiated and the resultant surfactant deficiency threatens the stability of end-expiratory lung volumes and significantly decreases lung compliance. This results in a risk of acute respiratory distress syndrome (ARDS) and subsequent bronchopulmonary dysplasia (BPD), a form of chronic lung disease. The risk of BPD is inversely proportional to gestational age with rates of 50% at 24-25 weeks, 26% at 26-27 weeks and 12% at 28-29 weeks.²⁹



Canalicular and Saccular Stages

- Respiratory airways develop accompanied by arteries and veins
- Thinning of epithelium by underlying capillaries allows gas exchange
- Surfactant first detectable at 24-25 weeks gestation
- Mesenchyme decreases
- Alveoli start to appear from 29 weeks

Alveolar Stage

- Cup shaped alveoli with double capillary loops at 34 weeks
- Alveoli multiple and increase in size until 2-3 years
- Capillary bed increases in area
- Airways, arteries and veins increase in size

Figure 4: Appearance and growth of the lung in the canalicular, saccular and alveolar stages

Note large surface area achieved by branching and septation and thin alveolar/vascular interface increasing efficiency of gas exchange. TB=terminal bronchiolus; sac=saccule; arrows=capillaries. AD=alveolar duct; a=alveolus. Reproduced with permission from: *Journal of Anatomy*. Volume 201, Issue 4, pages 325-334, 14 OCT 2002 DOI: 10.1046/j.1469-7580.2002.00097.x <http://onlinelibrary.wiley.com/doi/10.1046/j.1469-7580.2002.00097.x/full#f1>
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The original description of BPD was based on the pathological changes seen in moderately preterm infants exposed to high concentrations of oxygen and aggressive mechanical ventilation.³⁰ With the introduction of antenatal corticosteroids, surfactant, non-invasive respiratory support and oxygen saturation monitoring, the pathology of BPD has changed. Historically BPD was characterised by severe inflammation, fibrosis and airway damage. “New BPD” occurs in extremely premature infants and is characterised primarily by arrested or impaired pulmonary development with alveolar hypoplasia and malformed pulmonary vasculature (Fig. 5). Animal BPD models can be induced by exposure to hyperoxia.(Fig.5)

Intrauterine programming is likely to play a role in the development of BPD as intrauterine growth restriction and placental insufficiency, chorioamnionitis, maternal smoking and lack of antenatal steroids prior to preterm birth all influence risk of subsequent BPD.³¹⁻³³ The combination of inflammation and oxidative stress is central to the arrest of normal pulmonary development.^{34, 35}

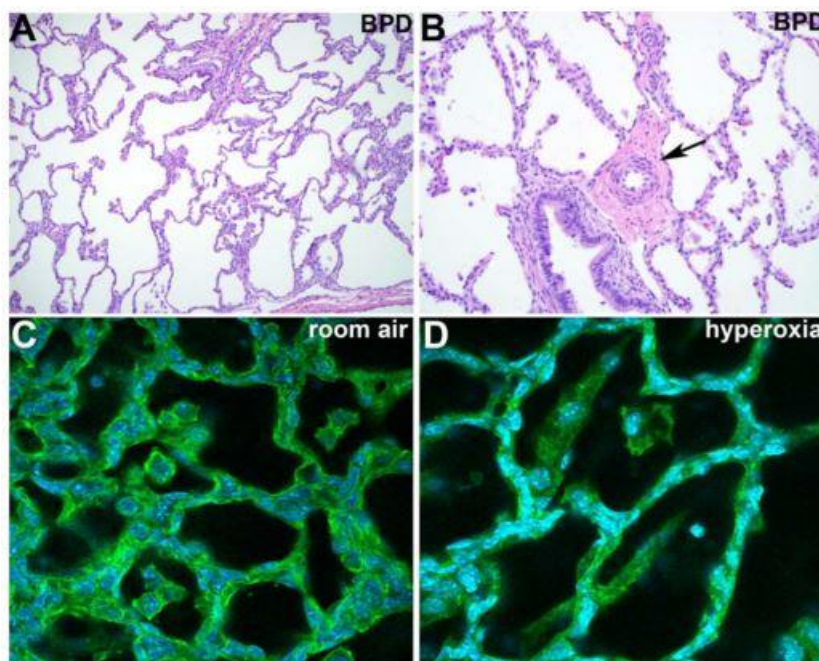


Figure 5: Histological presentation of new BPD

(A,B) Histology of a patient with fatal new BPD, demonstrating increased distal airspaces with decreased septation and reduced alveolarization (A); as well as pulmonary vascular wall thickness in small arteries (arrow in (B)), suggestive of pulmonary hypertension.

(C,D) Confocal images of postnatal day 7 mouse lungs exposed to either room air (C) or 85% O₂ hyperoxia (D) from birth onward. Note that the hyperoxic lung exhibits reduced alveolarization, exemplifying the alterations in distal airspace that mimics new BPD. Nuclei are stained with DAPI (blue) and green signal is extracellular matrix autofluorescence.

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The impact of prematurity on the immature heart

Heart and lung function are intimately related. Premature birth disrupts normal cardiopulmonary development. The underdeveloped heart, lungs and pulmonary vasculature of the prematurely born infant have to adapt and function in a way that is entirely different to that of the foetus.

The heart of an infant born very prematurely has an immature cellular structure that is less adapted to the physiological stressors of post-natal life. Myocytes are smaller, often with a single nucleus compared to the multinucleated myocytes of the term infant, there are fewer sarcomeres, immature contractile proteins and fewer mitochondria.^{36, 37 38} During foetal life cardiac size is increased by cardiomyocyte hyperplasia (increasing cell numbers), however, postnatally hypertrophy (increase in cell size) predominates.³⁹ The preterm heart is less compliant and contractile than that of a term born baby.⁴⁰

During foetal life the right ventricle (RV) predominates with a thicker ventricular wall and greater cardiac output than the left ventricle (LV).⁴¹ The pulmonary vasculature is a low flow, high resistance circuit. In contrast the LV encounters relatively low systemic pressure in foetal life with the placenta acting as a high flow, low resistance circuit. After birth the loss of the placenta leads to an immediate increase in systemic pressure and LV afterload. The inflation of the lungs and exposure to relative hyperoxia leads to a fall in pulmonary vascular resistance and decreased RV afterload. With premature birth a heart that is still developing encounters a sudden increase in systemic pressures and a decrease in pulmonary pressures dramatically altering the relationship between the left and right ventricle before the heart has fully matured.

Postnatal persistence of the ductus arteriosus (PDA), a foetal conduit between the main pulmonary artery (MPA) and aorta, increases with decreasing gestational age and may further complicate cardiac transition to postnatal life causing left to right shunting, higher flow through pulmonary vasculature, reduced pulmonary compliance, increased LV output and vascular steal phenomena reducing blood flow to vital organs.^{42 43} Premature birth also has long term effects on the cardiovascular system predisposing preterm-born adults to an increased risk of hypertension, coronary artery disease, dyslipidaemia and diabetes.⁴⁴⁻⁴⁶

Embryological development of the heart is coordinated by a foetal cardiac gene programme which leads to fundamental differences in the heart of the foetus compared to that of the neonate. The foetal heart's primary energy source is carbohydrate in the form of glucose or lactate.^{47 48} This provides a highly efficient source of energy and is maintained by the constant

supply of glucose via the placenta. This enables energy to be rapidly mobilised during times of stress. One third of the foetal cardiomyocyte volume is glycogen.^{47, 48} The foetal cardiomyocyte sarcomere has a higher ratio of myosin heavy chain (MHC) β to MHC α which renders it stiffer and less contractile.^{47, 48} Levels of B-type natriuretic peptide (BNP), a cardiac hormone, are high and thought to play a role in promoting foetal cardiac development with animal studies demonstrating that natriuretic peptide receptor knockout results in marked cardiac hypertrophy and fibrosis.⁴⁹⁻⁵¹

After birth this foetal cardiac gene programme undergoes a rapid switch, to the so-called “adult” cardiac gene programme, thought to be primarily mediated by exposure to higher oxygen concentrations, perinatal hormones and an abrupt discontinuation of glucose supply. This programme is characterised by a predominance of fatty acid oxidation for energy metabolism with only 2% of the cardiomyocyte volume being glycogen.⁴⁷ The MHC β :MHC α ratio decreases decreasing stiffness and increasing contractility.⁴⁸ BNP levels, after an initial rise during transitional circulation, subsequently fall.^{52, 53} This switch is however, potentially reversible. At subsequent times of cardiac stress the heart may revert to this foetal cardiac gene programme which, in the short term, has a cardioprotective effect.^{48, 54} The interaction between foetal gene programmes and modification by the in-utero and post-natal environments is currently an area of active research.

Pulmonary vascular disease after preterm birth

In addition to the disruption of the normal pattern of cardiopulmonary development, premature birth also puts infants at risk of pulmonary vascular maldevelopment. Indeed alveolar development appears to be dependent on pulmonary vascular development in what has been termed “the vascular hypothesis”.^{55, 56} In an example of synchronised development, respiratory epithelial cells secrete vascular growth factors that act on endothelial cell receptors to drive pulmonary vascular development (Fig.6).⁵⁶ The development of the pulmonary capillary network in turn drives alveolarization.

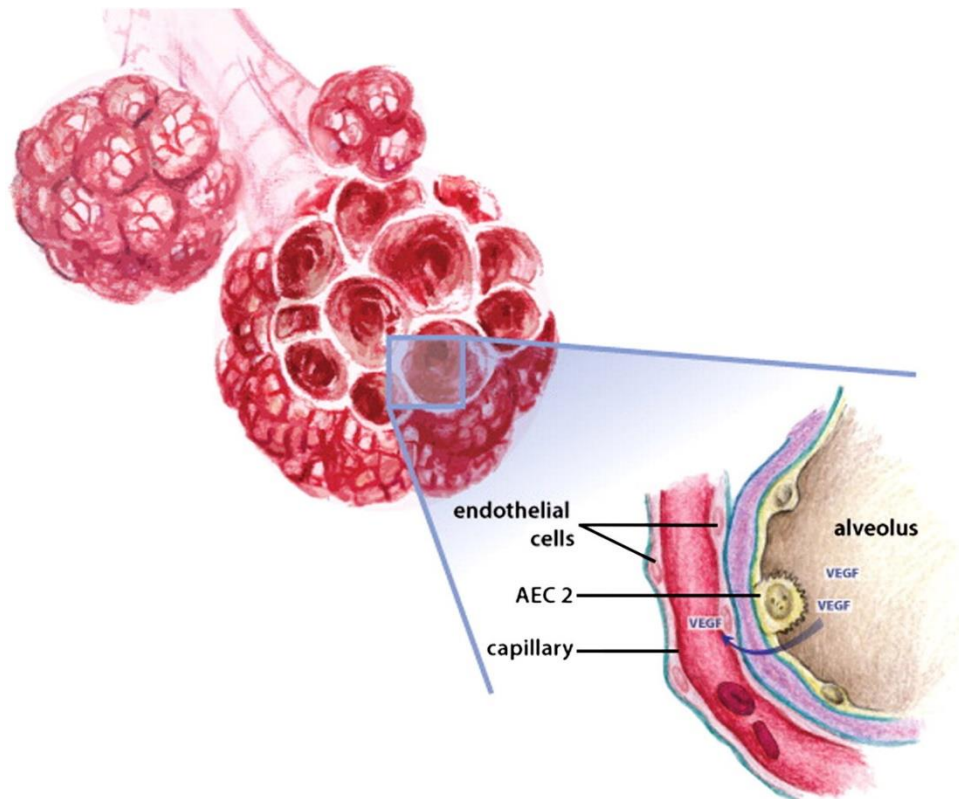


Figure 6: Schematic of proposed interactions between vascular growth factors and their receptors during alveolarization

Vascular growth factors are secreted by the respiratory epithelium and signal to their receptors located on the vascular endothelium to promote angiogenesis and drive alveolarization. (AEC2 = alveolar epithelial type 2 cell, VEGF = vascular endothelial growth factor).

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Pulmonary vascular disease, in premature infants, is characterised by a paucity of pulmonary vessels which are dysmorphic and dysfunctional. Histology of the lung shows pulmonary vessels that are reduced in number, maldistributed and dysmorphic with medial hypertrophy and distal muscularisation.⁵⁶ Functionally they are also abnormal with a tendency to vasoreactivity that compromises gas exchange.⁵⁷ These abnormal pulmonary vessels sit alongside alveoli that are larger but fewer in number.⁵⁸ Intrapulmonary arteriovenous anastamotic vessels (IAAV) have also been found in the lungs of infants who have died of BPD.⁵⁹ These connecting vessels are present in foetal life but should disappear postnatally. They shunt deoxygenated blood into the pulmonary venous network bypassing the gas-exchange interface. Inflammation and immune regulatory cells also appear to modify lung angiogenesis which may explain the relationship between chorioamnionitis and BPD.⁶⁰ In combination these features result in poorer gas

exchange and a propensity for precipitous drops in oxygen saturation necessitating respiratory support.

In foetal life the placenta is the major organ of gas exchange. Placental underperfusion, such as that seen with preeclampsia and other hypertensive disorders of pregnancy, is associated with a subsequent increased risk of BPD and particularly associated with an increased risk of BPD-associated pulmonary hypertension.⁶¹ This suggests foetal programming may be important in the subsequent development of the pulmonary vascular disease associated with premature birth.

The most severe end of the pulmonary vascular disease spectrum is pulmonary hypertension which may cause right heart failure. BPD-associated pulmonary hypertension may also be complicated by left ventricular dysfunction, systemic to pulmonary collaterals and pulmonary vein stenosis.^{62, 63} Prospective studies have suggested 18% of infants with a birthweight of <1000g will develop pulmonary hypertension with a significantly higher rate in those who develop BPD.⁶⁴

Infants with pulmonary vascular disease and in particular pulmonary hypertension are at increased risk of recurrent episodes of hypoxemia, prolonged hospitalisation and need for respiratory support including home oxygen therapy, increased risk of readmission with respiratory illness in the first few years of life, increased risk of death, neurodevelopmental impairment and potentially long term cardiorespiratory effects in adulthood.^{65, 66 62, 67-71} This condition has significant short and long term financial implications for families, the health system and society. Further research is needed to understand the pathophysiology of BPD-associated pulmonary hypertension, to develop better diagnostic tools and ultimately to develop preventative strategies of care.

Impact of oxygen on pulmonary vascular development

Hypoxia is a potent pulmonary vasoconstrictor. During foetal life the developing lung is exposed to a low oxygen tension. Hypoxia stimulates the release of vascular endothelial growth factor (VEGF) and consequently the development of lung vasculature and acts as a cofactor in the growth of pneumocytes.⁷²

Premature birth exposes an underdeveloped lung and respiratory network to relative hyperoxia compared to the in-utero environment. **Hyperoxia** (a supply of oxygen that is in excess of cellular requirements) is known to have deleterious effects on the immature lung with

prolonged exposure to high concentrations causing inflammation, cell death and consequent pulmonary oedema.^{73, 74} Exposure to excess oxygen and oxidative stress may be a potentially preventable cause of pulmonary hypertension in preterm infants due to the suppressive effects on vascular endothelial growth factors, activation of inflammatory pathways and cellular toxicity. The mechanisms by which this damage is triggered are complex (Fig.7). Hyperoxia reduces the transcription of VEGF which is vital for pulmonary vascular development.^{75, 76} Excess oxygen also generates reactive oxygen species and activates an inflammatory response. This triggers a cascade that ultimately damages mitochondria, the cellular power unit, leading to cell death.⁷³ The result of exposure of the developing lung to hyperoxia is a pulmonary vascular bed that has reduced numbers of small pulmonary arteries that are maldistributed and with increased distal smooth muscle leading to heightened vasoreactivity. In turn, as normal pulmonary vascular development is needed for the development of alveoli, inhibition of VEGF results in alveoli that are both simple in structure and decreased in number.^{55, 56} Exposure to other inflammatory events such as chorioamnionitis or post-natal sepsis will further aggravate this damage.³⁵

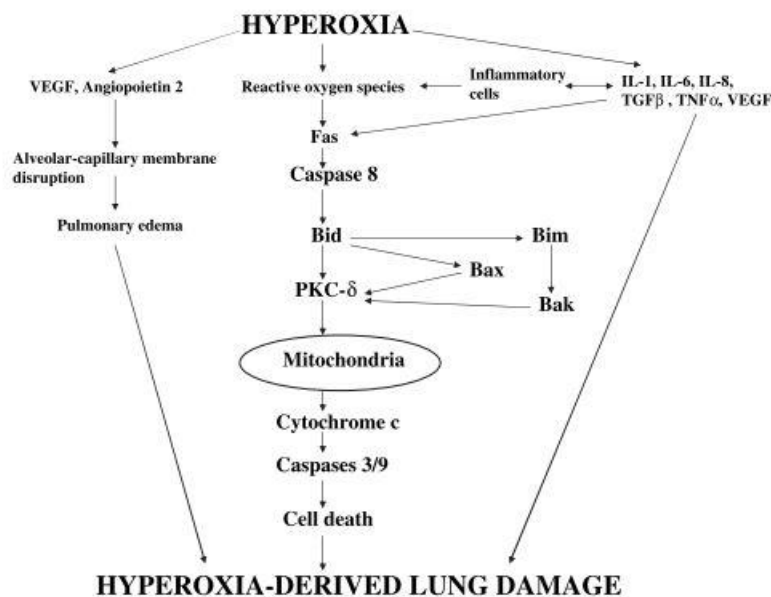


Figure 7: A proposed model of hyperoxia-derived lung damage in neonates delineating some of the pathways

Hyperoxia exposure leads to release of certain mediators [eg. VEGF and angiopoietin 2] that disrupt the alveolar-capillary membrane leading to pulmonary oedema which contributes to lung injury. Other cytokines [eg. interleukin (IL)-1, IL-6, IL-8, transforming growth factor (TGF) b, tumour necrosis factor (TNF) a, VEGF] are also released from lung cells that attract inflammatory cells to the lung. These inflammatory cells as well as hyperoxia per se release reactive oxygen species, which can initiate the mitochondrial-dependent cell death pathway. The cytokines and cell death mediators contribute to pulmonary injury resulting in hyperoxia-derived lung damage. (PKC=protein kinase C).

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Infants born prematurely are also at risk of chronic intermittent **hypoxemia** (where the supply of oxygen is inadequate to meet cellular requirements) due to intrinsic and extrinsic factors. This is known to increase the production of free radicals.⁷⁷ In premature infants recurrent hypoxemia is often accompanied by a subsequent period of hyperoxemia. Intermittent hypoxia-hyperoxia exposure in preterm animal models is associated with the down-regulation of hypoxic-inducible factor (HIF) and angiogenic gene expression and alveolar simplification.⁷⁸ It is likely to be a significant contributing pathway to the development of BPD and pulmonary vascular disease. Chronic intermittent hypoxemia may also have lasting effects on respiratory control causing a subsequent decreased response to acute hypoxia.^{27, 79} An understanding of the impact of hypoxia and hyperoxia is critical in order to support optimal lung development and reduce risk of pulmonary hypertension. The effects of too little and too much oxygen on the developing lung are complex and sometimes contradictory (Fig.8,9). Identifying just the right amount of oxygen for the very preterm infant has proved to be the holy grail of neonatal research.

	Structure	Key Effects
Hypoxia	Alveoli	<ul style="list-style-type: none"> • ↓ Alveolarization • ↓ Gas exchange • Altered angiogenesis
	Airway Smooth Muscle	<ul style="list-style-type: none"> • ↑ ASM thickness • ↑ Airway inflammation
	Airway Epithelium	<ul style="list-style-type: none"> • ↓ Na⁺ transport • ↑ Mucous secretion
Hyperoxia	Alveoli	<ul style="list-style-type: none"> • ↓ Alveolarization • ↑ Interstitial fibrosis • ↓ Gas exchange • ↑ Macrophage and PMN infiltration • ↑ Interstitial edema • ↑ epithelial cell death
	Airway Smooth Muscle	<ul style="list-style-type: none"> • ↑ AHR • ↑ ASM thickness • ↑ ASM proliferation • ↑ ECM remodeling • ↓ Airway relaxation • ↓ Bronchial-alveolar attachment • ↑ immune cell infiltration
	Airway Epithelium	<ul style="list-style-type: none"> • ↑ Cytokine and chemokine expression • ↑ Epithelial thickness

Figure 8: Effects of hypoxia and hyperoxia on the developing lung

Reproduced with permission from: Vogel E.R, Britt R.D, Trinidad M.C, Faksh A, Martin R.J, MacFarlane P.M, et al. Perinatal oxygen in the developing lung. *Can J Physiol Pharmacol.* 2015;93(2):119-27. Copyright Canadian Science Publishing or its licensors

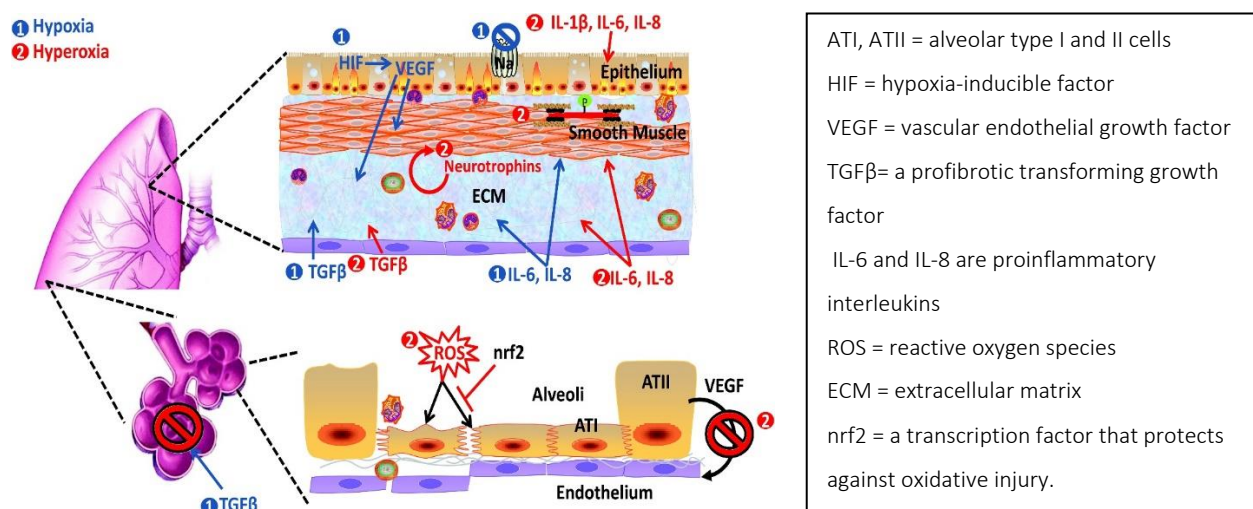


Figure 9: Potential mechanisms by which hypoxia and hyperoxia affect lung development

Hypoxia activates the HIF-VEGF pathway of vascular development. Hyperoxia can cause oxidative damage to alveolar epithelium. Hypoxia and hyperoxia can induce proinflammatory and profibrotic mediators. Reproduced with permission from: Vogel E.R, Britt R.D, Trinidad M.C, Faksh A, Martin R.J, MacFarlane P.M, et al. Perinatal oxygen in the developing lung. *Can J Physiol Pharmacol.* 2015;93(2):119-27. Copyright Canadian Science Publishing or its licensors

Epidemiology of late pulmonary hypertension in preterm infants

There are limited published data regarding the epidemiology of pulmonary hypertension associated with premature birth and even less regarding the spectrum of pulmonary vascular disease. It is estimated that the incidence of pulmonary hypertension in infants born prematurely ranges from 17-37%.^{64 80-82} Prospective studies suggest up to 14% of extremely premature infants with no or mild BPD but up to 29% of those with moderate to severe BPD have signs of pulmonary hypertension at 36 weeks corrected post-menstrual age (PMA).^{83, 84} However, there are wide variations in the population studied, the diagnostic tools and criteria used and the timing of assessment. Infants with BPD and pulmonary hypertension have significantly greater morbidity and health care utilisation than those with BPD alone.⁸⁵ Mortality has been estimated at 38% of infants diagnosed with bronchopulmonary dysplasia-associated pulmonary hypertension with a probability of survival 3 years after diagnosis of 52%.⁶²

One of the problems for the neonatologist with published clinical data is that the series tend to be from highly selected patients referred to centres specialising in pulmonary hypertension. It is difficult to know what the incidence is of pulmonary hypertension in a general population of premature infants as there are no large multicentre, prospective studies or standardised screening procedures although screening is recommended.⁸⁶⁻⁸⁸

Factors associated with an increased risk of pulmonary vascular disease include extreme prematurity, poor placental perfusion such as that seen with preeclampsia, chorioamnionitis, intrauterine growth restriction (IUGR), exposure to excessive oxygen, BPD, mechanical ventilation, pulmonary vein stenosis, aorto-pulmonary collaterals and large persistent patent ductus arteriosus.^{60-62, 67, 82, 89-92}

Further research is needed to establish the incidence of pulmonary hypertension in very premature infants. We also need to establish which risk factors should prompt screening investigations and to identify establish optimal timing of such screening in the hope that early treatment may improve outcomes. There is, however, a paucity of evidence regarding the treatment of late pulmonary hypertension in very premature infants.

Management of late pulmonary hypertension in preterm infants

The approach to managing pulmonary hypertension in premature infants is complex and requires a multi-faceted approach. There are a lack of randomised controlled trials of treatment in this population and recommendations are mostly based upon expert opinion.⁹³⁻⁹⁶ On the basis of this expert opinion: Lung disease should be evaluated and every attempt made to optimise lung function and enhance gas exchange. Hypoxia, which can precipitate a pulmonary hypertensive crisis, should be avoided. Equally, iatrogenic hyperoxia must be minimised to avoid further impairment of pulmonary vascular development. Structural airways anomalies such as vocal cord paralysis, sub-glottic stenosis, tonsillar/adenoidal hypertrophy and tracheomalacia should be excluded. Nutrition should be optimised. Gastroesophageal reflux or microaspiration may also contribute to pulmonary hypertension and should be managed.

Inhaled nitric oxide is a pulmonary vasodilator that mediates production of cyclic guanosine monophosphate (cGMP) which induces smooth muscle relaxation. Nitric oxide needs to be administered continuously due to the short half-life and is expensive and labour intensive. It is a very effective treatment for acute persistent foetal circulation with high pulmonary vascular tone in term infants.⁹⁷ Results for late pulmonary hypertension in very premature infants may be more mixed due to the fixed structural component of the disease. No long term benefit has been demonstrated in this population.^{98, 99}

Cardiac catheterisation is recommended prior to commencing chronic vasodilator therapies. Sildenafil is a phosphodiesterase-5 inhibitor that induces pulmonary vasodilation mediated through cGMP. It can be administered orally, has a longer half-life and is considerably cheaper

that nitric oxide. It can also be safely administered in a community setting. Sildenafil may be a safe and effective treatment for late pulmonary hypertension in very premature infants although not all studies have shown consistent improvements in oxygenation, it does not appear to prevent BPD and there are no data on long term outcomes.¹⁰⁰⁻¹⁰⁴

Prostacyclin analogues are second line therapy options for resistant pulmonary hypertension. These agents activate adenylate cyclase which increases cyclic adenosine monophosphate which results in smooth muscle relaxation. There is very limited data on safety and efficacy for their use in the preterm population and they are associated with significant side effects rendering them unsuitable for the outpatient setting.⁹⁵

Conditions of hypoxia cause the release of endothelin-1, a potent endothelial vasoconstrictor. Endothelin-1 receptor antagonists act to counter this. These agents can be given orally, however there are very little data on their use in the preterm population and side effects limit their use.

Animal studies suggest that treatment with bone marrow sourced mesenchymal stem cells or even just mesenchymal stem cell conditioned media, may protect against oxygen-induced lung injury and pulmonary vascular maldevelopment.¹⁰⁵ Although promising, this therapy is at the experimental stage only.

Treatment of pulmonary hypertension in premature infants is complex, challenging, expensive and potentially harmful. Therefore it is vital that there are clear diagnostic criteria and reliable diagnostic tests before embarking upon treatment.

Diagnosis of late pulmonary hypertension in preterm infants

The Pulmonary Vascular Research Institute Task Force has published a consensus classification for paediatric pulmonary hypertensive vascular disease which recognises BPD-associated disease as a separate subgroup.¹⁰⁶ Pulmonary hypertensive vascular disease can be defined as a mean pulmonary artery pressure at rest and at sea level of >25 mmHg and a pulmonary vascular resistance index >3.0 Wood units.m² for biventricular circulations.^{87, 107} However, this definition relies on cardiac catheterisation and is generally applied in children above the age of three months. In younger infants who are still completing the transition from foetal to neonatal circulation a systolic pulmonary artery pressure of > 36mmHg is generally considered elevated.^{108, 109} Cardiac catheterisation is the current gold standard test for evaluating the structure of the pulmonary vascular bed and measuring pulmonary vascular pressure. However,

this is an invasive procedure that is available only at specialist centres and the transport of fragile premature infant for this procedure carries a significant morbidity and mortality risk.

Echocardiography utilising ultrasound offers a non-invasive assessment of cardiac structure and function and an indirect assessment of pulmonary pressures. However, it still involves some handling of infants and accuracy is dependent on the skill of the scanner. Furthermore there is limited validated data on echocardiographic measures of late pulmonary hypertension in the preterm population and debate persists regarding an echocardiographic definition of pulmonary hypertension in this setting.^{64, 80, 81, 110-113} Because of the structural and functional differences between the premature heart and the mature heart more data needs to be collected to establish baseline reference echocardiographic indices in the preterm population. Only then can we validate optimal echocardiographic measures of late pulmonary hypertension. A simple, reliable and affordable screening test for at risk infants that would enable clinicians to triage which infants warrant echocardiographic assessment and referral for cardiac catheterisation would also be of great value.

Screening for late pulmonary hypertension

The development of late pulmonary hypertension is ominous for a preterm infant heralding a significant risk of death.^{62, 80, 81, 114, 115} As it develops insidiously the disease is usually well established by the time it is clinically apparent and irreversible structural changes have taken place in the pulmonary vasculature. Management strategies, primarily oxygen and pulmonary vasodilators, are at best designed to support the infant, acting on any reversible vasoconstriction while waiting for lung growth and maturation to ameliorate the structural component of the disease. Prolonged respiratory support or oxygen is often required. Moreover, after apparent clinical improvement, pulmonary hypertensive crises may occur when an infant is challenged by a respiratory infection or general anaesthetic.^{98, 116} Even with time and growth abnormal pulmonary artery pressures and subclinical ventricular dysfunction can persist throughout childhood and adolescence, affecting exercise tolerance, while in adulthood structural and functional abnormalities of the right ventricle become apparent and are associated with an increased risk of heart failure and cardiovascular death.^{71, 117 45, 98} These challenges and concerns have led to recommendations to screen preterm infants for late pulmonary hypertension in the hope that detecting the disease at an earlier stage of its evolution will facilitate earlier treatment and decrease mortality and morbidity.

To successfully screen for a disease it is necessary to:

1. Understand the pathophysiology such that a population at risk can be screened while those not at risk are not subjected to screening
2. Have a screening test that is low risk, not excessively invasive, cost effective and that exhibits an acceptable level of sensitivity / specificity
3. Have a treatment available that will improve outcomes
4. Time screening at a point that most cases will be detected and detection at this point will improve outcomes

A consensus statement has recently been released on the evaluation of BPD-associated pulmonary hypertension by the Paediatric Pulmonary Hypertension Network, a multidisciplinary group of North American pulmonary hypertension experts.⁸⁶ Other screening algorithms have also been proposed by the American Heart Association, the American Thoracic Society, the European Paediatric Pulmonary Vascular Disease Network and pulmonary hypertension research groups.^{62, 87, 88, 111-113, 118-120} However, a recent survey of American Academy of Pediatrics neonatology members revealed only 38% currently have an institutional screening protocol.¹²¹

There are currently no national screening recommendations for late pulmonary hypertension in preterm born infants in New Zealand. There is currently no international consensus on screening for late pulmonary hypertension in premature infants. Several groups have recently published screening recommendations. These are compared in Appendix A. These recommendations are American Heart Association class of recommendation level 1 and level of evidence B or C meaning the benefit of screening is considered greater than the risk but the recommendation is based upon evidence from a single randomised controlled trial or non-randomised studies or consensus expert opinion.⁸⁷

Who to screen

Current recommendations tend to adopt a broad, risk factor based approach or an approach that is more targeted to symptoms of pulmonary hypertension or a combination of the two. Where screening is based upon established risk factors it is recommended to screen infants born at extremely low gestational age, and/or extremely low birthweight and/or those with a diagnosis of BPD. The more symptom-based approach recommends screening infants with significant and persistent oxygen or respiratory support needs or unexplained poor growth.

When to screen

Most groups recommend initial screening at 36 weeks PMA which ties in with the time point at which infants are classified as having BPD or not. However, most groups also argue that screening could occur at any time in infants who were exhibiting signs and symptoms suggestive of pulmonary hypertension.

Mehler et al demonstrated that 41% of infants with pulmonary hypertension do not develop signs of pulmonary hypertension until after discharge from NICU demonstrating the need for follow-up of at-risk infants.¹¹² Whether to screen again and the timing of subsequent screening was variable in the studies reviewed. Timing ranged anywhere from 6 weeks after initial scan to at 1 year of age depending on whether pulmonary hypertension had been diagnosed on initial screen or not and the degree of ongoing respiratory support.

How to screen

There is currently no consensus criteria for the diagnosis of late pulmonary hypertension in premature infants although all reviewed screening algorithms agree that echocardiography should be the initial test. Only three studies offered definitive ultrasound criteria for diagnosis. The remainder suggested a range of parameters that could be used to evaluate for evidence of raised pulmonary pressures and right heart dysfunction. Common to all studies was interrogation of the TR jet if present and an evaluation for septal flattening. Most studies also included RV hypertrophy or dilatation and measures of RV dysfunction. Cardiac catheterisation was generally only recommended for severe disease or prior to the commencement of long-term vasodilator therapy.

The cardiac stress hormone BNP, or the terminal fragment (NTproBNP) were not recommended as a screening test in isolation due to lack of data but some proposed their use to monitor effects of treatment.^{86-88, 113}

Resource implications

The broader the chosen screening criteria the greater the likely cost of the programme and the higher the risk of exposing infants to unnecessary testing. However, restrictive criteria may decrease costs but increase the risk of missing infants with pulmonary hypertension.

The primary test costs associated with screening relate to echocardiography. Ideally screening echocardiograms should be performed by specialist cardiac sonographers. This is a limited human resource often with a significant test cost and may also have potential safety implications if it requires an unstable infant to be transported out of the NICU. Neonatologists

are increasingly acquiring heart ultrasound skills which, if certified and maintained, may allow cot side screening on the neonatal unit at reduced cost. However, by 36 weeks PMA some infants who may qualify for screening may have been transferred to regional centres without neonatal echocardiography services. The frequency of follow-up scans would also significantly add to the cost of any programme.

If BNP or NTproBNP blood testing is incorporated into screening protocols there will be a laboratory cost attached to this. This may be significant if the sample requires transportation to another centre as this test is not universally available. There are also potential ethical implications if blood testing was not otherwise indicated at this time. None of the reviewed studies performed a cost-benefit analysis. It is clear that more data is needed on both the role of BNP in detecting pulmonary hypertension in preterm infants and which heart ultrasound parameters offer the best predictive value.

B-type Natriuretic Peptide

Potential Biomarker?

Introduction

Biomarkers have been defined by the National Institutes of Health Biomarkers Definitions Working Group as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”.¹²² A useful biomarker is one that is inexpensive, easy to measure with minimal patient discomfort and consistently adds diagnostic or prognostic information to guide management. Examples of biomarkers currently in use include haemoglobin A_{1c} (HbA_{1c}), troponin T (TnT) and C-reactive protein (CRP). The level of HbA_{1c} (haemoglobin that is irreversibly bound to glucose over the lifespan of a red blood cell) is used to monitor long term glycemic control in adults and children with diabetes, to guide therapy and thereby lower the risk of long term complications.^{123, 124} Plasma cardiac TnT, a protein release by damaged heart muscle, is widely used to rule out myocardial infarction in patients presenting to the emergency department with chest pain and also helps clinicians prognosticate the risk of poor outcomes.^{125, 126} Plasma CRP is a non-specific inflammatory marker that is used in neonates to support or refute a diagnosis of sepsis.¹²⁷ Biomarkers should demonstrate high sensitivity and specificity in order to reduce the likelihood of false positive or false negatives. A biomarker is currently lacking for neonatal pulmonary vascular disease but B-type Natriuretic Peptide (BNP) has been proposed.

BNP is a hormone rapidly released predominantly by ventricular cardiac myocytes in response to the stretch induced by pressure and volume loading.^{128, 129} Smaller quantities are also released from cardiac atria and the brain where it was originally discovered. Hypoxia and ischaemia have also been shown to stimulate the release of BNP and other hormones and cytokines may influence levels.^{130, 131}

There is a minimal amount of stored BNP and it is essentially “made to order” with the gene, located on the short arm of chromosome 1, being transcribed in less than an hour in response to stimuli. BNP is initially released as **proBNP** then cleaved into signal peptide and a 108 amino acid (aa) prohormone (**proBNP**) (Fig.10). This is subsequently cleaved into the 32-aa active **BNP** hormone and the biologically inert 76-aa N-terminal proBNP fragment (**NTproBNP**)

by the actions of *corin*, a transmembrane cardiac protease, or *furin*, a convertase concentrated in the Golgi apparatus and expressed in a wide range of tissues.

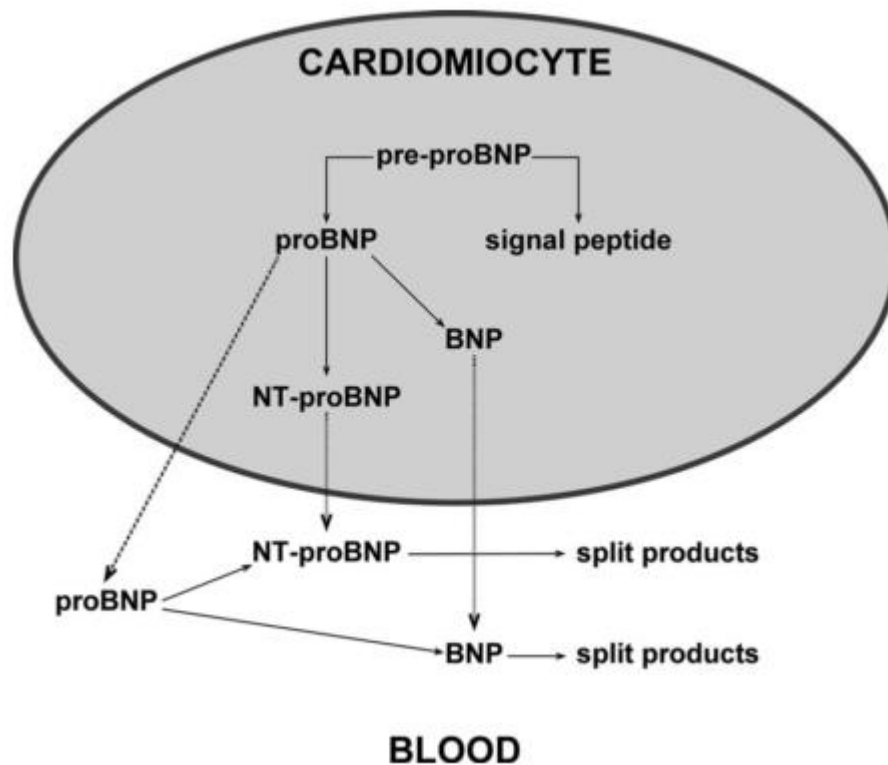


Figure 10: Synthesis and processing of BNP

Reproduced by permission from Springer Nature License: Springer. *Heart Fail Rev*. Originally published in: Cantinotti M, Law Y, Vittorini S, Crocetti M, Marco M, Murzi B, et al. The potential and limitations of plasma BNP measurement in the diagnosis, prognosis, and management of children with heart failure due to congenital cardiac disease: an update. *Heart Fail Rev*. 2014;19(6):727-42

BNP mediates its biological actions by binding to natriuretic peptide receptor A (NPR-A) and activating cyclic GMP. BNP acts on the kidney to increase glomerular filtration and inhibit sodium reabsorption producing a consequent natriuresis and diuresis.¹²⁹ BNP also has cardiovascular effects, reducing blood pressure and pre-load by inducing arterial and venous vasodilatation as well as having a direct effect on myocardial relaxation and inhibitory effects on the sympathetic nervous system.^{129, 132} In combination these effects are cardioprotective acting to reduce pressure and volume loading on the heart and reduce pathological remodelling. BNP also appears to play an anti-inflammatory role decreasing the production of oxygen free radicals by activated macrophages.¹³³

BNP is progressively broken down primarily by renal clearance pathways by the actions of natriuretic peptide clearance receptor C located in the vasculature and by the actions of neutral endopeptidases (Fig.11). Endopeptidases are expressed on renal tubules, vascular endothelium,

heart and lungs. In disease states, in addition to the bioactive 32-aa BNP, cleavage at other sites of the prohormone occurs and some of the BNP detected by immunoassays are these biologically inert fragments. Atrial natriuretic peptide, a related cardiac hormone, and BNP compete for the same receptors and so can effectively inhibit one another in terms of clearance and bioactivity. The actions of BNP are also antagonised by the actions of the renin-angiotensin-aldosterone system which encourages sodium and water retention and vasoconstriction.

NTproBNP has no known biological activity but is more stable than BNP with a longer half-life of 120 minutes compared to 20 minutes for BNP. The clearance of NTproBNP is less well understood but attributed to renal clearance.¹³⁴

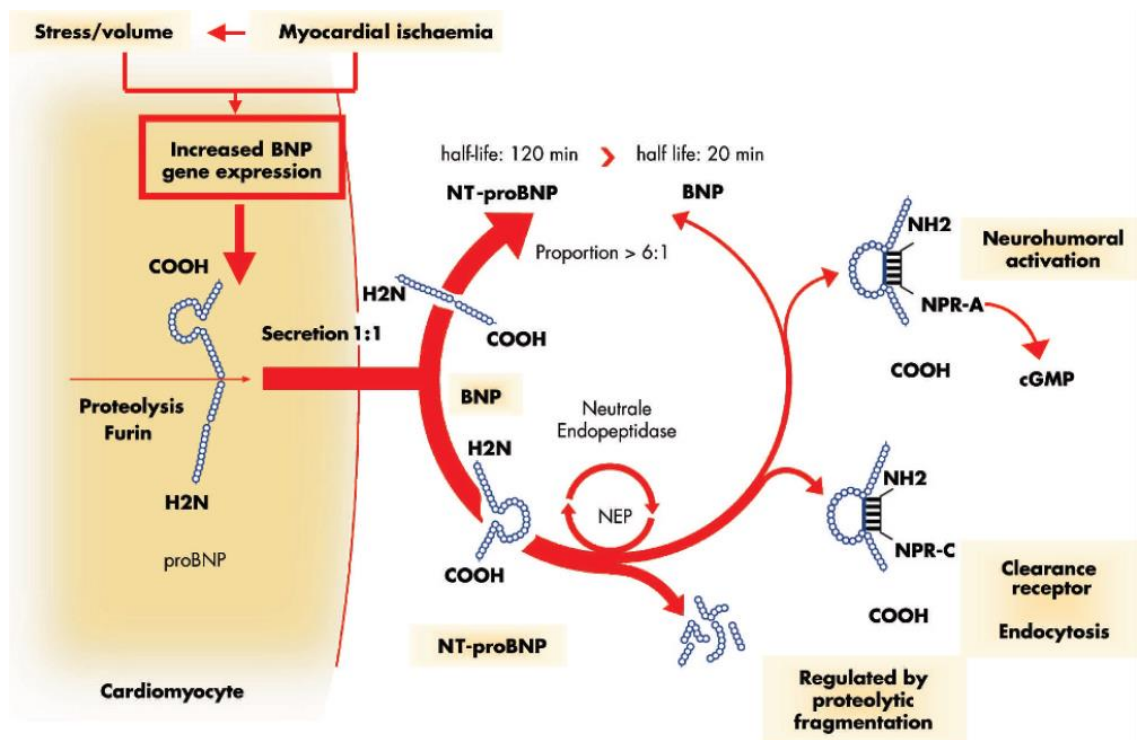


Figure 11: Release and degradation of B type natriuretic peptide

Reproduced from Weber M, Hamm C. Role of B-type natriuretic peptide (BNP) and NT-proBNP in clinical routine. *Heart*. 2006;92(6):843-9, with permission from BMJ Publishing Group Ltd.

As BNP and particularly NTproBNP are renally cleared they will be elevated in renal impairment.¹³⁵ BNP and NTproBNP have also been reported to be elevated in septic shock.¹³⁶⁻¹⁴⁰ This may be secondary to the cardiac effects of severe sepsis rather than the inflammatory response itself.¹⁴¹

BNP is part of the foetal cardiac gene programme that is switched off after birth but can be reactivated in times of cardiac stress later in life.⁴⁷ This allows a switch back to carbohydrates for cellular energy which is conducive in the short term to cellular survival, particularly in the context of an hypoxic environment.⁴⁸ Under these circumstances a rise in BNP acts as a cardioprotective agent to prevent pathological remodelling in a heart under stress.^{54, 142} However, if the stress persists ultimately these short term adaptive mechanisms will fail.

BNP in adult medicine

Plasma levels of BNP and NTproBNP have been evaluated in a number of settings using validated assays. In adults, increasing age is associated with increasing BNP levels with smaller differences seen due to ethnicity, gender and body mass.^{143, 144} BNP and NTproBNP have now been widely adopted in adult medicine where they are used to diagnose, prognosticate and monitor response to treatment for heart failure.¹⁴⁵⁻¹⁴⁷ The American Heart Association and European Society of Cardiology clinical practice guidelines endorse the use of BNP and NTproBNP in the evaluation of the patient with heart failure.^{148, 149} BNP and NTproBNP also have value in predicting poor outcomes in pulmonary hypertension and acute coronary syndrome.¹⁵⁰⁻¹⁵² An NTproBNP cut-off level of 35 pmol/L has high negative predictive value for heart failure in patients presenting to emergency department with acute breathlessness with heart failure being very likely if level is > 53 pmol/L.¹²⁹ High NTproBNP levels are also an independent predictor of short term cardiac risk in adults with acute coronary syndrome.^{152, 153} In adults with heart disease, natriuretic peptides play a role in reducing pathological cardiac remodelling.¹⁴² This led to recombinant BNP (nesiritide) being approved as a treatment for heart failure although it is no longer widely used despite its favourable haemodynamic effects as it offers no reduction of mortality or readmission rates.^{154, 155} Elevated BNP and NTproBNP levels in adults with pulmonary hypertension are associated with poorer prognosis and serial monitoring demonstrates a fall in levels with successful treatment.^{151, 156-158}

BNP in childhood disease

BNP and NTproBNP have proven to be useful adjunct to clinical assessment for the diagnosis, risk stratification and monitoring of children with congenital heart disease, heart failure, cardiomyopathy and pulmonary hypertension.^{159-161 162-164} A recent systematic review has highlighted the wide variation in levels of BNP/NTproBNP in children with pulmonary hypertension and the difficulties in using these markers to guide clinical practice.¹⁶⁵

BNP in neonates

BNP is expressed in the human placenta and may have a role in maintaining blood supply to the foetus by acting as a vasodilator.¹⁶⁶ Corin deficient pregnant animal models show characteristics of preeclampsia and markedly impaired trophoblast invasion with uterine spiral artery remodelling.¹⁶⁷ BNP also suppresses cardiac fibroblast growth and may have a role in cardiac organogenesis.¹⁶⁶ Animal models where natriuretic peptide actions are knocked out exhibit hypertension, cardiac hypertrophy and fibrosis.^{50, 51} Studies of circulating NTproBNP levels in the foetus have found levels 4-10 fold higher in foetuses where there is anaemia, cardiac or urinary tract malformations or intrauterine growth restriction.^{168 169, 170}

BNP and NTproBNP do not cross the placenta, but can be measured in cord blood and reflect foetal levels.^{171, 172} There are no differences in BNP or NTproBNP levels taken in arterial compared to venous cord blood. NTproBNP levels correlate highly with plasma BNP levels in plasma and umbilical cord blood.^{173 174 175} Although levels in adults show sex differences and also increase with advancing age, levels are not affected by gender in the neonatal or childhood period although some differences have been shown in levels at puberty.^{171, 172, 176}

BNP is purported to play a role in neonatal transitional circulation, rising immediately after birth to peak on day 1-2 before declining to steady state levels.^{53 177} It is likely this surge in BNP is stimulated by the sudden increased pressure on the left ventricle caused by cord clamping and loss of the low resistance placental circuit. BNP has been shown to be elevated in infants with persistent foetal circulation, also known as persistent pulmonary hypertension of the newborn (PPHN).¹⁷⁸ This condition has different pathophysiology, clinical course, response to therapy and outcome compared to late pulmonary hypertension in very premature infants. Persistence of a haemodynamically significant patent ductus arteriosus (HsPDA) will cause elevation of BNP levels as will any other condition causing pressure or volume loading of the heart.^{179, 180 181 52, 173} BNP and NTproBNP have proved to be a useful biomarker for the identification of HsPDA and monitoring response to treatment.¹⁸⁰

NTproBNP and late pulmonary hypertension in preterm infants

Despite improvements in the survival of preterm infants, BPD rates remain static and contribute to poor short term and long-term outcomes.¹⁸²⁻¹⁸⁴ Undetected BPD-associated pulmonary hypertension may contribute to respiratory morbidity, intensive care readmission and late deaths. Identifying infants who have pulmonary vascular disease may allow targeted monitoring and therapy for this high risk group. NTproBNP, a sensitive marker of cardiac stress,

offers theoretical promise as a low cost initial screening tool for the detection of early cardiac dysfunction secondary to rising pulmonary vascular pressure.

Two small studies have found higher levels of NTproBNP on day 3 and 28 in infants who go on to develop BPD even after adjusting for gestational age and PDA status.^{185, 186} The few studies of late pulmonary hypertension in preterm infants have used a range of assay kits and have varied significantly in terms of patient selection, timing of testing and diagnostic criteria used for determining the presence of pulmonary hypertension (Table 1).

Table 1: Studies of BNP or NTproBNP levels in premature infants with pulmonary hypertension (PH)

Study	Patient population (number)	Diagnostic criteria for PH	Testing	Results
Cuna 2013 ¹⁸⁷ Retrospective study comparing BNP levels in those with and without PH	Preterm infants with BPD undergoing screening echo for PH (25, 16 with PH, 9 without)	At least one of: 1. Tricuspid regurgitant jet velocity increased 2. Interventricular septum flattening 3. Right ventricular hypertrophy 4. Right to left shunting	Variable timing for echocardiographic diagnosis of PH BNP measured +/- 10 days of scan BNP assay not identified	Median BNP higher in those with PH higher (413 vs 55 pg/ml) p<0.001 BNP ≥117pg/ml had 93.8% sensitivity and 100% specificity for PH
Cuna 2014 ¹⁸⁸ Retrospective study comparing mortality and BNP in infants with BPD and PH	Preterm infants with BPD and PH (36)	At least one of: 1. Tricuspid regurgitant jet velocity increased 2. Interventricular septum flattening 3. Right ventricular hypertrophy 4. Right to left shunting	Initial echocardiogram and BNP between 4-6 weeks then monthly. ADVIA Centaur BNP assay (Siemens USA)	Peak BNP levels lower amongst survivors (128 vs 997 pg/ml) p <0.004 Peak BNP ≥220 pg/ml had 90% sensitivity and 65% specificity in predicting death before neonatal discharge
Montgomery 2016 ¹⁸⁹ Cross-sectional pilot study comparing NTproBNP levels in preterm infants with and without PH	< 27 weeks and or ≤750g with BPD at 36 weeks PMA (20, 5 with PH, 15 without)	1. Left ventricle systolic eccentricity index ≥1.5 or 2. Flat interventricular septum in systole	Echo at 36 weeks PMA and NTproBNP testing within 1 week of echo Assay not identified	NTproBNP level significantly higher in PH vs no PH (1650 vs 520pg/ml) p=0.001 NTproBNP >1000pg/ml predicted PH with 100% sensitivity and 94% specificity

Study	Patient population (number)	Diagnostic criteria for PH	Testing	Results
Dasgupta 2018 ¹⁹⁰ Prospective trial comparing serial NTproBNP levels in preterm infants with and without PH	<30 weeks GA and <1500g birthweight (52 infants, 8 with PH)	1. PA pressure >25mmHg estimated by tricuspid regurgitant jet velocity and 2. right ventricular hypertrophy and/or 3. flattening of the inter-ventricular septum On all scans at 28, 32, 36 weeks PMA	VITROS NTproBNP assay	28 weeks PMA cut-point of 2329pg/ml for detection of PH

Reference values and testing

Before NTproBNP or BNP can be considered as a possible biomarker for pulmonary hypertension normal values in healthy preterm infants over time must be established. There are a number of commercial assays available for both BNP and NTproBNP including point of care testing. Whole blood levels are stable at room temperature when collected in ethylene diamine tetra acetic acid (EDTA) additive tubes for at least 24 hours for BNP and at least 72 hours for NTproBNP. The levels also remain stable during the freezing and thaw process allowing batch processing.

Caution should be exercised in comparing BNP studies and interpreting “reference values” as there is known to be some variability depending on the assay type and the age at blood sampling.^{171, 172, 176, 191, 192} Roche Diagnostics (Basel, Switzerland) assays are the most widely used and have minimal variability.¹⁹³ Although there is a one to one ratio of active hormone to N-terminal fragment, NTproBNP levels are approximately six times higher in the blood due to the longer half-life. BNP and NTproBNP levels are reported in pg/ml or pmol/L. For NTproBNP there is an eight-fold difference with 1 pmol/L = 8.457pg/ml.¹⁹⁴ Differences in glycosylation may also affect levels as it may block the actions of corin and furin and also interfere with the ability of some assays to detect BNP or NTproBNP by competing for the binding site.¹⁹⁵

Reference values for amniotic NTproBNP levels for foetal life from 10-34 weeks have been reported showing a steady decline in NTproBNP from 10 weeks to a steady state by about 26 weeks.¹⁶⁸ Mean cord blood levels of NTproBNP in healthy neonates have been reported to be

578-670 pg/ml (71-79pmol/L) using a Roche testing kit.^{171, 172, 176} Neonatal reference levels in healthy term infants from four pooled studies using the Roche testing kit suggest a median(range) on day 0-2 of 3183 pg/ml (260-13224) or 374 pmol/L (31-1555) and a day 3-11 median(range) of 2210 pg/ml (28-7250) or 260 pmol/L (3.3-853).¹⁹² A further study reporting NTproBNP levels in healthy term neonates in the first few days of life reported a mean(SD) of 3042(1783)pg/ml or 360 pmol/L (210)¹⁷². The wide variation in the first few days of life reflects the dynamic variation in transitional circulation and variable age at testing and numbers of infants tested in most studies have been small.¹³⁸

Biological markers have inherent variability, exhibiting variability on serial testing even in conditions of apparent clinical stability. NTproBNP is no exception. A small component is due to analytic variation, but the remaining “biological” variability reflects a multitude of physiologic factors that influence secretion and clearance of these peptides. Understanding the degree of normal variability of any given biomarker within a stable physiological state is important to interpret serial levels. The biological variability (or relative change value) of NTproBNP is currently accepted to be 25% in adults with stable heart failure, meaning that a 25% change in NTproBNP levels is likely to represent a clinically significant change (either deterioration or improvement) in the patient’s physiological state.¹⁹⁶ The accepted relative change value for healthy adults is higher at approximately 50% reflecting lower levels and a greater percentage change. The degree of normal variability of NTproBNP in preterm neonates has not been established.

Summary

NTproBNP is a sensitive marker of cardiac wall stress that is widely used in the adult setting for diagnosis and prognosis. It can be easily tested in preterm infants and holds potential as a biomarker for rising pulmonary artery pressure causing heart strain. Serial testing of high risk infants may allow clinicians to identify those infants in need of further investigation by echocardiography and cardiac catheterisation, to identify infants who may benefit from medications that lower pulmonary pressures and to monitor the response to this therapy.

Gaps in current knowledge

There are, however, significant gaps in our current understanding. Further research is needed to establish the normal pattern of NTproBNP levels over time and the factors that influence NTproBNP in preterm infants, particularly factors that are prevalent such as patent ductus arteriosus and BPD. It is also important to establish whether there may be glycosylation or post-translational processing differences that may affect assay interpretation. If it is to be investigated as a marker to support the detection of late pulmonary hypertension in premature infants it will need to be paired with heart ultrasound ideally with objective, quantitative and widely accepted measures of raised pulmonary artery pressures.

Heart Ultrasound in the Detection of Late Pulmonary Hypertension in Preterm Infants

Introduction

Cardiac catheterisation is the gold standard test for measuring pulmonary pressures. The procedure involves accessing the vascular system, usually via the femoral vein, and feeding a catheter up into the heart and great vessels. It enables the direct measurement of pressures in the main pulmonary artery and right ventricle. The injection of contrast medium allows a pulmonary angiogram to provide detailed anatomical information. However, it is invasive and only available at specialist units necessitating the potentially hazardous transport of a fragile neonate to access this service. This makes cardiac catheterisation unethical and unacceptable as a screening tool. It is reserved for the most severe cases or when additional cardiac pathology is suspected.

Heart ultrasound is now widely available in neonatal intensive care units with many neonatologists now trained in clinician performed heart ultrasound (Fig.12). It is safe, non-invasive and well tolerated by the infant. It is used for assessing cardiac structure and function as well as monitoring disease progression and response to treatment. Heart ultrasound has been used to estimate pulmonary pressures and offers reasonable accuracy in the early period post-delivery.^{197, 198} There is less certainty about the use of heart ultrasound for the estimation of pulmonary pressures outside this period in very premature infants.



Figure 12: Photo of premature baby having a heart ultrasound. Permission to use photo for educational and teaching purposes granted by family

It would be valuable to have established and validated heart ultrasound parameters that could be used to detect evolving pulmonary hypertension in the context of BPD. Unfortunately there are no large, prospective trials comparing heart ultrasound against cardiac catheterisation for the diagnosis of late pulmonary hypertension in infants born prematurely. What we currently know is derived and inferred from adult studies and small, mostly retrospective, paediatric and neonatal studies using different ultrasound criteria at different time points.

There are no direct heart ultrasound measures of pulmonary artery systolic pressure.

Measures that have been used conventionally to assess for pulmonary hypertension have included tricuspid regurgitation (TR) jet peak velocity, ductal shunt pattern, shortened main pulmonary artery (MPA) acceleration time, interventricular septal flattening and the presence of right ventricular (RV) hypertrophy or dilatation. However, these traditional ultrasound techniques do not reliably predict pulmonary hypertension when compared directly to cardiac catheterisation.¹⁹⁹ Newer techniques including tissue Doppler imaging (TDI) and speckle tracking hold promise.^{200, 201} However, whatever the technique used it is important to understand what the parameter is measuring and potential pitfalls. Some measures, such as TR jet peak velocity and ductal shunt pattern **estimate RV systolic pressure**. Other measures such

as tricuspid valve inflow E/A wave ratio and right index of myocardial performance (RIMP) assess **RV dysfunction** while some demonstrate the **effects of prolonged raised pulmonary artery pressures** (RV hypertrophy/enlargement). Unfortunately not all measures are obtainable consistently in neonates where assessment is often hindered by high heart rates and rapid ventilation. The measurements also have a degree of intraobserver and interobserver variability and some are completely subjective rather than quantitative measures. Most do not have reference ranges established for the neonatal still less the preterm population nor account for the developmental changes in these parameters over time.^{202, 203} It is also important to be aware that pulmonary hypertension is also relative to left sided cardiac pressures. In the absence of a central arterial catheter, the measures of left ventricular (LV) pressure are also imprecise. LV systolic and diastolic dysfunction may also contribute to pulmonary hypertension. Hence clinicians must assess the heart using a broad range of ultrasound techniques in order to accumulate evidence to support or refute a diagnosis of pulmonary hypertension. In addition, preterm pulmonary vascular disease is a spectrum of disease which develops gradually over time. There is no defined time point at which at risk infants develop pulmonary hypertension. Serial assessments are therefore likely to be important to identify infants on a pulmonary hypertension trajectory.

Conventional heart ultrasound parameters to assess for pulmonary hypertension

A number of different heart ultrasound measures and assessments can be used for the evaluation of pulmonary hypertension in infants and children (Table 2), each with advantages and disadvantages.²⁰⁴

Table 2: Echocardiographic measures commonly used in the evaluation of late pulmonary hypertension (PH)

Echo Parameter	Information derived	Qualitative or quantitative	Reference ranges available or diagnostic cut off suggested?	Drawbacks
TR jet Doppler interrogation	Estimate of pulmonary artery pressure	Quantitative	> 2.7m/s consistent with elevated pulmonary pressure Use of Bernoulli equation to estimate PA pressure: ≥ 35 mmHg and $> \frac{1}{2}$ systemic considered significant	Not always present even with PH, very angle dependent, variable sensitivity and specificity
Interventricular septum position at end systole	Estimate of pulmonary artery pressure	Qualitative	Flat – approximately half systemic pressure Flat-posterior systolic right to left bowing –systemic pressure Severe post-systolic bowing – suprasystemic pressure	Interobserver variability
LV eccentricity index	Estimate of pulmonary artery pressure	Quantitative	Systolic eccentricity index > 1.3 ^{205, 206}	May also be increased by pure volume loading
Shortening of MPA acceleration time	Estimate of pulmonary artery pressure	Quantitative	Corrected AT/RVET < 0.31 ^{207, 208}	Variability in measurement Unable to assess if turbulence from PDA High pulmonary vascular resistance may prolong AT

Echo Parameter	Information derived	Qualitative or quantitative	Reference ranges available or diagnostic cut off suggested?	Drawbacks
MPA mid-systolic notching	Pulmonary vascular resistance	Qualitative	No	Subjective. Absence of notching may occur in pulmonary hypertension (suggestive of pulmonary venous hypertension)
RV dilatation	RV function	Qualitative	No	Subjective and may be biased by experience of reviewer. Dependent on duration of PH
RV hypertrophy	RV function	Qualitative	No	Subjective and may be biased by experience of reviewer. Dependent on duration of PH
TAPSE	RV lateral wall longitudinal performance	Quantitative	As per gestational age ²⁰⁹ <0.5cm is less -2SD for infants < 30 weeks	Reference ranges refer to preterm infants who are only 1-2 days old TAPSE may not reflect global RV function Angle and load dependent
PR jet Doppler interrogation	Estimate of mean and diastolic pulmonary artery pressure	Quantitative	Early peak and end diastolic velocity used with Bernoulli equation $(4 \times V^2) + RA \text{ pressure}^{210}$ $\geq 25 \text{ mmHg}$ diastolic pulmonary pressure consistent with pulmonary hypertension	Not always present, requires a holosystolic envelope

Echo Parameter	Information derived	Qualitative or quantitative	Reference ranges available or diagnostic cut off suggested?	Drawbacks
Shunt gradient (PDA, VSD)	Estimate of pulmonary artery pressure	Quantitative	Method to calculate for PDA shunt described by Musewe. ²¹¹ With unidirectional shunts difference between pulmonary and systemic systolic pressures can be calculated using $4 \times (\text{peak shunt velocity})^2$	Often complex to measure if shunts are bidirectional.
RV fractional area change	Estimate of pulmonary artery pressure	Quantitative	Levy 2015	Often difficult to define endocardium
Tricuspid E/A by pulsed Doppler	Right heart function	Quantitative	As per gestational age ²¹²	Waves often become fused at high heart rates. Affected by volume status and the use of inotropes. Progressive diastolic dysfunction will cause a rise in atrial pressure which can cause a "pseudonormal" ratio.
Tricuspid RIMP(Tei index)	Right heart function	Quantitative	As per gestational age ²¹²	Can be affected by heart rate. Angle dependent. May be unreliable in the presence of significant TR jet with elevated RA pressure

Echo Parameter	Information derived	Qualitative or quantitative	Reference ranges available or diagnostic cut off suggested?	Drawbacks
TV S' by TDI	Right heart function	Quantitative	By gestational age ^{213, 214}	Load dependent and may be pseudonormalised under conditions of volume loading. Angle dependent Can be affected by heart rate
TV E' by TDI	Right heart function	Quantitative	By gestational age ²¹³	Angle dependent E' and A' may fuse at high heart rates
TV A' by TDI	Right heart myocardial function	Quantitative	By gestational age ²¹³	Angle dependent E' and A' may fuse at high heart rates
LV dilatation	Left ventricular function	Qualitative	No	Intra and Interobserver variability
LV hypertrophy	Left ventricular function	Qualitative	No	Intra and interobserver variability
Mitral valve RIMP (Tei index)	Left ventricular function	Quantitative	As per gestational age ²¹²	Can be affected by heart rate. Angle dependent. Can be difficult to acquire.
Left ventricular ejection fraction by Simpsons Biplane method	Left ventricular function	Quantitative	55-70% ²¹⁵	Inaccurate if imaging planes are off-axis or foreshortened or in patients with regional variation in systolic function
Left ventricular shortening fraction	Left ventricular function	Quantitative	28-38% ²¹⁵	Angle dependent

Echo Parameter	Information derived	Qualitative or quantitative	Reference ranges available or diagnostic cut off suggested?	Drawbacks
Mitral valve E/A by pulsed Doppler	Left ventricular function	Quantitative	By gestational age ²¹²	Waves may be fused at high heart rates
Mitral valve S' by TDI	Left ventricular function	Quantitative	By gestational age ²¹³	Angle dependent. May be affected by heart rate.
Mitral valve E' by TDI	Left ventricular function	Quantitative	By gestational age ²¹³	Angle dependent. Waves may be fused at high heart rates.
Mitral valve A' by TDI	Left ventricular function	Quantitative	By gestational age ²¹³	Angle dependent. Waves may be fused at high heart rates

Tricuspid regurgitation jet

In the absence of pulmonary stenosis the peak velocity of a TR jet on conventional Doppler correlates with MPA systolic pressure.²¹⁶⁻²²⁰ Use of the Bernoulli equation allows estimation of the RV systolic pressure (RVSP= $4 \times (\text{peak TR jet velocity})^2$) which, in the absence of a gradient across the pulmonary valve, is equivalent to the MPA systolic pressure.²⁰⁴ The TR jet must demonstrate a complete, clearly defined envelope and peak velocity in order to be used to estimate MPA systolic pressure (Fig. 13, 14).

Concurrent measurement of the systemic pressure is needed, ideally with invasive blood pressure (BP) monitoring. A level of greater than one third of systemic pressure is indicative of raised right heart pressures. However, a significant TR jet may only be present in less than a third of preterm infants with a diagnosis of BPD-associated pulmonary hypertension.^{80, 199, 221}



Figure 13: Mild tricuspid regurgitation on Doppler ultrasound (flow shown below baseline)

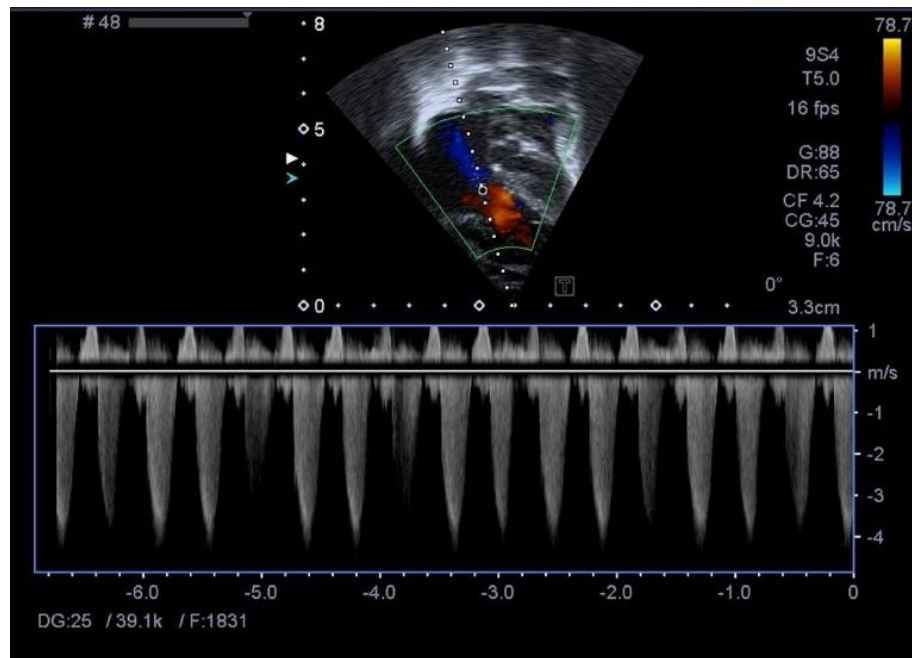


Figure 14: Significant tricuspid regurgitation on Doppler ultrasound

Ductal shunting pattern

Pulmonary pressures can be qualitatively estimated from the pattern of shunting through a patent ductus arteriosus using pulse wave Doppler from a high left parasternal view (Fig. 15). Low velocity bidirectional shunting can be considered to reflect systemic level pulmonary artery pressures whilst exclusive right-to-left shunting is suggestive of suprasystemic pulmonary artery pressure.^{211, 222, 223} As pulmonary pressures rise, the duration of right to left shunting will increase with <30% of the cardiac cycle close to but below systemic pressure, while right to left shunting of >30% reflects higher than systemic pressures.^{211, 223} It is possible to estimate the pulmonary pressures from the ductal shunt peak velocity using the modified Bernoulli equation but this is more complex than for the TR jet and not practicable for routine clinical use.²²³ Interrogation of the ductal Doppler pattern tends to be most useful in the context of PPHN where there is persistence of the foetal circulation and not in the assessment of late pulmonary hypertension in preterm infants as the duct has often closed by the time pulmonary pressures rise.

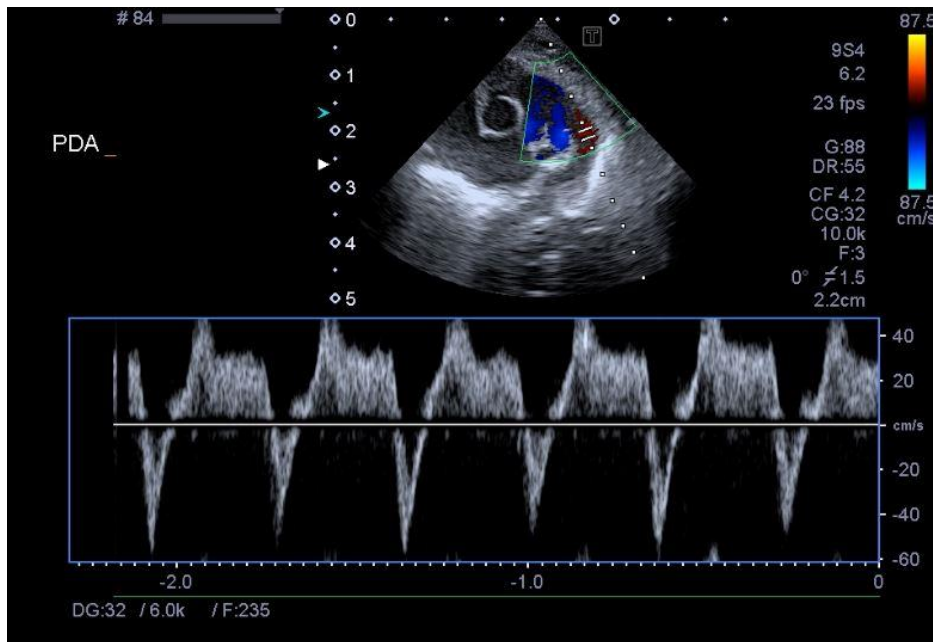


Figure 15: Bidirectional shunting through patent ductus arteriosus

Left to right above the baseline and right to left below

Shortened acceleration time on MPA Doppler

The time taken for the MPA systolic flow to reach its peak velocity tends to become shorter as the pressure and resistance to flow increases. In paediatric and adult populations the pulmonary artery acceleration time (AT) divided by the right ventricular ejection time (RVET) on Doppler ultrasound has been shown to have an inverse correlation with pulmonary artery pressure on cardiac catheterisation.^{224, 225} This ratio may be affected by heart rate. Corrected AT:RVET can be calculated by dividing by the square root of the R-R interval on electrocardiogram.

AT:RVET_c has been shown to rise in premature infants over the first seven days of life being most pronounced for the first three days (thought to reflect decreasing pulmonary artery pressure) then stabilise at approximately 0.54-0.57.^{226, 227} The rise over the first three days occurs more slowly in infants who go on to develop BPD and the mean AT:RVET_c after seven days is lower compared to those who do not develop BPD.²²⁶ Severe pulmonary hypertension will often cause a saw-tooth appearance to the MPA Doppler. However, it is sometimes difficult to accurately determine where to measure the peak of acceleration in some Doppler recordings. In particular it is often not able to be measured in the context of a hemodynamically significant duct due to the turbulence in MPA flow (Fig.16). At high heart rates this parameter can become less reliable and it is

crucial to consistently place the Doppler sampling gate at the midpoint of the MPA just below the pulmonary valve. RV dysfunction will also prolong the acceleration time such that even with significant pulmonary hypertension AT:RVETc may be normal.¹⁹⁸ It has been suggested that a ratio of < 0.23 is likely to represent high pulmonary artery pressure and an intermediate level of 0.24-0.35 to reflect moderate pressure but that pulmonary hypertension cannot be excluded if this ratio is normal.²²³ There is considerable debate about the reliability of this measure with many studies having small numbers of infants and wide confidence intervals.

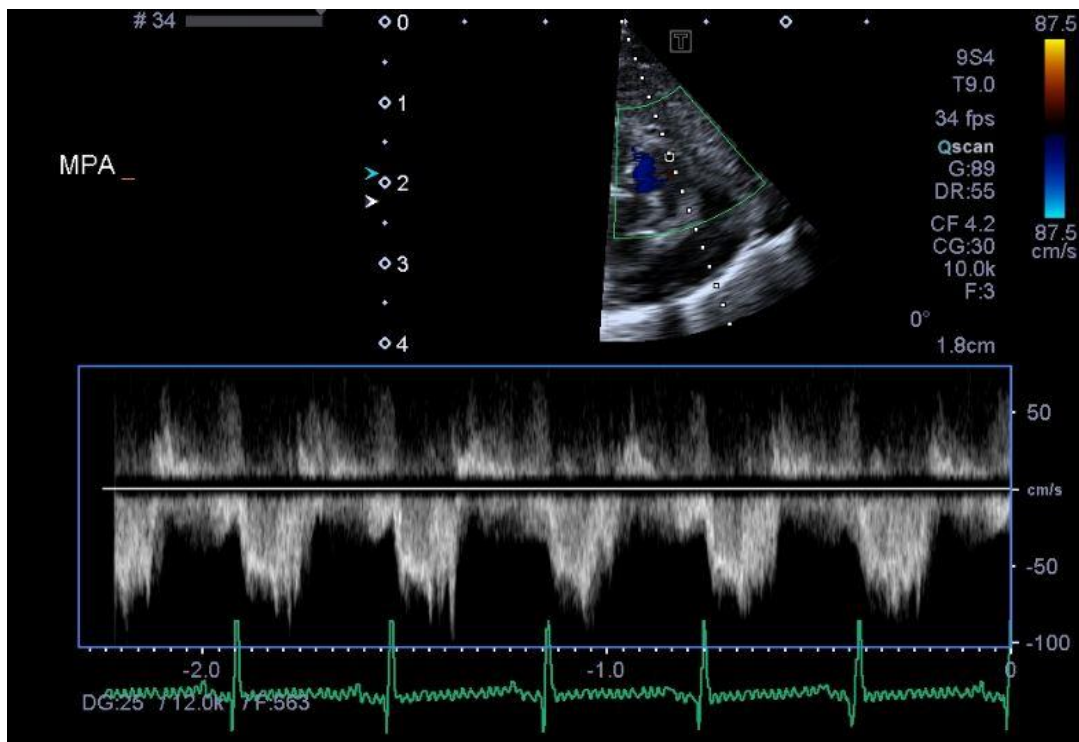


Figure 16: MPA Doppler with turbulent flow pattern secondary to PDA

Right ventricular hypertrophy and right ventricular dilatation

After chronic exposure to high pulmonary pressures the RV hypertrophies in an attempt to compensate. Eventually this fails and the RV begins to dilate and right ventricular failure ensues.^{228, 229} When RV hypertrophy or dilation is seen it is important to exclude RV outflow tract obstruction or causes of RV volume overload. This is a subjective assessment. Attempts to calculate RV volume are complicated by the geometry of the RV and the challenges of getting a consistent view using a two dimensional imaging modality. RV volume is best assessed by cardiac MRI. Although RV hypertrophy and

dilation are not sensitive measures of pulmonary hypertension and subjectivity may affect reliability, their presence signals severity and chronicity.

Septal wall flattening

The interventricular septal wall normally bows into the RV during systole. If pulmonary pressures are moderately raised then the septum will be flat at end systole. Further elevation of pulmonary pressures will cause the septum to bow into the LV. Septal wall flattening, has shown reasonable correlation with right ventricular systolic hypertension in children.²³⁰ This is sometimes represented as the eccentricity index, which is a measurement of the maximal LV diameter parallel to the interventricular septum divided by the LV diameter perpendicular to the septum (Fig.17). It can be measured at end-systole or end-diastole and is normally approximately 1, but in the presence of raised right sided pressures is >1, with RV function becoming impaired in premature infants at levels of ≥ 1.3 .²⁰⁶ Septal flattening is commonly present in very preterm infants with late pulmonary hypertension with many infants who demonstrate this sign on day 7 going on to have evidence of raised pulmonary pressures at 36 weeks PMA.⁸⁴

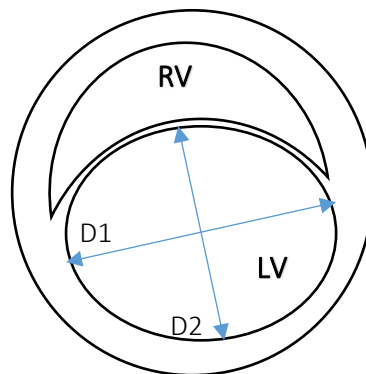


Figure 17: Left ventricular eccentricity index $LVEI = D1/D2$

Advanced Cardiac Ultrasound

Advanced ultrasound techniques that may be useful in assessing for pulmonary hypertension include the right index of myocardial performance, tricuspid annular plane of systolic excursion, tissue Doppler interrogation of the tricuspid valve and speckle tracking echocardiography.

Right Index of Myocardial Performance (RIMP)

RIMP or right Tei index is a marker of global RV function that is inversely correlated with RV output.²³¹ An image demonstrating RIMP can be found in methods chapter. RIMP

assesses both systolic and diastolic time intervals and is determined using conventional or tissue Doppler imaging (TDI). TDI interrogates a point on the myocardium measuring myocardial tissue velocities. TDI RIMP allows the measurements to be done on a single image enhancing accuracy. It has been shown to correlate well with RV ejection fraction.²³² It has been used widely to assess RV function in adults demonstrating prognostic value and utility in monitoring changes in pulmonary hypertension over time.²³³ In preterm neonates the RIMP has been shown to fall to a steady state level after 24 hours.²³⁴ RIMP is age dependent, decreasing with time after birth but may remain elevated in infants with evolving BPD.²³⁵ It does not appear to be affected by heart rate, blood pressure, PDA and gestational age and birth weight effects appear to drop out when infants with and without BPD are assessed separately.²³⁵

Tricuspid Annular Plane of Systolic Excursion (TAPSE)

The orientation of fibres in the right ventricle means that contraction occurs in a predominantly longitudinal plane. TAPSE is the measure of systolic displacement of the tricuspid annulus reflecting systolic right ventricular function and correlating with RV ejection fraction.²³⁶ TAPSE is measured by examining the total excursion of the tricuspid annulus during ventricular systole on M-mode using apical four chamber view. A sample image can be found in methods chapter. It is simple to perform and highly reproducible.²³⁶ Diminished TAPSE has been demonstrated in pulmonary hypertension and cardiac failure in adult and paediatric populations with diagnostic and prognostic implications.^{236, 237} TAPSE values increase with age and are affected by body surface area but not gender or heart rate.²³⁸ Reference ranges and z scores have recently been published for preterm neonates.²⁰⁹

Tricuspid annulus S', E', A', E/A, E/E'

Doppler interrogation of the inflow through the tricuspid valve generates a double wave form, the peak early diastolic flow velocity (E) and the peak atrial systolic flow velocity (A). An example image can be found in methods chapter. E corresponds to early diastolic relaxation, A corresponds with atrial contraction. The tricuspid valve E/A ratio has been used for evaluating RV diastolic function in adults. However, it is affected by heart rate and can be difficult to obtain in children and neonates due to high resting heart rates which often result in fusion of the waves. In adults a tricuspid E/A ratio of < 0.8 suggests impaired RV relaxation and a level of >2.1 suggests restricted filling.²¹⁸ Although there

are a few studies of these measures in preterm infants in the first few days of life there is a paucity of longitudinal data.^{213, 239, 240}

Because of the longitudinal plane of myocyte contraction the point of focus for right heart **tissue Doppler** interrogation is on the TV annulus along the RV free wall. TDI allows the systolic and diastolic function to be analysed simultaneously. A tissue Doppler tracing generates a systolic wave (S'), an early diastolic wave (E') and a late diastolic wave (A'). Sample image in methods chapter. TDI myocardial velocity measurements have been validated against cardiac catheter data and have been used to assess and monitor myocardial dysfunction in adult cardiology.²⁴¹ In the presence of pulmonary hypertension significant reductions in systolic (S') and early diastolic (E') tricuspid myocardial velocities are seen.²⁴² The E/E' ratio has been found to be a good marker for right atrial pressure and RV end-diastolic pressure in adults.²⁴³

Paediatric and neonatal reference values for tricuspid annulus S', also known as tricuspid annulus peak systolic velocity (TAPSV) have recently been published.^{214, 244} TAPSV correlates with RV systolic function and is reduced in pulmonary hypertension secondary to congenital heart disease.²⁴² Early diastolic tissue velocity (E') has been found to increase with age with preterm born infants reaching term equivalent age demonstrating tissue velocities that are comparable to term born infants.²²² Infants with moderate BPD have been shown to have a higher early Doppler inflow velocity to the early tissue Doppler velocity ratio (E/E').²⁴⁵ The E' wave has also been shown to be reduced or absent in infants with pulmonary hypertension.²⁴⁶ However, there is some overlap in myocardial velocities with some infants with pulmonary hypertension showing preserved myocardial velocities.²⁴⁶

Speckle tracking echocardiography

During contraction the myocardium undergoes deformation in a longitudinal, circumferential and rotational plane. When the myocardium is imaged by ultrasound a speckle pattern is visible due to interference and acoustic reflections with each part of the myocardium having a different speckle signature. Measurement of right ventricular longitudinal deformation using speckle tracking echocardiography appears to be a feasible and reproducible technique for assessing regional RV function in the preterm infant and it may be a useful addition to current tools for the diagnosis and monitoring of pulmonary hypertension, however it requires specialised software.^{200, 201 247}

Summary

Invasive cardiac catheterisation remains the only way to accurately measure pulmonary pressures and exclude anatomical abnormalities of the pulmonary vasculature.

Conventional heart ultrasound measurements may be inadequate to reliably detect the late pulmonary hypertension associated with prematurity. Newer, more advanced ultrasound techniques have not been widely studied in the preterm population.

Ultrasound provides, at best, indirect estimates of pulmonary pressure many of which are subjective and lack validity in the diagnosis of prematurity-associated late pulmonary hypertension. It is therefore important that clinicians interrogate the heart serially using multiple techniques to evaluate for rising pulmonary pressures.

Gaps in current knowledge

More research is needed to determine the optimum diagnostic protocol in the population of preterm infants at risk of late pulmonary hypertension.

The Role Oxygen Saturation Monitoring in the Prevention and Detection of Pulmonary Vascular Disease

Introduction

Respiratory support is a key component of care for babies born very prematurely. Improvements in perinatal care including oxygen saturation monitoring, antenatal steroids, surfactant treatment and non-invasive respiratory support have dramatically improved survival rates, yet rates of bronchopulmonary dysplasia remain static.^{69, 248} In-utero and post-natal factors are thought to contribute to BPD. Supporting developmentally immature lungs while avoiding the potentially harmful effects of therapy is a delicate balancing act.²⁴⁹ Understanding the relationship between oxygen and the developing lung is crucial to understanding the evolution of bronchopulmonary dysplasia and associated pulmonary vascular disease.

Pathological effects of hyperoxia

The foetal lung is programmed to develop in a low oxygen environment. The partial pressure of oxygen in the umbilical vein (delivering oxygen to the foetus) is approximately 4.7kPa compared to 10.5kPa in adult arterial blood and 19.6kPa in air at sea level.²⁵⁰ Foetal blood is at best only 80-90% saturated which is considerably lower than the >94% seen in healthy infants, children and adults.²⁵⁰ During the first stage of labour the mean blood oxygen saturation in the foetus may be as low as 50%±10%.²⁵¹ However, foetal haemoglobin is adapted to these low concentrations with enhanced oxygen uptake but decreased tissue delivery.

Premature birth suddenly exposes the developing lung to relative hyperoxia, even in room air, and a gradual switch from foetal to adult haemoglobin. Adult haemoglobin has decreased oxygen uptake but enhanced tissue delivery better suited to the relatively higher oxygen supply and increased tissue demands of post-natal life. Historically preterm infants were also exposed to high levels of supplemental oxygen with devastating effects on vision secondary to the development of retinopathy of

prematurity.²⁵² Retinopathy of prematurity develops in two phases. The first phase is triggered by excess oxygen which suppresses VEGF and inhibits normal retinal blood vessel growth. The second phase occurs when the insufficient retinal vessels cannot meet the oxidative metabolic demands of the retina inducing a rise in VEGF and an excessive proliferation of abnormal retinal vessels.

There are parallels that can be drawn between the pathophysiology of retinopathy of prematurity and pulmonary vascular disease with excess oxygen front and centre. Alveolar hyperoxia is known to have toxic effects on the lung, triggering an inflammatory cascade that results in damage to the alveolar-capillary barrier with endothelial cell death, resultant vascular leak, impaired gas exchange and ultimately epithelial cell death.^{74 253} Preterm infants have underdeveloped anti-oxidant strategies to combat the damaging effects of oxygen free radicals although the modulation of oxygen sensitivity is more complex than simply the relative levels of antioxidant enzymes.²⁵⁴

Many factors have been identified to be involved in both lung development and the inflammatory process induced by exposure to excess oxygen. HIF is a master transcription factor released in response to hypoxia and involved in the regulation of hundreds of genes including that of VEGF. VEGF is involved in endothelial cell development and remodelling of vascular tissue including pulmonary vessels. This process is essential for alveolar development.^{55, 56} HIF levels in the lungs of premature animals are reduced when mechanically ventilated.^{255, 256} Infants who go on to develop BPD have significantly lower levels of VEGF detected.²⁵⁷ Prolonged exposure to hyperoxia leads to an initial surge of VEGF, coinciding with the initial period of vascular damage, followed by a decrease in VEGF leading to poor vascular repair and regeneration and consequent poor alveolar development and malformed pulmonary vasculature.⁵⁶ Activation of VEGF in experimental models of BPD restores alveolar development with VEGF fulfilling Koch's alveolarization postulate (Fig.18).

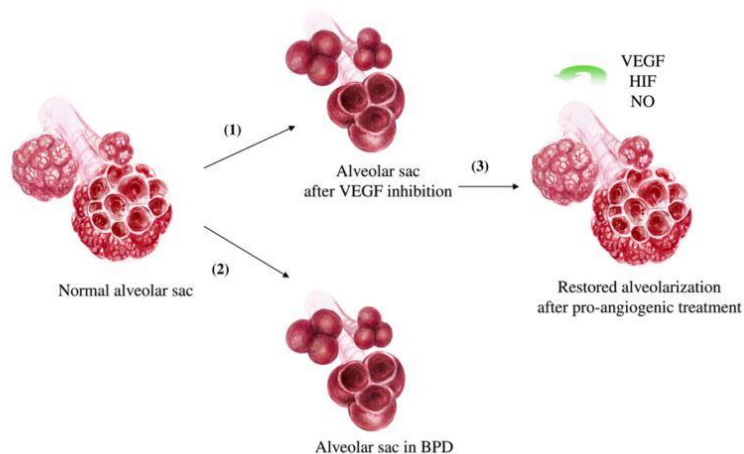


Figure 18: Illustration of VEGF's fulfilment of Koch's alveolarization postulate

- 1) Inhibition of VEGF signalling impairs alveolar development
- 2) Conversely impaired alveolarization in human and experimental BPD is associated with decreased VEGF signalling
- 3) Overexpression or activation of VEGF in experimental BPD restores normal alveolar development

Reprinted with permission of the American Thoracic Society. Copyright © 2018 American Thoracic Society. Thebaud B, Abman SH. Bronchopulmonary dysplasia: where have all the vessels gone? Roles of angiogenic growth factors in chronic lung disease. *Am J Respir Crit Care Med.* 2007;175(10):978-85. *The American Journal of Respiratory and Critical Care Medicine* is an official journal of the American Thoracic Society

Studies of oxygen toxicity have focused primarily on continuous exposure at high concentrations. Chronic exposure of newborn rodent and preterm baboon animal models to high levels of oxygen results in a BPD-like lung injury.^{258, 259} However, with modern intensive care premature babies are more likely to be exposed to intermittent alveolar hyperoxia. Surprisingly short exposures have been shown to cause oxidative lung damage.²⁶⁰ However, even with continuous exposure there is the propensity for recovery and an adaptive response in human newborns. The impact of hyperoxia is modifiable. Antenatal exposure to corticosteroids or inflammatory mediators may decrease the deleterious effects of oxygen exposure while intrauterine growth restriction or poor postnatal growth may increase sensitivity to oxygen toxicity.^{248, 261, 262} Prior exposure to oxygen can also prime the lung to tolerate the effects of prolonged hyperoxia. More work is needed to understand the impact of mild intermittent hyperoxia exposure.

Impact of hypoxia

Hypoxemia can also cause harm to the developing foetus and newborn. Foetuses with intrauterine growth restriction secondary to poor placental function are exposed to chronic hypoxemia in-utero. Hypoxemia in the preterm neonate is caused by the

complex interplay between an immature respiratory centre and mechano and chemo receptors (causing central apnoea), an underdeveloped airway and bronchial tube network (prone to obstruction) and an immature gas exchange interface (with smaller surface area, alveoli prone to oedema and inflammatory reaction, a greater distance between alveoli and pulmonary vessels and poorly developed pulmonary capillaries prone to vasoreactivity leading to ventilation-perfusion mismatch).²⁶³ In addition hypoxemia can have iatrogenic causes such as obstruction or displacement of endotracheal tube or nasal cannula or poor positioning leading to airway compromise.

Persistent severe hypoxemia ultimately leads to cell death. The foetus or neonate may be able to adapt to chronic sustained hypoxemia of less severity but there is a cellular cost. Poor placental function can result in chronic oxygen and nutritional deprivation. The chronic hypoxia associated with intrauterine growth restriction results in an increased risk of pulmonary hypertension in the neonatal period.^{92, 264} Adaptive mechanisms that occur in-utero, such as redistribution of blood flow to vital organs, result in myocardial and vascular remodelling which may persist and increase risk of cardiovascular disease in adulthood.²⁶⁵

Infants born very prematurely frequently experience chronic intermittent hypoxemia which has been linked to increased incidence of retinopathy of prematurity, need for prolonged respiratory support and adverse neurodevelopmental outcome.^{266-268 269, 66} A low baseline oxygen saturation of $\leq 90\%$ has also been shown to be associated with an increased risk of subsequent chronic intermittent hypoxemia.²⁶⁷

In the modern era of neonatal intensive care with carefully monitored oxygen saturations it is rare for an infant to be exposed to excessively high or low levels of oxygen for prolonged periods. It is more likely that infants will experience intermittent hyperoxia and hypoxia. Our understanding of the consequences of this, and how best to avoiding swinging saturations is still evolving. It is also sobering that babies born prematurely in resource poor countries remain at high risk of the completely preventable iatrogenic damage caused by excessive oxygen.

Oxygen saturation monitoring

Pulse oximetry monitors allow the non-invasive continuous estimation of blood oxygen saturation. Two light emitting diodes are used to transmit red and infrared light through the skin (Fig. 19). Deoxygenated haemoglobin absorbs more red light and oxygenated-

haemoglobin absorbs more infrared light. A photoreceptor placed opposite the diodes compares the ratio of absorbed light and uses a pre-programmed algorithm to calculate the peripheral capillary oxygen saturation (SpO₂). As oximeters detect pulsatile blood in order to estimate arterial rather than venous blood oxygen saturation, movement can cause artefact. Modern Masimo oximeters (Masimo Corporation, Irvine, California) utilise signal extraction technology which greatly reduces the signal noise caused by movement and gives a performance index (percentage time SpO₂ is within 7% of control value) of 94%.²⁷⁰ Hypothermia and poor skin perfusion may cause falsely low readings and the relative ratio of foetal to adult haemoglobin may also affect reported SpO₂ levels as will the quality of the stored normative data on which the algorithm is based. Masimo oximeters also have the capacity to store data which can be downloaded for analysis of trends over time using software such as PROFOX (PROFOX Associates Inc, Escondido, California).



Figure 19: Photo of oxygen saturation probe and monitor

Oxygen saturation targeting

The understanding that insufficient oxygenation increases mortality and neurodevelopmental morbidity and exposure to excessive oxygen causes pathological lung and eye injury has led to practice of oxygen saturation targeting. Identifying the range that offers the best cost-benefit ratio has proved challenging.^{249, 271} A number of large multicentre trials have attempted to answer the question of optimum saturation

targets in preterm infants to prevent the adverse effects of hyperoxia and hypoxia. The STOP-ROP trial looked at whether randomisation to two different levels of oxygen saturation targeting (89-94% or 96-99%) would affect the incidence and progression of retinopathy of prematurity. Although they found no difference in ROP, the group randomised to the higher oxygen saturations had a greater evidence of the effects of lung injury.²⁷² The BOOST trial demonstrated that infants randomised to higher oxygen saturation targets (95-98% versus 91-94%) were more likely to be dependent on oxygen at 36 weeks PMA and had a longer duration of oxygen therapy.²⁷³

Neonatal Oxygenation Prospective Meta-analysis (NeOProM) is a collaborative meta-analysis of the results from five similarly designed, large, international, multicentre trials (BOOST-II, COT, SUPPORT) targeting oxygen saturations of either 85-89% or 91-95% in infants born at < 28 weeks from birth to 36 weeks PMA.²⁷⁴⁻²⁷⁶ There were concerns raised over issues with the pulse oximeter algorithm and differences in the definition of neurological impairment.^{249, 277} Although there were differences in the outcomes of individual trials, the metaanalysis demonstrated that the higher oxygen target was associated with a lower risk of death and a lower rate of necrotising enterocolitis but a higher rate of oxygen use at 36 weeks PMA. Pulmonary hypertension was not a reported outcome of these trials. There is evidence from a retrospective cohort study that targeting higher versus lower oxygen saturations may lead to lower rates of prematurity-associated pulmonary hypertension.²⁷⁸ Debate continues, but on balance, current evidence suggests that targeting oxygen saturations of 91-95% in extremely preterm infants, with access to high quality ROP screening, offers the best risk-benefit profile for oxygen therapy. However, there is concern that this change in practice may lead to a rise in ROP emphasising the tremendous challenge in defining and maintaining optimal oxygen saturations in preterm neonates.²⁷⁹

Despite the practice of oxygen saturation targeting, the AVIOX trial reported preterm infants spend between 16-64% time within their targets with infants above the target range 20-73% of the time.²⁸⁰ Laptok et al also reported time in target to be 58%.²⁸¹ Hypoxemia may be multifactorial however, hyperoxemia is a potentially preventable source of iatrogenic harm. Pulse oximeters estimate the percentage oxygen saturation of haemoglobin but do not measure the partial pressure of dissolved and unbound oxygen. Due to the shape of the oxygen dissociation curve it is important to recognise that small changes in SpO₂ above 90% are associated with relatively large changes in arterial

oxygen tension.²⁸² An additional challenge with oxygen saturation targeting is that even with a clearly defined unit policy compliance with alarm limits has been shown to be poor and variability amongst nursing opinion and practice high.^{283 284}

In addition not all premature infants are alike with regards to oxygen saturation stability. Some infants develop a propensity for saturations that swing from sudden lows to highs that makes nursing care challenging. It is not clear why this subset of infants develop such variability in their oxygen saturation signature. There are likely to be many contributing variables including in-utero environmental programming however, one possibility is that these infants have dysfunctional pulmonary microvasculature prone to sudden vasospasm leading to areas of the lung that are ventilated but not perfused and/or shunting of deoxygenated blood into the arterial system.⁵⁹

Nursing observation charts, although meticulous, are blunt instruments for observing the second to second variability of oxygen saturations and heart rate. With the development of pulse oximeters with downloadable memory we now have the ability to review this with greater precision. Time above, below or within certain saturation levels can be analysed and mean saturation and standard deviation derived. The coefficient of variation (CV), which describes the ratio of the standard deviation to the mean, is a measure of instability that captures both variability around the mean and those infants whose mean is lowered by frequent hypoxic episodes. The CV appears to have more discriminating value than the mean or standard deviation.²⁸⁵ The optimal measures of oxygen saturation instability in premature infants are still unclear and further work needs to be done if we are to better evaluate our care and avoid iatrogenic harm from too little or too much oxygen.

Summary

Hyperoxia contributes to the development of pulmonary vascular disease in very premature infants. Improving our understanding of pulse oximetry instability may allow us to use pulse oximetry analysis to refine respiratory support for individual babies within the broad framework of oxygen saturation targeting guidelines and reduce risk of iatrogenic harm. Deeper analysis of individual pulse oximetry data over time may also potentially identify infants with evolving pulmonary vascular disease.

Key Points

What is known:

- Premature birth is common and is associated with poor growth and long term cardiorespiratory and neurodevelopmental sequelae
- Infants born very prematurely are at risk of pulmonary hypertension which carries a significant morbidity and mortality risk prompting calls for screening for this condition
- N-terminal pro-BNP is a cardiac hormone released by ventricles under volume or pressure stress that is cardioprotective and can be used for diagnosis and prognosis in adults with heart failure and pulmonary hypertension and has been shown to rise during transitional circulation in preterm infants
Clinician performed heart ultrasound is a useful tool for evaluating transitional circulation including persistent pulmonary hypertension of the newborn using conventional techniques
- Exposure of the developing lung to excessive oxygen is implicated in the development of pulmonary hypertension but maintaining oxygen saturations in an optimal target range is challenging in very preterm infants.

Gaps in current knowledge:

- Although screening is recommended the optimal approach to screening very premature infants for pulmonary hypertension is uncertain
- The evolution of NTproBNP levels over time in preterm infants beyond the period of transitional circulation and the factors that affect these levels are still unclear. The relative proportions of circulating BNP isoforms are unknown in preterm infants. Whether NT-proBNP can be used as a biomarker of late pulmonary hypertension is not yet established
- There is currently no consensus on heart ultrasound criteria for the diagnosis of late pulmonary hypertension in premature infants. The feasibility and utility of incorporating more advanced ultrasound techniques into clinician performed heart ultrasound has not been tested
- There is a lack of data on patterns of oxygen saturation instability over time in very preterm infants and the best way to assess instability. Human factors that may contribute to hyperoxia have been understudied

HYPOTHESIS & METHODS

Methods

Study rationale

Pulmonary vascular disease is an insidious complication of the lung disease of extreme prematurity and is associated with cardiac dysfunction, increased morbidity and mortality.^{62, 64, 67, 114, 115, 117} Early identification of pulmonary vascular disease or its impact on cardiac function may allow early intervention improve outcomes. Screening has been recommended but screening protocols are debated and consensus has not been reached, in part due to knowledge gaps.^{86, 87} The risk of BPD-associated or late pulmonary hypertension is uncertain in a general population of premature infants as published data have been drawn from highly selected populations referred to centres specialising in pulmonary hypertension.^{64, 80-82} A better understanding of the evolution of this disease is needed which necessitates serial observation of a population of at risk infants.

Very preterm infants with clinical risk factors for pulmonary hypertension would benefit from a sensitive and specific biomarker of cardiac stress that enables clinicians to identify early cardiac compromise and to triage infants who warrant further investigation. NTproBNP is a sensitive marker of myocardial stress caused by volume and pressure overload. Serial levels may have merit as a marker of rising pulmonary pressures in premature infants but this has not yet been fully evaluated. Changes in this hormone over time and factors that affect levels and processing in very prematurely born infants are understudied and not adequately described.

Cardiac catheterisation is the gold standard for measuring pulmonary vascular pressures but is invasive and carries risk. There is currently no consensus on heart ultrasound criteria for the diagnosis of late pulmonary hypertension in preterm infants.

Conventional heart ultrasound assessment alone may be inadequate for identifying very preterm infants with late pulmonary hypertension and more advanced ultrasound techniques that provide accurate quantitation of cardiac function are yet to be fully evaluated in this population.

Hyperoxia is a contributing factor to pulmonary hypertension in very premature infants and hypoxia can precipitate pulmonary hypertensive crises in affected infants.

Furthermore, oxygen saturation stability is perturbed by pulmonary vascular disease due to dysmorphic vessels exhibiting dysfunctional vasoreactivity. Changing patterns of oxygen saturation levels over time have not been studied as possible indicators of evolving pulmonary hypertension. More data is needed to better understand the oxygen saturation patterns in very preterm infants over time and how we can reduce iatrogenic hyperoxia that may contribute to pulmonary hypertension.

Hypotheses

NTproBNP

1. NTproBNP levels will change over time in very preterm infants and will be higher in infants with:

- haemodynamically significant patent ductus arteriosus
- bronchopulmonary dysplasia
- pulmonary hypertension

2. NTproBNP will not vary significantly with gestational age at birth, hypoxia, mechanical ventilation or haemoglobin levels but will be higher in the context of renal impairment

3. There will be additional circulating forms of BNP in very preterm infants, other than BNP(1-32) and NTproBNP (1-76), and the types of isoforms will differ in infants with BPD and/or HsPDA compared to infants without these conditions

Heart ultrasound

4. Specific quantitative markers of RV function will be measurable in very preterm infants by clinician performed heart ultrasound but some parameters will be more difficult to consistently attain and less useful than others

5. The temporal profile of these quantitative measures of RV function will reflect growth and developmental changes in cardiac function

Oxygen saturation

6. The pattern of oxygen saturation instability will vary over time in very preterm infants

7. Infants who develop BPD or later pulmonary hypertension will have evidence of greater oxygen saturation instability

8. Poor compliance with oxygen saturation alarm limits is a contributing factor to exposure to hypoxia and hyperoxia in neonatal units

Health and Neurodevelopmental Outcomes

9. Our very preterm cohort will experience high rates of respiratory morbidity in the first two years of life and morbidity will be greater in those with BPD

10. Our cohort will experience similar rates of neurodevelopmental impairment to rates reported by ANZNN for the time period of the study

11. Higher NTproBNP levels in the neonatal period will be associated with poorer cardiorespiratory health in the first two years of life and with higher rates of neurodevelopmental impairment at two years

12. Poorer right heart function in the neonatal period will be associated with higher rates of cardiorespiratory morbidity in the first two years of life and higher rates of neurodevelopmental impairment at two years

13. Greater oxygen saturation instability in the neonatal period will be associated with higher rates of cardiorespiratory morbidity in the first two years of life and higher rates of neurodevelopment impairment at two years

Aims

NTproBNP

1. To determine the range and temporal profile of NTproBNP levels in infants born at <30 weeks gestation at day of life 3, 10, 28 and at 36 weeks PMA

2. To determine the utility of NTproBNP levels to diagnose haemodynamically significant PDA, diagnosed by pre-specified ultrasound criteria

3. To determine the utility of NTproBNP levels to diagnose bronchopulmonary dysplasia

4. To determine the utility of NTproBNP levels to diagnose pulmonary hypertension on the basis of pre-specified conventional heart ultrasound criteria

5. To investigate whether NTproBNP levels are affected by gestational age, ventilation, hypoxia, renal impairment or haemoglobin level

6. To determine the types of circulating isoforms of BNP in very preterm infants and to investigate whether BNP isoforms differ in premature infants with BPD and/or HsPDA and those without these conditions

Heart ultrasound

7. To assess a cohort of infants born at < 30 weeks gestation for pulmonary hypertension using conventional heart ultrasound criteria

8. To evaluate the feasibility of clinician-performed quantitative heart ultrasound measures of RV function

9. To document the temporal changes in quantitative measures of RV function in a cohort of very preterm infants and investigate their relationship to body surface area and BPD

Oxygen saturation

10. To conduct detailed analysis of 72 hour recordings of oxygen saturation data using a variety of measures of instability at four time intervals from birth to 36 weeks PMA and to compare these measures in infants with and without BPD and/or pulmonary hypertension

11. To audit unit compliance with oxygen saturation alarm protocols and to survey nursing opinion of oxygen saturation targeting in the NICU

Health and neurodevelopmental outcomes

12. To describe the mortality, growth, health and neurodevelopmental outcomes in the first 2 years of life in a modern, well characterised cohort of preterm infants

13. To investigate whether early NTproBNP, TAPSE, TAPSV or oxygen saturation CV correlate with key outcomes, specifically:

- duration of NICU stay
- number of hospital readmissions in first 2 years
- duration of hospital readmissions in first 2 years
- neurodevelopmental impairment at 2 years

Study design

Prospective observational cohort study.

Setting and participants

This trial was conducted at the Christchurch Women's Hospital NICU, Christchurch, New Zealand. This is a tertiary level NICU that serves the region of Canterbury, that in 2013 had a population of 539,436 (annual births of 6,543) and the West Coast with a population of 32,148 (annual births of 385).²⁸⁶

Inclusion criteria:

Infants born at < 30 weeks gestation between March 2013 and April 2015 and cared for in Christchurch Neonatal Intensive Care Unit. Infants were enrolled by 72 hours of age.

Exclusion criteria:

Known structural airway or lung anomalies, congenital anomalies of the pulmonary arteries or pulmonary veins, major systemic vessel-to-pulmonary artery collateral vessels, congenital heart disease (except those with patent ductus arteriosus, patent foramen ovale or secundum atrial septal defect < 5mm or tiny restrictive muscular ventricular septal defect), severe liver disease, or persistent pulmonary hypertension of the newborn on day 3 heart ultrasound (defined as severe hypoxia associated with estimated main pulmonary artery pressure >25mmHg). If infants had a grave prognosis such that they were not expected to survive to day 3 they were also excluded.

Ethical considerations

This study complied with the Declaration of Helsinki regarding the ethical principles for medical research involving human participants. The parents of all eligible infants were approached about the study either prior to delivery or within 72 hours of delivery, offered a spoken and written explanation of the study and invited to consent to their child participating. A copy of the patient information and consent form is included in Appendix B. Written informed consent was obtained from the parents of all study participants prior to day 3 testing commencing. Ethical approval was granted by the University of Otago Human Ethics Committee (12/298). Local study approval was granted after consultation with Māori through Te Komiti Whakarite and the study was conducted in accordance with the principals of the Treaty of Waitangi.¹ The study team was granted

additional ethical approval to place a recordable pulse oximetry monitor on all eligible infants after delivery prior to written consent being granted as it was acknowledged that it is standard clinical practice to monitor oxygen saturations in this population after birth and on the understanding that any data collected would be deleted if parents subsequently refused consent.

Participation was entirely voluntary and no compensation was awarded for participation in this trial. Patient safety was paramount with all care taken to ensure patient comfort and well-being during testing. In the event of an injury secondary to participation in this study participants may be eligible for compensation by the New Zealand Accident Compensation Corporation (ACC) according to the provisions of the Injury Prevention, Rehabilitation, and Compensation Act 2001.

Outcomes

Primary outcome measures:

- 1) Bronchopulmonary dysplasia

This was defined using the following two definitions, the first being a binary definition and the second allowing grading by severity:

- 1) Persistent oxygen or respiratory support requirement at 36 weeks PMA (definition utilised by the ANZNN in 2013) OR
- 2) Oxygen requirement for at least 28 days and BPD graded at 36 weeks PMA according to the following National Institute of Child Health and Human Development (NICHD) criteria (Table 3).²⁸⁷

Table 3: NICHD criteria for BPD severity

Grading	Criteria
Mild BPD	Breathing room air
Moderate BPD	Need for <30% oxygen
Severe BPD	Need for ≥30% oxygen and/or positive pressure respiratory support

At the time of this study in the Christchurch NICU the need for oxygen or respiratory support at 36 weeks PMA was not determined by a standardised physiological challenge²⁸⁸ but by clinical decision usually after analysis of a 24 hour pulse oximetry study.

2) Pulmonary hypertension

This was defined by conventional heart ultrasound criteria (Table 4).^{81, 199}

Table 4: Criteria for diagnosis of pulmonary hypertension using conventional ultrasound parameters

Criteria if tricuspid regurgitation jet present:	Criteria if no tricuspid regurgitation jet present:
<p>1. Estimated RV pressure >33% systemic pressure:²⁸⁹</p> <p>RV pressure estimated using TR jet peak velocity and the modified Bernoulli equation (pressure gradient=4xjet velocity²).</p> <p>OR</p> <p>2. TR jet \geq 3m/s</p>	<p>Demonstration of at least 2 of the following echocardiographic findings in the absence of pulmonary stenosis or RV outflow tract obstruction (subjectively assessed):</p> <ol style="list-style-type: none"> 1. right ventricular enlargement 2. right ventricular hypertrophy 3. interventricular septal flattening or bowing right to left 4. abnormal pulmonary artery Doppler (saw-tooth pattern or shortened acceleration time)

Secondary outcome measures:

1) Haemodynamically significant PDA defined based on commonly accepted criteria:¹⁸⁰

1. Ductal diameter \geq 1.5mm (on B-mode greyscale, high parasternal true sagittal view)

AND

2. Pulsatile flow pattern (Fig.20)

PLUS at least one of the following:

A) left atrial to aortic diameter (LA:Ao) \geq 1.5 (M-mode through the aortic root and left atrium)

B) reversed diastolic flow in descending aorta (Doppler gate placed in descending aorta below insertion of duct)

C) turbulent MPA flow, diastolic peak $>$ 0.2m/s (Doppler gate place just below the pulmonary valve).

2) Duration of neonatal admission

3) Number and duration of readmissions to hospital in first two years of life

4) Neurodevelopmental impairment at 2 years

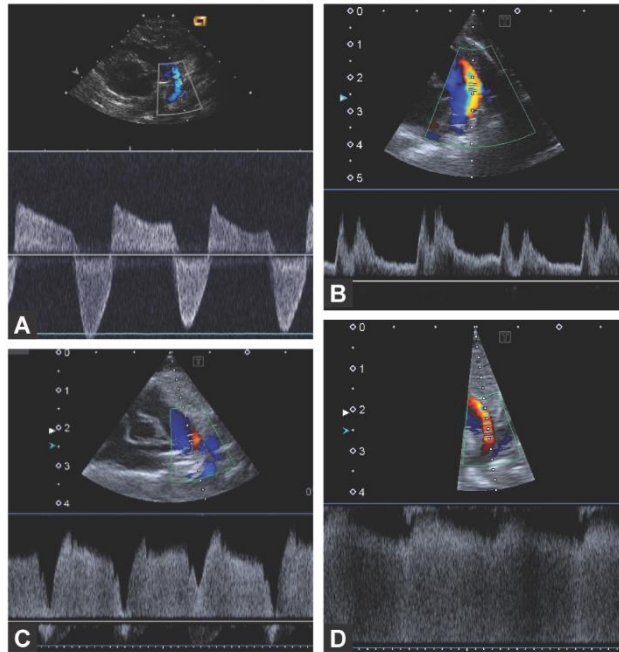
Neurodevelopmental impairment was determined by review of clinical notes and/or ANZNN data collected for results of formal neurodevelopmental evaluation examination by a paediatrician and either Bayleys III assessment by a developmental psychologist or equivalent developmental evaluation by an Early Intervention provider. Cognitive, language and motor delay was graded as mild, moderate or severe. Severe delay was defined as scores < -3 standard deviations (SD), moderate delay as scores -3 SD to < -2 SD, and mild delay as scores -2 SD to < -1 SD relative to the mean. The mean (SD) of these scales is 100 (15), giving cut-points of: severe < 55 , moderate 55–69, and mild 70–84. Children who did not qualify for formal testing had their hospital records reviewed for information relating to neurodevelopment at the nearest assessment point to 2 years.

Blindness was defined as vision $< 6/60$ in the best eye as assessed by an ophthalmologist. Significant hearing impairment was defined as requiring unilateral or bilateral hearing aids or cochlear implant. Cerebral palsy is a non-progressive motor disability present from birth. The functional level of disability can be graded from 1-5 using the Gross Motor Functional Classification System (GMFCS).²⁹⁰ In this study cerebral palsy was identified based upon a diagnosis of cerebral palsy by a paediatrician or paediatric neurologist.

Any neurodevelopmental impairment was defined as deficit in any domain requiring additional support or intervention. **Mild neurodisability** was defined as mild cerebral palsy (GMFCS < 3) or documented impairment in vision, hearing, cognitive, motor or language delay not meeting criteria for moderate to severe. **Moderate to severe neurodisability** was defined as: moderate or severe cerebral palsy (GMFCS ≥ 3), visual acuity $< 6/60$ in better eye, sensorineural deafness with hearing aids, developmental quotient less than -2 SD relative to reference standard.⁷

5) Growth at two years (weight, height, head circumference)

6) Death in the first 2 years of life



Doppler patterns

- A. Bidirectional shunting in pulmonary hypertension
- B. Pulsatile pattern in unrestrictive haemodynamically significant duct
- C. Growing pattern in transitional circulation
- D. Closing or continuous pattern in constricting ductus

Figure 20: Doppler flow patterns across ductus arteriosus (Images taken by author)

Testing

On day of life 3, 10 and 28 and at 36 weeks PMA participants had the following tests performed:

1. Plasma NTproBNP level
2. Clinician performed heart ultrasound
3. Pulse oximetry recording of heart rate and oxygen saturation for approximately 72 hours prior to blood test
4. Blood pressure recording

When there was a clinical suspicion of infection a blood C reactive protein was also checked to screen for possible sepsis.

NTproBNP testing

A minimum of 0.6ml of arterial, venous or capillary blood was taken for NTproBNP testing as close in time as possible to the heart ultrasound. Samples were coordinated with clinical care blood tests to minimise infant discomfort. Oral sucrose (0.1-0.2ml) was given for analgesia. Blood was collected in tubes containing ethylene diamine tetra acetic acid (EDTA). Samples were immediately refrigerated at 4° C until collection that day or within 72 hours if over a weekend. Samples were then centrifuged at 3000 rpm and plasma subsequently frozen at -80°C minimum.

NTproBNP levels were batch processed using an electro chemiluminescent sandwich enzyme-linked immunoabsorbant assay using a ROCHE e411 Cobas analyser. Levels are reported in pmol /L. This testing kit is unaffected by bilirubin level, haemolysis or lipaemia.

If infant was suspected of having sepsis or had CRP >10 then NTproBNP level was deferred until sepsis improved to prevent a confounding effect on NTproBNP levels. Renal function was assessed by urine output, blood pressure and urea and creatinine level was monitored as part of routine clinical care.

BNP isoform testing

Further testing was done to establish the relative proportions of circulating BNP isoforms in our cohort and the presence of any post-translational glycosylation. Plasma volume remaining after initial NTproBNP testing was used for isoform testing. Low available plasma volumes necessitated pooling of samples from several infants and across all four testing times. These groups were selected on the basis of the presence or absence of BPD and day 3 HsPDA. This grouping is detailed further in the BNP chapter isoforms results chapter. This testing was conducted using a combination of Luminex® Assay (Luminex Corp, Austin Texas) and High Performance Liquid Chromatography (HPLC).

Luminex assay and high performance liquid chromatography techniques

Residual unused plasma was pooled according to the clinical groupings detailed in the BNP isoforms results chapter. These samples were concentrated on SepPak C18 cartridges and assessed on the CHI Luminex assay system. They were then characterised using HPLC in order to determine the relative proportions of circulating forms of BNP.

Assays, Reagents and Antibody

Table 5 outlines the epitopes identified in BNP isoform testing. Reagents EDC [1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride] and Sulfo-NHS (N-hydroxysulfosuccinimide) were purchased from Thermo Scientific. MES [2-(N-morpholino)ethanesulfonic acid] was obtained from Sigma-Aldrich Corp. Color-coded carboxylated magnetic beads were from Luminex Corporation. Recombinant ProBNP1-108 from Hytest; BNP1-32 from Bachem, N-terminal pro-BNP1-76 from Phoenix. PBS containing 0.1% BSA, 0.04% Tween-20 and 0.05% sodium azide (PBS/TBN) was used as the assay and wash buffer. MES buffer (50 mmol/L, pH 5) was used for conjugating antibodies to beads. Monoclonal antibodies were purchased from Hytest, or raised by Christchurch Heart Institute. Phycoerythrin conjugates were purchased from Moss Inc.

Table 5: Epitopes identified in BNP isoform testing

Assay	Epitopes	Detection target and cross-reactivity
BNP	24C5: epitope BNP11-22 and 50E1: epitope BNP26-32	Cross reacts with BNP and proBNP
BNP H43	50E1: epitope BNP23-32 and polyclonal Ab rabbit H43: epitope BNP1-13	Specific for BNP
NTproBNP general	15F11: epitope NTproBNP13-24 and 24E11: epitope NTproBNP67-76	Cross reacts with NTproBNP and proBNP
NTproBNP specific	15F11: epitope ntBNP13-24 and polyclonal Ab rabbit J25: epitope NTproBNP62-76	Specific for NTproBNP
proBNP	50E1: epitope BNP23-32 and 16F3: epitope NTproBNP13-20	Detects glycosylated and non-glycosylated proBNP
RSproBNP	24C5: epitope BNP11-22 and 16F3: epitope NTproBNP13-20	Detects glycosylated and non-glycosylated proBNP with ring structure closed
T71proBNP	24C5: epitope BNP11-22 and 24E11: epitope NTproBNP67-76.	Detects intact non-glycosylated proBNP

Capture antibody (24C5, 50E1 or 15F11, 10 µg per 6.25×10^6 beads) was coupled to Luminex magnetic microspheres using the Luminex sample protocol for 2-step carbodiimide coupling of protein to carboxylated microspheres.²⁹¹ Confirmation of coupling was assessed using streptavidin-phycoerythrin (PE), or anti-rabbit phycoerythrin. Antibody coupled microspheres were stored in the dark at 2–8 °C until required.

Working standards of BNP1-32, NTproBNP1-76 were prepared by serial dilution of a stock standard with PBS/TBN. Working standards for proBNP were prepared by serial dilution of a stock standard with diluted stripped donated plasma.

Luminex BNP assay

For BNP, BNP H43 and NTproBNP general, specific standards, QC, or sample (50 μ L) were added to duplicate wells of a clear 96-well plate followed by appropriate coupled microspheres (2500 beads/25- μ L well), 25 μ L of diluted detection antibody was added and the plate was covered and mixed (750 rpm) on an orbital plate shaker for 25 h (\pm 10 min) at 4 $^{\circ}$ C.

After a wash step, anti-rabbit PE was added to each plate (50 μ L/well, 4 μ g/mL), and the plate incubated for a further 30 min with shaking.

After a subsequent wash step, the beads were resuspended in 100 μ L assay buffer before reading the plate on the Luminex 200 system. Median fluorescent intensity (MFI) data was uploaded to an immunoassay program (StatLia, Brendan Scientific), and results interpolated off a fitted standard curve.

Luminex NTproBNP assay

For NTproBNP general, specific standards, QC, or sample (50 μ L) were added to duplicate wells of a clear 96-well plate followed by appropriate coupled microspheres (2500 beads/25- μ L well) the plate was covered and mixed (750 rpm) on an orbital plate shaker for 3 h (\pm 10 min) at 4 $^{\circ}$ C.

After a wash step 50 μ L of diluted detection antibody was added and the plate was covered and mixed (750 rpm) on an orbital plate shaker for 30 minutes at Room Temperature.

After a wash step, streptavidin-PE was added to each plate (50 μ L/well, 4 μ g/mL), and the plate incubated for a further 30 min with shaking.

After a subsequent wash step, the beads were resuspended in 100 μ L assay buffer before reading the plate on the Luminex 200 system. Median fluorescent intensity (MFI) data was uploaded to an immunoassay program (StatLia, Brendan Scientific), and results interpolated off a fitted standard curve.

Luminex proBNP assay

For proBNP, RSproBNP and T71proBNP, specific standards, QC, or sample (50 μ L) were added to duplicate wells of a clear 96-well plate followed by appropriate coupled microspheres (2500

beads/25- μ L well) the plate was covered and mixed (750 rpm) on an orbital plate shaker for 16-18 h at 4 °C.

After a wash step 50 μ L of diluted detection antibody was added and the plate was covered and mixed (750 rpm) on an orbital plate shaker for 30 minutes at Room Temperature.

After a wash step, streptavidin-PE was added to each plate (50 μ L/well, 4 μ g/mL), and the plate incubated for a further 30 min with shaking.

After a subsequent wash step, the beads were resuspended in 100 μ L assay buffer before reading the plate on the Luminex 200 system. Median fluorescent intensity (MFI) data was uploaded to an immunoassay program (StatLia, Brendan Scientific), and results interpolated off a fitted standard curve.

High performance liquid chromatography

Plasma extracts were subjected to size-exclusion HPLC (SE-HPLC) at room temperature on a TSK-Gel G2000SW peptide column (Toyosoda, Tokyo, Japan) using isocratic conditions of 20% acetonitrile/0.1%trifluoroacetic acid (TFA) at a flow rate of 0.5ml/minute. Fractions were collected at 1 minute intervals and subjected to Luminex or Roche NTproBNP assay. The SE-HPLC column was calibrated using dextran blue (V_0), cytochrome C (~Mr 12,400), aprotinin (~Mr 6,500), urotensin II (~Mr 1,600) and glycine (V_t).

Heart Ultrasound Protocol

Personnel

Heart ultrasounds were performed by neonatologists with training in clinician performed cardiac ultrasound (Drs Harris, Dixon and More). Of the 202 scans performed, 90% were performed by Dr Harris to reduce interobserver variability. All three clinician-scanners had received basic and advanced training in neonatal cardiac ultrasound through the Australasian College of Ultrasound Medicine and Dr Harris received additional one on one training on TAPSE and tissue Doppler parameters with a cardiac ultrasonographer at Starship Children's Hospital. An average of 10-15 measurements were taken for each parameter where possible to reduce intraobserver variability. Off-line measurements were all done by Dr Harris using software on the heart ultrasound machine or if measurements were delayed measurements were done using Synapse[®] Cardiovascular software (Fujifilm, Australia). Heart ultrasound analysis was conducted by Dr Harris who was blinded to NTproBNP results and not involved in the clinical care of the infants but not blinded to the pulse oximetry results. A paediatric cardiologist from Starship Hospital in Auckland, New Zealand, Dr Clare O'Donnell, reviewed all 36 week PMA

scans for signs of pulmonary hypertension using pre-defined criteria (Table 4). Dr O'Donnell was blinded to NTproBNP results, pulse oximetry results, previous heart ultrasound scan results and clinical history of the infant.

Equipment

A Toshiba Aplio 500 ultrasound machine was used with neonatal cardiac presets and a 6.5MHz probe. No infant was on a muscle relaxant at the time of the heart ultrasound. No angle correction or respiratory gating was used. A concurrent electrocardiogram heart rate tracing was recorded through the ultrasound machine.

Infant Considerations

Scans were performed at the bedside after discussion with bedside nurse regarding current stability of the infant and throughout the scan infant comfort and health were paramount. Oral sucrose was administered for analgesia if required. Scans were terminated if the infant became clinically unstable or unduly distressed during the scanning.

Timing of Scans

Heart ultrasounds were performed on day 3 (as close to 72 hours of age as practicable), day 10, day 28 and when the infant reached 36 weeks PMA. Scans were paired as close as practicable in time to NTproBNP sampling.

Ultrasound Images

Standard views were imaged according to guidelines recommended by the Australasian Society of Ultrasound Medicine and the American Society of Echocardiography (Appendix C). A summary of views is presented in Table 6.²⁰⁴ More advanced quantitative measures were selected based on a review of literature in this field.²¹⁸ Further detail is provided on some of the advanced quantitative measures in Figs. 21-25 and equations 1-3. Several images or video clips were taken of each view with calculations averaged over 10-15 cycles. Measurements were conducted offline where possible to reduce scanning time.

Table 6: Summary table of cardiac ultrasound views

Window	Views	Modality	Detail
<i>Subcostal</i>	<i>Transverse situs view</i>	1. 2D grey scale 2. Colour Doppler	<ul style="list-style-type: none"> • to show normal situs of the abdominal great vessels
	<i>Aortic view</i>	1. 2D grey scale 2. Colour Doppler	<ul style="list-style-type: none"> • anterior sweep of great vessels to the subcostal view of ascending aorta.
	<i>Atrial septal view</i>	1. 2D grey scale 2. Colour/pulse Doppler	<ul style="list-style-type: none"> • zoomed image of right and left atrium and atrial septum • measurement of any septal defect • colour Doppler of atrial septum to interrogate atrial shunt • pulse Doppler of any interatrial shunt
<i>Apical</i>	<i>Four chamber view</i> <i>Tricuspid annulus focused view</i>	1. 2D grey scale 2. Pulse/continuous wave Doppler 3. M mode 4. Tissue Doppler	<ul style="list-style-type: none"> • Clip of atria and ventricles demonstrating relationship and relative size of all four chambers and atrioventricular valves • colour Doppler of flow across mitral and tricuspid valves (Fig. 21) • pulse/continuous wave Doppler of inflow across mitral and tricuspid valves • pulse/continuous wave Doppler of any tricuspid regurgitation, peak velocity measured off-line • M mode of tricuspid annulus free wall with cursor aligned perpendicular to the movement of the annulus, off-line measure tricuspid annular plane of systolic excursion (TAPSE) (Fig. 22). • tissue Doppler image (TDI) of tricuspid annulus, off-line measurement of E', A', S' and calculation of right index of myocardial performance (RIMP) using equation 1 (Figs. 23, 24).

Window	Views	Modality	Detail
<i>Parasternal Long Axis</i>	<i>Biventricular sweep</i> <i>Left ventricular view</i> <i>Right ventricular view</i>	1. 2D grey scale 2. Pulse and colour Doppler 3. M Mode	<ul style="list-style-type: none"> • <i>sweep from left ventricle, through intraventricular septum to right ventricle</i> • <i>image intraventricular septum, left ventricle, mitral valve, left atrium and aortic valve</i> • <i>measure any intraventricular septal defect</i> • <i>colour Doppler across intraventricular septum and pulse Doppler any defect</i> • <i>M –Mode across aortic root and left atrium, measure LA:Ao ratio off-line</i> • <i>M-Mode perpendicular to intraventricular septum at level of mitral valve, measure fractional shortening off-line using following equation 2</i> • <i>image RV, right atrium and tricuspid valve</i> • <i>colour Doppler across tricuspid valve</i> • <i>pulse/continuous wave Doppler across tricuspid valve</i>
<i>Parasternal short axis view</i>	1. <i>Aortic valve view</i> 2. <i>Biventricular view</i> 3. <i>Pulmonary valve and artery view</i>	1. 2D grey scale 2. Colour/pulse Doppler	<ul style="list-style-type: none"> • <i>2D grey scale image/clip of aortic valve demonstrating tri-leaflet arrangement</i> • <i>sweep from base to apex of ventricles and intraventricular septum</i> • <i>colour/pulse Doppler of any ventricular septal defects</i> • <i>clip to assess intraventricular septal position at end systole</i> • <i>2D grey scale image/clip of right ventricular outflow tract to exclude obstruction</i> • <i>colour Doppler clips of flow across pulmonary valve, main pulmonary artery and into left and right pulmonary arteries</i> • <i>pulse wave Doppler of main pulmonary artery flow just distal to pulmonary valve, offline measure acceleration time (time to peak velocity) and right ventricular ejection time and calculate ratio (AT:RVET) and correct for heart rate using equation 3 (Fig. 25)</i>

Window	Views	Modality	Detail
<i>High parasternal view</i>	<p>1. <i>Pulmonary artery/ductal view</i></p> <p>2. <i>Aortic arch view</i></p> <p>3. <i>Pulmonary vein view</i></p>	<p>1. 2D grey scale</p> <p>2. Colour/pulse Doppler</p>	<ul style="list-style-type: none"> • <i>Sweep across aorta and main pulmonary artery demonstrating patent ductus arteriosus if present, measure ductal diameter</i> • <i>Colour Doppler and pulse wave Doppler of ductus</i> • <i>2D grey scale image of aortic arch and origin of main branches</i> • <i>colour Doppler and pulse wave Doppler of aorta distal to origin of ductus</i> • <i>colour Doppler demonstrating four pulmonary veins entering left atrium</i>

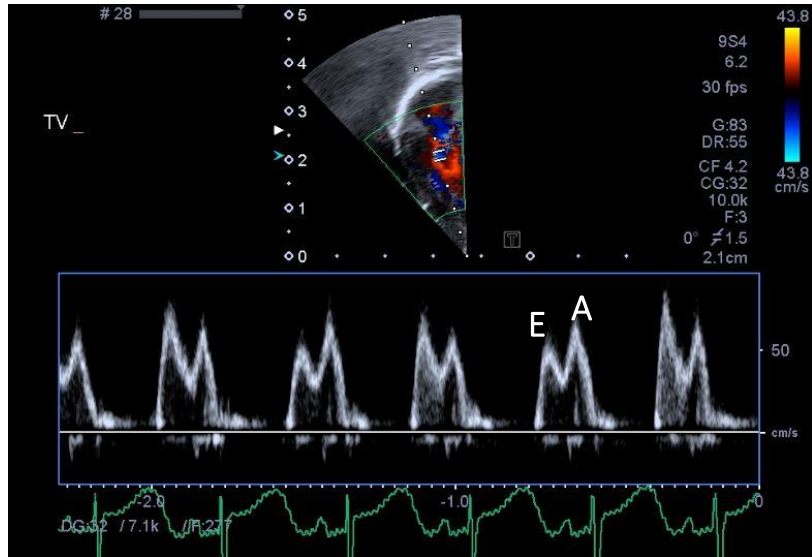


Figure 21: Tricuspid E (early) and A (late) diastolic waves by conventional Doppler

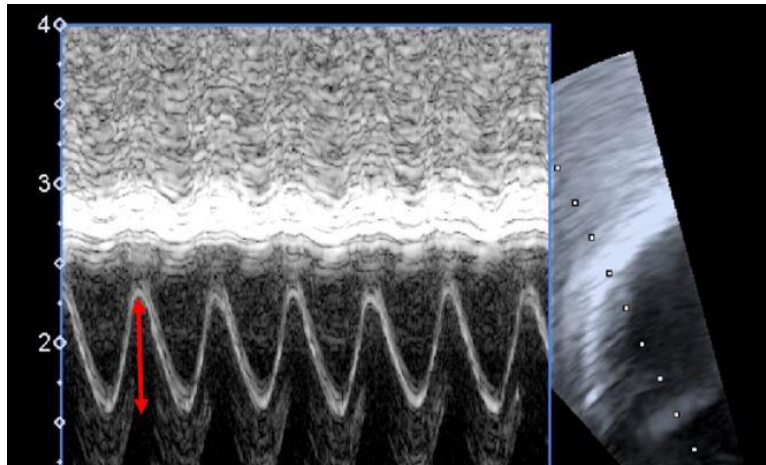


Figure 22: Tricuspid Plane of Systolic Excursion measurement using M mode

Cursor placed on tricuspid annulus and aligned perpendicular to the movement of annulus.

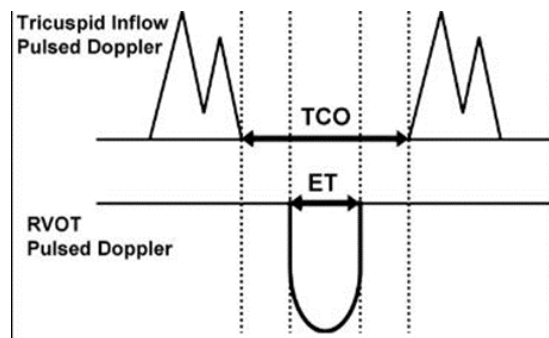


Figure 23: Indices for the measurement of RIMP. RVOT = RV outflow tract, TCO= tricuspid opening time. ET = RV ejection time

Reused with permission from Rudski LG et al. Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography. 2010;23(7):685-713

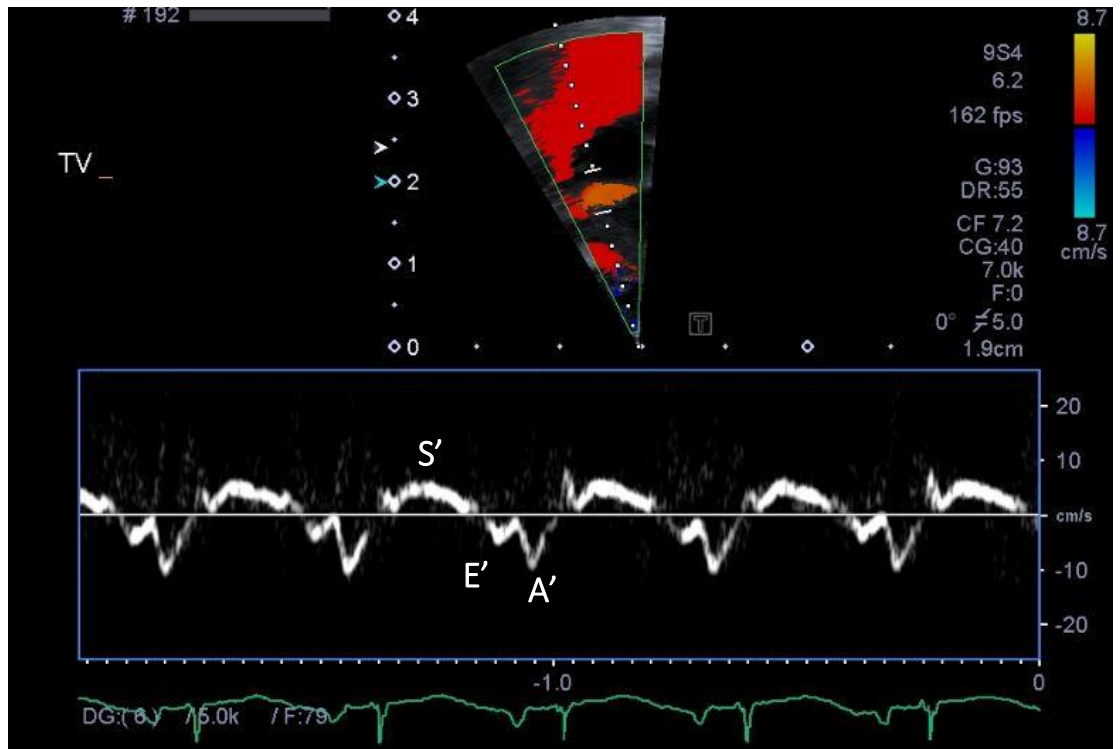


Figure 24: Tissue Doppler image of tricuspid annulus

E', A' peaks (below baseline) and S' wave (above baseline)

$$\text{RIMP} = (\text{IVCT} + \text{IVRT}) / \text{RVET} = \text{TCOT} - \text{RVET} / \text{RVET}$$

IVCT = isovolumetric contraction time
 IVRT = isovolumetric relaxation time
 RVET = right ventricular ejection time
 TCOT = tricuspid closure to opening time

Equation 1: Right Index of Myocardial Performance

$$\text{FS\%} = \frac{(\text{LVEDD} - \text{LVESD}) \times 100}{\text{LVEDD}}$$

LVEDD = left ventricular end-diastolic dimension
LVESD = left ventricular end-systolic dimension

Equation 2: Left ventricular Fractional Shortening



Figure 25 and Equation 3: Ratio of main pulmonary artery acceleration time to right ventricular ejection time(RVET) corrected for heart rate

Blood Pressure

At least two blood pressure measurements recorded on the day of the scan. Invasive recordings were used if an arterial catheter was in place otherwise non-invasive recordings were taken using an automated device.

Oxygen Saturation Monitoring

Eligible infants had pulse oximeter recordings commenced at birth and data was recorded for 72 hours. Subsequent recording periods were 72 hours prior to day 10, 28 and 36 weeks PMA NTproBNP blood testing.

Oxygen saturation data was collected using a Masimo Radical 7 Signal Extraction CO-oximeter (Masimo Corporation, Irvine, California) with upgraded software. Sample averaging time was set to 2 seconds. Masimo sensor probes designed for preterm infants were placed on the right wrist either side of the radial arterial pulsation (unless there was a contraindication to this). A cable connected the Masimo oximeter with the bedside cardiorespiratory monitor to allow heart rate and oxygen saturation data collected by the Masimo to be displayed on the bedside monitor which nurses used to guide care.

Oxygen saturation targets and alarm settings remained as per unit protocol at the time. Nurses responded to alarms as per the unit's standard of care at the time. Oxygen saturation targeting protocols changed in August 2013, detailed in Table 7, after publication of the BOOST II trial of oxygen saturation targeting.²⁹² The new protocol was a local decision based on unit consensus taking into consideration evidence at the time.

Table 7: Oxygen saturation targets before and after August 2013

Corrected GA	Old targets on oxygen	New targets on oxygen	Old targets in air	New targets in air
< 32 weeks	88-92%	90-92%	88-100%	90-100%
32-36 weeks	92-95%	92-95%	92-100%	92-100%
≥36 weeks	95-98%	95-98%	95-100%	95-100%

Oxygen saturation data was downloaded and analysed by Dr Harris using Profox software (PROFOX Associates Inc, Escondido, California). Prior to analysis the downloaded data was reviewed by Dr Harris and any large areas of aberrant data suggestive of a prolonged period of lack of contact (ie. where there was no heart rate detected) were manually deleted. The data was then scanned automatically for aberrant data and these points reviewed by Dr Harris and manually edited if they appeared to represent aberrant data (eg. where there is a complete gap in data collection or a single aberrant saturation that appeared artefactual).

Data analysis included mean oxygen saturation, 4% desaturation index (DSI4%), defined as the average number of times per hour that the oxygen saturation level decreased by ≥4%, DSI4% >10 seconds, coefficient of variation (CV = (standard deviation/mean of measurements) x 100), the percentage of time infants spent outside of the target saturation range, and percentage of time infants spent with oxygen saturations <88% and <80%.

The clinical team was not made aware of the pulse oximetry data for the day 3, 10 or 28 collections. A copy of the results of the 36 week data collection was given to the clinical team as collecting these data this was part of normal clinical care at the time.

Demographic and in-hospital clinical data

Clinical and demographic data was collected by chart review. Data collected is itemised in Table 8.

Table 8: Clinical and demographic data collected

General	Antenatal	Growth/Nutrition/GI	Respiratory	Cardiovascular	Infection	Neurology	Ophthalmology
Gestation at birth	IUGR	Birthweight & z score	Intubated at birth	Inotropic support	Episodes suspected sepsis	Intraventricular haemorrhage	Retinopathy (ROP) highest stage
Survived to discharge	Singleton/multiple pregnancy	Birth length & z score	Surfactant given	HsPDA day3	Episodes proven sepsis	Neurodevelopmental outcome:	ROP laser treatment
Duration of neonatal stay	Maternal smoking while pregnant	Birth head circumference & z score	Max oxygen in delivery suite	HsPDA day10	Ureaplasma treated	- any developmental support required	ROP cryotherapy
Gender	Antenatal steroids (any/complete)	Birth body surface area (BSA)	Respiratory distress syndrome	PDA day28		- blindness	ROP other surgery
Ethnicity	Magnesium sulphate given antenatally	Day 10 weight & z score	ANZNN BPD	PDA 36wks		- deafness	
	Rupture of membranes >24 hours before delivery	Day 10 length & z score	NICHD BPD grade	Medical treatment PDA		- cerebral palsy	
	Preeclampsia	Day 10 head circumference & z score	Diuretics for BPD	Surgical treatment PDA		- Bayleys III / Carolina	
	Chorioamnionitis	Day 10 BSA	Hours conventional ventilation	Diuretics for PDA		Curriculum assessment	
		Day 28 weight & z score	Hours high frequency ventilation				
		Day 28 length & z score	Total hours ventilation				
		Day 28 head circumference & z score	Hours CPAP				
		Day 28 BSA					

General	Antenatal	Growth/Nutrition/GI	Respiratory	Cardiovascular	Infection	Neurology	Ophthalmology
		36 week weight & z score 36 week length & z score 36weeks head circumference & z score 36 week BSA Breastfeeding at discharge Necrotising enterocolitis (NEC) NEC management	Hours BIPAP Hours HiFlow Home on oxygen Total hours oxygen Total hours respiratory support Caffeine given Maximum dose caffeine citrate Gestational age at which caffeine stopped Doxapram given Gestational age at which doxapram stopped Postnatal steroids Steroid course total dose				

Post-discharge data collection

1. Respiratory Health at 1 year

When participants were 1 year of age a respiratory health questionnaire designed for this study and not previously validated was sent to parents (Appendix D). The aim of this questionnaire was to identify frequency of presentations to primary and secondary health care and respiratory morbidity in the first year of life. Consent was also sought to check responses against general practice and hospital health records.

2. Hospital Encounters in first 2 years of life

Hospital records were checked for health encounters beyond discharge from the neonatal unit up to 2 years PMA.

The following data were collected:

1. Number of readmissions
2. Total days in hospital
3. Type of admission
4. Weight, height and head circumference at 2 years
5. Visual problems
6. Hearing problems
7. Chronic respiratory symptoms
8. Seizures
9. Hydrocephalus
10. Cerebral palsy

3. Neurodevelopmental Outcome

Outpatient clinic records were checked for neurodevelopmental assessments done as part of routine follow-up of very preterm infants and when completed results for Bayley Scale of Infant and Toddler Development (BSID-III) were recorded.²⁹³

Sample size calculations

A biostatistician was engaged to assist with statistical planning and analysis. At the time of study design there was very little published data on NTproBNP levels in preterm infants and the association of these with BPD and no data on NTproBNP and BPD-associated pulmonary hypertension.. Sample size calculations for the design of this study were based

on a small single unit sample of Israeli infants <34 weeks gestation, looking at the association between NTproBNP levels and BPD.¹⁸⁶ This study produced correlations between NTproBNP levels and measures of respiratory distress around day 28 in the region of 0.40-0.50. Based on this study and other published data suggesting an incidence of pulmonary hypertension of approximately one third of infants with BPD estimates from standard power tables²⁹⁴ suggested that for our study to have 80% power to detect correlations in excess of 0.40 with $\alpha = 0.05$ would require a minimum sample size of 45 infants. Allowing for a small potential sample loss due to failure to complete the full set of assessments such as due to transfer to another centre or withdrawal of infant from study a target sample size of $N = 50$ infants was recommended.

At study inception approximately 60% of infants <30 weeks gestation admitted to Christchurch Women's Hospital neonatal unit had ongoing oxygen/respiratory support requirements at 36 weeks PMA, and just under half were <28 weeks gestation. Consideration of sample power to detect mean differences in a continuous outcome between subgroups defined on the basis of these or similar characteristics suggests that with a sample size of 50 the sample has between 75%-80% power with $\alpha = 0.05$ to detect a mean difference between groups in excess of 0.80 SD. This corresponds to a 'large' effect size as defined by Cohen²⁹⁴

While the study is adequately powered to detect large effects, statistical power to detect more moderate effects is substantially reduced. For example, the sample has only 50% power to detect a correlation in excess of .25, and approximately 40% power to detect a 'moderate' effect size difference between groups in excess of 0.50 SD; and only 10% power to detect small effects (correlations in excess of .10, between group differences of 0.20 SD).

The availability of repeated measures data is likely to enhance the study's power to examine change in measures over time or to detect between group differences.²⁹⁵ However, the extent to which power is enhanced by the repeated measures design is dependent on the nature and type of the relationships being examined.

Statistical methods

General Principles

In general quantitative variables are described by mean and standard deviation (SD) or median and interquartile range (IQR) depending on whether they are normally distributed or not. Qualitative variables are described by frequency and percentages. For bivariate analyses parametric tests were used for normally distributed data and non-parametric tests for non-normally distributed data. Regression models were extended to control for relevant demographic, perinatal or clinical factors. Where data were non-normally distributed appropriate data transformations (eg logarithmic) were conducted. A p value of < 0.05 was considered statistically significant. Data was stored using Microsoft Excel and statistical analysis was performed using Microsoft Excel, Stata 15, SAS 9.4 and GraphPad InStat Software.

Specific Analyses

Distribution of NTproBNP levels and factors affecting NTproBNP

Standard graphical methods (eg box and whisker plots) were used to display the distribution of NTproBNP levels at each age (days 3, 10, 26 and 36 weeks PMA). As the distribution was skewed a natural log transformation was applied. Fitted regression models were used to examine the relative contributions of birth gestational age, ventilation status and renal function on day 3 and 10 NTproBNP levels. The strength of the association between hypoxia and NTproBNP and the association between haemoglobin and NTproBNP was calculated using the Spearman's rank correlation coefficient.

Associations between NTproBNP levels and clinical factors (HsPDA, BPD, pulmonary hypertension, gestational age, ventilation, hypoxia, renal impairment, haemoglobin level)

A combination of graphical and tabular methods were used to display the bivariate associations between NTproBNP levels and HsPDA or BPD or pulmonary hypertension outcomes depending on whether the outcome is continuous (eg severity of BPD) or dichotomous (eg diagnosis of BPD or HsPDA or pulmonary hypertension). In each case regression models (linear regression for continuous outcomes, logistic regression for dichotomous outcomes) were fitted to the data to examine the nature of the association and the strength of the association was summarised by the R-squared statistic. Regression models were extended to control for relevant demographic, perinatal or

clinical factors. Where data were non-normally distributed appropriate data transformations (eg logarithmic) were conducted. Receiver Operating Characteristic curve analysis²⁹⁶ was conducted to examine the extent to which it is possible to distinguish at an early age between those infants who do/do not have a haemodynamically significant PDA and those who go on to develop BPD by day 3 and day 10 NTproBNP. A predictive cut-off value was chosen to maximise sensitivity and specificity.

BNP isoform frequency

Frequency of BNP isoforms/fragments was described graphically according to clinical group status.

Feasibility of advanced heart ultrasound techniques and changes over time

The frequency of attainment of ultrasound parameters is described. Changes over time in quantitative parameters are described using mean (SD) or median (IQR). Comparisons of ultrasound parameters between sub-groups stratified by BPD status were tested for statistical significance using standard tests - Chi square test of independence for dichotomous outcomes; t-test for independent samples or analysis of variance for continuous measures; or Mann-Whitney rank-sum test in cases where the data was strongly non-normally distributed. Box and whisker plots were used to display results graphically.

Changes in measures of oxygen saturation instability and effect on clinical outcomes

Changes over time in quantitative measures are described using mean (SD) or median (IQR). Unpaired t tests were used to compare means. Repeated measures ANOVA analysis was used to assess effect of group and time interactions. Box and whisker plots or scatter plots were used to display results graphically. The strength of the association between measures of oxygen saturation instability and neurodevelopmental outcomes was assessed using Spearman correlations. Statistically significant associations were adjusted using linear regression methods to control for gender, birthweight z score, gestation, BPD status, antenatal steroids, maternal smoking and presence of HsPDA on day 3. Repeated measures ANOVA analysis was used to assess effect of BPD status and time interactions. Mean differences in oxygen saturation measures by BPD status over time adjusting for gender, gestation, birthweight z score and day 3 HsPDA were estimated from linear regression models fitted to repeated measures data for each fitted within a Generalising

Estimating Equation framework.²⁹⁷

Audit of alarm compliance and survey of oxygen saturation targeting

Audit results were tabulated according to relevant clinical subgroups with frequencies expressed as percentages. Survey results were described using percentages for frequency of particular responses.

RESULTS

Baseline Demographics and In-Hospital Outcomes

Background

Pulmonary hypertension is an important complication of premature lung disease that is associated with increased morbidity and mortality.^{62, 64, 67, 114, 115} The incidence of pulmonary hypertension in infants with BPD is estimated to be 17-37% but none of these studies were conducted in Australasia and regional differences in practice may alter this incidence.^{64, 80-82, 115} With recent guidelines suggesting screening for this condition local data is needed from prospective cohorts to establish the potential magnitude of this issue before screening can be considered.^{86, 87} Although cardiac catheterisation is the gold standard for diagnosis for pulmonary hypertension, it is invasive and only available at specialist centres. Hence heart ultrasound is used by most centres to identify raised pulmonary pressures but there are currently no validated diagnostic criteria for late pulmonary hypertension in very preterm infants. Prospective data on the feasibility and utility of advanced quantitative heart ultrasound measures for the evaluation of late pulmonary hypertension in very premature infants is needed. Investigation of the potential role for biomarkers such as NTproBNP and oxygen saturation instability is warranted. This chapter describes the baseline demographics and in-hospital outcomes for our study which attempts to address some of these questions.

Aims

1. To describe recruitment and retention for this study
2. To describe the data available for analysis at all assessment points
3. To describe baseline demographic characteristics and in-hospital outcomes of this cohort as a whole and by HsPDA and BPD status
4. To compare our cohort to contemporaneous national data and Australasian Neonatal Network data to evaluate how representative our cohort is of this data

Data on the incidence of pulmonary hypertension in our cohort (as diagnosed by pre-

defined heart ultrasound criteria described in methods chapter) is presented in chapter 6.

Methods

Setting

This study was conducted at the Christchurch Neonatal Unit, a tertiary neonatal intensive care unit, serving Canterbury and the West Coast and the neonatal surgical centre for the Canterbury, West Coast and Otago regions.

Participants

All infants born at < 30 weeks gestation between March 2013 and April 2015 and cared for at Christchurch Neonatal Unit were invited to participate.

Exclusion criteria were known structural airway or lung anomalies, congenital anomalies of the pulmonary arteries or pulmonary veins, major systemic vessel-to-pulmonary artery collateral vessels, congenital heart disease (except those with patent ductus arteriosus, patent foramen ovale or secundum atrial septal defect < 5mm or tiny restrictive muscular ventricular septal defect), severe liver disease, or persistent pulmonary hypertension of the newborn on day 3 heart ultrasound (defined as severe hypoxia associated with estimated main pulmonary artery pressure >25mmHg). If infants had a grave prognosis such that they were not expected to survive to day 3 they were also excluded.

Data collection

Participants had paired heart ultrasound, NTproBNP levels and oxygen saturation data collected on days 3, 10, 28 and at 36 weeks PMA. The testing protocol and heart ultrasound criteria for pulmonary hypertension are described in detail in methods chapter. Demographic and in-hospital clinical outcome data was sourced from hospital records and outcome data submitted to the Australian and New Zealand Neonatal Network and collated and analysed in a de-identified form by study number using Microsoft Excel.

Data Analysis

Data acquisition was described by frequency. Whole cohort descriptive statistics were performed using percentages, means (SD) or medians (IQR) where the data was non-

normally distributed. T tests were done for the comparison of means of independent samples and Chi squared test for the comparison of percentages.

Where possible contemporary data from the Australian and New Zealand Neonatal Network (ANZNN) report for 2014²⁹⁸ and national data from the New Zealand census²⁹⁹ and the Ministry of Health³⁰⁰ is presented for comparison.

Results

Recruitment

Figure 26 shows the flow diagram of recruitment and retention. Sixty-five infants were eligible on the basis that they were born at less than 30 weeks gestation Christchurch Women's Hospital during the study period. However, three babies had a grave prognosis and died before day three and were excluded on this basis. One infant was an out of area transfer who was excluded as they would be transferred back to their base hospital once stable and a bed became available making them unable to participate in the study. One infant had uncertain gestation and was thought to be close to 30 weeks and was excluded due to this uncertainty. Seven other families were approached but declined to participate. Of the fifty-three infants initially recruited two had to be excluded after the day three scan due to significant cardiac anomalies. The causes of death for the two children who died between day 28 and 36 week PMA testing were respiratory failure secondary to severe BPD and pulmonary haemorrhage secondary to Escherichia coli sepsis.

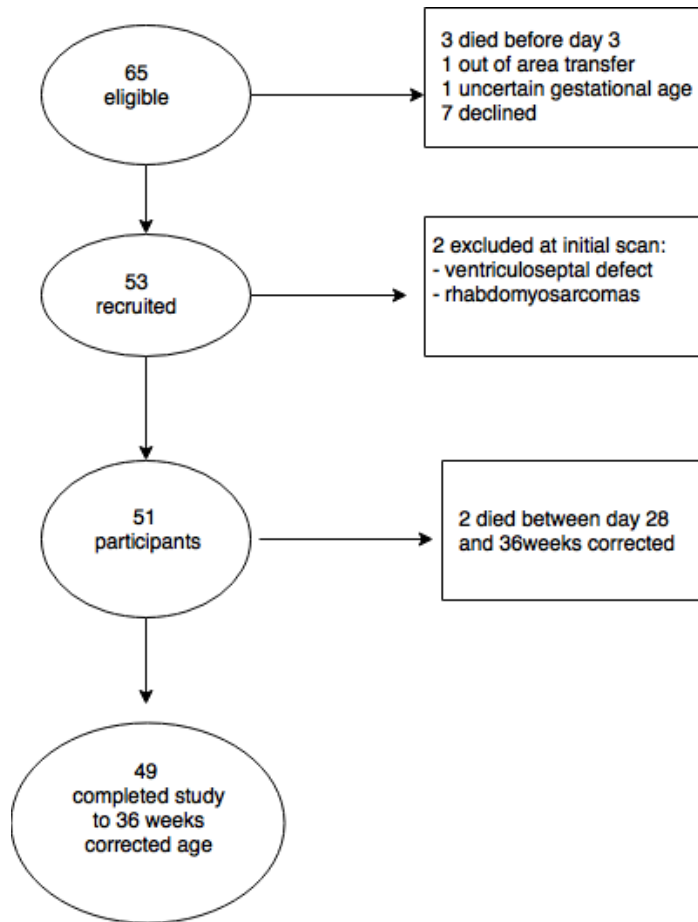


Figure 26: Recruitment and retention

Data collection

Table 9 shows the number of participants for which NTproBNP, heart ultrasound and pulse oximetry data were available for analysis.

Table 9: Number of patients for which data was available for analysis at each data collection point

	Day 3	Day 10	Day 28	36 weeks PMA
NTproBNP	50	51	51	48
Heart USS	51	51	51	49
Pulse oximetry	51	51	51	48

One infant missed their data collection at 36 weeks PMA and had all assessments done at 7 weeks post term. The median (IQR) time between heart ultrasound and NTproBNP

testing was 4 (1.5-7.3) hours. The mean (SD) hours of valid oxygen saturation data analysed was 68 (9.2) for day 3, 69 (9.4) for day 10, 70 (8.6) for day 28 and 70 (7) for 36 weeks PMA.

Clinical characteristics of cohort

Antenatal and post-natal clinical characteristics

Tables 10 and 11 present relevant antenatal variables, baseline demographic, clinical characteristics and in-hospital morbidities for the cohort as a whole. Mean gestational age was 27.8 weeks with 53% less than 28 weeks gestation (24weeks = 2, 25 weeks = 3, 26 weeks = 7, 27 weeks=15, 28 weeks=8 29 weeks=16). No infant in our cohort met our criteria for a diagnosis of pulmonary hypertension and this is discussed further in chapter 5. Tables 12 and 13 compares characteristics of the cohort by BPD status (ANZNN definition) and by day 3 HsPDA status as these are important clinical covariates.

Table 10: Antenatal characteristics (whole cohort)

Clinical Characteristic	n=47 pregnancies
Singleton pregnancy	84%
Maternal smoking while pregnant	19.6% (21.6% of infants exposed due to twins)
Antenatal steroids (any/complete)	92/61%
Magnesium sulphate given	58.8%
Rupture of membranes >24 hours before delivery	27.4%
Preeclampsia	15.7%
Chorioamnionitis suspected clinically	19.6%
Chorioamnionitis on placental histology	58% (28% mild-moderate, 30% severe)*
IUGR pregnancy	7.8%

* Placental histology available for 50 infants

Table 11: Infant characteristics and in-hospital morbidity (whole cohort)

Clinical Characteristic	n=51
Infant ethnicity (infants with more than one ethnic group identified were counted in each of these groups)	11.8 % Māori 56.9% New Zealand European 7.8% Pasifika 19.6% Asian 7.8% other European 3.9% other
% Male/Female	55/45
Mean(SD) GA(weeks)	27.8(1.5)
Mean(SD) birthweight(g)	1104(225)
Mean(SD) birthweight z score	-0.10(0.88)
Surfactant administered	82%
Never ventilated	13.7%
Postnatal steroids	5.9%
Median(IQR) duration of ventilation (hours)	16 (7-54)
Median(IQR) duration of all respiratory support (hours)	1087 (676-1628)
Median(IQR) duration of oxygen (hours)	631 (173-1325)
Discharged on home oxygen	15.7%
Mean(SD) duration of hospitalisation (days)	98 (34)
Alive at discharge	96%

Clinical Characteristic	n=51
Treated with caffeine	100%
Treated with doxapram for apnoea	17.6%
BPD (n=49)	63%
HsPDA day 3	49%
IVH any grade	17.6%
IVH grade 3 or 4	2.0%
NEC	0%
ROP	3.9 %(stage 1 only)
Culture positive sepsis	15.7%
Treated for gastroesophageal reflux	62.7%
First milk feed breastmilk	100%
Any breastfeeding at discharge* (n=45)	71%

** Breastfeeding at discharge may have been part of a mixed feeding plan and may have been given by bottle in infants who were not able to manage feeding at the breast.*

Clinical characteristics by BPD status

Table 12 shows the clinical characteristics of our cohort according to whether they developed BPD or not. In two infants death before 36 weeks PMA precluded determination of a diagnosis of BPD. However, both infants were grouped with BPD infants as at the time of death as one had evidence of severe lung disease and was ventilator dependent (died at 32 weeks PMA) and the other had persistent oxygen requirement and need for non-invasive respiratory support (died at 34 weeks). Infants with BPD had significantly lower gestational age and birth weight (but not birth weight z score) and longer duration of ventilation.

Table 12: Clinical characteristics of cohort by BPD status (ANZNN definition)

Measure	Whole cohort (n=51)	No BPD (n=18)	BPD (or death) (n=33)	<i>p</i> *
Mean(SD) gestational age (weeks)	27.8 (1.5)	28.4 (1.3)	27.4 (1.5)	0.02
Mean(SD) birth weight(g)	1104 (225)	1223 (158)	1039 (232)	0.004
Mean (SD) birth weight z score	-0.10 (0.88)	0.00 (0.72)	-0.16 (0.96)	0.54
% Male	55	50	58	0.60
% Any antenatal steroids	92	89	94	0.52
% Complete antenatal steroids **	61	56	64	0.57
% Maternal smoking in pregnancy	21.6	22.2	21.2	0.93
% Never ventilated	14	6	18	0.21
Median(IQR) hours ventilation	16 (6.5-53.5)	12.5 (4.7-48)	17 (8-55)	0.407
Median(IQR) hours any respiratory support	1076 (671-1651)	516 (307-835)	1498 (1034-1828)	<0.0001
% Received surfactant	82	78	85	0.70
% Treated with doxapram***	18	11	21	0.46
% HsPDA day 3	51	33	58	0.10
% Inotropic support	12	6	15	0.41

*Chi square or Fisher's exact tests for comparison of percentages and t-test for independent samples for comparison of means. Mann Whitney for comparison of medians where distribution was non-

Gaussian**Complete steroids defined by 2 doses < 7 days before delivery

***For prevention or treatment of apnoea of prematurity

Clinical characteristics by HsPDA status

Table 13 documents clinical characteristics of our cohort according to whether they had HsPDA on day 3 of life. There were no significant between group differences.

The changing PDA status of the cohort over time is displayed in Fig.27. By day 3 half of the cohort had a closed or non-haemodynamically significant duct. None of the infants who were classified as having closed or non-significant duct on day 3 received treatment. No infants had PDA treatment prior to day 3. 7/25 of those classified as having HsPDA at day 3 had medical treatment with 2 of those infants subsequently going on to have ligation after day 28. Only one of the 7 infants treated had a change in the haemodynamic significance of their duct by day 10. One infant who had a closed or non-significant duct on day 3 developed HsPDA by day 10. Of the 16 infants with HsPDA on day 10 a PDA was still detectable on day 28 in 10 of them.

Table 13: Clinical characteristics according to day 3 PDA status

Measure	No HsPDA (n=26)	HsPDA (n=25)	P
% (n) Male	46.2 (12)	64.0 (16)	0.20 ¹
Mean (SD) gestation (wk)	28.1 (1.5)	27.4 (1.4)	0.08 ²
Mean (SD) birthweight (g)	1132 (219)	1054 (230)	0.22 ²
Mean (SD) birthweight z-score	-0.13 (0.66)	-0.08 (1.08)	0.84 ²
Mean (SD) hours ventilation	43 (145)	224 (413)	0.04 ²
% (n) Bronchopulmonary dysplasia	53.8 (14)	70.8 (17)	0.22 ¹
% (n) Necrotizing enterocolitis	0.0 (0)	4.0 (1)	0.49 ³
% (n) Retinopathy of prematurity	3.9 (1)	4.0 (1)	0.51 ³
% (n) Intraventricular haemorrhage	11.5 (3)	24.0 (6)	0.24 ³
% (n) Death	0.0 (0)	8.0 (2)	0.24 ³

¹Chi-square test ²t-test for independent samples ³Fisher's exact test.

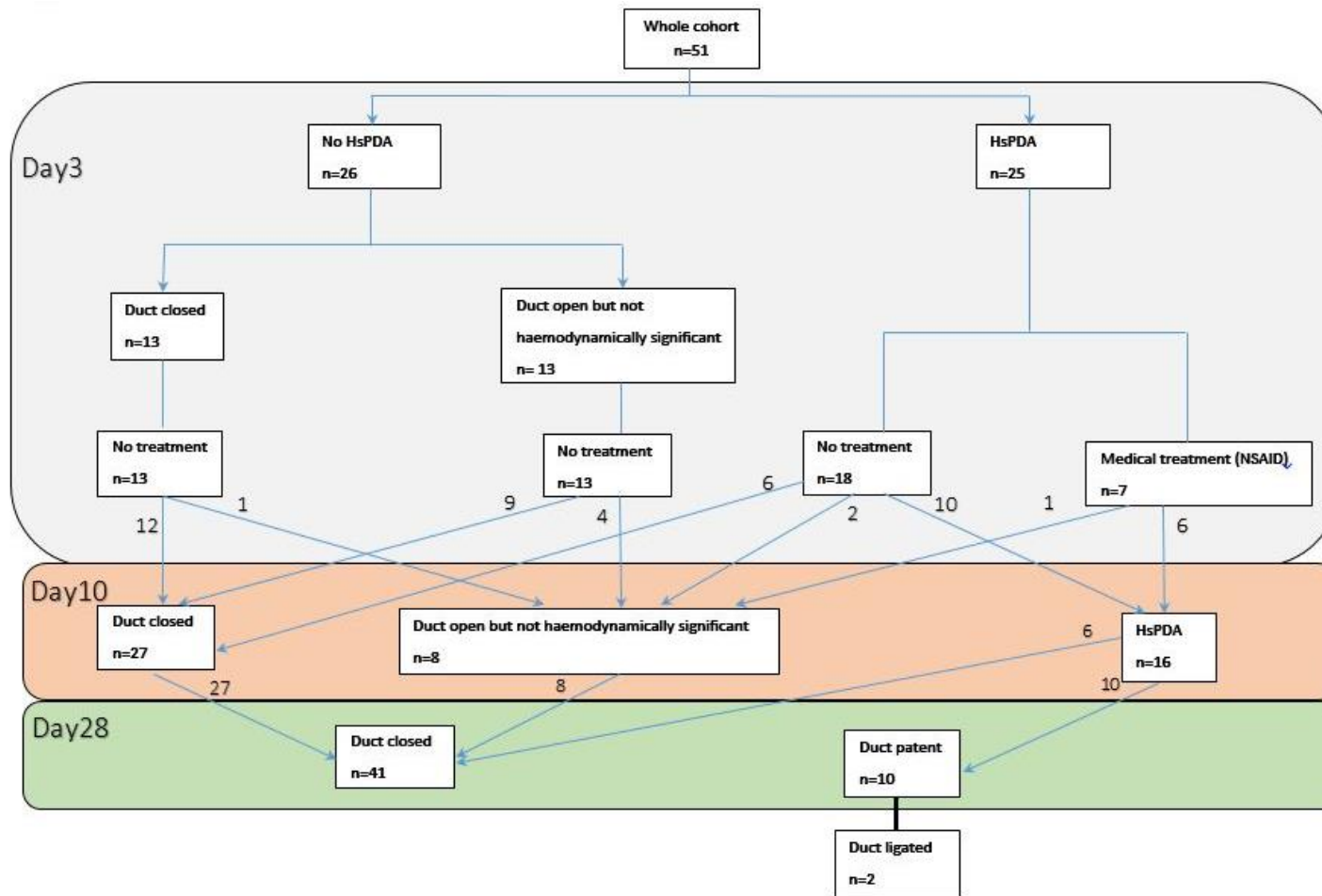


Figure 27: Flow chart of changing PDA status over time

Growth Outcomes

Mean z scores at birth, day 10, day 28 and 36 weeks PMA were calculated for weight and head circumference using the methods used by ANZNN and based upon British WHO 1990 data files (Table 14). Length z scores could not be calculated for day 3, 10 or 28 as for most of the infants their corrected age was out of range for the calculator. Weight and head circumference z scores fell from birth to day 10 and day 28 but recovered beyond this. All growth parameters were + or- <1 SD from mean at 36 weeks PMA.

Table 14: Growth parameters across time for whole cohort

Mean (SD) z score	Birth	Day 10	Day 28	36 weeks PMA
Weight	-0.10 (0.88)	-0.65 (0.76)	-0.66 (0.77)	0.15 (0.86)
Length	No z score data	No z score data	No z score data	-0.63 (0.98)
Head circumference	-0.67 (0.81)	-1.39 (0.79)	-1.35 (0.89)	-0.25 (0.86)

Discussion

Antenatal

The proportion of singleton to multiple births in our cohort is higher than that seen across ANZNN. In 2014 ANZNN reported 74% singletons in the 24-30 week gestational age bracket.²⁹⁸ Antenatal smoke exposure was in line with New Zealand census data from 2013 in which 20.6% of women aged 18-44 years smoked.²⁹⁹ Rates of antenatal steroid administration are similar with 89.5% of ANZNN mothers treated.²⁹⁸ A complete course of antenatal steroids is considered to be two doses 24 hours apart with delivery > 24 hours and < 7 days after last dose. This is often more difficult to achieve due to the rapidity of spontaneous preterm labour and safety considerations around delaying delivery if mother or baby are compromised. Despite this almost two thirds of our cohort achieved complete steroid cover prior to delivery. Rates of antenatal magnesium sulphate, a neuroprotectant, were almost the same with 53% of ANZNN mothers receiving this.²⁹⁸ We are unable to compare rates of intrauterine growth restriction because of the varying definitions of this condition. We recorded this if this was

documented in maternal notes as a clinical problem. Similarly preeclampsia is often recorded within the umbrella term of 'hypertensive disorders' and we were unable to directly compare the incidence. It is interesting to note the discrepancy between the rate of chorioamnionitis suspected antenatally and the three times higher rate confirmed histologically.

Postnatal

Race has been shown to influence the rate of preterm birth.³⁰¹⁻³⁰³ Maori, Pacific and Asian ethnicities were higher in our cohort than the general population. This may reflect differing fertility rates and the increased rate of poorer maternal health in some minority groups.

Māori were well represented in our infant sample with 11.8% identifying as Māori compared to 2013 Canterbury census representation of 7.8%.²⁹⁹ Those that identified as European (NZ European and other European) were underrepresented in our sample compared to the Canterbury and national data respectively (64.7% : 86.9% : 74.0%) while Pacific people (7.8% : 2.5% : 7.4%) and Asians (19.8% : 6.9% : 11.8%) were overrepresented.²⁹⁹ There were slightly more males in our cohort and it is important to note that male babies born preterm have worse outcomes than female babies.³⁰⁴ Male babies were also over-represented among NICU admissions in New Zealand in 2014 (58.1% of the New Zealand registrants compared to 51.7% of total live births in New Zealand).

The mean New Zealand Deprivation Index Decile ³⁰⁵ for our cohort was 6.7 where decile 1 represents low deprivation and decile 10 represents high deprivation. These deciles are equally distributed across the New Zealand population as a whole and are based on home address and as such are a crude estimate of social deprivation.

The rate of BPD in our cohort was unexpectedly high compared to ANZNN data.²⁹⁸ There is no global consensus regarding the definition of BPD although attempts have been made.²⁸⁷ This has made it difficult to compare studies. There has long been concern regarding the wide inter-unit and geographic variations in BPD rates. In addition traditional definitions of BPD do not correlate with long term respiratory outcomes calling into question the utility of this diagnostic label.³⁰⁶ At the time of this study a diagnosis of BPD was made if an infant had premature lung disease and had an ongoing need for oxygen or respiratory support at 36 weeks PMA. There was no standardised

protocol or physiological challenge performed to determine whether an infant needed the level of support they were on. In the Christchurch unit it was usual practice to perform a 24 hour oxygen saturation analysis and on the basis of the subjective interpretation of this a clinician would make a decision about ongoing respiratory support. It is likely that there were differences in practice between clinicians and across the units in the network with regards to determining a diagnosis of BPD. Because of this a “shift test” based on a modified Walsh test was introduced after this study was conducted.³⁰⁷ This involves a room air challenge for infants on low levels of oxygen or respiratory support. As this was not done for our cohort it is possible that infants with mild oxygen or respiratory support requirements would not have been classified as having BPD if they had had a physiological challenge. As the deleterious effects of invasive ventilation have become apparent, neonatology has been moving towards non-invasive respiratory support strategies. This has included continuous positive airway pressure (CPAP), humidified high flow and low flow oxygen. Of our cohort, 13% were never ventilated and those who were had low total hours of ventilation in line with this strategy.

Our cohort had a mean gestational age of approximately 28 weeks and a mean birthweight of just over 1100g. A gestational age of less than 28 weeks or a birthweight of less than 1000g is conventionally chosen to represent the most vulnerable group of preterm infants and as our cohort is relatively small we have only small numbers at the extremes of gestational age and birthweight. Small for gestational age babies were not over represented in our cohort with mean birthweight z score of -0.10. Survival was high in our cohort, however, infants who were not expected to survive the first few days of NICU admission were excluded from our study. This compares to ANZNN survival rates of 68.4% at 24 weeks gestation and up to 96.7% at 29 weeks gestation.²⁹⁸

Artificial surfactant administration, given to help stabilise preterm lung disease, was higher in our cohort than the 72% reported in 2014 by ANZNN.²⁹⁸ Only three of our infants received intratracheal surfactant but were not subsequently ventilated. Most babies in our cohort required a period of intubation and ventilation, although, the duration of assisted invasive ventilation varied widely. Our cohort had a shorter duration of invasive ventilation compare to the Network (ANZNN 2014: median(IQR) hours for 24-25 weeks =274.5 (97.5–611.5), 26-27 weeks = 64 (17–199) , 28-29 weeks =22 (10–71)).²⁹⁸ However, our cohort had a much higher rate of BPD (63% compared to 37%).²⁹⁸

Post-natal steroid use for severe lung disease, resistant hypotension or severe hypoglycemia was slightly lower than the 7.2% of all ANZNN registrants.²⁹⁸ All of our infants had caffeine therapy which was started early and prophylactically for the prevention of apnoea of prematurity and due to evidence of beneficial effects on short term respiratory function and long term neurodevelopmental outcome.³⁰⁸⁻³¹⁰ Doxapram, a respiratory stimulant, was also used to treat oxygen saturation instability in several of our cohort but there is no comparative ANZNN data for this.

A haemodynamically significant duct was a common problem affecting half of our cohort in the first three days which is not unexpected in this group of very to extremely preterm infants. It is difficult to compare rates due to differing definitions of haemodynamic significance.

Rates of intraventricular haemorrhage within the brain were low in our cohort with 17% having any degree of bleeding (compared to 23% in ANZNN) but only 2% having a significant bleed that may be associated with long term consequences.²⁹⁸

Rates of retinopathy were very low in our cohort but not dissimilar to rates in recent years. Only 3.9% of our cohort had ROP and this was only stage 1 with no stage 2-4 disease. ANZNN infants are eligible for the recommended screening if they are born at < 31 weeks gestation and/or have a birthweight of < 1250g. Of those ANZNN registrants who were screened 6.3% had stage 3-4 ROP.²⁹⁸

Sepsis rates were similar to the 14.7% of 2014 ANZNN 24 to < 30 weeks infants who had sepsis during their admission.²⁹⁸ ANZNN defined systemic sepsis as a clinical picture consistent with sepsis, and either a positive bacterial or fungal culture of blood and/or cerebrospinal fluid.

Breastmilk feeding is the preferred enteral nutrition for preterm infants with many short-term and long term health benefits including a reduction in the risk of necrotising enterocolitis. All of our babies received breastmilk as the first milk and there were high rates of breastmilk feeding at neonatal discharge that were comparable to the 72% seen in ANZNN.

We had no cases of necrotising enterocolitis, a gastrointestinal complication of prematurity that can result in death or devastating short gut syndrome and prolonged need for parenteral nutrition. This condition is known to have wide geographic variations in incidence but the incidence across ANZNN in 2014 was also low at 1.3%.

Our cohort generally had a healthy post-natal growth trajectory which has been shown to correlate with better short-term and long-term outcomes.^{264, 311-313} Our high rates of breastmilk feeding were likely contributed to by our Baby Friendly Hospital Initiative accreditation, specialised in-unit lactation consultants and the opening of a Human Donor Milk Bank in February 2014. The Baby Friendly Hospital accreditation is a WHO, UNICEF initiative that ensures that Maternity and Neonatal services adopt practices that protect, promote and support breastfeeding. Suspected gastroesophageal reflux disease was treated with milk thickeners and acid suppression in almost two-thirds of our cohort.

Summary

In comparison with the contemporaneous regional ANZNN neonatal network and New Zealand population data, infants recruited to this study were generally representative of babies born at less than 30 weeks gestation and admitted to NICU with the exception of a higher rate of BPD, lower rate of invasive ventilation and a low rate of ROP. However, approximately half of our cohort was >28 weeks with only small numbers of babies born at the extremes of prematurity which may reduce the likelihood of detecting morbidity. With regards to other risk factors for pulmonary hypertension our cohort had a high incidence of BPD, HsPDA and chorioamnionitis but only a handful of pregnancies were complicated by preeclampsia or growth restriction.

In conclusion, our cohort, although modest in size, is a fair representation of New Zealand NICU babies born at less than 30 weeks gestation in whom clinicians may consider screening for pulmonary hypertension. Outcomes for this cohort would not however, be generalizable to resource poor neonatal settings, populations with a low ethnically European population or NICUs where invasive ventilation is use is high and non-invasive respiratory support low.

N-Terminal pro B-type Natriuretic Peptide

Background

Biomarkers

Interpretation of biomarker assay results necessitates an understanding of the biology of the chosen marker within a selected patient population and an appreciation of the factors that may affect accurate quantification from sampling to analysis. This is particularly important when assays that have been developed and validated for an adult population are used in a paediatric setting.

BNP is a cardiac hormone that has a cardioprotective function mediating natriuresis, diuresis, vasodilation, inhibition of sympathetic activity and reducing pathological myocardial remodelling. It is released predominantly from the cardiac ventricles as a prohormone from which the biologically active BNP and the inert NTproBNP fragment are cleaved. Details of release and degradation of BNP are outlined in Fig. 11 in the background chapters. BNP is part of a foetal cardiac gene programme thought to be involved in cardiac development.¹⁶⁶ Reactivation of this foetal cardiac gene programme can occur as a cardioprotective mechanism during times of cardiac stress.^{48, 54} BNP and NTproBNP are released under conditions of increased wall stress due to ventricular volume or pressure loading and as such have proved useful as a diagnostic and prognostic biomarker of heart failure in adults.¹⁴⁵⁻¹⁴⁹ They may also have potential as biomarkers of cardiac dysfunction in preterm infants, however, targeted research in this population is needed due to the developmental differences in the preterm heart and the unique pathophysiology experienced after premature birth.

It has been suggested that the characteristics of an optimal cardiac biomarker are that they should be completely cardiac specific, well characterised, easy to measure accurately, stable when stored, not usually found in the normal circulation in high concentrations and for which a clinical role has been clearly defined.³¹⁴ Many of these questions remain unanswered for NTproBNP in the preterm population which has hindered use in this clinical setting. Reference levels, particularly beyond the period of transitional circulation, have not been well characterised in premature infants.^{53, 172, 176}

In-vivo factors that may affect NTproBNP levels in premature infants

There are many reasons why levels of NTproBNP in premature infants may be different from those in adults or indeed term-born infants. BNP is part of the foetal cardiac gene programme and high levels are known to be present in healthy foetuses early in gestation.¹⁶⁶ Levels subsequently decline prior to birth then rise again with transitional circulation before declining to steady state levels. However, cardiac maturation is not yet complete when premature infants are born and have to complete the transition from foetal to neonatal circulation.

In addition, clearance is also likely to be different due to the reduced renal function in prematurely born infants. More than 60% of nephrogenesis occurs in the final trimester of pregnancy and even in term infants glomerular filtration rate is reduced secondary to low perfusion pressure, increased tone of the efferent arteriole and relatively high blood viscosity.³¹⁵ Premature infants also face multiple potential insults to kidney function during the course of their hospital admission.

The biological variability (or relative change value) of NTproBNP levels is currently accepted to be 25% in adults with stable heart failure, meaning that a 25% change in NTproBNP levels is likely to represent a clinically significant change (either deterioration or improvement) in the patient's physiological state.¹⁹⁶ The accepted relative change value for healthy adults is higher at approximately 50% reflecting lower levels, meaning that smaller absolute changes in peptide levels produce a greater percentage change. The degree of normal variability of NTproBNP in preterm neonates has not been established.

We know that NTproBNP levels are higher in preterm infants compared to term infants. However, this does not appear to be related to gestational age per se but rather secondary to conditions that are more prevalent in premature infants.³¹⁶ Levels are elevated in infants with significant intrauterine growth restriction.¹⁷⁰ There is some evidence that levels may be predictive of persistence of a haemodynamically significant PDA, however, clinicians have been unable to adopt this biomarker into clinical practice due to inconsistencies in trial design, lack of consensus regarding the definition of a clinically significant PDA and variable predictive thresholds.^{176, 180, 317}

Other conditions that are specific to the preterm population and may affect levels have not been fully investigated. Some studies have suggested that BNP and NTproBNP levels are higher in infants who develop BPD, however, little is known about the effects of ventilation on these levels.^{185, 186, 318} Pulmonary hypertension may complicate BPD and cause right ventricular strain,

which could be a stimulus for BNP synthesis. Small, mostly retrospective studies of highly selected populations have suggested BNP or NTproBNP levels may be predictive of pulmonary hypertension and mortality.¹⁸⁷⁻¹⁸⁹ To date, no neonatal studies have examined the effect of renal function in premature infants on levels of NTproBNP. As hypoxia may stimulate the release of BNP via hypoxia-inducible factor the relationship between exposure to hypoxia and NTproBNP levels warrants examination.³¹⁹ Due to similarities in the BNP gene promotor and that of the globin gene it is plausible there may be a relationship between haemoglobin and NTproBNP levels.³²⁰ This is relevant as premature infants are at increased risk of anaemia and are transitioning from foetal to adult haemoglobin. The relationship between BNP/NTproBNP and the inflammatory cascade is not yet fully understood but there also appears to be a relationship between significant inflammation and BNP/NTproBNP levels.³²¹

Data regarding post-translational processing of BNP are now emerging with evidence of a number of other circulating forms of BNP in adults. There are currently no data on BNP isoforms in the preterm population and this is investigated further in the next chapter.

In-vitro factors that may affect NTproBNP levels in premature infants

In addition to in-vivo factors, in-vitro laboratory factors may affect NTproBNP levels. Assay manufacturers product information states that samples that are collected in EDTA are stable for 3 days at room temperature or longer at 4 °C. Levels are unaffected by haemolysis (haemoglobin <0.621 mmol/L or <1.0 g/dL), lipaemia (triglycerides <17.1 mmol/L), hyperbilirubinaemia (bilirubin <428 µmol/L), or common pharmaceuticals. If, however, the patient is on high-dose biotin therapy, sampling should be delayed at least 8 hours after the last dose as this interferes with the assay.

Gaps in current knowledge

The range and temporal profile of NTproBNP levels from transition to neonatal discharge are not well established. Although the cardiac effects of a large patent ductus arteriosus and renal impairment are known to increase NTproBNP levels the effects of gestational age, chronological age, mechanical ventilation, hypoxia, haemoglobin, BPD and late pulmonary hypertension are not clear. There are also no published data on relative forms of circulating BNP molecules in the preterm population.

Hypotheses

1. NTproBNP levels will change over time in very preterm infants and will be higher in infants with:

- haemodynamically significant patent ductus arteriosus
- bronchopulmonary dysplasia
- pulmonary hypertension

2. NTproBNP will not vary significantly with gestational age at birth, hypoxia, mechanical ventilation or haemoglobin levels but will be higher in the context of renal impairment

Aims

1. To determine the range and temporal profile of NTproBNP levels in infants born at <30 weeks gestation at day of life 3, 10, 28 and at 36 weeks PMA
2. To determine the utility of NTproBNP levels to diagnose haemodynamically significant PDA, diagnosed by pre-specified ultrasound criteria
3. To determine the utility of NTproBNP levels to diagnose bronchopulmonary dysplasia
4. To determine the utility of NTproBNP levels to diagnose pulmonary hypertension on the basis of pre-specified conventional heart ultrasound criteria
5. To investigate whether NTproBNP levels are affected by gestational age, ventilation, hypoxia, renal impairment or haemoglobin level

Methods

Participants and setting

This trial was conducted at the Christchurch Women's Hospital NICU, Christchurch, New Zealand.

Infants born at < 30 weeks gestation between March 2013 and April 2015 and cared for in Christchurch Neonatal Intensive Care Unit were eligible for enrolment by 72 hours of age. Infants were excluded if they had known structural airway or lung anomalies, congenital anomalies of the pulmonary arteries or pulmonary veins, major systemic vessel-to-pulmonary artery collateral vessels, congenital heart disease (except those with patent ductus arteriosus,

patent foramen ovale or secundum atrial septal defect < 5mm or tiny restrictive muscular ventricular septal defect), severe liver disease, or persistent pulmonary hypertension of the newborn on day 3 heart ultrasound (defined as severe hypoxia associated with estimated main pulmonary artery pressure >25mmHg). If infants had a grave prognosis such that they were not expected to survive to day 3 they were also excluded.

Testing

NTproBNP testing was paired with clinician-performed heart ultrasound assessment and three days of oxygen saturation monitoring as described in detail in methods chapter.

NTproBNP testing

Sample collection and storage

Serial NTproBNP levels were analysed from a minimum of 0.6ml plasma collected from capillary, arterial or venous blood. Sample collection occurred on days of life 3, 10, 28 and at 36 weeks PMA. Samples were initially stored at 4°C, then centrifuged at 3000rpm with plasma subsequently frozen at a minimum of -80°C prior to being batch processed.

Assay

NTproBNP levels were determined using a Roche E411 Cobas analyser which uses the Elecsys/Cobas ProBNP II assay. This assay contains two monoclonal antibodies which recognise epitopes located in the N-terminal part (1-76) of proBNP. The Cobas e411 electrochemiluminescence immunoassay (ECLIA) utilises a “sandwich” technique using a monoclonal capture antibody to amino acids 27-31 and a monoclonal detection antibody to amino acids 42-46.

Pulse oximetry

Oxygen saturations were recorded, as detailed in methods chapter, for approximately 72 hours prior to NTproBNP testing using a Masimo Radical 7 Signal Extraction CO-oximeter (Masimo Corporation, Irvine, California) with averaging time set to 2 seconds.

Heart Ultrasound

Heart ultrasound was performed by neonatologists, as detailed in methods chapter, on days 3, 10, 28 and 36 weeks PMA using a Toshiba Aplio 500 scanner with neonatal cardiac presets and a 6.5MHz probe.

Haemoglobin

Haemoglobin levels were not taken as part of this study so the closest levels taken to NTproBNP testing as part of routine clinical care were used in analysis.

Definitions

BPD was defined as persistent oxygen or respiratory support requirement at 36 weeks PMA (definition utilised by the Australian and New Zealand Neonatal Network in 2013) or graded according to the National Institute of Child Health and Human Development criteria as detailed in methods chapter.²⁸⁷ **Pulmonary hypertension** was defined according to the heart ultrasound criteria outlined in Table 4 in the methods section.^{81, 199} **HsPDA** was defined according to commonly accepted ultrasound criteria detailed in methods chapter.¹⁸⁰

Hypoxia

The percentage time spent with oxygen saturations less than 88% in the 3 days preceding NTproBNP testing was used as a marker of exposure to hypoxia.

Statistical analysis

Quantitative variables are described by mean and standard deviation (SD) or median and interquartile range (IQR). Box and whisker plots were used to display the distribution of NTproBNP levels at days 3, 10, 26 and 36 weeks PMA. As the distribution was skewed a natural log transformation was applied.

For bivariate analyses parametric tests were used for normally distributed data and non-parametric tests for non-normally distributed data. Regression models (linear regression for continuous outcomes, logistic regression for dichotomous outcomes) were fitted to the data to examine the nature of the association and the strength of the association were summarised by the R-squared statistic. These models were extended to control for relevant demographic, perinatal or clinical factors. A p value of < 0.05 was considered statistically significant.

Receiver Operating Characteristic curve analysis²⁹⁶ was conducted to examine the extent to which it is possible to distinguish at an early age between those infants who do/do not have a HsPDA and those who go on to develop BPD, by day 3 and day 10 NTproBNP. A predictive cut-off value was chosen to maximise sensitivity and specificity. The relationship between NTproBNP and BPD was analysed using both the dichotomous definition of BPD (need for oxygen or respiratory support at 36 weeks PMA) and the NICHD graded definition.²⁸⁷ As death before 36 weeks PMA precludes a diagnosis of BPD it was included in the dichotomous analysis.

For the analysis by BPD severity the infants with the two deaths before 36 weeks PMA were excluded as they could not be classified. Data was stored using Microsoft Excel and statistical analysis was performed using Microsoft Excel and either Stata 15, SAS 9.4 or GraphPad InStat Software.

Results

NTproBNP levels over time

Mean (SD) and median (IQR) NTproBNP levels at the four time intervals are shown in Table 15. Data are non-normally distributed and particularly skewed on days 3 and 10. A repeated measures analysis of variance using log transformation was performed to stabilise variance. There was a significant fall in NTproBNP over time ($p < 0.001$). Fig. 28 shows distribution of NTproBNP values over time using a logarithmic scale to facilitate data display. Initially there is a wide distribution of NTproBNP levels most noticeable on days 3 and 10. By day 28 most infants fall within a narrower range of levels.

Table 15: Mean (SD) and Median (IQR) NTproBNP (pmol/L) over time

	Day 3 n=50	Day 10 n=51	Day 28 n=51	36 weeks PMA n=48
Mean (SD)	1010 (1134)	607 (1030)	152 (296)	131 (129)
Median (IQR)	280 (148 – 1785)	149 (83 – 590)	73 (39 – 134)	97 (42 – 171)

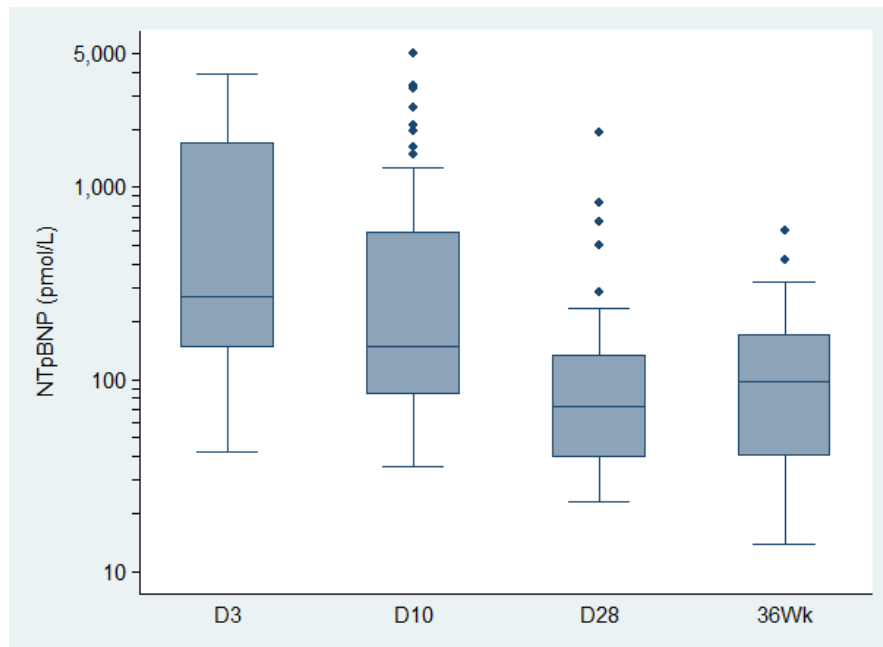


Figure 28: Distribution of log scale NTproBNP values over time.

NTproBNP by PDA Status

To explore what factors might be contributing to the wide distribution in NTproBNP levels on days 3 and 10 we examined the effect of HsPDA. Baseline characteristics of those infants with and without HsPDA on day 3 are documented in the methods chapter. The change in PDA status over time is detailed in Fig. 27 in the baseline demographics and in-hospital outcomes chapter.

Table 16 compares mean NTproBNP levels over time by day 3 HsPDA status. Significantly higher mean NTproBNP levels were seen on day 3 and day 10 in infants who had HsPDA identified on

day 3. By day 28 levels were not significantly different but by 36 weeks PMA the relationship had reversed with infants who had not had HsPDA on day 3 having significantly higher mean NTproBNP levels.

Table 16: Mean (SD) NTproBNP levels (pmol/L) over time by Day3 HsPDA status

NTproBNP	No D3HsPDA (n=26)	D3HsPDA (n=25)	p ¹
Day 3			
Median	147	1785	<0.001
IQR	89 – 211	1102 – 2608	
Day 10			
Median	91	590	<0.001
IQR	52 – 141	189 – 1622	
Day 28			
Median	75	56	0.21
IQR	39 – 95	37 – 185	
36 weeks PMA			
Median	140	68	0.01
IQR	78 – 194	30 – 115	

¹ t-test for independent samples using log-transformation to stabilise variance. Repeated measures analysis of variance for NTproBNP by HsPDA day 3: main effect of HsPDA $F(1,45)=25.4$ $p<0.001$; main effect of time $F(3,135)=47.0$ $p<0.001$; HsPDA x time interaction $F(3,135)=34.2$ $p<0.001$

Figure 29 compares the distribution of NTproBNP (log scale) over time between those who had HsPDA on day 3 and those who had a closed or haemodynamically non-significant PDA. This demonstrates a general decline from day 3 to day 28 which is steeper in those with HsPDA as they start at a higher level. There is an overlap in the range of levels on days 10, 28 and 36 weeks PMA. Levels appear to rise between day 28 and 36 weeks PMA in those without HsPDA. The median (IQR) day 3 NTproBNP of the infants who had day 3 HsPDA but no treatment and who subsequently had a closed or non-significant duct on day 10 was 931pmol/L (378-1395) compared to 1844pmol/L (1419-2433) in those infants with day 3 HsPDA and no treatment and a persistent HsPDA on day 10. The median (IQR) day 3 NTproBNP of the infants who had a closed or non-significant duct by day 10 was 192pmol/L (122-372) compared to 2272 (1435-3002) in those with HsPDA on day 10.

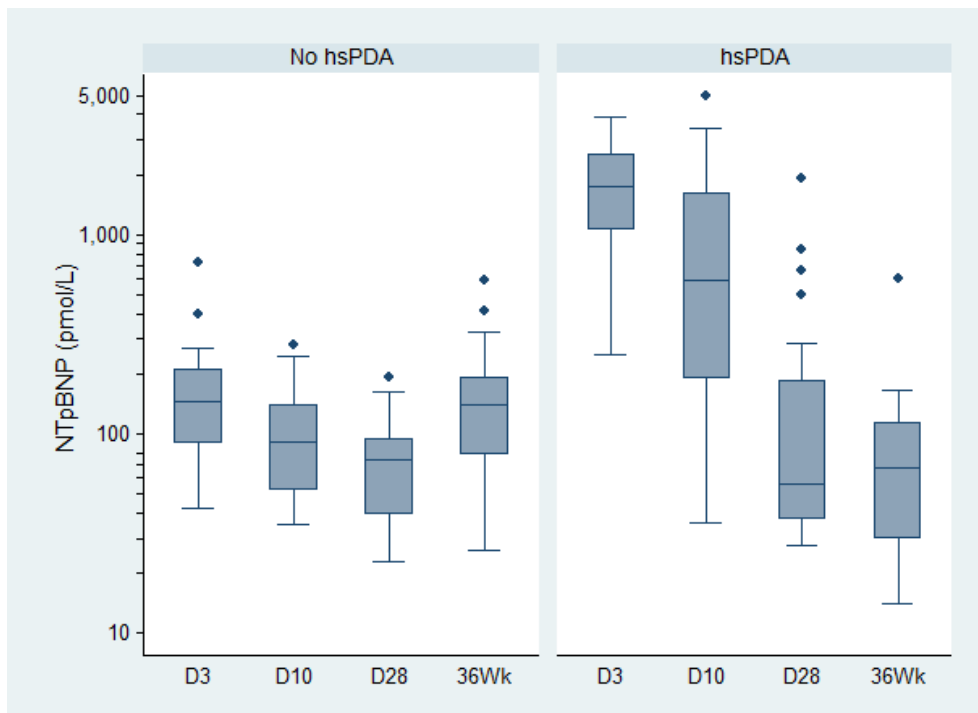


Figure 29: Distribution of NTproBNP values (log scale) over time by day 3 HsPDA status

Receiver Operating Curves (ROC) for the prediction of HsPDA on day 3 and day 10 by day 3 NTproBNP level are shown in Fig.30. A chosen cut-off day 3 NTproBNP value of ≥ 287 pmol/L for the prediction of haemodynamically significant PDA on day 3 correctly classified 92% with a sensitivity of 92% and a specificity of 92%. A chosen cut-off NTproBNP value of ≥ 1010 pmol/L on day 3 for the prediction of a hemodynamically

significant PDA on day 10 correctly classified 88% with a sensitivity of 94% and a specificity of 85%.

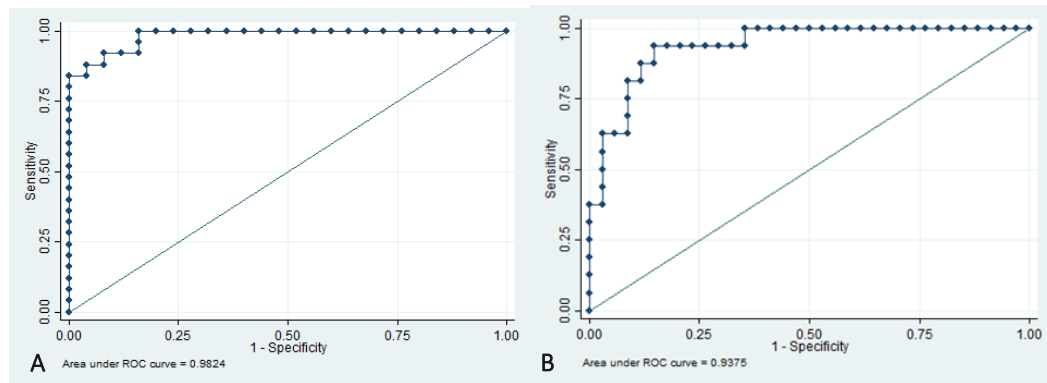


Figure 30: ROC curve for prediction of HsPDA

- A) On day 3 from day 3 NTproBNP
(SE 0.0127, 95% CI 0.96, 1.00)
- B) On day 10 from day 3 NTproBNP
(SE 0.0327, 95% CI 0.87, 1.00)

Table 17 reveals a small but statistically significant difference in mean (SE) \log_{10} NTproBNP levels at 36 weeks PMA by day 3 HsPDA status which persists after adjustment for gestation and birthweight z-score.

Table 17: Mean (SE) \log_{10} NTproBNP levels at 36 Weeks PMA by Day 3 HsPDA status unadjusted and adjusted for gestation, birthweight z-score

Measure	No HsPDA (n=25)	HsPDA (n=23)	r/ β	p
Unadjusted	2.09 (0.07)	1.79 (0.09)	-0.38	0.01
Adjusted	2.08 (0.08)	1.80 (0.08)	-0.35	0.02

Relative effect of gestational age, HsPDA, ventilation and creatinine level on NTproBNP levels

A fitted regression model (Table 18) was used to examine the relative contributions of gestation at birth, HsPDA status, ventilation on days 3 and 10 and day 3 creatinine level (as a surrogate for renal function) on day 3 and day 10 NTproBNP levels. The fitted model assumes that: (1) gestation is causally related to PDA status and need for

ventilation; (2) PDA status and ventilation are correlated over and above the causal effects of gestation; (3) all three variables are predictive of NTproBNP. This model demonstrates that although there is a negative correlation between gestation at birth and NTproBNP levels this is mediated predominantly via the effect of PDA status, but also ventilation and to some degree early renal function.

Table 18: Fitted regression models predicting NTproBNP levels on days 3 and 10

Predictor	β (SE)	p	β
Day 3 NTproBNP			
Gestation (wk)	-116.6 (80.4)	0.15	-0.15
HsPDA Day 3	1598.7 (218.6)	<0.0001	0.69
Ventilated Day 3	458.7 (319.1)	0.16	0.15
Creatinine Day 3 (umol/L)	-6.9 (8.9)	0.44	-0.08
	Adjusted R ² = 0.56		
Day 10 NTproBNP			
Gestation (wk)	-28.2 (65.4)	0.67	-0.04
HsPDA Day 10	1295.1 (187.6)	<0.0001	0.59
Ventilated Day 10	1483.1 (396.2)	<0.001	0.34
Creatinine Day 3 (umol/L)	20.4 (6.9)	0.005	0.26
	Adjusted R ² = 0.66		

NTproBNP and bronchopulmonary dysplasia

Table 19 shows higher NTproBNP values on days 3, 10, 28 in infants with BPD/death (ANZNN BPD definition) but lower levels at 36 weeks PMA. However, repeated measures ANOVA analysis demonstrated there was no significant overall effect of group status or group by time interaction, but there was a highly significant effect of time that was similar in both groups. Fig. 31 represents this using a box and whisker plot on a log scale. There is considerable overlap in values at all time periods.

Table 19: NTproBNP levels over time (pmol/L) by classification of BPD (ANZNN) or death

NTproBNP	No BPD (N=18)	BPD or Death (N=33)	p ¹
Day 3			
Median	240	1010	0.31
IQR	147 – 725	150 – 1976	
Day 10			
Median	97	225	0.09
IQR	88 – 185	83 – 910	
Day 28			
Median	57	83	0.13
IQR	40 – 79	39 – 156	
36 weeks PMA			
Median	156	78	0.07
IQR	78 – 190	34 – 140	

¹ t-test for independent samples using log-transformation to stabilise variance. Repeated measures ANOVA: Effect of Group (BPD or Death) $F(1,45)=1.37$, $p=0.25$; Effect of Time $F(3,135)=28.5$, $p<0.001$; Group x Time interaction $F(3,135)=3.0$, $p=0.03$

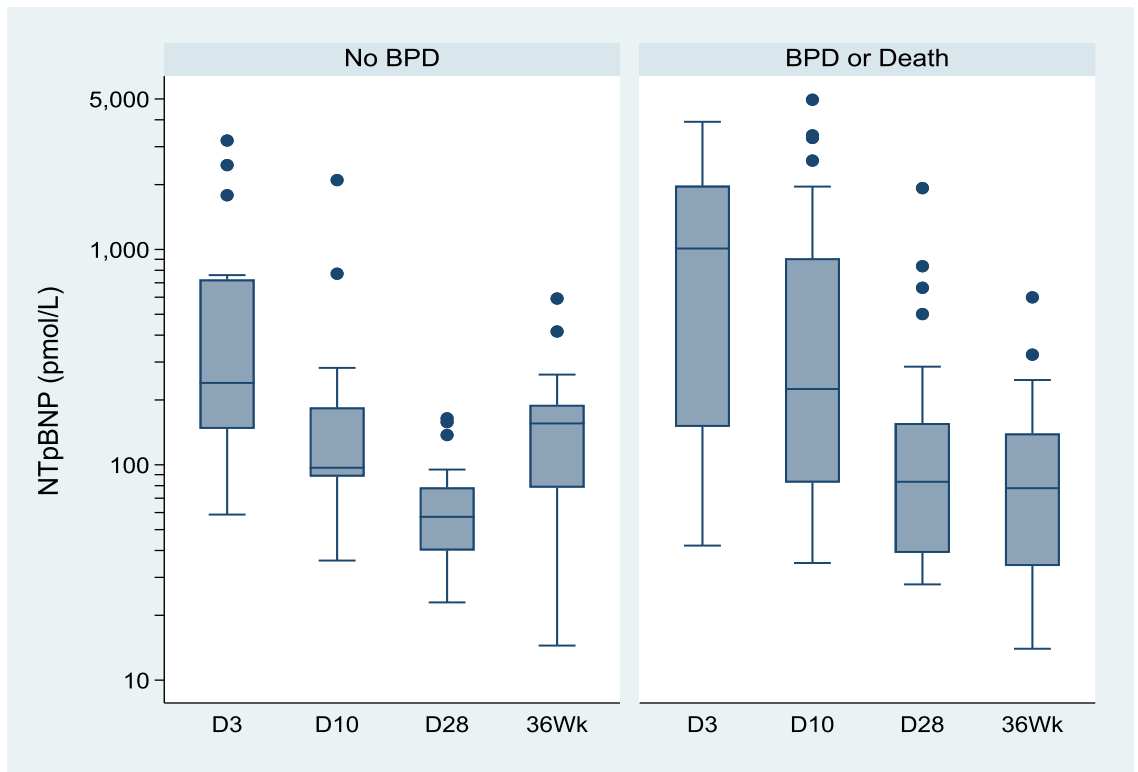


Figure 31: Distribution of NTproBNP values over time (log scale) by BPD status (ANZNN definition)

Table 20 shows the differences in NTproBNP levels by NICHD grade of severity of BPD. Fig. 32 shows log scale NTproBNP level over time according to NICHD BPD grade with those with severe BPD showed greater variability in levels and higher levels on day 3 and day 10 compared to those with no BPD. However, by 36 weeks PMA those with severe BPD had lower levels than those with no BPD. Mean NTproBNP levels varied significantly with BPD severity, time and severity by time.

Table 20: Mean (SD), median and IQR of NTproBNP levels (pmol/L) by NICHD scale severity of BPD

Measure	No BPD (N=27)	Mild/Moderate BPD (N=9)	Severe BPD (N=13)	p ¹
Day 3 Median (IQR)	244 (126 – 725)	184 (81 – 1010)	1443 (1102 – 2935)	<0.005
Day 10 Median (IQR)	95 (63 – 235)	117 (47 – 163)	910 (225 – 1958)	<0.001
Day 28 Median (IQR)	73 (40 – 109)	46 (39 – 61)	156 (34 – 501)	0.01
36 weeks PMAMedian (IQR)	112 (68 – 184)	89 (43 – 159)	72 (34 – 111)	0.30

¹ One way analysis of variance using log transformation to stabilise variance. Repeated measures analysis of variance for NTproBNP by NICHD severity: main effect of NICHD severity $F(2,44)=8.6$, $p<0.001$; main effect of time $F(3,132)=31.8$, $p<0.001$, NICHD severity x time interaction $F(6,132)=4.8$, $p<0.001$

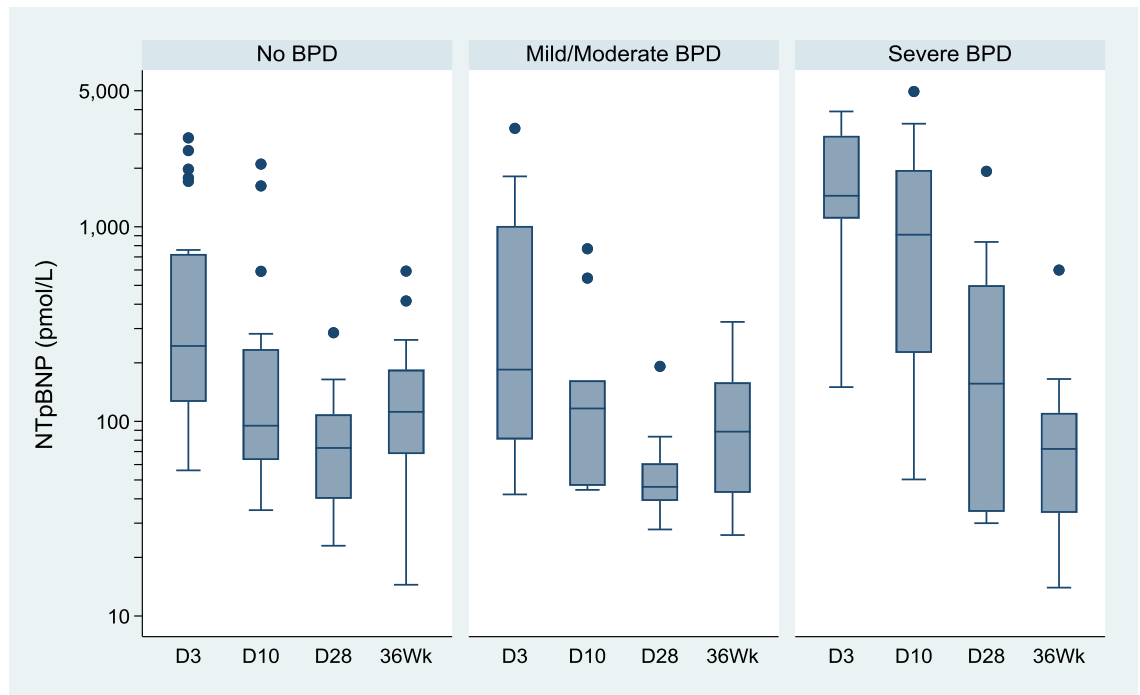


Figure 32: Distribution of NTproBNP levels over time (log scale) by BPD grade (NICHD definition)

Table 21 compares area under the curve (95% CI) for prediction of BPD status for varying definitions of BPD from NTproBNP levels at different time periods. Receiver operating curves for the prediction of severe BPD (NICHD) on the basis of day 3 and day 10 NTproBNP are shown in Fig. 33. The area under the curve for both day 3 and day 10 is modest at 0.80 (SE 0.07, 95% CI 0.66-0.94) and 0.83 (SE 0.07, 95% CI 0.69- 0.98) respectively. With a chosen cut-off value of ≥ 1102 pmol/L this analysis shows day 3 NTproBNP has a sensitivity of 76% and a specificity of 80% and correctly classifies 79% of infants with severe BPD. With a chosen cut-off value of ≥ 189.2 pmol/L. This analysis shows day 10 NTproBNP has a sensitivity of 84% and a specificity of 75% correctly classifying 77% of infants with severe BPD.

Table 21: Area under the curve (95% CI) for prediction of BPD status from NTproBNP level (Day 3, 10, 28) for varying definitions of BPD

	Definition of BPD			
NTproBNP	ANZNN BPD	NICHD Any BPD	NICHD Mod/Severe BPD	NICHD Severe BPD
Day 3	0.60 (0.44 – 0.77)	0.65 (0.49 – 0.81)	0.63 (0.45 – 0.80)	0.80 (0.66 – 0.94)
Day 10	0.63 (0.47 – 0.79)	0.69 (0.54 – 0.86)	0.70 (0.53 – 0.87)	0.83 (0.69 – 0.98)
Day 28	0.59 (0.43 – 0.75)	0.53 (0.35 – 0.71)	0.62 (0.43 – 0.81)	0.67 (0.45 – 0.89)

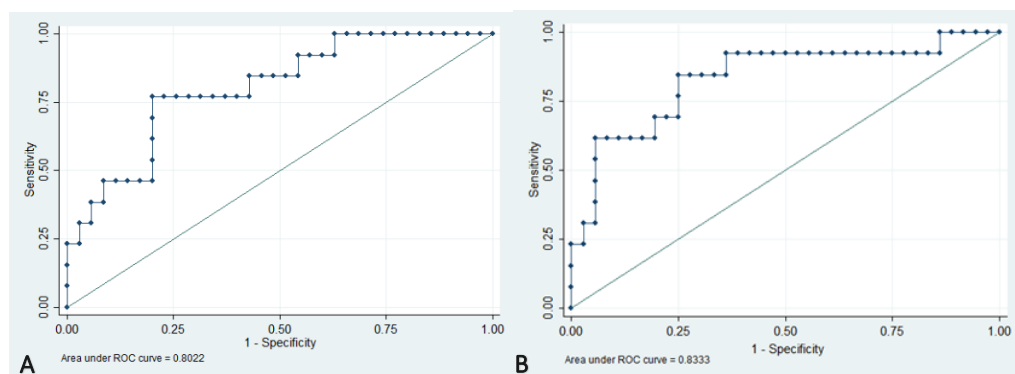


Figure 33: ROC curve for severe BPD (NICHD definition)

A) By day 3 NTproBNP (AUC 0.8022)

B) By day 10 NTproBNP (AUC 0.8333)

No significant difference in mean (SE) \log_{10} NTproBNP level across all time periods was found for infants classified as no BPD compared to those with BPD or death by 36 weeks PMA (Table 22). Mean (SE) \log_{10} NTproBNP levels on days 3, 10 and 28 did vary significantly by NICHD scale severity of BPD (Table 23). However, this was no longer significant once adjusted for gestation, birthweight z-score and day 3 PDA status. Adjusting for the total duration of ventilation made no difference.

Table 22: Mean (SE) log₁₀ NTproBNP levels by BPD status unadjusted and adjusted for gestation, birthweight z-score and day 3 HsPDA status

NTproBNP	No BPD (N=18)	BPD or Death (N=33)	p
Day 3			
Unadjusted	2.50 (0.12)	2.71 (0.11)	0.22
Adjusted	2.69 (0.08)	2.64 (0.06)	0.67
Day 10			
Unadjusted	2.11 (0.10)	2.45 (0.11)	0.05
Adjusted	2.29 (0.11)	2.35 (0.08)	0.68
Day 28			
Unadjusted	1.79 (0.06)	1.97 (0.08)	0.14
Adjusted	1.82 (0.10)	1.95 (0.07)	0.35
36 weeks PMA			
Unadjusted	2.08 (0.10)	1.87 (0.07)	0.08
Adjusted	2.03 (0.10)	1.90 (0.07)	0.30

Table 23: Mean (SE) log₁₀ NTproBNP levels by NICHD grade of BPD unadjusted and adjusted for gestation, birthweight z-score and day3 HsPDA status

Measure	No BPD (N=27)	Mild/Moderate BPD (N=9)	Severe BPD (N=13)	p
Day 3				
Unadjusted	2.48 (0.10)	2.43 (0.23)	3.06 (0.14)	0.007
Adjusted	2.63 (0.07)	2.55 (0.11)	2.70 (0.11)	0.66
Day 10				
Unadjusted	2.10 (0.09)	2.12 (0.16)	2.35 (0.17)	<0.001
Adjusted	2.22 (0.09)	2.17 (0.15)	2.57 (0.15)	0.14
Day 28				
Unadjusted	1.84 (0.05)	1.72 (0.09)	2.19 (0.17)	0.02
Adjusted	1.85 (0.09)	1.72 (0.13)	2.17 (0.13)	0.07
36 weeks PMA				
Unadjusted	2.02 (0.07)	1.95 (0.14)	1.81 (0.12)	0.31
Adjusted	1.95 (0.08)	1.92 (0.13)	1.96 (0.13)	0.96

Effect of late pulmonary hypertension on NTproBNP level

None of our cohort met the predefined criteria for pulmonary hypertension at the 36 week scan so no analysis could be undertaken to determine effects of this condition on NTproBNP levels.

Effect of haemoglobin on NTproBNP level

The mean number of days between haemoglobin testing and NTproBNP testing was 0.3. There was evidence of a significant ($p < .001$) negative bivariate correlation between haemoglobin and NTproBNP on days 3 and 10 but not at later ages (Spearman

correlation was -0.50 on day 3, -0.45 on day 10). However, haemoglobin was not a significant predictor of NTproBNP levels at these ages when other factors associated with NTproBNP levels were taken into account, notably HsPDA status.

Effect of hypoxia

The percentage time spent with oxygen saturations less than 88% in the 3 days preceding NTproBNP testing was used as a marker of exposure to hypoxia. The percentage time oxygen saturations were less than 88% showed a similar but less consistent pattern of association with NTproBNP levels to haemoglobin. Spearman correlation at day 3 (0.21, $p=.14$) and day 10 (0.52, $p<.001$), weaker and non-significant associations at later ages. In summary, exposure to hypoxia defined as percentage time less than 88% was not an independent predictor of NTproBNP when other factors, in particular PDA status, were taken into account.

Effect of chronological age on 36 week NTproBNP

Although the day 3, day 10 and day 28 NTproBNP levels were all taken at the same chronological age this was not the case for the 36 week PMA samples which varied according to gestation at birth. The median (IQR) NTproBNP level at this time point was 97 pmol/L (40-168) with 90% of the cohort having levels <250pmol/L. Fig. 34 shows that there is no relationship between NTproBNP levels at 36 weeks PMA and chronological age. This may have been influenced by four outliers (only one of whom had PDA persisting at 36 weeks and BPD, the remaining three had neither HsPDA or BPD). If these outliers are excluded there may be slight inverse relationship between chronological age and NTproBNP level which is consistent with the known gradual decline over childhood to puberty.¹⁷⁷

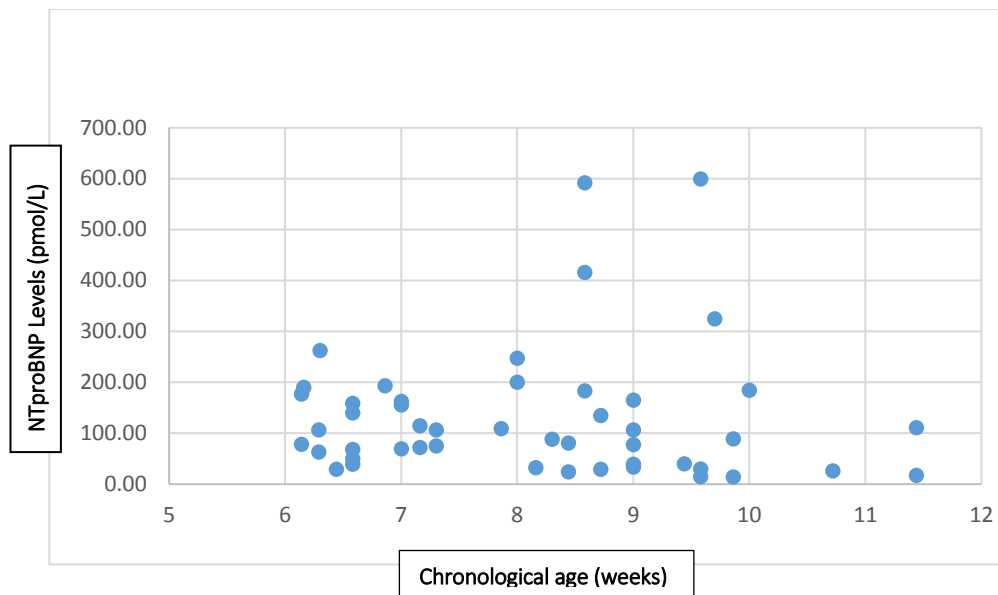


Figure 34: Relationship between NTproBNP levels at 36 weeks PMA and chronological age

Discussion

This study is the first to document temporal changes in NTproBNP in a cohort of very preterm infants and to investigate a range of potential factors that may influence levels. NTproBNP levels generally declined as has been shown in other studies.^{53, 177} The haemodynamic effect of a patent ductus arteriosus is a strong influence on levels with renal impairment also causing an increase in levels. The effect of evolving BPD appears to be modest with ventilation, exposure to hypoxia and haemoglobin not significantly influencing levels in our cohort.

Our study confirmed that gestational age has a negative correlation with early NTproBNP levels but that this is primarily mediated by PDA status. Although chronological age affected NTproBNP levels during the first month of life this again appeared to be mediated by transitional circulation and PDA status and, once a relative steady state had been reached, age alone was not a significant influence on levels. This is consistent with the very slow decline to adult levels over the first ten years shown in other studies.³²² Further study is needed on larger cohorts of premature infants from birth to beyond term equivalent age to better establish reference ranges over time for NTproBNP.

Although the overall trend for our four collection times was for NTproBNP levels to decline from a peak at day 3 to a more steady level, some infants had an increase in NTproBNP level between day 3 and day 10 (or an absence of a decline) and some babies

had an increase in NTproBNP between day 28 and 36 weeks PMA. Day 3 NTproBNP levels were significantly higher in infants with HsPDA and day 3 NTproBNP levels demonstrated high sensitivity and specificity for the prediction of HsPDA on day 3 and 10. Infants classified as having a closed or non-significant duct on day 3 reached low NTproBNP levels within the first few days of life whereas the decline was slower in those with HsPDA. Ductus arteriosus patency and increased left to right shunting appears to be the key factor influencing the wide distribution of NTproBNP levels on day 3 and day 10 and is likely to be the main reason behind any increase in levels between days 3 and 10. Any effect of gestational age in our cohort appeared to be primarily mediated by PDA status. The cause of a late rise in NTproBNP is unclear. Infants with no HsPDA on day 3 tended to have higher levels at 36 weeks PMA than those who had experienced HsPDA. This finding is unexpected, unexplained and warrants further investigation.

In this study only a third of infants classified as having HsPDA on day 3 were treated by clinicians. There remains considerable debate around the criteria for a clinically significant PDA and despite recognised short term consequences of PDA, treatments have failed to improved long term outcomes.³²³ The low numbers of infants treated in this cohort reflects this clinical uncertainty. The decision to treat was likely influenced by other factors such as gestational age, the clinical condition of the infant including ventilation status and relative contraindications to treatment. Heart ultrasound criteria alone may be inadequate to guide PDA triage and broader scoring systems have been suggested.^{324, 325} NTproBNP has potential to be a valuable adjunct to clinical and echocardiographic triage tools and should be considered in future PDA trial design.

Levels of NTproBNP in our cohort were considerably higher than those seen in the healthy adult population age even at 36 weeks PMA. In the adult setting an NTproBNP level of <300pg/ml (35pmol/L) in dyspnoeic patients has a negative predictive value of 99% for a diagnosis of congestive heart failure with levels of >53pmol/L making heart failure highly likely.³²⁶ In our cohort, at 36 weeks PMA, 34/48(71%) of levels were >53pmol/L. Published data from Nir et al suggest a median steady state NTproBNP level in healthy infants beyond the period of transitional circulation, using a Roche assay, of 17pmol/L with the 97.5th percentile being 118pmol/L.¹⁹² The study by Montgomery et al suggested levels above this at 36 weeks PMA predict pulmonary hypertension with a sensitivity of 100% and a specificity of 94% in a high risk population.¹⁸⁹ In our cohort 18/48(37%) at 36 weeks PMA had an NTproBNP level >118pmol/L but none met our ultrasound criteria for pulmonary hypertension. This may reflect the lower pre-test

probability in our general population of premature infants or differences in criteria used to define pulmonary hypertension.

The BPD rate in this cohort by the ANZNN definition at the time was 63%. This was higher than anticipated when compared to a BPD rate of 35% for those born 24-29 weeks in ANZNN units in 2013. A diagnosis of BPD at 36 weeks PMA without a physiological test may be influenced by unit policy regarding oxygen saturation targets and individual clinician opinion.^{288, 327} It is possible our BPD group may have included babies who may not have met the criteria for BPD by a physiological challenge. For this reason the cohort was also analysed according to the NICHD scale for BPD which classifies infants into no, mild, moderate or severe grades.²⁸⁷ ANZNN now incorporate a physiological challenge to prior to a diagnosis of BPD.

Modest differences in NTproBNP between the infants with and without BPD (ANZNN) did not achieve statistical significance. This was true for comparisons between BPD (NICHD) grade when adjusted for gestational age at birth, birthweight z score and day 3 PDA status. At 36 weeks PMA regardless of BPD grade NTproBNP levels are very similar. So even in the face of severe lung disease, provided it develops gradually, the preterm heart appears to adapt well. The long term cardiac effects of prematurity are currently an active area of research with both structural and functional differences found in the hearts of adults born prematurely.^{45, 328} It is possible that there may be a long-term trade-off for some of these early adaptive mechanisms.

NTproBNP levels in our cohort did not demonstrate good ability to predict BPD although day 10 NTproBNP did have a modest ability to predict severe BPD. Sellmer et al found the risk of death or BPD increased 1.7 times for every 1 unit increase of natural log NTproBNP on day 3 which held for infants with or without PDA.¹⁸⁵ Some of our infants had an unexplained increase in levels between day 28 and 36 weeks PMA. Infants with severe BPD are at greatest risk for developing pulmonary vascular disease and pulmonary hypertension although this often takes weeks to evolve and may not be clinically apparent until after term equivalent age. Whether NTproBNP levels at 36 weeks PMA and beyond are higher in infants who go on to develop pulmonary hypertension requires further studies that incorporate heart ultrasound monitoring and follow-up beyond hospital discharge.

Strengths of our study include its prospective design, paired NTproBNP and heart ultrasounds over four time intervals with criteria for HsPDA clearly defined and heart

ultrasound analysis blinded to NTproBNP. As entry criteria were broad our study population is of interest to clinicians. However, this is a small study powered only to detect moderate to large effects. Any unplanned subset analysis may be subject to bias. The lack of an association between ventilation, hypoxia and haemoglobin may reflect cohort size, but does suggest that these variables are not dominant determinants of NTproBNP. The heart ultrasounds and analysis in this study were performed by neonatologists, not cardiac ultrasonographers or cardiologists, however this is unlikely to have significantly weakened the quality of our ultrasound data. In addition the neonatologists were not blinded to the clinical status of the infant. A standardised treatment protocol would have allowed us to better assess the ability of NTproBNP to predict PDA closure. However, this is a modest sized but well described cohort strengthened by the combination of biochemical, ultrasound and oxygen saturation data with clinical data to 2 years of age and goes some way to filling gaps in knowledge regarding NTproBNP in very prematurely born infants.

Implications for practice

We are the first to show the temporal changes in NTproBNP in a very preterm cohort from day 3 through to 36 weeks PMA. We demonstrated that NTproBNP is a highly sensitive and specific biomarker for the prediction of ultrasound-defined HsPDA. NTproBNP may help stratify infants for PDA treatment by identifying those who not only have a significant duct but are also showing signs of cardiac compromise and those less likely to achieve spontaneous closure. NTproBNP therefore has potential as an adjunct blood marker to heart ultrasound to reduce the frequency of scanning of fragile infants. Given the significant impact of the transitional circulation on natriuretic peptide release, the known natural decline in BNP over childhood and the inherent biological variability of BNP and NTproBNP it is likely serial monitoring, supported by heart ultrasound, is going to offer the greatest value to clinicians.

Early NTproBNP was a modest predictor of severe BPD in our cohort and may have utility as part of a prognostic scoring system. Most of our cohort had NTproBNP levels within a relatively narrow range at 36 week PMA compared to the first month of life. This is useful information and deserves to be repeated in a larger cohort which would help inform clinicians on a normal range of NTproBNP at a time period when screening for pulmonary hypertension in at risk infants is recommended.⁸⁶⁻⁸⁸

Understanding the usual pattern of NTproBNP levels over time, and the factors that may influence levels, as we have demonstrated, will also help clinicians monitor cardiac function over time in preterm infants. This is important to better understand the developmental origins of cardiovascular disease in adulthood.

Conclusion

This study is the first to show the temporal changes in NTproBNP levels in very preterm infants from the period of cardiac transition to 36 weeks PMA, a time at which screening for pulmonary hypertension has been proposed. We have demonstrated the key determinants of NTproBNP levels and shown NTproBNP is a strong predictor of HsPDA and a modest discriminator of severe BPD. NTproBNP may help to better stratify infants with HsPDA in need of treatment by identifying those infants who not only have a large duct but are also have evidence of cardiac compromise.

BNP Isoforms

Background

It is now understood that post-translational processing of proBNP, NTproBNP and BNP results in a number of other circulating natriuretic peptide derived molecules, which can complicate the interpretation of assays.³²⁹ Validated commercial assays for the major circulating congeners BNP and NT-proBNP are available and widely used. Although our understanding of the post-translational processing of BNP and NTproBNP and the potential impact on bioactivity and assay performance in adults is emerging there are currently no published data in premature infants.

Studies of adults with chronic heart failure have shown significant variation in the circulating forms of the B-type Natriuretic Peptides.^{330, 331} Unprocessed proBNP is often present in high amounts in the circulation of adults with heart failure.^{332, 333} In adult patients with advanced heart failure proBNP is the predominant circulating form, and has greatly reduced biological activity compared to BNP.³³⁴ Peripheral processing of proBNP appears to be important in the regulation of BNP activity.³³⁵ As currently available commercial assays do not distinguish between proBNP and BNP, patients with chronic heart failure may in fact have an effective deficiency of BNP activity despite assay results suggesting high levels of circulating peptide. It is not clear whether this paradoxical relative abundance of proBNP is due to increased production of proBNP primarily, or to increased clearance of 1-32 BNP or impaired processing of proBNP, although all three mechanisms are relevant.

Processing of BNP congeners within the cardiomyocyte and circulation is detailed in Fig.35. The BNP gene is activated predominantly by ventricular wall stress but also by local myocardial ischaemia, cytokines, angiotensin II and endothelin-1. This activation stimulates translation of messenger RNA in the endoplasmic reticulum of cardiac ventricular myocytes to the 143 aa preproBNP. Cleavage of the proBNP (108 aa) molecule from its signal peptide occurs in the Golgi apparatus. Glycosylation of ProBNP may occur at this point. ProBNP is then cleaved into the 1-76 aa residue NTproBNP and the biologically active 77-108 aa BNP. Although then released in a 1:1 ratio, plasma level plasma levels of NTproBNP are several-fold higher than BNP and this is thought primarily to be due to differences in clearance – with NT-proBNP clearance primary being renal,

while BNP also undergoes clearance via the NPR-C receptor as well as enzymatic degradation by neutral endopeptidase.¹³⁴ Processing of proBNP is predominantly thought to occur at the moment it is released into the circulation via the actions of two convertase enzymes, furin and corin.¹⁹⁵ The action of furin produces BNP 1-32 and corin BNP 4-32. **Glycosylation** of proteins after translation is common and potentially serves multiple functions. ProBNP processing by corin and furin appears to be inhibited by the presence of **glycosylation of proBNP** at Thr71.³³⁶ Circulating proBNP can be found in a form that is glycosylated at Thr71 (and therefore not available for convertase processing) and a form that is not glycosylated at Thr71 (and therefore available for convertase processing). It is not clear why both forms of proBNP are released into the circulation. However, the non-glycosylated form suggests that some processing within the plasma may occur providing a circulating reserve of BNP.¹⁹⁵

In addition, **glycosylation of NTproBNP** can occur at a number of threonine and serine residues within the N-terminal region. Seferian et al identified glycosylation sites in the central region (aa residue 28-56) of NTproBNP.³³⁷ Significant inter-individual variability in glycosylation of proBNP and NTproBNP has been found and patients with chronic heart failure appear to have a greater degree of glycosylation when compared to patients with acute heart failure.^{331, 337, 338} Further processing of NTproBNP has also been demonstrated. **Fragments** which are smaller than the 76 aa NTproBNP molecule have been detected in the circulation of adults with heart failure demonstrating that NTproBNP undergoes extensive N- and C-terminal proteolytic processing.^{330 329} Assays should therefore avoid simultaneously targeting the extreme N and C-terminals and the central region of O-glycosylation in order to detect all NTproBNP in the sample.

The Roche Elecsys ProBNP II assay has been shown to detect non-glycosylated proBNP (1-108) and non-glycosylated NTproBNP (1-76) but not glycosylated proBNP or glycosylated NTproBNP.³³⁹ In addition, NTproBNP assays may not only detect free NTproBNP (1-76) but also proBNP (1-108) which has NTproBNP still attached. However, evidence to date suggests the major commercial NTproBNP assay, the Roche Cobas e411 or Elecsys, detects only NTproBNP.³⁴⁰

In premature infants BNP and NTproBNP have been shown to be elevated during transitional circulation but little is known about what isoforms or fragments of either molecule predominate during or beyond this early period of cardiac adaptation when,

due to chronic lung disease, the right ventricle, in particular, may be under chronic strain.

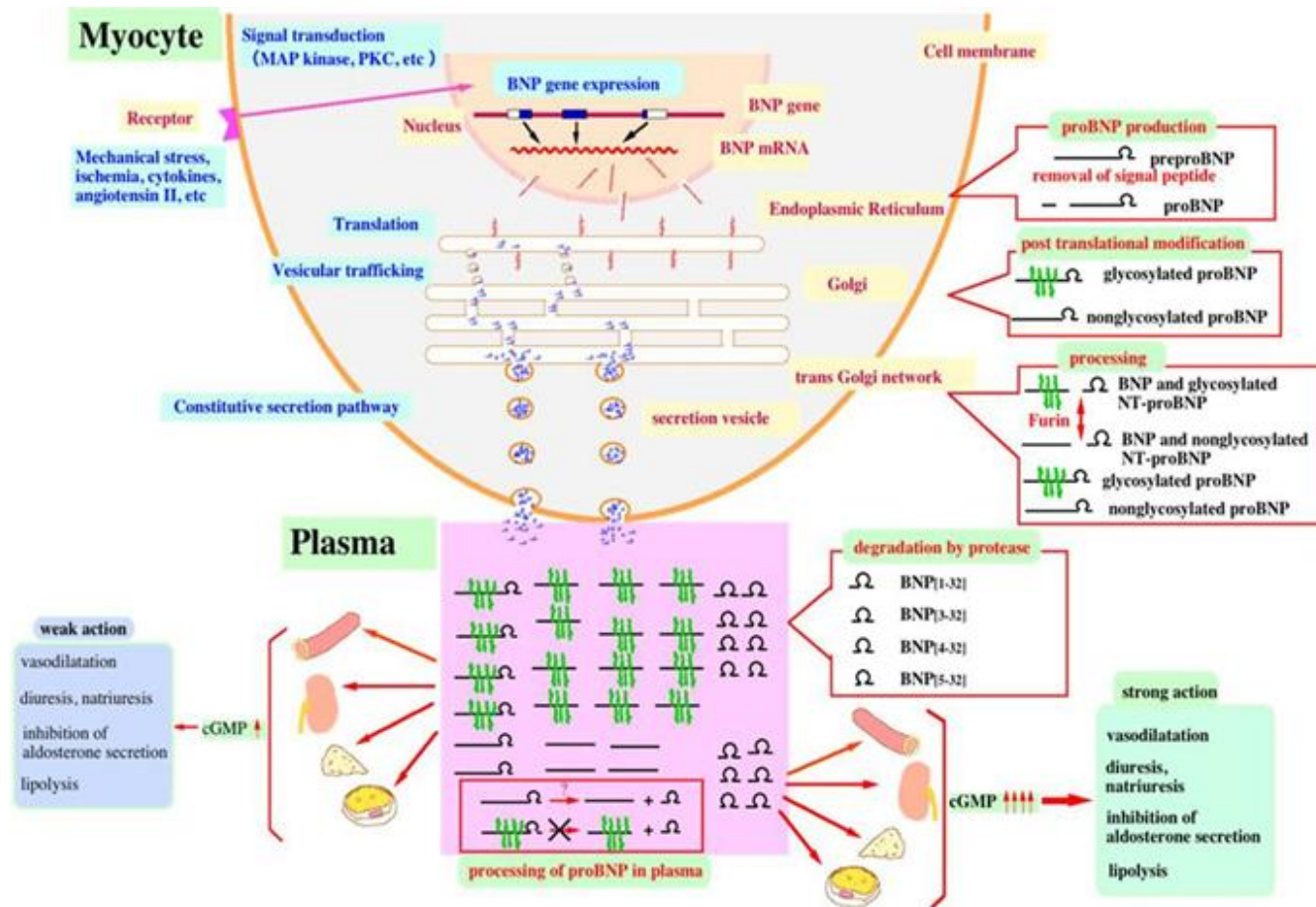


Figure 35: Schematic representation of BNP processing

PKC = protein kinase C, MAP kinase = mitogen-activated protein kinase, cGMP = cyclic guanosine monophosphate

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Gaps in current knowledge

There is currently no published data on circulating isoforms of BNP and BNP fragments in premature infants. We do not know whether there is significant glycosylation of BNP molecules in this population and whether this and relative proportions of BNP isoforms differ under different clinical conditions. As glycosylation can interfere with assay interpretation and fragments other than BNP (1-32) have weaker biological activity this is important information for clinicians when interpreting neonatal BNP results.

Hypotheses

1. There will be additional circulating forms of BNP other than BNP (1-32) and NTproBNP (1-76) in very premature infants
2. Post-translational processing of BNP and NTproBNP will differ in patients with BPD and/or HsPDA compared to those without

Aims

1. To determine the types of circulating isoforms of BNP in very preterm infants
2. To investigate whether BNP isoforms differ in premature infants with BPD and/or HsPDA and those without these conditions

Methods

Participants and setting

This trial was conducted at the Christchurch Women's Hospital NICU, Christchurch, New Zealand.

Infants born at < 30 weeks gestation between March 2013 and April 2015 and cared for in Christchurch Neonatal Intensive Care Unit were eligible for enrolment by 72 hours of age. Infants were excluded if they had known structural airway or lung anomalies, congenital anomalies of the pulmonary arteries or pulmonary veins, major systemic vessel-to-pulmonary artery collateral vessels, congenital heart disease (except those with patent ductus arteriosus, patent foramen ovale or secundum atrial septal defect < 5mm or tiny restrictive muscular ventricular septal defect), severe liver disease, or persistent pulmonary hypertension of the newborn on day 3 heart ultrasound (defined as severe hypoxia associated with estimated main pulmonary artery pressure >25mmHg). If infants

had a grave prognosis such that they were not expected to survive to day 3 they were also excluded.

Plasma volume remaining after initial NTproBNP testing was used for isoform testing. Low available plasma volumes necessitated pooling of samples from several infants and across all four testing times. Among 15 infants with adequate residual plasma, we identified sub-groups based on clinical features and performed analyses of pooled plasma from these subgroups. Table 24 details the groupings used for pooled testing and the total plasma volume available for testing. Group 1 were well babies who had low NTproBNP throughout all testing points, had no HsPDA and no BPD. Group 2 had high early NTproBNP levels, HsPDA on day 3 but no BPD. Group 3 were the sickest babies, had high early NTproBNP, HsPDA on day 3 and BPD.

Table 24: Clinical groupings for pooled plasma analysis of BNP isoforms

	HsPDA	BPD	Total pooled plasma volume (μl)
Group 1 n=4	No	No	3950
Group 2 n=3	Yes	No	4010
Group 3 n=8	Yes	Yes	6205

Definitions

BPD was defined as persistent oxygen or respiratory support requirement at 36 weeks PMA. HsPDA was defined according to ultrasound criteria detailed further in methods chapter.

NTproBNP isoform testing

Sample collection and storage

Plasma was collected from capillary, arterial or venous blood in a cohort of 51 infants born before 30 weeks gestation. Sample collection occurred on days of life 3, 10, 28 and at 36 weeks PMA. Samples were initially stored at 4°C until collection within 72 hours, then centrifuged at 3000rpm with plasma subsequently frozen at a minimum of -80°C prior to being batch processed.

Assays and HPLC

NTproBNP levels were ascertained by electro chemiluminescent sandwich enzyme-linked immunoabsorbant assay using a Roche E411 Cobas analyser. Residual plasma was then analysed for BNP isoforms and glycosylation using a combination of Luminex assay and high performance liquid chromatography as detailed in methods chapter. Details of the epitopes identified to detect isoforms of BNP are detailed in Table 25.

Table 25: Epitopes identified in BNP isoform testing

Assay	Epitopes	Detection target and cross-reactivity
BNP	24C5: epitope BNP11-22 and 50E1: epitope BNP26-32	Cross reacts with BNP and proBNP
BNP H43	50E1: epitope BNP23-32 and polyclonal Ab rabbit H43: epitope BNP1-13	Specific for BNP
NTproBNP general	15F11: epitope NTproBNP13-24 and 24E11: epitope NTproBNP67-76	Cross reacts with NTproBNP and proBNP
NTproBNP specific	15F11: epitope ntBNP13-24 and polyclonal Ab rabbit J25: epitope NTproBNP62-76	Specific for NTproBNP
proBNP	50E1: epitope BNP23-32 and 16F3: epitope NTproBNP13-20	Detects glycosylated and non-glycosylated proBNP
RSproBNP	24C5: epitope BNP11-22 and 16F3: epitope NTproBNP13-20	Detects glycosylated and non-glycosylated proBNP with ring structure closed
T71proBNP	24C5: epitope BNP11-22 and 24E11: epitope NTproBNP67-76.	Detects intact non-glycosylated proBNP

Statistical Analysis

Frequency or proportion of BNP isoforms/fragments was described graphically according to clinical group status.

Results

BNP Isoforms

Figures 36-38 show results of BNP isoform testing on pooled plasma.

Group 1 (low NTproBNP at all time points, no BPD, no HsPDA): showed a small amount of total, likely cleaved, BNP (dark circles). There was no intact BNP1-32 (red line, open circles). The green and yellow lines demonstrate that most of the immunoreactivity is

exclusively due to NT-proBNP with no proBNP at all.

Group 2 (high initial NTproBNP, HsPDA but no BPD): Similar to group 1, but lacking in the BNP form (dark circles). NTproBNP predominates with no proBNP.

Group 3 (high initial NTproBNP, HsPDA and BPD): The majority of immunoreactivity is NT-proBNP but on closer inspection the results are more complex than Group 1 and 2. The BNP assay detected both proBNP and BNP forms (approximately 50:50). The specific BNP1-32 assay also picked up a tiny amount of that peptide. Intact, non-T71 glycosylated proBNP is present (pale blue line). The T71 amino acid region is where proBNP gets cleaved by furin but in this group the proBNP is present and not cleaved. The yellow line shows that NT-proBNP predominates just like in Group 1 and 2.

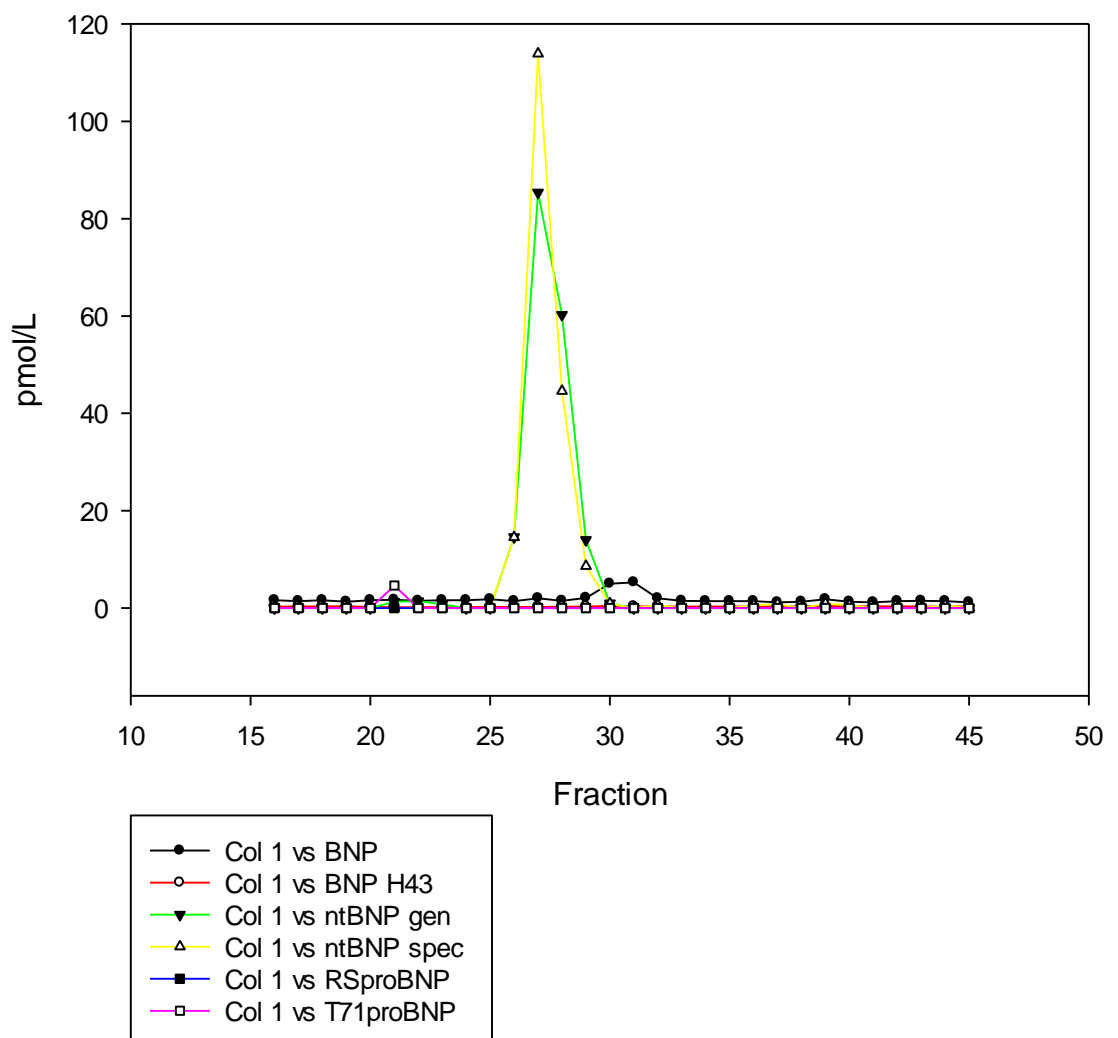


Figure 36: BNP isoform by HPLC testing: Group 1 (no HsPDA, no BPD)

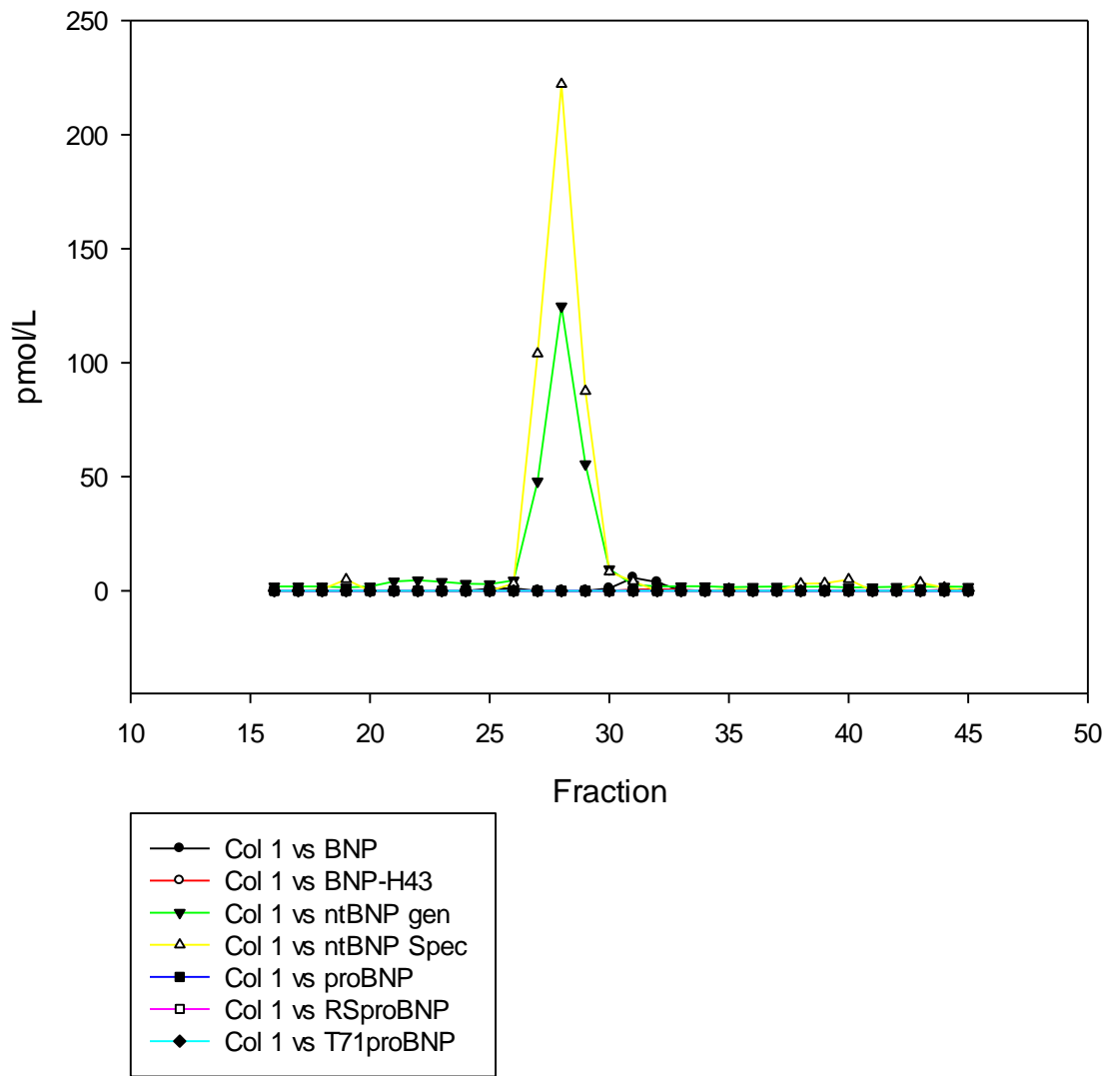


Figure 37: BNP isoform by HPLC testing: Group 2 (HsPDA, no BPD)

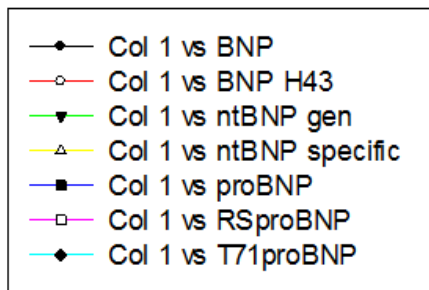
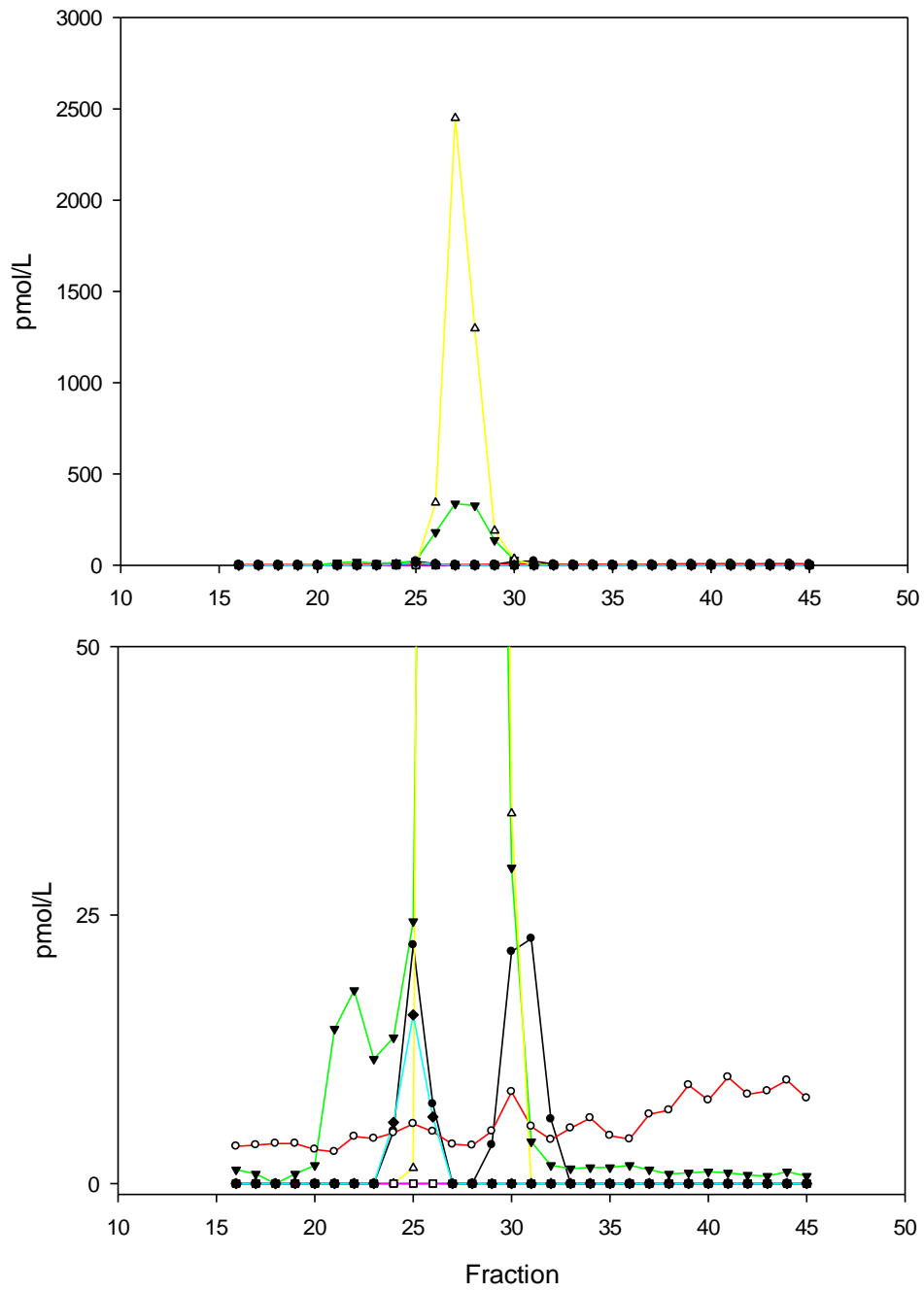


Figure 38: BNP isoform by HPLC testing: Group 3 (HsPDA, BPD)

Bottom graph shows enlarged detail of top graph

Discussion

NTproBNP appears to be the predominant isoform across each of our clinical groups in our pooled sample analysis. It is not possible to comment on any changes over time as these samples were grouped together across time periods due to low remaining sample volume. Based on this data, it does not look likely that the differences in NTproBNP levels between infants with and without BPD and HsPDA seen at 36 weeks PMA are primarily due to differences in glycosylation status, although this cannot be definitively ruled out as a contributing factor. The small amount of larger than proBNP material at Fraction 22 seen in group 3 could be a covalently bound, dimer or trimer of proBNP, possibly formed by opposing cysteines from each proBNP that link to an opposing proBNP, instead of to the other cysteine on their own peptide. Such observations have been made for a larger form of the related atrial natriuretic peptide molecule in human plasma.³⁴¹ This observed material eluting from the SE-HPLC column is unlikely to be immunoglobulin G-bound proBNP as the conditions of the HPLC column would necessarily separate an antibody-antigen complex. However, in a more benign SE-HPLC buffer, any immunoglobulin G bound to NTproBNP may still be present and should be the subject of further investigations.

The major weakness of this study is that BNP isoform testing was not part of our original study protocol and this necessitated using what plasma was remaining after standard NTproBNP testing. As sample volumes taken from very premature infants are minimised to reduce iatrogenic anaemia this meant we were unable to test for differences in isoforms in all patients and at all times separately and had to pool samples. Care was taken with the chosen techniques to ensure we had adequate volumes to produce reliable results. However, to better understand changes in post-translation BNP processing and the effects of clinical condition and time, analysis should be conducted on larger samples of individual patient plasma at different time intervals. An additional limitation is that we cannot exclude the possibility of some enzymatic degradation of peptide during the processing and storage of samples. As the majority of samples were promptly separated and frozen, the impact is likely to be small. The inventory system used for the study did not document processing times and therefore did not allow you to evaluate any impact of delays in processing. This is, however, to the best of our knowledge, the first time BNP isoforms have been investigated in a very preterm sample and we have produced some novel results. Although some parallels could be drawn clinically between the chronic ventricular wall stress seen in adult heart failure and

effects on the right heart of chronic lung disease (BPD) in very premature infants we did not find, as is seen in adults, high amounts of circulating unprocessed proBNP. This suggests that all circulating BNP is likely to have full biological activity.

In summary, this novel study of BNP isoforms in a very preterm population suggests NT-proBNP is the major circulating form and that there appears to be minimal degradation in plasma. This suggests it is likely to be a robust marker in this clinical setting. However, we did find some subtle differences in detected isoforms in the infant group who had experienced HsPDA and BPD and this deserves further study to determine whether infants who experience both significant left and right heart strain have differential processing of BNP.

Heart Ultrasound for the Diagnosis of Pulmonary Hypertension in Premature Infants

Background

The period after birth is a time of tremendous physiological change. Precipitous preterm delivery necessitates the rapid adaptation of an immature heart from a foetal circulation to neonatal circulation which may have long term consequences. Birth precipitates a switch in cardiomyocytes from hyperplastic to hypertrophic cardiac programming.^{44, 342} The preterm myocardium is stiffer and demonstrates relative diastolic dysfunction compared to the term heart making it vulnerable to rises in preload and afterload.^{343, 344} Premature birth exposes developmentally immature cardiomyocytes to relative hyperoxia combined with a sudden increase in systemic vascular resistance and a fall in pulmonary vascular resistance that alters the relationship between the left and the right side of the heart before cardiac maturation is complete. There is mounting evidence that these physiological changes or stressors cause alterations in cardiac structure and function that persist into adulthood.^{45, 328}

Premature infants have a more globular heart shape at birth with reduced heart mass and volume relative to body size.³⁴⁵ By three months of age biventricular hypertrophy is evident, the degree of which is negatively correlated with gestation at birth.³⁴⁵ At 6 years, the left ventricle and left ventricular outflow tract of extremely preterm born children is significantly smaller with concentric rather than longitudinal contraction and evidence of diastolic dysfunction.³⁴⁶ The changes in the right ventricle have not been well characterised and are of particular relevance due to the potential impact of bronchopulmonary dysplasia. The onset of right ventricular dysfunction in adults with heart disease is closely associated with symptomatic decline, loss of functional capacity and reduced survival.²³⁷ Cardiac magnetic resonance imaging studies in adults born preterm have revealed smaller right ventricular cavity size with evidence of right ventricular hypertrophy and dysfunction that is proportional to gestational age at birth.⁴⁵ A study of adolescents born preterm demonstrated higher pulmonary artery pressures than term born controls with those with BPD having the highest pressures.⁷¹ Swedish population registry data also suggests preterm born adolescents and young adults have a

several fold increased risk of pulmonary hypertension (OR 8.5) even after adjusting for congenital heart disease and chronic pulmonary disease.⁷⁰ Furthermore a study of adults born preterm with no evidence of resting pulmonary hypertension showed an exaggerated and abnormal rise in pulmonary artery pressure on exercise stress echocardiogram compared to term born controls but unexpectedly this was demonstrated primarily in those without a previous history of BPD.³⁴⁷ In addition a cardiac catheter study performed on well adults born preterm without known cardiorespiratory disease demonstrated preterm subjects had higher mean pulmonary arterial pressures with borderline pulmonary hypertension in 27% and overt pulmonary hypertension in 18%.⁶⁹ Those born preterm had evidence of a stiffer vascular bed at rest and were less able to augment cardiac index or right ventricular stroke work during exercise.⁶⁹ The long term impact of premature birth on cardiovascular health in adulthood demands further study of longitudinal preterm cohorts with particular attention to cardiac adaptation to preterm birth.

Cardiac catheterisation is the gold standard for identifying pulmonary hypertension but is rarely used in preterm neonates as it is not widely available, is invasive and carries a morbidity risk. Heart ultrasound is readily available and non-invasive and so the preferred modality for evaluating for pulmonary hypertension. The most commonly accepted surrogate measure of pulmonary artery pressure is the peak velocity of the TR jet. However, this is often not present meaning other evidence must be sought.^{199, 207} Alternative commonly used but subjective criteria if a TR jet is not present are RV hypertrophy, dilatation, shortening of the pulmonary artery acceleration time and evidence of interventricular septal flattening. Targeted neonatal echocardiography guidelines now recommend a quantitative assessment of the RV function as an important component of the serial assessment of cardiac function in premature infants, particularly when pulmonary hypertension is suspected.²⁰⁴ Our understanding of how the impact of premature birth on cardiac development can be detected or quantified by heart ultrasound parameters is still evolving and there is limited published data on the effect of gestational and chronological age, growth and disease processes on quantitative measures of RV function.

There are a number of factors that make heart ultrasound challenging in preterm infants: physiological instability with handling, high heart rates and respiratory rates, small acoustic windows with often hyperinflated lungs, vibration when on high frequency

ventilation and rapid circulatory changes after birth. Because of the shape of the RV, assessment of its size and function is also more difficult than for the LV. Historically, RV systolic function assessment has been largely qualitative and subjective. Interobserver agreement and reproducibility for subjective RV assessment are suboptimal. More recently there has been a push to adopt measures of RV function that are objective, quantitative and that have more acceptable reproducibility, retest variability and interobserver agreement. A number of these are now widely used in the assessment of adults but data is lacking in the preterm population. RV systolic function is complex and involves longitudinal, radial and circumferential components. No single index can adequately summarise RV function, but, as for the LV, it appears that changes in RV longitudinal function can be quantified and these are sensitive markers of overall function.

The additional quantitative measurements evaluated in this study were selected on the basis that they have all been shown to be affected by pulmonary hypertension in other clinical settings.

TAPSE can be measured from M-mode and is an excellent marker of longitudinal RV function that has excellent temporal resolution and so is helpful at higher heart rates. It can also be measured using 2D imaging when alignment with longitudinal motion is not possible, which allows differentiation of longitudinal shortening of the heart from motion due to translation. TAPSE is decreased in the context of pulmonary hypertension and heart failure in adult and paediatric settings and reference ranges have recently been published for preterm neonates.^{236, 237, 348, 349 209}

The ratio of main pulmonary artery acceleration time to RV ejection time (AT:RVETc) is a simply acquired measure that reflects pulmonary vascular resistance, pulmonary arterial compliance and RV function. A level of <0.31 is suggestive of raised pulmonary artery pressures with a level of <0.23 correlating well with severe pulmonary hypertension.^{350, 351} There is some debate over whether it needs to be corrected for heart rate. Both raw MPA acceleration time and AT:RVET have been shown to have high sensitivity and specificity for predicting pulmonary vascular disease and raised pulmonary pressures when compared to cardiac catheterisation.³⁵¹ Significant interatrial or ductal shunts However, may make it difficult to interpret this measure.

Early diastolic (E) and late diastolic (A) waves measured by conventional Doppler at tricuspid inflow or at the tricuspid annulus by TDI (E' and A') can be a useful measure of

diastolic function. The initial E wave reflects passive RV filling while the A wave reflects atrial contraction. In healthy children and adults the ratio of E:A is >1 . However, in preterm neonates this ratio may be <1 reflecting relative diastolic dysfunction.²¹³ The early diastolic wave is also reduced in children with pulmonary hypertension.²⁴⁶ Although easy to measure these waves often become fused and cannot be distinguished at high heart rates. They may also be affected by volume status and the use of inotropes. In addition progressive diastolic dysfunction will cause a rise in atrial pressure which can cause a “pseudonormal” ratio. Severe progressive diastolic dysfunction will cause the E wave to become much taller than the A wave although this will be accompanied by significant symptoms of heart failure. There is little published data on the normal range for this parameter in the preterm population or the changes in E/A over time.

RIMP or right Tei index has the advantage of evaluating RV systolic and diastolic function. Using TDI TV systolic and diastolic velocities can be measured simultaneously decreasing effect of time dependent variables. It has demonstrated good reproducibility, low interobserver variability and is independent of both blood pressure and heart rate.³⁵² It is higher in the presence of raised pulmonary pressures.²³⁵ There is little published data on the changes in RIMP over time in premature infants.

Another TDI measure, TV S', also known as the tricuspid annulus peak systolic velocity (TAPSV), is also a highly reproducible marker of RV longitudinal systolic function and is less dependent on acoustic window but can be affected by loading conditions. It is reduced with pulmonary hypertension and correlates with RV ejection fraction.^{242, 353} Limitations of TDI include aliasing if the myocardial velocities are higher than the velocity scale. It is also angle dependent and requires high frame rates to ensure temporal resolution. Normal ranges for preterm infants within the first 48 hours of life have been published.²⁴²

As ventricles are interdependent it is important to also consider left ventricular function when assessing for RV dysfunction. Many of the indices described are also used to evaluate LV function: Left ventricular index of myocardial performance, mitral valve annular plane of systolic excursion, E and A waves. In addition left ventricular output can also be calculated or an estimate of left ventricular ejection fraction made. For the purposes of our study we chose left ventricular fractional shortening (FS) in combination with subjective assessment of LV hypertrophy and dilatation. FS offers an estimate of LV function. However, there are limitations. Many would argue that left ventricular output

is a better measure in preterm infants and that more than one measure is needed, however, this would have lengthened scanning time which had to be limited in these fragile babies but we acknowledge a compromise was made.

Gaps in current knowledge

There is currently no consensus for diagnosing late pulmonary hypertension in preterm infants by heart ultrasound. There is a paucity of data regarding normal ranges for quantitative indices for evaluating RV function and the developmental changes in these measures over time.

Hypotheses

1. Specific quantitative markers of RV function will be measurable in very preterm infants by clinician performed heart ultrasound but some parameters will be more difficult to consistently attain and less useful than others
2. The temporal profile of these quantitative measures of RV function will reflect growth and developmental changes in cardiac function

Aims

1. To assess a cohort of infants born at < 30 weeks gestation for pulmonary hypertension using conventional heart ultrasound criteria
2. To evaluate the feasibility of clinician-performed quantitative heart ultrasound measures of RV function
3. To document the temporal changes in quantitative measures of RV function in a cohort of very preterm infants and investigate their relationship to body surface area and BPD

Methods

Patient factors

Infants born at < 30 weeks gestation at Christchurch Women's Hospital gestation between March 2013 and April 2015 were eligible. Any infants with persistent pulmonary hypertension of the newborn on day 3 or structural heart disease apart from PDA or patent foramen ovale or secundum atrial septal defect less than 5mm or tiny restrictive muscular ventricular septal defect was excluded from the study (see exclusions in methods chapter).

Machine factors and scan acquisition

Scans were performed by neonatologists trained in advanced neonatal heart ultrasound (SH, BD, KM) using a 6.5 MHz probe on a Toshiba Aplio500.

Infants were scanned in a supine position. No infants were scanned under general anaesthetic. All infants were given oral sucrose prior to or during scanning for comfort. Infants were scanned in an incubator or under a radiant heater to ensure thermoregulation was maintained. If an infant became physiologically unstable or distressed during a scan the scan was paused or terminated so that the infant could be stabilised or comforted. Infants were scanned under a range of respiratory support conditions: no support, nasal prong oxygen, high flow oxygen, continuous positive airway pressure, bi-level positive airway pressure, conventional ventilation and high frequency ventilation.

No respiratory gating was used. No angle correction was performed. Care was taken to optimise focal depth and cursor alignment and to minimise the acoustic window to enhance image quality. Several images were taken and the best quality images used for offline measurements. Several measurements were taken (10-15 over several cardiac cycles) and then averaged to reduce intraobserver variability. Most of the scans were performed by a single scanner (SH) to minimise interobserver variability. Interobserver variability was not formally assessed due to the impact of sequential scanning by a different operator on a medically compromised infant. A paediatric cardiologist who was blinded to clinical condition of the infant, the results of previous scans, NTproBNP testing and pulse oximetry analysis, independently reviewed all scans performed at 36 weeks PMA for evidence of pulmonary hypertension according to predefined criteria (documented in Table 4 in methods chapter).

Ultrasound imaging protocol

Cardiac structure was assessed using the clinician performed heart ultrasound protocol described in methods chapter and based on recommendations by Australasian Society of Ultrasound Medicine (Appendix C). When a patent ductus arteriosus was present it was classified as haemodynamically significant by predefined criteria defined based upon those commonly employed in neonatal studies (described in methods chapter).¹⁸⁰ Concurrent blood pressure was measured (invasively if an arterial line was in situ or non-invasively if not).

For our primary aim of assessing for pulmonary hypertension, conventional diagnostic heart ultrasound criteria were used based on the presence of a significant TR jet or alternative criteria if TR jet was absent. These criteria are described in detail in Table 4 in methods chapter.

Additional quantitative heart ultrasound indices that were assessed are outlined below. For more detail on how these parameters were acquired see methods chapter.

1. Tricuspid annular plane of systolic excursion (TAPSE):

TAPSE was assessed by measuring total excursion of tricuspid annulus during ventricular systole with M-mode using the apical four chamber view. Care was taken to align the cursor with the direction of longitudinal movement of the tricuspid annulus. TAPSE z score was calculated using an online calculator (<http://dev.parameterz.com/tapse>) based on the reference data provided in the paper by Koestenberger.²⁰⁹ This reference dataset did not provide z scores for infants born at <26 weeks. The calculator developer states the 1SD values are calculated by first determining if the measured value (score) is above or below the mean, then dividing by two the difference between the mean and the appropriate 2SD value, e.g. $SD = [+2SD - \text{mean}] / 2$. The z-score is then calculated in the conventional manner: $z = [\text{score} - \text{mean}] / SD$.

2. Conventional Doppler E and A velocities:

E and A wave velocities were evaluated using conventional pulse-wave Doppler on an apical four chamber view with the Doppler cursor sampling tricuspid inflow.

3. TV tissue Doppler E', A' and S' (TAPSV):

E', A' and S' velocities were interrogated using tissue Doppler imaging of the tricuspid annulus using an apical four chamber view.

4. Right index of myocardial performance (RIMP)

RIMP was interrogated using TDI of the tricuspid annulus and calculated using the

formula: $RIMP = (IVCT+IVRT)/RVET = TCOT-RVET/RVET$

(IVCT = isovolumetric contraction time. IVRT= isovolumetric relaxation time. RVET = right ventricular ejection time. TCOT= tricuspid closure to opening time).

5. Ratio of pulmonary artery acceleration time to RV ejection time (AT:RVETc):

The ratio of main pulmonary artery time to peak acceleration to right ventricular ejection time was evaluated using pulse Doppler with the cursor sampling the main pulmonary outflow immediately below the pulmonary valve. An electrocardiograph tracing was run concurrently to measure heart rate and this ratio was corrected for heart rate (by RR interval on concurrent electrocardiogram) using the formula:

$$\text{AT:RVETc} = \frac{\text{AT/RVET}}{\sqrt{\text{RR}}}$$

6. *LV fractional shortening (FS):*

Left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were evaluated using M-mode on a LV focussed parasternal long axis view with the M-mode cursor placed perpendicular to the intraventricular septum just below level of mitral valve. Fractional Shortening was then calculated using the formula:

$$\text{FS\%} = \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \times 100$$

Statistical analysis

Mean and standard deviation and/or median with interquartile ranges were performed for all quantifiable continuous heart ultrasound parameters. Dichotomous variables were reported as frequencies. The relationship between BPD and these ultrasound measurements was compared for both the dichotomous BPD definition of need for oxygen or respiratory support at 36 weeks PMA as well as the NICHD graded BPD definition.²⁸⁷ Analysis of associations between BPD status and ultrasound parameters was conducted using Chi square or Fisher’s exact tests for comparison of percentages and t-test for independent samples for comparison of means.

Changes in TAPSE were modelled using linear mixed effects regression in which the repeated assessments of TAPSE were modelled as a function of chronological age and predictors. Modelling was limited to the interval from day 3 to day 28 due to the high variability in chronological age at the 36 week assessment. A small number of observations (n=7) of body surface area at day 3 were missing due to missing data on length at birth. For the purposes of the regression modelling these observations were imputed using day 10 length to predict birth length. Marginal predicted trajectories (means and 95% CI) in TAPSE at varying centiles of body surface area were calculated

from the fitted model adjusting for other predictors. TAPSE at 36 weeks PMA was modelled using multiple linear regression. All analyses were conducted using Stata 15. With a sample size of 51 the study had 80% power at $\alpha=0.05$ to detect a correlation between TAPSE and a predictor in excess of .40, suggesting that the study was adequately powered to detect only moderate to strong associations.

A mixed effect regression model was used to predict TAPSV in which the repeated assessments of TAPSV were modelled as a function of chronological age and potential predictors of body surface area, gestational age at birth, birthweight and birthweight z score.

Results

Patients

53 infants were recruited. Two were excluded due to a large VSD and multiple cardiac rhabdomyomas. Two infants died between day 28 and 36 weeks PMA. 31 of 49 survivors developed BPD. Mean (SD) gestational age 27.8 (1.5) weeks.

Structural heart assessment findings

Table 26 shows a summary of structural findings over time focussing on the right heart and presence of shunts. No infant had any abnormality of aortic or mitral valvular structure or function. At 36 weeks PMA one infant had mild LV dilatation, no infants had LV hypertrophy and LV contractility appeared normal.

Table 26: Clinician performed heart ultrasound summary findings on day 3,10,28 and 36 weeks PMA

	Day 3 n=51	Day 10 n=51	Day 28 n=51	36 weeks n=49
%(n) Pulmonary stenosis	0(0)	0(0)	0(0)	0(0)
%(n) Pulmonary regurgitation*	16(8)	8(4)	16(8)	16(8)
%(n) Significant tricuspid regurgitation**	0(0)	0(0)	0(0)	0(0)
%(n) RV dilatation	0(0)	0(0)	0(0)	2 (1)*
%(n) RV hypertrophy	0(0)	0(0)	0(0)	2 (1)*
%(n) PFO/small ASD	74(38)	67(34)	57(29)	43(21)

	Day 3 n=51	Day 10 n=51	Day 28 n=51	36 weeks n=49
%(n)VSD	0(0)	0(0)	0(0)	0(0)
%(n) PDA	74(38)	51(26)	27(14)	24(12)
%(n) HsPDA	49(25)	31(16)	16(8)	4(2)

**All cases of PR were trivial and were not associated with any other signs of raised pulmonary pressure. All detected RV hypertrophy and dilatation was mild. ** TR jet considered significant if full envelope seen and peak velocity $\geq 3\text{m/s}$*

Conventional ultrasound criteria for pulmonary hypertension

Agreement between paediatric cardiologist (blinded to clinical details) versus neonatal paediatrician (not blinded) assessment for pulmonary hypertension, by predefined criteria, at 36 weeks PMA is summarised in Table 27. None of our study cohort met the predefined criteria for pulmonary hypertension at 36 weeks PMA as assessed by paediatric cardiologist or neonatologist. Some infants had interventricular septal wall flattening, however, there was disagreement on this between cardiologist and neonatologist (agreement occurring on only three infants).

Table 27: Comparison of paediatric cardiologist versus neonatal paediatrician assessment of heart ultrasound at 36 weeks PMA for pulmonary hypertension

Heart ultrasound parameter	Number of infants (paediatric cardiologist assessment) n=49	Number of infants (neonatal paediatrician assessment) n=49
Significant TR jet*	0	0
Right ventricular hypertrophy	0	1(mild)
Right ventricular enlargement	1 (mild)	1(mild)
Interventricular septal - flattening - bowing right to left	5 0	10 0
Shortening MPA acceleration time	2 (mild)	2(mild)
Meets criteria for pulmonary hypertension	0	0

* TR jet considered significant if full envelope seen and peak velocity $\geq 3\text{m/s}$

Summary Statistics for Quantitative Heart Ultrasound Parameters

The majority of quantitative heart ultrasound measures were reliably acquired in all subjects (Table 28). This was particularly true for TAPSE. The TDI E'/A' ratio was unable to be acquired consistently due to wave fusion at high heart rates. A significant discrepancy was noted between E/A and E'A' early in analysis so further assessment of E'/A' was abandoned.

Table 28: Data acquisition for quantitative heart ultrasound measures

Parameter	Day 3 n=51(%)	Day 10 n=51(%)	Day 28 n=51(%)	36 weeks PMA n=49 (%)
TAPSE	51(100)	50(98)	46(90)	48(98)
TAPSV	47(92)	44(86)	45(88)	45(92)
RIMP	46(90)	43(84)	45(88)	46(94)
E/A	40(78)	39(76)	29(57)	28(57)
AT:RVETc	33(65)	43(84)	45(88)	48(98)
LV FS	51(100)	49(96)	49(96)	47(96)

There were changes over time in some but not all ultrasound parameters of RV and LV function for the cohort as a whole (Table 29). When stratified by BPD status (Table 30), between group differences just reached significance for TAPSE at day 10 and 36 week PMA and AT:RVETc on day 3. There were no significant differences found in any of these measures by BPD severity.

Table 29: Summary statistics for heart ultrasound parameter values at days 3, 10, 28 and 36 weeks PMA

Measure		Day 3	Day 10	Day 28	36 Weeks
TAPSE	<i>Median</i>	5.60	6.45	7.95	10.6
	<i>IQR</i>	4.9 – 6.2	5.9 – 6.9	7.4 – 8.7	9.7 – 11.4
	<i>Mean (SD)</i>	5.56 (0.91)	6.45 (1.00)	8.00 (1.15)	10.50 (1.20)

Measure		Day 3	Day 10	Day 28	36 Weeks
TAPSE z-score ²⁰⁹	<i>Median</i>	0.53	1.38	2.31	3.93
	<i>IQR</i>	-0.67 – 1.69	0.55 – 2.66	1.11 – 3.65	2.71 – 4.93
	<i>Mean (SD)</i>	0.58 (1.56)	1.40 (1.69)	2.46 (1.97)	3.74 (1.71)
RIMP	<i>Median</i>	0.32	0.31	0.30	0.27
	<i>IQR</i>	0.26 – 0.45	0.25 – 0.39	0.23 – 0.37	0.23 – 0.35
	<i>Mean (SD)</i>	0.37 (0.14)	0.33 (0.11)	0.30 (0.09)	0.31 (0.11)
TAPSV	<i>Median</i>	5.8	6.7	7.9	9.2
	<i>IQR</i>	5.2 – 6.3	5.9 – 7.4	7.3 – 8.4	8.7 – 10.0
	<i>Mean (SD)</i>	5.73 (0.88)	6.70 (0.98)	7.81 (0.92)	9.09 (1.08)
AT: RVETc	<i>Median</i>	0.52	0.55	0.53	0.51
	<i>IQR</i>	0.45 – 0.58	0.47 – 0.64	0.44 – 0.59	0.46 – 0.59
	<i>Mean (SD)</i>	0.50 (0.10)	0.56 (0.13)	0.52 (0.10)	0.52 (0.11)
E/A (Doppler)	<i>Median</i>	0.64	0.70	0.68	0.80
	<i>IQR</i>	0.58-0.73	0.59-0.75	0.64-0.74	0.76-0.91
	<i>Mean (SD)</i>	0.64 (0.10)	0.70 (0.14)	0.70 (0.11)	0.84 (0.18)
LV FS	<i>Median</i>	39	38	36	37
	<i>IQR</i>	34 – 44	33 – 41	31 – 41	32 – 41
	<i>Mean (SD)</i>	38.5 (8.2)	37.8 (9.2)	35.9 (9.2)	35.5 (8.4)

Table 30: Mean (SD) heart parameter values at Days 3, 10, 28 and 36 Weeks PMA by ANZNN BPD or death classification

Measure		No BPD (n=18)	BPD or Death (n=33)	p
TAPSE	Day 3	5.7 (0.7)	5.5 (1.0)	0.50
	Day 10	6.9 (0.9)	6.2 (1.0)	0.02
	Day 28	8.3 (1.3)	7.9 (1.1)	0.22
	36 Weeks PMA	11.0 (1.1)	10.2 (1.2)	0.04
TAPSE z-score	Day 3	0.4 (1.3)	0.7 (1.7)	0.55
	Day 10	1.7 (1.7)	1.2 (1.7)	0.34
	Day 28	2.4 (1.9)	2.5 (2.0)	0.86
	36 Weeks PMA	4.2 (1.7)	3.5 (1.7)	0.16
RIMP	Day 3	0.36 (0.13)	0.37 (0.14)	0.94
	Day 10	0.34 (0.13)	0.32 (0.10)	0.48
	Day 28	0.33 (0.11)	0.29 (0.08)	0.20
	36 Weeks PMA	0.29 (0.14)	0.31 (0.10)	0.60
TAPSV	Day 3	5.7 (0.9)	5.7 (0.9)	0.94
	Day 10	6.4 (0.8)	6.8 (1.0)	0.18
	Day 28	8.0 (0.8)	7.7 (1.0)	0.29
	36 Weeks PMA	9.2 (1.0)	9.0 (1.2)	0.68
AT: RVETc	Day 3	0.56 (0.06)	0.47 (0.10)	0.01
	Day 10	0.60 (0.10)	0.53 (0.14)	0.08
	Day 28	0.54 (0.09)	0.51 (0.10)	0.23
	36 Weeks PMA	0.52 (0.11)	0.52 (0.10)	0.93

Measure		No BPD (n=18)	BPD or Death (n=33)	p
LV FS	Day 3	39.1 (8.2)	38.2 (8.4)	0.73
	Day 10	37.3 (7.8)	38.0 (10.1)	0.81
	Day 28	34.3 (7.8)	36.8 (9.9)	0.38
	36 Weeks PMA	34.6 (8.1)	36.0 (8.7)	0.59

Tricuspid Annular Plane of Systolic Excursion (TAPSE)

Ease of acquisition

TAPSE was relatively easily acquired once care was taken to align sample beam perpendicular to the motion of the tricuspid annulus. Only 7 out of 202 TAPSE measurements were not recorded, with three being poor quality images due to incorrect angle of insonation and four not done.

Change over time and factors affecting TAPSE

TAPSE values increased in linear fashion over the period day 3 to day 28 (Fig. 39). A linear mixed effects regression analysis was conducted to **model the change in TAPSE over time to day 28** (Table 31). This analysis showed statistically significant effects of age, gender and (time dynamically assessed) BSA. When other factors were taken into account mean TAPSE was higher for females and those with greater body surface area. However, increase in TAPSE was unrelated to gestation, use of antenatal steroids, BPD status or the presence of HsPDA on day 3. Tests for multiplicative age by predictor interactions showed no evidence of significant interactions, indicating that the rate of increase did not vary with levels of the predictor variables. From the fitted model estimates of average change in TAPSE at varying centiles of body surface area were made, adjusting for other predictors in the model (Table 32).

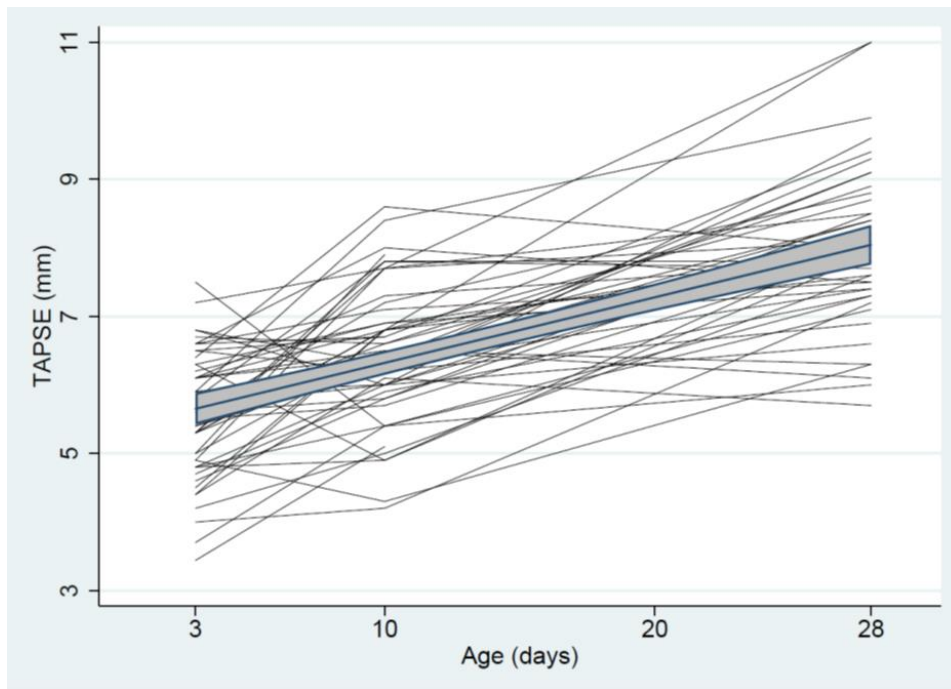


Figure 39: Individual TAPSE trajectories day 3 to day 28 and mean linear trajectory over time with 95%CI (shaded)

Table 31: Linear mixed effects regression model predicting change in TAPSE day 3 to day 28

Measure	<i>B</i> (<i>SE</i>)	<i>p</i>
Intercept	6.2 (2.2)	0.006
Age (days)	0.07 (.01)	<0.001
Female	0.41 (.18)	0.023
Birth weight z-score	0.23 (.14)	0.10
Gestation at birth (weeks)	-0.10 (.10)	0.33
Body surface area (m ²)	23.1 (11.3)	0.041
Antenatal steroids	-0.40 (.32)	0.22
BPD (or death)	-0.16 (.21)	0.46
HsPDA day 3	0.14 (.18)	0.44

Table 32: Observed centiles (25th, Mean, 75th) of body surface area (BSA) at days 3, 10, and 28, and predicted mean (95% CI) TAPSE(mm) by centiles of BSA adjusted for gender, gestation at birth, birthweight z-score, receipt of antenatal steroids, BPD status and presence of HsPDA day 3

Measure	Day 3	Day 10	Day 28
Centiles of Body Surface Area (m ²)			
25 th Centile	0.091	0.099	0.118
Mean	0.100	0.106	0.127
75 th Centile	0.107	0.115	0.139
Predicted Mean TAPSE (95% CI)			
25 th Centile	5.49 (5.21 – 5.76)	6.15 (5.91 – 6.40)	7.83 (7.51 – 8.17)
Mean	5.70 (5.50 – 5.90)	6.32 (6.15 – 6.49)	8.04 (7.77 – 8.32)
75 th Centile	5.86 (5.60 – 6.12)	6.53 (6.28 – 6.77)	8.32 (7.94 – 8.71)

Predictors of TAPSE at 36weeks PMA

As noted in methods, the wide variability in chronological age at the 36 week assessment made it difficult to incorporate data from this assessment in the mixed effects growth model above. A separate multiple regression analysis was conducted to examine predictors of TAPSE at 36 weeks PMA (Table 33). This showed that birthweight z-score was the only significant predictor of TAPSE ($p=0.01$). Other factors including BSA, gestation at birth, gender, receipt of antenatal steroids, BPD status, and presence of HsPDA were unrelated to TAPSE at 36 weeks PMA.

Table 33: Multiple regression model predicting TAPSE at 36 weeks PMA

Measure	B(SE)	p
Intercept	10.2 (4.9)	0.044
Female	-0.40 (.35)	0.26
Birth weight z-score	0.61 (.23)	0.013
Gestation at birth (weeks)	0.07 (.12)	0.54
Body surface area (m ²)	-3.5 (16.2)	0.83
Antenatal steroids	-0.63 (.82)	0.45

Measure	<i>B(SE)</i>	<i>p</i>
BPD (or death)	-0.56 (.35)	0.12
HsPDA day 3	0.22 (.34)	0.52

TAPSE z scores

TAPSE z scores based upon the Koestenberger²⁰⁹ dataset increased over the four time periods (Table 29, Fig. 40). In our cohort mean (SD) TAPSE z-score at 36 weeks PMA was not significantly different between those with and without BPD (Table 30).

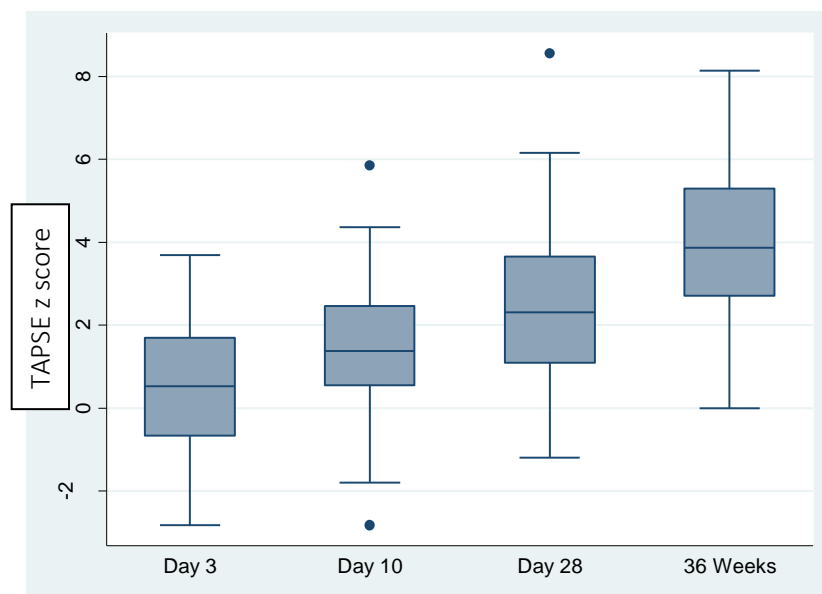


Figure 40: TAPSE z-scores over time

Right Index of Myocardial Performance (RIMP)

RIMP, interrogated by tissue Doppler imaging, was compared across the four time periods for the whole cohort and by BPD status. Technically this was a challenging parameter to acquire, being angle dependent and interfered with by high frequency ventilation vibrations, however, the overall RIMP data acquisition success rate was 89%. RIMP trended down over the four time intervals with no statistically significant difference seen between those who developed BPD and those who did not (Tables 29, 30).

E/A ratio tricuspid inflow

E/A ratio of Doppler-derived tricuspid inflow has been used in adults to look at RV dysfunction but due to high heart rates in our neonatal cohort the peaks were often fused on and it was often only measurable in approximately 2/3 of infants. Due to the high rate of missing E' and A' data (due to fusion of peaks at high rates) and a significant observed discrepancy between E/A and E'/A' where data was available, no further analysis has been undertaken of E'/A'. Overall there was a trend toward increasing E/A over our four assessments times but the ratio remained <1 (Table 29).

Tricuspid Annulus Peak Systolic Velocity (TAPSV) (S')

TAPSV by TDI relied on the same technique as RIMP and as such suffered the same technical difficulties in acquisition. In this cohort TAPSV increased over time, however, there was no significant difference in TAPSV by BPD status (Tables 29, 30). A mixed effect regression model (Table 34) for predicting TAPSV showed a highly significant effect for age and age-squared reflecting curvilinear growth but gestational age was non-significant. Birthweight z score was highly significant but BSA was not.

Table 34: Fitted model parameters from mixed effects regression models predicting TAPSV

Measure	TAPSV B(SE)	p
Age (days)	0.106 (0.014)	<0.001
Age ²	-0.00074 (0.00017)	<0.001
Body surface area (m ²)	-0.068 (8.914)	0.99
Gender	0.118 (0.158)	0.45
Gestation at birth (weeks)	0.041 (0.097)	0.67
Birthweight z-score	0.396 (0.119)	<0.001

AT:RVETc

This ratio was unable to be assessed when there was turbulence in the main pulmonary artery such as secondary to a haemodynamically significant PDA. This resulted in a large amount of missing data for the day 3 scan. This ratio remained stable across the four

assessment periods with no significant difference seen in infants with and without BPD (Table 29, 30).

Fractional Shortening (FS) of Left Ventricle

This measurement was easily acquired but care needed to be taken to align the M-mode cursor perpendicular to the intraventricular septum. FS remained stable over time and there were no significant differences seen between infants with and without BPD (Table 29, 30).

Discussion

General comments

It has been estimated that pulmonary hypertension occurs in 18-37% of infants born prematurely usually in association with moderate to severe BPD.^{64, 80-82} We present a study of a general population of infants born very preterm selected on the basis of gestational age rather than risk of pulmonary hypertension and followed prospectively. No infants in our study had pulmonary hypertension as defined by a set of conventional heart ultrasound parameters by 36 weeks PMA. It is likely the incidence is less in a general population of very preterm infants compared to higher risk or retrospectively selected populations and our study is in agreement with a similar study recently published in Australia. Stoecklin et al assessed for evidence of pulmonary hypertension and RV dysfunction at 36 weeks PMA in 197 infants born at <32 weeks gestation (73 with BPD) and found minimal evidence of pulmonary hypertension.³⁵⁴ Any screening or surveillance programmes will need to identify risk factors in addition to gestational age if they are to be cost-effective.

There is currently no consensus for diagnosis of late pulmonary hypertension in very preterm infants and some may argue that our criteria were too conservative. Conventional diagnostic criteria may not be sensitive enough for detecting late pulmonary hypertension, which, unlike acute PPHN, develops gradually beyond the period of transitional circulation, allowing time for the heart to adapt and potentially masking dysfunction. The most widely accepted proxy for main pulmonary artery pressure, the peak velocity of the tricuspid regurgitation jet, is not present in all infants with pulmonary hypertension and may be underestimated due to an inadequate or off-centre Doppler envelope.^{80, 199} Some pulmonary hypertension research groups would argue that simply the presence of RV hypertrophy or septal flattening is enough to

confirm abnormally high pulmonary pressures.^{80, 84} Septal flattening or bowing at end systole may hold promise as a marker of raised RV pressure which in the absence of pulmonary stenosis is likely to reflect raised pulmonary artery pressure. It is easily assessed and is also an indicator of the relative pressure difference between the left and right ventricle. However, evaluation is subjective and there was some disagreement between neonatologist and cardiologist on this parameter. It is likely that this finding will be more relevant if moderate to severe flattening is present although mild flattening may warrant a follow-up scan. Septal flattening may be better assessed using a quantitative measure such as LV eccentricity index which has been shown to have less interobserver variability.²⁰⁶

Our assessment time of 36 weeks PMA may arguably be too early to exclude pulmonary hypertension. Studies have suggested that pulmonary hypertension may take many weeks to evolve and studies that have included scanning beyond term equivalent age have shown a median age at diagnosis of 2-4 months of age but some not detected for several months beyond term equivalent.^{62, 80, 112} The optimum time for screening for BPD-associated pulmonary hypertension has not yet been established. The absence of significant pulmonary hypertension in our cohort does not preclude the existence of occult pulmonary vascular disease with dysfunctional pulmonary vessels at risk of decompensation when put under stress such as a respiratory infection or general anaesthetic.

When formulating a consensus definition of pulmonary hypertension using heart ultrasound criteria, the ease of acquisition and reproducibility must be considered. With some training we showed it was possible for more advanced, quantitative parameters to be incorporated into clinician performed heart ultrasound in preterm infants although some measures were more difficult to consistently acquire. It must be noted that acquiring these parameters considerably lengthens scanning time which may compromise the infant and behaves the clinician to only perform assessments that are of relevance to clinical care.

TAPSE

TAPSE is a simple and reproducible heart ultrasound parameter that can be mastered by neonatologists. TAPSE has been shown to have relatively low intraobserver variability and modest interobserver variability. Eriksen et al reported intraobserver variation of 5.5% and interobserver variation of 9.8%.³⁵⁵ Jain et al reported intraclass correlation coefficient

(ICC) of 0.97 (95% CI 0.93-0.99) and coefficient of variation (CV) of 3.4% for intraobserver variability and an ICC of 0.52 (95%CI 0.11-0.78), CV 10.9% for interobserver variability.²³⁹ Repeated examinations are therefore best done by the same examiner. This cohort demonstrated a linear trajectory for TAPSE. BPD had minimal effect on TAPSE in our cohort even when lung disease was severe. Although TAPSE z score at birth was similar to the Koestenberger cohort, z score subsequently and unexpectedly increased over time. This is unlikely to be explained by differences in growth as the reference dataset excluded infants who were small for gestational age and the mean birth weight z score of our cohort was -0.10 and 0.15 by 36 weeks PMA. TAPSE is affected by the angle of insonation. However, if the beam is not correctly aligned with the direction of annulus displacement this would be expected to lower rather than increase TAPSE. The reference z scores for TAPSE in preterm infants are based on TAPSE measured within 48 hours of birth in a low risk healthy preterm population without BPD. The impact of preterm birth and subsequent neonatal course on TAPSE is not known. Levy et al have demonstrated that RV Fractional Area Change, another measure of RV function, increases at twice the rate over the first month of life in preterm infants compared to term infants.³⁵⁶ The increasing TAPSE z score beyond the period of transitional circulation may reflect functional adaptive changes of an immature myocardium to the challenges of preterm birth. TAPSE may well have diagnostic and prognostic utility in monitoring the impact of preterm birth on cardiopulmonary outcomes, however, further research with larger preterm cohorts is needed to establish reference ranges for TAPSE over time and to establish whether a failure of TAPSE to increase at the expected rate is associated with pulmonary hypertension, poor respiratory outcomes or increased mortality.

RIMP

Tissue Doppler RIMP was technically difficult to acquire. Even in the hands of skilled clinician scanner it may be difficult to obtain in some infants and may be prone to interobserver variability. Previous studies have suggested that RIMP tends to fall after birth but may be elevated in infants with BPD.^{235, 357} RIMP in our cohort did not vary greatly across our four assessment times. The relative stability across time suggests it may be independent of body size which could be a useful feature in circumstances where there is rapid growth. However, larger cohorts have found a greater difference over time.²²² We could not demonstrate a significant difference between those with and without BPD consistent with the Di Maria cohort.²²² Low intraobserver variability (ICC

0.97 (95% CI 0.92-0.98), CV 9.5%) but greater interobserver variability (ICC 0.80 (95% CI 0.56-0.92), CV 20.9%) has been demonstrated for RIMP in term babies suggesting serial exams should be performed by the same person.²³⁹

TAPSV

TAPSV appears to be a useful measure of RV function over time. Koestenberger showed excellent ICC for interobserver (0.97) and intraobserver (0.98) variability.²¹⁴ Jain et al also reported low intraobserver variability (ICC 0.96 (95%CI 0.89 to 0.98), CV 3.7%) and low interobserver variability (ICC 0.94 (95%CI 0.86 to 0.98) CV 4.9%).²³⁹

TAPSV was able to be acquired for most scans in our cohort and increased over time in our cohort consistent with other studies.^{202, 214, 354} We could not demonstrate any significant differences in TAPSV in infants with and without BPD in agreement with the study by Stoecklin.³⁵⁸ The day 3 and 36 week values in our study were similar to that seen in a Scandinavian study of more mature premature infants scanned on day 3 and term equivalent age.²⁰² When compared to the Koestenberger cohort who were born at a similar gestational age but only scanned within 48 hours of birth, the day 3 TAPSV was similar, however, by 36 weeks PMA the TAPSV in our cohort was closer to that of a 2-3 month old term born infant.²¹⁴ This suggests that chronological age has an impact on TAPSV and that infants reaching a corrected gestation will have higher TAPSV than those born at that gestation. Whether this reflects cardiac adaptation to preterm birth, and whether this persists beyond the neonatal period, requires further study.

AT:RVETc

Although relatively easy to acquire in the absence of a PDA, AT:RVETc could not be measured when there was pulmonary artery turbulence which is common with concurrent PDA. The assessment of MPA Doppler acceleration time has the potential to be highly subjective as it may not have a sharply defined peak leading to interobserver variability.³⁵⁹ All but one of our cohort had an AT:RVETc at 36 weeks PMA within the currently accepted normal range. We were not able to demonstrate a difference between those with and without BPD. It is likely to be a parameter that is useful if significant shortening is present but a normal AT:RVETc will not exclude pulmonary hypertension. In our cohort it did not appear particularly discriminating and could not be recommended alone as a means of determining the presence of raised right heart pressures.

E/A and E'/A'

Due to the high heart rates seen in preterm infants causing fusion of the early and late diastolic waves this measure cannot be consistently measured making it less useful to the neonatologist. Furthermore we found a discrepancy between Doppler and TDI acquired ratios and surmise that this may relate to E' and A' being angle dependent. Our mean day 3 E/A ratio was similar to those seen on day 1 in another preterm cohort.²¹³ To the best of our knowledge there are currently no normal ranges in preterm infants outside of the first few days of life. As it may be affected by loading conditions it may be unreliable. However, it is interesting to note that across all time points we assessed the E/A ratio remained <1 suggesting some degree of diastolic dysfunction persists out to 36 weeks PMA.

Fractional shortening

Although left ventricular output or LV ejection fraction(calculated by biplane Simpson's or five-sixths area x length method) are often the preferred measures of LV function in premature newborns they are more time consuming to acquire than FS. In view of the large number of ultrasound assessments that were undertaken as part of this study FS was selected to decrease scanning time. FS can be affected by septal wall flattening or paradoxical movement secondary to raised RV pressures. In our cohort FS was stable and well preserved across all four assessments even in infants with BPD. Our results are similar to those published by Kozak-Barany in a more mature preterm cohort scanned in the first four days of life and at 1 month.³⁶⁰ It is acknowledged that FS does not taken into account preload or afterload conditions and mean velocity of circumferential fibre shortening or TDI strain measurements may be more accurate for assessment of LV function.

Strengths and weaknesses

Strengths of our analysis include the longitudinal assessment of multiple indices of cardiac function over four clinically meaningful time periods in a well characterised prospective cohort. Weaknesses include a small study population, lack of a local term control group for comparison, minimal left ventricular quantitative assessment and the scans being performed by a clinician-scanner rather than a cardiac sonographer or paediatric cardiologist. We acknowledge that having three investigators conduct the scans in a small cohort may have introduced interobserver variability. However, we did attempt to reduce this by having Dr Harris perform 90% of the scans. We acknowledge

some measurements, in particular TAPSE, should also have been indexed against ventricular length.^{203, 361} This recommendation came after we had completed most of our scans and it was felt that we could not guarantee that dedicated LV views had been imaged that would accurately represent LV length and so have not conducted this indexing. LV eccentricity index, RV fractional area change and speckle tracking are other parameters that hold promise that we did not assess these and warrant evaluation in a larger preterm population. With regards to the linear regression analysis for the prediction of change in TAPSE, our sample size may be too small to allow the inclusion of 8 predictors and there may be some collinearity between gestational age, BSA and birthweight z score. Further evaluation of all of our studied parameters in much larger preterm cohorts is needed.

Conclusion

It is feasible for advanced cardiac ultrasound parameters to be incorporated into clinician performed targeted neonatal echocardiography for the assessment of late pulmonary hypertension. However, consultation with paediatric cardiologist would be recommended if there is evidence of raised pulmonary pressures, particularly prior to commencing pulmonary vasodilators. It is essential the limitations of these measurements are understood and more work is needed to establish reference ranges in this population. In view of the complex three-dimensional orientation and movement of myocardial fibres we would recommend serial scanning utilising more than one type of tool to assess left and right ventricular function. In our cohort right ventricular function appeared to be surprisingly well preserved in the face of even moderate to severe premature lung disease. We speculate that the foetal heart develops adaptive strategies after premature birth which may offer a short term survival benefit but could potentially lead to premature cardiac dysfunction in adulthood.

Oxygen Saturation Targeting: Patterns of Instability and Optimising Care

Background

Premature infants are prone to periods of hypoxia and hyperoxia. The causes are multifactorial. An immature brain predisposes premature babies to central apnoea while the relative proportions of upper airway structures and low muscle tone make them susceptible to obstructive apnoea.²⁷ Both poor peripheral chemoreceptor sensitivity to hypoxia and upregulated peripheral chemoreceptor responses appear to contribute to ventilatory instability.³⁶²⁻³⁶⁴ An underdeveloped lung structure and surfactant deficiency create ventilation-perfusion mismatch and chronic inflammation further compromises gas exchange.³⁶⁵

Supplemental oxygen is frequently required to support respiration. However, oxygen therapy for premature infants is a delicate balancing act. Cellular function requires an adequate oxygen supply and yet an excess can result in oxidative cell damage.^{76, 248, 267, 366} Excess oxygen is also a key protagonist in the development of pulmonary vascular disease which further aggravates intermittent hypoxia due to pathological vasoreactivity.^{73, 74, 367, 368} The interaction between oxidative stress and inflammation appears to be a particularly toxic cocktail in the development of pulmonary morbidity in very preterm infants.

Our evolving understanding of this narrow therapeutic window has led to the practice of oxygen saturation targeting for premature infants. Pulse oximetry allows clinicians to continuously monitor fluctuations in oxygen saturations and heart rate. Optimal targets have been the subject of intense research scrutiny in recent years.^{89, 272, 273, 276, 277, 369, 370} However, despite oxygen saturation targeting, very premature infants in high level intensive care units often spend more than half of their time outside of their allocated range.^{280, 281} There are a number of factors which influence the amount of time infants spend within their target range. These include how narrow the set targets are, how close the alarm settings are to the targeted range, the nurse to patient ratio and the clinical risk profile of the population being studied.^{280, 281, 371, 372} Adherence to unit policies regarding oxygen saturation alarm limits is also variable. In a study by Clucas and

colleagues the lower alarm limit was set correctly 91% of the time but the upper alarm limit was only set correctly 23% of the time with only 22% of infants studied having both alarm limits set correctly.²⁸³ Implementing new education programmes for nursing staff may not significantly increase the time spent in target.^{281, 369, 372}

Software is now available that can process stored oxygen saturation and heart rate data. This facilitates the in-depth analysis of oxygen saturation instability. Clinicians can identify infants who may require more respiratory support or who are experiencing iatrogenic hyperoxia. Modern oximeters and cardiorespiratory monitors have multiple ways in which respiratory instability can be evaluated. These include histograms which can be set up to show time in target over a set period, mean oxygen saturation, coefficient of variation (CV) and desaturation event index (DSI).

Gaps in current knowledge

There has been little published on optimal measures of oxygen saturation instability that may be of use to the neonatologist.²⁸⁵ Further study of oxygen saturation instability patterns and the factors that contribute to this instability is needed in order develop strategies to improve stability and to understand what role, if any, oxygen saturation monitoring may have in identifying infants developing pulmonary vascular disease. More detailed analysis of oxygen saturations will also highlight iatrogenic hyperoxia, a known contributor to the development of late pulmonary hypertension in very premature infants.

Hypotheses

1. The pattern of oxygen saturation instability will vary over time in very preterm infants
2. Infants who develop BPD or later pulmonary hypertension will have evidence of greater oxygen saturation instability
3. Poor compliance with oxygen saturation alarm limits is a contributing factor to exposure to hypoxia and hyperoxia in neonatal units

Aims

1. To conduct detailed analysis of 72 hour recordings of oxygen saturation data using a variety of measures of instability at four time intervals from birth to 36 weeks PMA and to compare these measures in infants with and without BPD and/or pulmonary hypertension

2. To audit unit compliance with oxygen saturation alarm protocols and to survey nursing opinion of oxygen saturation targeting in the NICU

Methods

Participants

Infants born at < 30 weeks gestation between March 2013 and April 2015 and cared for at Christchurch Women's Hospital NICU were eligible for participation. Infants were excluded if they had known structural airway or lung anomalies, congenital anomalies of the pulmonary arteries or pulmonary veins, major systemic vessel-to-pulmonary artery collateral vessels, congenital heart disease (except those with patent ductus arteriosus, patent foramen ovale or secundum atrial septal defect < 5mm or tiny restrictive muscular ventricular septal defect), severe liver disease, or persistent pulmonary hypertension of the newborn on day 3 heart ultrasound (defined as severe hypoxia associated with estimated main pulmonary artery pressure >25mmHg). If infants had a grave prognosis such that they were not expected to survive to day 3 they were also excluded.

Clinical data collection

Clinical data was collected including gender, gestational age, birth weight, antenatal steroids, HsPDA day 3, inotropic support, maternal smoking in pregnancy, respiratory support and treatment for apnoea with the respiratory stimulants caffeine or doxapram. BPD was defined by either the definition used by the Australia and New Zealand Neonatal Network (ANZNN) at the time (persistent oxygen or respiratory support requirement at 36 weeks PMA) or graded using the National Institute of Child Health and Human Development (NICHD) grading system.²⁸⁷ Pulmonary hypertension was defined by conventional heart ultrasound as detailed in the methods chapter.

Oxygen saturation monitoring and data collection

72 hours of oxygen saturation and heart rate data recorded on day of life 0-3, 7-10, 25-28 and at 36 weeks PMA using a Masimo Radical 7 pulse oximeter (Masimo Corporation, Irvine, California) with a two second averaging time. PROFOX analysis software (PROFOX Associates Inc, Escondido, California) provided numerical oxygen saturation and heart rate summary data which included:

1. Percentage time spent below 88%, below 80% and above 95%

2. Percentage time spent in target range
3. Mean oxygen saturation level and standard deviation (SD)
4. Desaturation event index 4% (DSI4%) - defined as number of episodes per hour where oxygen saturation decreased by $\geq 4\%$
5. Desaturation Index 4% >10s - defined as number of episodes per hour where oxygen saturation decreased by $\geq 4\%$ lasting longer than 10 seconds
6. Coefficient of variation (CV) of oxygen saturation was calculated using the formula:

$$CV = SD / \text{mean}$$

These variables enabled us to compare relative instability during periods of assessment. Time spent in target range and in hyperoxia while on oxygen (defined in this case as oxygen saturations $>95\%$), has only been assessed for the first 3 days of life as this is a critical period of transition for the preterm infant. Target ranges were as specified by unit protocol, which was revised in August 2013, after publication of the BOOST II trial and differed depending on whether infant was receiving oxygen therapy or not (Table 35).²⁹² Time receiving oxygen was assessed using nursing observation charts that recorded fraction of inspired oxygen hourly. It had to be assumed that this was the inspired fraction for the whole hour even though it is possible there may have been some adjustments made during this period that were not documented.

Table 35: Oxygen saturation targets before and after revision of targets August 2013

PMA	Old targets (oxygen)	New targets (oxygen)	Old targets (air)	New targets (air)
< 32 weeks	88-92%	90-92%	88-100%	90-100%
32-36 weeks	92-95%	92-95%	92-100%	92-100%
≥ 36 weeks	95-98%	95-98%	95-100%	95-100%

An example of the graphical oxygen saturation and heart rate data for a clinically stable infant (DSI4% 15, CV 0.015) and a comparatively unstable infant (DSI4% 104, CV 0.067) are shown in Figs. 41 and 42 respectively. A low signal IQ reading is also displayed.

Masimo state in their product information that this is assessed using “advanced signal algorithms” to generate a confidence interval with “low signal IQ” warning displayed when there is <30% signal quality. The processing algorithms are commercially protected, however, the company maintain that it is still likely to represent a true recording with any complete disruption of data recorded as a gap in the tracing. Comparative studies have shown Masimo monitors employing signal extraction technology have high specificity and sensitivity for SpO2 even in the setting of movement or low perfusion.^{373, 374}

Our data was also manually reviewed for aberrant data points which were excised from the recording prior to analysis. This included any region at the start of the trace where readings were missing or erratic and data acquisition had not yet stabilised and any regions where either heart rate or SpO2 data were completely missing. Finally the recording was automatically scanned for aberrant data (defined as a difference of >10% in SpO2 or >20 beats per minute for heart rate from the previous or subsequent data point) and each identified point reviewed manually to determine whether it was likely to represent a real or artefactual data point.

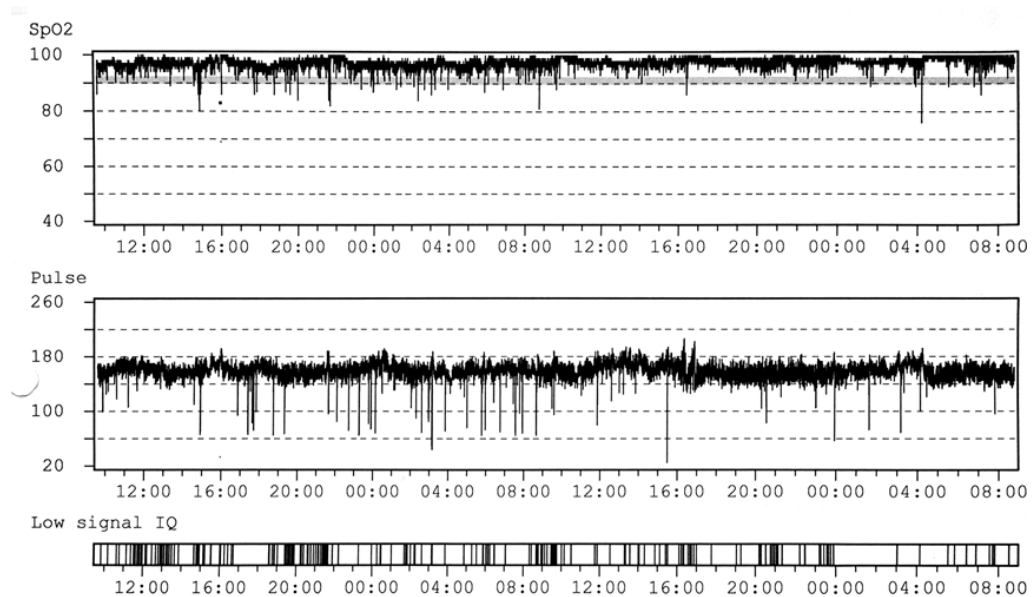


Figure 41: Snapshot of oxygen saturation and heart rate data over time in a stable infant

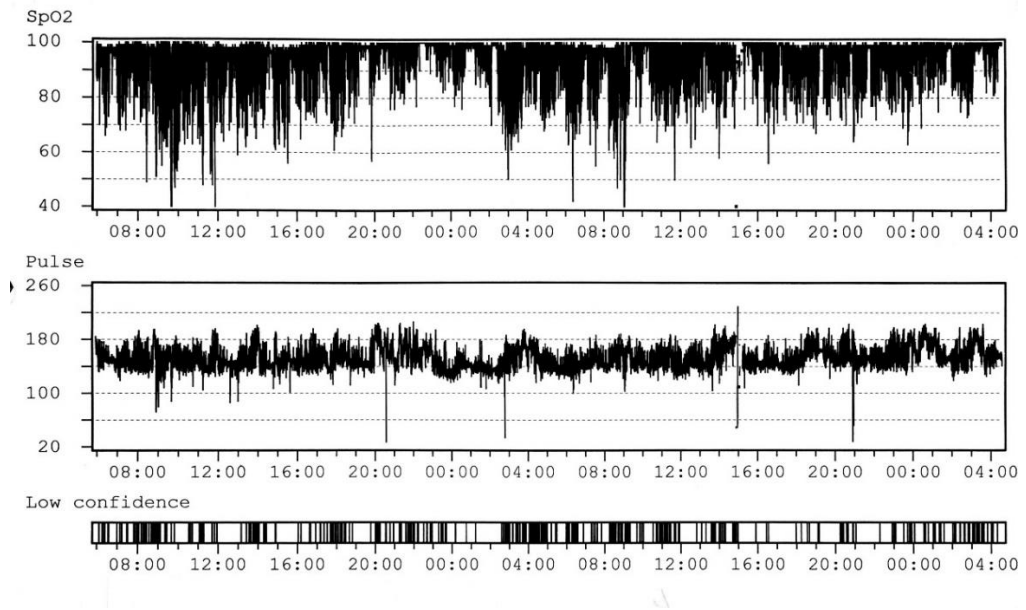


Figure 42: Snapshot of oxygen saturation and heart rate data in an unstable infant

Audit of oxygen saturation monitor alarm limits and nursing survey

Audit

Oxygen saturation monitor alarm settings were audited at Christchurch Women's Hospital Neonatal Intensive Care Unit, New Zealand, on randomly selected days and nights between June and October 2015. Nursing staff were not made aware of the audit. Monitor alarm setting and the gestation of the infant were recorded and whether the infant was on oxygen. These limits were then compared to unit policy (Table 36). Minor breeches of protocol were arbitrarily defined as $\pm \leq 2$ saturation points. Major breeches were defined as $\pm \geq 3$ saturation points.

Table 36: Oxygen saturation alarm limit policy during audit

PMA	Alarm limits in oxygen	Alarm limits in air
<32 weeks	88-94%	88-100%
32-36 weeks	90-96%	90-100%
>36 weeks	93-99%	93-100%

Survey

After these data were collected, during December 2015 to January 2016, we conducted an anonymous survey of bedside neonatal nursing opinion of oxygen saturation targeting in the same NICU (Appendix E). This survey was designed for this study and has not been validated. All neonatal nurses were invited to participate anonymously using either an electronic survey on Survey Monkey (www.surveymonkey.com) or in written form. The survey consisted of questions on nursing experience of participants and nursing practice, knowledge, and opinion of oxygen saturation targeting. The unit had a change in oxygen saturation target ranges in October 2015 after our audit period, in line with new national guidelines.³⁷⁵ The survey includes questions regarding both protocols. Survey responses were collated and analysed using Microsoft Excel

Statistical analysis

Changes over time in quantitative measures are described using mean (SD) and/or median (IQR). Chi square or Fisher's exact tests were used for comparison of percentages and t-test for independent samples for comparison of means. Mann-Whitney test was used to compare medians where distribution was non-Gaussian. If needed, some measures were log transformed to stabilise the variance. Repeated measure ANOVA was used to test significance of mean differences in measures of oxygen saturation instability over time. Repeated measures ANOVA analysis was used to assess effect of group and time interactions. Mean differences in oxygen saturation measures by BPD status over time adjusting for gender, gestation, birthweight z-score and presence of HsPDA on day 3 were estimated from linear regression models fitted to the repeated measures data for each fitted within a Generalised Estimating Equation (GEE) framework.²⁹⁷ Contrasts on the marginal predicted mean scores from the fitted models were calculated to test significance of covariate adjusted mean differences on oxygen measures over time. Box and whisker plots or scatter plots were used to display results graphically. Data were analysed using Stata 15, SAS 9.4 or GraphPad InStat software. The data for the oxygen saturation audit were collated and analysed using Microsoft Excel and results displayed descriptively as percentages.

Results

Clinical characteristics

51 infants had oxygen saturation monitoring on the first 3 time points and 47 at 36 weeks PMA (2 deaths and 1 missed, 1 infant was missed and had monitoring at 47 weeks

PMA but was included in analysis). Table 12 (Chapter 3) documents the clinical characteristics of our cohort as a whole and by BPD status. Two thirds of our cohort had BPD with 15.7% discharged on home oxygen. None of our cohort was diagnosed with pulmonary hypertension by our predefined criteria meaning no further analysis could be conducted with respect to this outcome. All infants received caffeine therapy for the prevention and treatment of apnoea of prematurity.

Oxygen saturation summary results

The median (IQR) hours of oxygen saturation data analysed was 71.3 (66.8-72.2) for day 0-3, 71.5 (67.7-73.6) for day 7-10, 71.6 (68.2-72.9) for day 25-28 and 70.5 (68.0-73.9) for 36 weeks PMA.

Mean (SD) maximum oxygen requirement in delivery suite was 70% (25) and this was the same for those who developed BPD and those who did not. Median (IQR) hours on oxygen during the 72 hour assessment period was 15 (3-44.7) on day 3, 14 (0-48.7) on day 10, 27 (2-67.3) on day 28 and 29 (0-70.1) at 36 weeks PMA.

Table 37 shows summary statistics for pulse oximetry measures over time. There was a trend towards all parameters worsening initially, peaking at day 28, and then improving by 36 weeks PMA. There was a wide variation in the data at all time points. Oxygen saturation instability at 36 weeks PMA was similar to that seen in the first 72 hours of life across most parameters.

Table 37: Summary statistics for pulse oximetry measures over time

Measure	Day 3	Day 10	Day 28	36 weeks	P*
<u>Oxygen saturation</u>					
Mean (SD)	94.0 (1.8)	93.7 (2.0)	93.6 (2.2)	95.9 (1.6)	<0.001
Median (IQR)	94.4	94.0	94.0	95.8	
IQR	92.9-95.1	91.8-95	92-95	95.2-96.9	
<u>% Time with SpO₂ <88</u>					
Mean (SD)	3.9 (3.4)	6.5 (6.1)	9.5 (7.5)	3.8 (2.7)	<0.001
Median	2.6	3.9	7.3	2.8	
IQR	1.6 – 5.9	1.8 – 9.6	4.6 – 10.9	1.8 – 5.1	

Measure	Day 3	Day 10	Day 28	36 weeks	P*
<u>% Time with SpO₂ <80</u>					
Mean (SD)	0.52 (0.48)	0.82 (0.96)	1.68 (1.55)	0.85 (0.84)	<0.001
Median	0.40	0.40	1.30	0.50	
IQR	0.20 – 0.74	0.10 – 1.20	0.70 – 2.20	0.30 – 1.20	
<u>Coefficient of Variation</u>					
Mean (SD)	0.033 (0.007)	0.036 (0.011)	0.045 (0.011)	0.036 (0.010)	<0.001
Median	0.031	0.035	0.045	0.033	
IQR	0.026 – 0.038	0.029 – 0.043	0.039 – 0.050	0.028 – 0.042	
<u>Desaturation Index 4%</u>					
Mean (SD)	20.8 (6.8)	36.0 (11.3)	56.9 (12.1)	51.3 (20.6)	<0.001
Median	20.1	36.5	57.6	50.8	
IQR	15.5 – 25.8	28.3 – 41.8	47.9 – 66.0	39.9 – 62.2	
<u>Desaturation Index 4% >10s</u>					
Mean (SD)	14.2 (4.9)	22.1 (6.0)	27.9 (6.9)	17.7 (6.0)	<0.001
Median	14.3	22.1	27.1	17.5	
IQR	(10.1-17.1)	(18.5-25.4)	(24.2-33.4)	(14.4-21.5)	

* Repeated measures ANOVA used to calculate significance

Time spent with oxygen saturations <88% or <80% (Figs. 43, 44)

Percentage time spent with oxygen saturations <88% or <80% rose over time peaking at day 28 before falling to similar levels to the first 3 days of life by 36 weeks PMA. The range was wide on days 10 and 28 with some infants spending up to one third of the time hypoxic. Across all data collection periods infants spent very little time with oxygen saturations <80%.

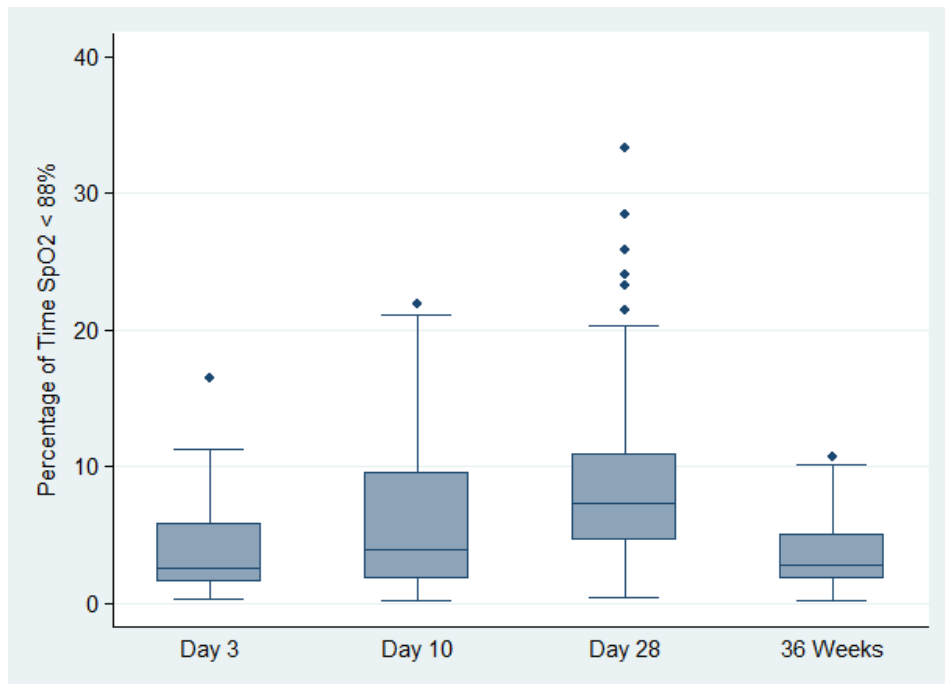


Figure 43: Percentage time spent with oxygen saturations <88%

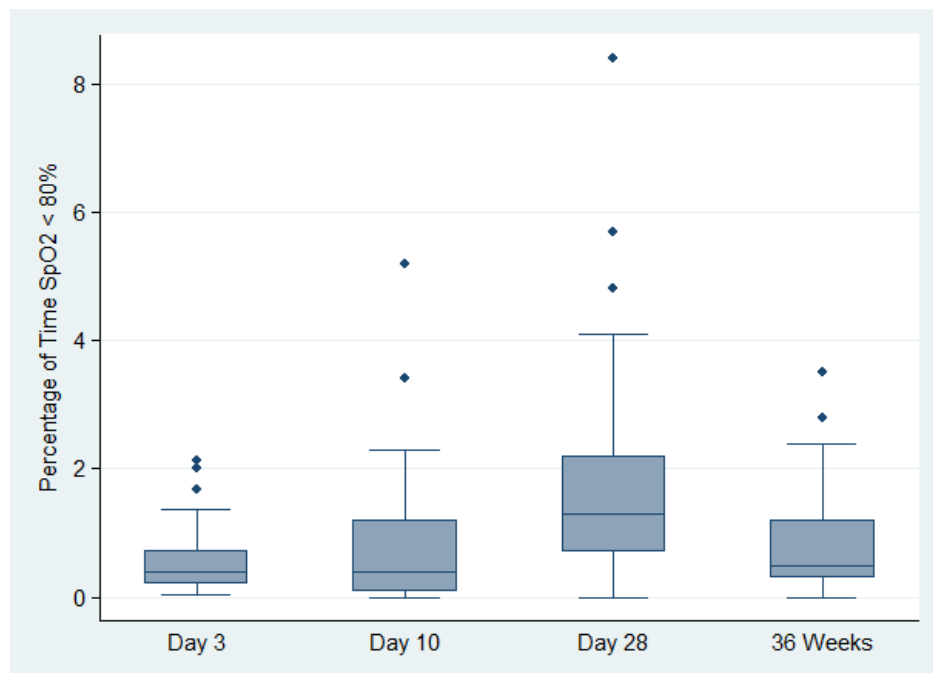


Figure 44: Percentage time spent with oxygen saturations <80%

Coefficient of variation and desaturation event index (Figs. 45-51)

Both the CV, DSI4% and the DSI4% >10 seconds increased over time to day 28 before falling (Figs. 47-49). Whereas the CV and DSI4%>10 seconds at 36 weeks PMA fell to levels similar to the first 3 days, the DSI4% remained higher than day 3 and day 10. There was considerable variation across the cohort for both of these parameters at all

time periods. If you follow the individual trajectories of CV (Fig. 50), it decreases for some infants between day 3 and day 10 and increases for others. Those infants who increased between day 3 and day 10 tended to have higher CV by day 28, however, there is considerable overlap. Only two infants in the whole cohort had a decrease in the CV between day 10 and day 28.

Figs. 51-53 show the relationship between PMA and CV on days 3, 10 and 28. On day 3 PMA appears to have little effect on CV but on day 10 and day 28 CV tended to be higher for infants with greater immaturity.

The median (IQR) day 10 CV of infants with HsPDA on day 10 was significantly higher than those without (0.048 (0.040-0.049) compared to 0.032 (0.025-0.035), $p < 0.0001$). The infants with the highest 10 CVs on day 10 all had HsPDA on day 10. None of the infants with the 15 lowest CVs on day 10 had HsPDA on day 10.

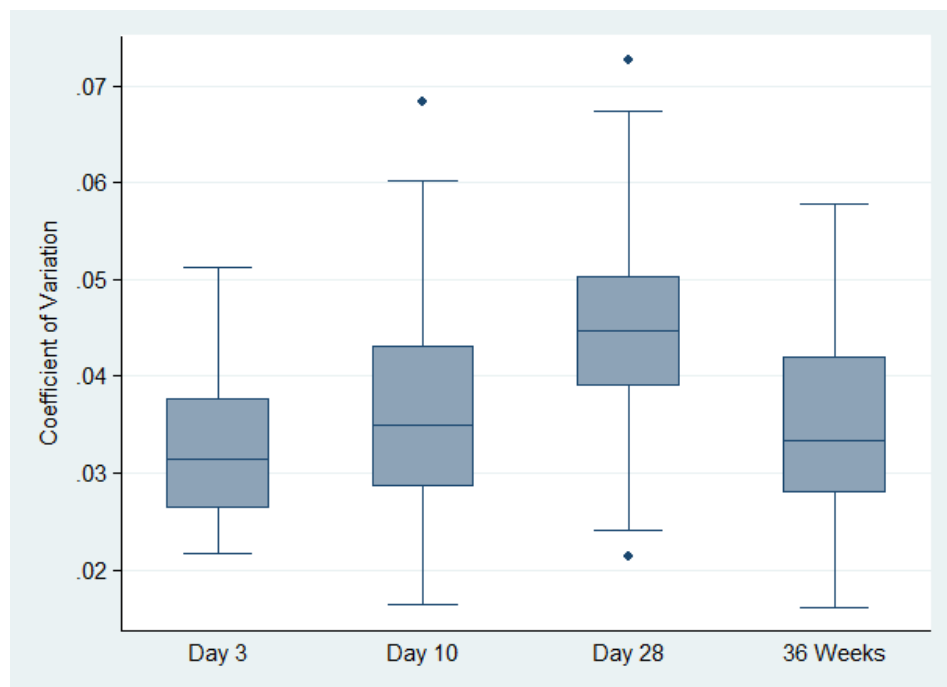


Figure 45: Coefficient of variation for oxygen saturations over time

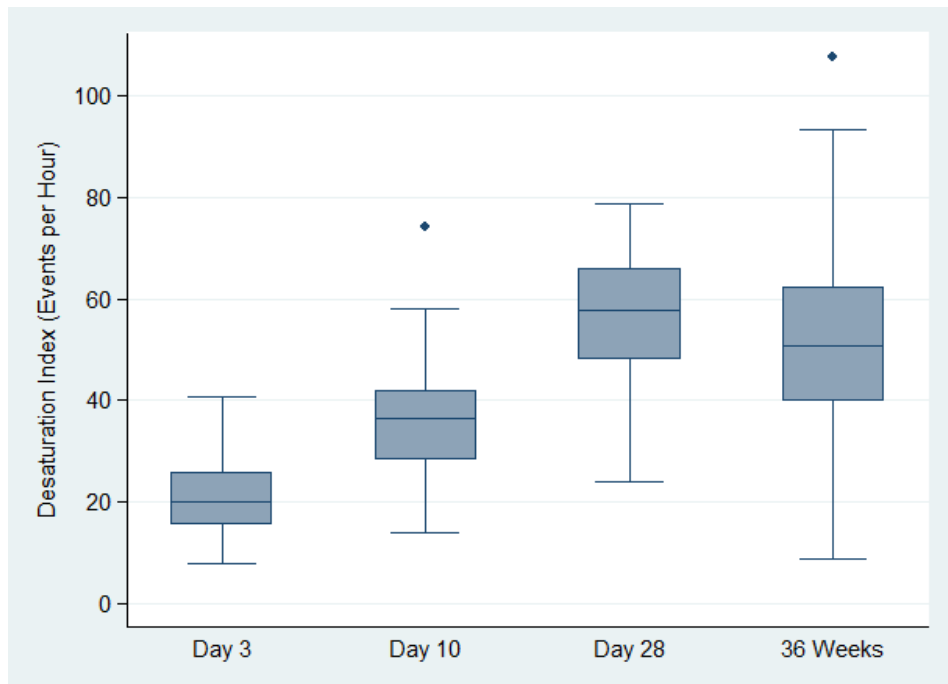


Figure 46: Desaturation event index 4% over time

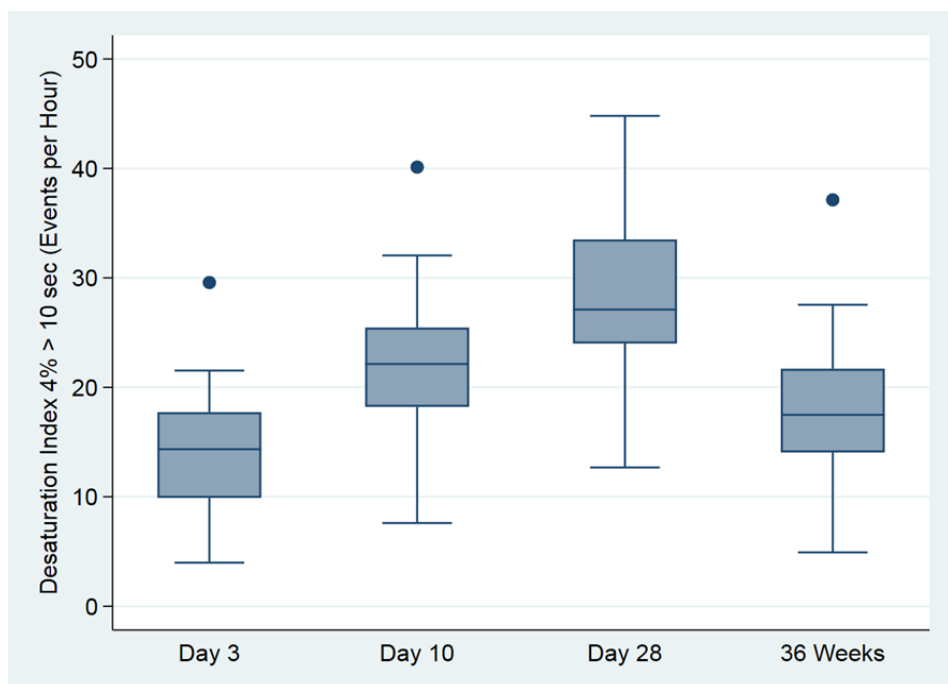


Figure 47: Desaturation Event Index 4% > 10 seconds over time

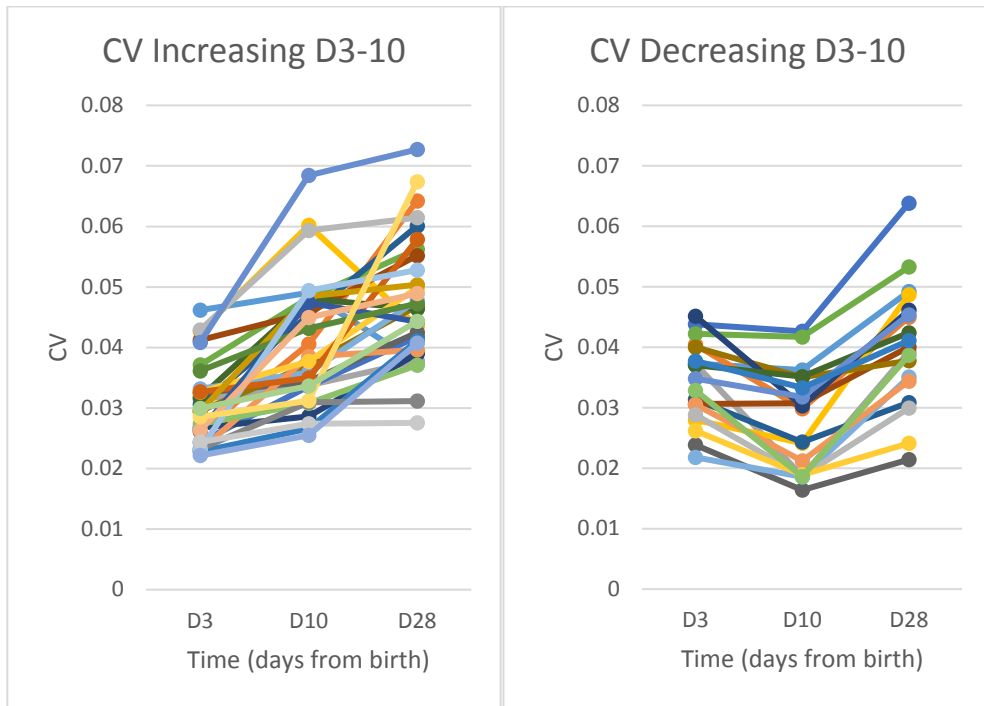


Figure 48: Individual trajectories of oxygen saturation CV over time

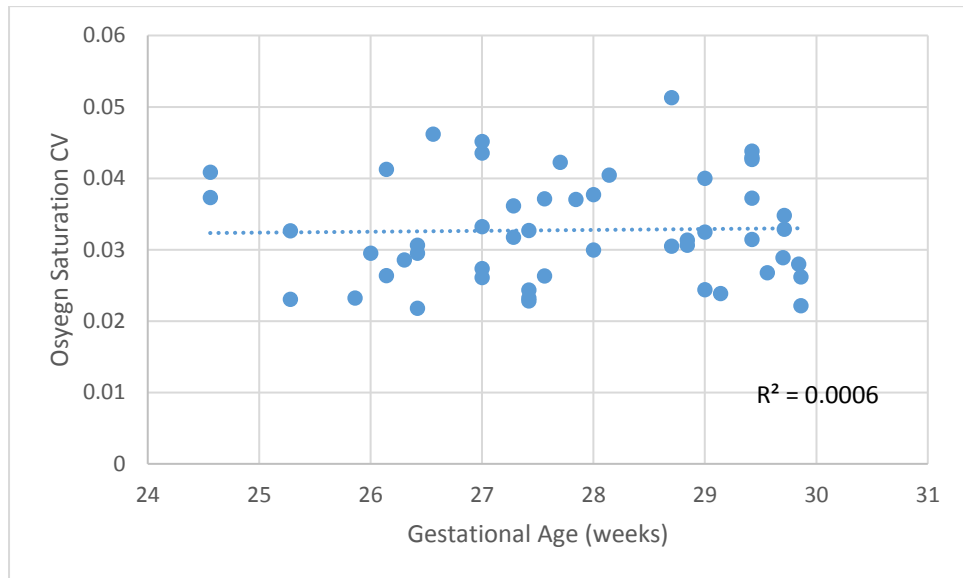


Figure 49: Day 3 coefficient of variation of oxygen saturation by gestational age

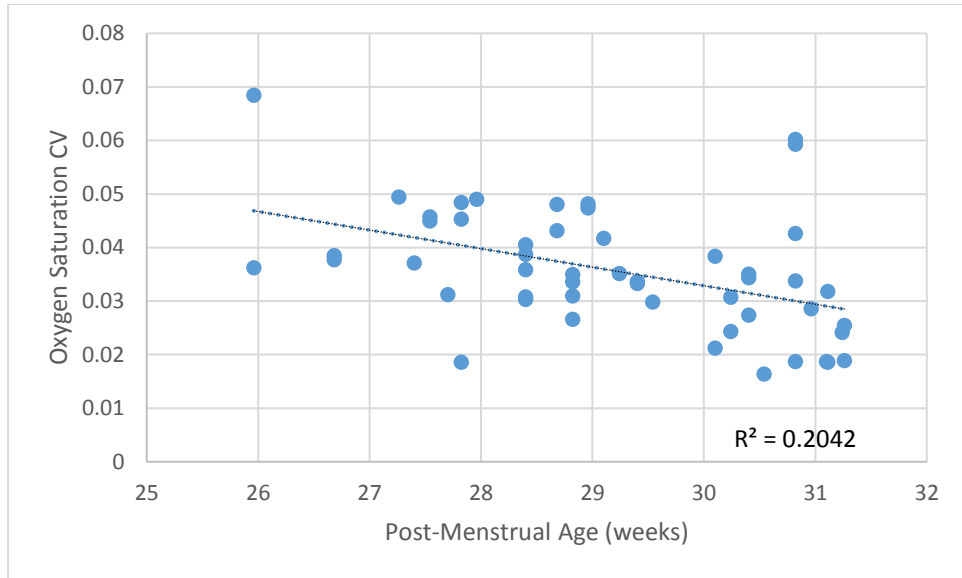


Figure 50: Day 10 coefficient of variation of oxygen saturation by post-menstrual age

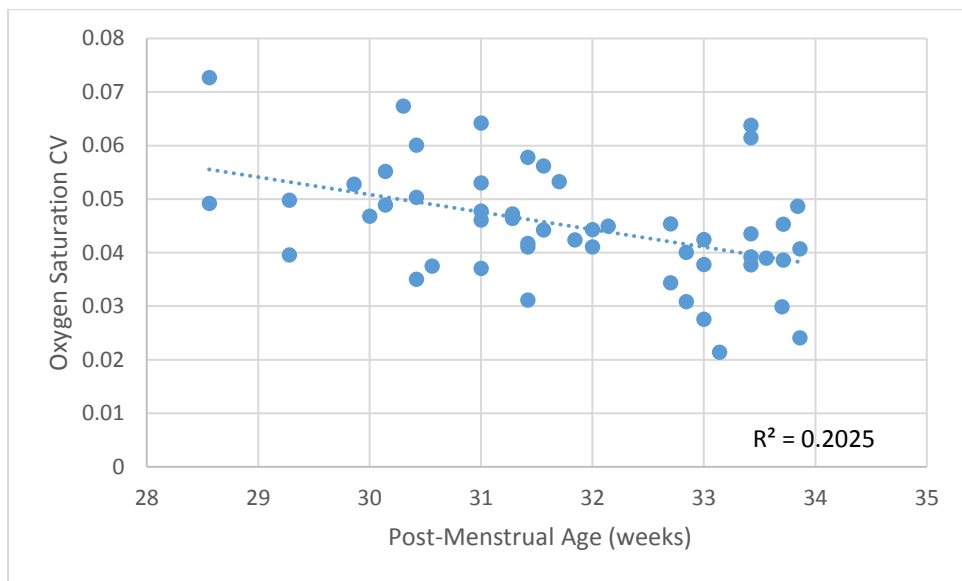


Figure 51: Day 28 coefficient of variation of oxygen saturation by post-menstrual age

Hyperoxia

The median (IQR) hours in oxygen during the first 72 hours of life was 15 (3-45). Seven infants did not have any oxygen after admission to NICU during the first 72 hours of pulse oximetry recording. The median (IQR) percent time spent in target oxygen saturation range while on oxygen during the first 72 hours was 20.4% (10.1-35.6). The median (IQR) percent time spent hyperoxic (oxygen saturations >95%) while on oxygen during the first 72 hours was 42.2% (17.1-61.5).

Bronchopulmonary dysplasia and time spent with oxygen saturations

<88% or < 80%

Percentage time spent with oxygen saturations <88% or <80% by BPD status (ANZNN definition) and BPD severity (NICHD definition) are shown in Tables 38-40 and Figs. 52 and 55. Significant within group differences are seen as well as between group differences at each time period. Infants who went on to develop BPD developed greater percentage time with saturations < 88% or <80% by day 10 and had a higher peak on day 28.

Table 38: Percentage time spent with oxygen saturations <88% over time by BPD status (ANZNN definition)

Measure	No BPD (n=18)	BPD or Death (n=33)	P
<u>Day 3</u>			
Mean (SD)	2.7 (2.1)	4.6 (3.8)	0.08
Median	2.0	2.9	
IQR	1.5 – 3.6	1.7 – 6.5	
<u>Day 10</u>			
Mean (SD)	3.4 (4.2)	8.2 (6.3)	0.01
Median	1.7	6.5	
IQR	0.7 – 4.0	2.9 – 12.6	
<u>Day 28</u>			
Mean (SD)	4.6 (2.4)	12.2 (8.0)	<0.001
Median	4.6	9.3	
IQR	2.3 – 5.9	6.8 – 17.2	
<u>36 weeks PMA</u>			
Mean (SD)	2.5 (1.9)	4.5 (2.9)	0.02
Median	1.9	3.4	
IQR	1.4 – 2.8	2.2 – 7.4	

Repeated measures ANOVA: Effect of Group (BPD or Death) $F(1,46)=12.2$, $p<0.01$; Effect of Time $F(3,138)=23.4$, $p<0.001$; Group x Time interaction $F(3,138)=4.9$, $p<0.005$

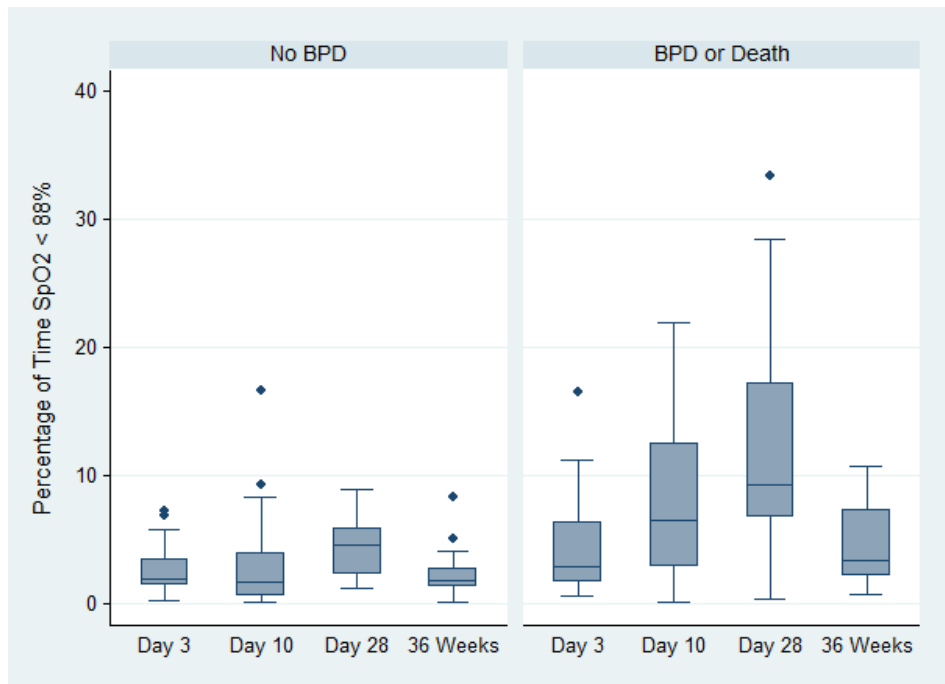


Figure 52: Percentage time oxygen saturations <88% by BPD status

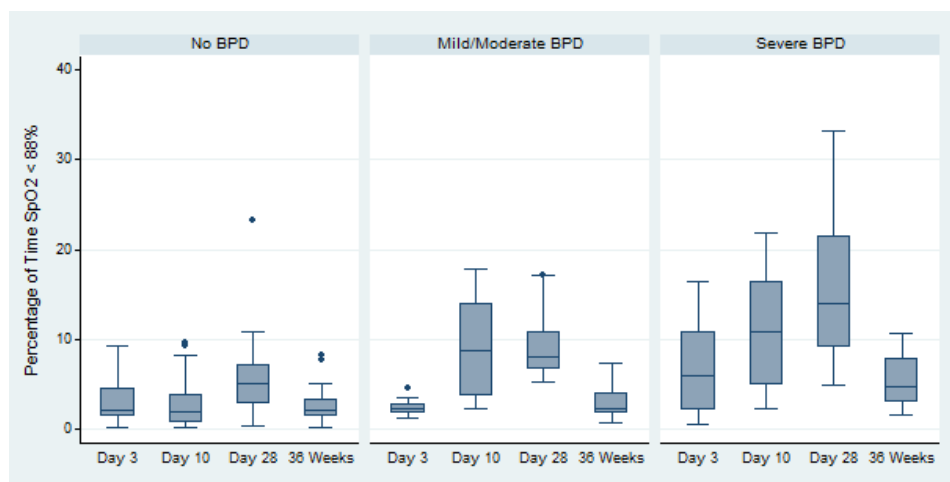


Figure 53: Percentage time oxygen saturations <88% by BPD grade

Repeated measures ANOVA: Effect of Group (NICHD BPD grade) $F(2,45)=16.2$, $p<0.001$; Effect of Time $F(3,135)=27.2$, $p<0.001$; Group x Time interaction $F(6,135)=7.1$, $p<0.001$

Table 39: Percentage time with oxygen saturations <80% over time by BPD status (ANZNN definition)

Measure	No BPD (n=18)	BPD or Death (n=33)	p
<u>Day 3</u>			
Mean (SD)	0.43 (0.43)	0.57 (0.50)	0.36
Median	0.24	0.43	
IQR	0.16 – 0.56	0.22 – 0.80	
<u>Day 10</u>			
Mean (SD)	0.41 (0.85)	1.04 (0.96)	0.05
Median	0.10	0.80	
IQR	0.00 – 0.20	0.40 – 1.40	
<u>Day 28</u>			
Mean (SD)	0.84 (0.67)	2.14 (1.69)	0.008
Median	0.75	1.80	
IQR	0.30 – 1.20	1.00 – 2.60	
<u>36 weeks PMA</u>			
Mean (SD)	0.46 (0.50)	1.07 (0.91)	0.01
Median	0.30	0.80	
IQR	0.20 – 0.60	0.37 – 1.60	

Repeated measures ANOVA: Effect of Group (BPD or Death) $F(1,46)=8.9$, $p<0.005$; Effect of Time $F(3,138)=18.6$, $p<0.001$; Group x Time interaction $F(3,138)=3.5$, $p=0.02$

Table 40: Percentage time spent with oxygen saturations <80% by BPD grade (NICHD definition)

Measure	No BPD (n=27)	Mild – Mod BPD (n=9)	Severe BPD (n=13)	p
<u>Day 3</u>				
Mean (SD)	0.47 (0.47)	0.60 (0.48)	0.60 (0.53)	0.73
Median	0.27	0.57	0.44	

Measure	No BPD (n=27)	Mild – Mod BPD (n=9)	Severe BPD (n=13)	p
IQR	0.20 – 0.67	0.16 – 0.80	0.35 – 0.81	
<u>Day 10</u>				
Mean (SD)	0.36 (0.44)	1.04 (1.05)	1.52 (1.25)	<0.001
Median	0.20	0.60	1.30	
IQR	0.00 – 0.50	0.40 – 1.10	0.80 – 1.70	
<u>Day 28</u>				
Mean (SD)	1.07 (0.99)	1.72 (1.29)	2.81 (2.10)	0.004
Median	0.80	1.30	2.00	
IQR	0.37 – 1.30	1.00 – 2.00	1.70 – 3.10	
<u>36 weeks PMA</u>				
Mean (SD)	0.61 (0.68)	0.79 (0.91)	1.38 (0.91)	0.02
Median	0.30	0.40	1.20	
IQR	0.20 – 0.70	0.20 – 1.10	1.00 – 1.70	

Repeated measures ANOVA: Effect of Group (NICHD status) $F(2,45)=8.6$, $p<0.001$; Effect of Time $F(3,135)=19.3$, $p<0.001$; Group x Time interaction $F(6,135)=3.3$, $p=0.005$

Bronchopulmonary dysplasia and coefficient of variation and desaturation index

Tables 42-44 and Figs. 54-59 show summary statistics for CV, DSI (4%) and DSI (4%) >10seconds by BPD status.

BPD and coefficient of variation

Overall there was a significant difference in CV between those with BPD and those without and a significant difference in CV over time. The differences in CV between BPD and no BPD were most noticeable on day 10 but continued and were still apparent at 36

weeks PMA. Although infants start out at a similar point for day 3 CV, for those who do not go on to develop BPD, the day 10 CV tends to remain stable or even fall where as it tends to rise for those who develop BPD (Fig. 54). The greatest rise tends to be seen in those who go on to develop severe BPD (Fig. 55). However, this does not hold true for all infants as there is some overlap in these parameters. Of note some infants without BPD still experience significant instability in oxygen saturations even at 36 weeks PMA.

Table 41: Coefficient of variation of oxygen saturation over time by BPD status (ANZNN definition)

Measure	No BPD (n=18)	BPD or Death (n=33)	P
<u>Day 3</u>			
Mean (SD)	0.030 (0.007)	0.034 (0.007)	0.12
Median	0.029	0.033	
IQR	0.024 – 0.033	0.029 – 0.033	
<u>Day 10</u>			
Mean (SD)	0.029 (0.011)	0.039 (0.010)	0.007
Median	0.027	0.038	
IQR	0.021 – 0.033	0.034 – 0.045	
<u>Day 28</u>			
Mean (SD)	0.038 (0.008)	0.049 (0.010)	0.002
Median	0.039	0.048	
IQR	0.031 – 0.043	0.042 – 0.054	
<u>36 weeks PMA</u>			
Mean (SD)	0.031 (0.008)	0.038 (0.010)	0.01
Median	0.028	0.038	
IQR	0.027 – 0.033	0.031 – 0.045	

Repeated measures ANOVA: Effect of Group (BPD or Death) $F(1,45)=12.3, p<0.001$; Effect of Time $F(3,135)=27.3, p<0.001$; Group x Time interaction $F(3,135)=1.58, p=0.20$

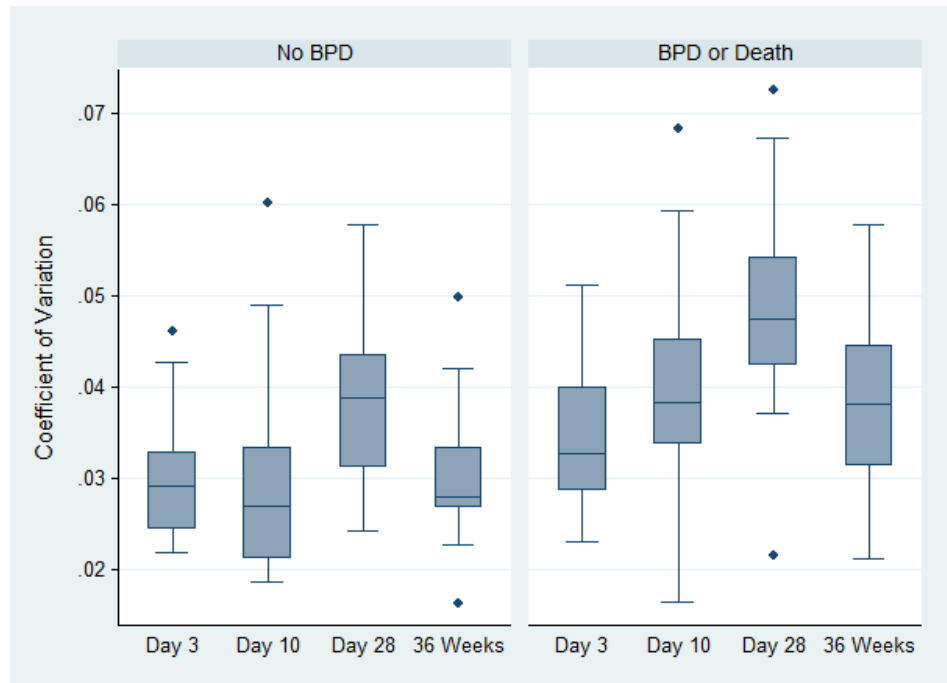


Figure 54: Coefficient of variation of oxygen saturation over time by BPD status

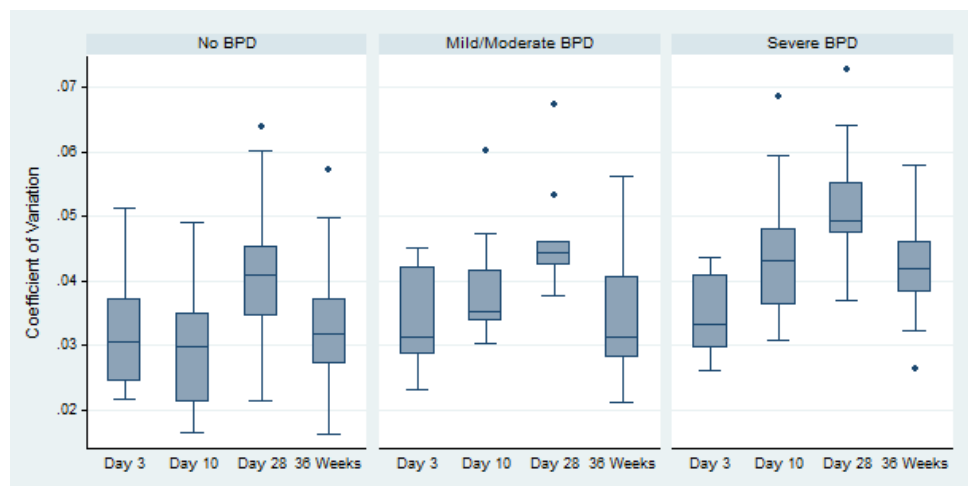


Figure 55: Coefficient of variation of oxygen saturation over time by BPD grade (NICHD definition)

Repeated measures ANOVA: Effect of Group (NICHD status) $F(2,44)=7.4, p=0.002$; Effect of Time $F(3,132)=28.5, p<0.001$; Group x Time interaction $F(6,132)=2.3, p=0.04$

BPD and desaturation event index

The DSI4% shows a clear and significant time trend with peak frequency around day 28 followed by a decline. There was only modest evidence of differences by BPD status (ANZNN) around day 10 with higher DSI4% in those with a subsequent diagnosis of BPD (Fig. 56, Table 42). However, elaboration by BPD severity (NICHD grade) revealed stronger differences on days 10 and 28, but reflected a tendency for those with mild to moderate BPD to have a higher frequency of events than those with severe BPD (Fig. 57).

These trends are echoed more strongly in the DSI4% >10sec data, with a clear peak at day 28 and the strongest differences by BPD status on days 10 and 28 with the mild to moderate BPD group peaking higher than the severe BPD group (Table 44)(Figs.58,59)

Table 42: Desaturation event index 4% over time by BPD status (ANZNN definition)

Measure	No BPD (n=18)	BPD or Death (n=33)	P
<u>Day 3</u>			
Mean (SD)	19.9 (7.4)	21.2 (6.5)	0.39
Median	18.7	21.9	
IQR	15.5 – 23.4	16.6 – 26.0	
<u>Day 10</u>			
Mean (SD)	30.9 (9.5)	38.9 (11.3)	0.02
Median	31.3	38.4	
IQR	23.7 – 37.2	32.6 – 43.8	
<u>Day 28</u>			
Mean (SD)	58.1 (10.1)	56.2 (13.2)	0.59
Median	58.6	56.6	
IQR	47.9 – 66.0	48.6 – 64.4	

36 weeks PMA			
Mean (SD)	50.2 (16.0)	51.8 (23.0)	0.79
Median	51.8	50.8	
IQR	42.3 – 60.5	39.0 – 64.9	

Repeated measures ANOVA: Effect of Group (BPD or Death) $F(1,46)=0.85$, $p=0.36$; Effect of Time $F(3,138)=79.6$, $p<0.001$; Group x Time interaction $F(3,138)=1.19$, $p=0.31$

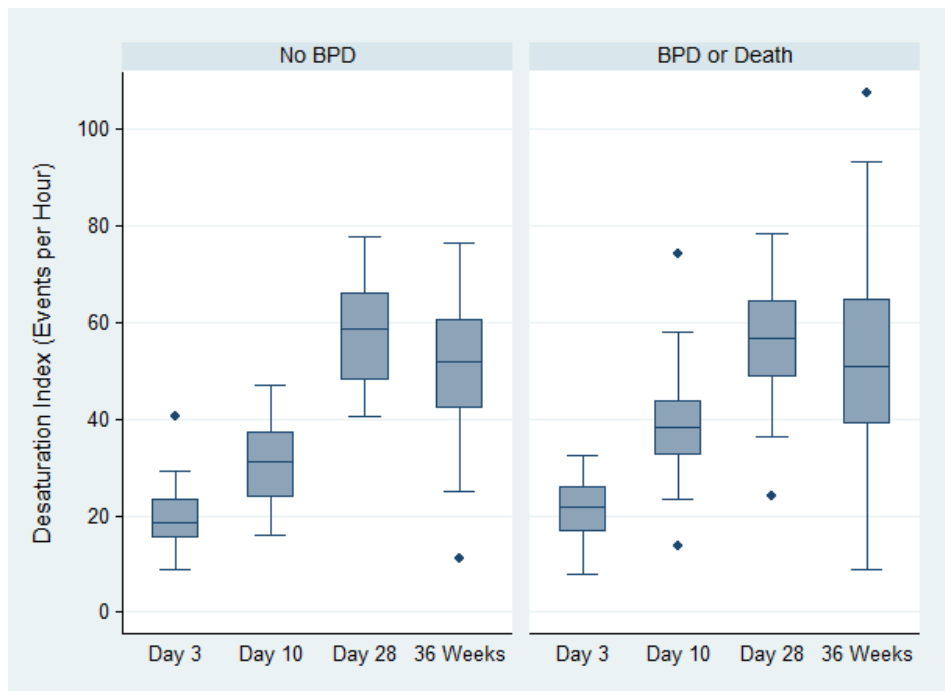


Figure 56: DSI4% over time by BPD status

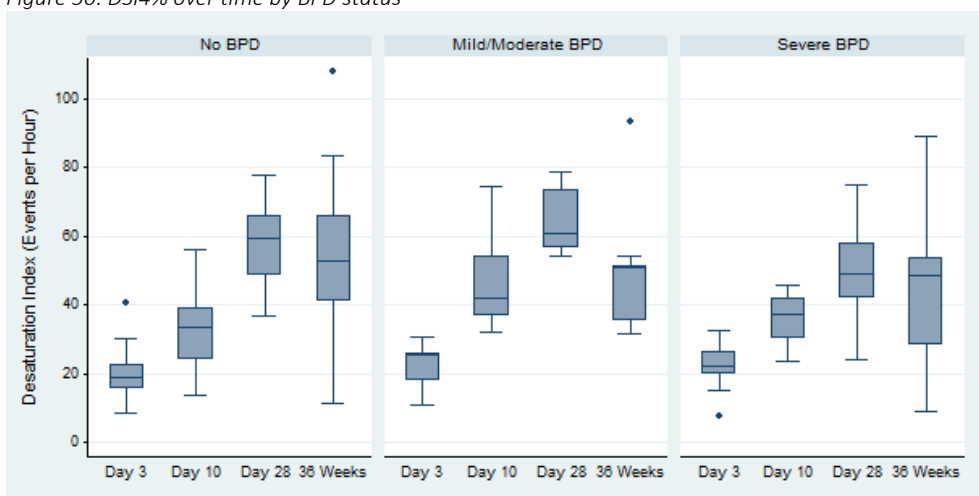


Figure 57: DSI4% over time by BPD grade

Repeated measures ANOVA: Effect of Group (NICHD BPD grade) $F(2,45)=2.6$, $p=0.09$; Effect of Time $F(3,135)=84.3$, $p<0.001$; Group x Time interaction $F(6,135)=2.50$, $p=0.03$

Table 43: Desaturation event index 4% >10 seconds by BPD (ANZNN definition)

Measure	No BPD (n=18)	BPD or Death (N=33)	P
<u>Day 3</u>			
Mean (SD)	13.7 (5.3)	14.4 (4.8)	0.64
Median	12.5	14.8	
IQR	10.2-15.8	10.4-17.8	
<u>Day 10</u>			
Mean (SD)	18.9 (5.2)	23.9 (5.8)	0.004
Median	18.5	23.5	
IQR	14.3-23.2	21.4-27.5	
<u>Day 28</u>			
Mean (SD)	23.8 (4.9)	30.2 (6.9)	0.001
Median	24.2	30.4	
IQR	20.6-26.3	26.1-35.1	
<u>36 weeks PMA</u>			
Mean (SD)	16.7 (5.0)	18.3 (6.5)	0.39
Median	16.5	18.1	
IQR	13.1-19.9	14.8-22.4	

Repeated measures ANOVA: Effect of Group (BPD or Death) $F(1,46)=8.2$, $p=0.006$; Effect of Time $F(3,138)=67.7$, $p<0.001$; Group x Time interaction $F(3,138)=2.4$, $p=0.07$

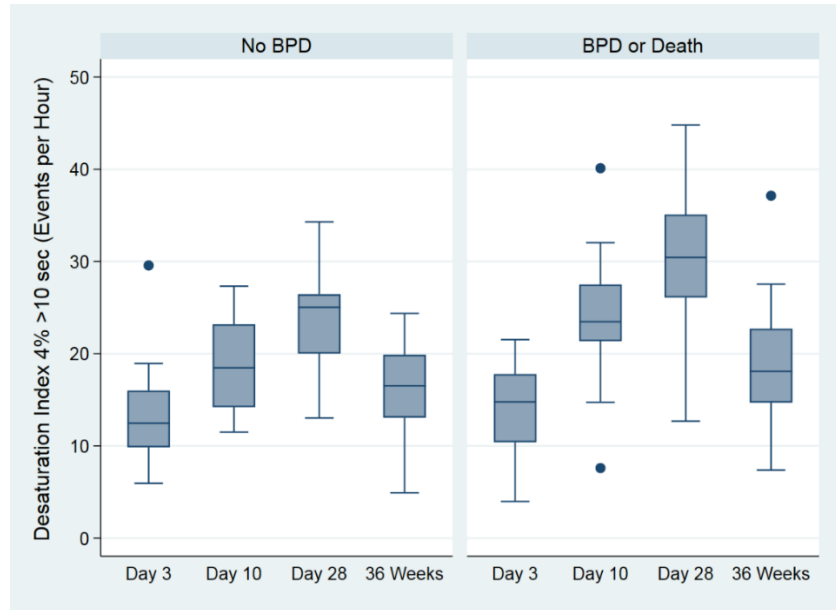


Figure 58: Desaturation Event Index 4% > 10 seconds over time by BPD status

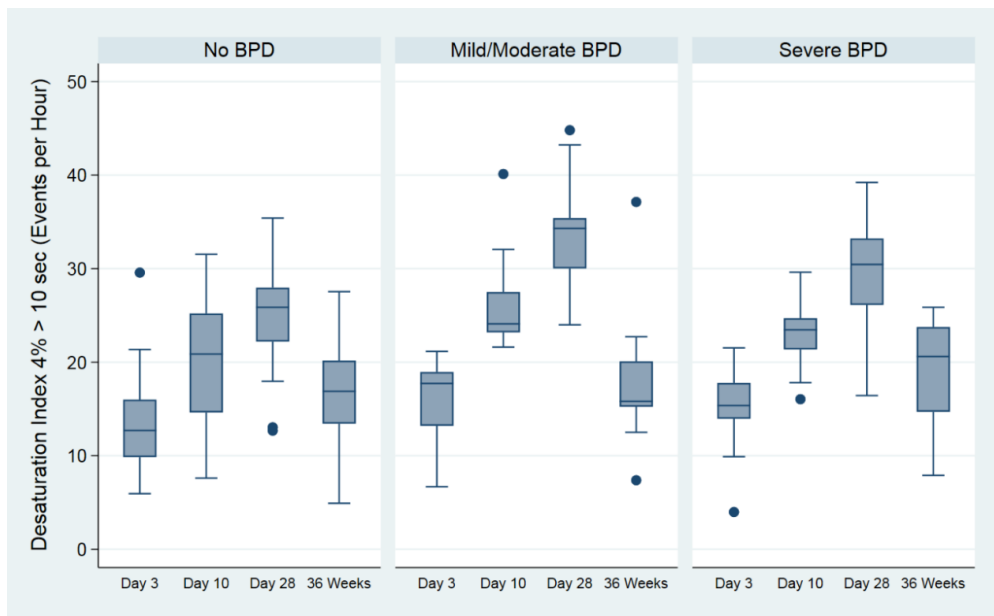


Figure 59: Desaturation Event Index 4% > 10 seconds by BPD grade

Repeated measures ANOVA: Effect of Group (NICHD BPD grade) $F(2,45)=5.9, p=0.005$; Effect of Time $F(3,135)=68.8, p<0.001$; Group x Time interaction $F(6,135)=2.1, p=0.06$

Adjustment for covariates

Table 44 demonstrates that when adjustments are made for the covariates of gender, birth gestation, birthweight z score and day 3 HsPDA status there is very little difference in measures of oxygen saturation instability between those with and without BPD/death by 36 weeks PMA with the exception of CV. More significant differences are seen in parameters on days 10 and 28 with CV, time spent with oxygen saturations <88% and DSI4%>10 seconds appearing to offer more reliable discrimination.

Table 44: Mean oxygen saturation parameters according to BPD status but adjusted for gender, birth gestation, birthweight z-score and HsPDA on day 3

Measure	No BPD (n=18)	BPD or Death (N=33)	P
Coefficient of Variation			
Day 3	0.032	0.033	0.54
Day 10	0.031	0.038	0.002
Day 28	0.040	0.048	0.001
36 weeks PMA	0.032	0.038	0.01
% Time SpO ₂ <88%			
Day 3	3.7	4.0	0.77
Day 10	4.3	7.7	0.009
Day 28	5.5	11.7	0.001
36 weeks PMA	3.3	4.1	0.05
% Time SpO ₂ <80%			
Day 3	0.60	0.48	0.66
Day 10	0.58	0.94	0.17
Day 28	1.01	2.05	0.001
36 weeks PMA	0.58	1.00	0.12

Measure	No BPD (n=18)	BPD or Death (N=33)	P
DSI4%			
Day 3	18.1	22.2	0.28
Day 10	29.1	39.9	0.005
Day 28	56.3	57.2	0.80
36 weeks PMA	48.4	52.7	0.28
DSI 4% > 10 seconds			
Day 3	13.3	14.6	0.44
Day 10	18.6	24.1	0.001
Day 28	23.4	30.4	<0.001
36 weeks PMA	16.2	18.5	0.17

Audit of alarm limit settings

Summary

The results of the audit of compliance with oxygen saturation alarm limit protocols are summarised in Tables 46, 47. In total 504 alarm settings were audited on a total of 91 different infants on 49 different days. The median (range) number of data points for each infant was 3(1-44). Of the 504 alarm settings audited, on 245 occasions infants were in air and in the remaining 259 on oxygen. Of the alarm limits audited 54% were during a day shift and 46% an evening or night shift. Of all audited alarm settings 45% were incorrect, 54% for those on oxygen. Both upper and lower alarm settings were incorrect at a higher frequency in infants on oxygen across all groups, compared to infants in air. It was uncommon for both limits to be incorrect.

Upper alarm limit

The upper alarm setting was incorrect more frequently in those infants on oxygen who also had a higher rate of major breeches. Overall 20% of infants on oxygen had their upper alarm limit set at 100%. Of infants on oxygen who were <32weeks PMA, 20% had upper limit set incorrectly at 100% compared to 15% of those 32-36 weeks PMA and 24% of those >36weeks PMA. Of the 39 infants <28 weeks PMA on oxygen, 44% had their upper alarm limit at 100%.

Lower alarm limit

The lower alarm setting was incorrect more frequently in infants who were in air and, with the exception of the 32-36 week PMA group infants in air also had a higher rate of major breeches of lower alarm limit.

Table 45: Overall comparison of alarm compliance according to post-menstrual age

	Overall n=504 (%)	<32 Weeks n=272 (%)	32-36 Weeks n=121 (%)	>36 Weeks n=111 (%)
Either limit incorrect	227 (45)	123 (45.2)	61 (50.4)	43 (38.7)
Both incorrect	23 (4.6)	5 (1.8)	14 (11.6)	4 (3.6)
Upper incorrect only	135 (26.8)	96 (35.3)	23 (19)	16 (14.4)
Major Breech	60 (11.9)	45 (16.5)	15 (12.4)	0 (0)
Lower incorrect only	115 (22.8)	32 (11.8)	52 (43)	31 (27.9)
Major Breech	53 (10.5)	15 (5.5)	18 (14.9)	20 (18)

Table 46: Alarm limit compliance by post-menstrual age and oxygen status

	<u>All</u> Oxygen n=259 <i>Air</i> n=245	<u><32 Weeks</u> Oxygen n=147 <i>Air</i> n=125	<u>32-36 Weeks</u> Oxygen n=58 <i>Air</i> n=63	<u>>36 Weeks</u> Oxygen n= 54 <i>Air</i> n=57
% Either incorrect				
Oxygen	54.4	62.6	58.6	27.8
<i>Air</i>	35.1	24.8	42.9	49.1
% Both incorrect				
Oxygen	15.6	3.4	24.1	5.5
<i>Air</i>	1.2	0	0	1.7
% Upper incorrect				
Oxygen	49.4	62.6	39.6	24.1
<i>Air</i>	2.8	3.2	0	5.3
%Major breech (upper)				
Oxygen	5	28.6	25.9	0
<i>Air</i>	1.2	2.4	0	0
% Lower incorrect				
Oxygen	13.5	3.4	43.1	9.2
<i>Air</i>	32.6	21.6	42.8	45.6
%Major breech (lower)				
Oxygen	5	1.4	15.5	3.7
<i>Air</i>	16.3	10.4	14.3	31.6

Survey of nursing opinion

Of the 100 nurses employed in the neonatal unit 50 nurses responded to our survey either in electronic or written form.

Nursing experience of participants

The majority of nurses worked rotating day and night shifts (72.5%) with 9.8% working only day shift and 17.6% working only night shift. Mean (SD) hours of work per week was 33.4 (8.7). The mean (SD) years of neonatal nursing experience of respondents was 14.1 (9.7) with 88.2% trained to work in the intensive care area of the neonatal unit.

Nursing knowledge of oxygen saturation targeting

Of respondents, 76.5% recalled being orientated to the unit policy on oxygen saturation targeting and alarm limit setting. Just over a quarter (26.9%) had attended an education session that included a discussion of oxygen saturation targeting within the last year, 9.6% 1-2 years ago, 40.4% more than 2 years ago and 23.1% stated they had never attended an education session where this was discussed.

Overall spontaneous recall of the old or new oxygen saturation targets was poor (Tables 48, 49). Most nurses (92.3%) understood that the gestational age based targets should be based on post-menstrual age not birth gestation.

Table 47: Recall of old oxygen saturation targets

<u>Old oxygen saturation targets</u>						
	<u><32 wks</u> <u>air</u>	<u><32 wks</u> <u>oxygen</u>	<u>32-36 wks</u> <u>air</u>	<u>32-36 wks</u> <u>oxygen</u>	<u>>36 wks</u> <u>air</u>	<u>>36 wks</u> <u>oxygen</u>
%Both Correct	56.3	34.4	43.8	21.9	14.7	11.8
%Lower Correct	65.6	65.6	50	50	14.7	23.5
%Upper Correct	78.1	40.6	87.5	34.4	100	44.1

Table 48: Recall of new oxygen saturation targets

<u>New oxygen saturation protocol</u>				
	<u><36 wks air</u>	<u><36 wks oxygen</u>	<u>>36 wks air</u>	<u>>36 wks oxygen</u>
%Both Correct	48.6	43.2	62.2	48.6
%Lower Correct	48.6	56.8	62.2	62.2
%Upper Correct	97.3	56.8	97.3	67.6

Nursing practice and opinion on managing oxygen saturation targets

The majority of nurses (76.9%) felt that both hypoxia and hyperoxia were potentially harmful to very preterm infants while 17.9% responded that hypoxia was more harmful and 5.1% responded that hyperoxia was more harmful. 70% of respondents cited the infant being too labile and desaturating too frequently as being the main reason why it was challenging to maintain infants within their targets. One fifth of nurses (20%) felt the targets were too narrow, 2.5% felt the targets were too high, none felt they were too low and 7.5% cited other reasons.

When asked what percentage of time they felt it was possible to keep infants < 30 weeks PMA within their targets nurses responses were variable (Fig. 60):

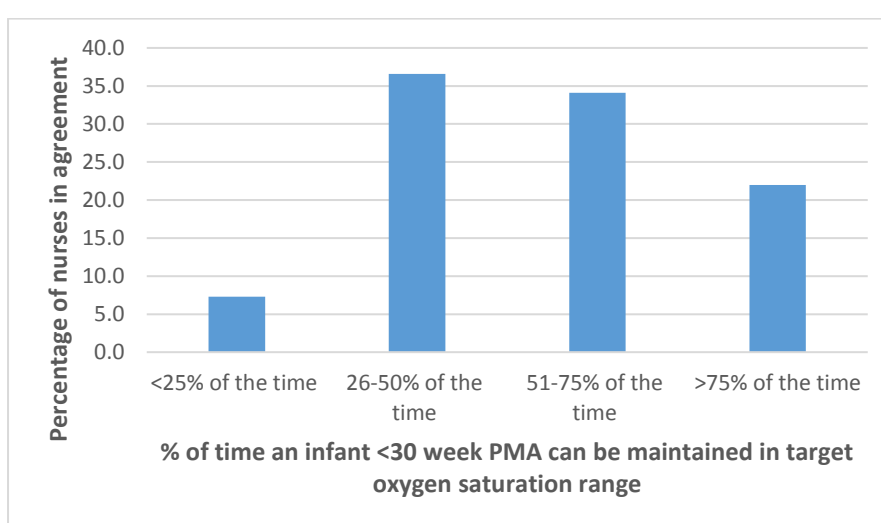


Figure 60: Nursing opinion on percentage of time it is possible to keep infants <30weeks gestation in target range

There was varying opinion on how what proportion of infants < 30 weeks PMA it is feasible to keep within their target range with only 17.1% stating this could be achieved in most infants (few infants= 29.3%, some infants = 31.7%, don't know 22%).

When asked if they would alter alarm limits if faced with an unstable infant who was frequently alarming 85.4% said no, 14.6% said yes. However, if an infant was doing well 22.2% said this would prompt them to be more lenient with their target range. 36.6% said gestational age influenced them being more lenient with targets.

Survey results suggested a discussion of oxygen saturation targets or a review of alarm settings was not a routine part of nursing or medical handover (Figs. 61, 62).

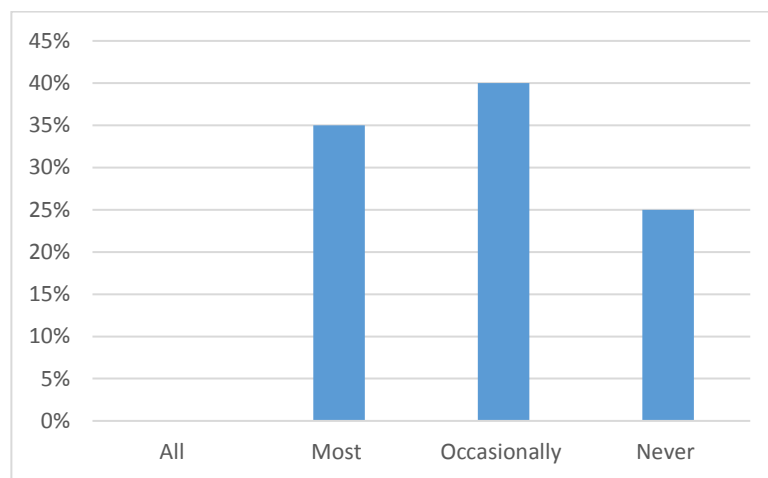


Figure 61: Nursing opinion on percentage of nursing handovers that oxygen saturation targets alarm settings are discussed

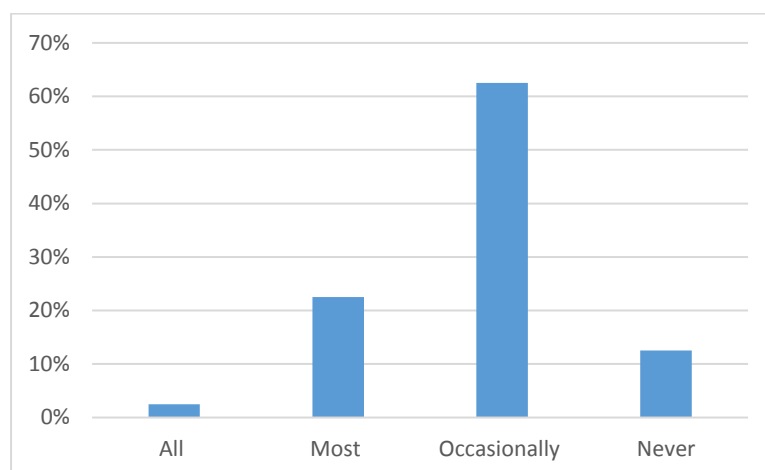


Figure 62: Nursing opinion on percentage of medical ward rounds where oxygen saturation targets or alarm settings are discussed

Discussion

Our study demonstrated that oxygen saturation instability tended to peak around day 28 but that there was wide variability in all parameters measured. Most indices were worse in those that developed bronchopulmonary dysplasia but this difference was only significant in some parameters on day 10 and 28. Despite an experienced nursing team and a clear unit protocol, compliance with oxygen saturation alarm limits was poor raising concern that premature infants may be exposed to iatrogenic hyperoxia. Staff find maintaining very premature infants in an optimal oxygen saturation range is challenging.

Measures of oxygen saturation instability

Oxygen saturation monitoring has become a fundamental part of the care of the preterm newborn. New technology now allows us to store and extract more detailed data and graph trends over time. In our cohort, measures of oxygen saturation instability all tended to worsen between the first 3 days of life and day 28 and then improve by 36 weeks PMA to a level similar to that seen on day 3. This is consistent with a study by DiFiore.²⁶⁷ Our findings at 36 weeks PMA are similar to a measures of instability studied in another New Zealand cohort of preterm infants at discharge.³⁷⁶

With the exception of DSI4%, all parameters (including DSI4%>10s) were worse in those babies who developed bronchopulmonary dysplasia with these differences becoming apparent by day 10. However, there was significant overlap between groups and over time. DSI4%>10 seconds, although reported by PROFOX reports may not be clinically meaningful in terms of neurodevelopmental outcomes as other groups only showed effects with oxygen saturations <80% for greater than 1 minute.⁶⁶ The most reliable and discriminating indices of oxygen saturation instability were the coefficient of variation and time oxygen saturations <88%.

Alarm limit compliance and nursing opinion of oxygen saturation targeting

Neonatal intensive care nurses are highly skilled practitioners and play a vital role in oxygen saturation targeting. However, review of oxygen saturation alarm limit compliance in our unit revealed similar findings to a study by Clucas et al.²⁸³ There was a high rate of incorrectly set alarm limits despite our nursing survey revealing an experienced nursing workforce. This non-compliance was higher in babies on oxygen with a high rate of alarms being set at 100% in infants less than 28 weeks PMA who are particularly vulnerable to the harmful effects of hyperoxia. Despite being displayed at cot side nursing staff found it difficult to recall alarm limit protocols. In addition three-

quarters of nurses surveyed had either never attended an education session on oxygen saturation targeting or attended one greater than 2 years ago. It was clear that routine review of oxygen saturation targeting on nursing and medical handovers was not part of unit culture. Despite knowledge about risks of hypoxia/hyperoxia the majority of nursing staff responded that moderately preterm infants are inherently unstable and challenging to keep within current targets. This is a known difficulty in nursing very premature infants.²⁸⁰ A study by Nghiem et al also showed that 71% of nurses cited infant lability as the primary reason for infants not being maintained in target.²⁸⁴ More research is needed on the effect of human factors on oxygen saturation targeting and whether technology can be better utilised to improve this practice.

Strengths and weaknesses

Strengths of our study include a prospective well characterised cohort of at risk infants that had detailed analysis of a number of measures of oxygen saturation instability over four time points from birth to near term. We also attempted to review human factors that might contribute to infants being exposed to preventable hypoxia and hyperoxia. Weaknesses include the discontinuous oxygen saturation data that does not allow a comprehensive analysis of trends in instability over the whole time course of admission. During the period of monitoring we also did not have continuous inspired oxygen concentration data and instead had to rely on assumptions based on hourly nursing observations. We only reported time in target for the first three days and only in infants on oxygen. One of the difficulties in reporting time in target is the wide variation in targets used across units and the variable targets for when breathing oxygen compared to air and changing targets as infants mature. This leads to data that is difficult to interpret and fraught with potential inaccuracy and we felt it was best omitted. Although an attempt was made to exclude artefact from the oximetry data it is acknowledged that pulse oximeters do have a degree of error and different methods for scanning for aberrant data can affect results.³⁷⁶ We also acknowledge that other researchers have used different set points for defining hypoxia which will need to be considered when comparing studies.⁶⁶ In addition our audit of alarm limits was performed on a different set of babies and our nursing survey conducted after data collection on our preterm cohort. We did not use a validated survey as we were unable to find one that met our needs and this highlights further work needed in this area. Ultimately we are unable to distinguish whether periodic hypoxemia promotes the development of BPD or whether evolving BPD makes infants more prone to periodic hypoxemia. This is observational data

only and intended to address some of the knowledge gaps regarding the comparison of various currently available measures of oxygen saturation instability in the preterm population overtime. Going forward, further study is needed on continuous oxygen saturation data on larger cohorts to identify which measures best correlate with clinically important outcomes.

Implications for practice

Our data showed that while on oxygen during the first three days of life infants spend only one fifth of their time in the target oxygen saturation range and almost half of this time hyperoxic. This is concerning because excess oxygen exposure may be a preventable cause of subsequent pulmonary vascular maldevelopment. Conversely although all infants tended to have increasingly unstable oxygen saturations up to day 28 some infants spent up to a third of their time with oxygen saturations below 88%. Not only might this increase the risk of poorer long term neurodevelopmental outcomes but in infants who have dysfunctional vasoactive pulmonary vessels hypoxia may precipitate a pulmonary hypertensive crisis. The common practice of hourly recordings of oxygen saturation and inspired oxygen concentration by nursing staff which informs clinical decision making does not capture detailed oxygen exposure and oxygen saturation instability data. We have demonstrated that pulse oximeters with downloadable memory offer the chance to use more discriminating data to further refine patient care.

Our findings suggest steps that could be taken to improve oxygen saturation targeting and alarm compliance. Very narrow targets that are not evidenced based may be very challenging for nursing staff prompting frequent alarms and risking alarm fatigue. A review of alarm settings and oxygen saturation instability should be part of nursing and medical handovers. Preset alarms according to unit protocol should be programmed into the cardiorespiratory monitor with birth gestation inputted on admission to ensure correct initial settings. Monitors should include a feature whereby if alarms are altered outside unit protocol staff are asked to confirm they want to override this and document a reason. Histograms of oxygen saturation data with time in target highlighted and a review of the coefficient of variation over time could provide a much more sophisticated look at respiratory stability than hourly recording charts.

Future directions

A number of research questions remain. Our cohort demonstrated, as others have, that the most unstable period for oxygen saturations for a preterm infant is not soon after delivery when the lungs are at their most immature and undergoing functional transition, but one month later.²⁶⁷ A similar pattern has been found in term infants.³⁷⁷ Oxygen saturation is a respiratory endpoint that has multiple contributing factors. The factors that lead one infant to be relatively stable while another has swinging saturations are still unclear and likely to be multifactorial. However, it seems that this propensity for instability begins in the first ten days of life. Whether antenatal factors, such as maternal smoking, intrauterine and post-natal growth restriction and chorioamnionitis contribute, requires further study. Gestational age is clearly important and represents the degree to which normal foetal development has been disrupted. These factors combined with postnatal factors such as, persistent ductus arteriosus, infection, inflammation, oxygen exposure, positive pressure ventilation and nutrition contribute to this variation in respiratory stability. Infant care factors such as handling, pain and environmental light and noise are also important. The contribution of evolving pulmonary vascular disease to oxygen saturation instability is uncertain and difficult to differentiate from other causes of intermittent hypoxia but merits further investigation.

It is clearly challenging to maintain infants with significant respiratory disease within recommended oxygen saturation targets. Automated oxygen delivery systems may offer greater refinement of oxygen saturation targeting. New algorithms for automated oxygen delivery to maintain oxygen saturation targets have shown promising results such that we are likely to see larger clinical trials soon.³⁷⁸⁻³⁸⁰ Better utilisation of bedside technology may allow clinicians and nursing staff to further refine oxygen therapy and respiratory support in preterm infants and reduce iatrogenic harm. A comprehensive oxygen therapy stewardship programme from birth to discharge should be considered to improve current practice and reduce iatrogenic harm. As the causes of instability are complex, achieving this is likely to require a multifaceted approach. The advancement of technology that permits continuous high quality data acquisition and analysis holds promise in advancing our understanding in this field but human factors also need to be studied.

Health and Neurodevelopmental Outcomes

Beyond Hospital Discharge

Introduction

Globally the complications of premature birth are the second leading cause of death in children under the age of five with half of all babies born before 32 weeks in resource poor settings dying.³⁸¹ However, relatively simple measures can lead to significant survival gains in under-resourced settings.³⁸² Reducing neonatal mortality globally has been at the heart of the Millennium Development Goals and the World Health Organisation Every Newborn Action Plan.^{383 384} In contrast survival rates of even extremely preterm infants with access to neonatal intensive care are high.³⁸⁵⁻³⁸⁸

As survival rates after premature birth increase, the medical profession has an ethical responsibility to strive to ensure that the cost of survival is not severe neurodevelopmental disability or major medical morbidity. Children born prematurely are at risk of impaired lung function and increased risk of respiratory disease secondary to the effects of the lung underdevelopment and secondary damage caused by positive pressure ventilation, excess oxygen and inflammation.³⁸⁹⁻³⁹¹ The brain is also particularly sensitive to the impact of prematurity due to the effects of intraventricular haemorrhage, hydrocephalus and diffuse white matter injury. This leads to a higher rate of cerebral palsy, intellectual impairment, hearing deficits as well as attentional and behavioural difficulties and educational underachievement.¹¹ The story of retinopathy of prematurity in which blindness is caused by the interaction between the developing retina and exposure to high levels of oxygen, has been one of the great tragedies of neonatal medicine.³⁹²

Regional neonatal networks now work collaboratively to collect and analyse data on short and medium term neonatal outcomes in order to improve clinical care.^{393, 394 395} In addition, several large longitudinal cohort studies have been tracking long term outcomes for those born premature with some of those studies now providing data on health in adulthood.³⁹⁶⁻⁴⁰⁰

There are a number of challenges in comparing outcome data for children born prematurely. Gestational age is the single greatest factor determining outcome. Accurate assessment of gestational age requires early pregnancy ultrasound or accurate last menstrual period date with a regular cycle. However, there is a margin of error even with early scans and not all pregnancies are dated by ultrasound and last menstrual period dates can be unreliable. There are significant differences in the lower gestational age limit at which life preserving treatment is offered and survival figures need to be explicit about whether those not offered resuscitation have been included.⁴⁰¹ Obstetric and neonatal care varies across centres and studies have shown outcomes can vary considerably even across intensive care units within the same country.^{388, 402}

Cardiorespiratory morbidity after premature birth

Beyond the neonatal unit premature-born children are prone to frequent readmissions secondary to respiratory illness, particularly in the first two years of life, and demonstrate impaired lung function that persists even into adolescence and early adulthood.^{25, 389, 390, 403, 404 405 406, 407} In the first year of life half of all children with BPD will be readmitted to hospital, more than double the rate seen in those without BPD and significantly higher than the rate in term born infants.^{408, 409} Because formal assessment of lung function is rarely possible below the age of six,⁴¹⁰ assessment of respiratory function in the first 2 years beyond neonatal discharge is therefore inferred from clinical outcome data such as respiratory morbidity and readmission due to respiratory causes and/or need for respiratory support. Important sequelae, such as raised pulmonary artery pressure and subclinical ventricular dysfunction, have been demonstrated in survivors of bronchopulmonary dysplasia in early childhood.^{116, 411, 412} However, the contribution of pulmonary vascular disease to late respiratory morbidity is unclear.

Morbidity from premature birth can be enduring with increasing evidence of poorer cardiorespiratory health in adulthood including pulmonary hypertension, cardiac dysfunction and an increased risk of metabolic syndrome which may shorten life expectancy.^{17, 18, 45, 69, 71, 413, 414} Longitudinal studies are vital to fully understand the adverse effects of premature birth and to develop treatment strategies to mitigate these effects. Better prognostic markers are also needed to help identify premature infants most at risk for adverse outcomes.

Neurodevelopmental morbidity after premature birth

Neurodevelopmental morbidity is an important adverse consequence of premature birth. A range of standardised tests have been validated and are now widely used to assess neurological outcomes for premature infants. Neurodevelopmental impairment rates can vary depending on the age at which the assessment is performed and the type of test administered.³⁹⁷ The Bayley Scale of Infant and Toddler Development (San Antonio, TX: PsychCorp) is a comprehensive neurodevelopmental assessment tool that has been widely validated for the evaluation of children born prematurely.⁴¹⁵ The Bayley assessment is designed to screen children in order to identify those in need of more intensive developmental support and is often used to compare neonatal outcomes in research studies. The Bayley third edition generates scores for three composite parameters (cognitive, motor and language) within which there are subtests of receptive and expressive language and fine and gross motor ability. Recently concern has been expressed that the revision of the tool from Bayley II to III has led to underdiagnosis of cognitive impairment and overdiagnosis of motor disability.⁴¹⁶⁻⁴²⁰ Despite this debate, Bayley III remains widely used across Australasian neonatal units, with results being interpreted with caution and many recommending that impairment should be identified based on cut-points based on local reference data.⁴²¹

Early markers to predict morbidity

In the absence of reliable tools for accurate prediction of longer term cardiorespiratory and neurodevelopmental morbidity, there is increasing interest in identifying biomarkers that improve early detection and could guide preventive management for these conditions. BNP has been shown to be a powerful independent predictor of functional outcome after stroke in adults.⁴²² Impaired cardiac function in the foetus and neonate has been linked to impaired brain development⁴²³ while BPD-associated pulmonary hypertension is an independent risk factor for poor neurodevelopmental outcomes.^{114, 424} Prolonged hypoxic episodes in extremely premature infants are also associated with poorer neurodevelopmental outcomes.⁶⁶ Biomarkers of cardiac function (NTproBNP) or echocardiographic indices of ventricular function (TAPSE, TAPSV) or clinical markers of respiratory instability (oxygen saturation coefficient of variation) may offer additional prognostic information to clinical assessment tools to guide early surveillance and management.⁴¹⁵

Gaps in knowledge

Prematurely born infants are known to have greater respiratory morbidity in the first few years of life compared to term born peers, but clinical tools do not adequately identify those most at risk, and the contribution of pulmonary vascular disease is unknown. Studies of the utility of BNP and NTproBNP for predicting poor outcomes in premature infants have focused on BPD or death before hospital discharge. There have been no studies of early NTproBNP (a cardiac stress hormone) or TAPSE and TAPSV (ultrasound markers of right heart function) or oxygen saturation CV (a measure of oxygen saturation instability) as predictive markers of poor neurodevelopmental or health outcomes beyond neonatal discharge. We aimed to review the health outcomes of our cohort of preterm infants during the first two years of life and neurodevelopment at two years corrected age and to determine whether these early indices correlated with later outcomes. This study was not designed to inform mechanistic links but rather to be hypothesis generating for future research on larger prospective cohorts.

Hypotheses

1. Our very preterm cohort will experience high rates of respiratory morbidity in the first two years of life and morbidity will be greater in those with BPD
2. Our cohort will experience similar rates of neurodevelopmental impairment to rates reported by ANZNN for the time period of the study
3. Higher NTproBNP levels in the neonatal period will be associated with poorer cardiorespiratory health in the first two years of life and with higher rates of neurodevelopmental impairment at two years
4. Poorer right heart function in the neonatal period will be associated with higher rates of cardiorespiratory morbidity in the first two years of life and higher rates of neurodevelopmental impairment at two years
5. Greater oxygen saturation instability in the neonatal period will be associated with higher rates of cardiorespiratory morbidity in the first two years of life and higher rates of neurodevelopment impairment at two years

Aims

1. To describe the mortality, growth, health and neurodevelopmental outcomes in the first 2 years of life in a modern, well characterised cohort of preterm infants

2. To investigate whether early NTproBNP, TAPSE, TAPSV or oxygen saturation CV correlate with key outcomes, specifically:

- duration of NICU stay
- number of hospital readmissions in first 2 years
- duration of hospital readmissions in first 2 years
- neurodevelopmental impairment at 2 years

Methods

Participants

Infants born at less than 30 weeks gestation between March 2013 and April 2015 and cared for at the Christchurch Neonatal Intensive Care Unit were eligible for inclusion. Infants were excluded if they had known structural airway or lung anomalies, congenital anomalies of the pulmonary arteries or pulmonary veins, major systemic vessel-to-pulmonary artery collateral vessels, congenital heart disease (except those with patent ductus arteriosus, patent foramen ovale or secundum atrial septal defect < 5mm or tiny restrictive muscular ventriculoseptal defect), severe liver disease, or persistent pulmonary hypertension of the newborn on day 3 heart ultrasound (defined as severe hypoxia associated with estimated main pulmonary artery pressure >25mmHg). If infants had a grave prognosis such that they were not expected to survive to day 3 they were also excluded.

Informed consent was obtained from parents of the participants and ethical approval was granted by the University of Otago Human Ethics Board (12/298). This consent included collecting data from clinical notes relevant to this trial, accessing follow-up data routinely collected for the Australian and New Zealand Neonatal Network (ANZNN) and a respiratory health questionnaire sent to parents/caregivers at the child's first birthday.

Testing and Data Collection

NTproBNP, heart ultrasound, oxygen saturations

Infants had NTproBNP levels and clinician-performed heart ultrasounds on days of life 3, 10, 28 and at 36 weeks corrected age with oxygen saturation data collected for approximately 72 hours prior to these testing points. This testing is described in detail in the methods chapter.

Mortality and cardiorespiratory morbidity

Mortality data in the first 2 years after neonatal discharge was collected from hospital records and the local ANZNN database. Electronic hospital databases were searched to identify all encounters with a hospital service in the first two years of life, all Emergency Department or Children's Acute Assessment Unit presentations and all overnight admissions. This data was available for infants seen in Canterbury, South Canterbury, West Coast and Dunedin Hospitals but not for any presentations at other hospitals although we are only aware of two children who moved out of this region before two years of age and for one of these we were able to establish that they had no hospital admissions. When participants were 1 year of age a respiratory health questionnaire designed for this study and not previously validated was sent to parents (Appendix D). The aim of this questionnaire was to identify frequency of presentations to primary and secondary health care and respiratory morbidity in the first year of life. Consent was also sought to check responses against general practice and hospital health records. Two year growth outcomes were collected from local ANZNN database or hospital records.

Neurodevelopment outcome

Neurodevelopmental outcomes were reviewed at 2 years corrected age. Children in this cohort qualified for formal neurodevelopmental testing if they met the ANZNN criteria for screening (born at <28 weeks gestation or < 1000g) or if their clinical course raised concern about the potential for adverse neurological outcome. Hospital records and/or the data submitted to the Australia and New Zealand Neonatal Network (ANZNN) were reviewed and the results of the closest neurodevelopmental assessment to two years corrected age (including paediatrician performed neurological examination and Bayley III or Carolina Curriculum assessment) were recorded.

If Bayley's III was performed, cognitive, language and motor delay was graded as mild, moderate or severe according to established criteria. Severe delay was defined as scores <-3 standard deviations (SD), moderate delay as scores -3 SD to <-2 SD, and mild delay as scores -2 SD to <-1 SD relative to the mean. The mean (SD) of these scales is 100 (15), giving cut-points are of: severe <55, moderate 55–69, and mild 70–84. Blindness was defined as vision < 6/60 in the best eye as assessed by an ophthalmologist. Significant hearing impairment was defined as requiring unilateral or bilateral hearing aids or cochlear implant. Cerebral palsy is a non-progressive motor disability present from birth. The functional level of disability can be graded from 1-5 using the Gross Motor

Functional Classification System (GMFCS).²⁹⁰ In this study cerebral palsy was identified based upon a diagnosis of cerebral palsy by a paediatrician or paediatric neurologist.

Overall moderate to severe neurodisability was defined as either:²⁹⁸

- moderate or severe cerebral palsy (GMFCS ≥ 3) or
- visual acuity $< 6/60$ in better eye or
- sensorineural deafness with hearing aids or
- developmental quotient less than -2 SD relative to reference standard.

Any neurodevelopmental impairment defined as deficit in any domain requiring additional support or intervention.

Comparative data

Outcomes were compared to ANZNN data for that period, the Growing Up in New Zealand (GUINZ) study or population based data from the Ministry of Health or Statistics New Zealand.^{298-300, 425, 426} Statistics New Zealand gathers population data for the New Zealand government. GUINZ is a longitudinal study tracking the health and development of approximately 7,000 New Zealand children, born 2009-2010, from before birth until they are young adults funded by the New Zealand government to inform future health and social policy. ANZNN is a collaborative network of neonatal units across Australia and New Zealand established in 1994 that monitors the care of high risk newborn infants by pooling data to ensure ongoing quality improvement in care. All level II and III neonatal units in New Zealand participate. Since its establishment the Network has developed a minimum data set and implemented a data collection that monitors the mortality and morbidity of infants admitted to neonatal intensive care units across Australia and New Zealand. Babies eligible for audit are those:

1. born at less than 32 weeks gestation, or
2. weighing less than 1500 grams at birth, or
3. received major surgery (surgery that involved opening a body cavity), or
4. received therapeutic hypothermia.

ANZNN recommend formal assessment for neurodevelopmental disability at 2 years corrected age in children born at < 28 weeks gestation and/or < 1000 g birthweight.

Analysis

Descriptive statistics of health outcomes are reported as means (SD) or medians (IQR or range). Z scores for the two year growth parameters were calculated according to the formula used by ANZNN. In the case of non-normal distributions Mann-Whitney test was used to evaluate for significant differences between those that did have BPD and those that did not. A p value of <0.05 was considered statistically significant.

The analysis of neurodevelopmental outcomes is based on a sample of 48 infants: two infants died before discharge; and one child with a rare genetic syndrome was excluded on the basis of preliminary analysis identifying the infant as an extreme outlier on all measures of neurodevelopmental outcome.

The strength of association between neurodevelopmental outcomes and measures of neonatal cardiorespiratory function was assessed using Spearman correlations. Statistically significant associations were adjusted using linear regression methods to control for gender, birthweight z-score, gestation, BPD status, antenatal steroids, maternal smoking and presence of HsPDA on day 3. Adjustments were conducted using multiple linear regression for duration of NICU stay; negative binomial regression for measures of hospital admission and duration of admission; logistic regression for neurodevelopmental delay. Spearman correlations assessed the strength of the association between measures of neonatal cardiorespiratory function (NTproBNP, TAPSE, TAPSV, oxygen saturation CV) and health and neurodevelopmental outcomes (duration of NICU stay, readmissions, neurodevelopmental outcome). Because of the large number of statistical tests reported and the possibility that some associations would be statistically significant simply by chance a Bonferroni correction was applied to determine statistical significance. The Bonferroni corrected p-value, calculated to take into account the correlation between measures, was $p < 0.008$. All analyses were conducted using SAS 9.4, Stata 15 or Graph Pad InStat software.

Results

Mortality in first two years of life

Two children died between day 28 and 36 weeks PMA giving a survival to discharge home of 96%. One child died of a pulmonary haemorrhage secondary to Escherichia Coli sepsis and the other child of respiratory failure secondary to severe BPD. No child died between 36 weeks PMA and 2 years of age.

Growth in first two years of life

The mean (SD) z score at 2 years corrected for weight was -0.24 (1.3) (n=48), for height was -0.22 (1.3) (n=44) and for head circumference was -0.70 (1.2) (n=26). The duration of breastmilk feeding during the first year of life was available for 45 infants: 17.8% had breastmilk for less than 6 weeks, 11.1% for 6 weeks to 3 months, 6.7% for 4-6 months, 35.5% for 7-12 months and 28.9% had breastmilk beyond 12 months.

Health in first year of life

After NICU discharge 98% of survivors had an encounter with some sort of hospital based service (inpatient or outpatient) in the first year of life. The median (range) of hospital service encounters (inpatient or outpatient) during the first year of life beyond neonatal discharge for the whole cohort was 8 (0-67). Two infants required intensive care admission, one for a respiratory arrest and another for severe apnoeas, both associated with a general anaesthetic. No children were diagnosed with pulmonary hypertension after discharge from neonatal unit.

Of the 49 surviving infants, 36 responded to the survey (73%). Table 49 summarises the social and demographic data of survey respondents while Table 50 details key survey results.

Table 49: Social and Demographic Data of survey respondents n=36

Characteristic	number or %
Ethnicity *	
NZ European	64%
Maori	11%
Pacific	5%
Asian	19%
Other	11%
Mean birth gestation	28.1 weeks
Gender F: M	41.7% : 58.3%
Bronchopulmonary dysplasia	64%
Ratio of rooms to people living in home**	1.5

Characteristic	number or %
Maternal smoking	25%
Smoke-free homes†	66.7%
Mean (range) NZ Deprivation Index Decile ^{305‡}	5.3 (1-10)
Attendance at daycare/early learning centre	16.7%
Mean number of children living in the home (<16 years)	1.9
Home heating	100%
Single parent household	8.3%

* Some infants identified as more than one ethnic group

**Hallways, bathrooms, toilets and laundry excluded. If living areas were open plan, the kitchen, dining area and lounge were counted separately.

† Defined as no smokers living in the home

‡Based on discharge address. 1 represents low deprivation and 10 high level of deprivation.

Table 50: Health in first year of life

Measure	% (n=36)
required CPAP after NICU discharge	13.9 (5)
ventilated after NICU discharge (excluding for surgery)	2.8 (1)
no respiratory illness	50 (18)
bronchiolitis	36 (13)

Measure	% (n=36)
pneumonia	2.8 (1)
recurrent wheeze	11.1 (4)
inhaler prescribed	13.9 (5)
steroids prescribed	5.5 (2)
cough lasting > 4 weeks	13.9 (5)
discharged on apnoea monitor	63.9 (23)
apnoea requiring resuscitation post-NICU discharge	5.5 (2)
Eczema	41.7 (15)
Hayfever	2.8 (1)
ear infection	13.9 (5)
nasogastric tube feeds post-NICU discharge	22 (8)
treated for gastroesophageal reflux	50 (18)
immunisation up to date	100 (36)
influenza vaccine	33.3 (12)
prophylaxis against respiratory syncytial virus	0 (0)

Hospital health outcomes in first two years of life

Of the 49 surviving children, 57% presented to the hospital children's acute assessment unit or the hospital emergency department in the first two years of life. The median (range) of presentations to the hospital children's acute assessment unit or the hospital emergency department in the first two years of life was 1 (0-11). Following index NICU discharge, 49% of our cohort were re-hospitalised for an overnight admission in the first 2 years of life. The median number (range) of readmissions in first 2 years of life was 0 (0-19). For those admitted there was a median 3.5 days of readmission (range 1-148 days). Cardiorespiratory causes accounted for 64% of readmissions. Of those without BPD (ANZNN definition) 55% were readmitted during the first 2 years (Median [range] of 1 [0-2] admissions) with a median duration (range) of stay was 1(1-5) day. In comparison 45% of those with BPD were readmitted during the first 2 years (Median 0 [0-19]). However, of those admitted the median stay was 5.5 (1-148) days. There was no significant difference in the number (p 0.813) or duration of readmissions (p 0.554) during first two years for those with or without BPD.

Neurodevelopment at two years

Neurodevelopmental information was available in all survivors between 1-3 years of age. 28 of the 49 surviving children had a Bayley Scale of Infant Development (III) performed. A further 10 infants had formal neurodevelopmental assessment performed by a multidisciplinary early intervention team. For the remaining 11 infants who did not have formal neurodevelopmental assessment outpatient records were reviewed for any reference to impairment. 25/28 Bayley assessments were done at 24-27 months corrected age. The remaining 3 were assessed at 30, 31 and 34 months. The 10 infants who had an alternative form of validated developmental assessment (Carolina Curriculum and Neuro-Sensory Motor Development Assessment) by an Early Intervention team were assessed between 12 and 37 months corrected.^{427, 428}

No child in our study was blind or had significant hearing impairment. One child had a diagnosis of hemiplegic cerebral palsy (GMFCS level 2). One child had a diagnosis of global developmental delay thought to be secondary to a syndrome not yet identified.

In our cohort of 49 survivors, moderate to severe neurodisability was identified in three children but in two of these children the area of impairment was language and it was reported by assessors that having English as a second language influenced these low

scores. Any neurodevelopmental impairment was reported in 11 (22%) children. More detailed results of the Bayley assessment are shown in Table 51.

Table 51: Bayley Scale of Infant Development (BSID) III Assessment (n=28)

Measure	Score or % (n=28)
Median composite cognitive score	102.5
Median composite language score	98.5
Median composite motor score	97
%(n) BSID cognitive composite score <55 (3SD below mean)	0 (0)
%(n) BSID cognitive composite score 55-69 (2SD below mean)	3.6 (1)
%(n) BSID cognitive composite score 70-84 (1SD below mean)	7.1 (2)
%(n) BSID language composite score <55 (3SD below mean)	0 (0)
%(n) BSID language composite score 55-70 (2SD below mean)*	10.7 (3)
%(n) BSID Language composite score 70-84 (1SD below mean)*	10.7 (3)
%(n) BSID motor composite score <55 (3SD below mean)	0 (0)
%(n) BSID Motor composite score 55-69 (2SD below mean)	3.6 (1)
%(n) BSID Motor composite score 70-84 (1SD below mean)	10.7 (3)

*Four children in the cohort had English as a second language

NTproBNP, TAPSE, TAPSV, oxygen saturation CV and outcome

Table 52 shows the Spearman correlations between our novel measures of neonatal cardiorespiratory function (NTproBNP, TAPSE, TAPSV, oxygen saturation CV) and key health and neurodevelopmental outcomes (duration of NICU stay, readmissions, neurodevelopmental outcome). There were statistically significant associations between:

1. Duration of NICU stay and:

- NTproBNP on day 3 ($r=.53$)

- day 10 TAPSE ($r=-.41$)

- oxygen saturation CV on days 10 and 28 ($r=.39, .38$).

Duration of NICU stay was higher amongst those with higher NTproBNP on day 3, those with smaller TAPSE on day 10, and those with higher oxygen saturation variability on day 10 or 28.

2. All measures of **hospital readmission** and:

- day 28 NTproBNP ($r= .41$ to $.51$)

All cause and cardiorespiratory readmissions and readmission days were higher amongst those with higher day 28 NTproBNP. Median (IQR) day 28 NTproBNP for those with no readmissions 40 pmol/L (31-87) compared to 83 pmol/L (60-166) for those who were readmitted in first 2 years.

3. **Neurodevelopmental impairment** at age 2 years and:

- day 28 TAPSE ($r=-.40$)

Those with developmental impairment had smaller TAPSE on day 28. Mean (SD) day 28 TAPSE for those without neurodevelopmental impairment at 2 years was 8.2mm (1.9) compared to 7.1mm (0.8) for those with impairment.

Table 52: Spearman correlations between health and neurodevelopmental outcomes (0-2 years) and measures of neonatal cardiorespiratory function (n=48)

Measure	Duration NICU admission (days)	Number of readmissions (all cause)	Total days readmission (all cause)	Number of cardio-respiratory readmissions	Number of cardio-respiratory acute assessments and admissions	Any neuro-developmental impairment
NTproBNP						
Day 3	.53*	-.10	-.09	-.13	-.24	.23
Day 10	.33	.08	.09	.14	.05	.22
Day 28	.06	.41*	.44*	.51*	.36	-.03
TAPSE						
Day 3	-.13	.15	.12	.01	.04	-.16
Day 10	-.41*	.08	.06	.03	.11	-.14
Day 28	-.20	-.05	-.08	.03	.03	-.40*
TAPSV						
Day 3	-.01	.09	.08	.00	-.03	-.10
Day 10	-.05	.22	.22	.39	.36	-.16
Day 28	-.20	.05	.01	.02	-.11	-.10
O ₂ Saturation CV						
Day 3	.14	.17	.19	.17	-.03	.33
Day 10	.39*	.03	.06	.13	.16	.18
Day 28	.38*	.11	.14	.13	.10	.10

* p<.008, Bonferroni corrected p-value

To identify whether our novel markers were independent predictors of key outcomes, the above associations were adjusted using linear regression methods to control for a range of established antenatal and perinatal factors including: gender, birthweight z-score, gestation, BPD status, antenatal steroids, maternal smoking and presence of HsPDA on day 3. These analyses showed that for duration of NICU stay and all of the measures of hospital readmission/duration up to 2 years the observed associations were reduced and became statistically non-significant after covariate adjustment. However, for the measure of neurodevelopmental impairment at age 2 the strength of the observed association with day 28 TAPSE was unaffected by covariate adjustment.

Table 53 illustrates the associations between day 28 TAPSE (by quartile) and neurodevelopmental impairment at age two. The table shows the rate of neurodevelopmental impairment before and after adjustment for covariates. The table also reports the regression coefficients and tests of significance from logistic regression models fitted to the data. The findings demonstrate a strong association between day 28 TAPSE and rates of neurodevelopmental impairment, with very strong similarity between the unadjusted and adjusted results. The adjusted association is statistically non-significant. However, this appears to be due to a slight inflation of the standard errors in the adjusted model rather than to a noticeable reduction in the adjusted model coefficient.

Table 53: Rates (%) of neurodevelopmental impairment (0-2 years) by quartiles of Day 28 TAPSE; CV before and after covariate adjustment for gender, birthweight z-score, gestation, BPD status, antenatal steroids, maternal smoking and presence of HsPDA on day 3

Measure	n ¹	Unadjusted %	Adjusted %
Day 28 TAPSE Quartile			
Q1 (low)	10	40.0	41.4
Q2	11	36.4	23.1
Q3	12	8.3	10.9
Q4 (high)	10	0.0	4.5
		$B(SE) = -1.09 (0.46);$ $p=0.02$	$B(SE) = -1.07 (0.58);$ $p=0.07$

¹ 5 infants were not assessed for TAPSE on Day 28

Discussion

We have presented growth, health and neurodevelopmental outcomes for our very preterm cohort out to two years corrected and demonstrated satisfactory growth and that respiratory symptoms are common but rates of significant neurodevelopmental impairment are low.

Health outcomes

Although most infants had only a few hospital service encounters during this period, a few children had high health needs and half of our cohort were readmitted to hospital during the first two years. This is despite our cohort demonstrating low levels of overcrowding, high rates of home heating, high levels of immunisation and low attendance at early childhood education or daycare centres.⁴²⁹ Although rates of readmission were similar between infants with BPD and those without, those with BPD tended to have a longer admission and those children with the highest health needs also had BPD. This is consistent with larger longitudinal preterm cohorts.^{408, 409, 430} A Norwegian cohort reported readmission rates of 49% at one year of while a local Christchurch cohort demonstrated a readmission rate of 55% in preterms in the first two years compared to 26% in term born controls.^{407, 431} The Growing Up in New Zealand (GUINZ) study reported only 20% of this predominantly term born cohort were admitted to hospital by age two.⁴²⁵

One third of our survey respondents had bronchiolitis in the first year of life with more than one in ten having recurrent wheeze. No infant received prophylaxis against respiratory syncytial virus, the main pathogen implicated in bronchiolitis. Maternal smoking rates during pregnancy were 16.7% However, by one year of age 25% of mothers were smoking and one-third of households were not smoke-free. Antenatal smoke exposure has been linked to adverse foetal lung development and antenatal and postnatal smoke exposure is associated with poor respiratory health.^{432-434 435-439} It is plausible that infants with premature lung disease are at even greater risk. Supporting women of childbearing age to quit smoking and maintain abstinence postnatally could have significant health benefits and reduced health costs for those children born prematurely.

All of our survey respondents had received all scheduled immunisations by one year of age. In the New Zealand schedule there is only one further vaccination beyond 12

months of age. The GUINZ cohort reported full immunisation rates of 92% at two years. Despite being a high risk population, only one third of our cohort received the influenza vaccination according to parental recall at one year. This vaccine is not part of the New Zealand schedule and is unfunded unless certain criteria are met. These criteria include children under the age of 5 years with significant respiratory disease which suggests more children could have been offered protection.

In our cohort there were no late pulmonary hypertension diagnoses reported beyond neonatal discharge. However, pulmonary vascular disease is a spectrum of dysfunction and two children had significant respiratory events associated with general anaesthetic, necessitating admission to intensive care for respiratory support. It is possible this may reflect some pulmonary vasoreactivity. Studies have shown that children with pulmonary hypertension have a significantly greater risk of pulmonary hypertensive crisis, cardiac arrest or dying with anaesthetic induction.⁴⁴⁰ Without detailed follow-up of high risk children we risk missing occult cardiopulmonary dysfunction that could put children at significant risk.^{117, 441}

Neurodevelopmental outcomes

Rates of cognitive, language and motor delay in our cohort were similar to that seen in contemporary extremely low birth weight cohorts born in Victoria Australia.^{396, 442}

Neurological impairment rates reported by ANZNN in 2014 were higher, however, these data include infants born at less than 24 weeks and also term infants with hypoxic ischaemic encephalopathy making comparison difficult. ANZNN reported cerebral palsy in 7.4% in all children followed up. Any cognitive delay was reported in 15.1%, any language delay in 27% and any motor delay in 17.5%.²⁹⁸ One of the difficulties in assessing language and cognitive outcomes in our cohort was the assessment of children for who English is a second language where it was unclear how much this may have affected their performance. We also know that rates of neurodevelopmental impairment differ depending on the type of assessment tool, the quality of the assessment and the timing of assessment with motor impairments often decreasing over time while more subtle cognitive deficits reveal themselves.

Growth outcomes

All of our cohort received some breastmilk in the first year of life. This is similar to the GUINZ cohort which had 97% initiating breastfeeding at birth. 82% of our cohort sustained breastfeeding until at least 6 weeks but this had fallen to 29% beyond one year

of age compared to 37% in the GUINZ cohort. The high rates of initiating breastfeeding in our unit are likely the result of a supportive breastfeeding policy and culture, lactation consultant support and the establishment of a human milk bank. It is important to continue this support beyond hospital discharge in order to sustain breastfeeding. Growth rates were also good in our cohort which is important as postnatal growth can affect neurodevelopmental outcome.^{311, 313}

Early markers to predict morbidity

We demonstrated strong univariate associations between our novel markers of cardiorespiratory status and adverse outcomes within the first two years. There was a strong positive association between day 3 NTproBNP and duration of NICU stay. There were equally significant positive associations between day 28 NTproBNP and subsequent hospital readmission rates. As the presence of HsPDA is the primary factor driving high levels of NTproBNP on day 3 this supports early physiological instability having a significant impact on subsequent neonatal course. This is further evidence of the importance of supporting physiological stability from birth. Persistently high NTproBNP out to day 28, beyond the period of cardiac transition, is a marker of poorer cardiovascular adaptation. NTproBNP is an integrative marker of cardiorespiratory status that also reflects renal function and volume status. Early NTproBNP may help predict very preterm infants who are likely to have high needs during their NICU stay and a higher hospital readmission rate.

TAPSE was negatively correlated with duration of NICU stay on day 10 and with neurodevelopmental impairment on day 28. Oxygen saturation CV was positively correlated with duration of NICU stay on day 10 and 28. No significant associations were found between TAPSV and health and neurodevelopmental outcomes. However, after adjusting for covariates these associations became non-significant apart from day 28 TAPSE and neurodevelopmental outcome. There is biological plausibility that poorer cardiac function and greater oxygen saturation instability may affect brain development. These findings emphasise the importance of close evaluation of cardiorespiratory function and striving to maintain physiological stability from birth.

Strengths and weaknesses

A significant weakness of our study is the size of the cohorts and the impact on statistical analyses and the ability to demonstrate an independent ability of NTproBNP, TAPSE, TAPSV or oxygen saturation CV to predict outcomes. The strong univariate associations

(even after Bonferroni correction) seen in this cohort suggest there is a strong likelihood that markers such as NT-proBNP and TAPSE may provide independent predictive value in adequately powered cohorts. Further studies of larger cohorts are therefore needed. Our findings may not be relevant to other cohorts and would need testing in that setting. Our cohort was well characterised and we provide comprehensive follow-up data to 2 years. Despite our rigorous attempts, we cannot exclude that some follow-up data is missing, due to events occurring out of region. We were unable to collect consistent data of family doctor visits and so have not reported this. The response rate to our survey was 73% and as we do not have data for the non-responders it is possible that there may be some selection bias in these results. The NZ social deprivation decile was also lower for survey respondents than the whole cohort suggesting some of the non-responders had greater levels of social deprivation which may adversely affect health outcomes. In addition the social deprivation index is a crude tool for estimating social deprivation.

Implications for practice

In summary, our cohort is similar to regional cohorts and demonstrated high health utilisation in the first two years of life but low levels of significant neurodevelopmental impairment. Gestational age, birthweight and BPD are likely to be the strongest determinants of poor health and neurodevelopmental outcomes. We have shown some significant univariate associations for novel markers of cardiorespiratory function but acknowledge a much larger cohort is needed to demonstrate independent predictive value. However, these data suggest that there would be value in exploring novel prognostic markers further. NTproBNP and TAPSE in particular may be useful as part of a prognostic scoring system and warrant further study.

CONCLUSION

Conclusion

Background

To be born prematurely is to experience an interruption of development, triggering a range of adaptive responses that may offer a short term survival benefit but have long term consequences. Infants born very prematurely are at risk of lung maldevelopment including pulmonary vascular maldevelopment which can have lasting effects.^{57, 114, 391, 443}

The pulmonary vascular disease-pulmonary hypertension spectrum is an adaptive path of pulmonary development that is influenced by baseline genetic risk, currently poorly understood, interacting with a multitude of in-utero and postnatal factors.^{57, 443}

Identifying this pathological pattern of development, and the factors that contribute to its development, is critical to the timely implementation of management that could prevent the significant associated morbidity and mortality. The complex aetiology of late pulmonary hypertension in very premature infants means that prevention and treatment will require a multifaceted approach. Due to the insidious development of pulmonary hypertension, screening and surveillance is needed to improve outcomes.^{86, 87, 221} Clinical information, heart ultrasound evaluation, pulse oximetry data and biomarkers may all have a role to play. This thesis describes a coordinated series of studies that evaluated modern markers of cardiac function and their ability to detect abnormality in a real world population of preterm infants. We sought to evaluate tools that could improve detection of pulmonary hypertension and could be used for screening. Careful characterisation of clinical, biomarker and novel ultrasound indices has provided important novel findings in regard to the temporal profile of these markers, the feasibility of their acquisition and their value in detecting cardiopulmonary abnormality and predicting outcomes.

Summary of findings

NTproBNP

This study investigated whether the cardiac stress hormone NTproBNP could be used as a *biomarker to detect rising pulmonary artery pressure* in very prematurely born infants. Prior to commencing this study, estimates of the incidence of pulmonary hypertension varied from 18% of infants with a birthweight of <1000g, to 25% of infants with BPD to

37% in those with BPD and prolonged ventilation.^{64, 80, 81} However, none of our cohort born at less than 30 weeks gestation had pulmonary hypertension at 36 weeks PMA according to our definition using conventional ultrasound criteria.^{120, 444} Nor did any infant have a subsequent diagnosis of pulmonary hypertension by two years of age. This precluded testing of our primary hypothesis. This low incidence of pulmonary hypertension in very preterm infants was replicated in a recent Australian study and suggests that the overall prevalence of pulmonary hypertension in a modern setting may be lower than rates from highly specialised quaternary centres.³⁵⁴ However, it is possible that we have missed pulmonary hypertensive disease by using criteria that were too stringent. In the absence of cardiac catheterisation data we also cannot exclude lesser degrees of pulmonary vascular disease. We only used one quantitative measure (TR jet peak velocity) which is often not detectable in late pulmonary hypertension in preterm infants.^{199, 207} Our alternative criteria were subjective and so may have been affected by the experience or bias of the assessor. Due to the low sensitivity of any one heart ultrasound parameter for detecting pulmonary hypertension it is likely that a wide range of indices will need to be evaluated.²⁰⁷ However, our cohort also had low rates of intrauterine growth retardation, good post-natal growth and predominantly non-invasive respiratory support with few infants at the extremes of prematurity. All of these factors are likely to have contributed to a lower than expected rate of pulmonary hypertension. We cannot exclude lesser degrees of pulmonary vascular disease which may cause increased morbidity during challenges such as respiratory illness or general anaesthesia.

This is, to the best of our knowledge, the first study to investigate *temporal changes in NTproBNP*, paired with heart ultrasound and oxygen saturation data, in a general cohort of infants born at <30weeks gestation at multiple time points from day 3 to 36 weeks PMA – a time point at which many recommend screening for pulmonary hypertension. We demonstrated that NTproBNP levels are high on day 3 and tend to fall over time. We showed that levels in very premature infants are considerably higher than those seen in adults and are highly variable on day 3 and day 10 of life, primarily due to the effects of transitional circulation. NTproBNP proved to be a *highly sensitive and specific predictor of HsPDA*.

BPD is characterised by both a paucity of alveoli and a paucity of pulmonary vessels and is often complicated by pulmonary hypertension.⁵⁶ *Bronchopulmonary dysplasia did not have a significant effect of on NTproBNP levels after adjustment for covariates such as*

HsPDA. This is in contrast to other studies.¹⁸⁵ NTproBNP is clearly very sensitive to acute ventricular pressure and volume overload as demonstrated by the effect of HsPDA. However, the more slowly evolving chronic stress that chronic lung disease, such as BPD, may confer predominantly on upon the right ventricle may not be such a strong stimulus to BNP release. In the very preterm infant the development of pulmonary vascular collateral pathways may mitigate some of the potential cardiac effects of BPD. In the similar adult setting of chronic obstructive pulmonary disease in the absence of heart failure, NTproBNP levels are also not very high.⁴⁴⁵ Acute exacerbations of chronic obstructive pulmonary disease do cause a rise in NTproBNP although its performance is better for negative predictive value than positive.⁴⁴⁵ Therefore NTproBNP may still be a useful biomarker of acute decompensation due to pulmonary hypertension either due to an acute crisis or because compensatory cardiac mechanisms fail.

We investigated *other factors affecting NTproBNP levels* in this population. Although impaired renal function increased levels, haemoglobin levels, recent exposure to intermittent hypoxia and mechanical ventilation did not. Chronological age but not gestational age influenced levels. Unexpectedly, levels at 36 weeks PMA were higher in those without a history of HsPDA and without a diagnosis of BPD. We explored whether high levels post-partum were in part due to processing of the peptide and performed additional analyses to identify circulating isoforms of BNP. Findings from this analysis demonstrated that NT proBNP1-76 was the dominant circulating form and there was no evidence of significant glycosylation or of circulating fragments. Hence the difference seen at 36 weeks PMA could not be explained by glycosylation differences. Our findings also support a high degree of biological variability in NTproBNP as has been shown in the adult population with levels being driven predominantly by factors stimulating secretion rather than by altered post processing or clearance.¹⁹⁶

Heart ultrasound

In the absence of invasive cardiac catheterisation, estimating main pulmonary artery pressure and assessing RV function using heart ultrasound is critical to detecting pulmonary hypertension. However, conventional ultrasound criteria for diagnosing pulmonary hypertension may not be sufficiently sensitive to identify late pulmonary hypertension in premature infants that evolves slowly and beyond the period of transitional circulation.

Clinician performed heart ultrasound in the NICU is currently primarily being used to monitor transitional circulation. Our study demonstrated that it is *feasible for more advanced quantitative measures of right ventricular function to be incorporated into clinician performed heart ultrasound*. This may be of value to a pulmonary hypertension screening programme as utilising clinician scanners is likely to increase the availability of scanning, reduce costs and allow infants to be scanned in the NICU.

We also investigated the *effects of time, growth and BPD on these heart ultrasound measures* which had not previously been reported. Our study revealed some important results and demonstrated that some parameters performed better than others in terms of ease of acquisition, reproducibility and discriminating qualities with TAPSE performing particularly well. Our study also *highlighted the importance of incorporating developmental changes* in these indices over time into reference ranges. Our results need to be further validated in larger preterm cohorts and developmental changes compared to term born infants.

With very to extremely premature birth the immature heart has to adapt to post-natal circulatory changes and compensate for an underdeveloped pulmonary vascular system. Initially this is likely to be through increased contractility, which may explain the unexpected increases in TAPSE seen in our cohort. Eventually, if RV preload or afterload stress persists, systolic and diastolic dysfunction will occur with structural changes in the RV seen as hypertrophy and/or dilatation.³⁴³ This persisting chronic stress on the developing heart may be the reason adults born very prematurely have evidence of RV hypertrophy, RV dysfunction and sub-clinical pulmonary vascular disease.^{45, 69, 347} The absence of overt pulmonary hypertension in the neonatal period does not exclude the presence of cardiac adaptations that may have consequences to cardiovascular health in adulthood.

Oxygen saturation monitoring

Supporting developmentally immature lungs often requires oxygen to overcome the effects of an underdeveloped gas-exchange interface which contributes to chronic intermittent hypoxemia in very premature babies. However, hyperoxia can have a toxic effect on the developing lung and in particular interfere with pulmonary vascular development. A great deal has been published on the importance of oxygen saturation targeting in premature infants. Comparatively little has been published on how oxygen

saturation instability changes over time and the utility of various measures of instability now available to the clinician with modern oximeters.²⁸⁵

Our study demonstrated that babies born at less than 30 weeks are *most unstable on days 25-28 of life* compared to the other time points we studied. *Oxygen saturation CV and percentage time spent with oxygen saturation less than 88% appeared to offer the most consistent discriminating value* when comparing infants with and without BPD. Babies who went on to be diagnosed with BPD became more unstable than those who did not from about day 7-10 highlighting the critical importance of early care. *Time in target during the first 72 hours, a critical period of physiological transition, was low and the incidence of exposure to hyperoxia unacceptably high.* In our neonatal intensive care unit there was a *high rate of incorrect alarm settings* identified putting particularly extremely preterm infants on oxygen therapy at risk of undetected and potentially harmful hyperoxia.

Health and neurodevelopmental outcomes

Rates of respiratory disease in the first two years of life were high in our very preterm cohort. Half required readmission to hospital during this period predominantly due to respiratory illness. In contrast, *rates of moderate to severe neurodevelopmental impairment were low.* This is consistent with other studies and regional network data.^{7, 407 406, 430}

Early life NTproBNP predicted duration of NICU stay and hospital readmission while TAPSE strongly predicted any neurodevelopmental impairment by two years. These findings further emphasise the impact of early cardiorespiratory stability on later outcomes.

Implications for practice

Use of NTproBNP in premature infants

We showed that NTproBNP is a highly sensitive and specific marker of HsPDA by our definition. This is consistent with a recent systematic review.¹⁸⁰ However, incorporation into clinical practice has been impeded by the lack of consensus definition of HsPDA in relation to clinically meaningful outcomes and the ever shifting opinion on whether to treat PDA or not. These issues will need to be resolved before NTproBNP can be used routinely, however, *NTproBNP should be considered during the design of in any future PDA trials* as it may help to better stratify infants who would benefit from treatment.

There is *insufficient evidence to recommend NTproBNP as a standalone biomarker for pulmonary hypertension screening* in very premature infants. However, it may be useful as an adjunct to heart ultrasound particularly to monitor response to treatment. A baseline level should therefore be considered in at risk infants at 36 weeks PMA as part of a pulmonary hypertension surveillance programme.

Optimal heart ultrasound parameters for diagnosing late pulmonary hypertension

There is *currently no consensus on optimal heart ultrasound criteria for the diagnosis of late pulmonary hypertension* in very premature infants. Our findings support *further training for neonatologists in quantitative measures of right heart function* that could be incorporated into clinician performed heart ultrasound under the oversight of a paediatric cardiology service. This would enable better study of the changes in right heart function over time in response to preterm birth. On the basis of our review of current literature and our study findings we *recommend the inclusion of TAPSE, tissue Doppler indices and evaluation of the LV eccentricity index.*^{205, 206, 209, 214, 446, 447}

Oxygen saturation targeting

Our study highlights the challenge of managing oxygen saturation instability in very premature infants. We also demonstrated that an increase in instability is usual for all infants but that some infants are particularly unstable and that this often becomes apparent in the first 7-10 days. While this may suggest some in-utero programming this *emphasises the importance of striving to maintain physiological stability from birth.* We propose that more attention needs to be paid to the human factors contributing to poor time in target and *suggest an oxygen saturation stewardship programme* should be evaluated to reduce iatrogenic harm.

Optimising health and neurodevelopmental outcomes

The high level of health utilisation of our cohort in the first two years of life and the significant burden of respiratory disease emphasise the importance of *preventative care.* Supporting mothers to *stop smoking in pregnancy* and not return to smoking in the post-partum period and homes to be smoke free in addition to *supporting breastfeeding* may help to reduce the burden of respiratory illness in infants born prematurely.

Two infants in our cohort had life threatening respiratory events requiring intensive care associated with a general anaesthetic after discharge from NICU. Other studies have demonstrated the high risk of anaesthesia to children in the context of pulmonary

hypertension.^{440, 448} This highlights the importance of identifying infants with a propensity to respiratory instability prior to discharge to allow careful *pre-operative work-up for occult pulmonary hypertension* and judicious anaesthetic planning to reduce this risk.

In the absence of a vaccine against respiratory syncytial virus which is responsible for a great deal of the respiratory morbidity, *prophylaxis with a virus-specific neutralising antibody* is the only available option to reduce morbidity. This is recommended by the American Academy of Pediatrics for use in infants born at <29 weeks gestation.⁴⁴⁹ Unfortunately the cost of this therapy is high and it is not subsidised by the government in New Zealand. Clinicians should consider applying for exceptional circumstances funding for high risk infants.

Screening for pulmonary hypertension

No screening programme for late pulmonary hypertension in premature infants currently exists in New Zealand. International practice is also highly variable.¹²¹ Many international guidelines and pulmonary hypertension research groups now recommend screening.⁸⁶⁻⁸⁸ Currently published recommendations for screening show a lack of consensus although some common ground does exist. We have reviewed these recommendations and *recommend a national surveillance programme* should be developed to better identify the incidence and reduce the associated morbidity/mortality of this condition. A suggested protocol for screening in the New Zealand setting, based on published recommendations, is included in Appendix F. In order to be cost-effective this programme should be based upon relative risk and embedded within existing national and Australasian neonatal networks. This would inform prevalence of this condition in our population and identify any locally relevant ethnic differences, allow audit of the programme design and monitoring of any adverse events. Studies to establish reference ranges for heart ultrasound parameters and NTproBNP should be conducted alongside this surveillance programme with a view to refining this protocol informed by these data. International collaboration and networking should be encouraged.

Who to screen

Our review of recommendations and screening algorithms published by a variety of experts shows some variability as to which infants should be screened but *all bar one protocol included those with a diagnosis of BPD*.^{62, 84, 86-88, 111-113, 119, 120, 207} Some groups recommended screening only infants with severe BPD while others acknowledge that

pulmonary hypertension may occur in infants with mild BPD or without BPD and expand criteria to include all infants with BPD, infants below a specified birthweight or gestational age or any infant displaying symptoms suspicious for pulmonary hypertension. A recent systematic review by Nagiub and metanalysis by Al-Ghanem provide valuable information on odds ratios for a *variety of risk factors that should be considered when developing a screening programme*.^{115, 450}

When to screen

Early detection of pulmonary hypertension has been shown to improve survival in adults and children.^{108, 451-453} Screening for pulmonary hypertension in premature infants needs to be planned at a time when most infants will be detected and yet early enough that detection can still improve outcomes. There is *consensus across most groups that screening should be conducted around 36 weeks PMA*. Studies that have looked at screening earlier, such as that done by Mourani et al on day 7, have shown that the presence of pulmonary hypertension on day 7 ultrasound is associated with a significantly increased risk of BPD and late pulmonary hypertension but also that some cases of early pulmonary hypertension resolve by 36 weeks PMA.⁸⁴ Further support for screening at 36 weeks PMA is provided by Bhat et al who showed that assessment at 4 weeks diagnoses only one third of affected infants.⁶⁴ We also know that a percentage of infants will present after 36 weeks PMA necessitating the inclusion in the guidelines of indications for reassessment if initial assessment is negative.^{62, 112} *The optimum timing for reassessment is uncertain* although most agree that serial monitoring while oxygen dependent is advisable.

What tests to use

All protocols we reviewed *recommended an echocardiogram* for screening with cardiac catheterisation reserved for those with severe disease or prior to considering long term pulmonary vasodilator therapy. It is important to note that although an echocardiogram is reasonably good at detecting pulmonary hypertension it is not reliable for classifying severity when compared to cardiac catheterisation.¹⁹⁹ There is some variation regarding which ultrasound parameters to include in echocardiographic assessment. All would agree that a full evaluation for any structural heart disease is essential. The most commonly included parameters are *TR jet and MPA flow Doppler interrogation, RV dilatation/hypertrophy and septal flattening either assessed qualitatively or quantitatively using the LV eccentricity index*. Using shunt gradients across PDA or ventricular septal

defect, if present, to estimate RV pressures is recommended by some as a useful adjunct. Some algorithms suggest use of more advanced techniques such as *TAPSE and tissue Doppler indices* (for example RIMP, E', E'/A', S') for assessing RV function, however, many of these indices need further validation in the preterm population.

The Pediatric Pulmonary Hypertension Network recommend at a minimum:

1. A complete evaluation of cardiac anatomy including interrogation of shunts and the identification of pulmonary veins
2. An assessment of RV and LV size, hypertrophy and function
3. Evaluation of intraventricular septal position
4. Interrogation, if present, of tricuspid/pulmonary regurgitation jet
5. Simultaneous measurement of blood pressure

Echocardiogram findings must therefore be interpreted in the context of the clinical situation. A diagnosis of pulmonary hypertension is therefore based on cumulative risk factors combined with clinical suspicion and supported by the cumulative weight of echocardiographic evidence.

Although most NICUs in New Zealand have ready access to an ultrasound machine that can be used at the bedside, and many neonatologists have some training in heart ultrasound, the experience and capability is variable. Clinician performed heart ultrasound is not equivalent to an echocardiogram performed by a cardiac ultrasonographer or cardiologist and has traditionally been used to evaluate basic structure and function in the period of transitional circulation. Any proposed change to this scope would need to be discussed with paediatric cardiology and the Australasian Society of Ultrasound Medicine. If neonatologists were to conduct the heart ultrasound at 36 weeks PMA then further training specialising in the assessment of late pulmonary hypertension would be needed.

Some, but not all guidelines, recommend serum *BNP or NTproBNP to be used at baseline and follow-up* to help monitor response to treatment. As BNP or NTproBNP levels can be highly variable with no clear diagnostic cut-off point, serial assessment is recommended. Pulse oximetry is not included in any screening protocol but is highlighted in some as a component of the management in the context of targeting oxygen saturations and avoiding hypoxia.

Strengths and Weaknesses

Strengths of this study are that it is a well described cohort representative of a population at risk of pulmonary hypertension. Paired biomarker, heart ultrasound and pulse oximetry data were evaluated over four clinically relevant time points. Subjects were followed up to two years of age with health and neurodevelopmental data. Interpretation of heart ultrasound and pulse oximetry data was performed blinded to NTproBNP results. The paediatric cardiologist completing the evaluation for pulmonary hypertension at 36 weeks PMA was blinded to clinical condition of the infant and test results. An extensive review of literature in this field was conducted and current evidence was incorporated in discussions of this study's findings, recommendations made and to highlight gaps in knowledge.

A key weakness of our study was a cohort size that, in hindsight, was inadequate to test our primary hypothesis. Although we recruited the minimum number recommended by our initial power calculations the incidence of pulmonary hypertension was less than expected and we were unable to extend our recruitment due to limited funding and personnel. We also recognise that infection may affect BNP secretion and thereby affect NTproBNP levels. As 58% of our cohort had histological evidence of chorioamnionitis it is possible that this may have influenced our day 3 NTproBNP levels. However, we think this is unlikely to have been a major confounder in the absence of any clinical evidence of significant sepsis and in the absence of a raised CRP. With regards to BNP isoform testing we also had to pool samples across clinical groups and time due to small sample volumes. Taking a larger sample volume for NTproBNP testing would have allowed analysis of individual differences in isoforms and glycosylation patterns over time. However, we were able to demonstrate the predominant circulating isoform in the very preterm population and that glycosylation is low. We demonstrated some novel data on more advanced quantitative ultrasound measures of right heart function. However, there is a need for larger cohort studies to establish reference ranges for the heart ultrasound parameters discussed. It would have been valuable to have been able to compare the changes in these parameters over time, and those for oxygen saturation, to a control group of term-born infants. Continuous oxygen saturation data combined with contemporaneous continuous inspired oxygen concentration data would give a more accurate picture of time in target. Ideally ultrasound estimates of pulmonary artery pressure should be compared contemporaneously with cardiac catheterisation data but this would not have been ethical in our population. It would also have been better to

have had a more detailed ultrasound evaluation of the left ventricle due to the interdependence of RV and LV. Renal function was not evaluated at all NTproBNP testing time points and as such we cannot exclude the possibility that renal dysfunction may have contributed to higher levels of NTproBNP. Agitation, hypoxia, hypercarbia, and changes in airway pressure can cause transient changes in pulmonary vascular pressures so snapshot assessments may not accurately reflect pulmonary vascular function. BPD rates differ by ethnicity so risk of pulmonary hypertension is also likely to differ and our findings may not be generalizable to populations with a different ethnic make-up.

Future Directions

There are a number of important questions that demand further research.

In order to better compare the incidence of pulmonary hypertension in very premature infants, an *ultrasound based consensus definition* using advanced quantitative techniques is needed. National and international collaboration should be encouraged to develop and refine surveillance/screening recommendations. *National registries*, especially if embedded within already existing neonatal data collection networks, would help our understanding of the natural history of this disease. Furthermore these data could better identify risk factors that may inform trials of preventative strategies and treatments.

There is currently insufficient evidence to recommend NTproBNP in isolation for screening for pulmonary in very premature infants. However, it may be a useful adjunct to heart ultrasound evaluation for diagnosis and serial monitoring to evaluate the effect of treatment strategies. *Evaluation of NTproBNP at 36 weeks PMA as part of a national pulmonary hypertension surveillance programme* would better inform us of the potential predictive value of this cardiac marker.

Exposure of a developmentally immature lung to hyperoxia is a potentially preventable contributor to pulmonary vascular disease. Aspiring to optimal target saturations is not enough. We need to examine the human factors that may contribute to sub-optimal time in target and harness technology to assist us in better delivering this goal. An *oxygen saturation stewardship programme* aiming to reduce exposure to excess oxygen from the birthing suite to discharge should be developed within neonatal units. This programme should examine both human and technological aspects of oxygen delivery and utilise analysis of continuous oxygen saturation monitoring to enhance care.

There is also an urgent need for better *early markers of pulmonary vascular disease* if we are to reduce morbidity and mortality from this condition. We know from adult studies that more than 50% of the pulmonary circulation has to be obstructed before we see an elevation in resting pulmonary artery pressure.⁴⁵⁴ Current ultrasound markers are not sensitive enough to detect early disease. B-type natriuretic peptide rise appears to be a late phenomenon related to failure of cardiac adaptive mechanisms. Advanced cardiorespiratory imaging techniques such as three dimensional echocardiography and cardiac magnetic resonance imaging may help fill this void. A number of other potential biomarkers have been investigated in other settings but are yet to be tested in the preterm population.⁴⁵⁵ These include endothelial cell markers, inflammatory markers, markers of oxidative stress, micro-RNA, heart function-related markers, extracellular matrix related proteins and metabolic biomarkers.

More work is needed to further our understanding of the *mechanisms of pulmonary vascular development* and in particular the interaction between antenatal factors such as intrauterine growth restriction, preeclampsia, chorioamnionitis and maternal smoking and postnatal factors such as mechanical ventilation, hyperoxia, poor growth and infection. Genetic and epigenetic factors are also poorly understood. Unravelling these mechanisms will help the development of novel therapies which are likely to impact not only on pulmonary hypertension but also bronchopulmonary dysplasia. There is also a need for further study of the *developmental changes and adaptive mechanisms of the preterm heart* with particular attention paid to the long term effects of premature birth on cardiovascular function. To advance this field cross-disciplinary collaboration is essential.⁴⁵⁶

Finally it is imperative that we use what is currently known to improve care. To this end we need to *optimise respiratory care* to minimise invasive ventilation, avoid hyperoxia, minimise hypoxic episodes and utilise all available data to deliver precision management. It is also vital that we consider the global impact of this disease. As low and middle income countries upscale neonatal care more children born very prematurely will survive. Given the high rates of intrauterine and post-natal infection and antenatal and post-natal growth restriction in under-resourced populations, these surviving infants and are at significant risk of pulmonary hypertension if exposed to unregulated oxygen. It is vital that these countries are supported to develop strategies to mitigate this risk. The story of pulmonary hypertension highlights the importance of consensus diagnostic

criteria, data validated specifically for premature infants and collaboration to address an under recognised but important condition.

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APPENDICES

Table 54: Summary of recommendations for screening preterm born infants for pulmonary hypertension (PH)

Study	Patient selection	Timing	Initial screening test	Follow-up screening (even if initial screen negative)	Indication for cardiac catheterisation
Krishnan 2017 ⁸⁶ Expert consensus from Pediatric Pulmonary Hypertension Network	1. Persistent ventilator support at day 7 2. Moderate to severe BPD or no/mild BPD who have worsening respiratory course/prolonged oxygen requirement 3. Sustained need for respiratory support with recurrent hypoxemia	<ul style="list-style-type: none"> • Day 7 if meet criteria 1 • 36 weeks PCA if meet criteria 2 • Any age if meet criteria 3 	Echocardiogram BNP/NTproBNP if echo positive for PH	Echocardiogram 4-6 monthly if oxygen requirement persists or any time there is worsening of oxygen requirement or need for increased respiratory support. 2-4 monthly if PH confirmed	Echocardiogram suggests >2/3 systemic arterial pressure with severe septal flattening OR Echocardiographic measures of PH and BNP/NTproBNP fail to improve
Abman 2015 ⁸⁷ American Heart Association and American Thoracic Society guidelines	1. Severe respiratory distress syndrome especially in context of oligohydramnios or IUGR 2. BPD requiring ventilator support or not weaning from oxygen or oxygen requirement out of proportion to respiratory disease 3. Any infant with slow clinical improvement requiring high levels of respiratory support and oxygen or recurrent hypoxemia or unexplained poor growth or persistently high PaCO ₂	<ul style="list-style-type: none"> • Early if meet criteria 1 • At 36 weeks PCA if meet criteria 2 • Any time if meet criteria 3 	Echocardiogram BNP/NTproBNP at diagnosis and follow-up	4-6 monthly echocardiogram	Persistent signs of severe cardiorespiratory disease or Before commencement of vasodilator therapy or Recurrent pulmonary oedema

Study	Patient selection	Timing	Initial screening test	Follow-up screening (even if initial screen negative)	Indication for cardiac catheterisation
Naguib 2015 ²⁰⁷ Systematic review	Infants with BPD – particularly those with moderate to severe BPD	<ul style="list-style-type: none"> Not stated 	Echocardiogram	Not stated	Not stated
Weismann 2017 ¹¹³ Single centre, prospective	<ol style="list-style-type: none"> BPD OR Birthweight \leq 750g OR Clinical suspicion of PH 	<ul style="list-style-type: none"> 36-38 weeks PCA 	Echocardiogram Consider NTproBNP	1 year of age unless clinical concern	Persistent significant pulmonary hypertension prior to pulmonary vasodilator drugs
Mehler 2018 ¹¹² Single centre, prospective	Birth weight <1000g AND <ol style="list-style-type: none"> BPD or No BPD or Clinical signs of pulmonary hypertension 	<ul style="list-style-type: none"> 36 weeks PCA if meet criteria 1 At discharge for criteria 2 Any time for criteria 3 	Echocardiogram	3-6 months post discharge (6 weeks post discharge if pulmonary hypertension diagnosed)	Not specified
Altit 2017 ¹¹¹ Single centre, expert consensus	<ol style="list-style-type: none"> < 28 weeks GA at birth or Birthweight \leq1250g 	<ul style="list-style-type: none"> 36 weeks PCA 	Echocardiogram	Every 2 months if persistent oxygen requirement	On a case by case basis after consultation with cardiology, anaesthesiology and respiratory specialist
Hilgendorff 2016 ⁸⁸ Report of European Pulmonary Vascular Disease Network	<ol style="list-style-type: none"> Severe BPD or <28 GA at birth or Consider if prolonged oxygen requirement, frequent desaturations or poor growth 	<ul style="list-style-type: none"> Once or twice a month if suspicion of pulmonary hypertension 	Echocardiogram NTproBNP if pulmonary hypertension confirmed	Beyond discharge 3 monthly if BPD and need for oxygen. 3-6 monthly if growth retardation/failure to thrive, VLBW, BPD without ongoing oxygen, ventilation or CPAP beyond 28 days of age	Persistent pulmonary hypertension despite adequate treatment of lung disease, before starting pulmonary vasodilators

Study	Patient selection	Timing	Initial screening test	Follow-up screening (even if initial screen negative)	Indication for cardiac catheterisation
Mourani 2015 ²²¹ Expert opinion	<ol style="list-style-type: none"> 1. Moderate or severe BPD or 2. Severe respiratory disease and/or suspicion of pulmonary hypertension 	<ul style="list-style-type: none"> • 36 weeks if criteria 1 • Any time for criteria 2 	<p>Echocardiogram</p> <p>Baseline BNP</p>	<p>Repeat echocardiogram a2-4 monthly while on oxygen or</p> <p>Echocardiography and serial BNP more frequently according to severity of PH</p>	<p>Severe PH (RV systolic pressure >35mmHg and RV systolic pressure/systemic systolic blood pressure >2/3 or severe septal flattening</p>
Mourani 2009 ¹²⁰ Expert opinion	<ol style="list-style-type: none"> 1. Infants with severe RDS who require high levels of oxygen and ventilator support, especially if IUGR or oligohydramnios or 2. Infants with slow rate of clinical improvement with persistent or progressive needs for respiratory support or 3. Infants with BPD who require positive pressure ventilation or are oxygen dependent at 36 weeks CGA or have recurrent cyanotic episodes 	<ul style="list-style-type: none"> • Early if criteria 1 or • Any time pulmonary hypertension is suspected such as criteria 2 or • 36 weeks CGA if criteria 3 • 	<p>Echocardiogram</p>	<p>2-4 monthly echocardiogram while on oxygen then 2 months after weaning from oxygen</p>	<p>Echo confirmed pulmonary hypertension AND</p> <ol style="list-style-type: none"> 1. Persistent signs of severe cardiorespiratory disease or clinical deterioration not directly related to airways disease or 2. Suspected of significant pulmonary hypertension despite optimal management or 3. Prior to starting chronic vasodilator therapy or 4. Unexplained, recurrent pulmonary oedema

Study	Patient selection	Timing	Initial screening test	Follow-up screening (even if initial screen negative)	Indication for cardiac catheterisation
Khemani 2007 ⁶² Single centre recommendations based on retrospective review	BPD AND 1. Birth GA ≤ 25 weeks or 2. Birthweight <600g or 3. Small for gestational age or 4. Prolonged mechanical ventilation or 5. Increased oxygen requirements that cannot be explained by lung pathology or 6. Poor growth despite adequate caloric intake	Not specified	Echocardiogram	Not specified	Severe respiratory disease and severe pulmonary hypertension or if known or suspected cardiovascular anomalies
Meau-Petit 2013 ¹¹⁹ Multi-centre, prospective	Birth GA <33 weeks and BPD	<ul style="list-style-type: none"> • 3 months of age 	Echocardiogram	<p>If not on oxygen 6 months, 12 months and then every 2 years</p> <p>If on oxygen assess at 8-10 days after cessation of oxygen, 3,6 and 12 months then every 2 years</p>	Prior to treatment with pulmonary vasodilators

Table 55: Proposed heart ultrasound criteria for diagnosis (**bold**) or suggested measures for evaluation of pulmonary hypertension in reviewed studies

Study	Criteria/measure
Krishnan 2017 ⁸⁶	Elevated TR jet velocity if present Septal flattening RV dilation/hypertrophy TAPSE RV fractional area change Tissue Doppler E/E', S', E'/A', RIMP
Abman 2015 ⁸⁷	Elevated TR jet peak velocity Raised systolic pulmonary pressure: systemic systolic blood pressure Septal flattening qualitatively or by LVEI Evidence of right to left shunting RV dilatation TAPSE Evaluation of LV function and for pulmonary vein stenosis
Naguib 2015 ²⁰⁷	If TR jet present: - estimated pulmonary artery systolic pressure >40 mmHg - ratio of systolic time to diastolic time (SD/DD) >1.15 -ratio of TR velocity to tissue velocity time integral of pulmonary valve Doppler (TVR/VTI) >0.14 If no TR and no PDA: - RIMP >0.38 - LPA flow analysis (inflection time <4.3 msec, AT/RVET <0.31, deceleration index >0.4) If no TR but PDA present with left to right shunt: - LPA flow analysis (inflection time <4.3 msec, AT/RVET <0.31, deceleration index >0.4) - eccentricity index <0.81 - TAPSE<0.5cm If no TR but PDA present with right to left shunt: - mean pulmonary artery pressure>systolic pulmonary artery pressure -eccentricity index <0.81. - TAPSE<0.5cm
Weismann 2017 ¹¹³	TR jet gradient of >36 mmHg or presence of septal flattening in systole
Mehler 2018 ¹¹²	TR jet peak velocity of ≥ 2.8 m/s or systolic pulmonary pressure*:systemic systolic blood pressure ≥ 0.5
Altit 2017 ¹¹¹	TR jet velocity gradient Pulmonary regurgitation gradient Direction of PFO/ASD/PDA/VSD shunts End-systolic septal position qualitatively or by LVEI AT/RVET ratio Tricuspid systolic to diastolic time ratio RIMP TAPSE E/A and E'/A' of tricuspid valve by conventional and tissue Doppler TAPSV (S') Pulmonary veins Doppler: rule out pulmonary vein stenosis Right-sided structural measurements and pulmonary valve size Combined with comprehensive review of left ventricle

Study	Criteria/measure
Hilgendorff 2016 ⁸⁸	Refer to echocardiographic recommendations by European Paediatric Pulmonary Vascular Disease Network ⁴⁵⁷ Interrogation of tricuspid or pulmonary regurgitation jet if present TAPSE RV strain RV volume TV S', E', A' LVEI Pulmonary artery acceleration time Evaluation of left ventricle
Mourani 2015 ²²¹	Estimated RV systolic pressure >35mmHg by interrogation of TR jet* or septal flattening
Mourani 2009 ¹²⁰	Elevated systolic pulmonary artery pressure by interrogation of TR jet* Right heart dilatation Septal flattening AT:RVET RIMP
Khemani 2007 ⁶²	Estimated pulmonary artery pressure by interrogation of TR jet Septal flattening
Meau-Petit 2013 ¹¹⁹	Estimated pulmonary artery pressure >25mmHg by interrogation of TR jet* or pulmonary regurgitation jet Septal flattening Dilatation of the right atrium or main pulmonary artery RV hypertrophy or dysfunction

* calculated using TR jet peak velocity and Bernoulli equation

Canterbury DHB

District Health Board

Te Poari Hauora o Waitaha



Participant Information Sheet: Neonatal BNP Study			
Study title:	Investigating BNP levels in extremely preterm babies: Can BNP help to identify babies at high risk of the complications of premature lung disease?		
Locality:	Christchurch Women's Hospital	Ethics committee:	University of Otago Ethics Committee Approval 12/298
Lead investigator:	Dr Sarah Harris	Contact phone number:	0211544835

Your child is invited to take part in a study on BNP (B-type natriuretic peptide) levels in preterm babies born at less than 30 weeks gestation. Whether or not your child takes part is your choice. If you don't want them to take part, you don't have to give a reason, and it won't affect the care your baby receives. If you do want them to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you'd like your child to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. We expect this will take about 20 minutes. You may also want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 7 pages long, including the Consent Form. Please make sure you have all the pages.

Why are we doing the study?

Babies who are born very prematurely have very immature cardiovascular (heart and blood vessels) and respiratory (lung) systems. Neonatal intensive care aims to support these systems until they mature over time such that the baby can cope on their own. Some infants do remarkably well while others struggle with complications related to the immaturity of their heart and lungs. A few infants may develop a potentially life threatening complication called *pulmonary hypertension* where the blood vessels do not develop normally in the lungs and place strain on the heart. This complication can lead babies to have repeated sudden drops in the level of oxygen in the blood and require prolonged breathing support. Doctors do not know exactly how many babies are susceptible to the complication because it is not routinely looked for. Current estimates in the medical literature suggest up to one in three babies who develop chronic lung disease due to their prematurity may have pulmonary hypertension.

This study aims to test the level of a protein in the blood over the first few weeks of life to see if it can help us to predict which babies are more likely to develop pulmonary hypertension. The protein occurs naturally in the body and is called *N-terminal pro B type natriuretic peptide* (NTpBNP for short). This protein has been extensively studied in the adult population and there are many studies in older children but comparatively little study has been done in the neonatal age group. It has already been shown that NTpBNP levels increase with increasing severity of neonatal lung disease and that levels are high in term babies born with pulmonary hypertension. We would like to compare levels in babies born very prematurely to see whether they are higher in babies who develop complications of premature lung disease, in particular, pulmonary hypertension. If this protein proves to be useful in this setting it may open up further studies to address how we can prevent babies from developing this complication.

This study aims to recruit 80 patients over 2 years.

This study is being conducted as part of a Masters of Medical Science, University of Otago. It has been reviewed and approved by the University of Otago Human Ethics Committee.

My supervisors are:

Associate Professor Nicola Austin, Neonatologist, Christchurch Women's Hospital.

Associate Professor Richard Troughton, Cardiologist, Christchurch Hospital

This study is funded through a Masonic Paediatric Fellowship with assistance from the Maurice and Phyllis Paykel Trust.

A Maori Health Consultation has been conducted and the study will be conducted in a manner that honours the principles of the Treaty of Waitangi.

What would participation involve?

Babies who take part in this study will have the following blood tests, echocardiograms (heart ultrasound) and oxygen saturation monitoring performed:

1. Blood tests:

- On days 3, 10, 28 and when infants reach the equivalent of 36 weeks gestation (ie. 4 weeks before their original due date).
- This test will be for the NTpBNP protein level in their blood.
- Babies born prematurely routinely need blood tests to monitor their health and we would aim to do the study test at the same time as routine blood tests so your child would not be exposed to any additional discomfort.
- The amount of blood needed for this test is very small (0.6ml) so that the whole amount for the study would total half a teaspoon of blood.
- A C-reactive protein test is done at the same time to check for any signs of infection as this can falsely elevate the NTpBNP level. This test is often part of the routine tests premature babies have as part of their care. The amount of blood needed for this test is 0.1-0.2ml. If your child is unwell with an infection at the time the NTpBNP level may have to be deferred or repeated at another time.
- If there is sample left over after testing we seek separate consent to store this for future testing for some of the new bio-markers that may be of relevance in the development of heart dysfunction or

pulmonary hypertension.
(This does NOT involve genetic testing).

2. Echocardiograms (heart ultrasound):

- On days 3, 10, 28 and when they reach the equivalent of 36 weeks gestation (ie. 4 weeks before their original due date).
- The ultrasound is similar to a pregnancy scan except that a small ultrasound probe will be moved over the infant's chest to obtain a picture of the structure and function of the heart and major blood vessels.
- Ultrasound involves sound waves and is used routinely in neonatal care and considered safe for premature babies.
- The heart ultrasound will take approximately 30 minutes.

3. Oxygen saturation monitoring:

- It is routine for premature babies to have the oxygen levels monitored continuously via a band that is placed around a hand or foot.
- For this study we will use a more sophisticated monitor that has the ability to detect changes in oxygen levels every 2 seconds and store that information so that we can download it later for analysis.
- This will be performed for 72 hours around days 3, 10, 28 and when they reach the equivalent of 36 weeks gestation (ie. 4 weeks before their original due date).

We will also be collecting background data about your infant's health from the clinical notes that are relevant to the interpretation of the results of the study.

Your baby's health and well-being are paramount to the research team and we will always put their needs above that of our research. As the principal investigator I will make myself available throughout the study to answer any questions or address any concerns you may have.

What are the possible benefits and risks to you of participating?

There are no clear benefits to your baby in participating in this study. However, it is hoped this study will help us provide better care for premature babies in the future just as our care of your child today is guided by the results of past research. There is no financial payment for participation. We do not anticipate that this study will put your child at any additional risk. If at any point during the blood testing or heart ultrasounds your infant does not appear to be coping with the handling we will stop and wait until they are more stable.

What would happen if you were injured in the study?

In the unlikely event of a physical injury as a result of your child's participation in this study, they may be covered by ACC under the Injury Prevention, Rehabilitation, and Compensation Act 2001. ACC cover is not automatic, and your child's case would need to be assessed by ACC according to the provisions of the Injury Prevention, Rehabilitation, and Compensation Act 2001. If your child's claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors, such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses, and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators.

If you have any questions about ACC, contact the nearest ACC office or the investigator.

What are the rights of participants in the study?

Your child's participation in this study is entirely voluntary. It is up to you to decide whether or not to take part. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care your child receives.

This is a cohort study, which means we study a selected group of patients over time. To avoid bias in the study the investigators and clinical team will not know the results of the NTpBNP level until the analysis phase. The oxygen saturation data will be downloaded at a later time and so we recommend you discuss any concerns you have regarding your baby's oxygen saturation levels with the clinical team caring for your baby. We will be able to discuss the results of the heart ultrasound with you.

Patient confidentiality will be protected. Documents will be stored securely and patient data de-identified (your baby will be known in the study by a study number rather than by their name or NHI number).

We would normally inform your baby's General Practitioner of their participation in the study. If you would not like their GP to be informed this can be stated on the consent form.

What will happen after the study ends, or if you pull out?

Once the study is complete we plan to submit the results for presentation at a medical conference and for publication in a medical journal. As in the study no individual patient will be identified during either presentation or publication.

If you would like to receive a copy of the results of the research when it is complete you can request this on the consent form. It may take 3 years for the final study results to become available to you.

The study records will be kept in a secure hospital location for 15 years. The secure storage of these records is the responsibility of the neonatal research team. After this time the study records will be destroyed.

Where can you go for more information about the study, or to raise concerns or complaints?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Principal Investigator

Dr Sarah Harris
Neonatal Pediatrician
Christchurch Women's Hospital

If you have any complaints about the study you are entitled to take these to the Health and Disability Commissioner or voice them through the hospital complaints process.

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800555050
Fax: 08002SUPPORT(080027877678)
Email: advocacy@hdc.org.nz

You can also contact the University of Otago Human Ethics Committee that approved this study on: Phone 03 479 8256

Consent Form: Neonatal BNP Study

Declaration by participant: *(signed by parent/legal guardian as participant is a child)*

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.

I understand that this study involves my child having blood tests, heart ultrasounds and oxygen saturation monitoring.

I have had the opportunity to ask questions and I am satisfied with the answers I have received.

I have been given a copy of the Participant Information Sheet and Consent Form to keep.

I understand that my child's participation is voluntary and that I am free to withdraw my child from the study at any time, without giving any reason, without my medical care or legal rights being affected.

I understand the results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) and that no personal or identifying information about my child will be included.

I freely agree for my child to participate in this study.

I agree / do not agree (delete one) for any blood left over after testing to be stored for up to 5 years for future testing for cardioendocrine biomarkers.

I consent to any remaining samples being disposed of using:

Standard disposal methods, OR;

Disposed with appropriate karakia

I agree / do not agree (delete one) for my child's GP to be informed of participation in this study.

I would / would not (delete one) like to receive a copy of the results of the study upon completion.

Participant's name: *(signed by parent/legal guardian on behalf of child)*

Signature:

Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature:

Date:

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.



CCPU Neonatal Outline of (Advanced) Cardiac Scan

The following descriptions provide details of the quality and content of scans candidates are expected to achieve for both basic and advanced components of the CCPU qualification. Candidates should be aiming to produce a scan that can be readily interpreted by others.

The advanced cardiac scan should build on the skills elucidated in the basic scan. An advanced scanner will be expected to perform all the elements of a basic scan and include additional elements that may be determined by the clinical circumstances. An advanced scanner will also be expected to respond to ultrasound findings that would indicate additional views, measurements and procedures.

While an individual scan will not necessarily include all the elements described below the scanner should be competent in all elements and include examples of each in the portfolio of logged scans.

Items in italics are additional elements for the Advanced level cardiac scan.

Windows	Views	Modality	Detail
Parasternal long axis	Left ventricular	2D	Produce an image clip of the left ventricle clearly showing LA, Ao and LV. The LV should be horizontal on the screen and the clip allow visual inference of LA:Ao and the relative size and function of the LV. <i>Measure Ao diameter at level of valve leaflets.</i>
		Colour Doppler	Produce a colour Doppler clip demonstrating ventricular inflow across MV and outflow across Ao. <i>Demonstrate pulmonary veins.</i>
		<i>M-mode</i>	<i>Produce an M-mode image through Ao and LA and use measurement package to calculate LA:Ao</i>
			<i>Produce M-mode image perpendicular to the LV through the tips of the MV and use measurement package to calculate LV systolic and diastolic diameters, shortening and ejection fractions. If appropriate measure myocardial thickness.</i>
	Tricuspid	2D	Produce an image clip demonstrating the RA, TV and RV showing the

	view		opening and closing of the TV.
		Colour Doppler	Produce a colour Doppler clip demonstrating RV inflow and TR if present.
Windows	Views	Modality	Detail
		<i>PW / CW</i>	<i>Image and measure TR velocity.</i>
	Pulmonary valve view	2D	Produce an image clip of the RV outflow demonstrating the patency of the PV <i>Measure MPA diameter at level of valve leaflets.</i>
		<i>Colour Doppler</i>	<i>Produce a colour Doppler clip demonstrating blood velocity across pulmonary valve and into MPA.</i>
		<i>PW/CW</i>	<i>Interrogate MPA and measure VTI to calculate RVO.</i>
Parasternal short axis	Aortic valve view	2D	Produce a clear image of the AV demonstrating three cusps (Mercedes Benz sign).
	Pulmonary artery view	2D	Produce an image clip of the MPA and bifurcation into RPA and LPA.
		Colour Doppler	Colour Doppler clip demonstrating blood velocity in MPA, showing bifurcation and reverse or turbulent ductal flow if present.
	Short axis LV views	2D	Produce an image clip sweeping through LV from outlet through MV to apex.
		<i>Colour Doppler</i>	<i>Image while sweeping ventricular septum to exclude VSDs.</i>
		<i>M-mode</i>	<i>Optionally measure ventricular contraction in short axis.</i>
Apical	Four chamber view	2D	Image clip showing a clear four chamber image demonstrating relationship and relative size of all four main chambers and the AV valves. The image should have the septum near vertical.

		Colour Doppler	Produce colour Doppler clips demonstrating blood velocity across each AV valve. Image ventricular and atrial septae. Demonstrate pulmonary veins.
		PW/CW	If indicated interrogate flow velocities across AV valves and calculate pressure gradients.
	Long axis view	2D	Image clip demonstrating LV outflow tract and relationship to LV.
Windows	Views	Modality	Detail
		Colour Doppler	Colour Doppler interrogating velocity across AV.
		PW/CW	Interrogate Ao and measure VTI to calculate LVO.
High Parasternal	Aortic arch view	2D	Image clip demonstrating complete aortic arch and origin of main branches.
		Colour Doppler	Colour Doppler clip of aortic arch demonstrating flow velocity to exclude coarctation and possible presence of retrograde ductal flow.
		PW/CW	Trace aortic arch with PW to exclude coarctation, interrogate distal arch for ductal steal (if the ductus is patent).
	Pulmonary artery/ductal view	2D	Image clip demonstrating anatomy of MPA and origin of LPA showing ductus if present.
		Colour Doppler	Colour Doppler clip of blood velocity for above image excluding or confirming ductal flow velocity.
		PW / CW	If ductal shunt demonstrated PW or CW (as appropriate) interrogation of ductal velocity pattern.
Subcostal	Short axis Situs View	2D	Confirm normal Situs
	Atrial septal view	2D	Image clip demonstrating RA, LA and atrial septum at level of foramen ovale.
		Colour Doppler	Colour Doppler clip of above to demonstrate size and direction of atrial

			shunt.
		<i>PW</i>	<i>Interrogate atrial shunt to determine velocity and direction.</i>
	<i>RA SVC cut</i>	<i>Colour Doppler</i>	<i>Demonstrate SVC flow</i>
		<i>PW</i>	<i>Interrogate SVC velocity, measure and calculate VTI.</i>
	<i>Aortic view</i>		
<i>Mid SE SVC Diam View</i>		<i>2D</i>	<i>Image SVC and measure diameter.</i>



Respiratory Health Questionnaire¹

Name of Child:

Study Number: BOPP.....

How to complete the questionnaire:

These questions refer to your child's health **AFTER discharge from the neonatal unit until NOW.**

Please tick the appropriate box



Some questions may ask you to write an answer

If you are unsure of an answer, tick or write "UNSURE".



There are 48 questions.

It should be completed by a parent/caregiver who lives with the child.

If you have difficulty understanding this questionnaire contact:

Sarah (phone) **0211544835** or email **sarah.harris@otago.ac.nz**

and we will arrange to go through it with you (with an interpreter if needed).

¹ Approved by University of Otago Human Ethics Committee

Background Information:

Date questionnaire completed:

day ____ month _____ year _____ *(please fill in today's date)*

Person completing questionnaire:

Mother

Father

Other

If you ticked "other" please state your relationship to the child

Main language spoken at home:

English

Other

If you ticked "other", please tell us which language:

.....

CONSENT TO ACCESS MEDICAL RECORDS:

I give consent for the BOPP Study research team to access the General Practitioner and hospital medical records of my child.....

for the purposes of cross-checking the information given in this questionnaire.

Name.....

Signature.....

Date...../...../.....

1. Since discharge from the neonatal unit, how many times has your child seen a GP or family doctor? (if unsure give an estimate)

- Never
- 1-3 times
- 4-6 times
- 7-10 times
- >10 times

2. Since discharge from the neonatal unit, has your child been seen in the hospital emergency department, CAA (Children's Acute Assessment) or been admitted to hospital overnight?

- No
- Yes
- Unsure

If YES: How many times? *(please write number)*

What was the reason your child was seen in the emergency department, CAA or admitted to hospital?

Reason:

.....
.....
...

3. Since discharge from the neonatal unit, has you child needed CPAP breathing support?

- No
- Yes
- Unsure

4. Since discharge from the neonatal unit, has your child needed *ventilator* breathing support?

No

Yes

Unsure

5. After discharge from neonatal unit has your child ever been admitted to the *Intensive Care or High Dependency Units*?

No

Yes

Unsure

If yes what was the reason for this?

.....
.....

6. Since discharge from the neonatal unit has your child had any of the following conditions diagnosed by a doctor? (*tick as many as apply*)

Bronchiolitis

Pneumonia

Croup

Whooping cough

Wheezing

Chest infection

None of the above

7. Since discharge from the neonatal unit has your child been diagnosed with asthma?

No

Yes

Unsure

8. Since discharge from the neonatal unit has your child used an *inhaler* to treat wheezing or breathlessness?

No

Yes

Unsure

If NO skip to question 10.

9. How often would your child usually need to take this inhaler?

Every day

1- 6 times a week

1-3 times every month

Less than once a month

Only with a cold/respiratory infection

10. Since discharge from the neonatal unit, has your child used an inhaler to prevent wheezing (usually taken every day)?

No

Yes

Unsure

11. Since discharge from the neonatal unit, has your child required treatment with steroids (prednisone or prednisolone)?

No

Yes

Unsure

If NO, skip to question 13.

12. Since discharge from the neonatal unit, how many times has your child required treatment with steroids?

.....(write number)

13. Has your child had a cough that has lasted more than 4 weeks?

No

Yes

Unsure

14. Since discharge from neonatal unit has your child had difficulty feeding due to breathlessness?

No

Yes

Unsure

15. Since discharge from the neonatal unit, has your child required tube feeds (either via a nasogastric tube or a gastrostomy tube)?

No

Yes

Unsure

If NO, skip to question 17.

16. How old were they when they no longer required tube feeds?

Stopped tube feeds at(please state in months)

Still requiring tube feeds now

17. Does your child snore when they sleep?

No, never.

Yes, occasionally

Yes, most of the time

18. When your child went home from the neonatal unit did they use an apnoea monitor (a machine that alarms when babies stop breathing)?

No

Yes

Unsure

19. Since discharge from the neonatal unit, has your child had any episodes where they have paused or stopped breathing (apnoea)?

No

Yes

Unsure

If NO skip to question 21.

20. When they had the apnoea did your child require resuscitation?

.....
.....
.....
.

21. Since discharge from the neonatal unit, has your child been diagnosed (by a doctor) with *eczema* (itchy red rash requiring moisturisers and sometimes steroid creams)?

- No
- Yes
- Unsure

22. Since discharge from the neonatal unit, has your child been diagnosed (by a doctor) with *hayfever* (recurrent sneezing, itchy and watery nose and eyes)?

- No
- Yes
- Unsure

23. Since discharge from the neonatal unit, has your child had ear infections?

- No
- Yes
- Unsure

If NO, skip to question 26.

24. How many ear infections has your child had since discharge from the neonatal unit?

.....(please fill in number of infections)

25. Has your child required grommets (ventilation tubes) to treat ear infections?

No

Yes

Unsure

26. Is your child immunised?

No

Yes

Unsure

If NO, skip to question 28.

27. Which immunisations have they had so far?

6 week vaccines

3 month vaccines

5 month vaccines

15 month vaccines

28. Has your child had the flu vaccine?

No

Yes

Unsure

29. Has your child been given *palivizumab* (this is an injection to prevent the RSV infection which causes bronchiolitis)?

No

Yes

Unsure

30. Has your child ever had breast milk feeds (including when they were in NICU)?

No

Yes

Unsure

If NO skip to question 32.

31. How long did your child have breast milk?

<6weeks

6 weeks-3 months

4-6months

7-12months

12-15months

Continues to have breast milk

32. Has your child been diagnosed with *gastroesophageal reflux disease* (sometimes called “reflux”)?

No

Yes

Unsure

If NO, skip to question 34.

33. What treatment have they needed for this? (tick as many as apply)

- Gaviscon
- Ranitidine
- Omeprazole
- Surgery ("Nissen fundoplication")
- Antireflux formula milk

34. Does your child attend a child care centre (daycare, kindergarten, crèche etc)?

- No
- Yes
- Unsure

35. How many adults (16 years or older) live in your home?

.....(please write number)

36. How many children (< 16 yrs) live in your home?

.....(please write number)

If ZERO skip to question 38.

37. How many of the children are under 5 years?

.....(please write number)

38. How many rooms are there in your house?

(If your living areas are open plan, count kitchen, dining area and lounge separately. Do not count hallways/bathrooms/toilets/laundry)

.....(please write number)

39. How do you heat your home? (tick as many as apply)

- central heating
- electrical heaters
- gas heaters in rooms
- coal or wood fire
- no heating source
- other

40. Do you keep any household pets?

- No
- Yes
- Unsure

If NO skip to question 42.

41. Do you keep any of these pets? (tick as many as apply)

- dog
- cat
- other furry pets
- bird

42. Does the child's mother smoke cigarettes?

- No
- Yes
- Unsure

If NO skip to question 45.

43. How many cigarettes per day does the child's mother smoke?

1 to 10

11 to 20

more than 20

Unsure

44. Did the child's mother smoke during the pregnancy with this child?

No

Yes

Unsure

45. Does the child's father smoke cigarettes?

No

Yes

Unsure

If NO, skip to question 47.

46. How many cigarettes per day does the child's father smoke?

1 to 10

11 to 20

more than 20

Unsure

47. Of the other people living at your house, how many are smokers?

.....(please write number)

48. Did you have problems understanding this questionnaire?

No

Yes

Please write any comments you have about your child's health or about the questionnaire in the space below:

Thank you for completing the questionnaire.

Oxygen Saturation Targeting Questionnaire

With the change in saturation alarm limits recently we conducting a survey of nursing opinion on oxygen saturation targeting. Please note that these surveys are completely confidential and anonymous and are designed to reflect unit rather than individual practice. Please answer all questions.

Thank you very much for taking the time to do this survey. Please circle (or write) your answer:

1. Do you work primarily:
 - a. Day shift
 - b. Evening/night
 - c. Rotating

2. How many hours on average do you work a week?

3. Are you level three trained?
 - a. Yes
 - b. No

4. How many years of NICU nursing experience do you have?

5. Do you recall being orientated our unit protocol regarding oxygen saturation targeting and alarm limit setting?
 - a. Yes
 - b. No

6. When did you last attend an education session that included discussion of oxygen saturation targeting?
 - a. < 1 year ago
 - b. 1-2 years ago
 - c. > 2 years ago
 - d. Never

For these questions please use your own recall and do not confer with colleagues or look up protocol

7. The oxygen saturation targets in the unit changed at the end of October this year. What were the OLD oxygen saturation alarm limit settings?
 - a. <32 weeks in air
 - b. < 32 weeks in oxygen
 - c. 32-36 weeks in air
 - d. 32-36 weeks in oxygen
 - e. >36 weeks in air
 - f. >36 weeks in oxygen

8. B. What are the NEW oxygen saturation alarm limit settings?
 - a. <36 weeks in air
 - b. <36 weeks in oxygen
 - c. 36 weeks in air
 - d. 36 weeks in oxygen

9. On each monitor in the unit there is a sign with gestational age and target ranges on it. With regards to the OLD guidelines, did you use the infant's birth gestational age or their corrected gestational age to set alarm limits?
- Birth gestational age
 - Corrected gestational age
10. What % of time do you think infants < 30 weeks spend in their target saturation ranges on our unit?
- <25% of the time
 - 26-50%
 - 51-75%
 - >75%
11. In your opinion, how feasible was it to maintain infants born at <30 weeks gestation within the OLD oxygen saturation range?
- The policy range was feasible for MOST of these infants
 - The policy range was feasible for SOME of these infants
 - The policy range was feasible for FEW of these infants
 - Don't know
12. In your opinion, what is the MAIN reason why infants born <30 weeks gestation CANNOT be maintained within the oxygen saturation range as specified by your NICU's written Unit Policy/Guideline? Choose one of the following answers.
- Oxygen saturation range TOO LOW
 - Oxygen saturation range TOO HIGH
 - Oxygen saturation range TOO NARROW
 - Infants TOO LABILE and desaturate too frequently
 - Other

13. If a baby tends to fluctuate in their oxygen saturation level, frequently activating the alarms, do you change the alarm setting?
- Yes
 - No
14. If a baby is doing well does this prompt you to be more lenient with their target range?
- Yes
 - No
15. Does the gestational age of an infant prompt you to be more lenient on their target range?
- Yes
 - No
16. Are oxygen saturation targets and alarm limits discussed as part of bedside nursing handover?
- All the time
 - Most of the time
 - Occasionally
 - Never
17. Are oxygen saturation targets and alarm limits discussed as part of medical ward round?
- All the time
 - Most of the time
 - Occasionally
 - Never
18. In your opinion what is potentially more harmful for an infant born at < 28 weeks gestation?
- Hypoxia
 - Hyperoxia
 - Hypoxia and hyperoxia both equally important

19. In your opinion what is potentially more harmful for an infant born at 34 weeks gestation?

- a. Hypoxia
- b. Hyperoxia
- c. Hypoxia and hyperoxia both equally important

This concludes your survey. Please encourage other nurses in your unit to complete this survey without discussing the questions or your answers. Thank you very much for your contribution and time.

Proposal for the Surveillance of Pulmonary Hypertension in Premature infants

There are currently no national screening recommendations for late pulmonary hypertension in preterm born infants in New Zealand. There is currently no international consensus on screening for late pulmonary hypertension in premature infants. In the absence of international consensus this proposal is based on a review of recently published proposed guidelines for the screening/surveillance of later pulmonary hypertension in infants born very prematurely.^{62, 84, 86-88, 111, 112, 119, 120, 207} These recommendations are American Heart Association class of recommendation level 1 and level of evidence B or C meaning the benefit of screening is considered greater than the risk but the recommendation is based upon evidence from a single randomised controlled trial or non-randomised studies or consensus expert opinion. This proposal is designed to be practical in the New Zealand setting and to be a starting point for a national discussion on surveillance for this condition. Interpretation of echocardiographic indices should be based upon published reference data.

Who should undergo screening/surveillance:

Criteria A	Criteria B
<ul style="list-style-type: none">• All infants born at < 28 weeks gestation• Any very preterm infant who has persistent need for mechanical ventilation or non-invasive respiratory support and oxygen(excluding low flow nasal prong oxygen) at 36 weeks PMA	<ul style="list-style-type: none">• Any infant in whom pulmonary hypertension is suspected eg. Oxygen requirement out of keeping with the degree of lung disease, slow clinical improvement with persisting high levels of respiratory support and/or poor growth

Criteria A	Criteria B
<ul style="list-style-type: none"> • Any infant with IUGR/SGA who has persistent oxygen requirement or recurrent hypoxemia at 36 weeks PMA • Any infant who on any earlier scanning (at or after day 7) had signs of raised pulmonary pressures 	

Timing of screening tests:

1. Initial screen

Criteria A - 36 weeks PMA

Criteria B - Any time pulmonary hypertension suspected

2. Follow-up scans

1. If signs of pulmonary hypertension on initial scan

- Repeat scans according to level of clinical concern to guide management

2. If no signs of pulmonary hypertension on initial scan

- Repeat 3 months after 36 week PMA scan
- If persistent oxygen requirement at this second scan then repeat scans 3 monthly while on oxygen
- If signs of pulmonary hypertension at this second scan then repeat scans 3 monthly until resolution

Screening/surveillance testing:

Echocardiography

Components considered essential are marked with *. Other parameters should be done if scanner expertise allows.

1. A complete evaluation of cardiac anatomy including evaluation for tricuspid regurgitation, pulmonary regurgitation, interrogation of shunts and the identification of pulmonary veins*
2. If significant tricuspid regurgitation present then calculate estimated pulmonary artery pressure using Bernoulli equation and compared to systolic systemic pressure*
3. Qualitative evaluation of RV and LV for dilatation and hypertrophy*
4. Left ventricular eccentricity index*
5. Evaluation of right ventricular function (TAPSE, tricuspid valve E'/A' and S', right index of myocardial performance)
6. Evaluation of left ventricular function (consider calculating LV output by interrogation of aortic outflow or LV ejection fraction by Biplane Simpson's method, mitral annular plane of systolic excursion, mitral valve E'/A' and S' and left index of myocardial performance)

N-Terminal -proBNP

- If pulmonary hypertension confirmed do baseline NTproBNP and repeat at follow-up

3. Blood pressure

- Measured at the same time as heart scan