

1-1-2014

Newborn Heart Study - Novel Echocardiographic and Biomarker Measurements in Health & Disease

Kathryn Armstrong

Royal College of Surgeons in Ireland

Citation

Armstrong K. Newborn Heart Study - Novel Echocardiographic and Biomarker Measurements in Health & Disease [MD Thesis].
Dublin: Royal College of Surgeons in Ireland; 2014.

This Thesis is brought to you for free and open access by the Theses and Dissertations at e-publications@RCSI. It has been accepted for inclusion in MD theses by an authorized administrator of e-publications@RCSI. For more information, please contact epubs@rcsi.ie.

— Use Licence —

Creative Commons Licence:



This work is licensed under a [Creative Commons Attribution-Noncommercial-Share Alike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/).

RCSI LIBRARY

000701928

ROYAL COLLEGE OF SURGEONS IN IRELAND
701 928
11 JUN 2014
LIBRARY

**Newborn Heart Study- Novel Echocardiographic and Biomarker Measurements in
Health & Disease**

Dr Kathryn Armstrong

MBChB, Bsc Med Sci, MRCPCH

**Submitted for the degree of Doctor of Medicine to Royal College of Surgeons in
Ireland**

November 2012.

Royal College of Surgeons in Ireland

Supervisors: Professor Eleanor J Molloy

Dr Oral Franklin

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree, MD, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed  _____

Student Number 20131122 _____

Date January 7th 2014 _____

TABLE OF CONTENTS

CHAPTER 1: SECTION I:

NEONATAL TRANSITIONAL CIRCULATION AND CARDIAC FUNCTION

1.1.1	Introduction	1
1.1.2	Fetal Circulation	1
1.1.3	Circulatory Changes in the Perinatal Period	2
1.1.4	Myocardial Structure	4
1.1.5	Left Ventricular Development	4
1.1.6	Physiology of Rotational Movement	5
<i>1.1.6a</i>	<i>Myocardial Force Development</i>	<i>7</i>
<i>1.1.6b</i>	<i>Myocardial Deformation</i>	<i>8</i>

CHAPTER 1: SECTION II

PRETERM TRANSITIONAL CIRCULATION AND CARDIAC FUNCTION

1.2.1	Circulatory Changes in the Preterm Infant	9
1.2.2	Systemic Effects of the Patent Ductus Arteriosus	10
1.2.3	Preterm Myocardial Structure	10
1.2.4	Myocardial Performance in the Preterm Infant	11
1.2.5	Vitamin D Levels and Myocardial Function in the Preterm Infant	11

CHAPTER 1: SECTION III

CARDIOVASCULAR FUNCTION IN INFANTS WITH NEONATAL ENCEPHALOPATHY

1.3.1	Introduction	12
1.3.2	Pathogenesis of Myocardial Hypoxic Injury	12
1.3.3	Consequences of Hypothermia on Cardiac Function	15
1.3.4	Novel therapies for Cardiovascular dysfunction secondary to hypoxia ischaemia	15

CHAPTER 1: SECTION IV

2-DIMENSIONAL ECHOCARDIOGRAPHIC ASSESSMENT OF VENTRICULAR FUNCTION

1.4.1	Introduction	19
1.4.2	Assessment of Systolic Function	19
1.4.3	Assessment of Systolic Function in Preterm Infants	20
1.4.4	Assessment of Systolic Function in Neonatal Encephalopathy	22
1.4.5	Assessment of Diastolic Function	22
1.4.6	Assessment of Diastolic Function in Healthy Normal Term Infants	24
1.4.7	Assessment of Diastolic Function in Preterm Infants	24
1.4.8	Assessment of Diastolic Function in Neonatal Encephalopathy	24
1.4.9	Conclusion	27

CHAPTER 1: SECTION V:

TISSUE DOPPLER IMAGING

1.5.1	Introduction	28
1.5.2	TDI Measurements	29
1.5.3	TDI in Healthy Normal Term Infants	29
1.5.4	TDI in Preterm Infants	31
1.5.5	TDI in Neonatal Encephalopathy	31
1.5.6	Pitfalls of TDI	32

CHAPTER 1: SECTION VI

SPECKLE TRACKING IMAGING

1.6.1	Introduction	33
1.6.2	Speckle Tracking Imaging in Adults	36
1.6.3	Speckle Tracking Imaging in Children	36

CHAPTER 1: SECTION VII

SERUM BIOMARKERS OF CARDIAC FUNCTION

1.7.1	Introduction	37
1.7.2	Physiology of Troponin T	37
1.7.2a	<i>Troponin T in Clinical Practice</i>	38
1.7.2b	<i>Troponin in Neonates</i>	40
1.7.2c	<i>Troponin T in Preterm Infants</i>	40
1.7.2d	<i>Troponin T in Neonatal Encephalopathy</i>	41
1.7.3	Physiology of N Terminal Pro-B-Type Natriuretic Peptide (NTproBNP)	42
1.7.3a	<i>Clinical use of BNP/NTpro BNP</i>	43
1.7.3b	<i>NTproBNP in Neonates</i>	43
1.7.3c	<i>NTproBNP in Preterm Infants</i>	44
1.7.4	Point of Care Assessment of Troponin T/NTproBNP	45

CHAPTER 1 :SECTION VIII

1.8.1	Hypothesis	46
1.8.2	Aims	46

CHAPTER 2

EXPERIMENTAL DESIGN AND METHODS

2.1	Ethical Approval	47
2.2	Patient Population	47
2.2 a	<i>Term Control Infants</i>	47
2.2 b	<i>Preterm Infants</i>	48
2.2 b(i)	<i>Sample Size calculation for Preterm Infants</i>	48
2.2 b(ii)	<i>Antenatal History & Delivery details for Preterm Infants</i>	49
2.2 b(iii)	<i>Clinical Parameters for Preterm Infants</i>	50
2.2 b(iv)	<i>Preterm Outcomes</i>	51

2.2 c	<i>Infants with Neonatal Encephalopathy</i>	52
2.2 c(i)	<i>Grading of Neonatal Encephalopathy</i>	52
2.2 c(ii)	<i>Clinical Parameters for Term Infants with NE</i>	53
2.2 c(iii)	<i>Investigations in Infants with Neonatal Encephalopathy</i>	54
2.2 c(iv)	<i>MRI</i>	54
2.3	Biochemical Parameters for Point of Care Testing	
2.3a	<i>Validation of Cobas h232 Analyser</i>	54
2.3b	<i>Analysis using Cobas h232 Analyser</i>	55
2.4	Echocardiographic Parameters	57
2.4.1	M mode measurement of Left Ventricular Systolic Function	57
2.4.2	Dimensional LV Diastolic Function	58
2.4.3	Presence of Patent Ductus Arteriosus	58
2.4.4	Tissue Doppler Imaging	62
2.4.5	Speckle Tracking Imaging	64
2.5	Echocardiographic Measurements for each group	64
2.5.1	Term Control Infants	64
2.5.2	Preterm	64
2.5.3	Infants with Neonatal Encephalopathy	65
2.6	Statistical Analysis	65
2.6a	<i>TDI Measurement Validation</i>	65
2.6a(i)	<i>Intra-Observer Variability for TDI Measurements</i>	66
2.6a(ii)	<i>Inter –Observer Variability for TDI Measurements</i>	66

CHAPTER 3

TISSUE DOPPLER IMAGING QUANTIFIES CHANGES IN PRETERM MYOCARDIUM.

3.1	Introduction	71
3.2	Hypothesis	74
3.3	Aims	74

3.4	Results	75
3.5	Discussion	85

CHAPTER 4

TISSUE DOPPLER IMAGING MAY PREDICT OUTCOMES IN PRETERM INFANTS

4.1	Introduction	88
4.2	Hypothesis	90
4.3	Aims	90
4.4	Results	92
4.5	Discussion	98

CHAPTER 5

VITAMIN D LEVELS AND MYOCARDIAL FUNCTION IN PRETERM INFANTS

5.1	Introduction	102
5.2	Hypothesis	104
5.3	Aims	104
5.4	Results	105
5.5	Discussion	107

CHAPTER 6

SPECKLE TRACKING IMAGING IN TERM INFANTS

6.1	Introduction	109
6.2	Hypothesis	111
6.3	Aims	111
6.4	Results	112
6.5	Discussion	113

CHAPTER 7

CARDIOVASCULAR FUNCTION IN INFANTS WITH NEONATAL ENCEPHALOPATHY

7.1	Introduction	116
7.2	Hypothesis	118
7.3	Aims	118
7.4	Results	119
7.5	Discussion	127

CHAPTER 8

POINT OF CARE TESTING FOR CARDIAC BIOMARKERS IN THE NEONATAL INTENSIVE CARE UNIT

8.1	Introduction	131
8.2	Hypothesis	133
8.3	Aims	133
8.4	Results	134
8.4.1	Validation	134
8.5	Discussion	140

CHAPTER 9

DISCUSSION

9.1	Introduction	142
9.2	Tissue Doppler Imaging	142
9.3	Impact of Serial Echocardiography in the NICU	143
9.4	Speckle Tracking Imaging	144
9.5	Cardiovascular Function in Neonatal Encephalopathy	144
9.6	Point of Care Testing for Troponin T and NT pro BNP in Neonates	146
9.7	Future Research	147
9.7.1	<i>Cardiovascular Follow up</i>	147

9.7.2	<i>Vitamin D and cardiac Function</i>	149
9.7.3	<i>Sepsis and Cardiac Function</i>	149
9.7.4	<i>Stem cell research in Cardiovascular Disease in Infants</i>	150
9.7.5	<i>Future Echocardiographic Training</i>	150
9.8	Conclusion	151
REFERENCES		153

SUMMARY OF THESIS

Neonatal myocardial dysfunction may impact negatively on survival outcomes in preterm and term infants. Early identification and subsequent intervention may improve outcomes in preterm infants and term infants following a hypoxic ischaemic injury. Current echocardiographic measures for assessing Left Ventricular systolic and diastolic function have limitations in preterm infants. Newer techniques such as tissue Doppler Imaging (TDI) and Speckle Tracking Imaging (STI) provide a global assessment of myocardial systolic and diastolic function and have been introduced in adult cardiology. However there is a paucity of data on these modalities in children and neonates. Cardiac biomarkers, Troponin T and NT pro BNP reflect myocardial dysfunction and are elevated in preterm infant with a Patent Ductus Arteriosus and in term infants with neonatal encephalopathy.

Sixty healthy term control infants were recruited who underwent echocardiography on Day 2-4 of life. Normal data for Left and Right ventricular diastolic velocities were obtained using Tissue Doppler Imaging. A further 20 healthy term infants underwent echocardiography for longitudinal strain analysis using Speckle Tracking Imaging.

Infants < 32 weeks or < 1500kg underwent serial echocardiography to compare measures of shortening and ejection fraction with Tissue Doppler velocities. There was a significant increase in the RV systolic and diastolic velocities over the first week of life. There was a significant increase in the LV diastolic velocities over the first week of life. There was no significant increase in either LV shortening or ejection fraction over the first week of life. Tissue Doppler velocities were significantly lower

over the first week of life in infants who developed Chronic Lung Disease, Necrotising Enterocolitis and in those infants who required surgical PDA ligation. Vitamin D levels were low in preterm infants enrolled in the study, and Tissue Doppler systolic and diastolic velocities were significantly lower in preterm infants compared to term infants on Day one of life.

Our final part of the study was the validation of the Cobas h232 Point of Care Analyser for analysis of Troponin T and NT pro BNP. We found that the Cobas h232 had correlation with the gold standard laboratory Roche 800 analyser

In conclusion serial echocardiography in the NICU may improve outcomes in preterm infants by allowing early therapeutic intervention. Analysis of Troponin T and NT pro BNP at the bedside in conjunction with echocardiography may aid in the diagnosis of myocardial dysfunction, more timely therapy and ultimately improve survival and neurodevelopmental outcomes in preterm infant and in those with Neonatal Encephalopathy.

ACKNOWLEDGEMENTS

I would like to start by thanking two people, for their help, support, encouragement and mentorship without which I couldn't have gotten through the last two years. Firstly Professor Eleanor Molloy who has been the driving force behind my research. No matter what time of day or night, she has been there to answer questions, be constantly enthusiastic and supportive. Dr Orla Franklin for her continued enthusiasm for this project, her help in guiding my career and for her ever positive feedback about achievements obtained from this project.

I would like to thank all of the staff, patients and parents in the National Maternity Hospital who participated in this study. Without you it would not have been possible. A special thank you to Ms Marie Culleton and Catherine Doughty in the laboratory for facilitating me in every way and making the point of care analysis possible.

I would also like to thank The National Children's Research Centre and in particular Professor Blanco, Dr O'Donnell and Dr Kelly for funding my MD and for their continued commitment and enthusiasm to achieving a high standard of research in Paediatrics in Ireland. Tim Grant from C-Star has been an integral part of this project with his statistical knowledge. I have learnt so much from him over the last two years.

I wish to thank my colleagues Lisa, Deirdre and Emily, firstly for their help in patient recruitment and for their encouragement, friendship and fun in the research office over the last two years.

Lastly but not least there are no words to sum up the encouragement, love, support and belief that I have received from my husband Tadhg and my family over the last two years – Thank you.

ABBREVIATIONS

A'	Late diastolic velocity
AEDF	Absent end-diastolic flow
ANP	Atrial Natriuretic Peptide
Ao	Aorta
APH	Antepartum haemorrhage
BiPAP	Biphasic Intermittent Positive Airway Pressure
BMC	Bone Marrow progenitor Cells
BNP	B-type Natriuretic peptide
BP	Blood pressure
c TNT	Cardiac Troponin
CK MB	Creatinine Kinase (muscle& brain type)
CLD	Chronic Lung Disease
CPAP	Continuous positive airway pressure
E:A	Early atrial to late atrial diastolic ratio
E'	Early diastolic velocity
ECG	Electrocardiogram
EEG	Electroencephalogram
EF	Ejection Fraction
HsPDA	Haemodynamically significant Patent Ductus Arteriosus
HUCBSC	Human Umbilical Cord Blood Stem Cells
IVH	Intraventricular Haemorrhage
IVS	Intraventricular Septum
LA	Left Atrium
LA:Ao	Left Atrial:Aortic ratio
LOS	Late Onset Sepsis
LV	Left Ventricle
LVEDD	Left Ventricular End diastolic Dimension

LVESD	Left Ventricular End Systolic Dimension
MI	Myocardial Infarction
MV	Mitral Valve
NE	Neonatal Encephalopathy
NEC	Necrotising Enterocolitis
NICU	Neonatal Intensive Care Unit
NP O ₂	Nasal Prong Oxygen
NSAIDS	Non steroidal anti inflammatory Drugs
NT pro BNP	N Terminal Pro B Type Natriureic Peptide
PA	Pulmonary Artery
PDA	Patent Ductus Arteriosus
PET	Pre-eclampsia
PFO	Patent Foramen Ovale
PICU	Paediatric Intensive Care Unit
POCT	Point of Care Testing
PPHN	Persistent Pulmonary Hypertension of the Newborn
PROM	Prolonged rupture of the Membranes
PVR	Pulmonary Vascular Resistance
REDF	Reversed End Diastolic flow
ROP	Retinopathy of Prematurity
RV	Right Ventricle
S'	Systolic velocity
SD	Standard deviation
SF	Shortening Fraction
SIMV	Synchronised intermittent Mandatory Ventilation
SIRS	Systemic Inflammatory Response Syndrome
STI	Speckle Tracking Imaging
SVD	Spontaneous Vaginal Delivery

SVR	Systemic Vascular Resistance
TDI	Tissue Doppler Imaging
TV	Tricuspid Valve
VLBW	Very low birth weight

PRIZES ASSOCIATED WITH THIS THESIS

- **Irish Paediatric Association Gold Medal for best Oral Presentation**, Tissue Doppler Imaging Quantifies Early Change in Preterm Myocardium, April 2012
- **National Children's Research Centre Open Day Prize Winner**, May 2012

PEER REVIEWED PUBLICATIONS ASSOCIATED WITH THIS THESIS

1. **K.Armstrong**, E J Molloy. Doing a higher Medical Degree. 'The Way I see it' BMJ Careers 29/6/2011 Accepted for publication in BMJ Careers
2. **K.Armstrong**, O.Franklin,D Sweetman, EJ Molloy.Cardiovascular dysfunction in infants with Neonatal Encephalopathy. Accepted for publication in Archives of Disease in Childhood
3. D.Sweetman, **K.Armstrong**, J Murphy, EJ Molloy. Cardiac Biomarkers in Neonatal Hypoxic Ischaemia. Under Review. Acta Paediatrica

BOOK CHAPTER

Congenital Diaphragmatic Hernia and Congenital Heart Disease. **K Armstrong**, O.Franklin, EJ Molloy. Diaphragmatic Hernia: Diagnostics and Treatment", ISBN 978-953-307-720-8

PUBLISHED ABSTRACTS

1. Novel Myocardial Velocity Measures in Preterm Infants - Speckle Tracking Imaging. **K Armstrong**, E J Molloy, O Franklin. *Pediatric Research* 70, 261-261
2. Changes in Myocardial Velocities in the 1st week of life in preterm infants. **K Armstrong**, E J Molloy, O Franklin. *Cardiology in the Young*.2011
3. Tissue Doppler Imaging Quantifies early change in Preterm Myocardium. **K Armstrong**, E J Molloy, O Franklin. *Pediatric Research*, 2012.
4. Vitamin D levels and Myocardial Function in Preterm Infants **K Armstrong**, C Onwuneme, O Franklin, E J Molloy. *Pediatric Research*, 2012.
1. Cardiovascular Dysfunction in Infants with Neonatal Encephalopathy in the 1st week of life. **K Armstrong**, D Sweetman, O Franklin, E J Molloy. *Pediatric Research*, 2012.

PRESENTATIONS ASSOCIATED WITH THIS THESIS

INVITED SPEAKER

- Functional Assessment of the Heart. **Point of Care Ultrasound for Neonatologists. The Coombe Woman's and Children's Hospital, October 2011**
- The 10 Commandments for Surviving an MD. **Research Symposium, Irish Paediatric Association, April 2012**

INTERNATIONAL SCIENTIFIC MEETINGS

1. Novel Myocardial Velocity Measure in Preterm Infants – Speckle Tracking Imaging. **K Armstrong**, E J Molloy, O Franklin. **October 2011, European Society of Paediatric Research**
2. Changes in Myocardial Velocities in First week of life in Pre Term Infants. **K Armstrong**, E J Molloy, O Franklin. **May 2011, Association of European Paediatric Cardiology**
3. Tissue Doppler Imaging Quantifies early change in Preterm Myocardium. **K Armstrong**, E J Molloy, O Franklin. **October 2012, European Academy of Paediatric Societies**
4. Vitamin D levels and Myocardial Function in Preterm Infants **K Armstrong**, C Onwuneme, O Franklin, E J Molloy. **October 2012, European Academy of Paediatric Societies**
5. Cardiovascular Dysfunction in Infants with Neonatal Encephalopathy in the 1st week of life. **K Armstrong**, D Sweetman, O Franklin, E J Molloy. **October 2012, European Academy of Paediatric Societies**

NATIONAL SCIENTIFIC MEETING

1. Tissue Doppler Imaging Quantifies Early Change in Preterm Myocardium. **K Armstrong**, E J Molloy, O Franklin. **April 2012, Irish Paediatric Association Meeting, Belfast**

2. Novel myocardial velocity measures in term infants – speckle tracking echocardiography. **K Armstrong, E J Molloy, O Franklin. June 2011, Irish Paediatric Association Meeting, Kilkenny**

3. Changes in Myocardial Velocities in Preterm Infants in the first week of life. **K Armstrong, E J Molloy, O Franklin Irish Perinatal Society, March 2011, Galway**

4. **Inaugural Irish Paediatric Echo Meeting, Dublin, May 2011. Assistant Director**

CHAPTER 1: SECTION 1

NEONATAL TRANSITIONAL CIRCULATION AND CARDIAC FUNCTION

1.1.1 Introduction

Newborn infants undergo a number of haemodynamic changes during the fetal to neonatal transitional period which may have a global impact on myocardial function. Preterm infants with a patent ductus arteriosus (PDA) and term infants who have suffered a hypoxic ischaemic event may have poor cardiac function which may have a negative impact on both morbidity and mortality. The usual measures of assessment of systolic myocardial function are the echocardiographic measures of ejection and shortening fraction. However newer modalities such as Tissue Doppler (TDI) and Speckle Tracking Imaging (STI) exist which may provide a better assessment of not only of systolic but also of diastolic myocardial function. Early identification of myocardial dysfunction in both preterm infants with a PDA and infants with neonatal encephalopathy may improve outcomes, allow intervention and help to monitor treatment response.

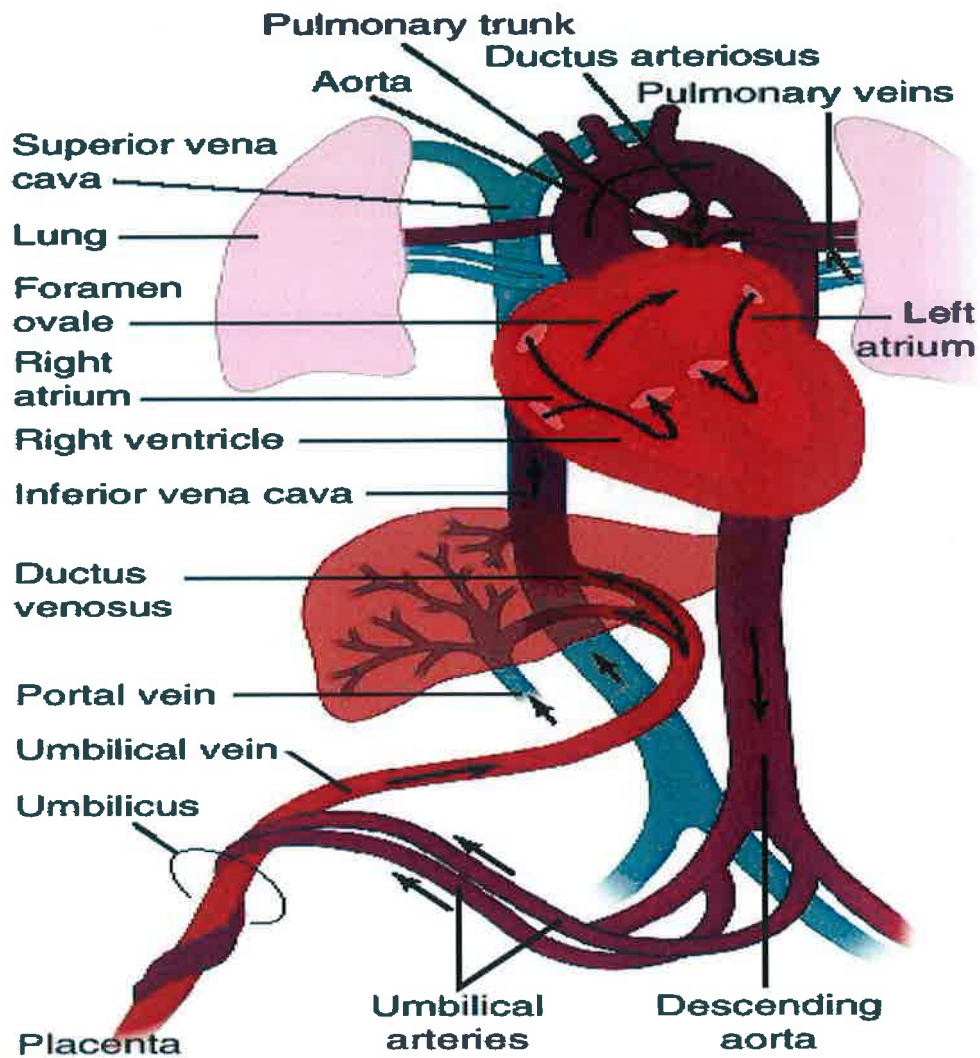
1.1.2 Fetal Circulation

The fetal circulation differs from the neonatal circulation. In fetal life the placenta is the site of gas exchange, following delivery this role is taken over by the lungs. The fetal circulation has adapted to this by having ventricles which work in parallel and not in series. The Right ventricle (RV) contributes approximately two-

thirds of the combined ventricular output which predominantly supplies the placenta and shunts exist through the ductus arteriosus, foramen ovale and ductus venosus. (Figure 1.1.1) There is high pulmonary vascular resistance (PVR) and low systemic vascular resistance (SVR) and equal pressures in the aorta and the pulmonary artery due to the large patent ductus arteriosus (PDA) (Stopfkuchen 1987).

1.1.3 Circulatory Changes in the Perinatal Period

During the transition period from fetal to neonatal life, the neonate undergoes a number of haemodynamic changes. The cessation of umbilical venous return causes the preload to the RV to decrease and the ductus venosus to close. The pulmonary vascular resistance and pulmonary artery pressure decrease, with a resulting increase in pulmonary blood flow, leading to decreased RV pressure. The removal of the low resistance placental circulation increases the SVR. There is increased return of blood to the left atrium causing a rise in left atrial pressure allowing closure of the patent foramen ovale (PFO). There is functional closure of the PDA. The increase in SVR and the dramatic fall in PVR cause a reversal of blood flow through the ductus arteriosus. This in turn contributes to an increase in pulmonary blood flow and an increase in left ventricular preload. (Stopfkuchen 1987)



■ Oxygenated Blood
 ■ Deoxygenated Blood
 ■ Mixed Blood

Figure 1.1.1. Fetal Circulation. Oxygen rich blood from the placenta passes through the umbilical veins across ducts venosus, to the inferior vena cava before entering the right ventricle (black arrows). The ductus arteriosus diverts blood away from the lungs to the descending aorta. Deoxygenated blood then returns to the placenta via the umbilical arteries. (source – adapted from – <http://medicaldictionary.thefreedictionary.com/fetal+circulation>)

1.1.4 Myocardial Structure

Throughout the process of maturation there are changes in the underlying structure and function of the myocyte, with an increase in size and number.

Myocytes in the myocardium are composed of individual striated muscle fibres.

Under the light microscope each fibre contains multiple cross-banded strands (myofibrils) which run the length of the fibre and are composed of sacromeres, which themselves are made up of a thick and thin myofilament. (Braunwald 1971) These myofibrils are in a spiral/helical arrangement, which emerges throughout several sequential embryonic stages.

1.1.5 Left Ventricular Development

In the early tubular heart the wall of the Left Ventricle (LV) develops two layers of epithelial cells, the inner layers forms sheets of chords that develop gradually into inner centripetally aligned trabeculae. Later there is proliferation and compaction of the outer layers and the invasion of the coronary vessels into the mantle of muscle fibres from the epicardial surface. In the final stage the morphogenesis of transmural helical layers is complete, with the coronary vascular tree as seen in the adult heart. The fibre direction is predominantly longitudinal in the endocardial region, circumferential in the mid walls and longitudinal again over the epicardial surface (Sengupta, *et al* 2007).

The size of the human heart increases from infancy to adolescence. However the helically orientated myocardial fibre architecture, which creates LV torsion, has been observed in human hearts from neonates to adults and is independent of sex and age (Notomi, *et al* 2005).

The LV is described as ellipsoid in shape, with the long axis directed from the apex to the base. The posterolateral wall is significantly thicker than the septum with a gradual thinning of the LV observed towards the apical segments (Rankin, *et al* 1976).

The contractile elements of the myocardium are embedded in the LV with specific geometry and elastic properties. By the initial force development of the contractile elements, the ventricle can increase its internal pressure up to a point that this is high enough to open the aortic valve. From then on the shortening of the contractile elements decrease the cavity size so that its internal blood is ejected (Bijnens, *et al* 2009).

1.1.6 Physiology of Rotational Movement

Leonardo Da Vinci was among the first to observe that during the cardiac cycle the mammalian heart rotates (Burns, *et al* 2008a). The helical orientation of myocardial fibres of the LV wall provides an equal distribution of regional stress and strain. However their complex geometry mandates that systolic mechanics occur in circumferential, radial and longitudinal directions (Greenbaum, *et al* 1981) (Vendelin, *et al* 2002). As understanding of the architecture of the heart has improved, it is apparent that torsion or the twisting motion which results from the rotation of the apex and base of heart in different directions is integral to normal cardiac function (Burns, *et al* 2008b). (Figure 1.1.5)

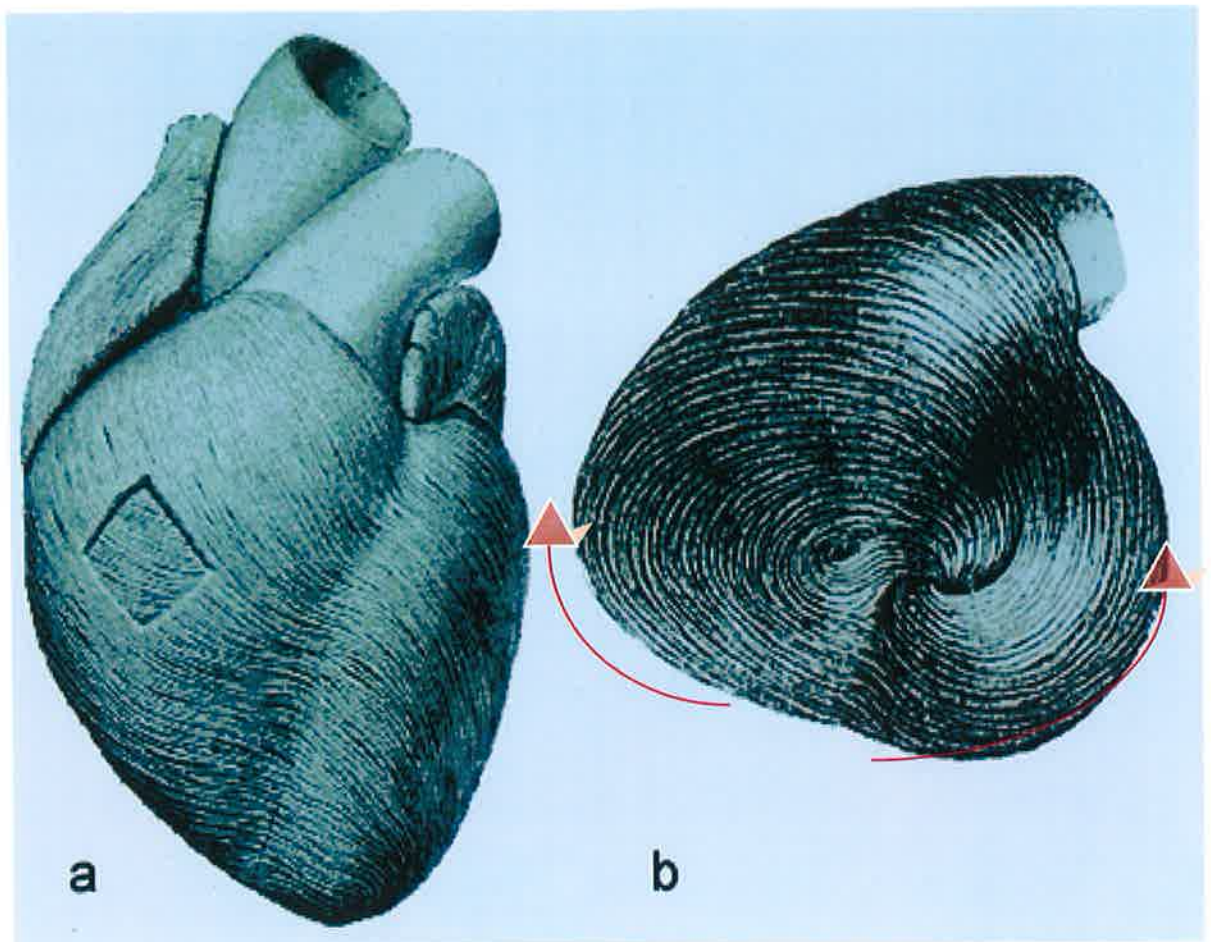


Figure 1.1.5 : a) Showing the helical arrangement of myocardial fibres from the apical view. B) Clockwise and counter clockwise arrangement of the helical fibres (red arrows). Adapted from (<http://jtcs.ctsnetjournals.org/cgi/c>)

In the evaluation of systolic cardiac function, the intrinsic properties of the fibres and myocytes are important. This determines the effect of chronic conditions on the myocardium. The interaction between force and deformation are vital in understanding how the myocardium functions (Bijnens, *et al* 2009).

1.1.6a Myocardial Force Development

The force of contraction depends on initial muscle length (Braunwald 1971). The relationship between initial muscle length and the developed force is important for the function of heart muscle. This forms the basis of the Frank-Starling Law which states that 'the energy of contraction, however measured, is a function of the length of the muscle fibres, prior to contraction (Sarnoff and Berglund 1954). The contractile activity of the myocardium may be readily altered under differing loading conditions by changing the resting fibre length and by changes in contractility, both of which shift the myocardial velocity curve.

Several parameters of myocardial mechanics can be described. Rotation which is defined as the angular displacement of a myocardial segment in short axis view around the LV longitudinal axis measured in a single plane and is measure in degrees (Blessberger and Binder, 2010). Twist/torsion which is the net difference between apical and basal rotation (calculated from two short axis cross-sectional planes of the LV - degrees) (Notomi, *et al* 2005). Torsional gradient which is defined as twist/torsion normalised to ventricular length from base to apex and accounts for the fact that a longer ventricle has a larger twist angle (degrees/cm) (Blessberger and Binder, 2010).

1.1.6b Myocardial Deformation

Strain is defined as a dimensionless quantity of myocardial deformation and measures the magnitude of myocardial fibre contraction and relaxation (Pavlopoulos and Nihoyannopoulos 2008). Lagrangian strain (given the Greek letter Epsilon (ϵ)) is mathematically defined as the change of myocardial fibre length during stress at end systole compared to its original length in a relaxed state at end diastole and is expressed as percentage (%). In more simple terms it is a measure of tissue deformation (Marwick 2006). Strain rate (the temporal derivative of strain) is the change of strain per unit time. Negative strain indicates fibre shortening or myocardial thinning in the longitudinal or circumferential plane whereas a positive value describes lengthening or thickening in the radial plane (Marwick 2006) (Pavlopoulos and Nihoyannopoulos 2008).

CHAPTER 1 SECTION II

PRETERM TRANSITIONAL CIRCULATION AND CARDIAC FUNCTION

1.2.1 Circulatory Changes in the Preterm Infant

The normal haemodynamic changes described above which occur during the first 48 hours of transition from fetal to neonatal life differ in preterm infants who are less than 32 weeks gestation. The patent ductus arteriosus (PDA) differs from that in the term infant (>37 weeks gestation) in that it may close, remain patent or close and then reopen. The PDA allows left to right sided shunting of blood. The patent foramen ovale (PFO) which also may remain open allows shunting at the atrial level from left to right. The presence of either shunt may allow increased pulmonary blood flow, ultimately leading to a reduction in systemic blood flow and increased LV Preload. The presence of the PDA essentially allows shunting between the systemic and pulmonary circulation, which is defined as ductal steal. This results in systemic hypoperfusion and pulmonary overcirculation putting preterm infants at risk for complications such as pulmonary haemorrhage (Kluckow and Evans 2000).

1.2.2 Systemic Effects of the Patent Ductus Arteriosus

The presence of a left-right shunt across a PDA may have an effect on systemic perfusion and organ flow, in which instance it is referred to as an haemodynamically significant ductus arteriosus (hsPDA). Therefore it is the effect of the transductal shunt and the ability of the preterm myocardium to adapt that may have an impact on various organ systems and not just the presence of a PDA itself (Sehgal and McNamara 2009). Ductal steal means that blood is shunted from the systemic circulation to the pulmonary circulation, resulting in systemic hypoperfusion and pulmonary overcirculation. This process may contribute to Necrotising Enterocolitis (NEC), pulmonary haemorrhage and intraventricular haemorrhage (IVH) (Clyman 2006).

1.2.3 Preterm Myocardial Structure

Preterm infants have immature myocytes and decreased ventricular mass compared to term infants which may impact on global myocardial function. In fetal life the myocardium has fewer sarcomeres per unit mass and different isoforms of contractile proteins (Nadal-Ginard and Mahdavi 1989). The myofibre architecture of the RV and LV is likely to change throughout gestation, with the RV contributing more to combined ventricular function than the left during fetal life (Kiserud and Acharya 2004). Throughout the first weeks of life ventricular muscle mass grows and thereafter remains constant throughout childhood (Kozak-Barany, *et al* 2001). Echocardiography has been used to determine the geometry of the LV in the preterm infant and as they grow, the LV mass increases and assumes a circular cross-sectional shape (Lee, *et al* 1992).

1.2.4 Myocardial Performance in the Preterm Infant

Ventricular performance and cardiac output is determined by heart rate, contractility, preload and afterload. Contractility is the intrinsic ability of the myocardium itself to contract. Preload is the amount of blood present in the ventricle at end diastole and may be affected by the diastolic compliance of the ventricle. Afterload is the resistance against which the ventricle contracts and relies on vascular resistance or other ventricular outflow tract obstructions (Kluckow 2005).

The preterm heart is less compliant and contractile than a term newborn or adult heart. It therefore has limited ability to increase its stroke volume (contractility, preload and afterload) by increasing the end diastolic filling pressure and relies on its ability to increase heart rate to increase cardiac output (Kiserud and Acharya 2004). The preterm heart has less adaptation to cope with changes in preload and afterload.

1.2.5 Vitamin D Levels and Myocardial Function in the Preterm Infant.

Vitamin D deficiency has been linked to a number of chronic diseases including cardiac failure and cardiomyopathy in the paediatric population (Gupta, *et al* 2011). Case reports exist of infants presenting with cardiomyopathy and subsequent cardiac failure caused by hypocalcaemia as a result of vitamin D deficiency (Tomar, *et al* 2010). Agarwal *et al* reported low Vitamin D levels in up to 80% of very low birth weight infants (Agarwal, *et al* 2012). Preterm infants may be at risk for poor cardiac function as a result of low vitamin D levels.

CHAPTER 1 SECTION III

CARDIOVASCULAR FUNCTION IN INFANTS WITH NEONATAL

ENCEPHALOPATHY

1.3.1 Introduction

A hypoxic -ischaemic insult occurring around the time of birth may result in an encephalopathic state characterised by the need for resuscitation at birth, neurological depression, seizures and electroencephalographic abnormalities (Azzopardi, *et al* 2008). Any interruption of the placental blood or oxygen supply may cause multi organ dysfunction (Martin-Ancel, *et al* 1995) (Shah, *et al* 2004). Cardiovascular dysfunction has been increasingly recognised in these infants as a result of hypoxic ischaemic damage to the myocardium (Evans 2006) (Barberi, *et al* 1999) (Fatemi, *et al* 2009). Neonates with asphyxia have low cardiac output with decreased myocardial contractility, systemic hypotension and pulmonary hypertension (Evans, *et al* 1998). Cardiac abnormalities in asphyxiated neonates may be often under diagnosed due to the difficulty in accessing urgent and serial echocardiography in many neonatal centres.

1.3.2 Pathogenesis of Myocardial Hypoxic Injury

Oxygen homeostasis is critical for survival and function of all cells and in particular the myocardium (Calbet, *et al* 2009). Hypoxia is characterised by inadequate oxygen delivery to the myocardium and elicits both indirect and direct

effects on the heart which are mediated by neurohormonal mechanisms (Abdel, *et al* 2009). In the acute or chronic situation it is likely that a reduction in arterial blood oxygen partial pressure is responsible for the mismatch between oxygen supply and delivery and this in turn may result in a reduction in myocardial contractility (Calbet, *et al* 2009).

At the cellular level anoxia ablates adenosine triphosphatase synthesis leading to cell death by induction of apoptosis (Asano, *et al* 2003). Mammalian cells respond to hypoxia by activating transcription factors and hypoxia-inducible factors which bind to hypoxia responsive elements and consensus sequences in the promoter region of more than 100 genes. The transcription of these genes allows the cell to adapt to and survive the hypoxic environment (Calbet, *et al* 2009).

Although hypoxia is thought to exhibit a negative effect on myocardial contractility, several neural and humoral changes act conjointly to increase myocardial contractility in hypoxia. The increase in sympathetic activity and the release of apelin has a potent positive inotropic effect mediated in part by enhanced myofilament sensitivity to calcium which increases contractility. In contrast adenosine produced from the cardiac myocyte and endothelium in response to the hypoxic injury reduces the contractile responsiveness of the myocardium to adrenergic stimulation. However the production of nitric oxide may reduce or increase contractility depending on the micro-environmental circumstances (Calbet, *et al* 2009).

Antenatally, even in infants with non-acidemia related neurological impairments, 74% had intrapartum non-reassuring fetal heart rate patterns (Kodama, *et al* 2009). This is also mirrored in the persistent bradycardia often seen in infants with neonatal encephalopathy. However changes in baseline heart rate variability was found to be insensitive for detecting seizures in asphyxiated neonates (Leutmezer, *et al* 2003) but diminished heart rate variability may be a poor prognostic sign (Cherian, *et al* 2006).

Martin-Ancel *et al* found cardiac involvement in 29% of neonates with birth asphyxia (n= 72). Electrocardiogram (ECG) changes consistent with myocardial ischaemia were found in 19% and 21% had a transient systolic murmur (Martin-Ancel, *et al* 1995). Barberi *et al* graded ECG changes in asphyxiated newborns from 1-4 based on T, ST and Q wave measurements taken from a standard 12 lead ECG (Jedeikin, *et al* 1983). Infants with the most severe hypoxic damage had grade 3 – 4 ECG changes (Barberi, *et al* 1999). Similarly Kanik *et al* found that 38% of perinatally asphyxiated neonates had ECG changes consistent with myocardial ischaemia (Kanik, *et al* 2009). Normally the pulmonary vasculature relaxes after birth. However hypoxia prevents this normal physiological process resulting in increased pulmonary versus systemic vascular resistance and causes deoxygenated blood to be shunted to the systemic vasculature (Dakshinamurti 2005). Persistent pulmonary hypertension of the newborn (PPHN) precipitated by hypoxia is characterised by impairment of nitric oxide synthase, and is treated with exogenous inhaled Nitric Oxide therapy (Davidson, *et al* 1998).

1.3.3 Consequences of Hypothermia on Cardiac Function

Therapeutic hypothermia is now standard practice as a neuroprotective therapy following perinatal asphyxia, for infants who meet the inclusion criteria (Gebauer, *et al* 2006) (Battin, *et al* 2009) (Thoresen and Whitelaw 2000) (Jacobs, *et al* 2007).

Cardiovascular side effects have been noted in these infants with sinus bradycardia and hypotension with an increased need for inotropic support during the hypothermia therapy (Jacobs, *et al* 2007). During mild hypothermia cardiac output is reduced to approximately 67% of the post hypothermic level with a concurrent decrease in heart rate and stroke volume. (Gebauer, *et al* 2006) (Battin, *et al* 2009). Meta analysis of six clinical trials of hypothermia did not demonstrate a significant effect of hypothermia on cardiac arrhythmia requiring medical treatment (Jacobs, *et al* 2007). However no study has specifically looked at LF/RV function using echocardiography in patients undergoing therapeutic hypothermia.

1.3.4 Novel therapies for Cardiovascular dysfunction secondary to hypoxia

ischaemia Acute myocardial infarction (MI) in adults leads to cardiac remodelling, which includes thinning of the infarct wall, and cardiac dilatation. At the cellular level this is associated with apoptosis, necrosis of cardiac myocytes, hypertrophy, fibrosis and infiltration of inflammatory cells (Singla 2009). A similar process may occur following ischaemia secondary to hypoxic injury in newborns.

No single treatment to date allows complete regeneration of the injured myocytes whose abnormal state may ultimately lead to cardiac failure. However stem cell research is under investigation to treat heart failure due to myocardial hypoxic injury (Singla 2009).

A stem cell is clonogenic, self-renewing and multipotent. In response to intercellular signalling or environmental stimuli they can regenerate into cells derived from any of the three primary germ layers, ectoderm, endoderm and mesoderm. A cardiac progenitor cell can differentiate into any of the cardiac cell lineages, including endothelial cells and cardiomyocytes (Kaushal, *et al* 2009). In animal models embryonic and adult cardiac stem cells have been transplanted and demonstrated successful engraftment and differentiation into tissue specific cell types. Transplanted mouse embryonic stem cells in the infarcted heart also show significantly improved cardiac function up to three months following transplantation (Behfar, *et al* 2007). Similar results were noted in an engrafted sheep heart, suggesting that embryonic stem cells have the potential to engraft into cardiac myocytes in small and large animal models (Kaushal, *et al* 2009).

Transplantation of bone marrow mononuclear cells in adults following acute myocardial infarction, increased myocardial contractility (Dimmeler and Zeiher 2009). In adult studies the published data demonstrates that intracoronary infusions of autologous bone marrow progenitor cells (BMC) is safe and feasible in patients with acute myocardial infarction with improvement in global left ventricular ejection fraction by up to 9% (Fernandez-Aviles, *et al* 2004) (Wollert, *et al* 2004). The REPAIR-AMI (Intracoronary bone marrow derived progenitor cells in acute myocardial infarction) trial used BMC in adult patients following acute

myocardial infarction and demonstrated a significant improvement of global and regional ejection fraction in the BMC group (+5.5%) compared to placebo (+3%) during 4 months of follow-up (Schachinger, *et al* 2006) (Losordo, *et al* 2007) (Dimmeler and Zeiher 2009). In children with congenital heart disease cardiac failure is a cause of considerable morbidity. Prat Vidal *et al* found that human umbilical cord blood stem cells (Human UCBSs) may represent an alternative source of stem cells for myocardial-cell replacement (Prat-Vidal, *et al* 2007). HUCBSC were injected into a neonatal ovine heart and detected in the myocardium up to six weeks after transplantation following pulmonary artery banding, transplantation was accompanied by right ventricular functional improvement (Davies, *et al* 2010).

Apparent neonatal myocardial recovery following severe hypoxia-ischaemia may be related to the large number of HUCBSC naturally present. This is currently being evaluated in phase I/II clinical trials for the treatment of children with traumatic brain injury and cerebral palsy (Pimentel-Coelho and Mendez-Otero 2010). However there is a paucity of studies on long term cardiovascular function in infants with neonatal encephalopathy. Therefore future advance in stem cell therapy and evaluation of long term cardiac outcomes are required. (Figure 1.3.4)

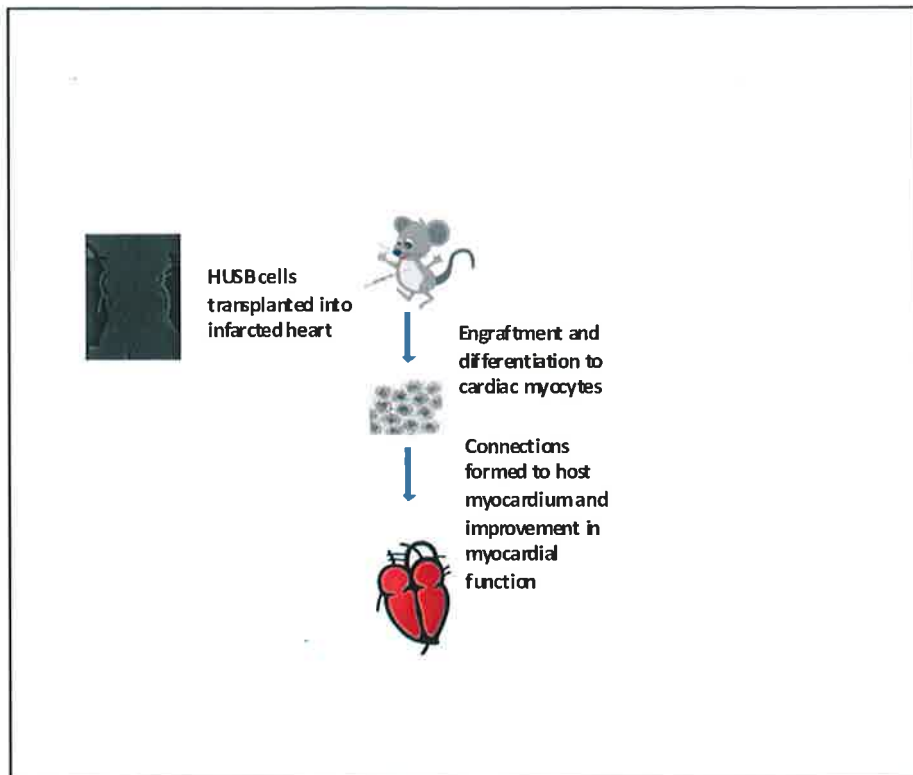


Figure 1.3.4. Mechanism of HUSB cell infusion into murine heart, with evidence of improvement of myocardial function as represented by black to red colour change

CHAPTER 1 SECTION IV

2-DIMENSIONAL ECHOCARDIOGRAPHIC ASSESSMENT OF VENTRICULAR FUNCTION

1.4.1 Introduction

Echocardiography is a painless, non invasive modality, first introduced by Edler and Hertz in 1954 (Edler and Hertz 2004). Echocardiography is now the standard tool used to assess myocardial function. This is vital for the clinical evaluation of cardiovascular disease, both congenital and acquired (Nesbitt, *et al* 2009).As well as providing information about structural cardiac abnormalities it provides information about global myocardial function.

1.4.2 Assessment of Systolic Function

Subjective qualitative assessment of LV systolic function is often used, however it is prone to inter and intraobserver variability, therefore quantitative measures are preferred (Hoffmann, *et al* 1996).The current measure for systolic assessment of the LV is the M mode measure of shortening (SF) and ejection fraction (EF). These measures are based on the assessment of the LV dimensions or size (Greenbaum and Gibson 1981).(Figure 1.4.2) These measures provide information on radial myocardial performance, but neglect the contribution of longitudinal contraction and deformation (Nesbitt, *et al* 2009). Systolic function is

determined by preload, afterload, heart rate and myocardial contractility. Using 2 D echocardiography measurements shortening fraction reflects contractility but does not account for preload and afterload. Wall stress analysis provides an assessment of preload, afterload and contractility (Tabbutt 2001). (Figure 1.4.2)

1.4.3 Assessment of Systolic Function in Preterm Infants

Assessment of systolic LV function in the preterm infant using M mode measures of SF and EF has a number of limitations. Firstly there may be flattening of the septal wall or paradoxical motion of the ventricular septum due to the interaction between the relatively high pressure RV and the LV. Poor imaging windows may also exist as a result of lung interference from preterm infants who are on continuous positive airways pressure (CPAP) ventilation (Mertens, *et al* 2011). Changes in SF and EF are also likely in preterm infants who have changes in preload and afterload. This is particularly important in preterm infants pre and post PDA closure, as the removal of the left-to-right shunt reduces preload, and removal of the low resistance pulmonary circulation increases mean arterial pressure and LV afterload (McNamara, *et al* 2010).

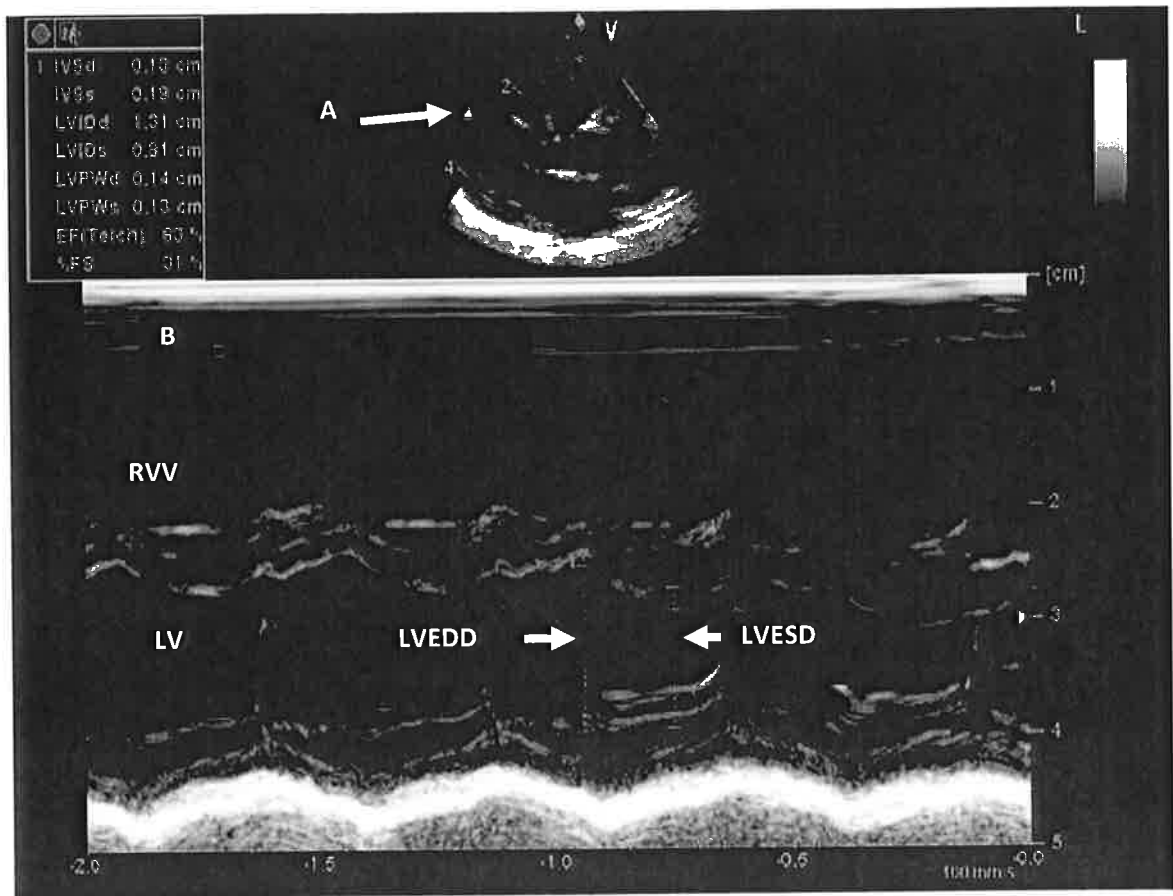


Figure 1.4.2. M mode of Left ventricle. A) Parasternal long axis view through the left ventricle. B) M mode of left Ventricle. RV – right ventricle, LV – left ventricle, LVEDD - arrow pointing to the left ventricular end diastolic dimension, LVESD – arrow pointing to the left ventricular end systolic dimension.

1.4.4 Assessment of Systolic Function in Neonatal Encephalopathy

Traditionally left ventricular systolic function is evaluated using M Mode to measure ejection (EF) and shortening fraction (SF) (Kadivar, *et al* 2008) (Lopez, *et al* 2010). Barberi *et al* found that SF decreased in severely versus mildly asphyxiated newborns (Barberi, *et al* 1999). Wei *et al* also demonstrated lower EF and SF in infants with severe asphyxia compared to those who were mildly asphyxiated and controls (Wei, *et al* 2009).

1.4.5 Assessment of Diastolic Function

Signs of diastolic dysfunction may precede systolic dysfunction to evaluate diastolic function. Diastolic function can be evaluated by a pulsed wave Doppler measurement at the mitral inflow tract, from an apical four chamber view where the A wave represents atrial contraction at the end of diastole and the E wave represents blood flow across the mitral valve in early diastole (Lopez, *et al* 2010) (Figure 1.4.5). The E:A ratio of early to late diastolic transmitral flow velocity can then be calculated. Abnormalities of LV diastolic function are often subclinical and are quantified by a decreased E:A ratio. Marbela *et al* studied LV diastolic dysfunction in adult patients with chronic obstructive pulmonary disease (COPD). Left ventricular diastolic dysfunction was present in patients with COPD (n=55) with a decreased E:A ratio suggesting impairment of LV filling (Malerba, *et al* 2011). Abnormalities of diastolic function in children and infants are usually non specific and have been correlated with prognosis.

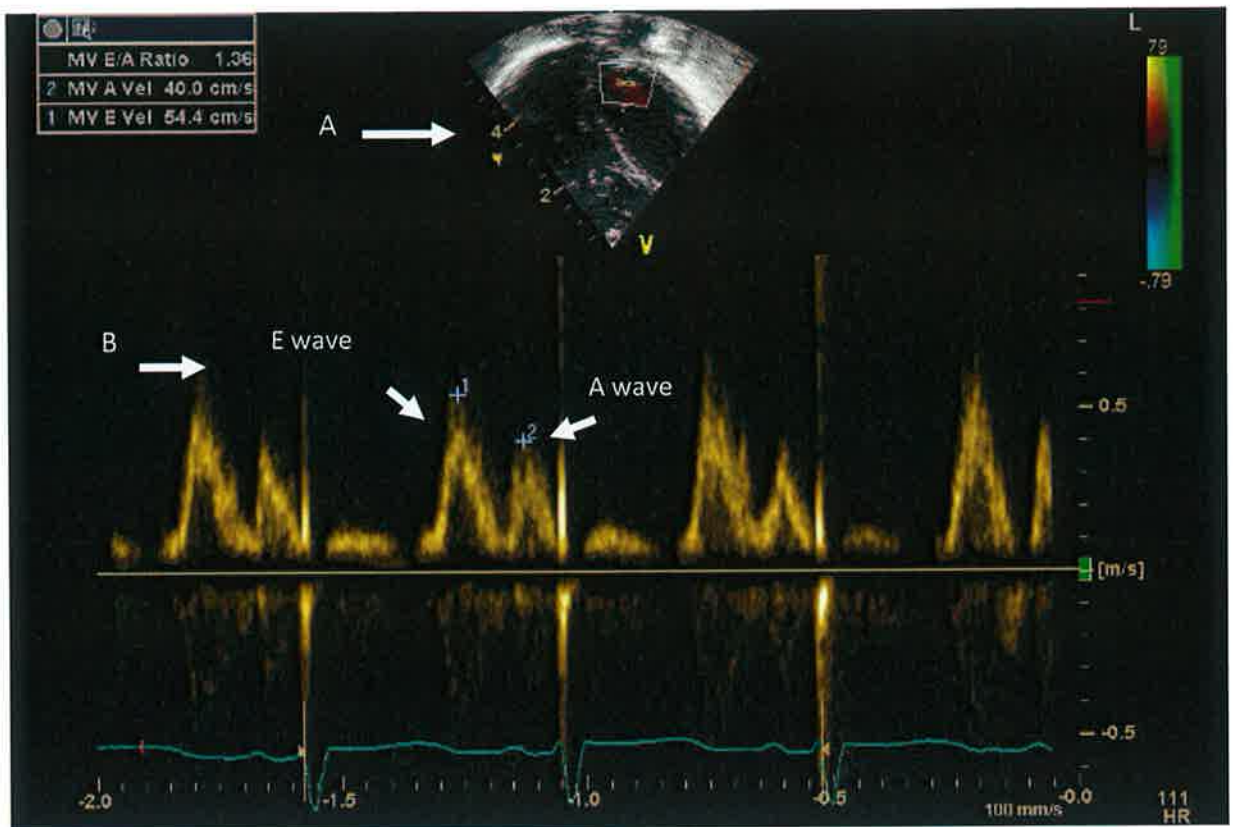


Figure 1.4.5. Doppler E:A Ratio Measurement of Normal Left ventricular diastolic function. A) Apical 4 chamber view with arrow pointing to mitral valve. B) Doppler trace across mitral valve. E wave – arrow pointing to E wave demonstrating blood flow across the mitral valve in early diastole. A wave - arrow pointing to A wave demonstrating blood flow across the mitral valve in late diastole. The ratio E:A is calculated by dividing the E wave measurement by the A wave measurement.

1.4.6 Assessment of Diastolic Function in Healthy Normal Term Infants

In term infants in the first week of life, there is a change from the fetal filling type with more dependence on filling during the atrial contraction (A wave), toward a more mature pattern with a higher early filling pattern (E wave)

1.4.7 Assessment of Diastolic Function in Preterm Infants

To date diastolic function has been difficult to measure in preterm infants with the majority of research focusing on mitral inflow patterns or the E: A ratio. In preterm infants this results in a pronounced increase in the E wave (Harada, *et al* 1994).(Figure 1.4.6). This method can be influenced by preload as in patients with a PDA, and sensitive to changes in heart rate which may limit its usefulness in this population (Stoddard, *et al* 1989)

1.4.8 Assessment of Diastolic Function in Neonatal Encephalopathy

Infants who suffer asphyxia have decreased LV systolic function and may therefore have decreased diastolic function. Limited data exists for this group and current Doppler measures of diastolic function i.e. mitral E: A ratio may not detect subtle changes in diastolic function. Ichihashi *et al* studied a small cohort of neonates with mild asphyxia finding no difference between the mitral E:A ratio in infants with mild asphyxia (n=20) compared to patients with no asphyxia (ilchihashi,

et al 2005). Matter et al found no significant difference between mitral E:A ratio in infants with severe asphyxia versus healthy controls (1.22 versus 1.26) (Matter, *et al* 2010).

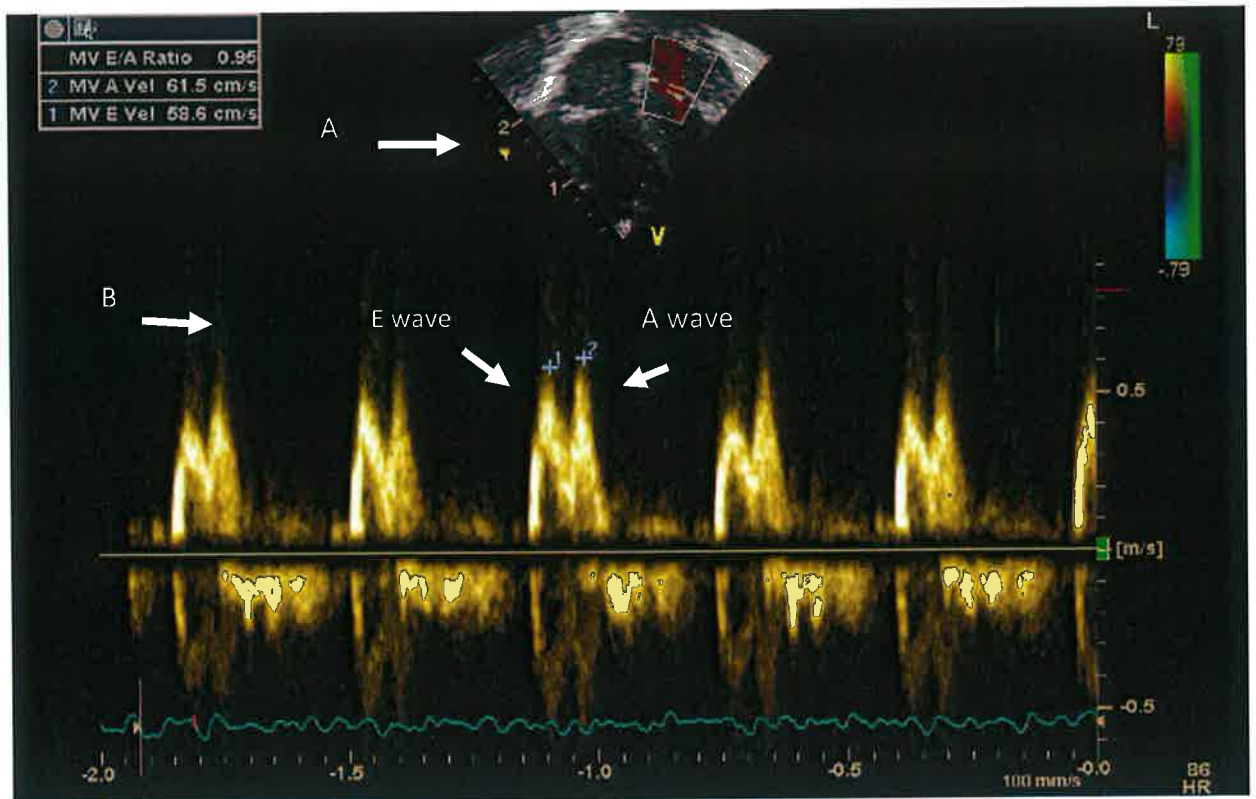


Figure 1.4.6. Doppler measurement of Left ventricular diastolic function in preterm Infant .

A) Apical 4 chamber view, arrow pointing to mitral valve. B) Doppler flow across the mitral valve. E wave – arrow pointing to the pronounced E wave of early diastole, A wave, arrow pointing to the more pronounced A wave of late diastole. The E:A ratio is calculated by dividing the value obtained for the E wave by that of the A wave.

1.4.9 Conclusion

Newer echocardiographic measures are evolving in order to overcome deficiencies in standard estimates of . LV torsion is now thought to be a sensitive indicator of not only systolic but also diastolic performance. Speckle Tracking Imaging (STI) and Tissue Doppler Imaging (TDI) are novel techniques which allow assessment of regional ventricular function based on myocardial motion and deformation.

CHAPTER 1 SECTION V

TISSUE DOPPLER IMAGING

1.5.1 Introduction

Christian Andreas Doppler discovered the Doppler effect over one and a half centuries ago. However it was 100 years later before it was utilised in medical ultrasound procedures (Pavlopoulos and Nihoyannopoulos 2008). Doppler techniques are based on the frequency shift of the ultrasound waves when reflected from a moving target and were first described as a useful measure of blood flow (Kostis, *et al* 1972). Detection and quantification of blood flow velocity is based on the scattered echo from red blood cells. The colour Doppler blood algorithm is set to detect the high velocity/low amplitude of blood. Myocardium on the other hand is a low velocity/high amplitude structure. TDI uses Doppler to detect the low frequency, high amplitude signals of myocardial tissue motion, thus providing information on the velocity of the tissue as well as systolic and diastolic function (Van de Veire, *et al* 2008). TDI has been in widespread use since the latter half of the century. The introduction of colour coded tissue velocity imaging in the 1990s has allowed measurement of displacement, strain and strain rate (McDicken, *et al* 1992).

1.5.2 TDI Measurements

TDI can be performed in either pulsed wave or colour modes. Pulsed wave TDI is used to measure peak myocardial velocities from the apical 4 chamber view where long axis ventricular motion is parallel to the ultrasound beam. Pulsed wave measurements can be taken at the mitral and tricuspid annuli and the ventricular septum. The cardiac cycle is then represented by 3 wave forms, S', which is the early systolic myocardial velocity, E', which is the early diastolic myocardial velocity and A', which is the late diastolic myocardial velocity (Ho and Solomon 2006). (Figure 1.5.2). TDI allows measurement of systolic and diastolic velocities in a single waveform. Systolic and diastolic velocities have been shown to be accurate and reproducible in adult patients (Garcia, *et al* 2006). In adult patients with chronic heart failure TDI measurement S' velocities were more useful at identifying myocardial dysfunction than ejection fraction (Garcia, *et al* 2006).

1.5.3 TDI in Healthy Normal Term Infants

TDI has been successfully used to measure myocardial velocities in preterm and term infants. Mori *et al* measured myocardial velocities in term infants over the first week of life in an aim to establish normal values (n=130). They concluded that right sided myocardial velocities were higher than left, and the early diastolic velocities of both the mitral and intraventricular septum increased over the first few days following birth, most likely as a result of improved diastolic function of the left ventricle (Mori, *et al* 2004).

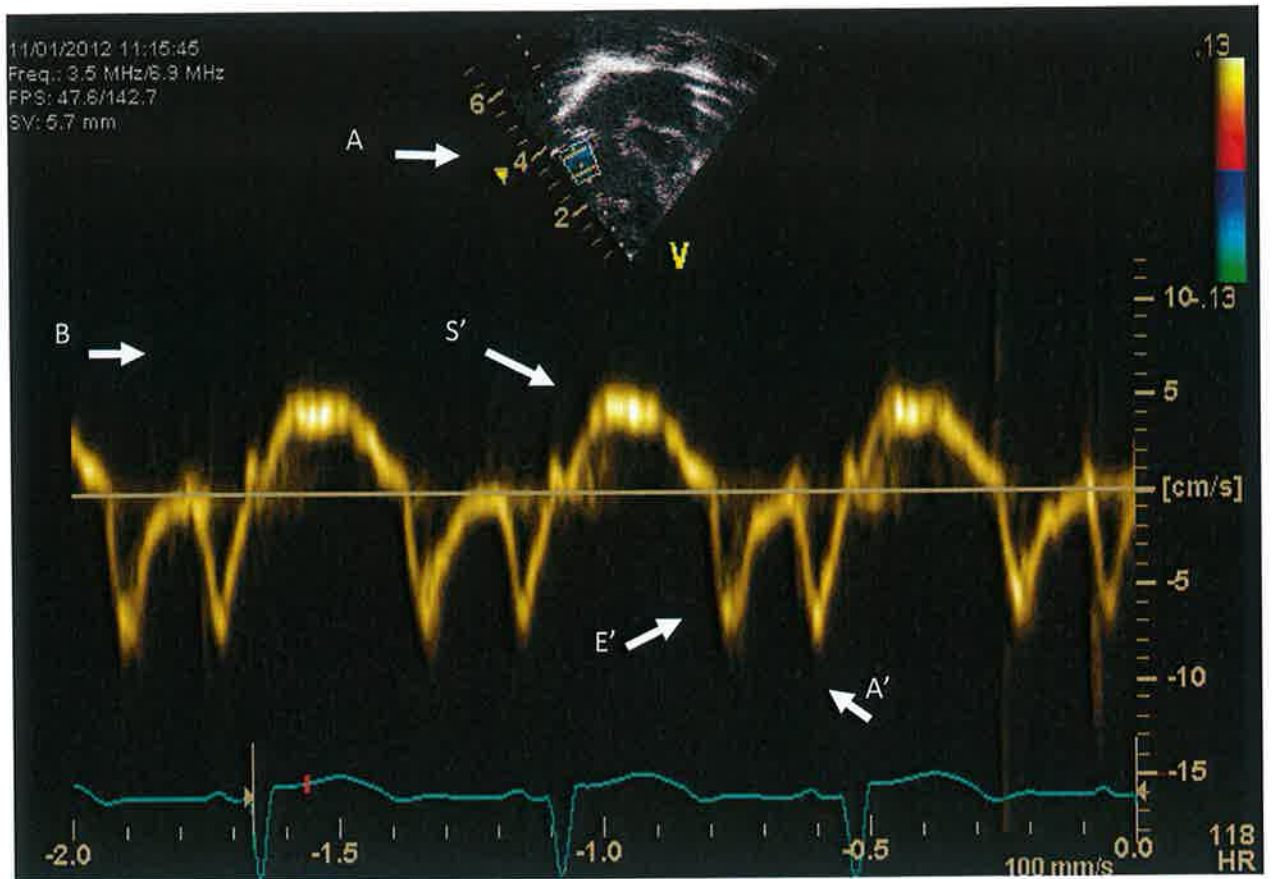


Figure 1.5.2. Tissue Doppler Imaging of the Right Ventricle in Term Infant. A) Apical 4 chamber view . Arrow pointing to Tricuspid valve annulus. Pulsed wave Doppler box at the Right ventricular wall. B) Tissue Doppler trace at the right ventricular wall. S' - arrow pointing to the S wave demonstrating early systolic velocity measurement, E' arrow pointing to the E wave of early diastolic velocity measurement, A' arrow pointing to A wave of late diastolic velocity measurement

1.5.4 TDI in Preterm Infants

As TDI is less preload and afterload dependent than pulsed wave Doppler, it may be useful in the study of systolic and diastolic myocardial function in preterm infants. Negrine & Ciccone et al have studied TDI in preterm infants and have found higher right sided ventricular velocities than left (n=20 & n=20). Both systolic and diastolic myocardial velocities increase with gestational age (Negrine, *et al* 2012) (Ciccone, *et al* 2011).

1.5.5 TDI in Neonatal Encephalopathy

Tissue Doppler imaging (TDI) is evolving as a technique to allow measurement of myocardial contraction and relaxation velocity from the myocardium and could allow recognition of myocardial dysfunction related to hypoxic injury (Negrine, *et al* 2012) (Mori, *et al* 2004). Wei et al used tissue Doppler imaging to serially evaluate left ventricular systolic function in newborns with mild to severe asphyxia (n=31). The peak systolic velocity of the anterior mitral valve leaflet (*S'* wave) was measured with TDI. The LVEF and SF of the severe asphyxia group were significantly lower than in the mild and control groups. The *S'* wave of the asphyxia group was significantly lower than that of control group ($p < 0.001$). (See figure 1.5.2 for demonstration of *S'* wave). In the severe asphyxia group, the *S'* wave at 24 h was significantly lower than that at 48 or 72 h ($P < 0.001$). The findings of this study suggest that *S'* by TDI is a more sensitive indicator of left ventricular systolic function than left ventricular ejection or SF measured by M-mode echocardiography (Wei, *et al* 2009).

1.5.6 Pitfalls of TDI

TDI in clinical practice has a number of pitfalls such as the angle of insonation, signal noise and measurement variability which may limit its use. If the angle between the velocity direction and the ultrasound beam is > 20 degrees, the real velocity is underestimated. Since correct alignment is not always possible, TDI derived measures lose validity at the apical segments (Storaa, *et al* 2003)

CHAPTER 1 SECTION VI

SPECKLE TRACKING IMAGING

1.6.1 Introduction

Speckle tracking echocardiography is a novel technique which allows offline calculation of myocardial velocities, specifically strain and strain rate (Blessberger and Binder 2010) (Teske, *et al* 2007). Strain as previously described is the dimensionless quantity of myocardial deformation and measures the magnitude of myocardial fibre contraction and relaxation (Pavlopoulos and Nihoyannopoulos 2008). Strain rate (the temporal derivative of strain) is the change of strain per unit time. Negative strain indicates fibre shortening or myocardial thinning in the longitudinal or circumferential plane whereas a positive value describes lengthening or thickening in the radial plane (Pavlopoulos and Nihoyannopoulos 2008) (Marwick 2006).

The principle of measuring myocardial velocities based on tissue tracking was first introduced by Reisner and Leitman in 2004 and relies on acquisition of a pattern of grey speckles on the echo ultrasound image (Leitman, *et al* 2004) (Reisner, *et al* 2004). The speckle artefacts in the echo image are generated at random due to reflections, refraction and scattering of echo beams. This is referred to as a speckle pattern, and characterises the underlying myocardial tissue. It is assumed to be unique for each myocardial segment and in this unique way has been compared to a human fingerprint (Blessberger and Binder 2010). These speckles stay stable throughout the cardiac cycle and can be used as natural

acoustic markers for tagging myocardial motion in the LV wall. STI is performed as an offline analysis from digitally recorded and ECG triggered cine loops. Post processing software defines a 'kernel' (cluster of speckles) and follows this kernel from frame to frame. Detection of spatial movement of this kernel during the cardiac cycle allows direct calculation of Lagrangian strain (the expression of the instantaneous deformation relative to the initial length) (D'Hooge, *et al* 2000).

Myocardial velocity is estimated from the shift of individual speckles divided by the time between successive frames. Strain rate can also be calculated. In order to commence analysis the endocardial borders must be accurately traced to define the Region of Interest (ROI). This can be undertaken in both the apical four chamber view and at in the parasternal short axis view at the papillary muscle level. The apical 4 chamber view provides information on LV Longitudinal strain, whereas the short axis view at the papillary muscle provides information on both LV circumferential and radial strain.

The post processing analysis automatically divides the ventricle into six equally distributed segments to generate values for each region for example at the LV lateral and septal, basal, mid and apical regions. Data is then displayed in several formats providing information on strain and strain rate. (Figure 1.6.1).

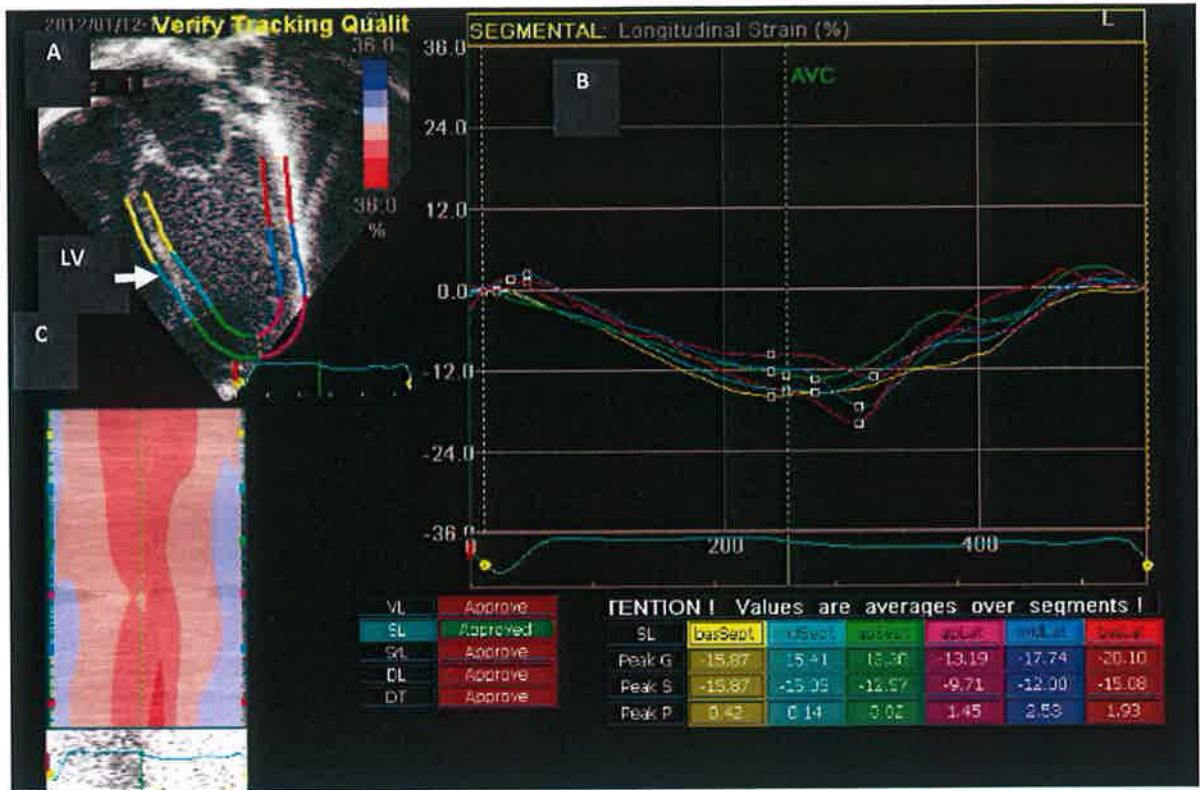


Figure 1.6.1. Echocardiographic indices of Left Ventricular (LV) Longitudinal Strain.

A – Apical 4 chamber tracing of LV to derive longitudinal strain (%). EchoPAC software calculates longitudinal strain by tracking acoustic kernels over the cardiac cycle.

B & C - Longitudinal strain averaged over six standard segments to give time – strain plots. Colour codes according to individual segment represented.

1.6.2 Speckle Tracking Imaging in Adults

STI has been validated in both experimental and human studies with normal values for LV systolic function established (Amundsen, *et al* 2006) (Helle-Valle, *et al* 2005). In clinical practice it has been used to assess the LV following acute or chronic ischaemia, asynchrony and in different loading and inotropic conditions (Edvardsen, *et al* 2006).

1.6.3 Speckle Tracking Imaging in Children

Traditional echocardiographic measures such as EF and SF used to evaluate LV systolic function may not be applicable to children with complex congenital cardiac malformations. These measures are based on LV dimensions and may not be applicable to the ventricular arrangements in children with complex congenital heart disease (CHD). Strain and strain rate imaging provide detailed assessment of regional alterations in ventricular myocardial contraction. Normal values have been established for young children (Ages 1-19 years) (Marcus, *et al* 2011). In clinical practice the use of STI has been limited by lack of normative data. In preterm and term infants no normative data exists to date, as STI may have limitations in infants with higher heart rates.

CHAPTER 1 SECTION VII

SERUM BIOMARKERS OF CARDIAC FUNCTION

1.7.1 Introduction

Cardiac biomarkers may be defined as biological analytes that are detectable in the bloodstream at elevated levels during the continuum of cardiovascular disease or in the immediate aftermath of myocardial damaged. They should ideally be specific for cardiac tissue and absent from non-myocardial tissue and need to be easily accessible to achieve high diagnostic sensitivity (McDonnell, *et al* 2009). The gold standard for detecting myocardial necrosis has been an elevated level of the cardiac-specific isoform of Creatine Kinase (CK-MB). In the last decade newer cardiac markers such as Troponin T, I and C have been the cornerstone laboratory medicine measurement of myocardial infarction in suspected acute coronary syndrome (Goldmann, *et al* 2001). B-type natriuretic peptide (BNP) and its inert metabolite N-terminal-pro BNP (NT pro BNP) are used clinically in the context of heart failure (Wu, *et al* 2007).

1.7.2 Physiology of Troponin T

Troponin is an inhibitory protein complex forming part of the contractile apparatus of all striated muscle, including the heart. It regulates the excitation contraction coupling within the cardiac myocytes which are triggered by intracellular calcium. Troponin T, I and C are subunits of the thin filament associated troponin-tropomyosin complex which is involved in regulating muscle

contraction. Their nomenclature refers to their physical properties, Troponin T (Tropomyosin binding), Troponin C (calcium binding) and Troponin I (Inhibitory). (Clark, *et al* 2004) (Figure 1.7.2). The majority of cardiac troponins are bound in the contractile apparatus, however Troponin C and I are thought to be cardiac specific and are released in response to myocardial ischaemia (Fromm 2007).

1.7.2a Troponin T in Clinical Practice

Since 2000 cardiac troponins have replaced the traditional CK MB as the diagnostic marker for acute myocardial infarction as more sensitive for the detection of myocardial injury (Alpert, *et al* 2000). Prolonged myocardial ischaemia leads to myocardial cell death and release of Troponin T and I peaking within 4 – 12 hours after myocardial necrosis and reaching peak levels between 12-48 hours from symptom onset (Fromm 2007). Cardiac Troponins have been noted to be increased in patients with pulmonary embolism, renal insufficiency, and acute stroke (Fromm 2007). In adults the diagnostic and prognostic impact of elevated cardiac troponin levels in patients with sepsis has been established. Both increased severity of sepsis and poor short term prognosis are associated with increased Troponin levels (Spies, *et al* 1998) (Maeder, *et al* 2006). However uncertainty remains as to whether troponin release reflects irreversible myocardial damage or reversible myocardial depression (Wu, *et al* 1999).

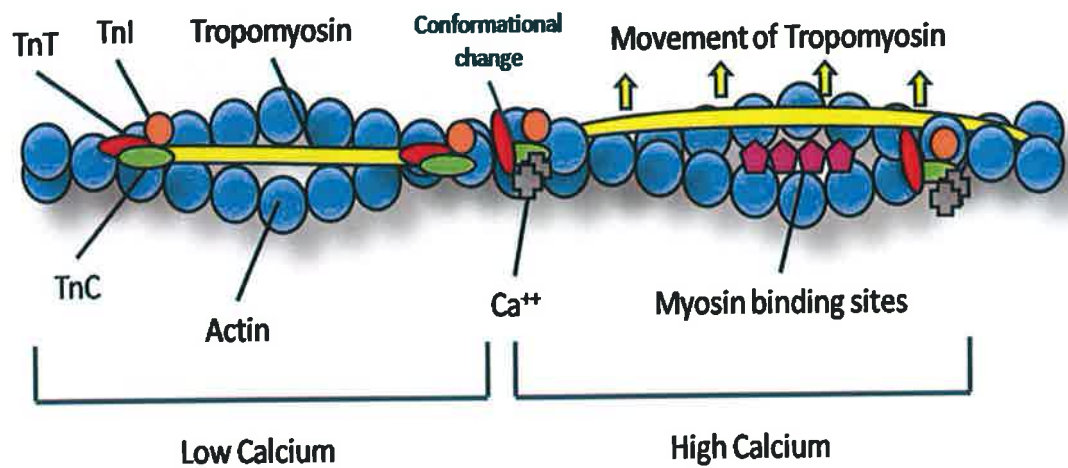


Figure 1.7.2. Schematic representation of the myofibrillar thin filament. In the absence of calcium ion, tropomyosin blocks the myosin binding sites present on actin. Calcium binding to cTnT induces conformational change in the troponin complex exposing the myosin binding sites and facilitating muscle contraction.

1.7.2b Troponin in Neonates

Troponin T is detected in the blood of healthy neonates. Clark et al have found that concentrations of Troponin T are higher in neonates with respiratory distress using a regression model (Clark, *et al* 2004). Abdel-Hady et al correlated Troponin T with left and right ventricular Tei Index in septic term infants. They found that non survivors had significantly higher serum cardiac troponin T concentrations and left ventricular Tei index than survivors (Abdel-Hady, *et al* 2012). Fenton et al found increased Troponin I on admission in 57% of patients and at 12 hrs in 46% of pediatric intensive care patients admitted with septic shock and cardiovascular failure (Fenton, *et al* 2004). Admission Troponin I inversely correlated with measures of ejection fraction and fractional shortening and is directly proportional to wall stress. Patients who had increased admission Troponin I had lower heart rate corrected mean velocity of circumferential fibre shortening (preload and heart rate independent measure of left ventricular systolic function) and higher wall stress (measure of afterload) compared with patients with normal Troponin I. Admission Troponin I also correlated with mortality in children admitted to the intensive care unit (Fenton, *et al* 2004).

1.7.2c Troponin T in Preterm Infants

Levels of Troponin T are higher in premature infants with respiratory distress syndrome (RDS) and sick hypotensive infants requiring inotropic support (Clark, *et al* 2004). Troponin T is also elevated in the presence of a PDA in preterm

and falls following successful treatment (El-Khuffash, *et al* 2008a). El Khuffash *et al* demonstrated the use of NTpBNP and Troponin as an adjunct to echocardiography to predict poor neonatal outcome (grade III/IV intraventricular haemorrhage (IVH) or death) in preterm infants with a PDA. They concluded that NTpBNP and Troponin T may have a role in screening infants with a PDA and monitoring response to treatment. Infants with severe IVH had a statistically significantly higher NTpBNP level (9209 pmol/l versus 1664 pmol/l p value <0.001) than those with mild IVH. Troponin T levels were 2.3µg/l in those with severe IVH versus 0.19µg/l (p value < 0.001) in those mildly affected at 48 hours. They also correlated elevated Troponin T levels with death before discharge and poor neurodevelopmental outcome at 2 years (El-Khuffash, *et al* 2011).

1.7.2d Troponin T in Neonatal Encephalopathy

Cardiac Troponin I and T are well established markers of myocardial ischaemia and cardiac failure in adults and children (Goldmann, *et al* 2001) (Karras and Kane 2001) (Fontes, *et al* 1999) (Alpert, *et al* 2000) (Hirsch, *et al* 1997). They have also been linked to cardiovascular dysfunction in sepsis (Spies, *et al* 1998) (Clark, *et al* 2001) (Maeder, *et al* 2006) (El-Khuffash, *et al* 2008). Troponin I levels in have been studied in asphyxiated newborn piglets and Borke *et al* confirmed that release of Troponin I was associated with myocardial damage (Borke, *et al* 2004). Troponin T is a predictor of myocardial injury in asphyxiated neonates (Gunes, *et al*

2005) (Szymankiewicz, *et al* 2005) and Troponin I is elevated in asphyxiated neonates (Trevisanuto, *et al* 2009).

1.7.2c Physiology of N Terminal Pro B-Type Natriuretic Peptide (NTproBNP)

Natriuretic peptides play an important role in the regulation of cardiovascular homeostasis and fluid volume (Brueckmann, *et al* 2005). Atrial natriuretic peptide (ANP) is secreted from the atria in response to increased left and right atrial pressure and volume loads and brain natriuretic peptide (BNP) is secreted from the ventricles in response to ventricular pressure and volume load (Nir, *et al* 2009). B-type Natriuretic peptide is a 32 amino acid ring structure which has its sequence present on chromosome 1. BNP causes diuresis, natriuresis and venous vasodilation. The net effect of which reduces intravascular volume and ventricular preload and afterload. Pro BNP is the inactive precursor and is cleaved into BNP, the active component and N-terminal pro-BNP (NTproBNP), an inactive byproduct. The half life of BNP is 20 minutes, whereas that of NTproBNP is 60 minutes making it more stable and useful clinically (El-Khuffash and Molloy 2007).

Good correlation has been shown between BNP and NTproBNP plasma levels in adult studies, with both being elevated in patients with left ventricular systolic and diastolic dysfunction, ischaemic heart disease and hypertrophic cardiomyopathy (Mair, *et al* 2001).

1.7.3a Clinical Use of BNP/ NTproBNP

BNP is linked to impaired left ventricular dysfunction in the critically ill adult population, echocardiographically confirmed by decreased ejection fraction and is a prognostic marker in those with congestive heart failure, ischaemic heart disease and acute coronary syndromes (Maeder, *et al* 2006). Adult studies have shown good correlation between BNP and NTproBNP (Mueller, *et al* 2005). In the largest adult study of BNP levels in the emergency department Chen *et al* demonstrated that adults with systemic inflammatory response syndrome (SIRS) or sepsis had a significantly positive detection rate of BNP when compared to normal adult controls ($p < .01$). An elevated BNP level was also an independent predictor of death in septic patients (Chen and Li 2009).

1.7.3b NTproBNP in Neonates

Biomarkers such as BNP are well established markers of myocardial ischaemia and cardiac failure in adults who have a structurally normal heart and in children with underlying congenital or acquired heart disease (Koch and Singer 2003) (Spies, *et al* 1998). In children with congenital heart disease both BNP and NTpBNP levels are elevated in keeping with a failing ventricle (Ohuchi, *et al* 2003). Ozhan *et al* have found a positive correlation between plasma concentration of BNP and the magnitude of the shunt in patients with an atrial or ventricular septal defect as derived from Doppler Qp/Qs evaluation (n=35) (Ozhan, *et al* 2007). Nir *et al* have reported normal ranges of NTproBNP in children (Nir, *et al* 2009).

1.7.3c NTproBNP in Preterm Infants

Levels of NT pro BNP surge at birth and reach a plateau on day 3-4, following which they fall, whereby they remain constant throughout infancy (Mir, *et al* 2006). In preterm infants, Pro BNP and NT-pro BNP have been introduced as a screening tool for the presence of a PDA and levels correlate well with echocardiographic evidence of reductions in ductal size (El-Khuffash, *et al* 2007). Murine experimental studies show that fetal ductal tissue shows a changing pattern in the expression of natriuretic peptides receptors. Fetal tissue has a higher level of natriuretic peptide receptors responsible for maintaining ductal patency and newborn ductal tissue has a higher ratio of natriuretic peptide receptors responsible for ductal degradation (Reynolds, *et al* 2004). Therefore BNP may have a role in maintaining ductal patency after birth. El Khuffash et al demonstrated the use of NTproBNP and Troponin as an adjunct to echocardiography to predict poor neonatal outcome (grade III/IV IVH or death) in preterm infants with a PDA. In addition NTproBNP and Troponin T may have a role in screening infants with a PDA and monitoring response to treatment (El-Khuffash and Molloy 2009) (El-Khuffash, 2011). Normal reference ranges for BNP and NTproBNP have been established but vary depending on age of neonate and the testing kit used (El-Khuffash and Molloy 2009) (Koch and Singer 2003).

1.7.4 Point of Care Assessment of Troponin T/NTproBNP

In the acute clinical setting the immediate availability of results is essential for therapeutic decision making. Point of care testing (POCT) is any test performed outside of the hospital central laboratory, and its development allows investigations to be carried out at the bed/cotside (Santrach and Burritt 1995). The majority of POCT testing can be performed on small volumes of blood.

Blood glucose monitoring was the first point of care test and is still the most widely used in the paediatric population. However bedside coagulation monitoring and BNP assessment are now widely used in children with congenital heart disease. Koch et al correlated POCT BNP testing and increasing levels of BNP with decreasing LV SF, increasing left to right shunt volume, increasing right ventricular pressure and mean pulmonary artery pressure (Koch, *et al* 2006). Advantages of POCT are a decreased turnaround time for clinical sampling which optimises the decision making process (Mor and Waisman 2000). Sampling is an important issue in the Neonatal Intensive Care unit (NICU), POCT offers the need for small venous samples therefore reducing the risk of iatrogenic anaemia (Hinds, *et al* 2007). Point of care testing for BNP has been used in neonates to determine PDA size with higher levels of BNP associated with larger PDA (Kalra, *et al* 2011). BNP levels were also used to monitor response to PDA treatment with trend analysis suggesting a strong association between PDA size and BNP level (Kalra, *et al* 2011). To date no study has used POCT for Troponin T or NT pro BNP testing in infants.

CHAPTER 1: SECTION VIII

1.8.1 Hypothesis:

Newer echocardiographic modalities in combination with biochemical markers to measure neonatal cardiac function may guide therapy and predict neonatal outcome in both term and preterm infants.

1. 8.2 Aims:

1 – To evaluate the use of Tissue Doppler Imaging in Preterm infants over the first week of life and correlate findings with clinical outcomes.

2 – To establish the normal range of Tissue Doppler Imaging for Healthy Term infants

3 – To determine the feasibility of Speckle Tracking Imaging in Healthy Term Infants

4 – To evaluate cardiac function in Infants with Neonatal Encephalopathy using standard echocardiographic measures, serum Troponin T and Tissue Doppler Imaging

5 – To validate Point of care Testing for Cardiac biomarkers – Troponin T and NT pro BNP in neonates.

CHAPTER 2

EXPERIMENTAL DESIGN AND METHODS

2.1 Ethical Approval

The study protocol was approved by the National Maternity Hospital's Ethics Committee. Patient information leaflets were given to each parent involved in the study and written informed consent was obtained.

2.2. Patient Population

The National Maternity Hospital is a tertiary referral centre with approximately 10 000 deliveries per year. Three different patient populations were included in this study: Term controls, Preterm infants and infants with Neonatal Encephalopathy. Infants with congenital abnormalities (including cardiac malformations other than a PDA) were excluded from this study. The study period was from July 2010 – July 2012.

2.2 a Term Control Infants

Term infants > 37 weeks gestation, with Apgars > 7 at five minutes and with a normal neonatal course were eligible for inclusion. Following discussion with the study statistician (TG) and evaluating previous observational studies, 60 term control infants was considered an adequate sample size for the TDI analysis. On review of the published literature for the observational study of longitudinal strain in term infants it was felt that 30 term infants would be an adequate sample size.

Delivery and clinical parameters recorded included:

1. Pregnancy Complications
2. Mode of delivery
3. Apgar Score at 1 & 5 minutes
4. Gestational Age
5. Birth weight
6. Length
7. Body surface area (BSA) (Haycock, *et al* 1978)

2.2 b. Preterm Infants: Preterm infants who were less than 1500g and/or < 32 weeks gestation were eligible for inclusion.

2. 2 b(i) Sample Size Calculation for Preterm Infants

The sample size was calculated for this study to have at least 80% power to detect with a level of significance of 0.05 a change in proportion of 0.42.

As long as less than half of the surviving infants have abnormal cardiac function, it can be concluded that there is an association between abnormal cardiac function and survival. SPSS assistance was provided by the study statistician (TG) – CSTAR.

2.2 b(ii) Antenatal History & Delivery details for Preterm Infants

Information regarding the antenatal course of the pregnancy and delivery were recorded, specifically:

1. Antenatal steroid administration. We defined a complete course as 12.5mg Betamethasone given 12 hours apart.
2. Presence/absence of preeclampsia
3. Antepartum haemorrhage
4. Absent or reversed end diastolic flow in the umbilical artery.
5. Maternal Pyrexia
6. Prolonged Rupture of the Membranes > 18 hours
7. Mode of delivery
8. Apgar Score at 1 and 5 minute
9. Resuscitation requirement
10. Surfactant administration

2.2 b (iii) Clinical Parameters for Preterm Infants

Patient data were recorded at time of echocardiogram and included:

1. Gestational Age
2. Gender
3. Birth weight
4. Medication – including Antibiotics, Caffeine, Morphine, Inotropes, Ibuprofen
5. Type of enteral feed
6. Vitamin D levels (in a subset of 10 patients)

At Time of Echocardiogram the following were recorded

7. Length
8. Heart Rate
9. Systolic Blood Pressure (Central arterial blood pressure was recorded using arterial line if insitu, if not cuff measurement was taken (mmHg)
10. Diastolic Blood Pressure
11. Mean Arterial Blood Pressure
12. Mode of Ventilation – SIMV, CPAP, BiPAP, NP O₂
13. Oxygen Requirement
14. Clinical cardiac Examination
15. Total fluid volume (ml/kg/day)
16. Urine Output (ml/kg/hr)

2.2 b (iv) Preterm Outcomes:

1. Neonatal death (within 28 days of delivery).

2. Intraventricular Haemorrhage (IVH)

This was defined and graded with the Papile classification (Papile, *et al* 1978). The cranial ultrasound scans were performed on/within the first 24 hours of life, with a second scan repeated at 48 – 72 hours and again on day 7 of life by a consultant paediatric radiologist. We grouped the infants into those who developed grades 0-II (mild-moderate IVH) and those with grades III/IV (severe IVH).

3. Chronic Lung Disease (CLD)

This was defined as the need for oxygen supplementation at 36 weeks corrected gestational age (Bancalari and Claure 2006).

4. Necrotising Enterocolitis (NEC)

This was based on Bell's criteria and graded from stage I - III (Bell 1978).

5. Late onset Neonatal Sepsis (LOS)

This was defined as a positive blood culture at > 72 hours of age.

6. Retinopathy of Prematurity (ROP)

This was defined according to the International Classification of Retinopathy of Prematurity. (Halliday 1984)

2.2 c Infants with Neonatal Encephalopathy

Term infants admitted to the Neonatal Unit who had evidence of Mild/Moderate/Severe Neonatal Encephalopathy were eligible for inclusion. Following discussion with the study statistician (TG) and looking at previous studies in this area a sample size of 35 - 40 was adequate. The patient group was defined using the following criteria: (Huang, *et al* 1999)

1. Abnormal neurological signs such as hypotonia or seizures in the immediate postnatal period, and or other organ dysfunction (kidneys, liver, and at least 2 of the following 3 criteria. Evidence or suspicion of hypoxic-ischaemic injury based on a history of fetal distress
2. Need for resuscitation after birth
3. Base deficit of >15mmol/L or PH <7.2 from cord blood or admission arterial sample.

2.2 c(i) Grading of Neonatal Encephalopathy

Neonatal Encephalopathy was graded as per Sarnat & Sarnat (Sarnat and Sarnat 1976).

i) Mild encephalopathy (NE I): Mild encephalopathy: hyperalertness, decreased spontaneous motor activity, activation of sympathetic functions lasting less than 24 hours with a normal electroencephalogram (EEG).

ii) Moderate/Severe encephalopathy (NE II/III): NE Stage II: generalised muscular hypotonia, strong distal flexion, multifocal seizures and/or pathological EEG. HIE Stage III: stuporous level of consciousness, flaccid muscle tone, suppression of brain stem and autonomic

functions, severely pathological EEG with a periodic pattern and isopotential phases.

2.2 c(ii) Clinical Parameters for Term Infants with NE

1. Pregnancy Complications
2. Mode of delivery
3. Apgar score at 1 & 5 minutes
4. Resuscitation
5. Therapeutic Hypothermia
6. Gestational Age
7. Birth weight
8. Medication – antibiotics, morphine, inotropes,
9. Seizure onset

Variables recorded at Time of Echocardiogram

10. Heart rate
11. Systolic & Diastolic Blood Pressure
12. Mode of Ventilation – SIMV, CPAP,
13. Oxygen Requirement
14. Cardiac Examination
15. Total fluid volume (ml/kg/day)
16. Urine Output
17. Medication – antibiotics, morphine, inotropes
18. Seizure onset

2.2 c(iii) Investigations in Infants with Neonatal Encephalopathy

1. Cranial USS – Day 1 of life
2. MRI Brain prior Day 5-10 of life
3. Serum Troponin T sample taken at time of echocardiogram

2.2c(iv) MRI

Infants with NE underwent MRI of the brain on Day 5-10 of life. All of the scans were scored independently by a paediatric radiologist. A standardised scoring system developed by Barkovich was used (Barkovich, *et al* 1998).

2.3 Biochemical Parameters for Point of Care Testing

2.3a Validation of Cobas h232 Analyser

In order to test the intra assay variation of the Cobas H232 analyser (figure 2.3a) a preliminary study was carried out using both QC Test material (high and low level) and repeated serum analysis using both the Troponin T and NT pro BNP test strips. Blood samples were collected in lithium heparin containers at one time point from an adult subject. Repeated measures were carried out using both Troponin T and NT pro BNP test strips consecutively over a 24 hour period. Samples were stored at room temperature and at 4 degrees Celsius. A total of twenty samples were repeated consecutively and then at 8, 10, 12 and 24 hours.

2.3b Analysis using Cobas h232 Analyser

For analysis of both Troponin T and NT pro BNP a 1 ml lithium heparin blood sample was taken from the patient. The POCT analysis was performed following sampling at the cot side using the Roche Diagnostics h 232 point of care machine. A cardiac test strip for Troponin T (Roche CARDIAC T QUANTITATIVE) was inserted. A pipette was then used to draw exactly 150µL from the blood sample which was then applied to the test strip. The measurement then took approximately 8-12 minutes to provide the result. Exactly the same process was carried out using NT pro BNP test strip (Roche CARDIAC pro BNP). Quality Control (QC) checks using both high and low QC material were performed at regular intervals.

The remainder of the blood sample (minimum of 500µL) was sent to the biochemistry laboratory where the sample was centrifuged and the plasma frozen at -20 degrees Celsius for later batch analysis. The sample was analysed using Roche Cobas 8000 analyser.

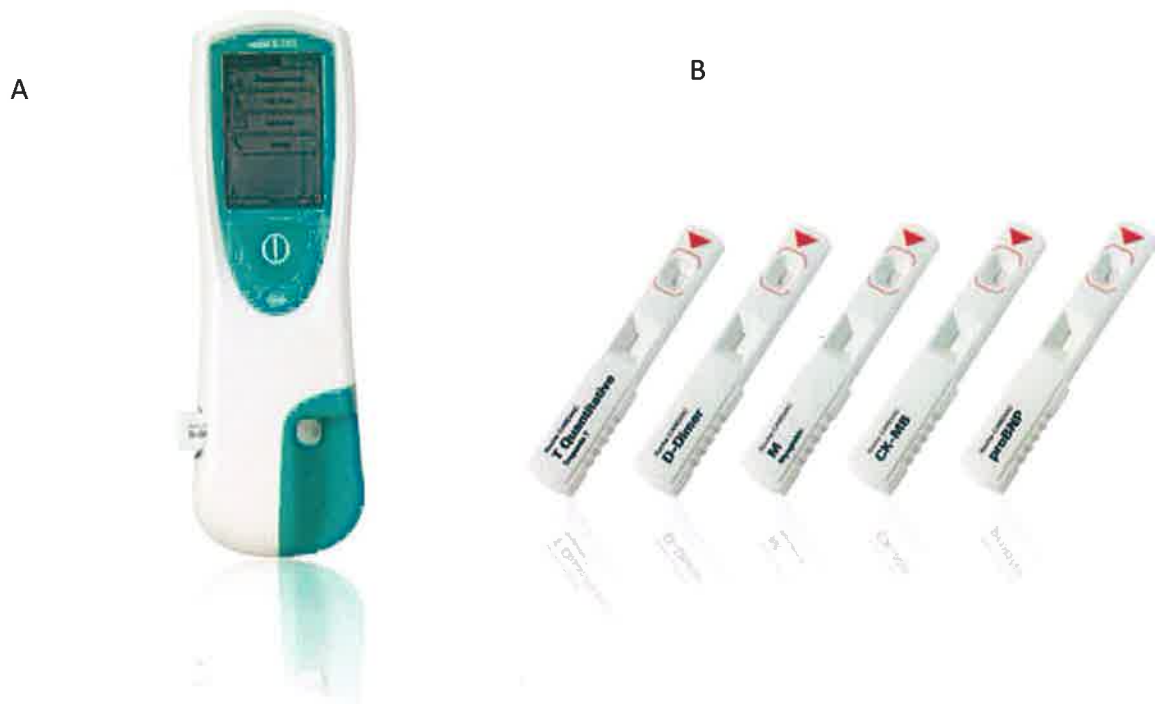


Figure 2.3a. Cobas h 232 Point of Care Testing kit.

A) Cobas h 232 Point of care bedside machine. B) Point of care Test strips for bedside analysis of Troponin T and N T pro BNP

2.4 Echocardiography Parameters

The GE Vivid I (General Electric, with a 7MHz probe) was used throughout this study. Studies were carried out by a single observer (KA). Images were recorded from standard echocardiographic views including subcostal, apical four-chamber, parasternal short and long axis and aortic arch. A detailed transthoracic echocardiogram was carried out for each patient according to the recommendations of the American Society of Echocardiography (Lopez, *et al* 2010). The scans were carried out with the infant during a quiet time in the supine position. All scans were stored on the echo machine before being downloaded to ECHOPAC 6 system for post processing analysis and validation.

2.4.1 M mode measurement of Left Ventricular (LV) Systolic Function

Standard measurement of LV systolic function is based on geometric quantification either by linear measurement of percentage shortening fraction (SF) which reflects contractility or volumetric measurement by calculating ejection fraction (EF). This can be measured from a parasternal long axis view using M Mode (Figure 1.4.2). It is then calculated by measuring left ventricular end diastolic volume (LVEDD) and left ventricular end systolic diameter (LVESD) and using the formula

- $SF (\%) = \frac{LVEDD - LVESD}{LVEDD} \times 100$
- $EF (\%) = \frac{LVEDD - LVESD}{LVEDD}$

Normal values exist for SF – 28-40% and EF > 55%. Assessment of systolic LV function in the preterm infant using M mode measures of SF and EF has a number

of limitations due to septal wall flattening or paradoxical motion of the ventricular septum due to interaction between the relatively high pressure RV and the LV (Mertens, *et al* 2011). These measures were recorded for preterm, normal term infants and those with evidence of Neonatal Encephalopathy.

2.4.2 Dimensional LV Diastolic Function.

There is limited available data on assessment of diastolic function in infants, and what exists is based on analysis of mitral inflow patterns. These measures are taken using a pulsed wave Doppler from the apical four-chamber view at the mitral and tricuspid valve level. (Figure 1.4.5) The early E wave reflects early diastolic filling and the A wave reflects atrial contraction. Maximum E and A measurements are then compared as a ratio (E:A). An E:A ratio < 1 indicates diastolic dysfunction. During the first week of life there is a change from the fetal filling type with more dependence on the filling during atrial contraction toward a more mature filling pattern with higher early filling (Harada, *et al* 1994). These measures were recorded for all three patient groups.

2.4.3 Presence of Patent Ductus Arteriosus

This was determined by 2-D echocardiography and colour pulsed wave Doppler from a high parasternal short axis view. Left atrial to aortic root diameter (LA:Ao) was measured from the parasternal long axis view using M mode. A PDA diameter of > 1.5 and an LA:Ao ratio of $>1.4:1$ was considered significant. This was measured for all preterm infants (El Hajjar, *et al* 2005). The PDA diameter was

measured from a high parasternal view using colour flow Doppler. A diameter of >1.5mm was considered significant (Kluckow and Evans 1995)(Figure 2.4.3 (i &ii)).

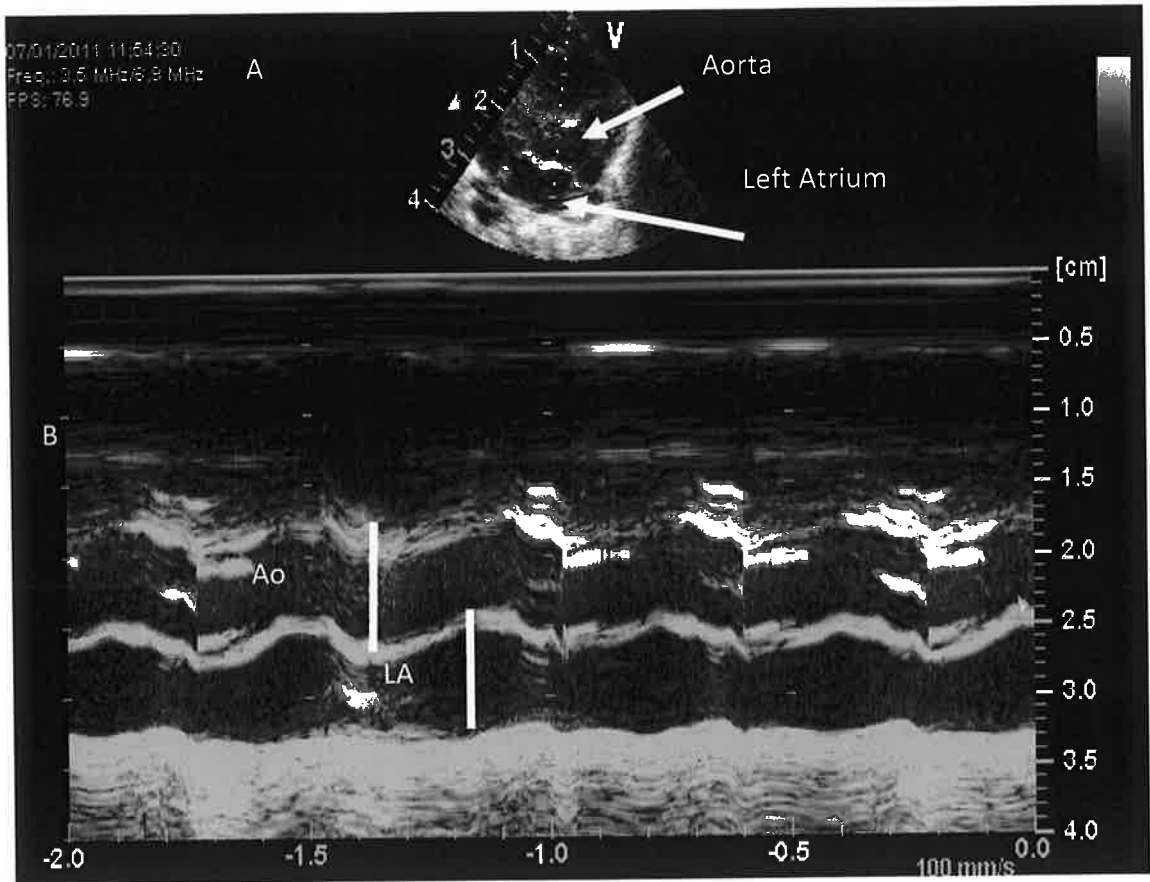


Figure 2.4.3(i) M mode Demonstrating Left Atrium and Aorta. A) Parasternal long axis view. Arrows pointing to left atrium and aorta. B) M mode of Left ventricle. Ao – Aorta, LA – left atrium. LA:Ao ratio calculated from measurement of Left Atrium divided by measurement of the aorta. Measurement taken from the white lines.

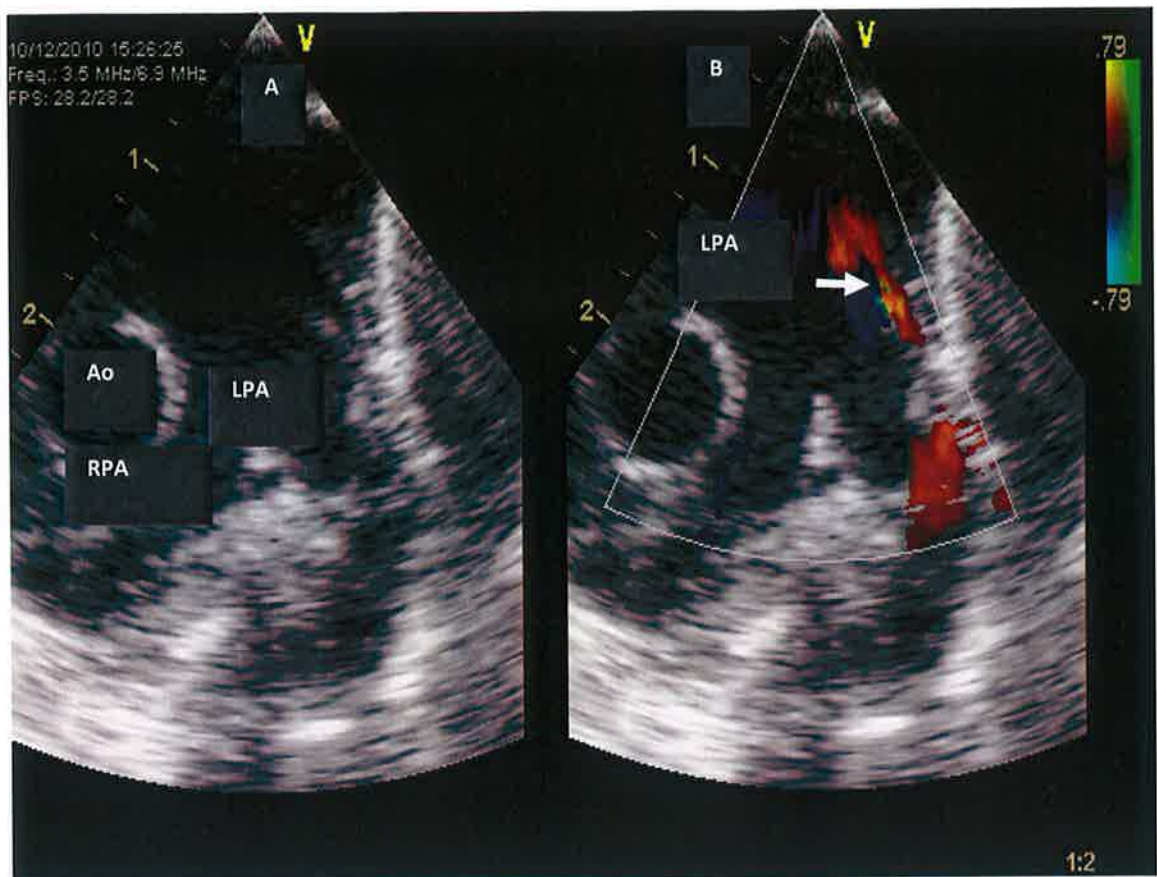


Figure 2.4.3(ii) Parasternal short axis (PSSA) view demonstrating Patent Ductus Arteriosus. A) PSSA view without colour flow Doppler. Ao- Aorta, RPA – right pulmonary artery. LPA – left pulmonary artery. B) Colour Doppler of the PDA – patent ductus arteriosus allows visualisation of left to right flow across the PDA. Red flow indicates left to right shunting across the ultrasound probe.

2.4.4 Tissue Doppler Imaging

TDI measures were taken from an apical 4 chamber view using a colour flow pulsed wave Doppler sample. Frame rates of 180 were used for each study with a Pulsed Wave Doppler (PWD) Gate sample of 0.12cm. TDI is used to measure longitudinal myocardial velocities and provides information on both systolic and diastolic velocities. The sample volume is placed in the ventricular myocardium adjacent to the mitral and tricuspid annulus and at the base of the intraventricular septum. The cardiac cycle is represented by three waveforms – S' the early systolic myocardial velocity, E' the early diastolic myocardial velocity and A' the late diastolic myocardial velocity (Figure 1.5.2) (Ho and Solomon 2006). These measures were recorded for preterm, term controls and infants with evidence of Neonatal Encephalopathy.

2.4.5 Speckle Tracking Imaging

2 D echocardiography was used to acquire images from the apical four-chamber view. ECG leads were insitu at time of echocardiogram and images were taken over three cardiac cycles triggered by the R wave of the QRS complex. The timing of the aortic valve closure with respect to peak systolic strain was manually obtained from a pulsed wave Doppler image of the left ventricular outflow tract. Frame rates of 70-90 Hz were used for all images recorded for STI. Images were stored on the Vivid I at the time of echocardiogram prior to being downloaded for offline speckle tracking analysis which was performed using EchoPAC (Version 6.1.0 for PC, GE Healthcare.AS).

The endocardial borders of the left ventricle were then manually traced within the end systolic frame for both the apical 4 chamber images. The second epicardial tracing is then automatically generated by the computer strain algorithm, however can be manually readjusted to cover the entire myocardial wall. Following satisfactory inspection that tracking covered the entire myocardium, and once EchoPAC software indicated adequate tracking by indicating a green box was tracking of the LV accepted (Figure 1.6.1).The strain software then automatically divides the cross sectional image into six segments. For the LV four chamber view the LV is divided into - the left lateral apical wall, left lateral middle wall, left lateral basal wall, the left septal apical wall, left septal middle wall and left septal basal wall (Blessberger and Binder 2010) (Marcus, *et al* 2011). From this the myocardial longitudinal strain values were obtained. All offline measurements were performed by a single observer (KA).

2.5 Echocardiographic Measurements

2.5.1 Term Control Infants

The healthy term infants had one echocardiogram on Day 1 – 4 of life. In all patients included the PDA was closed. Measurements recorded included:

1. Shortening Fraction
2. Ejection Fraction
3. Mitral E:A ratio
4. Tricuspid E:A ratio
5. TDI measurements at RV/IVS/LV

2.5.2 Preterm

Preterm infants had echocardiograms performed on Day 1, day 3-4 and day 7 of life. Measurements recorded included:

1. Shortening Fraction
2. Ejection Fraction
3. Mitral E:A ratio
4. Tricuspid E:A ratio
5. PDA Diameter
6. LA:Ao Diameter
7. TDI measurements at RV/IVS/LV

2.5.3 Infants with Neonatal Encephalopathy

The infants with evidence of Neonatal Encephalopathy had echocardiograms performed on Day 1-2, with a second scan repeated if the infant was still in the NICU on day 7. Measurements included:

1. Shortening Fraction
2. Ejection Fraction
3. Mitral E:A ratio
4. TDI measurements at RV/IVS/LV

2.6 Statistical Analysis

Data was analysed using SPSS for Windows version 18.0 (SPSS, Inc., Chicago, IL). Values are expressed as mean (SD). Results were analysed with paired Students t-test for normative data and independent T test for unrelated variables. Where significant association were found, a logistic regression model was used to explore the significance. Significance was set at $p < 0.05$.

2.6 a TDI Measurement Validation

The Bland Altman analysis was used to test Intra/interobserver variability. Twenty neonates were re evaluated after a ten minute rest period, each observer was blinded to the other results. A total of sixty measurements were compared. Repeated measurements were taken by observer 1(KA) and then observer 2 (OF). Bland Altman analysis was used to assess agreement between the two measurements (Bland and Altman 1986).

2.6a(i) Intra - Observer Variability for TDI measurements

The Variability expressed as mean percentage error (absolute difference between two measurements divided by the mean of the observations).

Measurements were compared for all systolic and diastolic velocities at the RV/IVS/LV. Repeated measurements were taken by observer 1(KA). On Bland Altman plots for intra observer error 1, represent the first measurement, 2 represents the repeated measurement. Bland Altman analysis was used to assess agreement between the two measurements (Bland and Altman 1986)

(Figure 2.6 a,b). The bias at the RV S' was 0.08cm , RV E' was 0.09cm, RV A' was 0.01cm, and the LV S' was 0.16cm, LV E' was - 0.11cm and LV A' was 0.02cm.

2.6(ii) Inter - Observer Variability for TDI measurements

The Variability expressed as mean percentage error (absolute difference between two measurements divided by the mean of the observations).

Measurements were compared for all systolic and diastolic velocities at the RV/IVS/LV. Repeated measurements were taken by observer 1(KA) and then observer 2 (OF). Bland Altman analysis was used to assess agreement between the two measurements (Bland and Altman 1986) (Figure 2.6 c,d). The bias at the RV S' was -0.11cm, RV A' was 0.16cm, RV E' was - 0.02cm and the LV S' was -0.11cm, LV E' was 0.02sm and LV A' was -0.11cm.

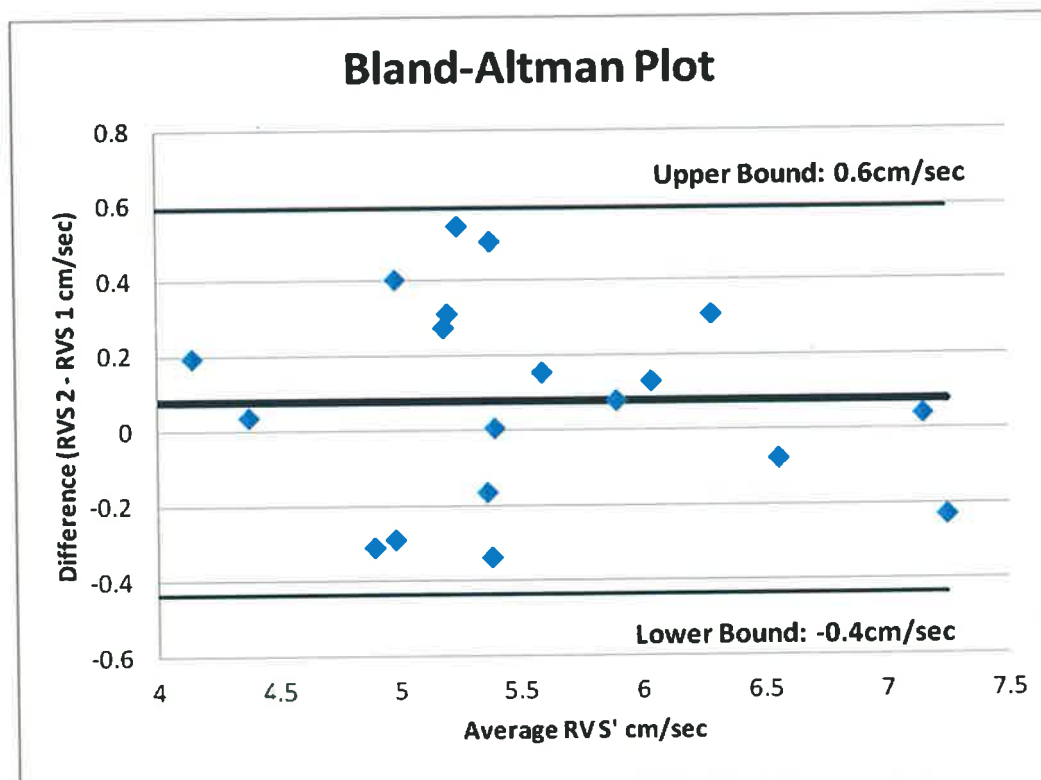


Figure 2.6 a. Intraobserver Agreement between RV S' velocities. RVS 1 – Right Ventricular systolic velocity measured by observer 1 (KA). RVS2 – Repeated Right ventricular systolic measurement by observer 1 (KA). Bias 0.08 cm/s.

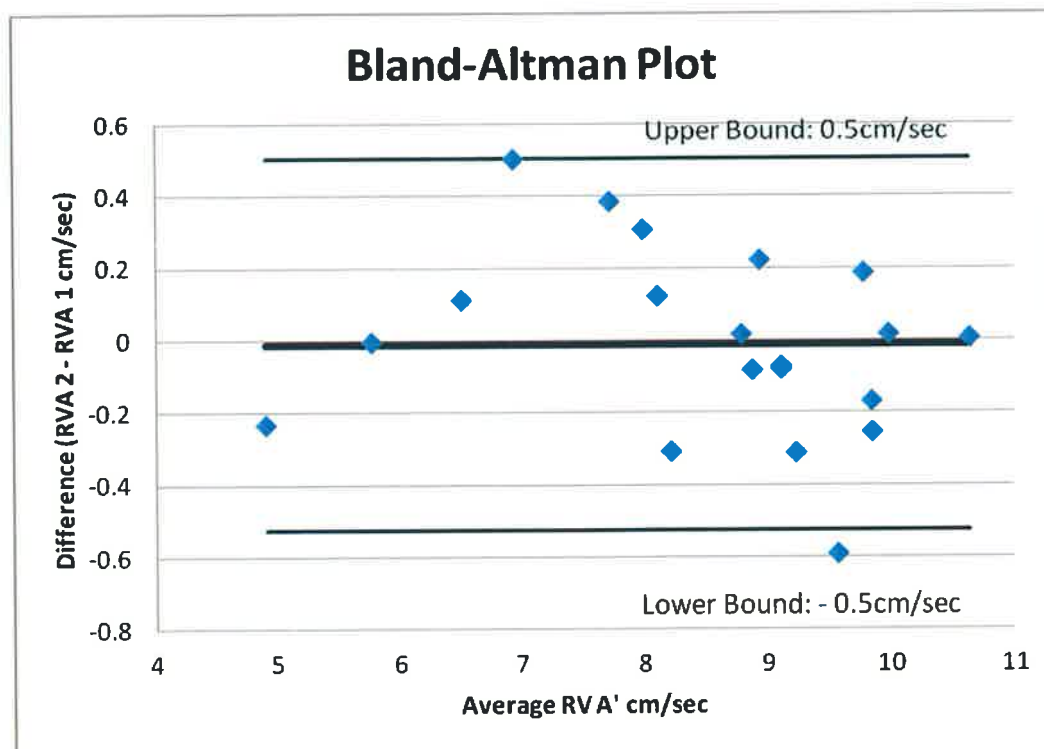


Figure 2.6 b. Intraobserver Agreement between RV A' velocities. RVA 1 – Right Ventricular late diastolic velocity measured by observer 1 (KA). RVA2 – Repeated Right ventricular late diastolic measurement by observer 1 (KA). Bias - 0.01cm/s.

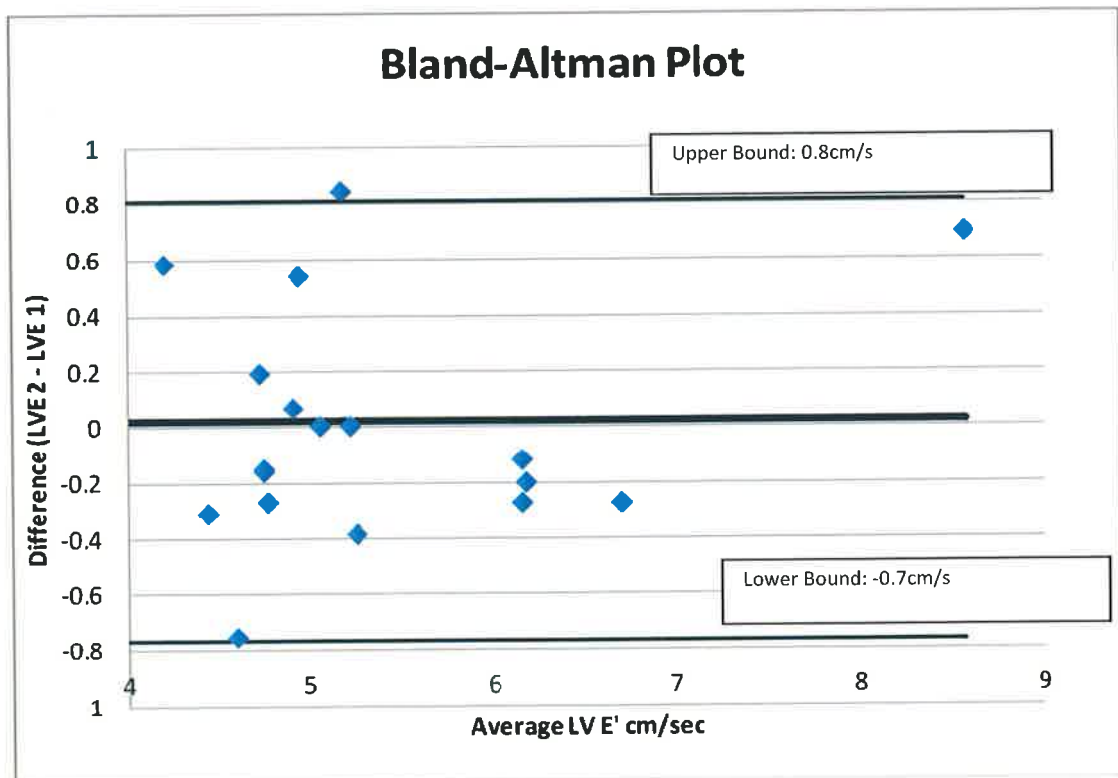


Figure 2.6 c. Interobserver Agreement between LV E' velocities. LVE 1 –Left Ventricular early diastolic velocity measured by observer 1 (KA). RVE2 – Repeated Left Ventricular early diastolic measurement by observer 2(OF). Bias - 0.02cm/s.

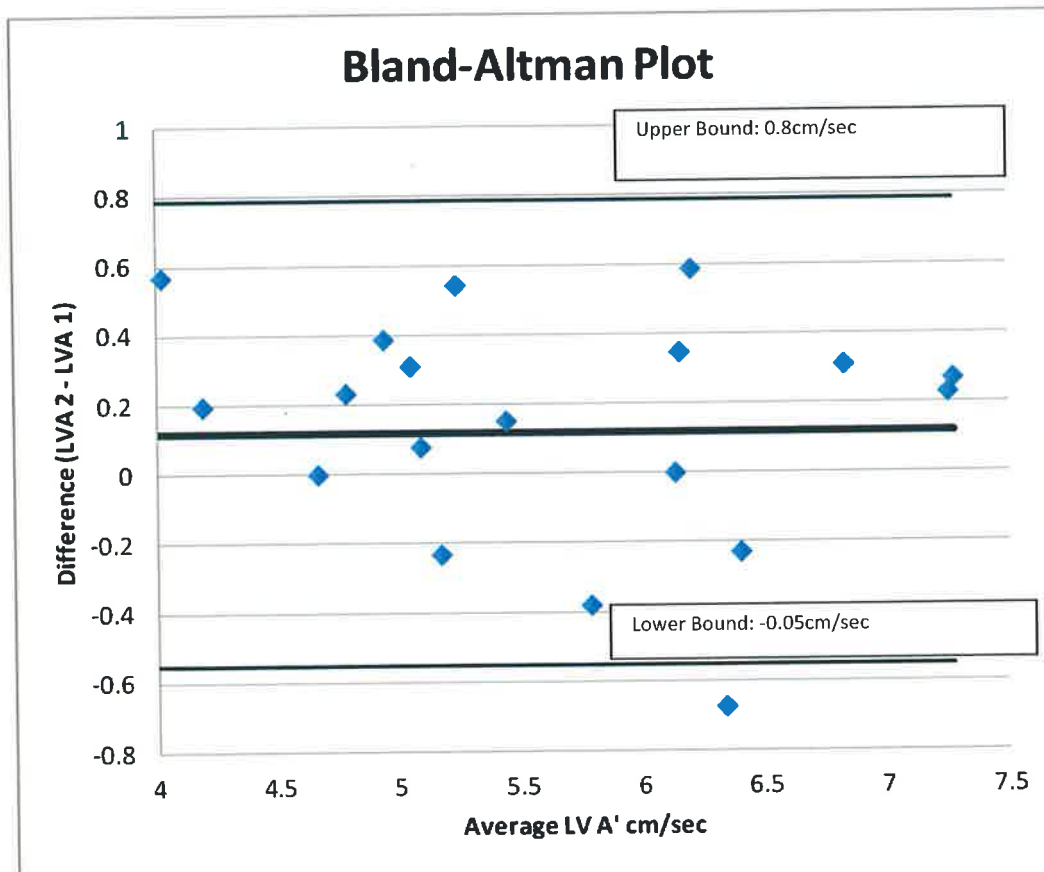


Figure 2.6d. Interobserver Agreement between LV A' velocities. LVA 1 –Left Ventricular late diastolic velocity measured by observer 1 (KA). LVA2 – Repeated Left Ventricular early diastolic measurement by observer 2 (OF). Bias - 0.11cm/s

CHAPTER 3

TISSUE DOPPLER IMAGING QUANTIFIES CHANGES IN PRETERM MYOCARDIUM

3.1 Introduction

The newborn heart undergoes changes to its histological structure and ventricular contractility during the transition from fetal to neonatal life (Stopfkuchen 1987). Throughout this process of maturation there are changes in the underlying structure and function of the myocyte, which increases in both size and number. Myocyte cells in the myocardium are composed of individual striated muscle fibres. The contractile activity of the myocardium may be readily altered under differing loading conditions by changing the resting fibre length and by changes in contractility, both of which shift the myocardial velocity curve. The preterm heart is less compliant and contractile than a term newborn or adult heart. It therefore has limited ability to increase its stroke volume (contractility, preload and afterload) by increasing the End diastolic filling pressure and relies on its ability to increase heart rate to increase cardiac output (Kiserud and Acharya 2004). The preterm heart has less adaptation to cope with changes in preload and afterload.

Changes also occur in the geometry and wall motion of the Left ventricle (LV) during the fetal to neonatal transition, with septal wall flattening resolving over the first few weeks of life and the LV assuming a circular cross sectional shape. Left ventricular mass also increases during this time (Lee, *et al* 1992). In the evaluation

of systolic cardiac function, the intrinsic properties of the fibres and myocytes are important, as they determine the effect on the myocardium of chronic conditions and reflect irreversible damage (Bijnens, *et al* 2009). The standard measurement for systolic assessment of the LV is the M mode measure of shortening (SF) and ejection fraction (EF) (Greenbaum, *et al* 1981). Both of these are volume based parameters. They measure radial myocardial performance but neglect the contribution of longitudinal contraction and deformation (Nesbitt, *et al* 2009). Ejection Fraction also has limited ability to reflect contractility in the context of abnormal loading conditions, for example in the preterm infant with a patent ductus arteriosus (PDA) or neonates with persistent pulmonary hypertension. From a practical point both SF and EF can be difficult to measure in preterm infants who are ventilated on Continuous Positive Airway Pressure (CPAP) as a result of poor echocardiographic windows due to the lung interference.

To date diastolic function has been difficult to measure in preterm infants with the majority of research focusing on mitral inflow patterns or the E/A ratio. This requires a pulsed wave Doppler measurement at the mitral inflow tract, from an apical four chamber view where the A wave represents atrial contraction at the end of diastole and the E wave represents blood flow across the mitral valve in early diastole (Lopez, *et al* 2010). In normal infants in the first week of life, there is a change from the fetal filling type with more dependence on filling during the atrial contraction (A wave), toward a more mature pattern with a higher early filling pattern (E wave). In preterm infants this results in a pronounced increase in the E wave (Harada, *et al* 1994). This method can be influenced by preload (end diastolic

pressure of the LV) as in patients with a PDA, and is sensitive to changes in heart rate which may limit its usefulness in this population (Stoddard, *et al* 1989).

Newer echocardiographic measures are evolving in order to overcome these deficiencies. Tissue Doppler Imaging (TDI) uses the same principles as conventional Doppler techniques to quantify the high amplitude, low velocity signal of myocardial tissue motion (Ho and Solomon 2006). It allows assessment of regional longitudinal ventricular contraction and relaxation velocities based on myocardial motion and deformation and provides measurements of instantaneous peak myocardial velocities (Vinereanu, *et al* 1999). It therefore not only provides information about the systolic but also on the diastolic myocardial performance.

3.2 Hypothesis

Tissue Doppler Imaging may be a superior modality to assess myocardial function in term and preterm infants.

3.3 Aims

1 – To establish normative data for Tissue Doppler Imaging in healthy term control infants

2 – To quantify changes in myocardial contractility in preterm infants (< 32 weeks gestation) during the first week of life and compare with healthy term controls using Tissue Doppler Imaging

3.4 Results

Normal Term Infant Controls

Sixty five normal term infants with an uncomplicated pregnancy, delivery and postnatal course had echocardiography carried out on Day 2-4 of life. Echocardiograms were carried out at this time, as it is likely that the PDA will have closed and pulmonary vascular resistance will have started to decrease. Five children were excluded from the analysis due to minor structural anomalies: Small Atrial septal defect (3), small muscular ventricular septal defect (2). No infant had clinical or echocardiographic evidence of a PDA. The mean gestational age (SD) was 39 (1.9) weeks and birth weight 3.64 (0.6) kg. There was equal sex distribution. Patient characteristics are outlined in Table 3.1. There was no significant difference between ejection and shortening fraction or Mitral/Tricuspid E:A ratio between the term and preterm infants.(Table 3.2) The TDI velocities were higher for all systolic and diastolic measurements at the right ventricle compared to the left. (Table 3.3)

Preterm Infants

Sixty four preterm infants were recruited. Infants with major congenital malformations including cardiac lesions other than PDA were excluded. Four infants with small – moderate atrial septal defects were excluded from analysis. Sixty preterm infants had one hundred and fifty echocardiograms. Their mean gestational age (SD) was 28 (1.9) weeks and birth weight was 1.2 (0.4) kg There were 38 male infants and 3 preterm infant deaths in the first 48 hours of life. Patient characteristics are highlighted in Table 3.1.

There was a significant increase in the heart rate ($p < 0.001$) and the systolic blood pressure ($p < 0.001$) over the first week of life in preterm infants (Table 3.4.)

On day 1 of life 73% (44) of the blood pressure measurements were from central arterial lines, with the remainder taken peripherally at the time of echocardiogram. When comparing preterm infants who were < 28 weeks gestation with those > 28 weeks there was a significant increase in all systolic blood pressure measurements and on pulse pressure measurements between day 3 and day 7 (Table 3.5). There was no difference between the mode of ventilation on Day 1 and Day 7. Thirty nine infants were on caffeine on day 1 compared to 36 on day 7 and only one infant was on inotropes at the time of echocardiogram.

There was no significant difference between the systolic measures of shortening and ejection fraction over the first week of life in preterm infants. (Table 3.2). There was a significant increase in the Tricuspid early diastolic filling velocity (E) wave ($p=0.014$.)

There was no significant difference between the Left Atrial to Aortic (LA:Ao) ratio over the first week of life irrespective of the presence of a PDA (as determined by colour flow Doppler)(Table 3.2). On day 1, 35 % ($n=21$) of infants had a haemodynamically significant PDA (LA:Ao ratio > 1.4), (El Hajjar, *et al* 2005) this had decreased to 32% ($n=19$) by day 7.

There was an increase in tissue Doppler myocardial velocities across all measurements in the first week of life, with right ventricular systolic and diastolic velocities increasing significantly ($p<0.001$ & $p=0.034$). (Table 3.6). In all preterm infants peak velocities were higher in the right than the left ventricle. There was a significant increase in the early diastolic filling of the intraventricular septum ($p<0.001$) and a significant increase in the left ventricular late diastolic velocities over the first week of life ($p=0.022$). There was a trend towards higher myocardial

velocities across all measures when comparing neonates who were less than 28 weeks gestation with those who were over 28 weeks on Day 7 however this did not reach statistical significance (Table 3.7). By the end of week one the preterm myocardial velocities were similar to the term infant myocardial velocities (Table 3.3).

	Term	Preterm	p value
Birth weight (kg)	3.64 [2.7 5.01]	1.23[0.53-2.0]	<0.001
Males	30(50)	38(63)	<0.202
Caesarean Section	21(35)	36(60)	<0.001
Maternal Antibiotics	5(8)	18(30)	<0.001
APH	None	4(7)	N/A
PET	None	9(15)	N/A
AEDF/REDF	None	8(13)	N/A
PROM	None	15(25)	N/A
ANS [Any	None	13(22)	N/A
Complete	None	47(78)	N/A
Apgar at 1 minute	9[4-9]	6.[2-9]	<0.001
Apgar at 5 minutes	9[7-10]	81[4-9]	<0.001
DR Resuscitation	4 (7)	53(88)	N/A

Table 3.1. Patient Characteristics. Values are represented as mean [range] or as number (percent). Independent Students t test was used to compare means & Chi square test for proportions. A/REDF – Absent/Reversed end diastolic flow, APH – Antepartum haemorrhage, PET – Pre eclamptic toxemia, PROM – Prolonged rupture of the membranes, ANS– Antenatal steroids, DR Resuscitation – Delivery room resuscitation. Significant results are in bold.

	Term Infants	Preterm Infants			p value	
		Day1	Day 3	Day 7		
SF(%)	32.0(6.7)	31.0 (9.6)	34.0(5.7)	34.0 (6.3)	0.3	
EF(%)	64.0(10)	62.0 (12.4)	67.0(7)	68.0 (9.0)	0.1	
LA:Ao	N/A	1.4(0.4)	1.4(0.4)	1.32(0.4)	0.7	
PDA	N/A	1.3(0.3)	1.3(0.4)	1.2(0.5)	0.5	
Diameter						
MV	E	50.9(10.0)	38.1(13.7)	39(16)	50.98(14.0)	0.3
	A	48.12(12.0)	48.74(16.8)	45 (19)	47.4(18.0)	0.06
	E:A	1.14(0.3)	0.85(0.2)	0.8(0.3)	0.9(0.43)	0.4
TV	E	41.57(9.0)	39.35(19.1)	32.6(18.6)	33.6(16.1)	0.01
	A	54.1(10.0)	48.21(21.6)	39.2(17.6)	42.4(26.4)	0.2
	E:A	2.2(0.9)	0.9(0.4)	0.9(0.6)	0.78(0.39)	0.3

Table 3.2: Standard Doppler Measures of Systolic and Diastolic Function. LA:Ao – Left atrial to aortic root ratio. PDA – Patent ductus arteriosus, MV – Mitral valve, TV – Tricuspid valve, E – Early diastole, A – Late diastole, E:A Early to late diastolic ratio. Values are expressed as mean (SD). Independent Students t test used for analysis to compare Preterm infants on Day 1 to Day 7. Significant results are in bold.

		Term	Preterm Infants		
		Controls	Day 1	Day 7	P value
RV	S'	6.3(1.3)	5.4(0.9)	6.2(1.0)	<0.001
	E'	7.5(1.8)	5.6(2.0)	6.4(1.7)	0.05
	A'	8.1(2.3)	7.4(1.6)	8.9(1.8)	0.03
IVS	S'	3.7(0.8)	3.6(0.9)	3.8(0.6)	0.7
	E'	4.1(1.2)	3.9(1.0)	4.6(1.2)	0.05
	A'	4.3(1.0)	4.4(1.1)	4.8(1.2)	0.04
LV	S'	5.4(1.3)	4.5(0.7)	4.8(0.7)	0.41
	E'	5.7(1.8)	5.0(1.4)	5.7(1.7)	0.71
	A'	5.4(1.5)	5.4(1.2)	6.0(0.9)	0.23

Table 3.3. Tissue Doppler Measurements in Term Controls and Preterm infants.

Measurements are in cm/s. Values are mean (SD.) RV – Right Ventricle, LV- Left ventricle, IVS – Intraventricular septum. S' – Early systolic velocity, E'- Early diastolic velocity, A' - Late diastolic velocity. Independent Students t test was used for analysis between term infants and preterm infants on Day 7. Significant results are in bold.

	Day 1	Day 3	Day 7	p value
Heart Rate	154(15)	160(15)	164(11)	<0.001*
Systolic BP	54(14)	62(13)	66(12)	<0.001*
Diastolic BP	37(10)	40(11)	39(11)	0.507
Pulse Pressure	44(11)	48(11)	48(10)	0.093
SaO ₂ (%)	95(3)	93(4)	96(4)	0.233
FiO ₂ (%)	25(7)	25(8)	23(5)	0.230

Table 3.4.: Clinical Characteristic of the Preterm Population. Values are mean (SD.)

SaO₂ - Oxygen saturations, FiO₂ - Inspired oxygen concentration. Paired students T test was used for the analysis. * Statistical significance p<0.05 compares significance between clinical characteristics on Day 1 and Day 7.

		<28 weeks	>28 weeks	p value
HR	Day 1	159(17)	152(16)	0.148
	Day 3	160(15)	159(15)	0.884
	Day 7	165(12)	166(12)	0.890
SBP	Day 1	49(13)	64(14)	<0.001
	Day 3	58(11)	71(13)	<0.001
	Day 7	62(12)	74(8)	<0.001
DBP	Day 1	35(11)	39(13)	0.185
	Day 3	36(9)	47(12)	<0.001
	Day 7	36(10)	42(12)	0.157

Table 3.5. Differences between Heart rate and Blood pressure in those < 28 weeks gestation. HR – Heart rate, SBP – Systolic blood pressure, DBP, Diastolic blood pressure. Values are mean (standard deviation). The figures in bold represent a statistically significant difference. Students Independent t test was used to compare means.

		Day 1	Day 3	Day 7	p value
RV	S'	5.4(0.9)	5.5(0.8)	6.2(1.0)	<0.001
	E'	5.6(2.0)	6.2(2.0)	6.4(1.7)	0.03
	A'	7.4(1.6)	8.5(1.7)	8.9(1.8)	<0.001
IVS	S'	3.6(0.9)	3.7(0.6)	3.8(0.6)	0.2
	E'	3.9(1.0)	4.3(1.3)	4.6(1.2)	<0.001
	A'	4.4(1.1)	4.8(1.1)	4.8(1.2)	0.08
LV	S'	4.5(0.7)	4.4(0.8)	4.8(0.7)	0.06
	E'	5.0(1.4)	4.7(0.9)	5.7(1.7)	0.12
	A'	5.4(1.2)	5.2(1.0)	6.0(0.9)	0.02

Table 3.6 : Changes in Myocardial Velocities in the first week of life in Preterm Infants. Measures are in cm/s. Values are expressed as mean (SD.) RV – right ventricle, LV – left ventricle, IVS – Intraventricular septum. S' – early systolic velocity, E' – early diastolic velocity, A' Late diastolic velocity. Mean values were compared using Paired Students t test was to compare velocities on day 1 with those on day 7. Significant values are in bold.

		Preterm Infants < 28		Preterm Infants > 28		
		weeks		weeks		
		Day1	Day 7	Day 1	Day 7	p value
RV	S'	5.3(0.8)	5.9(1.1)	5.6(1.0)	6.3(0.9)	0.37
	E'	4.9(1.9)	6.4(1.7)	5.9(1.7)	6.4(1.7)	0.98
	A'	7.7(1.6)	8.7(1.2)	7.7(1.6)	9.0(1.9)	0.67
IVS	S'	3.0(0.5)	3.5(0.6)	3.7(0.9)	3.6(0.7)	0.19
	E'	3.5(0.6)	4.1(1.3)	4.0(1.1)	4.5(1.2)	0.34
	A'	4.0(1.0)	5.0(1.4)	4.5(1.0)	4.6(1.1)	0.36
LV	S'	4.0(0.6)	4.4(0.5)	4.5(0.7)	4.7(0.8)	0.24
	E'	4.6(1.2)	4.7(1.3)	5.0(1.4)	5.8(1.1)	0.36
	A'	4.9(1.1)	5.5(1.1)	5.2(1.1)	5.8(1.1)	0.57

Table 3.7. Tissue Doppler Measurements in Preterm infants < 28 weeks gestation.

Measurements are in cm/s. Values are mean (SD.) RV – Right Ventricle, LV- Left ventricle, IVS – Intraventricular septum. S' – Early systolic velocity, E'- Early diastolic velocity, A' - Late diastolic velocity. Independent Students t test was used for analysis between preterm infants < 28 weeks gestation on Day 7. Significant results are in bold.

3.5 Discussion

There was a significant increase in the RV systolic and diastolic myocardial velocities over the first week of life in Very Low Birth Weight (VLBW) infants. There were also significant differences between all systolic and diastolic velocities, when comparing the right and left ventricle. By the end of week one the TDI measures in preterm infants at the LV were similar to term infants at birth which may reflect the ability of the preterm myocardium to remodel itself. However the RV and IVS systolic and diastolic velocities remained significantly lower in the preterm infants on Day 7 compared to the term infants. Both Negrine et al & Ciccone et al used tissue Doppler Imaging to assess myocardial velocities in preterm infants (Negrine, *et al* 2012) (Ciccone, *et al* 2011). Both reported similar values for both systolic and diastolic velocities to our study and concluded that TDI was reproducible in preterm infants (Negrine, *et al* 2012) (Ciccone, *et al* 2011). Similarly Mori et al successfully measured myocardial velocities over the first week of life in term infants in an attempt to establish normal values (Mori, *et al* 2004). Ours is the largest study to date to use Tissue Doppler Imaging to measure myocardial velocities in the preterm infant serially over the first week of life.

Evaluation of the RV should form an integral part of a neonatal echocardiogram. However to date there are no reliable reproducible quantitative parameters for the assessment of RV function (Mertens, *et al* 2011). We found that all right sided velocities were higher for systolic and diastolic measures compared to the LV, this is similar to TDI velocities in fetal life. Gardiner et al measured tissue Doppler velocities in preterm infants and found that right sided measures were higher than the left and that there was an increase in myocardial velocities with

increasing gestational age (Gardiner, *et al* 2006). We also demonstrated an increase in all myocardial velocities with increasing gestational age. However there was no statistically significant increase when comparing infants who were greater or less than 28 weeks gestation. The rise in RV systolic velocities may reflect an improvement in the contractility of the preterm myocardium over the first week of life that is not described by conventional echocardiographic measures.

Many pulsed wave diastolic Doppler measures are load dependent. Pulsed Doppler mitral inflow velocities – E:A ratio are altered by changes in preload (Stoddard, *et al* 1989). This may limit its use in the preterm population as changes in preload occur as the ductus arteriosus closes.

In this study we used the LA:Ao ratio and PDA diameter as a measure of PDA significance, and although there are limitations with these measures as with all PDA measurements they are accepted measures of a significant PDA (El Hajjar, *et al* 2005). A PDA diameter > 1.5mm and LA:Ao ratio of > 1.4:1 was deemed significant (El Hajjar, *et al* 2005). There was no significant difference in the LA:Ao ratio or the PDA diameter measurements over the first week of life. When comparing systolic and diastolic tissue velocity measurements from day 1-7 there was no significant difference in any measurement at the RV, IVS or LV in infants who had a significant ductus arteriosus. As there were significant changes in the RV systolic, IVS diastolic and LV early diastolic velocities the presence of a PDA may have no effect on myocardial wall motion velocities, making TDI a useful preload independent measure.

There was a significant increase in the systolic blood pressure over the first week of life. As the normal range for blood pressure in preterm infants is still widely

debated, this cohort provides further evidence of the significant haemodynamic changes occurring in preterm infants during the first week of life. Hegyi et al established normative data for systolic and diastolic blood pressure measurements in a large cohort of preterm infants, and described an increase in systolic blood pressure over the first week (Hegyi, *et al* 1996). Fleming et al reported that in term infants heart rate peaks at one month of age (Fleming, *et al* 2011), preterm infants may also follow this pattern. There was a significant increase in heart rate in our group of preterm infants over the first week. Preterm infants use an increase in heart rate to enhance cardiac output and this may correlate with the rise in the systolic velocities over the first week.

In conclusion Tissue Doppler Imaging offers a reliable measure of myocardial velocities over the first week of life. We have demonstrated normative data for healthy term infants. In preterm infants the rise in RV systolic/diastolic and LV early diastolic velocities to term values may reflect an improvement in the contractility of the preterm myocardium over the first week of life which is not reflected by measures of shortening and ejection fraction.

CHAPTER 4

TISSUE DOPPLER IMAGING MAY PREDICT OUTCOMES IN PRETERM INFANTS

4.1 Introduction

Preterm infants < 32 weeks gestation have a high risk group of mortality and morbidity. It is difficult to predict neonatal outcomes in preterm infants (Jobe 2001). Major morbidities among preterm survivors include Chronic Lung Disease (CLD), severe Intraventricular Haemorrhage (IVH), Necrotising Enterocolitis (NEC), Late onset Sepsis and Retinopathy of Prematurity (ROP). The majority of predictors of neonatal outcomes utilise gestational age at birth, birth weight or sex to predict survival (Shah, *et al* 2012).

The use of point of care ultrasound in the Neonatal Intensive Care Unit (NICU) has changed the approach to cardiovascular care of preterm infants, particularly during the postnatal transitional period (Sehgal and McNamara 2009). Guidelines now exist for functional echocardiography in the NICU which allow experienced Neonatologists to assess the ductus arteriosus, myocardial performance and pulmonary and systemic haemodynamics (Mertens, *et a* 2011). The use of serial echocardiography has been used to allow early identification of a significant PDA and to allow early commencement of therapy without waiting for clinical signs (O'Rourke, *et al* 2008). However while point of care ultrasound has been used to guide therapeutic decision making, there is no

evidence to date that it helps to improve neonatal outcomes (Sehgal and McNamara 2008).

4.2 Hypothesis

Tissue Doppler Imaging may identify infants at risk of multiorgan dysfunction in preterm infants < 32 weeks gestation

4.3 Aims

1 - To determine if Tissue Doppler Imaging measurements of systolic and diastolic myocardial velocities are altered in preterm infants who will develop

- a) Chronic Lung Disease
- b) Necrotising Enterocolitis
- c) Late onset Sepsis
- d) Surgical PDA Ligation
- e) Intraventricular Haemorrhage
- f) Neonatal Death

4.4. Results

Sixty four preterm infants were recruited. Infants with major congenital malformations including cardiac lesions other than PDA were excluded. Four infants with small – moderate atrial septal defects and were excluded from analysis. Sixty preterm infants had one hundred and fifty echocardiograms. Their mean gestational age (SD) was 28 (1.9) weeks and birth weight was 1.2 (0.4) kg There were 38 male infants and 3 preterm infant deaths in the first 48 hours of life. Patient characteristics are highlighted in Table 3.1.

In preterm infants, the RV early diastolic velocity measurements on Day 1, late diastolic velocity on Day 3 and LV systolic and early diastolic velocities on Day 7 were all significantly lower in preterm infants who subsequently developed Chronic Lung Disease (n=11) (Figures 4.1-4.4). LV systolic velocities on day 1 and RV late diastolic velocities were significantly lower in those who developed Retinopathy of Prematurity (n=5) (p=0.031 and p=0.032). Infants who developed Late onset Sepsis had significantly lower early diastolic velocities on day 7 (n=12) (p=0.033). Preterm infants who required PDA ligation had significantly lower RV diastolic velocities on day 1 (p=0.015), systolic velocity on day 7 (p = 0.002) and LV systolic and diastolic velocities on day 7 (n=3) (p = 0.045 & p= 0.05). Tissue Doppler systolic and diastolic velocities at the intraventricular septum on day 3 and 7 were significantly lower in those who developed Necrotising enterocolitis (Figure 4.5 – 4.6). Neither systolic nor diastolic tissue Doppler velocities over the first week of life were significantly lower in those who developed intraventricular haemorrhage (n=4) or in the preterm infants who died (n=3).

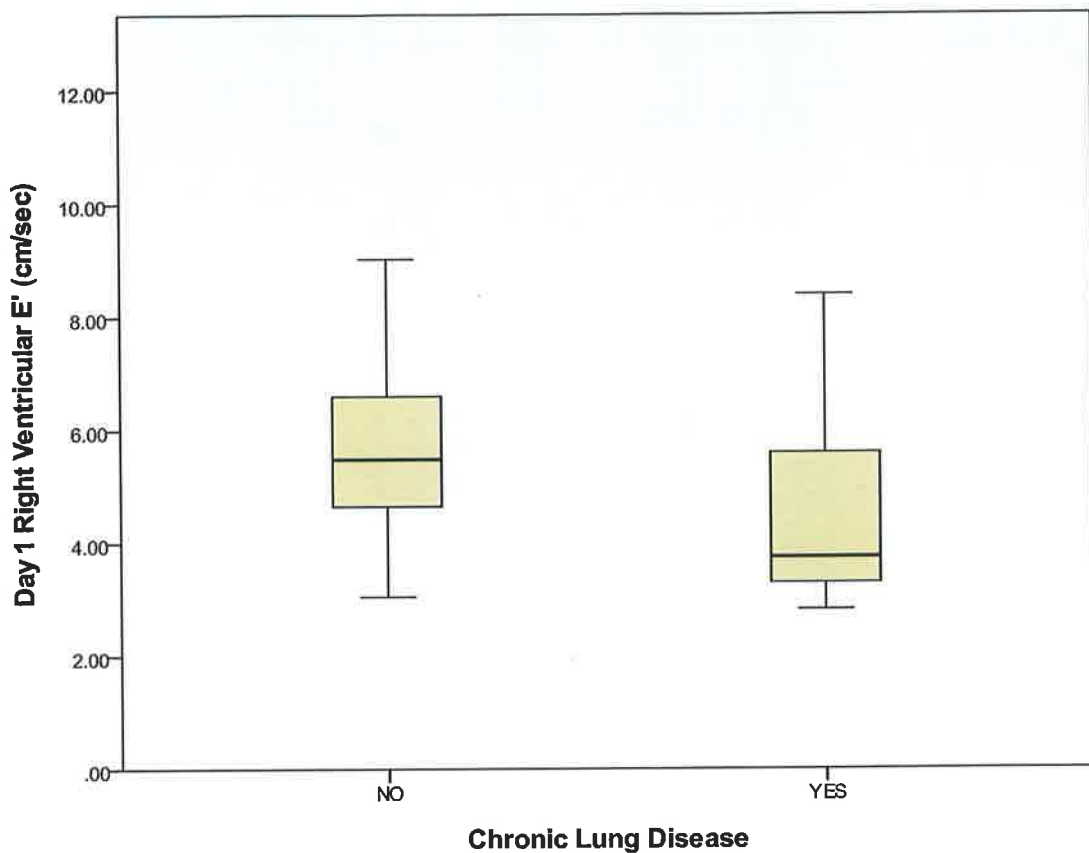


Figure 4.1. Day 1 Right Ventricular Early Diastolic (E') Velocity. This is a box and whisker plot which represents shows location, variation and skewness. The solid bar in the centre of the box is a measure of location with median represented; the box shows the 25th – 75th percentiles the whiskers show the mean +/- 3 Standard deviations. Infants who develop Chronic Lung Disease had significantly lower right ventricular early diastolic velocities on day 1 of life $p=0.032$. An Independent Students t test was used for analysis.

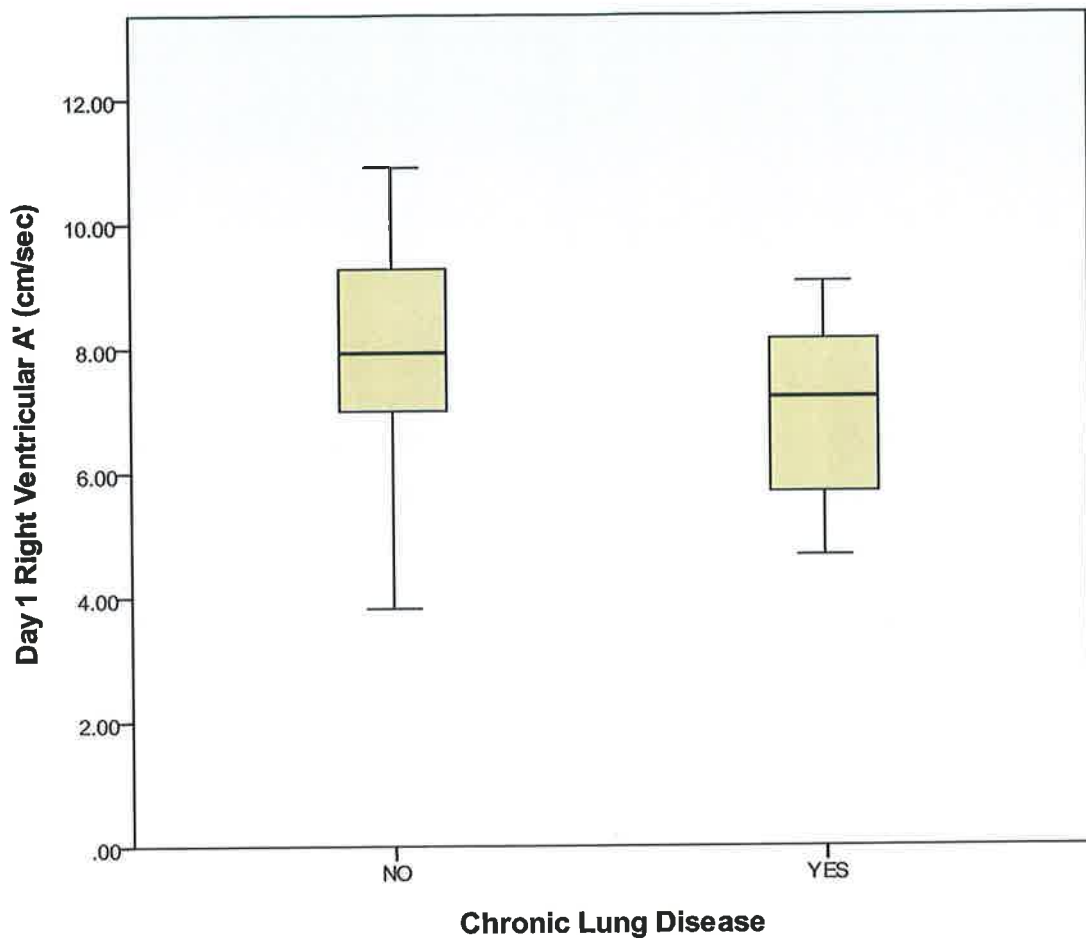


Figure 4.2. Day 3 Right ventricular Late Diastolic (A') velocity. Infants who develop Chronic Lung Disease had significantly lower right ventricular late diastolic velocities on day 3 life $p = 0.049$. An Independent Students t test was used for analysis.

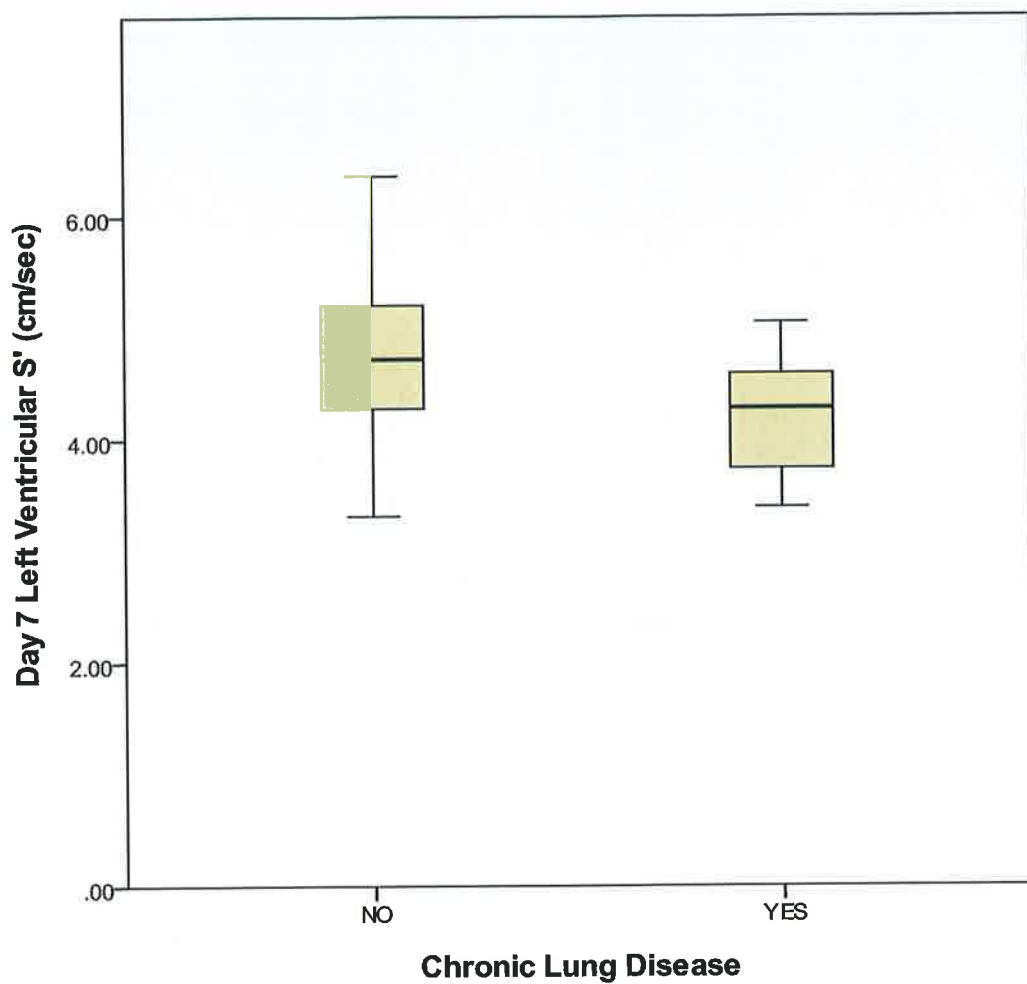


Figure 4.3 Day 7 Left ventricular Early Systolic (S') velocity. Infants who develop chronic lung disease had lower left ventricular systolic measurements on day 1 life $p = 0.05$. An Independent Students t test was used for analysis.

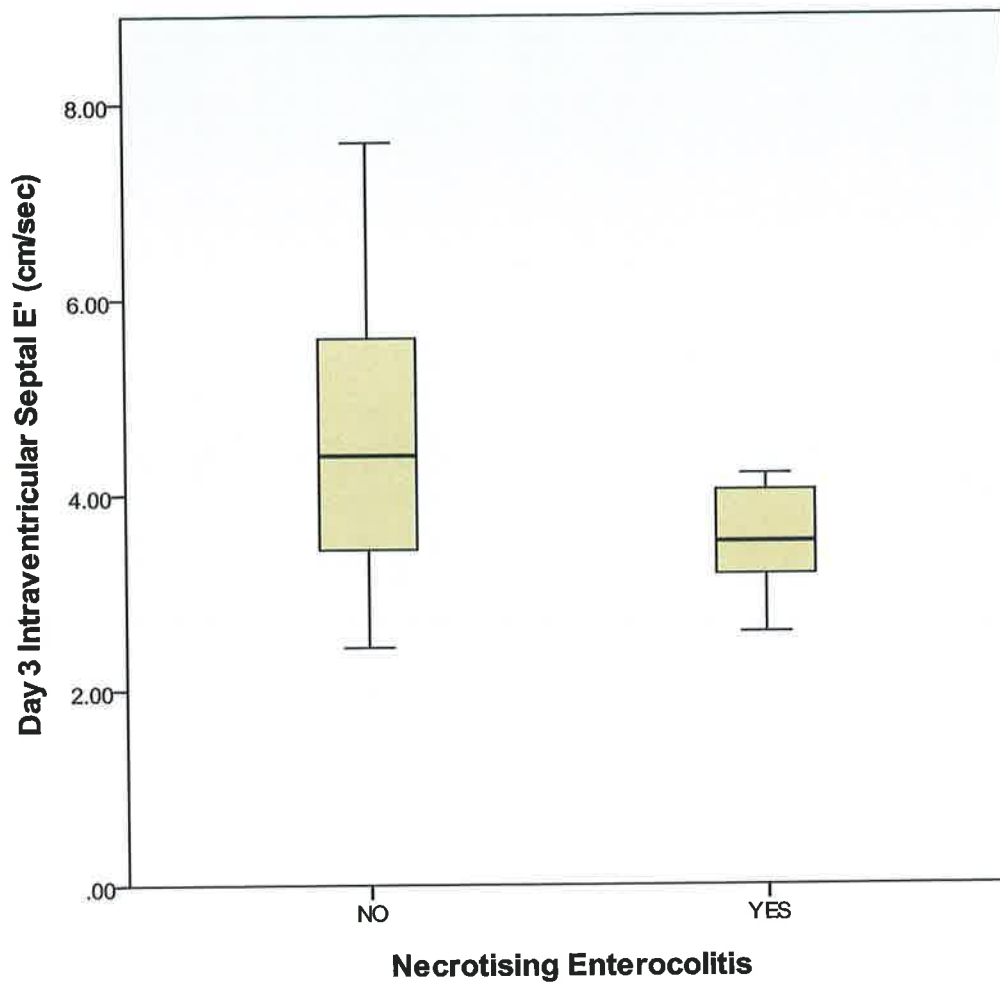


Figure 4.4. Day 3 Intraventricular Septal Early Diastolic (E') velocity. Infants who developed Necrotising Enterocolitis had lower early diastolic velocities at the Intraventricular septum on day 3 life $p = 0.05$. An Independent Students t test was used for analysis.

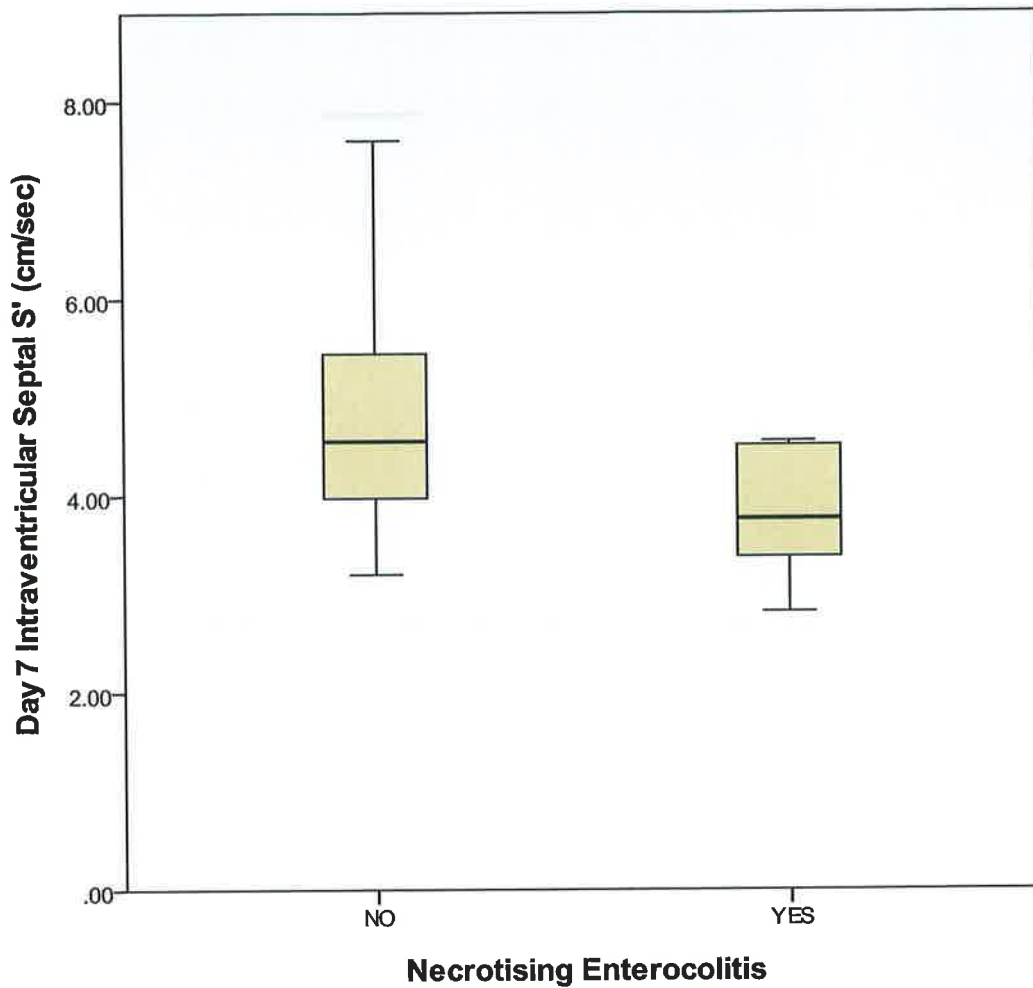


Figure 4.5. Day 7 Intraventricular Septal Early Systolic (S') velocity. Early systolic velocities on day 7 of life were significantly lower in preterm infants who developed Necrotising Enterocolitis $p= 0.04$. An Independent Students t test was used for analysis.

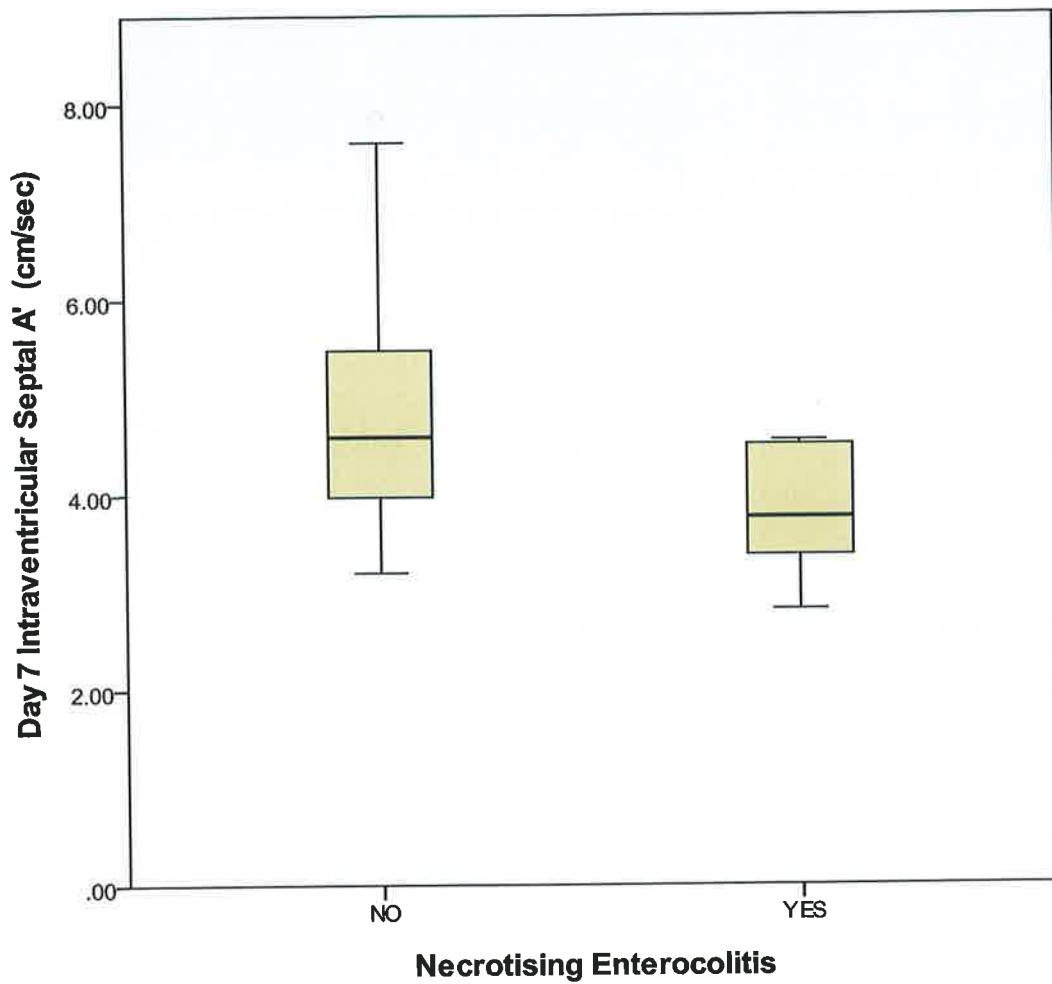


Figure 4.6. Day 7 Intraventricular Septal early diastolic velocity. On day 7 of life preterm infants who developed Necrotising Enterocolitis had significantly lower late diastolic velocities than those who did not $p = 0.03$. An Independent Students t test was used for analysis.

4.5 Discussion

We have found that significant changes in TDI systolic and diastolic velocities in preterm infants in the first week are associated with illnesses including Chronic Lung Disease and Necrotising Enterocolitis.

Perinatal factors such as low gestational age, low birth weight and male sex have been shown to predict those who will develop chronic lung disease (Henderson-Smart, *et al* 2006) (Messerschmidt, *et al* 2011, Shah, *et al* 2012). However despite the use of antenatal steroid, surfactant and other advances in NICU, CLD still remains a significant preterm morbidity. Barotrauma induced by mechanical ventilation, oxygen toxicity and management of the PDA have all been shown to increase the risk of developing CLD (Shah, *et al* 2012). Tissue Doppler measurements of both systolic and diastolic velocities on day 1, 3 & 7 were significantly lower in preterm infants who develop CLD. Turhan *et al* have demonstrated decreased right ventricular systolic velocities in adult patients with Chronic Obstructive Pulmonary Disease (COPD). In 58 patients with COPD they found that RV systolic velocities were significantly lower in COPD patients with right sided heart failure than in age matched controls ($p=0.025$) (Turhan, *et al* 2007). Our study has shown that measures of systolic and diastolic velocities are lower in the first week of life in infants who develop CLD. A decrease in myocardial velocities therefore reflects impairment in overall myocardial contractility that is not reflected in echocardiographic measures of shortening or ejection fraction.

The use of TDI measurement as part of routine functional assessment of the preterm infant in the NICU could therefore allow early recognition of those at risk for CLD. Careful use of mechanical ventilation, the early use of surfactant and the

avoidance of fluid overload may help to prevent CLD. However for infants with established CLD the importance of adequate oxygenation to prevent hypoxic episodes, provision of adequate nutrition and the used of diuretics may be helpful and could be guided by TDI measurements (Shah 2003).

The development of Necrotising Enterocolitis is multi factorial, and is thought to be associated with enteral feeding, inflammation and ischaemia (Carter and Holditch-Davis 2008). Multiple episodes of nosocomial infection and increased periods of mechanical ventilation are associated with an increased likelihood of developing NEC (Guthrie, *et al* 2003). Mechanical ventilation may reduce venous return causing pressure on the myocardium preventing effective contraction (Noori and Seri 2005). In our study we have shown decreased systolic and diastolic myocardial velocities in those who subsequently develop NEC on day 7. NEC may also be linked to an inflammatory process (Emami, *et al* 2012).

Retinopathy of Prematurity which is caused by the incomplete vascularisation of the retina, is a morbidity of prematurity and is associated with low birth weight and gestational age, and may be linked to high levels of oxygen supplementation (McGinnity and Halliday 1993) (Quinn 2005). In our study infants who had lower systolic and diastolic velocities in the first week of life had increased chance of developing ROP.

Diastolic dysfunction occurs in infants with neonatal sepsis. Abdel – Hady *et al* used a Doppler index of myocardial performance (Tei Index) to assess myocardial function in septic neonates. They concluded that neonatal sepsis is associated with systolic and diastolic myocardial dysfunction. Tissue Doppler imaging appears to be more sensitive than conventional echocardiography in the detection of this

dysfunction (Abdel-Hady, *et al* 2010). In our study we found evidence of diastolic impairment in the myocardial velocities at the RV wall on day 7 of life in infants with late onset sepsis. In experimental studies impaired cardiac function has been linked to Lipopolysaccharide (LPS) exposure. Neonatal mice injected with LPS to cause cytokine activation had increased levels of interleukin – 6 and 10 with concurrent decreased left ventricular diastolic function (Mukherjee, *et al* 2011). Neonatal morbidities such as CLD, NEC and ROP may be related to an inflammatory process similar to that which occurs in LOS and the inflammatory process may be what causes impaired myocardial function.

Targeted neonatal echocardiography has been introduced in the NICU, and one of its many uses is to demonstrate the presence of an haemodynamically significant PDA, allowing early recognition and to guide therapy (Mertens, *et al* 2011). Preterm infants who required surgical PDA ligation had significantly lower systolic and diastolic velocities over the first week of life. In the hands of an experienced echocardiographer measurement of myocardial velocities may predict those who may require PDA ligation and allow optimisation of their medical management.

One weakness of this study is that when we controlled for gestational age and gender on multivariate analysis none of the TDI velocities were significantly associated with predicting any of the morbidities described however the study was not powered to pick up a significant association. We feel that there is still a significant relationship between lower TDI velocities in the first week of life and neonatal morbidity.

In conclusion this preliminary data has demonstrated an association between Tissue Doppler measurements of myocardial velocities and neonatal morbidities such as CLD, NEC and ROP. Decreased tissue Doppler velocities at the RV/ IVS and LV are evidence of impaired myocardial contractility which is not picked up by current measures of ejection and shortening fraction. In the future a larger study would be valuable to use serial echocardiography and TDI velocity measurement to follow Very Low Birth Weight (VLBW) infants throughout their inpatient stay.

CHAPTER 5

VITAMIN D LEVELS AND MYOCARDIAL FUNCTION IN PRETERM INFANTS

5.1 Introduction

Vitamin D is necessary for the maintenance of calcium and phosphorous homeostasis, for bone mineralisation and may play a role in innate immune and auto immune responses (Dawodu and Nath 2011). Vitamin D is absorbed from the intestine (Holick 2005).

To date it has been accepted that the Vitamin D levels of preterm infants are dependent on the status of the Vitamin D status of the mother in pregnancy. However Argawal et al have recently reported the Vitamin D levels in preterm infants and have found 80% of preterm infants to have decreased levels (Agarwal, *et al* 2012). Giapros et al measured serum serum 25-hydroxyvitamin D (25(OH)D) levels in late preterm infants (32-36 weeks) over the first year of life in formula fed infants. Vitamin D levels were relatively low in all infants at 2 and 6 weeks and at six months remaining stable thereafter (n=128) (Giapros, *et al* 2012).

In adult patients Vitamin D deficiency is being linked to the development of cardiovascular disease. Wang et al studied measured Vitamin D levels in adult patients (n=1739). Patients were followed up over a five year period. Those with a vitamin D level of <39nmol/mL had a multivariable adjusted hazard ratio of 1.62 for cardiovascular events compared to those with a level of > 39nmol/mL (Wang, *et al* 2008). Ginde et al prospectively measured Vitamin D levels in older adults enrolled

in the Third National Health and Nutrition Examination Survey (NHANES III). There was a strong association between cardiovascular mortality and Vitamin D level <25nmol/L (adjusted hazard ratio 2.36) (Ginde *et al*, 2009).

Subclinical Vitamin D deficiency in infancy and following preterm births are associated with severe acute respiratory tract infection requiring hospitalisation and intensive care admission (McNally, *et al* 2009). Belderbos *et al* measured Vitamin D levels in cord blood correlating low Vitamin D levels in neonates with increased risk of bronchiolitis in the first year of life (Belderbos, *et al* 2011). Tomar *et al* looked at severe left ventricular dysfunction in babies and found that 16% had severe hypocalcaemia, with the primary cause related to low Vitamin D levels. Myocardial function improved in response to Vitamin D therapy (Tomar, *et al* 2010).

5.2 Hypothesis

Preterm infants with low Vitamin D levels may have altered myocardial function

5.3 Aims

1) To correlate Vitamin D levels with TDI velocities in preterm infants and compare to healthy term controls.

5.4. Results.

A subset of twenty infants was included in the analysis. Twenty echocardiograms were carried out on 10 preterm infants and 10 healthy term controls on day one of life. Mean (SD) gestational age was 28 (1.7) weeks and birthweight 1.29 (0.3)kg for the preterm group. Mean (SD) gestational age was 39 (1.3) weeks and birthweight 3.54 (0.9) kg for the control group.

All preterm infants in our cohort were Vitamin D deficient i.e.(Vitamin D levels < 50nmol/L). Mean (SD) Vitamin D levels in preterm infants correlated with maternal Vitamin D levels (29.2nmol/L (17.4) versus 28.79nmol/L (14.81) Five of the preterm infants had levels <30nmol/L and 2 infants had levels < 15nmol/L.

Right and Left ventricular systolic velocities were significantly lower in the preterm infants compared to controls (5.0 cm/s versus 6.8cm/s; $p=0.001$) and (3.8cm/s versus 4.5 cm/s; $p=0.015$). Early diastolic velocities at both the RV and LV were also significantly lower in the preterm group (4.7cm/s versus 8.1 cm/s; $p=0.014$) and (4.03cm/s versus 6.0 cm/s; $p=0.015$) (Table 5.1).

There was no significant increase in Right ventricular systolic (5.1 cm vs 4.8cm/sec) or diastolic myocardial velocity measures (5.2cm/sec vs 5.1 cm/sec) or left myocardial velocity systolic (3.7cm/sec vs 3.9cm/sec) or diastolic (4.1cm/sec vs 4.0cm/sec) measures between those with severe Vitamin D deficiency (< 30 nmol/L) and those with low normal levels of Vitamin D.

		Term		
		Controls	Preterm Infants	p value
		(n=10)	(n=10)	
RV	S'	6.8(0.8)	5.0(0.5)	0.001
	E'	8.2(2.3)	4.8(1.8)	0.014
	A'	8.8(2.0)	7.9(2.0)	0.432
IVS	S'	3.4(0.5)	3.5(1.0)	0.740
	E'	4.0(0.5)	3.6(0.8)	0.0361
	A'	4.3(0.8)	4.2(0.8)	0.912
LV	S'	4.5(0.7)	3.8(0.4)	0.015
	E'	6.0(1.7)	4.0(0.9)	0.015
	A'	5.6(1.2)	4.8(0.9)	0.187

Table 5.1 Tissue Doppler Velocities in Preterm infants with Vitamin D deficiency

compared to Healthy Term Controls. Measures are in cm/s. Values are expressed as mean (SD). RV – right ventricle, LV – left ventricle, IVS – Intraventricular septum.

S' – early systolic velocity, E' – early diastolic velocity, A' Late diastolic velocity.

Mean values were compared using paired Students t test. Significant values are in bold.

5.5 Discussion.

Preterm infants with Vitamin D deficiency have significantly lower RV and LV systolic and diastolic velocities than healthy term controls. All of the preterm infants were Vitamin D deficient (10nmol/L – 48.4nmol/L).

Vitamin D deficiency is associated with increased cardiovascular risk and myocardial disease in the adult population, the underlying mechanisms of which have yet to be defined (Lee, *et al* 2008). Vitamin D receptors have a broad tissue distribution that includes cardiomyocytes (Holick 2006). Gotsman et al found levels of Vitamin D in adults with heart failure to be significantly lower than adult controls (36.9nmol/L versus 40.7nmol/L, $p < 0.0001$) (n=3009). Patients who had Vitamin D supplementation had reduced mortality compared to those who did not (Gotsman, 2012). In children cardiac failure has been linked to hypocalcaemia, with myocardial function improving in response to Vitamin D therapy (Tomar, *et al* 2010).

The preterm infants in our study had low levels of Vitamin D and have low systolic and diastolic myocardial velocities compared to controls, recognising that the study was not powered to allow for confounders such as gestational age on multivariate analysis. Low Vitamin D levels in preterm infants may therefore cause functional cardiac impairment in preterm infants. In the future it would be valuable to have Vitamin D levels for the term infants, and also to serially echo the infants with low Vitamin D levels to determine if there is an improvement in myocardial function when Vitamin D levels are normal.

In conclusion in this prospective pilot study we have measured vitamin D levels in preterm infants. All infants were Vitamin D deficient and Tissue Doppler systolic and diastolic velocities were significantly lower in preterm infants than healthy term controls. It would be valuable to repeat this study in the future with larger numbers and to follow them serially to assess TDI velocities during hospital stay and following supplementation with Vitamin D.

Chapter 6

SPECKLE TRACKING IMAGING IN NORMAL TERM INFANTS

6.1 Introduction

Speckle Tracking Imaging (STI) is a promising new imaging modality that allows offline angle independent calculation of regional myocardial velocities and deformation parameters such as strain and strain rate. Echocardiography is the commonest approach for assessing cardiac function but is limited by operator dependence and has low sensitivity for detecting subtle abnormalities in myocardial contraction (Blessberger and Binder 2010). Global Left Ventricular Ejection Fraction (LVEF) reflects the sum of all regional shortening in the LV, however impairment of regional function may not cause a reduction in global EF unless several segments are involved. Methods which measure regional LV may be more sensitive than global EF to identify systolic dysfunction (Edvardsen, *et al* 2006). Both EF and SF are influenced by the preload and afterload of the myocardium and therefore may be altered in the presence of a PDA or in those with altered volume status and vascular resistance.

STI uses speckles which are natural acoustic markers which occur as small bright elements in conventional grayscale ultrasound images. These speckles are equally distributed around the myocardium and can be identified and followed through consecutive frames in the cardiac cycle. Post processing software allows a ROI to be traced, and allows calculation of myocardial strain and strain rate in the longitudinal plane from the Apical 4 chamber view and in the radial and

circumferential planes in the short axis view at the papillary muscle level (Leitman, *et al* 2004).

Normal values have been established in the adult and paediatric populations. However to date no normative data exists for healthy term infants (Helle-Valle, *et al* 2005) (Amundsen, *et al* 2006) (Marcus, *et al* 2011).

6.2 Hypothesis

In this study we hypothesised that STI is feasible in term control infants

6.3 Aims

1 - To quantify measurement of left ventricular strain in term infants

6.4 Results

Thirty normal term infants with an uncomplicated pregnancy, delivery and postnatal course had echocardiography carried out on Day 2-4 of life. The mean gestational age (SD) was 41 (0.7) weeks and birth weight 3.87(0.27) kg. There was equal sex distribution. Ten children were delivered by elective caesarean, the remainder by spontaneous vaginal delivery.

Twenty infants had images that were suitable for analysis of Left Ventricular strain. The ten excluded had incomplete echocardiographic data, or sub optimal imaging quality. Tracking was feasible in 90% of all segments in the apical 4 chamber view. Mean (SD) longitudinal peak systolic strain is represented as a percentage. At the Left ventricular basal septum was -17.29 (5.3)% , at the LV mid septum -16.78 (5.2)% at the LV apical septum was -15.26 (7.9)%, at the LV lateral septal wall -15.4(4.5)% at the LV mid lateral wall 16.1 (5.8)% and at the LV basal septum 16.8 (5.91)%. We attempted to measure LV radial circumferential and radial strain however no infant had suitable echo images for analysis.

6.5 Discussion

In this observational study we aimed to explore the feasibility of STI in term infants. Of thirty infants recruited, twenty had images suitable for analysis and we report normative values for Left ventricular strain in term infants.

Marcus et al in the largest retrospective paediatric cohort to date measured longitudinal, radial and circumferential strain (n=139) throughout childhood. In their cohort only 24 infants were less than one year old and the youngest child included was three months old. They found low systolic peak strain in infants compared to older children (Marcus, *et al* 2011). There is currently no consensus on myocardial contractility in infants, who are thought to have a higher basal contractile state and where SF is thought to underestimate ventricular function in the newborn (Rowland and Gutgesell 1995).

In clinical practice STI is used in the adult population to assess systolic function in patients with heart failure. Edvardsen et al have demonstrated alterations in systolic function and myocardial deformation in adults with heart failure who have normal ejection fraction (Edvardsen, *et al* 2006). Strain rate imaging has also been used to quantify LV radial dyssynchrony in adults with LV failure and to predict immediate and long term response to cardiac resynchronisation therapy (Suffoletto, *et al* 2006).

In the paediatric population STI has been used to evaluate strain rate in septic children in the Paediatric Intensive Care Unit (PICU). Strain rate was significantly reduced in septic patients compared to healthy controls, there was no significant difference in SF/EF between the two groups (Basu, *et al* 2012).

STI has none of the limitations described for TDI. It is angle independent and if 2D echocardiographic images are recorded and stored with good resolution it can be used retrospectively with frame rates of 50-70 Hz thought to be optimum as this increases spatial resolution (Teske, *et al* 2007).

However in our study we have found a number of limitations, which may be related to the study population. To use STI with minimum frame rates of between 50 – 70 Hz may result in under sampling in patients with tachycardia (Sitia, *et al* 2010). In our cohort of term infants we found it difficult to get consistently good images which may be why no normative data exists for term infants. It was technically difficult to complete a full echocardiogram with the infant at rest before they became upset, a problem not encountered when imaging older children or adults. At rest a term infants heart rate is 140 - 160 beats per minute, which can increase up to 180 beat per minute when they are crying. This may have lead to under sampling due to the low frame rates which are required for STI analysis. On review of the echocardiographic images a number were also unsuitable due to cardiac translation in the chest wall. Another problem we experienced may be software related. For the purpose of our study a GE Vivid I was available for use. Marcus *et al* in their study used the GE Vivid 7 (GE Health care) machine for echocardiography which may be a superior tool. Koopman *et al* compared the GE Vivid 7 against the Philips iE33 (Philips Medical Systems) and their respective post processing systems, EchoPAC and QLAB to assess longitudinal strain in children with congenital heart disease. They found good correlations between the two systems for longitudinal strain.(Koopman, *et al* 2010). In the future it would be useful to measure both LV radial and circumferential strain.

In conclusion, STI is a promising imaging modality with normative data being established for both the adult and the paediatric population. We experienced a number of limitations with regards to infant heart rate and the frame rates required for optimal image acquisition which may limit its used in term infants at present. Improvement in technology in the future may overcome these limitations.

CHAPTER 7

CARDIOVASCULAR FUNCTION IN INFANTS WITH NEONATAL ENCEPHALOPATHY

7.1 Introduction

A hypoxic -ischaemic insult occurring around the time of birth may result in an encephalopathic state characterised by the need for resuscitation at birth, neurological depression, seizures and electroencephalographic abnormalities (Azzopardi, *et al* 2008). These neurological manifestations are likely to be only part of the underlying pathology, as following a hypoxic insult maintenance of adequate tissue oxygenation is dependent on the oxygen content of blood, blood flow to the organ and the tissues ability to extract and use oxygen (Kluckow 2011).

Cardiovascular dysfunction has been increasingly recognised in infants with encephalopathy as a result of hypoxic ischaemic damage to the myocardium (Evans 2006) (Barberi, *et al* 1999) (Fatemi, *et al* 2009) and has been reported in up to 62-72% of cases (Shah, *et al* 2004). During a hypoxic ischaemic event, the myocardium functions to increase blood flow however if there is decreased myocardial perfusion ischaemic changes may occur in the papillary muscle and subendocardial tissue (Kanik, *et al* 2009). As a result neonates with asphyxia have a low cardiac output with decreased myocardial contractility, systemic hypotension and pulmonary hypertension (Evans, *et al* 1998).

Echocardiographic evidence of ischaemic myocardial injury is not well documented in infants with Neonatal Encephalopathy. Tissue Doppler imaging (TDI) is evolving as a technique to allow measurement of myocardial contraction and relaxation velocity from the myocardium and could allow recognition of myocardial dysfunction related to hypoxic injury (Negrine, *et al* 2012) (Mori, *et al* 2004). However to date studies have looked at TDI measurements in the first day of life and not serially over the first week.

Troponin T is an established marker of myocardial injury (Alpert, *et al* 2000). Newborns with severe neonatal asphyxia have significantly higher levels of Troponin T concentrations than healthy neonates on day one of life (Gunes, *et al* 2005) (Szymankiewicz, *et al* 2005). Elevated Troponin T levels persist for days following an asphyxia event, decreasing over the first week of life (Gunes, *et al* 2005).

7.2 Hypothesis

Infants with evidence of Neonatal Encephalopathy may have altered myocardial function in the first week of life compared to healthy term controls.

7.3 Aims

- 1 - To assess myocardial function using measures of contractility (SF & EF) and Tissue Doppler Imaging
- 2 - To correlate findings with serum Troponin T levels
- 3 – To determine if TDI measures could predict outcomes such as:
 - 1) Survival to discharge
 - 2) Clinical Neurological Status
 - a) Neurological Status
 - b) Sarnat Grade of Neonatal Encephalopathy
 - c) Seizures
 - d) MRI Brain

7.4 Results

A total of 30 echocardiograms were performed during the first week of life on 19 infants with evidence of Neonatal Encephalopathy (NE). Seventeen infants had paired serum Troponin T measurement at the time of echocardiography. Twenty healthy term control infants were used as controls for echocardiographic measures on day 1 of life but did not undergo venous sampling for Troponin T measurement.

Infants with Neonatal Encephalopathy

There were 14 males and 5 females in the NE cohort. Mean (SD) gestational age was 40.37 (1.5) weeks and birth weight 3.6 (0.87)kg. Nine infants were delivered by caesarean section. Mean Apgar score was 2 (2) at one minute and 4 (3) at five minutes. Sixteen patients were treated with therapeutic hypothermia and 18 patients survived to discharge. One infant died, three infants had NE Grade 0/I and sixteen had Grade II/III NE. Although only two infants had abnormal MRI brain scans. Eleven infants had abnormal Cranial ultrasound scans on Day one of life. The majority of these ultrasounds had poor grey white matter differentiation (n=2) with areas of increased ecogenicity (n=9).

Control Infants

There were 11 girls and 8 males in the healthy term controls. Mean (SD) gestational age 39.7 (1.9) weeks, birth weight 3.69 (0.59)kg. Seven infants were delivered by caesarean section. Mean Apgar score was 9(1) at one minute and 9(1)

at five minutes. None of the infants were admitted to the NICU and all were discharged home with their mothers.

Myocardial Function

Myocardial systolic and diastolic velocities at the right and left ventricle increased over the first week of life in infants with NE (Table 6.1). Right ventricular early systolic and late diastolic velocities were significantly higher in term infants on day one compared to those with NE ($p < 0.001$ & $p = 0.04$ respectively). Term infants had significantly higher TDI velocities across all measures compared to those who with mild or moderate-severe NE. There was a non significant trend towards lower systolic and diastolic velocities in infants with moderate-severe NE compared to those who were mildly affected. (Table 6.2)

Infants with Neonatal Encephalopathy who developed seizures (on either EEG or clinically) had significantly lower LV systolic velocities on day 3 (4.4cm/sec vs 5.4cm/sec, $p = 0.046$) (Figure 5.1). There was no significant difference in the TDI systolic or diastolic velocities of either the RV or LV in infants who had an abnormal MRI brain or cranial ultrasound scan. Similarly there was no significant difference in the TDI velocities in the one infant who died compared to those who survived.

There was no significant difference between systolic measures of shortening or ejection fraction between healthy controls and those with evidence of NE during the first week of life. Similarly there was no significant increase in either the mitral or tricuspid E: A ratios over the first week of life. (Table 6.3) There was no significant increase in heart rate, systolic or diastolic blood pressure measurements over the first week of life in infants with NE (Table 6.4).

Troponin T levels on day 1 were significantly higher than on day 7 in infants with NE (0.53ng/ml vs 0.34ng/ml, $p = 0.026$). Troponin T levels were significantly higher in the two infants with abnormal MRI Brain scans compared to 17 with normal MRIs (1.12 versus 0.33 ng/mL, $p=0.049$). There was no significant association between Troponin level on day 1 and abnormal cranial ultrasound on Day 1 ($p = 0.164$). Troponin T levels on day 1 were significantly higher in infants who were cooled compared to those who were not (0.47ng/mL versus 0.10 ng/mL, $p=0.005$). Troponin T levels on day 1 were significantly higher in the infant who died compared to those who survived to discharge (1.91 ng/mL versus 0.34ng/mL, $p=0.001$). There was no significant difference in the RV/LV systolic and diastolic TDI velocities in infants with NE who had an elevated Troponin T on Day 1 or Day 3 compared to those infants with a normal level.

	Control	NE			p value	
		Day 1	Day 3	Day 7		
RV	S'	6.6(0.8)	4.0(1.1)	4.8(1.0)	5.4(1.2)	<0.001
	E'	7.5(2.)	4.0(1.3)	4.8(1.4)	4.1(2.2)	0.04
	A'	8.3(2.3)	4.6(1.5)	6.1(1.4)	6.8(1.9)	0.01
IV	S'	3.7(0.6)	2.0(0.6)	2.4(0.7)	2.5(0.9)	0.01
	E'	3.8(0.8)	2.7(0.8)	2.6(0.9)	2.3(1.2)	0.224
	A'	4.3(0.7)	2.51(1.0)	3.4(0.6)	2.7(1.6)	0.268
LV	S'	4.9(0.7)	3.4(0.9)	4.0(0.8)	4.1(0.3)	0.156
	E'	6.1(1.8)	2.4(1.4)	3.9(0.8)	3.1(1.4)	0.126
	A'	5.7(1.2)	3.6(0.9)	4.6(0.8)	4.2(0.7)	0.19

Table 7.1. Tissue Doppler Measurements in Healthy Controls compared to those with Neonatal Encephalopathy(NE). Measurements are in cm/s. Values are mean(SD.) RV – Right Ventricle, LV- Left ventricle, IVS – Intraventricular septum. S' – Early systolic velocity, E' Early diastolic velocity, A' Late diastolic velocity. Independent Students t test was used for analysis between term infants and infants with NE on Day 1. Significant results are in bold.

	Control	Sarnat Grade of NE		p value	
		Mild (0/I)	Moderate-Severe (II/III)		
RV	S'	6.6(0.8)	6.4(1.0)	5.6(1.1)	0.26
	E'	7.5(2.0)	6.3(1.7)	6.6(1.2)	0.76
	A'	8.3(2.3)	7.3(0.9)	6.5(1.5)	0.42
IV	S'	3.7(0.6)	3.5(1.0)	3.0(0.5)	0.20
	E'	3.8(0.9)	3.8(0.7)	3.6(0.8)	0.6
	A'	4.3(0.7)	4.1(0.3)	4.0(1.1)	0.94
LV	S'	4.9(0.75)	4.5(1.0)	4.4(1.0)	0.84
	E'	6.1(1.8)	4.8(0.8)	5.1(1.5)	0.75
	A'	5.7(1.2)	4.9(0.6)	5.1(0.9)	0.77

Table 7.2. Tissue Doppler Measurements in Healthy Controls compared with NE divided by Sarnat Score on Day 1. Measurements are in cm/sec. Values are mean(SD.) RV – Right Ventricle, LV- Left ventricle, IVS – Intraventricular septum. S' – Early systolic velocity, E' Early diastolic velocity, A' Late diastolic velocity. Independent Students t test was used for analysis.

	Control	Neonatal Encephalopathy			p value	
		Day1	Day 3	Day 7		
Shortening	32.0(6.7)	31.9(7.7)	33.6(7.7)	34.2(7.6)	0.59	
Fraction (%)						
Ejection	64.0(10.0)	62(11.1)	65.6(11.0)	66.2(9.0)	0.442	
Fraction (%)						
MV	E	0.87(10.0)	45.7(7.7)	49.3(10.6)	48.6(4.1)	0.11
	A	48.1(12.0)	44.3(11.8)	51.5(9.0)	51.5(11.3)	0.18
	E:A	1.1(0.3)	1.1(0.3)	1.14(0.4)	1.0(0.1)	0.89
TV	E	41.6(9.0)	40.7(8.6)	36.6(7.7)	36.3(2.6)	0.36
	A	54.1(10.0)	48.2(6.9)	47.6(9)	48.1(13.6)	0.12
	E:A	2.2(0.9)	0.9(0.3)	0.8(0.1)	0.8(0.5)	0.17

Table 7.3: Standard Doppler Measures of Systolic and Diastolic Function MV –

Mitral valve, TV-Tricuspid valve. E – Early diastole, A – Late diastole, E:A Early to late diastole ratio. Values are expressed as mean (SD). The paired Students t test used for analysis to compare controls and infants with NE on infants on Day 1.

	Day 1	Day 3	Day 7	p value
Heart Rate	105(16)	113(26)	123(36)	0.234
Systolic BP	62.4(12.4)	60.1(7)	66(6)	0.132
Diastolic BP	42.2(8.5)	38.7(8.5)	57(8.0)	0.960
Pulse Pressure	48.5(6)	47.0(8.5)	51.0(6.0)	0.709
Sa O₂	98(2)	98(2)	98(2)	0.871

Table 7.4: Vital Signs of Infants with Neonatal Encephalopathy at time of Echocardiography. Values are mean (SD.) SaO₂ - Oxygen saturations. Paired Students t test was used for the analysis. Statistical significance p<0.05 compares significance between clinical characteristics on Day 1 and Day 3.

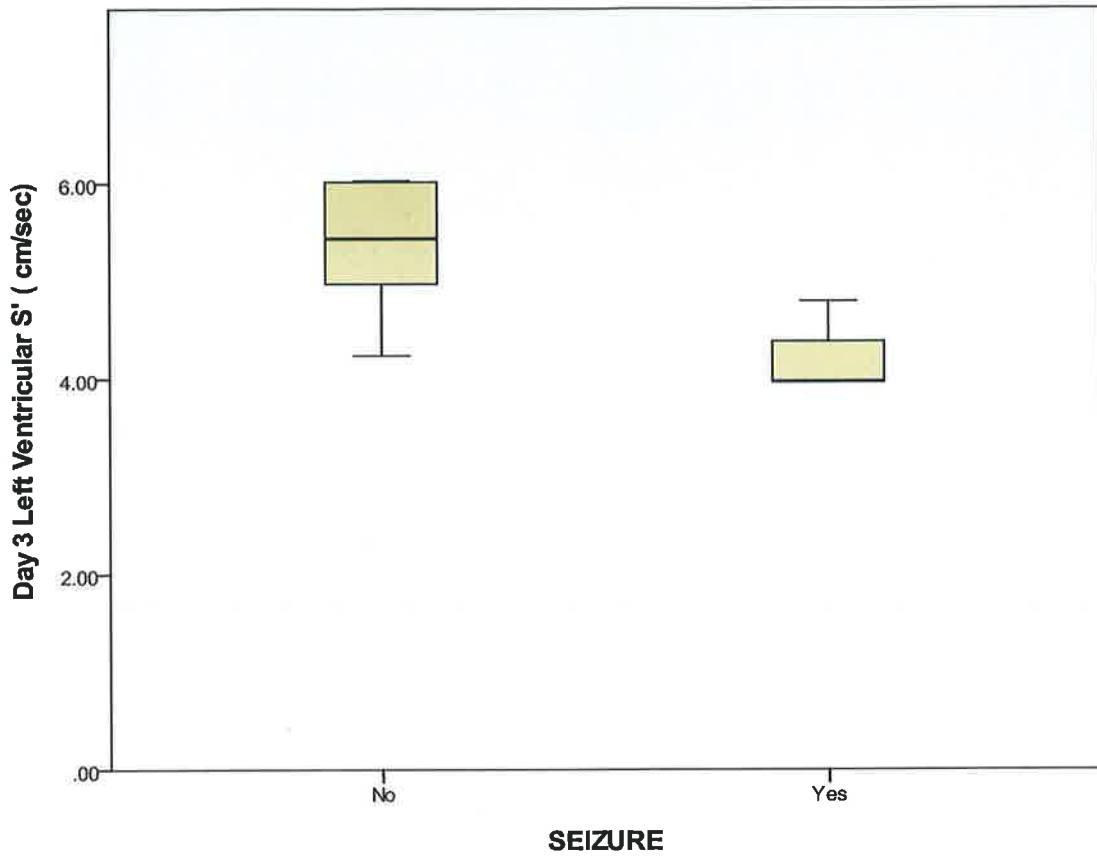


Figure 7.1 Day 1 Right ventricular early diastolic velocity in Infants with Neonatal Encephalopathy. Infants who developed seizures had significantly lower left ventricular systolic measurements on day 3 of life $p = 0.041$. An independent Students t test was used for analysis.

7.5 Discussion

On day one right ventricular systolic and diastolic velocities in those with evidence of NE were significantly lower in both the RV and LV compared to term controls. There was no difference between EF/SF between the two groups on day one. Although the TDI measurements did not reach a statistically significant difference the trend suggests that TDI measurements in infants with NE are lower on Day one of life compared to day 7 and to healthy term controls. When comparing term infants to those with mild NE and to those with moderate to severe NE there TDI velocities were significantly lower in infants with NE. There was no significant difference in TDI systolic and diastolic velocities in infants with mild NE compared to those with moderate – severe NE. However the trend did show that those with moderate-severe NE have lower TDI velocities across all measurements in the first week of life. By day 7 TDI velocities across all systolic and diastolic velocities had increased suggesting that on day 1 infants with NE have suppressed myocardial function compared to healthy term controls. By day 7 the LV systolic and diastolic TDI velocities were still lower than those of healthy term controls on day 1, but due to the small number in our cohort this may be why this did not reach statistical significance. This suggests that there may be residual evidence of myocardial dysfunction on Day 7 in infants with NE.

Tissue Doppler imaging (TDI) is evolving as a technique which allows measurement of myocardial contraction and relaxation velocity from the myocardium and could allow recognition of myocardial dysfunction related to hypoxic injury (Negrine, *et al* 2012) (Mori, *et al* 2004). TDI filters the low amplitude

Doppler signal from blood to display the high amplitude signals from the myocardium to produce systolic and diastolic velocity waveforms (Mori, *et al* 2004).

The majority of studies to date have focused on the difference between TDI measurements in infants with NE on day one of life, drawing comparisons with healthy term controls. Wei et al used tissue Doppler imaging to serially evaluate left ventricular systolic function in newborns with mild to severe asphyxia. The peak systolic velocity of the anterior mitral valve leaflet was measured with TDI. The LVEF and SF of the severe asphyxia group were significantly lower than in the mild and control groups. The systolic wave of the asphyxia group was significantly lower than that of control group ($p < 0.001$). In the severe asphyxia group, the systolic wave at 24 h was significantly lower than that at 48 or 72 h ($P < 0.001$). The findings of this study concur with our study findings and suggest that systolic measurement by TDI is a more sensitive indicator of left ventricular systolic function than left ventricular EF/ SF measured by M-mode echocardiography (Wei, *et al* 2009).

In our study LV systolic velocities on Day 3 were lower in infants who developed seizures, demonstrating a link between low systolic function and neonatal morbidity. There was no significant difference between TDI measurements in those infants who had abnormal neuroimaging (either cranial ultrasound or MRI brain) or in those who died.

Troponin T levels were significantly increased on Day 1 in infants with Neonatal Encephalopathy compared to levels on day 7 suggesting evidence of myocardial injury. This is supported by the decreased TDI systolic and diastolic

velocities in the NE infants compared to the term controls. This association, along with the evidence that there was no difference between EF/SF measures between the groups suggests that TDI and Troponin T in combination may be a superior measure to assess global myocardial dysfunction in these infants.

Many studies have now confirmed that Troponin T levels are increased in asphyxiated infants, (Wei, *et al* 2009) (Szymankiewicz, *et al* 2005) (Matter, *et al*) with elevated levels thought to predict poor prognosis. In our study the infant who died had a significantly higher Troponin T level than those who survived. Matter *et al* demonstrated that Troponin T levels were significantly increased in NE survivors versus non survivors (0.03 μ g/l vs 0.16 μ g/l). Similarly those infants who required inotropic support had higher Troponin T levels (Szymankiewicz, *et al* 2005). This current study supports the evidence that elevated Troponin T levels in infants with NE is evidence of subclinical myocardial injury which may be best evaluated by TDI. Matter *et al* correlated TDI measurements with Troponin T in asphyxiated infants versus term controls. Mitral and Tricuspid systolic velocities were significantly lower in asphyxiated neonates than in term controls (5.06cm/s vs 6.89cm/s) and (5.78cm/s vs 6.69cm/s) respectively. Troponin T levels were also significantly higher in asphyxiated neonates than term controls.

Both systolic and diastolic velocities were significantly decreased on day 1 of life in infants with Neonatal Encephalopathy with no significant impairment in measures of SF/EF. Studies to date have examined TDI measures in infants with NE on day 1 and controls and this is the first to serially measure myocardial function in infants with evidence of neonatal encephalopathy over the first week of life. Our

study demonstrated that Troponin T levels are significantly elevated in infants with NE who do not survive or who have abnormal neuroimaging. Tissue Doppler velocities were decreased in infants with NE on day one, suggesting myocardial dysfunction in these infants. Following up patients with Neonatal Encephalopathy into childhood is required to determine if there are any long term cardiovascular sequelae from a hypoxic event at birth. In addition two year developmental outcomes and MRI results could be correlated with early cardiovascular changes.

CHAPTER 8

POINT OF CARE TESTING FOR CARDIAC BIOMARKERS IN THE NEONATAL INTENSIVE CARE UNIT.

8.1 Introduction

Cardiac biomarkers such as Troponin T are accurate, sensitive and specific determinants of myocardial injury and are now considered gold standard in the diagnosis of acute myocardial infarction (Alpert, *et al* 2000) (Goldmann, *et al* 2001). POCT for Troponin T has evolved during the last decade with rapid assays providing results in the Emergency Department and out in the field as used by paramedics to allow prompt diagnosis of myocardial ischaemia and subsequent treatment (Goldmann, *et al* 2001). NTproBNP is synthesised in the ventricles and produced in increased amounts by the heart in response to congestive cardiac failure. Testing has been used to not only confirm diagnosis of heart failure but to monitor response to treatment (McDonnell, *et al* 2009).

In preterm infants Troponin T and NT pro BNP have been established as markers of the presence of a PDA (El-Khuffash, *et al* 2008b). Levels of both decrease in response to PDA treatment and as such may act as surrogates in the management of a PDA in the absence of echocardiography (Choi, *et al* 2005). El khuffash *et al* have developed a PDA scoring system that utilises both echocardiography, Troponin T and NT pro BNP to predict outcomes such as IVH and death in preterm infants (El-Khuffash, *et al* 2011).

Troponin T is also useful in infants with Neonatal Encephalopathy where it has been shown to be a predictor of myocardial injury (Gunes, *et al* 2005). Normal

ranges for both NT Pro BNP and Troponin T have been established in the preterm infant (Nir, *et al* 2009)(El-Khuffash, 2008).

Many Neonatal Intensive care units do not have access to an out of hours echocardiography service to confirm either the presence of a PDA in preterm infants or to assess myocardial function in infants with evidence of Neonatal Encephalopathy. Point of care analysis of Troponin T and NT pro BNP could alert the Neonatologist to early signs of a PDA or the presence of myocardial dysfunction and help to guide treatment.

A major advantage of POCT is the decreased turnaround time which optimises the decision making process (Dirks 1996)(Despotis, *et al* 1997). The majority of POCT can be done on small volumes of whole blood making them particularly useful in neonates, where repeated sampling of blood can lead to iatrogenic anaemia (Tan, *et al* 2006). Due to the unacceptably high number of unsuitable Troponin T results obtained from our laboratory due to haemolysis and clotting and as Troponin T is a time sensitive measure we decided to validate POCT for Troponin T. In our study 150 micro litres of blood are required for analysis using the Cobas h232 analyser compared to 500 microlitres required for the Roche 8000 analyser.

8.2 Hypothesis

To determine if point of care testing is a reliable and accurate method to assess Troponin T and NT pro BNP in the Neonatal Intensive Care Unit.

8.3Aims

1 - To validate the use of the Cobas h232 bedside analyser to test serum Troponin T and NT pro BNP in the Neonatal Intensive care unit.

8.4 Results

Reproducibility of the Cobas h232 was tested. Venous samples were obtained from one adult subject for measurement of Troponin T level. The same sample from this subject was tested over a 24 hour period with samples stored at 4°C and at room temperature. The Troponin levels were < 50 ng/L and remained stable over a 24 hour period at 4°C (Figure 8.1) and at room temperature. The same test was performed for NT pro BNP levels at 4°C and at room temperature (Figure 8.2). The NT pro BNP levels also remained constant over the 24 hour period at both temperatures.

8.4.1 Validation

To validate the Cobas h 232 analyser for use in the NICU, the Bland Altman method was used to assess agreement between the results of Troponin T and NT pro BNP measurements from the cobas h 232 analyser and the Roche 8000 analyser. A total of 40 samples (20 NT pro BNP and 20 Troponin T) were recorded. Samples were taken from term infants with evidence of Neonatal Encephalopathy (n=8) with the remainder taken from healthy adult controls (n=12) All samples tested provided a result for both Troponin T and NT pro BNP. The Bland Altman test was used to assess agreement. Figure 8.3 highlights the Cobas h232 NT pro BNP measurement and the Cobas 8000 measurement. The majority of the results are between the 95% limits of agreement. The bias is 40.65pg/mL. Troponin T measurements between the Coabs h232 and the Cobas 8000 Analysers are highlighted in figure 8.4. The majority of measurements fall between the 95% limits of agreement. The bias was 27.59ng/mL. Only 4 data points are shown as 10

measurements had an average of 27 with a difference of 46 and 8 measurements had an average of 26.5 with a difference of 47 so plot in the same positions.

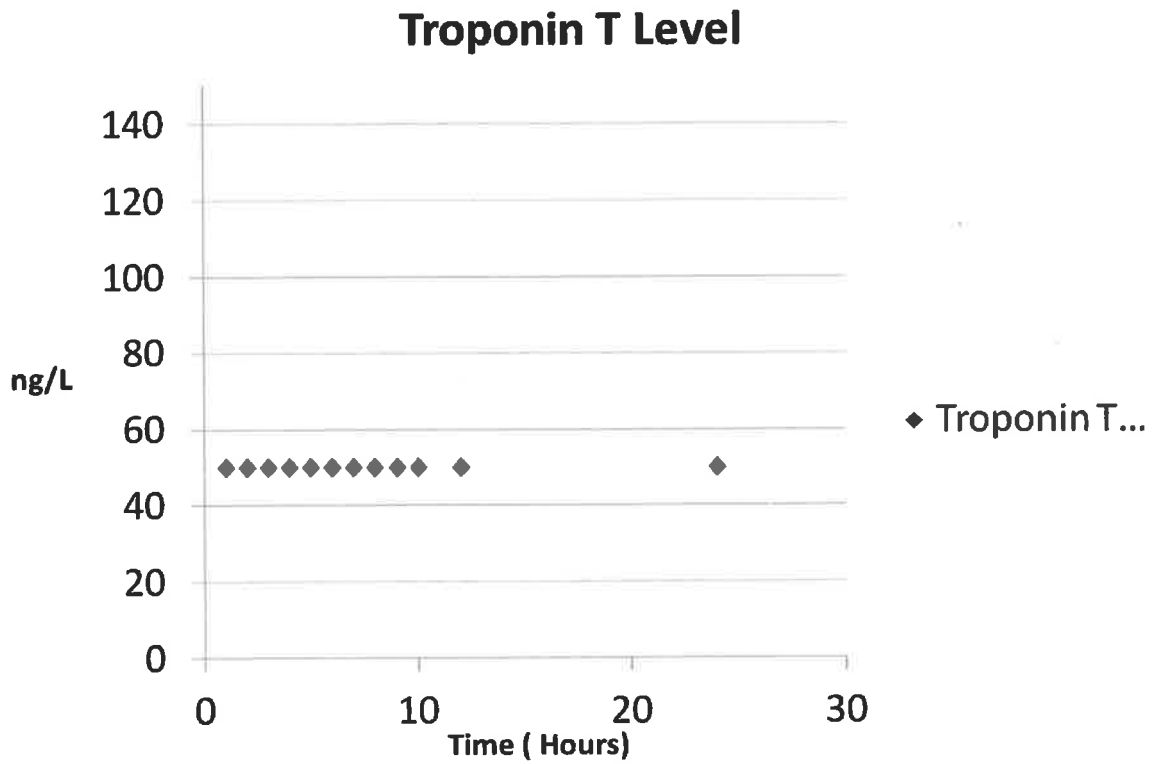


Figure 8.1. Reproducibility of Troponin T levels stored at 4 degrees Celsius over a 24 hour period. This scatter plot shows that Troponin T levels remained stable over a 24 hour period.

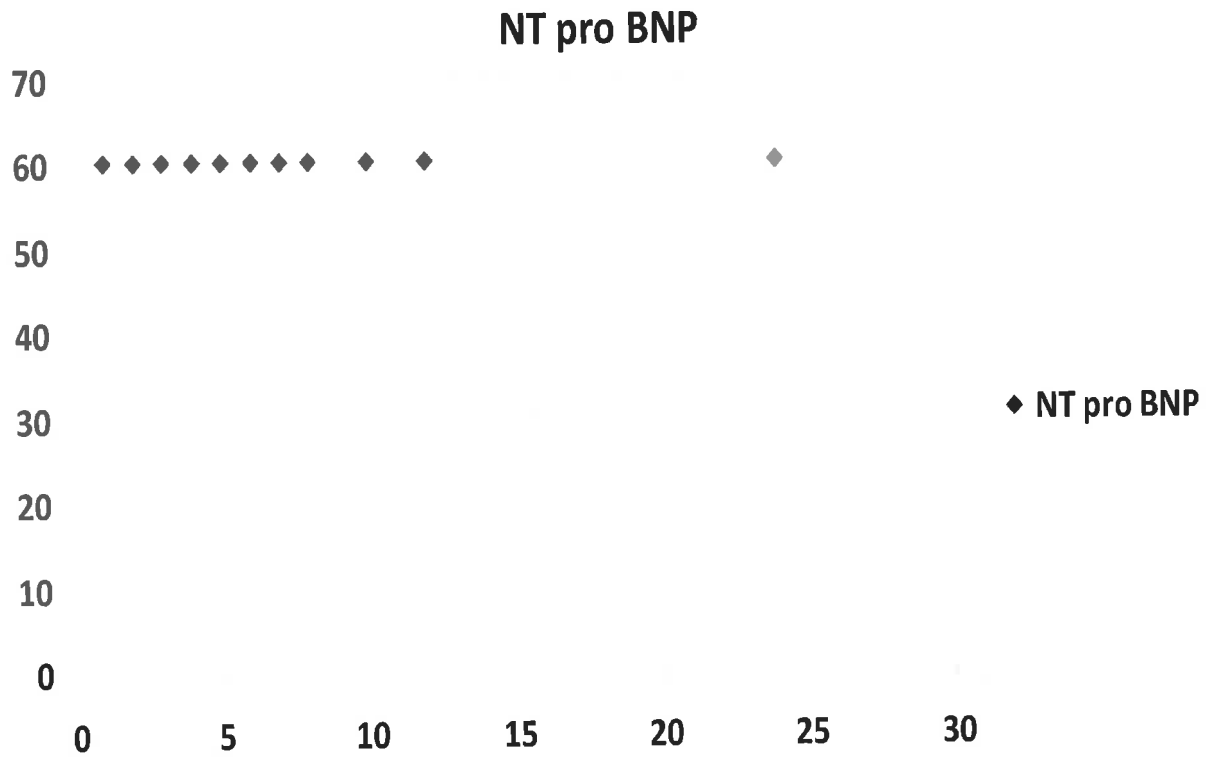


Figure 8.2. Demonstrating reproducibility of NT pro BNP levels over a 24 hour period. This scatter plot shows that NT pro BNP levels remained constant over a 24 hour period in a sample stored at room temperature.

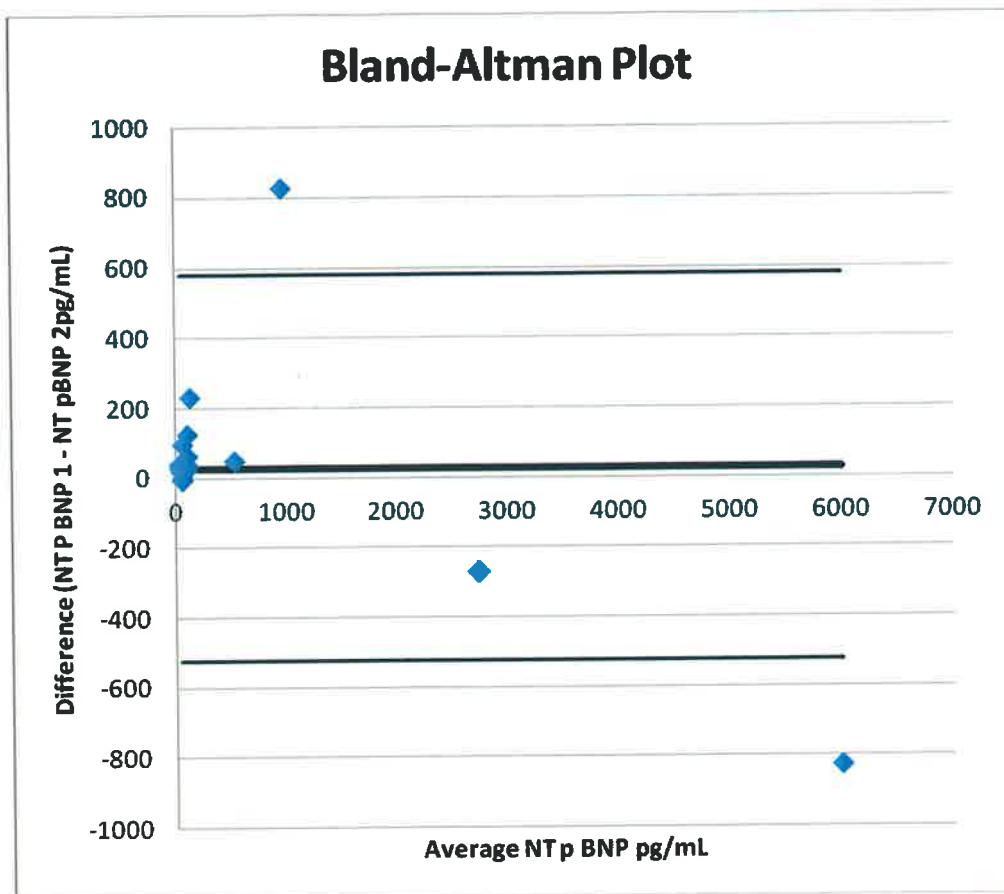


Figure 8.3 Comparison between NTpBNP 1 & NTpBNP2. NTpBNP1 – NT pro BNP result from the Cobas h232 analyser (pg/mL). NTpBNP2 – NT pro BNP results from the Cobas 8000 Analyser (pg/mL). Bias 40.65pg/mL. The majority of measurements were between the upper and lower limits of agreement.

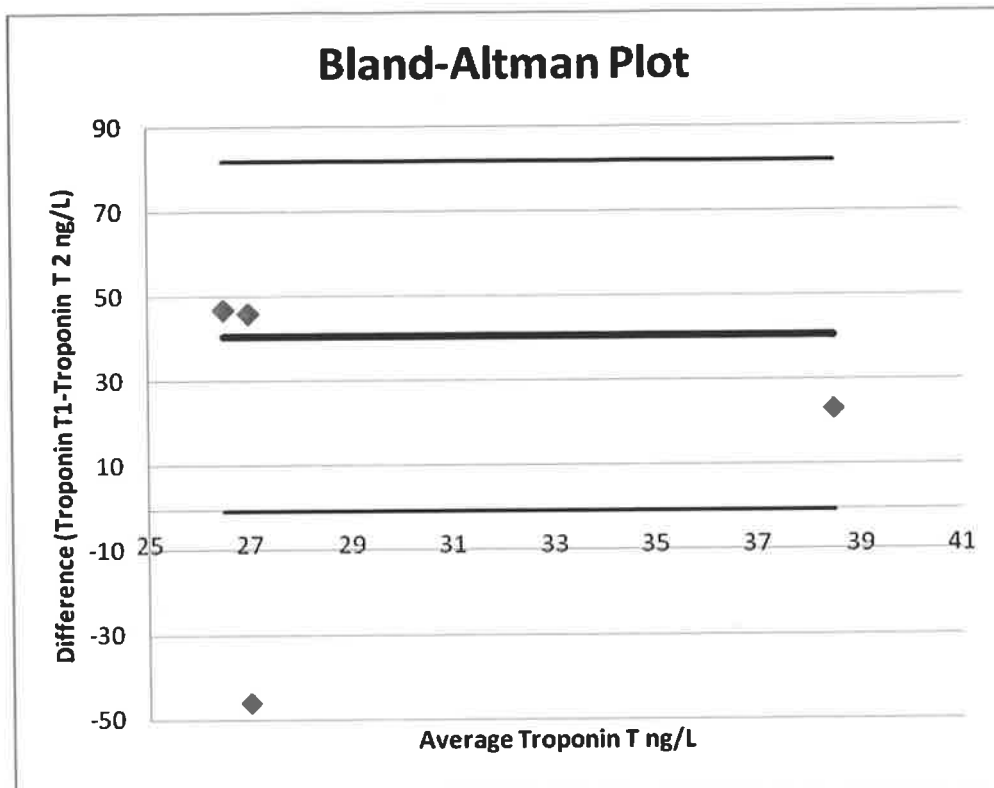


Figure 8.4 Comparison between Troponin T 1 & Troponin T2. Troponin 1 – Troponin T result from the Cobas h232 analyser (ng/mL). Troponin T2 –Troponin T results from the Cobas 8000 Analyser(ng/mL). Bias 27.59ng/mL. The majority of the measurements were between the upper and lower levels of agreement.

8.5 Discussion

There was agreement between the Cobas h232 analyser and the Roche 8000 analyser for both Troponin T and NT pro BNP levels. The Cobas 8000 analyser provides an accurate number for both Troponin T and NT pro BNP measurement with <14 ng/mL normal for Troponin T and < 300pg/mL normal for NT pro BNP. However the Cobas h232 analyser does not provide a numerical value and uses a cut off of < 50ng/mL as normal for Troponin T and <60pg/mL for NT pro BNP. As the majority of samples were taken from normal controls this explains the large bias result for both. All of the Troponin T and NT pro BNP measurements which were normal from the Cobas h232 analyser were also normal from the Cobas8000 analyser

The Cobas analyser is accurate for POCT testing for Troponin T and NT pro BNP, is quicker and requires a smaller blood sample than that of the Cobas 8000. In our laboratory up to 50% of the Troponin T samples that are taken from infants are unsuitable for analysis due to haemolysis or clotting. None of the samples analysed on the Cobas h232 analyser became haemolysed or clotted.

POCT for cardiac biomarkers has been successfully implemented in the clinical setting for diagnosis of acute Myocardial Infarction (MI) and to distinguish between respiratory and cardiac failure in adult patients. Both Troponin T and NT pro BNP are highly utilised specific cardiac biomarkers and their levels are strong indicators of myocardial damage (McDonnell, *et al* 2009).

In the preterm population, POCT has the advantage that it requires small volumes of blood which reduces the risk of iatrogenic anaemia, whilst allowing

timely availability of results which can enhance quick decision making at the cotside (Mor and Waisman 2000).

Point of care testing for BNP in preterm infants has shown that elevated levels are associated with a haemodynamically significant PDA and that monitoring BNP levels is valuable in determining the clinical course of a PDA (Choi, *et al* 2005). Troponin T and NT pro BNP have been linked to the presence of a PDA in preterm infants and their analysis at the cotside along with clinical assessment in the absence of or in adjunct to echocardiography may allow timely treatment of those individuals who require PDA treatment.

NT pro BNP may be a better marker than BNP as it is more stable and has a longer half life. Troponin T also rises in asphyxiated neonates, and in our study we have shown that levels peak on Day one and continue to fall over the first week, conversely during this time TDI measures of systolic and diastolic myocardial velocities continue to rise, suggesting that ischaemic myocardial damage caused by a hypoxic event improves during the first week of life.

Point of care testing is a simple, rapid method for testing Troponin T and NT pro BNP at the cotside. This study confirms the reliability of the Cobas h232 analyser. Future work should include integrating POCT testing of NT pro BNP and Troponin T into practice. A larger study utilising cotside measurement of Troponin T and NT pro BNP at time of echocardiogram in preterm infants with a PDA and to monitor response to treatment would be valuable. If Troponin T and NT pro BNP levels correlate not only with the presence of a duct but also in response to treatment these markers could be introduced as a surrogate to echocardiography in units where this service is not available.

CHAPTER 9

DISCUSSION

9.1 Introduction

Echocardiographic measures of systolic and diastolic function are evolving for both preterm and term infants. Tissue Doppler Imaging (TDI) and Speckle Tracking Imaging (STI) are emerging as measures of regional systolic and diastolic myocardial velocities and multiplane strain and strain rate. TDI is a reproducible and reliable measure of regional systolic and diastolic myocardial velocities. TDI measures overcome the shortfalls of measures of shortening and ejection fraction which are preload and afterload dependent and which have limited use in the neonatal population. STI has a number of limitations in this population which may be related to heart rate and to adequate image acquisition.

9.2 Tissue Doppler Imaging

We have demonstrated normative ranges for left and right ventricular systolic and diastolic myocardial velocities using TDI in preterm infants over the first week of life and also report normal ranges of TDI velocities in healthy term infants. We have shown that myocardial velocities increase in all measures over the first week of life in preterm infants, with significant increases in all measures of RV systolic and diastolic velocities, IVS early diastolic velocities and LV diastolic velocities. The presence of a PDA appears to have no significant effect on TDI measures, making them a preload independent measure and therefore more useful

than SF/EF measures in preterm infants. The major criticism of TDI measures are that they are angle dependent and cannot be used retrospectively. However we have shown them to be reproducible, and repeatable measures at the bedside.

9.3 Impact of Serial Echocardiography in the NICU

In our study TDI measures were recorded over the first week of life for preterm infants. We have shown that at different times TDI systolic and diastolic velocity measurements were significantly lower in infants who developed chronic lung disease, necrotising enterocolitis, retinopathy of prematurity and in those who required PDA ligation. Using TDI as a part of serial echocardiography in preterm infants may highlight those who are at risk of developing morbidities such as Chronic Lung Disease which may allow optimisation of ventilation, fluid balance and used of diuretics.

With the establishment of normative data for TDI measures in the preterm population, it may be useful to use TDI to assess myocardial function in a number of clinical scenarios. Over half of preterm infants who are hypotensive and acidotic have echocardiographic evidence of LV dysfunction using M Mode measures of SF and EF (Gill and Weindling 1993). As we have established TDI is more sensitive at detecting subtle changes in myocardial contractility therefore it is likely that impairment in myocardial contractility is under reported in this population. TDI may allow earlier diagnosis of myocardial dysfunction and therefore therapeutic intervention.

9.4 Speckle Tracking Imaging

Speckle tracking Imaging is being established to assess LV strain and strain rate in the adult population. We have attempted to establish normal data for a small cohort of term infants. The major limitation of STI is the need for high quality images due to the accuracy needed to track the endocardial border. The optimal frame rate for STI is 50 – 70 frames/minute and this could account for difficulty obtaining images in term infants as their resting heart rate is approximately 160 beats per minute. Marcus et al in the largest study to date created normative STI data for healthy children. However the youngest patient included in their study was 3 months old (Marcus, *et al* 2011). STI is now used in adult cardiology in patients to assess improvements in regional wall motion following cardiac resynchronisation therapy and in those with LV failure. Future work is required to establish normal data for LV strain in term infants as newer technologies develop.

9.5 Cardiovascular Function in Neonatal Encephalopathy

Infants with Neonatal Encephalopathy are at increased risk of myocardial dysfunction (Gunes, *et al* 2005) (Matter, *et al* 2010). In chapter 6 we examined the relationship between Troponin T levels and TDI systolic and diastolic velocities in infants with NE. On day 1 of life Troponin T levels are significantly higher than those on day 7. Troponin T levels were also significantly higher in the infants with Neonatal Encephalopathy who died and in those who had abnormal MRI imaging. Using Tissue Doppler Imaging we have demonstrated a trend toward increasing systolic and diastolic velocities in infants with NE. This suggests that initial myocardial dysfunction resulting from a hypoxic event at the time of birth event

may improve over the first week of life. During this time there was no significant increase in either the shortening or ejection fraction measurements. Early detection of myocardial dysfunction would allow more timely therapeutic intervention. Following fluid resuscitation as recommended by the American College of Critical Care guidelines 2007 , (Brierley, *et al* 2009) and the 2010 international Liaison Committee Recommendations (ILCOR) (Biban, *et al* 2011), supportive therapies to treat shock and improve hypotension include dopamine, epinephrine and dobutamine, these agents may improve cardiac function via β_1 adrenoceptor stimulation. However adverse effects such as tachycardia, increased oxygen consumption and altered tissue perfusion complicate their use (Evans 2006).

Dopamine is an endogenous catecholamine which improves blood pressure, cardiac output and stroke volume on starting doses of 10mcg/kg/min. However there is insufficient evidence to suggest that the use of dopamine in term infants with perinatal asphyxia either improves mortality or long term neuro developmental outcome (Hunt and Osborn 2002). Adrenaline has both α and β receptor agonist effects. At low doses it is a potent inotrope, chronotrope, systemic and pulmonary vasodilator (Paradis and Osborn 2004). Dobutamine is an inotrope with predominantly β receptor effects and up to up to 20 micrograms/kg/min is the dose range used to increase cardiac output (Paradis and Osborn 2004).

Milrinone is a phosphodiesterase III inhibitor and has been used post cardiac surgery for neonates with low cardiac output syndrome and also in the treatment of pulmonary hypertension (Chang, *et al* 1995). In animal models the vasodilatory effects of milrinone have been found to alleviate pulmonary hypertension in

newborn piglets. Joynt et al found that epinephrine, dobutamine and milrinone increase cardiac output, stroke volume and systemic oxygen delivery without aggravating pulmonary hypertension in asphyxiated newborn piglets (Joynt, *et al* 2010). Paradisis et al have studied the use of milrinone in preterm infants and have found no adverse effects with its use however it did not help to improve systemic flow (Paradisis, *et al* 2009).

The trend in our study was towards a significant improvement in myocardial velocities in infants with NE over the first week of life. It would be useful to look at TDI measures of systolic and diastolic velocities in a larger group of infants with Neonatal Encephalopathy and to undertake cardiovascular follow up to ensure complete recovery as myocardial damage in the newborn period may predispose to adult cardiovascular disease. In the future correlating Troponin T levels with MRI results may be useful to help predict developmental outcome.

9.6 Point of Care Testing for Troponin T and NT pro BNP in Neonates.

In this study we have validated the use of the Cobas h232 analyser for cotside analysis of Troponin T and NT pro BNP in the NICU. Troponin T and NT pro BNP are established markers of the presence of a PDA (Choi, *et al* 2005, El-Khuffash, *et al* 2007). Troponin T as we have demonstrated is elevated on day one of life in infants with Neonatal encephalopathy who have echocardiographic evidence of myocardial dysfunction. In the future it would be useful to use serial echocardiography and POCT testing of Troponin T to demonstrate evidence of myocardial injury and subsequent recovery. In both infants with NE and preterm

infants it would be helpful to correlate NT pro BNP and Troponin T with multiorgan outcomes.

9.7 Future Research

9.7.1 Cardiovascular Follow up

Advances in Neonatal Intensive care continue to improve the survival and short and long term outcomes of preterm infants. Neonatal cardiovascular research tends to focus on the presence and treatment of a haemodynamically significant patent ductus arteriosus (PDA) (Benitz 2010) and newer techniques to assess global myocardial function such as tissue Doppler Imaging (Ciccone, *et al* 2011) without looking at longer term cardiovascular outcomes.

Cardiovascular disease is the leading cause of death worldwide, (Muller-Nordhorn, *et al* 2008) and systemic hypertension remains one of the major cardiovascular risk factors. The Barker Hypothesis links low birth weight and growth restriction in utero to adult cardiovascular disease (Barker and Osmond 1986). A number of studies have linked high blood pressure in adulthood to extreme prematurity, (Kistner, *et al* 2005) (Doyle, *et al* 2003). Johansson *et al* have observed that systolic blood pressure increases progressively with decreasing gestational age at birth (Johansson, *et al* 2005).

During the neonatal period the structure and function of the neonatal cardiac myocyte and myocardium continue to mature (Louey and Thornburg 2005). This coupled with haemodynamic changes occurring during the transitional period with increasing systemic vascular resistance and decreasing pulmonary

vascular resistance are likely to have an overall effect on shape and function of the left ventricle (Azancot, *et al* 1983). Preterm infants have decreased ventricular mass compared to term infants, in the first weeks of life the ventricular muscle mass grows thereafter ventricular mass remains constant throughout childhood (Kozak-Barany, *et al* 2001). In animal models neonatal porcine hearts appear to be more sensitive to global ischaemia than adult hearts, with neonatal hearts having a significantly shorter time between the onset of ischaemia and myocardial damage (Wittnich, *et al* 1987). Neonates who suffer a hypoxic ischaemic event may have damage to the myocardium, making them more vulnerable to developing cardiovascular disease in later life.

No studies to date have looked at long term cardiovascular function in preterm infants with evidence of systolic and diastolic dysfunction, the assumption being that the myocardium makes a full recovery. There is also paucity of data on functional cardiac changes in the first few days of life.

Preterm infants are followed up with regards to their development over the first few years of life. We suggest that preterm infants who have evidence of myocardial dysfunction also be followed up throughout childhood. This would allow evaluation of ventricular function not only in the preterm period but also in the early childhood years. This could highlight those at risk for long term cardiovascular disease, allowing counselling about the avoidance of cardiovascular risk factors. However insufficient paediatric cardiology services may prevent cardiovascular follow up.

9.7.2 Vitamin D and cardiac function

In our study we have found that preterm infants have deficient Vitamin D levels. They have decreased myocardial systolic and diastolic velocities compared to term infants. This may suggest a relationship between Vitamin D levels and cardiac function. A larger prospective study correlating TDI measures of systolic and diastolic velocities with Vitamin D levels in preterm infants throughout their inpatient stay and following discharge may be helpful.

9.7.3 Sepsis and cardiac function

Sepsis remains an important cause of mortality and morbidity in the neonatal population with neonatal sepsis accounting for 45% of late deaths in the neonatal intensive care unit (NICU) (Meadow, *et al* 2002). Little is known about the cardiovascular response to sepsis in the neonate. However clinically they show signs of circulatory compromise with pallor, tachycardia and hypotension (Luce, *et al* 2007). Cardiac dysfunction may result from increased cardiac myocyte production of Tumour necrosis factor (TNF), nitric oxide, and peroxynitrite, which leads to further DNA damage and ATP depletion resulting in secondary energy failure (Carcillo 2003). The underlying cause of death in neonates with sepsis is likely multifactorial, however cardiovascular collapse is frequently observed. Impaired systolic function is often identified as the major cause with the contribution of diastolic dysfunction remaining unclear (Sturgess, *et al* 2010). We have demonstrated that TDI may be a superior tool for assessment of myocardial

systolic and diastolic velocities in preterm infants and therefore may be useful in detecting early myocardial dysfunction allowing timely therapeutic intervention.

9.7.4 Stem cell research in Cardiovascular Disease in infants.

We have shown that TDI values in preterm infants in the first week of life, increase significantly. By day 7 of life systolic and diastolic velocities are similar to those of term infants. Similarly infants with Neonatal Encephalopathy have TDI velocities that increase over the first week. This may indicate that initial cardiac dysfunction resolves over the first few days of life. In adults stem cell therapy is being explored as a treatment for patients with left ventricular dysfunction (Murry and Keller 2008) (Chong, Nelson, *et al* 2009). Human umbilical cord blood stem cells (Human UCBSs) provide a rich source of stem cells (Bizzarro, *et al* 2007) (Prat-Vidal, *et al* 2007) with remarkably high levels noted in peripheral blood in preterm term infants (Bizzarro, *et al* 2007). Apparent neonatal myocardial recovery following severe hypoxia-ischaemia may be related to the large number of HUCBSC naturally present. However there is a paucity of studies on long term cardiovascular function in infants with neonatal encephalopathy.

9.7.5 Future Echocardiographic Training

We have demonstrated that the use of serial echocardiography in the NICU may help to assess global myocardial function in conditions such as Neonatal Encephalopathy. As well as establishing normative data TDI measures of systolic and diastolic function in preterm infants in the first week of life may help to predict

neonatal morbidities. To enable functional echocardiography to be integrated into routine practice structured training needs to be undertaken in order to avoid misdiagnosis. In Australia and New Zealand training and accreditation in functional echocardiography has been developed with the Australasian Society for Clinician Performed Ultrasound, resulting in the Certificate of Clinician Performed Ultrasound (Neonatal). This involves 18-24 months of structured training with mentoring by experienced neonatal echocardiographers/paediatric cardiologists (Evans, *et al*). Training in this way allows expertise to be established in the field of functional echocardiography. Guidelines now exist regarding the use of targeted neonatal echocardiography in the NICU (Mertens, *et al* 2011) and hopefully in the future this will lead to accurate assessment of cardiac function in preterm and term infants.

9.8 Conclusion

In this project we have established normal data for tissue Doppler imaging myocardial systolic and diastolic velocities in preterm infants. We have established normal data for preterm infants and monitored myocardial function in this population over the first week of life. We have demonstrated that myocardial velocities in the first week of life can predict neonatal morbidities such as chronic lung disease, necrotising enterocolitis and those infants who require PDA ligation. Infants with evidence of Neonatal encephalopathy have significantly elevated Troponin T levels on Day one of life and this is significantly associated with poor outcome. The validation of a point of care bedside analyser for Troponin T in this project will allow efficient results to be obtained which may guide medical

treatment in these patients. We have highlighted the difficulties with new techniques such as speckle tracking imaging in healthy term infants, and with the development of improved software in the future, establishing normal data for healthy newborns may be possible. In conclusion new and evolving echocardiographic techniques for global myocardial assessment may be superior to existing measures.

REFERENCES

- Abdel, G.A., Abdullah, S.H. & Kordofani, A.Y. Plasma homocysteine levels in cardiovascular disease, malaria and protein-energy malnutrition in Sudan. *East Mediterr Health J*, 2009; 15: 1432-1439.
- Abdel-Hady, H.E., Matter, M.K. & El-Arman, M.M. Myocardial dysfunction in neonatal sepsis: A tissue Doppler imaging study. *Pediatr Crit Care Med*. 2012;3 :318-23
- Agarwal, R., Virmani, D., Jaipal, M.L., Gupta, S., Gupta, N., Sankar, M.J., Bhatia, S., Agarwal, A., Devgan, V., Deorari, A. & Paul, V.K. Vitamin D Status of Low Birth Weight Infants in Delhi: A Comparative Study. *J Trop Pediatr*. 2012 E pub ahead of print
- Alpert, J.S., Thygesen, K., Antman, E. & Bassand, J.P. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol*, 2000 ; 36: 959-969.
- Amundsen, B.H., Helle-Valle, T., Edvardsen, T., Torp, H., Crosby, J., Lyseggen, E., Stoylen, A., Ihlen, H., Lima, J.A., Smiseth, O.A. & Slordahl, S.A. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. *J Am Coll Cardiol*, 2006 ; 47:789-793.
- An international classification of retinopathy of prematurity. The Committee for the Classification of Retinopathy of Prematurity. *Arch Ophthalmol*, 1984; 102: 1130-1134.
- Asano, G., Takashi, E., Ishiwata, T., Onda, M., Yokoyama, M., Naito, Z., Ashraf, M. & Sugisaki, Y. Pathogenesis and protection of ischemia and reperfusion injury in myocardium. *J Nippon Med Sch*, 2003; 70: 384-392.

- Azancot, A., Caudell, T.P., Allen, H.D., Horowitz, S., Sahn, D.J., Stoll, C., Thies, C., Valdes-Cruz, L.M. & Goldberg, S.J. Analysis of ventricular shape by echocardiography in normal fetuses, newborns, and infants. *Circulation*, 1983; 68: 1201-1211.
- Azzopardi, D., Brocklehurst, P., Edwards, D., Halliday, H., Levene, M., Thoresen, M. & Whitelaw, A. The TOBY Study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: a randomised controlled trial. *BMC Pediatr*, 2008; 8: 17.
- Bancalari, E. & Claure, N. Definitions and diagnostic criteria for bronchopulmonary dysplasia. *Semin Perinatol*, 2008; 30: 164-170.
- Barberi, I., Calabro, M.P., Cordaro, S., Gitto, E., Sottile, A., Prudente, D., Bertuccio, G. & Consolo, S. Myocardial ischaemia in neonates with perinatal asphyxia. Electrocardiographic, echocardiographic and enzymatic correlations. *Eur J Pediatr*, 1999; 158: 742-747.
- Barker, D.J. & Osmond, C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet*, 1986; 1: 1077-1081.
- Barkovich, A.J., Hajnal, B.L., Vigneron, D., Sola, A., Partridge, J.C., Allen, F. & Ferriero, D.M. Prediction of neuromotor outcome in perinatal asphyxia: evaluation of MR scoring systems. *AJNR Am J Neuroradiol*, 1998; 19:143-149.
- Basu, S., Frank, L.H., Fenton, K.E., Sable, C.A., Levy, R.J. & Berger, J.T. Two-dimensional speckle tracking imaging detects impaired myocardial performance in children with septic shock, not recognized by conventional echocardiography. *Pediatr Crit Care Med*. 2012;13:259-64
- Battin, M.R., Thoresen, M., Robinson, E., Polin, R.A., Edwards, A.D. & Gunn, A.J. Does head cooling with mild systemic hypothermia affect requirement for blood pressure support? *Pediatrics*, 2009; 123: 1031-1036.
- Behfar, A., Perez-Terzic, C., Faustino, R.S., Arrell, D.K., Hodgson, D.M., Yamada, S., Puceat, M., Niederlander, N., Alekseev, A.E., Zingman, L.V. & Terzic, A. Cardiopoietic programming of embryonic stem cells for tumor-free heart repair. *J Exp Med*, 2007; 204: 405-420.

- Belderbos, M.E., Houben, M.L., Wilbrink, B., Lentjes, E., Bloemen, E.M., Kimpen, J.L., Rovers, M. & Bont, L. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics*, 2011;127:1513-1520
- Bell, M.J. Neonatal necrotizing enterocolitis. *N Engl J Med*, . 1978; 298: 281-282.
- Benitz, W.E. Treatment of persistent patent ductus arteriosus in preterm infants: time to accept the null hypothesis? *J Perinatol*, 2010; 30: 241-252.
- Biban, P., Filipovic-Grcic, B., Biarent, D. & Manzoni, P. New cardiopulmonary resuscitation guidelines 2010: Managing the newly born in delivery room. *Early Hum Dev*.2011;87:S9-11
- Bijmens, B.H., Cikes, M., Claus, P. & Sutherland, G.R. Velocity and deformation imaging for the assessment of myocardial dysfunction. *Eur J Echocardiogr*, 2009; 10: 216-226.
- Bizzarro, M.J., Bhandari, V., Krause, D.S., Smith, B.R. & Gross, I. Circulating stem cells in extremely preterm neonates. *Acta Paediatr*, 2007; 96: 521-525.
- Bland, J.M. & Altman, D.G. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1986 ; 1: 307-310.
- Blessberger, H. & Binder, T. NON-invasive imaging: Two dimensional speckle tracking echocardiography: basic principles. *Heart*, 2012; 96: 716-722.
- Borke, W.B., Munkeby, B.H., Morkrid, L., Thaulow, E. & Saugstad, O.D. Resuscitation with 100% O₂ does not protect the myocardium in hypoxic newborn piglets. *Arch Dis Child Fetal Neonatal Ed*, 2004; 89: F156-160.
- Braunwald, E. Structure and function of the normal myocardium. *Br Heart J*, 1971; 33; Suppl:3-8.
- Brierley, J., Carcillo, J.A., Choong, K., Cornell, T., Decaen, A., Deymann, A., Doctor, A., Davis, A., Duff, J., Dugas, M.A., Duncan, A., Evans, B., Feldman, J., Felmet, K., Fisher, G., Frankel, L., Jeffries, H., Greenwald, B., Gutierrez, J., Hall, M., Han, Y.Y., Hanson, J., Hazelzet, J., Hernan, L., Kiff, J., Kissoon, N., Kon, A., Irazuzta, J., Lin, J., Lorts, A., Mariscalco, M., Mehta, R., Nadel, S., Nguyen, T., Nicholson, C., Peters, M., Okhuysen-Cawley, R., Poulton, T., Relves, M., Rodriguez, A., Rozenfeld, R., Schnitzler, E., Shanley, T., Kache, S., Skippen, P., Torres, A., von Dessauer, B., Weingarten, J., Yeh, T., Zaritsky, A., Stojadinovic, B., Zimmerman, J. & Zuckerberg, A. Clinical practice

- parameters for hemodynamic support of pediatric and neonatal septic shock: 2007 update from the American College of Critical Care Medicine. *Crit Care Med*, 2009 ;37: 666-688.
- Brueckmann, M., Huhle, G., Lang, S., Haase, K.K., Bertsch, T., Weiss, C., Kaden, J.J., Putensen, C., Borggrefe, M. & Hoffmann, U. Prognostic value of plasma N-terminal pro-brain natriuretic peptide in patients with severe sepsis. *Circulation*, 2005 ; 112: 527-534.
- Burns, A.T., La Gerche, A., Maclsaac, A.I. & Prior, D.L. Augmentation of left ventricular torsion with exercise is attenuated with age. *J Am Soc Echocardiogr*, 2008 ; 21: 315-320.
- Burns, A.T., McDonald, I.G., Thomas, J.D., Macisaac, A. & Prior, D. Doin' the twist: new tools for an old concept of myocardial function. *Heart*, 2008 ;94: 978-983.
- Calbet, J.A., Robach, P. & Lundby, C. The exercising heart at altitude. *Cell Mol Life Sci*, 2009 ; 66:3601-3613.
- Carcillo, J.A. Pediatric septic shock and multiple organ failure. *Crit Care Clin*, 2003; 19: 413-440, viii.
- Carter, B.M. & Holditch-Davis, D. Risk factors for necrotizing enterocolitis in preterm infants: how race, gender, and health status contribute. *Adv Neonatal Care*, 2008; 8: 285-290.
- Chang, A.C., Atz, A.M., Wernovsky, G., Burke, R.P. & Wessel, D.L. Milrinone: systemic and pulmonary hemodynamic effects in neonates after cardiac surgery. *Crit Care Med*, 1995; 23: 1907-1914.
- Chen, Y. & Li, C. Prognostic significance of brain natriuretic peptide obtained in the ED in patients with SIRS or sepsis. *Am J Emerg Med*, 2009; 27: 701-706.
- Cherian, P.J., Blok, J.H., Swarte, R.M., Govaert, P. & Visser, G.H. Heart rate changes are insensitive for detecting postasphyxial seizures in neonates. *Neurology*, 2006; 67: 2221-2223.
- Choi, B.M., Lee, K.H., Eun, B.L., Yoo, K.H., Hong, Y.S., Son, C.S. & Lee, J.W. Utility of rapid B-type natriuretic peptide assay for diagnosis of symptomatic patent ductus arteriosus in preterm infants. *Pediatrics*, 2005; 115: e255-261.

- Chong, J.J. Cell Therapy for Left Ventricular Dysfunction: An Overview for Cardiac Clinicians. *Heart Lung Circ.* 2012;21: 532-42
- Ciccone, M.M., Scicchitano, P., Zito, A., Gesualdo, M., Sassara, M., Calderoni, G., Di Mauro, F., Ladisa, G., Di Mauro, A. & Laforgia, N. Different functional cardiac characteristics observed in term/preterm neonates by echocardiography and tissue doppler imaging. *Early Hum Dev*, 2011; 87: 555-558.
- Clark, S.J., Newland, P. & Thorburn, K. Troponin I versus troponin T: is there actually a debate in children with sepsis? *Crit Care Med*, 2001; 29: 1093-1094.
- Clark, S.J., Newland, P., Yoxall, C.W. & Subhedar, N.V. Concentrations of cardiac troponin T in neonates with and without respiratory distress. *Arch Dis Child Fetal Neonatal Ed*, 2004; 89: F348-352.
- Clyman, R.I. Mechanisms regulating the ductus arteriosus. *Biol Neonate*, 2006; 89: 330-335.
- Dakshinamurti, S. Pathophysiologic mechanisms of persistent pulmonary hypertension of the newborn. *Pediatr Pulmonol*, 2005; 39: 492-503.
- Davidson, D., Barefield, E.S., Kattwinkel, J., Dudell, G., Damask, M., Straube, R., Rhines, J. & Chang, C.T. Inhaled nitric oxide for the early treatment of persistent pulmonary hypertension of the term newborn: a randomized, double-masked, placebo-controlled, dose-response, multicenter study. The I-NO/PPHN Study Group. *Pediatrics*, 1998 ;01: 325-334.
- Davies, B., Elwood, N.J., Li, S., Cullinane, F., Edwards, G.A., Newgreen, D.F. & Brizard, C.P. Human cord blood stem cells enhance neonatal right ventricular function in an ovine model of right ventricular training. *Ann Thorac Surg*, 2012; 89; 585-593,
- Dawodu, A. & Nath, R. High prevalence of moderately severe vitamin D deficiency in preterm infants. *Pediatr Int*, 2011; 53: 207-210.
- Despotis, G.J., Joist, J.H. & Goodnough, L.T. Monitoring of hemostasis in cardiac surgical patients: impact of point-of-care testing on blood loss and transfusion outcomes. *Clin Chem*, 1997; 43: 1684-1696.
- D'Hooge, J., Heimdal, A., Jamal, F., Kukulski, T., Bijnens, B., Rademakers, F., Hatle, L., Suetens, P. & Sutherland, G.R. Regional strain and strain rate

- measurements by cardiac ultrasound: principles, implementation and limitations. *Eur J Echocardiogr*, 2000; 1: 154-170.
- Dimmeler, S. & Zeiher, A.M. Cell therapy of acute myocardial infarction: open questions. *Cardiology*, 2009; 113: 155-160.
- Dirks, J.L. Diagnostic blood analysis using point-of-care technology. *AACN Clin Issues*, 1996; 7: 249-259.
- Doyle, L.W., Faber, B., Callanan, C. & Morley, R. Blood pressure in late adolescence and very low birth weight. *Pediatrics*, 2003; 111: 252-257.
- Edler, I. & Hertz, C.H. The use of ultrasonic reflectoscope for the continuous recording of the movements of heart walls. 1954. *Clin Physiol Funct Imaging*, 2004; 24: 118-136.
- Edvardsen, T., Helle-Valle, T. & Smiseth, O.A. Systolic dysfunction in heart failure with normal ejection fraction: speckle-tracking echocardiography. *Prog Cardiovasc Dis*, 2006; 49: 207-214.
- El Hajjar, M., Vaksmann, G., Rakza, T., Kongolo, G. & Storme, L. Severity of the ductal shunt: a comparison of different markers. *Arch Dis Child Fetal Neonatal Ed*, 2005; 90: F419-422.
- El-Khuffash, A., Barry, D., Walsh, K., Davis, P.G. & Molloy, E.J. Biochemical markers may identify preterm infants with a patent ductus arteriosus at high risk of death or severe intraventricular haemorrhage. *Arch Dis Child Fetal Neonatal Ed*, 2008; 93: F407-412.
- El-Khuffash, A., Davis, P.G., Walsh, K. & Molloy, E.J. Cardiac troponin T and N-terminal-pro-B type natriuretic peptide reflect myocardial function in preterm infants. *J Perinatol*, 2008; 28: 482-486.
- El-Khuffash, A. & Molloy, E. The Use of N-Terminal-Pro-BNP in Preterm Infants. *Int J Pediatr*, 2009; 175: 216.
- El-Khuffash, A. & Molloy, E.J. Are B-type natriuretic peptide (BNP) and N-terminal-pro-BNP useful in neonates? *Arch Dis Child Fetal Neonatal Ed*, 2007; 92: F320-324.
- El-Khuffash, A.F., Amoruso, M., Culliton, M. & Molloy, E.J. N-terminal pro-B-type natriuretic peptide as a marker of ductal haemodynamic significance in

- preterm infants: a prospective observational study. *Arch Dis Child Fetal Neonatal Ed*, 2007; 92: F421-422.
- El-Khuffash, A.F., Slevin, M., McNamara, P.J. & Molloy, E.J. Troponin T, N-terminal pro natriuretic peptide and a patent ductus arteriosus scoring system predict death before discharge or neurodevelopmental outcome at 2 years in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2010;140:150-7
- Emami, C.N., Chokshi, N., Wang, J., Hunter, C., Guner, Y., Goth, K., Wang, L., Grishin, A. & Ford, H.R. Role of interleukin-10 in the pathogenesis of necrotizing enterocolitis. *Am J Surg*, 2012; 203; 428-435.
- Evans, N. Which inotrope for which baby? *Arch Dis Child Fetal Neonatal Ed*, 2006; 91: F213-220.
- Evans, N., Gournay, V., Cabanas, F., Kluckow, M., Leone, T., Groves, A., McNamara, P. & Mertens, L. Point-of-care ultrasound in the neonatal intensive care unit: international perspectives. *Semin Fetal Neonatal Med*, 2011; 16:61-816, 61-68.
- Evans, N., Kluckow, M. & Currie, A. Range of echocardiographic findings in term neonates with high oxygen requirements. *Arch Dis Child Fetal Neonatal Ed*, 1998; 78: F105-111.
- Fatemi, A., Wilson, M.A. & Johnston, M.V. Hypoxic-ischemic encephalopathy in the term infant. *Clin Perinatol*, 2009; 36: 835-858, vii.
- Fenton, K.E., Sable, C.A., Bell, M.J., Patel, K.M. & Berger, J.T. Increases in serum levels of troponin I are associated with cardiac dysfunction and disease severity in pediatric patients with septic shock. *Pediatr Crit Care Med*, 2004; 5: 533-538.
- Fernandez-Aviles, F., San Roman, J.A., Garcia-Frade, J., Fernandez, M.E., Penarrubia, M.J., de la Fuente, L., Gomez-Bueno, M., Cantalapiedra, A., Fernandez, J., Gutierrez, O., Sanchez, P.L., Hernandez, C., Sanz, R., Garcia-Sancho, J. & Sanchez, A. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. *Circ Res*, 2004;95: 742-748.
- Fleming, S., Thompson, M., Stevens, R., Heneghan, C., Pluddemann, A., Maconochie, I., Tarassenko, L. & Mant, D. Normal ranges of heart rate and

- respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. *Lancet*, 2011; 377; 1011-1018.
- Fontes, J.P., Goncalves, M. & Ribeiro, V.G. [Serum markers for ischemic myocardial damage]. *Rev Port Cardiol*, 1999; 8: 1129-1136.
- Fromm, R.E., Jr. Cardiac troponins in the intensive care unit: common causes of increased levels and interpretation. *Crit Care Med*, 2007; 35: 584-588.
- Garcia, E.H., Perna, E.R., Farias, E.F., Obregon, R.O., Macin, S.M., Parras, J.I., Agüero, M.A., Moratorio, D.A., Pitzus, A.E., Tassano, E.A. & Rodriguez, L. Reduced systolic performance by tissue Doppler in patients with preserved and abnormal ejection fraction: new insights in chronic heart failure. *Int J Cardiol*, 2006; 108: 181-188.
- Gardiner, H.M., Pasquini, L., Wolfenden, J., Barlow, A., Li, W., Kulinskaya, E. & Henein, M. Myocardial tissue Doppler and long axis function in the fetal heart. *Int J Cardiol*, 2006; 113: 39-47.
- Gebauer, C.M., Knuepfer, M., Robel-Tillig, E., Pulzer, F. & Vogtmann, C. Hemodynamics among neonates with hypoxic-ischemic encephalopathy during whole-body hypothermia and passive rewarming. *Pediatrics*, 2006; 117: 843-850.
- Giapros, V.I., Schiza, V., Challa, A.S., Cholevas, V.K., Theocharis, P.D., Kolios, G., Pantou, C. & Andronikou, S.K. Vitamin D and parathormone levels of late-preterm formula fed infants during the first year of life. *Eur J Clin Nutr*, 2012; 66: 224-230.
- Ginde AA, Liu, MC, Camargo CA. Demographic differences and trends of Vitamin D insufficiency in the US Population 1988-2004. *Arch Intern Med* 2009;109-626-32
- Gill, A.B. & Weindling, A.M. Echocardiographic assessment of cardiac function in shocked very low birthweight infants. *Arch Dis Child*, 1993; 68: 17-21.
- Goldmann, B.U., Christenson, R.H., Hamm, C.W., Meinertz, T. & Ohman, E.M. Implications of troponin testing in clinical medicine. *Curr Control Trials Cardiovasc Med*, 2001; 2: 75-84.
- Gotsman I, Shauer A, Zwas DR, Hellman Y, Keren A, Lotan C, Admon D. Vitamin D deficiency is a predictor of reduced survival in patients with heart

- failure;vitamin D supplementation improves outcome. *Eur J Heart Fail* 2012;14:357-66
- Greenbaum, R.A. & Gibson, D.G.Regional non-uniformity of left ventricular wall movement in man. *Br Heart J*, 1981; 45: 29-34.
- Greenbaum, R.A., Ho, S.Y., Gibson, D.G., Becker, A.E. & Anderson, R.H. Left ventricular fibre architecture in man. *Br Heart J*, 1981; 45: 248-263.
- Gunes, T., Ozturk, M.A., Koklu, S.M., Narin, N. & Koklu, E. Troponin-T levels in perinatally asphyxiated infants during the first 15 days of life. *Acta Paediatr*, 2005; 94: 1638-1643.
- Gupta, P., Tomar, M., Radhakrishnan, S. & Shrivastava, S. Hypocalcemic cardiomyopathy presenting as cardiogenic shock. *Ann Pediatr Cardiol*, 2011; 4: 152-155.
- Guthrie, S.O., Gordon, P.V., Thomas, V., Thorp, J.A., Peabody, J. & Clark, R.H. Necrotizing enterocolitis among neonates in the United States. *J Perinatol*, 2003; 23; 278-285.
- Harada, K., Shiota, T., Takahashi, Y., Tamura, M., Toyono, M. & Takada, G. Doppler echocardiographic evaluation of left ventricular output and left ventricular diastolic filling changes in the first day of life. *Pediatr Res*, 1994; 35: 506-509.
- Haycock, G.B., Schwartz, G.J. & Wisotsky, D.H. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr*, 1978; 93: 62-66.
- Hegy, T., Anwar, M., Carbone, M.T., Ostfeld, B., Hiatt, M., Koons, A., Pinto-Martin, J. & Paneth, N.Blood pressure ranges in premature infants: II. The first week of life. *Pediatrics*, 1996; 97: 336-342.
- Helle-Valle, T., Crosby, J., Edvardsen, T., Lyseggen, E., Amundsen, B.H., Smith, H.J., Rosen, B.D., Lima, J.A., Torp, H., Ihlen, H. & Smiseth, O.A. New noninvasive method for assessment of left ventricular rotation: speckle tracking echocardiography. *Circulation*, 2005; 112: 3149-3156.
- Henderson-Smart, D.J., Hutchinson, J.L., Donoghue, D.A., Evans, N.J., Simpson, J.M. & Wright, I. Prenatal predictors of chronic lung disease in very preterm infants. *Arch Dis Child Fetal Neonatal Ed*, 2006; 91: F40-45.

- Hinds, L.E., Brown, C.L. & Clark, S.J. Point of care estimation of haemoglobin in neonates. *Arch Dis Child Fetal Neonatal Ed*, 2007; 92: F378-380.
- Hirsch, R., Landt, Y., Porter, S., Canter, C.E., Jaffe, A.S., Ladenson, J.H., Grant, J.W. & Landt, M. Cardiac troponin I in pediatrics: normal values and potential use in the assessment of cardiac injury. *J Pediatr*, 1997; 130: 872-877.
- Ho, C.Y. & Solomon, S.D. A clinician's guide to tissue Doppler imaging. *Circulation*, 2006; 113: e396-398.
- Hoffmann, R., Lethen, H., Marwick, T., Arnese, M., Fioretti, P., Pingitore, A., Picano, E., Buck, T., Erbel, R., Flachskampf, F.A. & Hanrath, P. Analysis of interinstitutional observer agreement in interpretation of dobutamine stress echocardiograms. *J Am Coll Cardiol*, 1996; 27: 330-336.
- Holick, M.F. The vitamin D epidemic and its health consequences. *J Nutr*, 2005; 35: 2739S-2748S.
- Holick, M.F. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc*, 2006; 81: 353-373.
- Huang, C.C., Wang, S.T., Chang, Y.C., Lin, K.P. & Wu, P.L. Measurement of the urinary lactate:creatinine ratio for the early identification of newborn infants at risk for hypoxic-ischemic encephalopathy. *N Engl J Med*, 1999; 341: 328-335.
- Hunt, R. & Osborn, D. Dopamine for prevention of morbidity and mortality in term newborn infants with suspected perinatal asphyxia. *Cochrane Database Syst Rev*, 2002 CD003484.
- Ilchihashi, K., Yada, Y., Takahashi, N., Honma, Y. & Momoi, M. Utility of a Doppler-derived index combining systolic and diastolic performance (Tei index) for detecting hypoxic cardiac damage in newborns. *J Perinat Med*, 2005; 33: 549-552.
- Jacobs, S., Hunt, R., Tarnow-Mordi, W., Inder, T. & Davis, P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev*, 2007 CD003311.
- Jedeikin, R., Primhak, A., Shennan, A.T., Swyer, P.R. & Rowe, R.D. Serial electrocardiographic changes in healthy and stressed neonates. *Arch Dis Child*, 1983; 58: 605-611.

- Jobe, A.H. Predictors of outcomes in preterm infants: which ones and when? *J Pediatr*, 2001; 138: 153-156.
- Johansson, S., Iliadou, A., Bergvall, N., Tuvemo, T., Norman, M. & Cnattingius, S. Risk of high blood pressure among young men increases with the degree of immaturity at birth. *Circulation*, 2005; 112: 3430-3436.
- Joynt, C., Bigam, D.L., Charrois, G., Jewell, L.D., Korbitt, G. & Cheung, P.Y. Milrinone, dobutamine or epinephrine use in asphyxiated newborn pigs resuscitated with 100% oxygen. *Intensive Care Med*, 2010; 36: 1058-1066.
- Kadivar, M., Kiani, A., Kocharian, A., Shabaniyan, R., Nasehi, L. & Ghajarzadeh, M. Echocardiography and management of sick neonates in the intensive care unit. *Congenit Heart Dis*, 2008; 3: 325-329.
- Kalra, V.K., DeBari, V.A., Zauk, A., Kataria, P., Myridakis, D. & Kiblawi, F. Point-of-care testing for B-type natriuretic peptide in premature neonates with patent ductus arteriosus. *Ann Clin Lab Sci*, 2011; 41: 131-137.
- Kanik, E., Ozer, E.A., Bakiler, A.R., Aydinlioglu, H., Dorak, C., Dogrusoz, B., Kanik, A. & Yaprak, I. Assessment of myocardial dysfunction in neonates with hypoxic-ischemic encephalopathy: is it a significant predictor of mortality? *J Matern Fetal Neonatal Med*, 2009; 22: 239-242.
- Karras, D.J. & Kane, D.L. Serum markers in the emergency department diagnosis of acute myocardial infarction. *Emerg Med Clin North Am*, 2001; 19: 321-337.
- Kaushal, S., Jacobs, J.P., Gossett, J.G., Steele, A., Steele, P., Davis, C.R., Pahl, E., Vijayan, K., Asante-Korang, A., Boucek, R.J., Backer, C.L. & Wold, L.E. Innovation in basic science: stem cells and their role in the treatment of paediatric cardiac failure--opportunities and challenges. *Cardiol Young*, 2009; 19: Suppl 2, 74-84.
- Kiserud, T. & Acharya, G. The fetal circulation. *Prenat Diagn*, 2004; 24: 1049-1059.
- Kistner, A., Celsi, G., Vanpee, M. & Jacobson, S.H. Increased systolic daily ambulatory blood pressure in adult women born preterm. *Pediatr Nephrol*, 2005; 20: 232-233.
- Kluckow, M. Functional echocardiography in assessment of the cardiovascular system in asphyxiated neonates. *J Pediatr*, 2011; 158: e13-18.

- Kluckow, M. Low systemic blood flow and pathophysiology of the preterm transitional circulation. *Early Hum Dev*, 2005; 81: 429-437.
- Kluckow, M. & Evans, N. Early echocardiographic prediction of symptomatic patent ductus arteriosus in preterm infants undergoing mechanical ventilation. *J Pediatr*, 1995; 127: 774-779.
- Kluckow, M. & Evans, N. Ductal shunting, high pulmonary blood flow, and pulmonary hemorrhage. *J Pediatr*, 2000; 137: 68-72.
- Koch, A. & Singer, H. Normal values of B type natriuretic peptide in infants, children, and adolescents. *Heart*, 2003; 89: 875-878.
- Koch, A., Zink, S. & Singer, H. B-type natriuretic peptide in paediatric patients with congenital heart disease. *Eur Heart J*, 2006; 27: 861-866.
- Kodama, Y., Sameshima, H., Ikeda, T. & Ikenoue, T. Intrapartum fetal heart rate patterns in infants (> or =34 weeks) with poor neurological outcome. *Early Hum Dev*, 2009; 85: 235-238.
- Koopman, L.P., Slorach, C., Hui, W., Manlhiot, C., McCrindle, B.W., Friedberg, M.K., Jaeggi, E.T. & Mertens, L. Comparison between different speckle tracking and color tissue Doppler techniques to measure global and regional myocardial deformation in children. *J Am Soc Echocardiogr*, 2010; 23: 919-928.
- Kostis, J.B., Mavrogeorgis, E., Slater, A. & Bellet, S. Use of a range-gated, pulsed ultrasonic doppler technique for continuous measurement of velocity of the posterior heart wall. *Chest*, 1972; 62: 597-604.
- Kozak-Barany, A., Jokinen, E., Saraste, M., Tuominen, J. & Valimaki, I. Development of left ventricular systolic and diastolic function in preterm infants during the first month of life: a prospective follow-up study. *J Pediatr*, 2001; 139: 539-545.
- Lee, J.H., O'Keefe, J.H., Bell, D., Hensrud, D.D. & Holick, M.F. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? *J Am Coll Cardiol*, 2008; 52: 1949-1956.
- Lee, L.A., Kimball, T.R., Daniels, S.R., Khoury, P. & Meyer, R.A. Left ventricular mechanics in the preterm infant and their effect on the measurement of cardiac performance. *J Pediatr*, 1992; 120: 114-119.

- Leitman, M., Lysyansky, P., Sidenko, S., Shir, V., Peleg, E., Binenbaum, M., Kaluski, E., Krakover, R. & Vered, Z. Two-dimensional strain-a novel software for real-time quantitative echocardiographic assessment of myocardial function. *J Am Soc Echocardiogr*, 2004; 17: 1021-1029.
- Leutmezer, F., Scherthaner, C., Lurger, S., Potzelberger, K. & Baumgartner, C. Electrocardiographic changes at the onset of epileptic seizures. *Epilepsia*, 2003; 44: 348-354.
- Lopez, L., Colan, S.D., Frommelt, P.C., Ensing, G.J., Kendall, K., Younoszai, A.K., Lai, W.W. & Geva, T. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. *J Am Soc Echocardiogr*, 2012; 23: 465-495; quiz 576-467.
- Losordo, D.W., Schatz, R.A., White, C.J., Udelson, J.E., Veereshwarayya, V., Durgin, M., Poh, K.K., Weinstein, R., Kearney, M., Chaudhry, M., Burg, A., Eaton, L., Heyd, L., Thorne, T., Shturman, L., Hoffmeister, P., Story, K., Zak, V., Dowling, D., Traverse, J.H., Olson, R.E., Flanagan, J., Sodano, D., Murayama, T., Kawamoto, A., Kusano, K.F., Wollins, J., Welt, F., Shah, P., Soukas, P., Asahara, T. & Henry, T.D. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation*, 2007; 115: 3165-3172.
- Louey, S. & Thornburg, K.L. The prenatal environment and later cardiovascular disease. *Early Hum Dev*, 2005; 81: 745-751.
- Luce, W.A., Hoffman, T.M. & Bauer, J.A. Bench-to bedside review: Developmental influences on the mechanisms, treatment and outcomes of cardiovascular dysfunction in neonatal versus adult sepsis. *Crit Care*, 2007; 11: 228.
- Maeder, M., Fehr, T., Rickli, H. & Ammann, P. Sepsis-associated myocardial dysfunction: diagnostic and prognostic impact of cardiac troponins and natriuretic peptides. *Chest*, 2006; 129: 1349-1366.
- Mair, J., Hammerer-Lercher, A. & Puschendorf, B. The impact of cardiac natriuretic peptide determination on the diagnosis and management of heart failure. *Clin Chem Lab Med*, 2001; 39: 571-588.

- Malerba, M., Ragnoli, B., Salameh, M., Sennino, G., Sorlini, M.L., Radaeli, A. & Clini, E. Sub-clinical left ventricular diastolic dysfunction in early stage of chronic obstructive pulmonary disease. *J Biol Regul Homeost Agents*, 2011; 25: 443-451.
- Marcus, K.A., Mavinkurve-Groothuis, A.M., Barends, M., van Dijk, A., Feuth, T., de Korte, C. & Kapusta, L. Reference values for myocardial two-dimensional strain echocardiography in a healthy pediatric and young adult cohort. *J Am Soc Echocardiogr*, 2011; 24: 625-636.
- Martin-Ancel, A., Garcia-Alix, A., Gaya, F., Cabanas, F., Burgueros, M. & Quero, J. (Multiple organ involvement in perinatal asphyxia. *J Pediatr*, 1995; 127: 786-793.
- Marwick, T.H. Measurement of strain and strain rate by echocardiography: ready for prime time? *J Am Coll Cardiol*, 2006; 47: 1313-1327.
- Matter, M., Abdel-Hady, H., Attia, G., Hafez, M., Seliem, W. & Al-Arman, M. Myocardial performance in asphyxiated full-term infants assessed by Doppler tissue imaging. *Pediatr Cardiol*, 2010; 31: 634-642.
- McDicken, W.N., Sutherland, G.R., Moran, C.M. & Gordon, L.N. Colour Doppler velocity imaging of the myocardium. *Ultrasound Med Biol*, 1992; 18: 651-654.
- McDonnell, B., Hearty, S., Leonard, P. & O'Kennedy, R. Cardiac biomarkers and the case for point-of-care testing. *Clin Biochem*, 2009; 42: 549-561.
- McGinnity, F.G. & Halliday, H.L. Perinatal predictors of ocular morbidity in school children who were very low birthweight. *Paediatr Perinat Epidemiol*, 1993; 7: 417-425.
- McNally, J.D., Leis, K., Matheson, L.A., Karuananyake, C., Sankaran, K. & Rosenberg, A.M. Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediatr Pulmonol*, 2009; 44: 981-988.
- McNamara, P.J., Stewart, L., Shivananda, S.P., Stephens, D. & Sehgal, A. Patent ductus arteriosus ligation is associated with impaired left ventricular systolic performance in premature infants weighing less than 1000 g. *J Thorac Cardiovasc Surg*, 2012; 140: 150-157.

- Meadow, W., Frain, L., Ren, Y., Lee, G., Soneji, S. & Lantos, J. Serial assessment of mortality in the neonatal intensive care unit by algorithm and intuition: certainty, uncertainty, and informed consent. *Pediatrics*, 2002; 109: 878-886.
- Mertens, L., Seri, I., Marek, J., Arlettaz, R., Barker, P., McNamara, P., Moon-Grady, A.J., Coon, P.D., Noori, S., Simpson, J. & Lai, W.W. Targeted Neonatal Echocardiography in the Neonatal Intensive Care Unit: practice guidelines and recommendations for training. Writing Group of the American Society of Echocardiography (ASE) in collaboration with the European Association of Echocardiography (EAE) and the Association for European Pediatric Cardiologists (AEPC). *J Am Soc Echocardiogr*, 2011; 24: 1057-1078.
- Messerschmidt, A., Olischar, M., Birnbacher, R., Sauer, A., Weber, M., Puschnig, D., Unterasinger, L., Pollak, A. & Leitich, H. Is it possible to make a reliable prognosis within the first hour of life for very low birth weight infants delivered after preterm premature rupture of membranes? *Neonatology*, 2011; 99: 146-152.
- Mir, T.S., Flato, M., Falkenberg, J., Haddad, M., Budden, R., Weil, J., Albers, S. & Laer, S. Plasma concentrations of N-terminal brain natriuretic peptide in healthy children, adolescents, and young adults: effect of age and gender. *Pediatr Cardiol*, 2006; 27: 73-77.
- Mor, M. & Waisman, Y. Point-of-care testing: a critical review. *Pediatr Emerg Care*, 2000; 16: 45-48.
- Mori, K., Nakagawa, R., Nii, M., Edagawa, T., Takehara, Y., Inoue, M. & Kuroda, Y. Pulsed wave Doppler tissue echocardiography assessment of the long axis function of the right and left ventricles during the early neonatal period. *Heart*, 2004; 90: 175-180.
- Mueller, T., Gegenhuber, A., Poelz, W. & Haltmayer, M. Diagnostic accuracy of B type natriuretic peptide and amino terminal proBNP in the emergency diagnosis of heart failure. *Heart*, 2005; 91: 606-612.
- Mukherjee, R., McQuinn, T.C., Dugan, M.A., Saul, J.P. & Spinale, F.G. Cardiac function and circulating cytokines after endotoxin exposure in neonatal mice. *Pediatr Res*, 2010; 68: 381-386.

- Muller-Nordhorn, J., Binting, S., Roll, S. & Willich, S.N. An update on regional variation in cardiovascular mortality within Europe. *Eur Heart J*, 2008; 29: 1316-1326.
- Murry, C.E. & Keller, G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*, 2008; 132: 661-680.
- Nadal-Ginard, B. & Mahdavi, V. Molecular basis of cardiac performance. Plasticity of the myocardium generated through protein isoform switches. *J Clin Invest*, 1989; 84: 1693-1700.
- Negrine, R.J., Chikermane, A., Wright, J.G. & Ewer, A.K. Assessment of myocardial function in neonates using tissue Doppler imaging. *Arch Dis Child Fetal Neonatal Ed*. 2012;97:304-6
- Nelson, T.J., Martinez-Fernandez, A., Yamada, S., Mael, A.A., Terzic, A. & Ikeda, Y. pluripotent reprogramming from promiscuous human stemness related factors. *Clin Transl Sci*, 2009; 2: 118-126.
- Nesbitt, G.C., Mankad, S. & Oh, J.K. Strain imaging in echocardiography: methods and clinical applications. *Int J Cardiovasc Imaging*, 2009; 25: Suppl 1, 9-22.
- Nir, A., Lindinger, A., Rauh, M., Bar-Oz, B., Laer, S., Schwachtgen, L., Koch, A., Falkenberg, J. & Mir, T.S. NT-pro-B-type natriuretic peptide in infants and children: reference values based on combined data from four studies. *Pediatr Cardiol*, 2009; 30: 3-8.
- Noori, S. & Seri, I. Pathophysiology of newborn hypotension outside the transitional period. *Early Hum Dev*, 2005; 1: 399-404.
- Notomi, Y., Lysyansky, P., Setser, R.M., Shiota, T., Popovic, Z.B., Martin-Miklovic, M.G., Weaver, J.A., Oryszak, S.J., Greenberg, N.L., White, R.D. & Thomas, J.D. Measurement of ventricular torsion by two-dimensional ultrasound speckle tracking imaging. *J Am Coll Cardiol*, 2005; 45: 2034-2041.
- Ohuchi, H., Takasugi, H., Ohashi, H., Okada, Y., Yamada, O., Ono, Y., Yagihara, T. & Echigo, S. Stratification of pediatric heart failure on the basis of neurohormonal and cardiac autonomic nervous activities in patients with congenital heart disease. *Circulation*, 2003; 108: 2368-2376.

- Reynolds, E.W., Ellington, J.G., Vranicar, M. & Bada, H.S. Brain-type natriuretic peptide in the diagnosis and management of persistent pulmonary hypertension of the newborn. *Pediatrics*, 2004; 114: 1297-1304.
- Rowland, D.G. & Gutgesell, H.P. Noninvasive assessment of myocardial contractility, preload, and afterload in healthy newborn infants. *Am J Cardiol*, 1995; 5: 818-821.
- Santrach, P.J. & Burritt, M.F. Point-of-care testing. *Mayo Clin Proc*, 1995; 70: 493-494.
- Sarnat, H.B. & Sarnat, M.S. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol*, 1976; 33: 696-705.
- Sarnoff, S.J. & Berglund, E. Ventricular function. I. Starling's law of the heart studied by means of simultaneous right and left ventricular function curves in the dog. *Circulation*, 1954; 9: 706-718.
- Schachinger, V., Erbs, S., Elsasser, A., Haberbosch, W., Hambrecht, R., Holschermann, H., Yu, J., Corti, R., Mathey, D.G., Hamm, C.W., Suselbeck, T., Assmus, B., Tonn, T., Dimmeler, S. & Zeiher, A.M. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*, 2006; 355: 1210-1221.
- Sehgal, A. & McNamara, P.J. ho. *J Perinatol*, 2008; 28: 729-735.
- Sehgal, A. & McNamara, P.J. Does echocardiography facilitate determination of hemodynamic significance attributable to the ductus arteriosus? *Eur J Pediatr*, 2009; 168: 907-914.
- Sengupta, P.P., Krishnamoorthy, V.K., Korinek, J., Narula, J., Vannan, M.A., Lester, S.J., Tajik, J.A., Seward, J.B., Khandheria, B.K. & Belohlavek, M. Left ventricular form and function revisited: applied translational science to cardiovascular ultrasound imaging. *J Am Soc Echocardiogr*, 2009; 20: 539-551.
- Shah, P., Riphagen, S., Beyene, J. & Perlman, M. Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed*, 2004; 89: F152-155.
- Shah, P.S. Current perspectives on the prevention and management of chronic lung disease in preterm infants. *Paediatr Drugs*, 2003; 5: 463-480.

- Wang, T.J., Pencina, M.J., Booth, S.L., Jacques, P.F., Ingelsson, E., Lanier, K., Benjamin, E.J., D'Agostino, R.B., Wolf, M. & Vasan, R.S. Vitamin D deficiency and risk of cardiovascular disease. *Circulation*, 2008; 117: 503-511.
- Wei, Y., Xu, J., Xu, T., Fan, J. & Tao, S. Left ventricular systolic function of newborns with asphyxia evaluated by tissue Doppler imaging. *Pediatr Cardiol*, 2009; 30: 741-746.
- Wei, Y., Xu, T., Xu, J.X., Fan, J., Tao, X.Y. & Wang, K. [Evaluation of cardiac function after neonatal asphyxia by Doppler tissue imaging]. *Zhonghua Yi Xue Za Zhi*, 2009; 89: 117-120.
- Wittnich, C., Peniston, C., Ianuzzo, D., Abel, J.G. & Salerno, T.A. Relative vulnerability of neonatal and adult hearts to ischemic injury. *Circulation*, 1987; 76: V156-160.
- Wollert, K.C., Meyer, G.P., Lotz, J., Ringes-Lichtenberg, S., Lippolt, P., Breidenbach, C., Fichtner, S., Korte, T., Hornig, B., Messinger, D., Arseniev, L., Hertenstein, B., Ganser, A. & Drexler, H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*, 2004; 364: 141-148.
- Wu, A.H., Holtman, V., Tsongalis, G.J. & Macer, J. Homocysteine screening of a female Hispanic population. *Int J Mol Med*, 1999; 4: 295-297.
- Wu, A.H., Jaffe, A.S., Apple, F.S., Jesse, R.L., Francis, G.L., Morrow, D.A., Newby, L.K., Ravkilde, J., Tang, W.H., Christenson, R.H., Cannon, C.P. & Storrow, A.B. National Academy of Clinical Biochemistry laboratory medicine practice guidelines: use of cardiac troponin and B-type natriuretic peptide or N-terminal proB-type natriuretic peptide for etiologies other than acute coronary syndromes and heart failure. *Clin Chem*, 2007; 53: 2086-2096.