Longitudinal Assessment of Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome

AUTHOR(S)

Aisling Smith

CITATION

Smith, Aisling (2022): Longitudinal Assessment of Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome. Royal College of Surgeons in Ireland. Thesis. https://doi.org/10.25419/rcsi.16834534.v1

DOI

10.25419/rcsi.16834534.v1

Longitudinal Assessment of Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome

Aisling Mary Smith

Thesis submitted for the degree of Doctor of Philosophy

2021

Department of Neonatology, The Rotunda Hospital, Parnell Square, Dublin, Ireland.

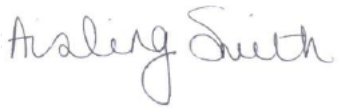
Royal College of Surgeons in Ireland, St Stephen's Green, Dublin, Ireland.

Supervisor: Professor Afif EL-Khuffash

Co-Supervisors: Professor Naomi McCallion & Doctor Orla Franklin

Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree Doctor of Philosophy, 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: 

Scholar Number: 17180911

Date: 18th of October 2021

Table of Contents

Abbreviations & Symbols

A:	Pulse wave Doppler late filling velocity
a':	Tissue Doppler late diastolic velocity
ANOVA:	Analysis of variance
ASD:	Atrial septal defect
AVSD:	Atrioventricular septal defect
Ca++:	Calcium
cGMP:	Cyclic guanosine monophosphate
CHD:	Congenital heart disease
CPAP:	Continuous positive airway pressure
CpG:	5'—C—phosphate—G—3'
CS:	Caesarean Section
DS:	Down Syndrome
DSCAM:	Down syndrome cell adhesion molecule
DS-CHD:	Infant with Down syndrome and congenital heart disease
DSCR:	Down syndrome critical region
DS-no CHD:	Infant with Down syndrome and no congenital heart disease
DS-no Surg:	Infants with DS who did not require surgical correction for congenital heart disease
DS-Surg:	Infants with DS who required surgical correction for congenital heart disease
e.g.:	Exempli gratia
E:	Pulse wave Doppler early filling velocity
e':	Tissue Doppler early diastolic velocity
ea':	Tissue Doppler early diastolic to late diastolic velocity ratio
ECG:	Electrocardiogram
ECMO:	Extracorporeal Membrane Oxygenation
Ee':	Transmitral pulse wave Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity ratio
eNOS:	Endothelial Nitric Oxide Synthase
FBC:	Full blood count

GLS:	Global longitudinal strain
ICU:	Intensive care unit
iNO:	Inhaled nitric oxide
IQR:	Interquartile range
IVCT:	Isovolumic contraction time
IVCV:	Isovolumic contraction velocity
IVRT:	Isovolumic relaxation time
IVRV:	Isovolumic relaxation velocity
Kg:	Kilograms
LA:	Left atrium
LV EI:	Left ventricular eccentricity index
LV:	Left ventricle
m/s:	meters per second
mm:	Millimetres
ms:	Milliseconds
N:	No
NCRC:	National Children's Research Centre
NO:	Nitric oxide
p:	p value
PAAT:	Pulmonary artery acceleration time
PAH:	Pulmonary arterial hypertension
PBF:	Pulmonary blood flow
PDA:	Patent ductus arteriosus
PEG:	Percutaneous endoscopic gastrostomy
PH:	Pulmonary hypertension
PICU:	Paediatric intensive care unit
PPHN:	Persistent pulmonary hypertension of the newborn
PVR:	Pulmonary vascular resistance
r:	Correlation coefficient
RA:	Right atrium
RV FW LS:	Right ventricular free wall longitudinal strain
RV:	Right ventricle

RVET:	Right ventricular ejection time
RV-PV:	Right ventricular pulmonary vascular
S:D:	Systolic to diastolic ratio
s':	Tissue Doppler peak systolic velocity
SD:	Standard deviation
SPSS:	Statistical package for the social sciences
SR:	Strain rate
STE:	Speckle tracking echocardiography
SVR:	Systemic vascular resistance
TAPSE:	Tricuspid annular plane systolic excursion
TDI:	Tissue Doppler imaging
TOF:	Tetralogy of Fallot
TPN:	Total parenteral nutrition
TR:	Tricuspid regurgitation
TV:	Tricuspid valve
VEGF:	Vascular endothelial growth factor
VIT:	Velocity time integral
Vs:	Versus
VSD:	Ventricular septal defect
Y:	Yes
ε:	Myocardial strain

List of Figures

List of Tables

Summary

Introduction: Infants with Down syndrome (DS) are at an increased risk of cardiopulmonary morbidity from birth. However, there is a dearth of information regarding longitudinal assessment of myocardial performance and pulmonary haemodynamics in infants with DS, both with and without structural cardiac disease, over the first two years of age.

Hypothesis & Aim: Our study hypothesis was that babies with DS have both persistent myocardial impairment and develop pulmonary hypertension (PH) more frequently and to a more severe extent than their non-DS counterparts, independent of structural cardiac disease. The aim of this study was to serially assess left ventricular (LV) and right ventricular (RV) performance and measurements of pulmonary haemodynamics in babies with DS, with and without congenital heart disease (CHD), utilising advanced echocardiography techniques over the first two years of age and to compare those measurements to a cohort of non-DS controls.

Methods: This was a prospective observational cohort study performed across the three tertiary neonatal units of Dublin. All infants with a diagnosis of DS made antenatally or postnatally were eligible for inclusion. Echocardiography was performed at six time points over the first two years of age for both the DS and control cohorts using novel echocardiography modalities including tissue Doppler imaging and speckle tracking echocardiography.

Results: Seventy babies with DS and 60 control infants were enrolled. Forty-eight (69%) of infants with DS were born with CHD. There is sustained impairment of biventricular systolic and diastolic performance and indices of PH in infants with DS, irrespective of structural cardiac disease, compared to controls over the first two years of age.

Conclusion: This work provides new insights into the evolution of LV and RV function and pulmonary haemodynamics in infants with DS over the first two years of age.

Acknowledgements

PhD Supervisor

Professor Afif EL-Khuffash: Department of Neonatology, The Rotunda Hospital, Dublin, Ireland.

I will be forever grateful for the expert leadership, huge kindness, and friendship of my supervisor Professor Afif EL-Khuffash. Thank you for a brilliant three years and the endless cups of coffee.

PhD Co-Supervisors

Professor Naomi McCallion: Department of Neonatology, The Rotunda Hospital, Dublin, Ireland.

I wish to sincerely thank Professor Naomi McCallion for her warmth, enthusiasm, and ongoing mentorship.

Doctor Orla Franklin: Department of Cardiology, Children's Health Ireland at Crumlin, Dublin, Ireland

I wish to sincerely thank Doctor Orla Franklin for her essential cardiology expertise, continuing guidance, and kind encouragement.

PhD Collaborators

- Professor Jan Miletin: The Coombe Women & Infants University Hospital, Dublin, Ireland.
- Doctor Anna Curley: The National Maternity Hospital, Holles Street, Dublin, Ireland.
- Professor Philip Levy: Department of Paediatrics, Boston Children's Hospital, Boston, Massachusetts, USA.
- Professor Eleanor Molloy: The Coombe Women & Infants University Hospital, Dublin, Ireland.

I am extremely grateful for the tremendous support of Professor Jan Miletin, Doctor Anna Curley, Professor Philip Levy and Professor Eleanor Molloy for this work. Thank you all.

Research Group

- Doctor Neidin Bussmann: Department of Neonatology, The Rotunda Hospital, Dublin, Ireland. Dr. Bussmann performed echocardiography assessments and data collection. She contributed 2% of the overall thesis work.
- Doctor Colm Breatnach: Department of Cardiology, Children's Health Ireland at Crumlin, Dublin, Ireland. Dr. Breatnach performed echocardiography assessments and data collection. He contributed 2% of the overall thesis work.

I wish to acknowledge the valued contributions of Doctor Neidin Bussmann and Doctor Colm Breatnach to this work and to thank them sincerely for their enthusiasm and camaraderie.

I wish to acknowledge the Neonatologists of The Rotunda Hospital; Professor Michael Boyle, Professor David Corcoran, Doctor Breda Hayes, Professor Adrienne Foran, Doctor Katie Cunningham and Doctor Margaret Moran, the Advanced Nurse Practitioners of The Rotunda Hospital; Christine McDermott, Professor Edna Woolhead and Mark Hollywood, and all of the amazing neonatal nursing staff for their outstanding support, kindness and encouragement throughout this study. Thank you all very much!

Most importantly, I wish to sincerely thank all the beautiful babies and their fantastic parents who took part in this study! I am deeply grateful for the enthusiasm, goodwill, and dedication you had for this work. Your altruism made this project possible, and it was my privilege to meet you.

Finally, I wish to thank my wonderful family, my parents James and Pauline and my sister Aoife, for their love and unwavering support throughout this PhD.

Academic Institutions

The Rotunda Hospital, Parnell Square, Dublin, Ireland.
Royal College of Surgeons in Ireland, St Stephen's Green, Dublin, Ireland.

Project Funding

This project received funding from the Health Research Board (NCHF-2017-005) and National Children's Research Centre (NCRC) Research and Education Support Grant (D/17/7).

Dedication

This work is dedicated to the memory of Declan McNamara

19th December 1954 – 5th April 1958

Chapter 1 : Introduction

1.1 Preamble

Down syndrome (DS) is the most common chromosomal abnormality of live born babies globally. The estimated incidence of DS worldwide is 1 in 700 newborns[1].The incidence of DS in Ireland is 1:546 live births, the highest rate in Europe[2]. DS was first described in 1866 by the English physician John Langdon Down and is characterised by over 80 clinical features with significant variability in their frequency and severity[3].

Babies with DS are a vulnerable patient cohort who require increased respiratory support, intensive care and longer hospital admissions when compared to the general neonatal population[4-7]. A 2018 study identified the most common reason for neonatal unit admission of infants with DS to be low oxygen saturations, the differential diagnosis of which in an infant with DS includes sepsis, congenital heart disease (CHD) and pulmonary hypertension (PH)[8]. There is also growing recognition within the neonatal and paediatric communities of reduced myocardial function at all stages of development in the DS population, even in individuals with structurally normal hearts[9-12]. However, the specific impact of the diagnosis of DS in the presence and absence of CHD, and associated impact of PH on myocardial performance over the first two years of age, remain poorly documented. There is a distinct lack of literature detailing serial measurements of pulmonary haemodynamics in babies with DS over the early newborn period. In addition, there is a dearth of information regarding longitudinal changes in both myocardial performance and pulmonary haemodynamics in infants with DS, with and without CHD, over the first two years of age. With the availability of advanced echocardiography techniques including tissue Doppler imaging (TDI), deformation analysis and left ventricular rotation mechanics it is now possible to accurately detect myocardial impairment in the neonatal and paediatric populations before the appearance of clinically overt disease. In Ireland we are uniquely poised to study this vulnerable population with one of the highest incidences of DS in the western world[2]. Further insight into the specific pathophysiological basis of myocardial function and PH evident in the babies and children with DS could aid our ability to diagnose

cardiorespiratory morbidity, provide earlier opportunities for therapeutic intervention, improve prognostication, and potentially impact positively on clinical outcome.

1.2 Principles of Cardiac Function

Prior to discussion of cardiac performance, pulmonary haemodynamics and the echocardiography techniques utilised to assess relevant parameters, a brief review of the principles of myocardial function is warranted. Myocardial function is the composite result of three physiological variables: intrinsic contractility, preload and afterload[13] **(Figure 1.1)**. Intrinsic contractility describes the force generated at myofiber level due to crosslinking of thick and thin filaments within the myofibril contractile unit at a certain stretch. Preload refers to the myocardial sarcomere length just prior to ventricular contraction, the best approximation for which the volume of blood in the ventricular cavity at end diastole[14]. Afterload refers to the resistance against which the ventricle must deform to eject blood.

Myocardial function may be impacted by various alterations in preload and afterload. The Frank-Starling relationship dictates that increasing preload will result in higher cardiac output up to a critical point, after which persistent over-stretching of the myocardium results in a reduction in contractility[15]. Preload is influenced by several physiological variables including hydration status, venous return and ventricular diastolic compliance[14, 16]. An increase in afterload is initially met with an increase in contractility (coupling) up to a certain threshold beyond which contractility cannot increase (uncoupling). Once uncoupling has occurred ongoing increases in afterload will cause a reduction in myocardial deformation and cardiac output[16]. Important physiological variables dictating the degree of afterload to which the ventricle are exposed is vascular resistance, the viscosity of blood and the patency of ventricular outflow tracts[16]. In summary, the term 'cardiac contractility' is not synonymous with 'cardiac function' but rather specifically refers to the force generated within myofibril contractile unit. The term 'cardiac function' encompasses the amalgamated interactions between intrinsic contractility, preload and afterload and therefore may be altered by forces imposed on any one of these three components.

With regards to the specific event times during the cardiac cycle; systole is the phase of the cardiac cycle when ventricular contraction and ejection of blood from the ventricles into the arteries occurs. Diastole is the phase of the cardiac cycle when ventricular relaxation occurs and ventricular filling from the atria to the ventricles occurs. Diastole is biphasic with an early (E) and late phase (A). Active ventricular relaxation occurs during early phase diastole and atrial contraction occurs in late phase diastole. Isovolumic contraction time is the interval of the cardiac cycle between the closing of atrioventricular valves (mitral and tricuspid valves) and the opening of the semi-lunar valves (aortic and pulmonary valves). Isovolumic relaxation time is the interval of the cardiac cycle between aortic valve closure and mitral valve opening.

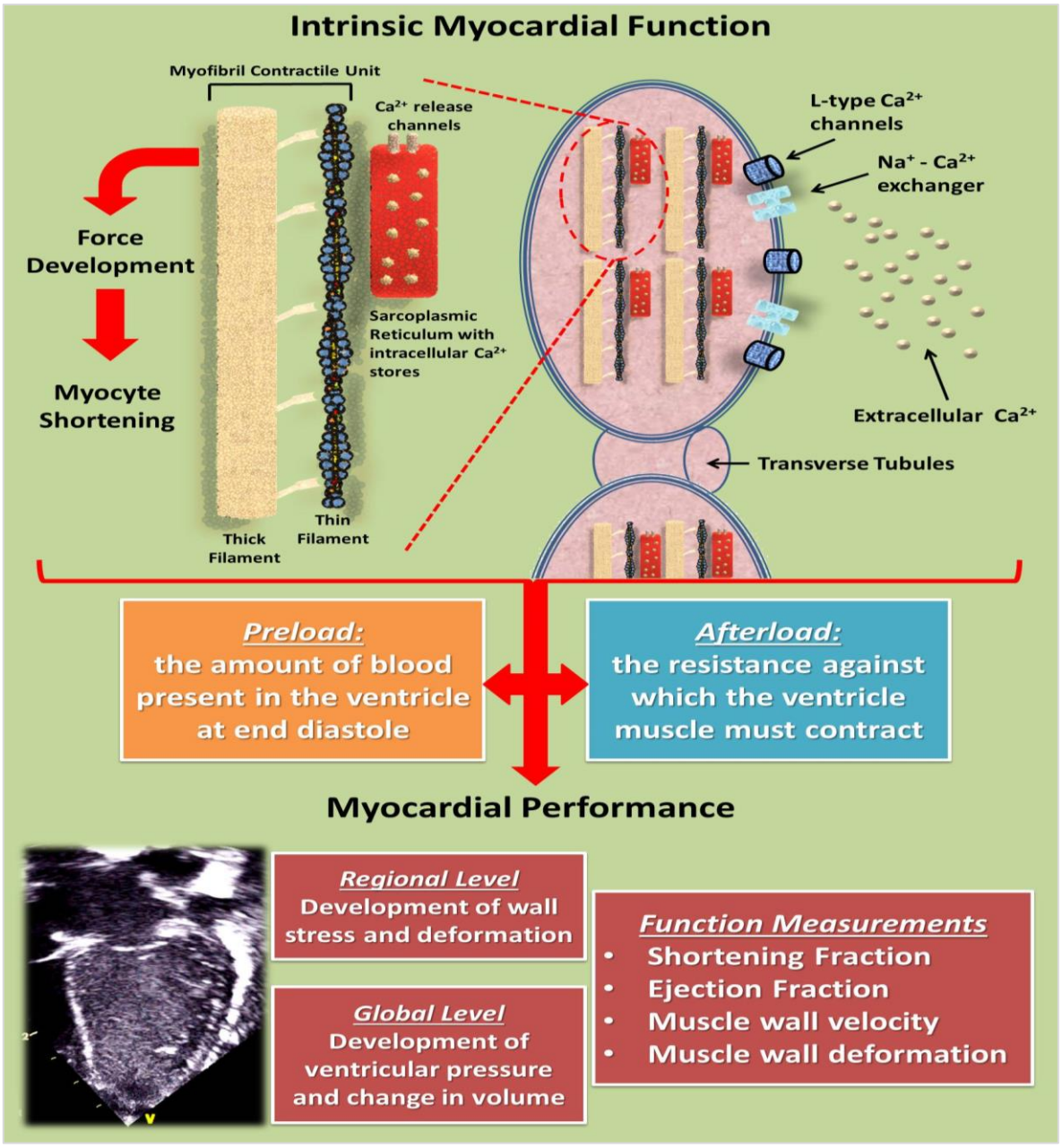


Figure created by Prof. Afif EL-Khuffash. Figure used with permission.

Figure 1.1: Myocardial performance.

The top panel of the illustration depicts the myofibril contractile unit. Extracellular calcium entry triggers intracellular calcium release from the sarcoplasmic reticulum causing crosslinking of the thick (myosin) and thin (actin) filaments. This crosslinking within the myofibril contractile unit generates contractile force. The bottom panel of the illustration depicts the interplay between preload, afterload and intrinsic function in determining overall myocardial function and echocardiography measurements used to quantify myocardial performance.

1.3 Foetal Circulation and Post-Natal Circulatory Transitioning

An understanding of foetal circulation and post-natal circulatory transitioning is necessary to fully appreciate the implications of abnormalities in this process and their impact on neonatal cardiovascular performance and pulmonary haemodynamics.

In utero oxygenated blood from the placenta courses through the umbilical vein, through the ductus venosus, inferior vena cava and enters then the right atrium. Much of this oxygen rich blood is shunted from the right atrium (RA) directly across the patent foramen into the left atrium (LA) allowing oxygenated blood to flow directly into the foetal systemic circulation. From the LA, blood enters the left ventricle (LV) from which it is pumped via the aortic arch branches to supply oxygen rich blood predominantly to the brain and upper body of the foetus. Deoxygenated blood returning from the upper body of the foetus via the superior vena cava mixes with the oxygenated blood entering the RA. Blood in the RA which is not shunted across the PFO flows into the right ventricle (RV). The RV is dominant during foetal development and is responsible for approximately 60% of blood flow from the foetal heart[17]. Only an estimated 10% of foetal RV output passes through the high resistance pulmonary vascular bed. The vast majority of RV cardiac blood flow passes from the pulmonary artery across the patent ductus arteriosus (PDA) to the aorta providing systemic blood flow to the lower body of the foetus. While the foetus is attached to the placenta the foetal heart exists in a low afterload environment. In utero the right-sided pulmonary circuit connected to the foetal lungs is the high pressure system, while the left-sided systemic circuit attached to the placenta is the low pressure system. However, the RV is protected from the high afterload of the pulmonary vascular bed due to the PDA, which preferentially shunts blood flow from the high pressure pulmonary circuit to the low pressure systemic circuit[18] **(Figure 1.2)**.

Major circulatory changes occur at birth. Following delivery, the baby takes their first breath and the umbilical cord is detached from the placenta. There is a significant increase in blood flow to the lungs to facilitate circulatory oxygenation and gas exchange with an associated drop in pulmonary vascular resistance (PVR)[19]. PVR is defined as resistance against blood flow from the pulmonary artery to the left atrium[20]. Following umbilical cord

clamping there is an associated rapid increase in systemic vascular resistance (SVR). Combined, these events result in the right sided pulmonary circuit becoming the low pressure system and the left-sided, systemic circuit becoming the high pressure system[21]. Now there is increased blood return from the lungs to the LA, increasing LA pressure, and a consequent change in direction of blood flow across the PFO. The previously right to left shunt which supported systemic blood flow in utero now flips to a left to right direction in postnatal life. Similarly, the drop in PVR and rise in SVR reverses the PDA shunt. In healthy, term babies the reversal of PDA flow to a left to right (systemic to pulmonary) shunt typically occurs by 24 hours of age[22]. Collectively, the circulatory adaptations to ex-utero life cause significant changes in the loading conditions to which the RV and LV are exposed **(Figure 1.3)**. The neonatal heart adjusts gradually in the weeks and months after delivery to these new loading conditions via myocardial remodelling[23]. With age, LV hypertrophy occurs to match the rise in SVR and there is a reduction in RV myocardial thickness in line with decreasing PVR[24].

For the vast majority of newborn infants, post-natal circulatory transitioning occurs without fault. However, in the presence of foetal, maternal or peri-partum pathology, normal post-natal circulatory transitioning may be interrupted. Abnormal transitioning results in the maintenance of high PVR. PVR remains high as the pulmonary vasculature has not responded adequately to normal post-natal adaptive processes, the vascular network remains tight and blood flow through the lungs is significantly impeded. This has several important effects on cardiorespiratory function; sustained elevation of PVR places the RV in a high afterload environment which can progress to RV dysfunction, LV preload is severely reduced due to inadequate blood flow return from the lungs to the LA and ongoing high PVR will maintain right to left shunting across PDA. Together, such consequences of failed transitioning can result in poor oxygenation, low cardiac output and impaired end organ perfusion. This state of failed post-natal circulatory transitioning is called pulmonary hypertension (PH)[25]. PH varies in severity but can be life threatening if not identified early and managed appropriately. Babies with DS have specific genetic and anatomical characteristics which place them at increased risk for the development of PH in the neonatal period which will be fully described in later paragraphs.

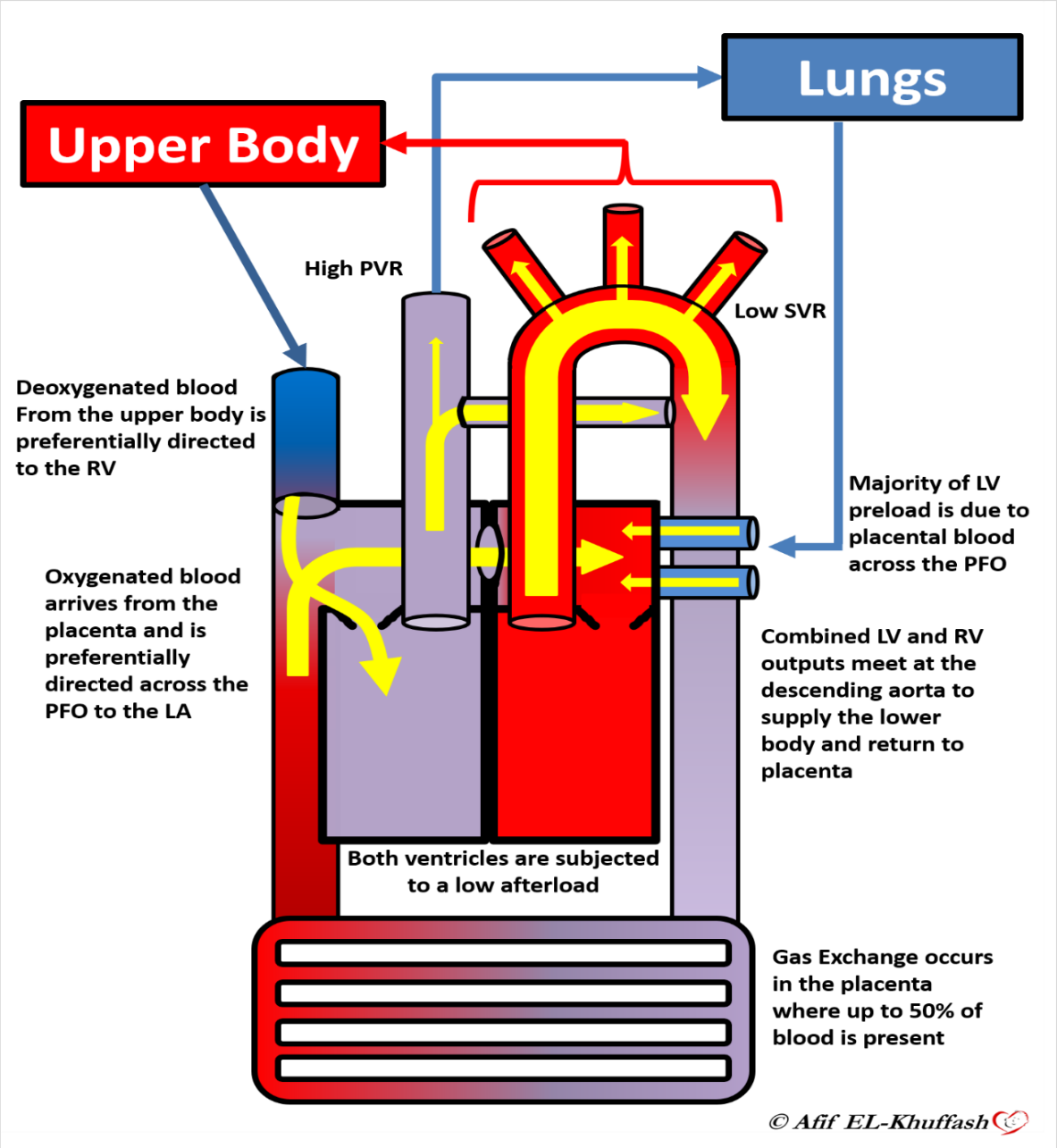


Figure created by Prof. Afif EL-Khuffash. Figure used with permission.

Figure 1.2: Foetal circulation.

This illustration depicts the unique features of the foetal circulation. LA: Left atrium; LV: left ventricle; PFO: patent foramen ovale; PVR: pulmonary vascular resistance; RV: right ventricle; SVR: systemic vascular resistance

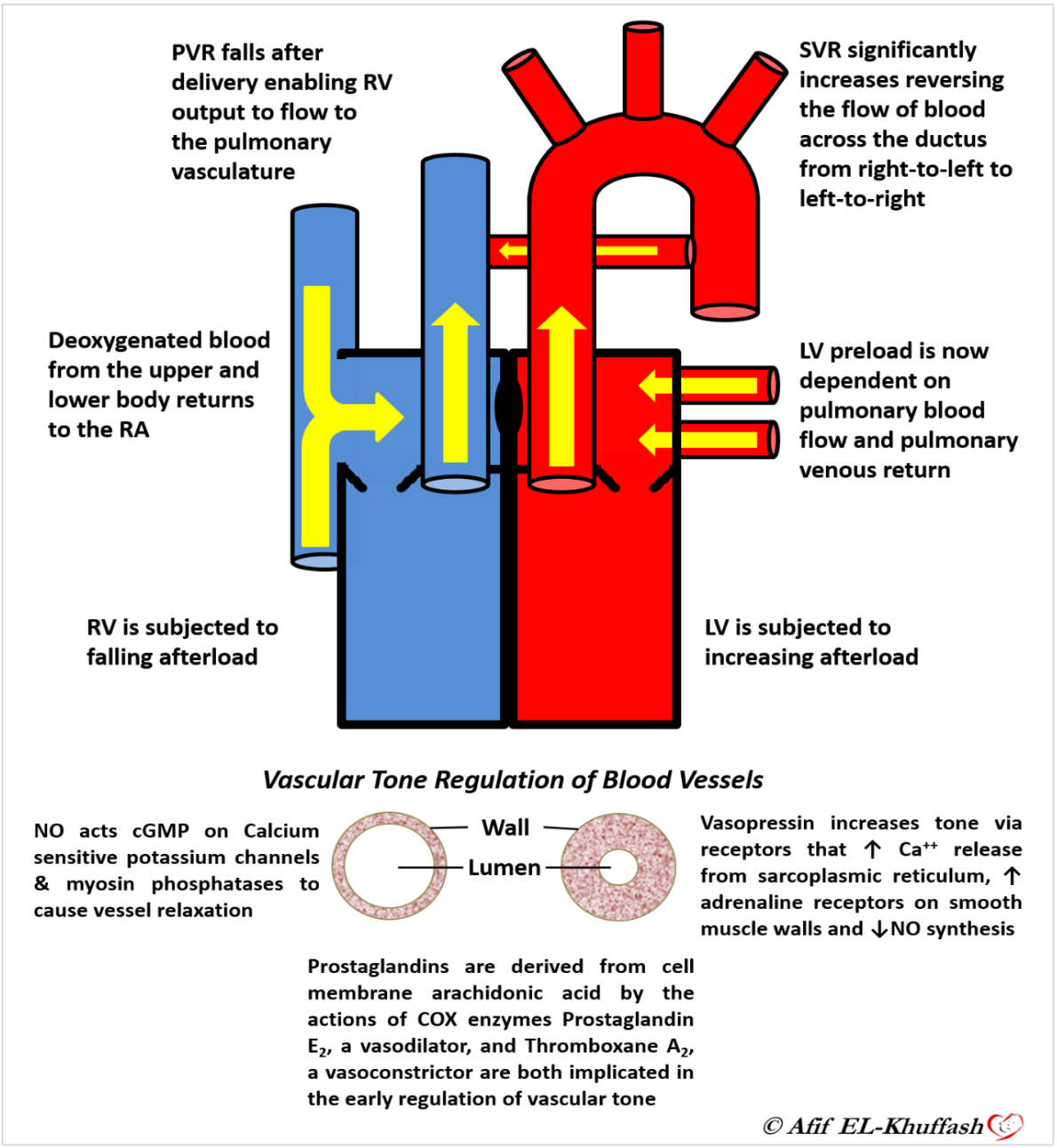


Figure created by Prof. Afif EL-Khuffash. Figure used with permission.

Figure 1.3: Neonatal circulation following birth.

This illustration depicts features of normal neonatal circulation following birth. Ca++: calcium; cGMP: cyclic guanosine monophosphate; LV: left ventricle; NO: nitric oxide; PFO: patent foramen ovale; PVR: pulmonary vascular resistance; RA: right atrium; RV: right ventricle; SVR: systemic vascular resistance

1.4 Novel Echocardiography Techniques

Monitoring cardiovascular wellbeing in many neonatal disease states remains challenging due to the insensitivity of clinical parameters in defining adequate systemic perfusion and identifying cardiac dysfunction and PH[26-28]. In addition, conventional echocardiography techniques such as shortening fraction and ejection fraction have known limitations in accurately appraising myocardial function[29]. However, in recent years major advances have been achieved in refining objective measures of biventricular myocardial performance and pulmonary haemodynamics in the neonatal and paediatric populations[30, 31]. Novel echocardiography techniques including deformation imaging, tissue Doppler imaging (TDI) and left ventricular (LV) rotation mechanics are accurate, objective techniques which can provide superior insight into the pathophysiological basis for cardiac impairment[32]. Right ventricular (RV) specific measurements such as RV fractional area change (FAC) and tricuspid annular plane systolic excursion (TAPSE) also enable quantitative assessment of RV function[33-36]. Reference ranges for STE and TDI are emerging in neonatal and paediatric populations[37-43]. Evaluation of pulmonary arterial pressure via echocardiography is also now possible. Pulmonary artery acceleration time (PAAT) is a validated echocardiographic measurement which is inversely correlated to pulmonary artery pressure and pulmonary vascular resistance[44].

Systolic and diastolic myocardial function may be assessed utilising conventional and novel echocardiography techniques, full explanations for which are detailed in the methodology chapter (Chapter 2). Briefly, deformation analysis is an advanced echocardiography technique which evaluates strain and strain rate measurements via STE. Myocardial strain (ε) is the term which quantifies the degree of myocardial deformation occurring during systole and is expressed as a percentage. Strain rate (SR) refers to the rate at which myocardial deformation occurs (s^{-1}). Myocardial strain is influenced by loading conditions; however, strain rate is relatively load independent and therefore a closer surrogate of inherent myocardial contractility[45-48]. Diastolic function may be interrogated using strain rate assessment, tissue Doppler evaluation of early (E) and late (A) diastolic velocities and E:A ratio measurements. In healthy term neonates the majority of LV filling occurs in the early phase of diastole with the early phase E wave possessing a higher

velocity than the late phase A wave. This phenomenon may be quantified using the E:A ratio, an assessment of diastolic function. These echocardiography methods have been utilised in previous studies to measure biventricular performance and pulmonary haemodynamics in individuals with DS at various ages, both with and without CHD.

1.5 Congenital Heart Disease and Myocardial Function in Infants with Down Syndrome

Approximately 50% of babies with DS will have some form of congenital heart disease (CHD)[49]. Atrioventricular septal defect (AVSD) is the most common cardiac anomaly in infants with DS (~45%) followed by ventricular septal defect (VSD)(~35%), secundum atrial septal defect (ASD) (~8%) and Tetralogy of Fallot (TOF) (4%) respectively[50]. Physical examination alone will not suffice for accurate detection of CHD in all individuals and echocardiography is recommended to identify any cardiac anomalies early in their neonatal course[28]. Structural cardiac disease confers a high risk of morbidity and mortality with CHD being the most frequent cause of death in children with DS over the first two years of age[51, 52]. CHD, even following surgical correction, carries long term health implications including the risk of re-operation, Eisenmenger syndrome and bacterial endocarditis[53]. CHD also impacts on neurodevelopmental outcomes with lower scores for receptive, expressive and composite language in infants and toddlers with DS who require corrective surgery in the first year of age compared to those children with DS and no CHD[54].

Structural abnormalities of foetal cardiac development in DS, including valve dysplasia and shorter ventricular septal length, are well recognised, both in those with and without AVSDs[55]. However, recent studies indicate that myocardial function in foetuses with DS is often abnormal, irrespective of the presence of CHD. Mula *et al* demonstrated evidence of foetal diastolic dysfunction via increased ductus venosus pulsatility index and increased tricuspid regurgitation in 28 foetuses with DS when compared to other aneuploidal foetuses and control foetuses[56]. Clur *et al* documented both systolic and diastolic dysfunction in foetuses with DS compared to controls, which manifest as reduced tricuspid valve (TV) A-wave velocity, reduced mitral valve A-wave velocity, reduced aortic valve peak velocity, reduced pulmonary valve peak velocity and reduced stroke volume[57].

In the neonatal population, our group have previously demonstrated that neonates with DS and structurally normal hearts have significantly lower RV strain values on day 2 and days 5-7 of age when compared to non-DS controls[9].

Similar studies have illustrated ongoing biventricular impairment with advancing age in children, adolescents, and adults with DS. A 2013 echocardiography study of 85 children aged between 7 and 13 years with DS and structurally normal hearts reported sub-clinical LV diastolic dysfunction and RV systolic and diastolic dysfunction when compared to age and sex matched controls[11]. This was demonstrated by significantly reduced LV tissue Doppler derived ea' ratio and prolonged LV isometric relaxation time (IVRT), and significantly reduced tissue Doppler derived RV peak systolic wave, prolonged RV isometric contraction time (IVCT), reduced RV ea' ratio and prolonged RV IVRT in children with DS compared to controls. As IVCT is inversely correlated with myocardial contractility and IVRT is inversely correlated to myocardial relaxation, prolongation of RV IVCT, LV IVRT and RV IVRT indicate impaired RV contractility and impaired LV and RV myocardial relaxation respectively[11]. Balli *et al* utilised STE in their study of 115 children with DS and structurally normal hearts who were aged between 6 and 13 years old to demonstrate significantly reduced early and late diastolic strain rate (SR) and early/late SR ratios compared to age and sex matched controls[10]. This myocardial impairment may impose a longstanding impact into later life, with an Italian study of adults with DS (mean age 36.1 +/-9.7 years) reporting that individuals with LV diastolic dysfunction demonstrated lower cognitive scores compared to those with preserved diastolic function[58].

1.5.1 Biological Mechanisms of Congenital Heart Disease and Myocardial Dysfunction in Down Syndrome

Dr. Jerome Lejeune confirmed the suspected association of DS with a chromosomal abnormality in Paris in 1959[1]. In 95% of cases DS occurs secondary to whole chromosomal aneuploidy, with the remaining 5% of cases resulting from translocations and mosaics[10, 59]. Trisomy of chromosome 21 results in an extra set of approximately 200 to 300 genes, however only ~30% of these genes are significantly overexpressed[51]. As approximately half of people with DS have structurally normal hearts, trisomy 21 alone is insufficient to cause CHD. This suggests that environmental and/or genetic modifiers interact with chromosome 21 to cause CHD, potentially in a dose dependant manner[60, 61]. There is growing appreciation of how such insights could contribute to our understanding of foetal cardiac development and structural cardiac disease[62, 63]. The DS critical region (DSCR) located on the long arm (q) of chromosome 21 is has been proposed as part of the molecular signal transduction pathway responsible for cardiac valve formation[51]. Barlow *et al* have also suggested the DS- cell adhesion molecule (DSCAM) gene of chromosome 21 as a potential genetic source of CHD in DS, as this 840kb region encodes a cell adhesion molecule expressed in the heart during cardiac development. Polymorphisms of the Endothelial Nitric Oxide Synthase (eNOS) gene are significantly associated with CHDs in DS[64, 65]. Evidence of abnormal angiogenesis has been detected in DS foetal heart tissue showing that mRNA expression of angiogenic factors, including vascular endothelial growth factor and placental growth factor, is significantly elevated compared to controls[66] Hypermethylation of the GATA4 gene, the translated protein of which is involved in myocardial differentiation and function, has also been identified in DS foetal heart tissue, indicating that epigenetic modifications and consequent deregulation of genetic expression may contribute to cardiac malformations and dysfunction[67]. In addition, environmental influences may potentiate or ameliorate the occurrence of CHD in DS through their interaction with the trisomic genome. For example, maternal cigarette smoking has been associated with AVSD, TOF and ASD while maternal folic acid supplementation in pregnancy may be associated with a reduced risk of CHD[68, 69]. Trisomy 21 may also negatively affect mitochondrial function through the downregulation of nuclear encoded mitochondrial genes as demonstrated in T21 foetal heart and brain tissues[70, 71]. Such disruption of expression of genes involved in

mitochondrial pathways can lead to decreased oxygen consumption, reduced ATP content and reactive oxygen species production[72]. Overall, such data emphasises that the underlying molecular interactions governing the DS cardiac phenotype are intricate, complex, and likely to exert multilevel effects on genetic regulation and expression.

1.6 Pulmonary Hypertension in Down Syndrome

This section of the introduction pertaining to pulmonary hypertension in Down syndrome, study rationale and study hypothesis has been published as a review article in The Archives of Disease and Childhood in 2020[73].

Pulmonary Hypertension (PH) is prevalent in children with DS with and without CHD, although the underlying aetiology and the timing of presentation may be different between these two groups. Early case series on PH in DS significantly underestimated the prevalence of PH in the setting of DS without CHD. PH in the DS population is a heterogeneous entity which results from multiple overlapping aetiologies. People with DS have specific anatomical, physiological and genetic tendencies that place this population at increased risk of developing PH in the early neonatal period (early PH) or at an older age with evolution of the pulmonary vascular disease (late PH). DS-related PH is currently categorised into two groups, WHO classification group I or III PH but the variable clinical presentation of PH in infants and children with DS, the frequent overlap between early and late PH and the multifactorial basis of PH in DS necessitate assuming a broader view of PH than is represented by traditional classification systems[74]. A more clinically relevant approach to diagnosis and management is to consider the timing of onset and the underlying pathogenesis of PH as an aberrant process of lung development.

1.6.1 Incidence of Pulmonary Hypertension in Infants with Down Syndrome

In the general neonatal population the rate of PH is estimated at 0.2%, significantly lower than that documented in neonates with DS[75, 76]. Early literature assessing PH in children with DS reported an incidence ranging between 1% and 5%; the majority of those infants were classified as having pulmonary arterial hypertension (PAH), however more recent data suggest that the true figure is significantly higher, ranging between 27% and 34%[77, 78]. Martin *et al* documented the overall incidence of echocardiography-confirmed PH in a cohort of 121 babies with DS at 34%, with no difference between those with and without CHD[8]. Bush *et al* reviewed records of 1242 children from birth to 21 years and showed an overall incidence of PH at 28%[77]. From the 346 patients with DS and PH, 241 (70%) had early PH, 53 (15%) had persistent PH and 52 (15%) had recurrent PH, by a median of 2.5 years of age. The differences in PH rates between early studies and recent reports in children with DS may be partially attributed to the varying clinical and echocardiographic diagnostic criteria applied to identify PH.

1.6.2 Early Pulmonary Hypertension

The paediatric task force at the world symposium on PH classified neonates with DS and CHD who present with early persistent PH of the newborn (PPHN) as Group I[74]. DS neonates without CHD with early PH are also clinically labelled as PPHN, but are within the Group III classification as a developmental lung disease[74].This distinction was made because of the recognition that infants with DS have high rates of PPHN and abnormalities of developmental lung disorders (e.g., reduced alveolarisation, decreased vessel density, persistence of the double-capillary network and hypertensive arterial remodelling)[77]. During the transitional period following birth, early PH in infants with DS is significantly associated with an increased necessity for invasive ventilation, more days of inhaled nitric oxide therapy, longer hospital admission and higher mortality before discharge[8, 79]. The Extracorporeal Membrane Oxygenation (ECMO) Registry reported that its use has increased in patients with DS over time with a higher likelihood of death following ECMO compared with non-DS ECMO patients (35% vs 25%) but that patients with a cardiac indication for ECMO have a higher mortality compared with those supported for respiratory indications[80,

[81]. Neonates with DS requiring ECMO have an increased incidence of bronchopulmonary dysplasia compared with neonates with DS not exposed to ECMO, adding further to their respiratory morbidity[82].

1.6.3 Biological Mechanisms of Early Pulmonary Hypertension in DS

The biological underpinnings of early PH in the context of DS are related to genetic and anatomical abnormalities identified in this population **(Figure 1.4)**. Neonates with DS are born with structural anomalies of the lung tissue including smaller total number of alveoli, decreased number of alveoli in relation to acini, and subpleural cysts [83-85]. Lung histology samples from 13 children with DS who died between the ages of 0 to 8 years demonstrated alveolar simplification, features of pulmonary arterial hypertensive remodelling, prominent bronchial vessels and persistence of a double capillary network in the distal lung[83]. Galambos *et al* observed that lung tissue from foetuses with DS overexpresses antiangiogenic factors, including collagen type XVIII alpha1, Collagen type IV alpha3 and tissue inhibitor of metalloproteinases metallopeptidase inhibitor 3, and suggested that impaired pulmonary vascular growth might subsequently cause impaired alveolarisation and progression to PAH[86, 87]. Collectively this can block capillary vasculature growth, a mechanistic perturbation that is associated with PH in adults [87]. Such inherent biological traits explain the significantly higher incidence of early PH that is clinically observed in this population of neonates.

1.6.4 Late Pulmonary Hypertension

With increased realisation of the significant association of DS with long-term cardiovascular and pulmonary morbidity, there is a growing interest in understanding how perinatal and postnatal physiological maladaptation predispose this high-risk population to an increased risk of late PH over time. It is likely that all the factors related to pulmonary vascular underdevelopment with early PH still play an important part in the persistence of PH in this population. Similarly, the aetiologies described below are more likely to

contribute to late PH but may also be implicated in the development of early PH during the early neonatal period.

Late PH is a major contributing factor to adverse outcomes in children and adults with DS[77]. A prospective study of 102 infants and children with DS undergoing corrective heart surgery demonstrated that those who required prolonged mechanical ventilation postoperatively (>72hours) had a higher incidence of PH at a younger age (83% vs 23%, p=0.012) and half of these patients required chronic treatment for chronic pulmonary hypertension on discharge home[88]. Iwaya *et al* reported lower preoperative pulmonary arterial compliance in individuals with DS and CHD when compared with non-DS individuals with CHD, and a significant association between low preoperative pulmonary arterial compliance in individuals with DS and the requirement for postoperative home oxygen therapy[89]. PAH is also associated with impaired linear growth during childhood, particularly in the presence of CHD[90].

In contrast to early PH associated with delayed transition following birth, late PH in children with DS is characterised by sustained and often progressive elevation of pulmonary arterial pressures that is diagnosed beyond the first few weeks of age. Late PH may develop in individuals originally diagnosed with early PH during the neonatal period, but can often develop in individuals with DS with initial successful postnatal transition that never manifest early PH. It is likely that some of the mechanisms of late PH are superimposed on the aforementioned congenital abnormalities of respiratory tissue and lung antiangiogenic factor expression, but other late PH aetiologies are linked to CHD, chronic hypoxaemia, recurrent respiratory illness, upper airway obstructions, sleep disorders, gastro-oesophageal reflux and altered immune function[86,91].

1.6.5 Aetiology of Late Pulmonary Hypertension

Children with DS are predisposed to upper airway obstruction and sleep disordered breathing due to a combination of midface hypoplasia, micrognathia, relative tongue enlargement, tracheal anomalies and generalised hypotonia [92-95]. Sleep disordered breathing is estimated to affect 30% to 75% of children with DS, compared with 2% of the general paediatric population[96]. In addition, children with DS have a reduced sympathetic drive and compromised cardiorespiratory response to sleep disordered breathing compared with healthy controls[97]. Pulmonary hypertension and cor pulmonale may be accelerated by this attenuated sympathetic response and concomitant chronic, intermittent hypoxia and respiratory acidosis induced by obstructive sleep apnoea.

Persistent systemic-to-pulmonary shunts secondary to CHD may contribute to the evolution of PH with increased pulmonary blood flow (PBF) contributing to a rise in mean pulmonary artery pressure **(Figure 1.4)**. In addition, the media of pulmonary arterioles is thinner in DS and will respond to the increased mechanical force and shear stress of systemic-to-pulmonary shunts by triggering an inflammatory response. This reaction will promote endothelial cell proliferation and subsequent thickening of the pulmonary arterial wall[98, 99]. Such adaptations may lead to permanent remodelling of the pulmonary vasculature and diminished production of nitric oxide, predisposing to a vasoconstrictive state[100, 101].

The immune system in infants with DS is known to function suboptimally, resulting in a lower total number of leucocytes, lymphocytes and monocytes as well as impaired maturation of T lymphocytes [102-104]. Chaushu *et al* demonstrated that individuals with DS and recurrent upper respiratory tract infections had significantly reduced immunoglobulin salivary secretion[105]. These immunological characteristics place patients with DS at higher risk of repeated upper and lower respiratory tract infections, which may exacerbate evolving PH.

Children with DS are prone to gastro-oesophageal reflux and episodes of aspiration due to structural abnormalities of the oropharyngeal airway, delayed oromotor function and

generalised hypotonia[92, 106]. Recurrent, chronic aspiration can result in significant respiratory morbidity including pneumonia, chronic cough, wheezing and the progression to pre-existing PH.

Pulmonary haemosiderosis, a rare disease of childhood, can also present with a more severe course in the context of DS.[107] It has been shown that children with DS younger than 20 years of age were more likely to present with an earlier onset of the disease, worse dyspnoea at diagnosis, more frequent secondary pulmonary hypertension and increased risk of fatal evolution[107].

Therefore, a complex interplay between structural, genetic an immunological deficiency exist in people with DS which can result in the evolution of PH.

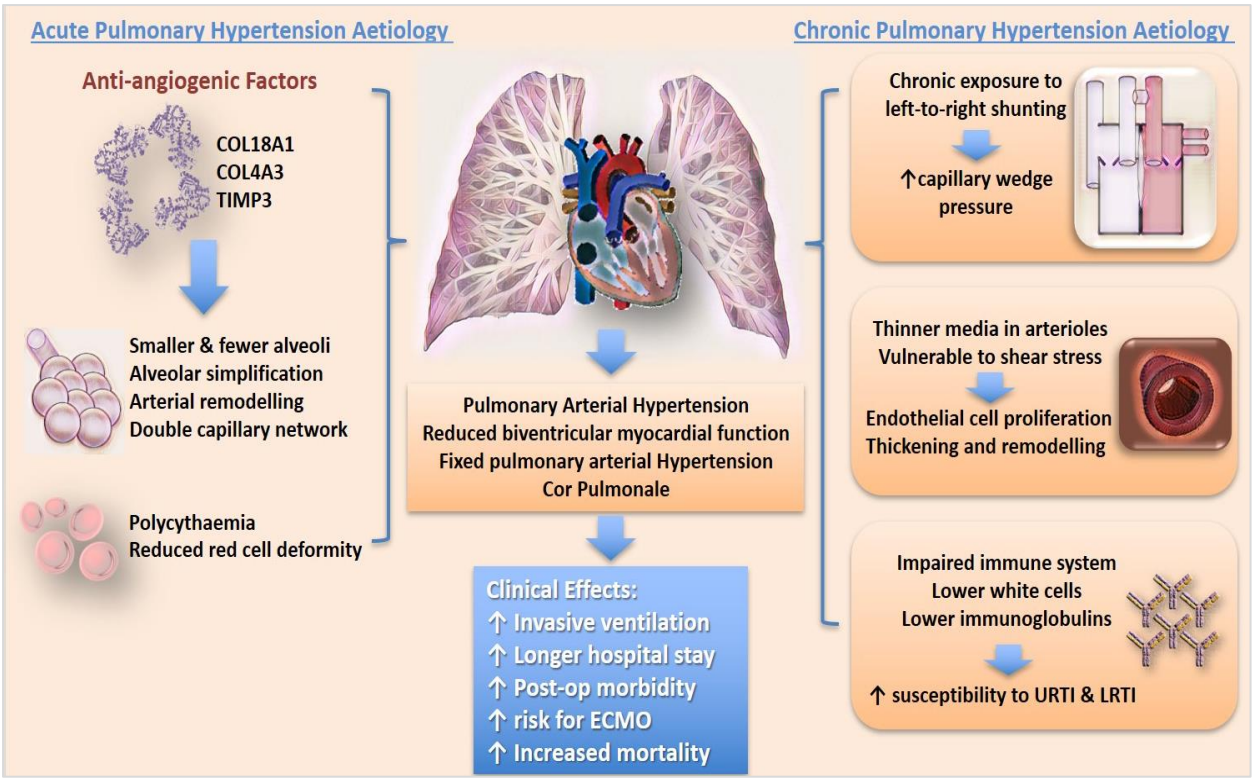


Figure 1.4: Aetiology of early and late pulmonary hypertension in Down syndrome.

This illustration depicts the aetiological factors of early and late pulmonary hypertension in infants and children with Down syndrome[73].

1.7 Impact of PH on Myocardial Function in Infants and Children with Down syndrome

PH results from a spectrum of cardiopulmonary factors and interactions that can impair the cardiac response to changes in loading conditions[108]. The challenge for the right ventricle (RV) and left ventricle (LV) in children with DS is to remain haemodynamically coupled to their respective circulations. Initially, the RV adapts to the increasing vascular load by enhancing contractility to maintain pulmonary blood flow (PBF). Important adaptive mechanisms that enhance contractile capabilities of the RV include muscle hypertrophy with increased wall thickness. Prolonged exposure to increased pulmonary vascular resistance and progressive pressure loading on the RV can lead to maladaptive ventricular remodelling, in which the RV dilates, stroke volume decreases, and heart rate increases to maintain cardiac output. RV systolic and diastolic dysfunction decrease stroke volume and lead to an overall reduction in cardiac output. Additionally, LV filling may also be affected by RV dilatation and septal bowing, with varying degrees of LV dysfunction manifesting due to the phenomenon of interventricular dependence.

Biventricular myocardial impairment is evident from infancy to adulthood in patients with DS, irrespective of the presence of CHD[9-11]. Breatnach *et al* documented that infants with DS and structurally normal hearts had more severe echocardiography-derived measures of early PH, including altered RV longitudinal strain values when compared with a healthy infant population without a diagnosis of DS[9]. Interestingly, RV longitudinal strain rate (SR) values were preserved in this study[9]. Strain measurements are influenced by myocardial loading conditions, and SR values are relatively load independent and a more accurate indicator of inherent myocardial contractility[30]. Therefore, the decrease in the magnitude of RV longitudinal strain values with preserved RV longitudinal SR values suggested that adverse loading conditions were the predominant cause for impaired function. Left ventricular rotation mechanics were also adversely affected in infants with DS over the first week of age in this 2019 study which demonstrated impaired LV basal rotation, resulting in reduced LV twist, torsion and twist rate[9].

1.8 Rationale for the Study

Life expectancy for individuals with DS is increasing with a median survival now estimated at 60 years of age[96, 109]. At present, cardiovascular and pulmonary disease account for an estimated 75% of mortality in individuals with DS. While most of the deaths occur in young adults, neonatal and infant mortality rates are higher in DS than the general population [110-113]. Furthermore, children with DS account for the most hospitalisations among all individuals with DS [110].

Neonates with DS represent a vulnerable patient cohort with a heterogeneous PH phenotype that confers a significant risk of morbidity and mortality. Overall, there is a growing appreciation of the high prevalence of PH in neonates, infants and children, both acute and chronic, and its association with serious adverse outcomes[8, 77, 100]. Despite the increased acknowledgement of the development of recurrent and later PH in children with DS, with and without CHD, there are no formalised guidelines that address how infants with DS with structurally normal hearts should be screened and followed as they mature. Bush *et al* recommended annual echocardiograms until school age for all individuals with CHD, early PH in the neonatal period, or who developed respiratory morbidities including chronic aspiration, recurrent pneumonias or obstructive sleep apnoea[77]. With increased recognition that the true incidence of PH is much higher than previously reported, the detrimental impact of PH on myocardial function in children with DS requires further clarification. Countries with a high incidence of DS are distinctly primed to evaluate this vulnerable population further.

The rationale for utilising novel echocardiography modalities including tissue Doppler imaging (TDI) and 2D STE is that such tools provide a more objective, sensitive, and accurate appraisal of myocardial performance than conventional echocardiography measurements. TDI and STE have been validated in adult, paediatric and neonatal populations, and offer the ability to assess systolic and diastolic function of both ventricles. Normative reference values now exist for preterm and term infants[31, 33, 37, 39, 40]. In addition, assessment of pulmonary pressures via echocardiography surrogates is also feasible. The PAAT is a validated echocardiographic measurement which is inversely correlated to

pulmonary artery pressure and pulmonary vascular resistance[44]. Such new echocardiography techniques provide an opportunity for the critical early detection and quantification of sub-clinical myocardial impairment and PH, with potential for therapeutic intervention prior to the evolution of fulminant global myocardial impairment[114, 115].

1.9 Study Hypothesis

Our study hypothesis was that babies with DS both have persistent myocardial impairment and develop PH more frequently and to a more severe extent than their non-DS counterparts independent of structural cardiac disease. Preliminary research suggested that PH impacts negatively on myocardial function, LV basal rotation and LV twist overall. This may be attributed to adverse loading conditions; however, impaired intrinsic contractility can also play a vital role.

1.10 Study Aim

The aim of this prospective, tri-centre, observational cohort study was to serially assess left ventricular (LV) and right ventricular (RV) performance and measurements of pulmonary haemodynamics in babies with DS with and without CHD utilising advanced echocardiography techniques over the first two years of age and to compare those measurements to a cohort of non-DS healthy controls. I also sought to evaluate the ability of echocardiography derived markers to predict important clinical outcomes including respiratory morbidity, clinically evident PH, hospital admissions, admission to a paediatric intensive care unit, necessity for ECMO and death.

Chapter 2 : Methodology

2.1 Ethical Approval

Ethical approval for this study was sought for both the Down Syndrome (DS) and control cohorts from the ethics committees of each of the three participating maternity hospitals in Dublin, Ireland: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. Ethical approval was granted from each site following application on the 'Standard Application Form for the Ethical Review of Health-Related Research Studies, which are not Clinical Trials of Medicinal Products for Human Use as defined in S.I. 190/2004'.

2.2 Study Design and Study Population

This was a prospective observational cohort study performed across the three tertiary neonatal intensive care units of Dublin: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. Each of the three maternity hospitals deliver 8,000 – 8,500 infants per year of which approximately 80 infants with DS are born annually. As such a city wide approach to recruitment of babies with provided the best opportunity for study enrolment. Consent, enrolment, and the initial echocardiography scans over the first week of age were performed in the neonatal department of recruitment. Longitudinal scans at 6 months, 1 year and 2 years of age were carried out in The Rotunda Hospital.

- Down Syndrome population

  All infants with a diagnosis of DS made antenatally or postnatally (later confirmed with Karyotyping) were eligible for inclusion. This included babies with DS with both congenital heart defects (CHD) and structurally normal hearts.

❖ Control population

In addition, a cohort of healthy term infants without a diagnosis of DS were enrolled in The Rotunda Hospital to serve as a control population. Healthy controls were infants born without clinical features of DS (and birth > 37 weeks gestation) and had no congenital anomalies, no maternal history of illness in pregnancy (diabetes of any type, pre-eclampsia, hypertension, clinical chorioamnionitis, polyhydramnios, oligohydramnios, pre-labour rupture of membranes, antepartum haemorrhage,) and did not require neonatal unit admission. The control cohort underwent echocardiography assessments at the same six time points as the DS cohort.

2.2.1 Sample Size

I planned a study of a continuous outcome variable from independent control and experimental subjects with 1 control per experimental subject. In our study, the continuous outcome variable used to determine the sample size was PAAT. In a previous pilot study, we observed a difference of 6ms in PAAT between infants with and without Down syndrome at birth (a 10% relative difference)[9]. PAAT within each group was normally distributed with a standard deviation of 10ms. If the true difference in the experimental and control means is 6ms, we will need to study 59 experimental subjects and 59 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.9. The Type I error probability associated with this test of this null hypothesis is 0.05. Based on this estimation, the number of infants recruited in each group was more than enough to demonstrate significant differences.

2.2.2 Information and Consent

Following approval from the consulting physician, parents of infants with DS and parents of control infants were approached and provided with verbal and written information regarding the study. Parents were given up to 12 hours to consider the participation of their infant in the study and written, informed consent was obtained prior to enrolment.

2.2.3 Exclusion Criteria

Infants were excluded if there was a lack of parental consent, or if there was a high likelihood of death over the first week of age.

2.3 Clinical Team

When a significant clinical finding was identified on echocardiography assessment in either the DS or control cohort I notified the treating physician and parents immediately and referred the patient to a consultant cardiologist for further evaluation.

2.4 Clinical Parameters

Clinically relevant parameters such as age (hours post delivery) and cardio-respiratory parameters were recorded including systolic blood pressure, diastolic blood pressure, mean blood pressure, mode of respiratory support, supplemental oxygen status, oxygen saturation measurements, intravenous fluid management and enteral feeding method. These were recorded during each echocardiography assessment over the first week of age.

2.5 Clinical Characteristics and Outcomes

Important antenatal, birth and neonatal characteristics as well as outcome data were collected for both the DS and control cohorts. This data collection was performed in the local neonatal unit of recruitment **(Table 2.1, Table 2.2)**. Longitudinal echocardiograms were performed in The Rotunda Hospital at 6 months, 1 year and 2 years of age for both the DS and control populations. Clinical data was recorded as per parental response at each follow up assessment **(Table 2.3)**.

Table 2.1: Clinical characteristics and outcome data collected over the first week of age.

Y: Yes; N: No; Kg: Kilograms; DS: Down Syndrome

Clinical Characteristics & Maternal Data	
Data	Output
Gestational age (Weeks)	
Birth weight (Kg)	
Maternal age (Years)	
Parity (Number)	
Male (Y/N)	
Multiple pregnancy (Y/N)	
Caesarean section (Y/N)	
1 and 5 Minute Apgar score (1-10)	
Cord pH (Number)	
Surfactant administration (Y/N)	
Cardiopulmonary resuscitation at delivery (Y/N)	
Adrenaline at delivery (Y/N)	
Chorioamnionitis (Y/N)	
Pre-eclampsia (Y/N)	
Absent end diastolic flow (Y/N)	
Polyhydramnios (Y/N)	
Oligohydramnios (Y/N)	
Antepartum haemorrhage (Y/N)	
Prolonged rupture of membranes (Y/N)	
Antenatal steroids x 2 (Y/N)	
Antenatal diagnosis of DS (Y/N)	
Atrioventricular Septal Defect (AVSD) (Y/N)	
Ventricular Septal Defect (VSD) (Y/N)	
Atrial Septal Defect (ASD) (Y/N)	
Other Cardiac Lesion (Narrate)	

Table 2.2: Outcome data collected over the first week of age.

Y: Yes; N: No

Outcome Data	
Data	Output
Culture positive sepsis (Y/N)	
Inotropes (Y/N)	
Inhaled Nitric Oxide (iNO) (Y/N)	
Intubation (Y/N)	
Ventilation (Y/N)	
Ventilation days (Number)	
Continuous Positive Airway Pressure (CPAP) Days (Number)	
Oxygen days (Number)	
Total Parenteral Nutrition Days (TPN) (Number)	
Days to full enteral feeds (Number)	
Hospital days (Number)	
Extracorporeal Membrane Oxygenation (ECMO) referral (Y/N)	
First Full Blood Count (FBC) (Haemoglobin/Platelets/ White Cell Count/Neutrophils)	
First oxygen saturation (Number)	
Discharge medications (Narrate)	
Died pre-discharge home (Y/N)	

Table 2.3: Clinical characteristics and outcome data collected over the first two years of age.

Y: Yes; N: No; Kg: Kilograms.

Data	Output
Age (months)	
L-thyroxine (Y/N)	
Normal FBC (Y/N)	
Hospital Admission (Y/N)	
Reason for Hospital Admissions (Narrate)	
Intensive Care Unit Admission (Y/N)	
Surgical Correction of Heart Defect (Y/N)	
Weight (Kg)	
Echocardiogram Findings (Narrate)	

2.6 Echocardiography Assessment

Echocardiography scans were performed at six time points over the first two years of age for both the DS and control cohorts. Three echocardiograms were carried out over the first week of age: Day 1, Day 2 and Day 3-5 and at three further time points over the first two years of age: 6 months, 12 months, and 24 months. Evaluations were performed using the Vivid echocardiography system (GE Medical, Milwaukee) and a cardiology multi-frequency probe. Echocardiography image acquisition was compliant with previously published guidelines with haemodynamically stable infants' supine position[116]. Echocardiograms were recorded on the echocardiography machine's internal hard drive and subsequently transferred to the Echo PAC archiving system for offline analysis.

The first echocardiogram of each infant included formal assessment for any congenital heart disease. Standard neonatal echocardiography windows including subcostal, parasternal long axis, parasternal short axis, apical, high parasternal, aortic arch and ductal views were utilised to perform a thorough evaluation of myocardial structure and function. No sedation was used during image acquisition at any age.

Examination of myocardial structure and function, pulmonary haemodynamics and patent ductus arteriosus (PDA) shunt characteristics were performed. Myocardial performance evaluation included both conventional and novel echocardiography techniques. Our research group and collaborators have previously reported the feasibility, reliability, and validity of tissue Doppler Imaging (TDI), deformation analysis by STE and rotation mechanics in the neonatal population[40, 41]. Details of the requisite techniques to obtain the echocardiography images and methods of offline analysis have been previously published[40, 41, 117-124]. All echocardiography images were used with permission from the 'Neonatologist Performed Echocardiography Teaching Manual' 2019 Edition by Prof. Afif EL-Khuffash[125].

2.7 Echocardiography Measurements

Anthropometric Measurements:

1. Aortic root diameter
2. Pulmonary artery diameter
3. Left ventricular dimensions; mitral valve annulus diameter and left ventricular length
4. Right ventricular dimensions; tricuspid valve annulus diameter, right ventricular basal diameter, right ventricular mid-cavity diameter and right ventricular length

Novel measures of myocardial performance:

1. Tissue Doppler velocities of the mitral, septal and tricuspid annuli
2. Tissue Doppler measured event timings; systolic time, diastolic time, isovolumic relaxation time and isovolumic contraction time
3. Tissue Doppler measured isovolumic relaxation velocity and isovolumic contraction velocity
4. Left ventricular longitudinal strain and longitudinal strain rate by speckle tracking echocardiography
5. Left ventricular rotation mechanics; basal rotation, apical rotation, left ventricular twist, left ventricular torsion, left ventricular twist rate and left ventricular untwist rate
6. Right ventricular longitudinal strain and longitudinal strain rate by speckle tracking echocardiography
7. Right ventricular pulmonary vascular coupling

Assessment of pulmonary haemodynamics:

1. Pulmonary artery acceleration time (PAAT)
2. Right ventricular ejection time (RVET)
3. PAAT:RVET ratio
4. Left ventricular eccentricity index

Assessment of patent ductus arteriosus parameters:

1. Patent ductus arteriosus diameter size in 2D
2. The direction and peak velocity of flow across the patent ductus arteriosus
3. Pressure gradient across the patent ductus arteriosus shunt

2.8 Anthropometric Measurements

2.8.1 Aortic Root Diameter

The aortic root diameter was measured in millimetres from the long axis parasternal view at the valve tips at the start of systole. Normal values for term infants on day one of age are 6.9 ±0.6 mm (mean (SD)) [31].

2.8.2 Pulmonary Artery Diameter

The pulmonary artery diameter was measured in millimetres from the long axis parasternal view at the valve tips at the start of systole. Normal values derived from the term control cohort of this project are 9 ± 1 mm.

2.8.3 Left Ventricular Measurements

The mitral valve annulus diameter was obtained in the 4 chamber view by measuring the distance between the mitral valve leaflets at the point of maximum opening in diastole in millimetres. The left ventricular (LV) length was obtained in the 4 chamber view by measuring the distance from the mitral valve and the LV apical wall at end diastole. Measurements were recorded in millimetres and normative data on LV dimensions have been previously published[31].

2.8.4 Right Ventricular Measurements

The tricuspid valve annulus diameter was obtained in the 4 chamber view by measuring the distance between the tricuspid valve leaflets at the point of maximum opening in diastole. The right ventricular (RV) basal diameter was obtained in the 4 chamber view by measuring the distance between RV base at the point of maximum tricuspid valve opening in diastole. The RV mid-cavity diameter was obtained in the 4 chamber view by measuring the mid-cavity distance at the point of maximum tricuspid valve opening in

diastole. The RV length was obtained in the 4 chamber view by measuring the distance between the tricuspid valve and the right ventricular apical wall at end diastole. Measurements were recorded in millimetres and normative data on RV dimensions have been previously described[33]

2.9 Novel Measures of Myocardial Performance

2.9.1 Tissue Doppler Velocities of the Mitral, Septal and Tricuspid Annuli

Pulse wave tissue Doppler velocities provide an objective and accurate evaluation of biventricular systolic and diastolic function. Tissue Doppler velocities may be obtained from the apical 4 chamber view at the basal segment of the left ventricular (LV) lateral wall, the interventricular septum and the right ventricular (RV) free wall using pulse wave **(Figure 2.1)**. This technique determines the systolic and diastolic velocities of myocardial motion from the base to the apex at the point of the mitral valve annulus, interventricular septum and tricuspid valve annulus thereby providing assessment of left and right ventricular function. The peak systolic velocity (s'), early diastolic velocity (e') and late diastolic velocity (a') were recorded in centimetres/second. The e' and a' diastolic velocities reflect active ventricular relaxation and atrial contraction phases of diastole respectively. If fusion of the e' and a' waves occurred, the single wave was documented as an a' wave. Details on image acquisition, measurement, interpretation, and reference ranges have been previously published[31, 40].

Measurements including the ea' ratio and Ee' ratio are also possible via tissue Doppler assessment. The early diastolic to late diastolic velocity ratio (ea') is an assessment of diastolic function with a low ea' ratio indicating the presence of diastolic impairment. The Ee' ratio provides an estimate of left atrial filling pressure utilising the ratio of transmitral pulse wave Doppler early filling velocity (E) to tissue Doppler derived early diastolic mitral annular velocity (e')[126, 127]. Higher values of Ee' ratio indicate elevated left atrial pressures.

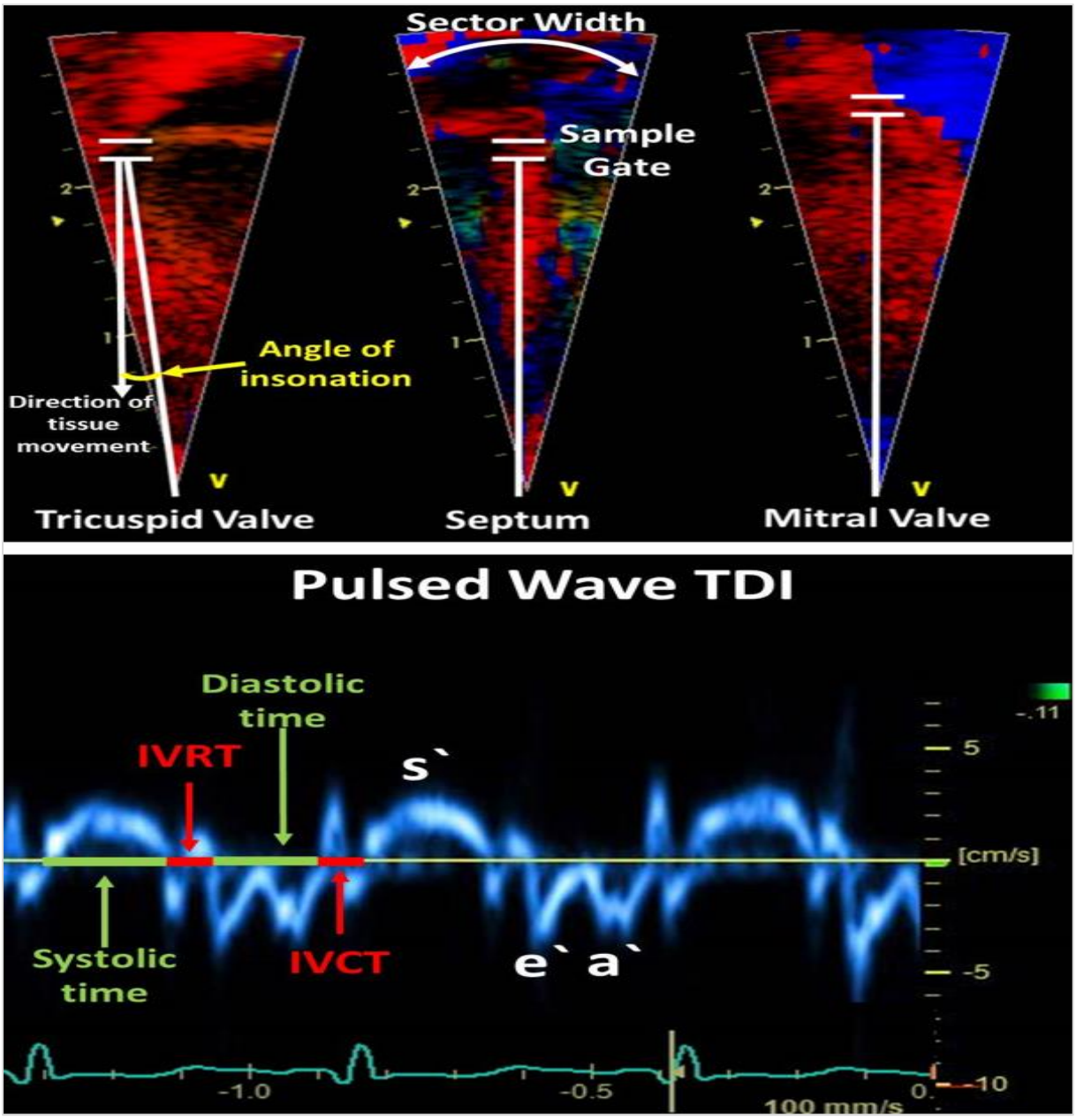


Figure 2.1: Measurement of tissue Doppler velocities.

Pulse wave tissue Doppler imaging (TDI) provides accurate assessment of myocardial velocities during systole (s'), early diastole (e') and late diastole (a') in the apical 4 chamber view at the left ventricular lateral wall, the interventricular septum and the right ventricular free wall. Myocardial movement towards the echocardiography probe is displayed as a positive wave and movement away from the probe is depicted as a negative wave. TDI also measures cardiac cycle event timings including systolic time, diastolic time, isovolumic relaxation time and isovolumic contraction time and their associated velocities of isovolumic relaxation velocity and isovolumic contraction velocity.

2.9.2 Tissue Doppler Measured Event Timings

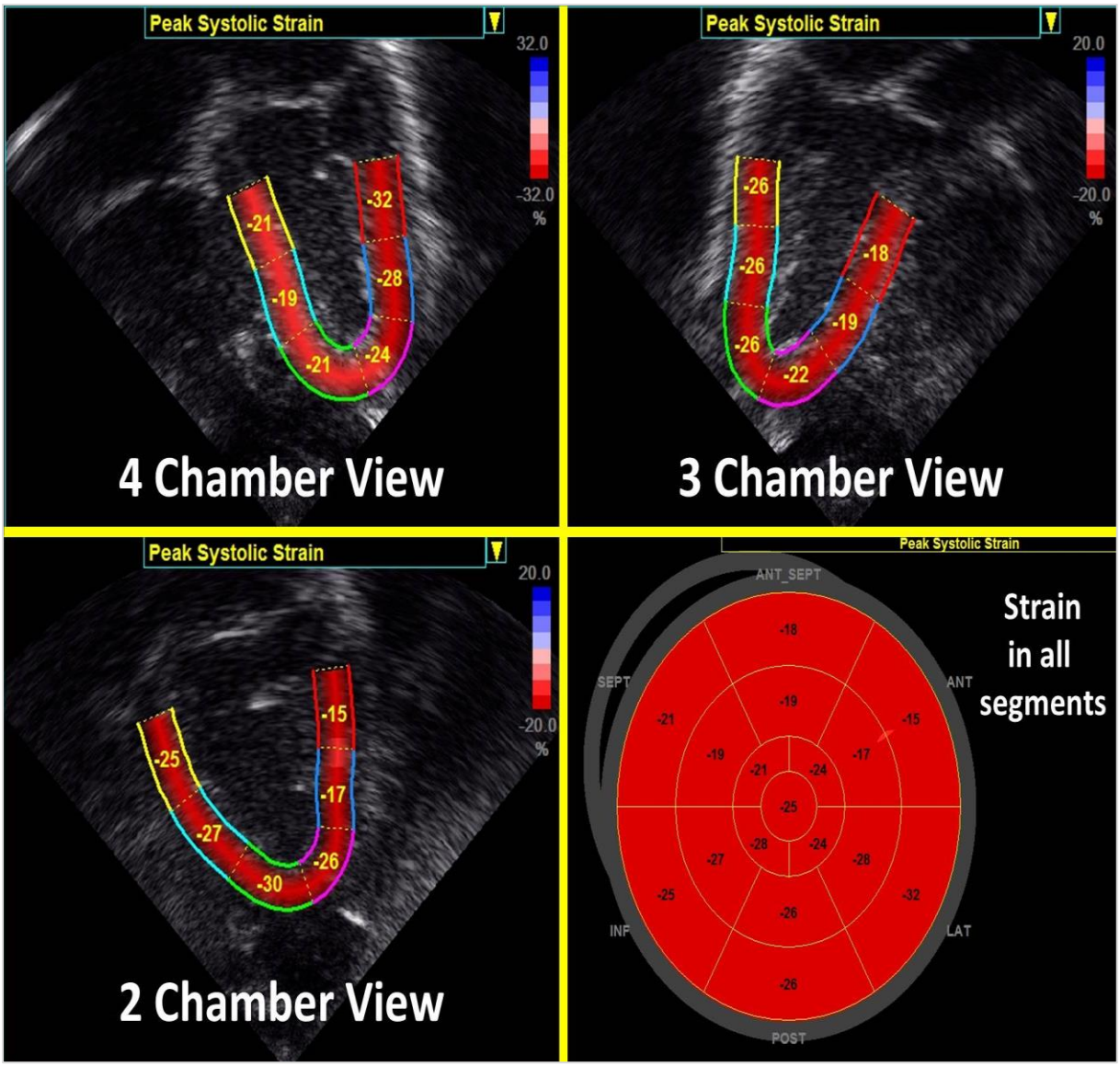
Tissue Doppler imaging (TDI) also provides cardiac cycle event timings including systolic time, diastolic time, isovolumic relaxation time (IVRT) and isovolumic contraction time (IVCT) **(Figure 2.1)**. Isovolumic contraction time is the interval of the cardiac cycle between the closing of atrioventricular valves (mitral and tricuspid valves) and the opening of the semi-lunar valves (aortic and pulmonary valves). Isovolumic relaxation time is the interval of the cardiac cycle between aortic valve closure and mitral valve opening. Evaluation of cardiac timing events is a method of myocardial performance evaluation. For example, an elevated systolic time: diastolic time ratio is a marker of diastolic dysfunction and a short IVRT may indicate the presence of a haemodynamically significant PDA which is forcing premature opening of the mitral valve in diastole due to left atrial volume loading[128]. Normative ranges of TDI event timings for term neonates are available[31].

2.9.3 Left Ventricular Longitudinal Strain and Longitudinal Strain Rate

Deformation refers to the change in shape of the myocardium from diastole to systole. 2D STE is a novel echocardiography technique which provides an objective and reliable evaluation of left and right ventricular systolic and diastolic function through deformation analysis. Myocardial strain (ε) is the term which quantifies the degree of myocardial deformation occurring during systole and is expressed as a percentage. Strain rate (SR) refers to the rate at which myocardial deformation occurs (s^{-1}). Myocardial strain is influenced by loading conditions; strain will increase with increasing preload and will decrease with an increase in afterload[45]. However, strain rate is relatively load independent and therefore a closer surrogate of inherent myocardial contractility[45-48].

To derive the deformation indices of strain and strain rate specialised 2D STE software is utilised which tracks the motion of myocardial speckles. Myocardial speckles result from acoustic backscatter generated by the ultrasound beam. By tracking the movement of myocardial speckles frame by frame over the cardiac cycle 2D STE software will provide strain (the relative change in distance between those speckles) and strain rate (the speed at which the change in distance is occurring) measurements. Left ventricular (LV)

longitudinal deformation evaluation uses standard B-mode images of the LV in the 4, 3 and 2 chamber views. Each of the three views bisects the LV in a different plane to permit global assessment of LV performance **(Figure 2.2)**. To ensure accurate 2D STE evaluation the echocardiography images clearly outlined the cardiac walls, the frame rate was optimised, and a steady ECG signal was available to assess the event timings of the cardiac cycle. During offline analysis the endocardial border of the LV wall was traced in each of the 4, 3 and 2 chamber views. The 2D STE software algorithm divides the LV myocardial wall of the 4, 3 and 2 views into six segments. Six coloured curves for each view are generated based on the timing of the opening and closure of the semilunar valves. Each coloured curve represents the individual strain measurement of each of the six myocardial segments (basal septum, mid-septum, apical septum, basal lateral, mid-lateral, and apical lateral) and provides measurements of strain, systolic strain rate, early diastolic strain rate and late diastolic strain rate. The white dashed curve represents the average, global value strain or strain rate value calculated across the six myocardial segments within each specific echocardiographic view. The software calculates the global LV strain values as the average of the peaks from the curve for the entire region of interest from each of the 4 chamber, 2 chamber and 3 chamber views. A similar process was applied to the RV for calculation of RV longitudinal strain and longitudinal strain rate measurements. Detailed reports on echocardiography image acquisition, optimisation, interpretation, and normative ranges for neonatal deformation analysis have been previously published[31, 39, 41, 129]. Throughout the remainder of the thesis, we will report peak systolic strain and strain rate values without the negative sign for ease of graphical presentation. Therefore, a higher value indicates better myocardial function.



El-Khuffash A, Schubert U, Levy PT, Nestaas E, de Boode WP. Deformation imaging and rotational mechanics in neonates: a guide to image acquisition, measurement, interpretation, and reference values. Pediatr Res. 2018;84(Suppl 1):30-45

Figure 2.2: Measurement of 2D speckle tracking echocardiography derived strain of the left ventricle.

The speckle tracking echocardiography software divides the myocardial wall of the 4, 3 and 2 left ventricular (LV) views into six segments each and provides a global appraisal of LV strain by averaging the measurements across all the 17 segments[41]. The strain values from the individual segments are displayed in the 'bulls eye' illustration in the bottom right section of the diagram.

2.9.4 Left Ventricular Rotation Mechanics

Left ventricular (LV) rotation mechanics are an emerging echocardiography technique for further evaluation of LV performance. Rotation mechanics measure the degree of clockwise, basal rotation at the level of the mitral valve and the degree of anti-clockwise, apical rotation at the LV apex. The net effect of these two opposing rotation forces is called LV twist. LV twist supports ejection of blood from the LV during systole. LV untwist augments early diastolic filling by generating a negative pressure gradient and a 'suction' effect which promotes early diastolic LV filling. The speed at which the LV twists is termed the LV twist rate and the speed at which the LV untwists at is termed the LV untwist rate.

Measurements for LV rotation mechanics are obtained from the short axis parasternal view at the level of the mitral valve and apex with an appropriate frame rate **(Figure 2.3)**. Offline analysis of the echocardiography images permits evaluation of both basal and apical rotation. Clockwise rotation is depicted as a negative deflection while anti-clockwise rotation is depicted as a positive deflection. LV twist is calculated as the difference between clockwise, basal rotation and anti-clockwise apical rotation and is expressed in degrees of rotation. LV torsion is LV twist indexed to LV length and permits comparison of LV twist across varying LV sizes. Detailed information on echocardiography image acquisition and interpretation for LV rotation mechanics is available[41].

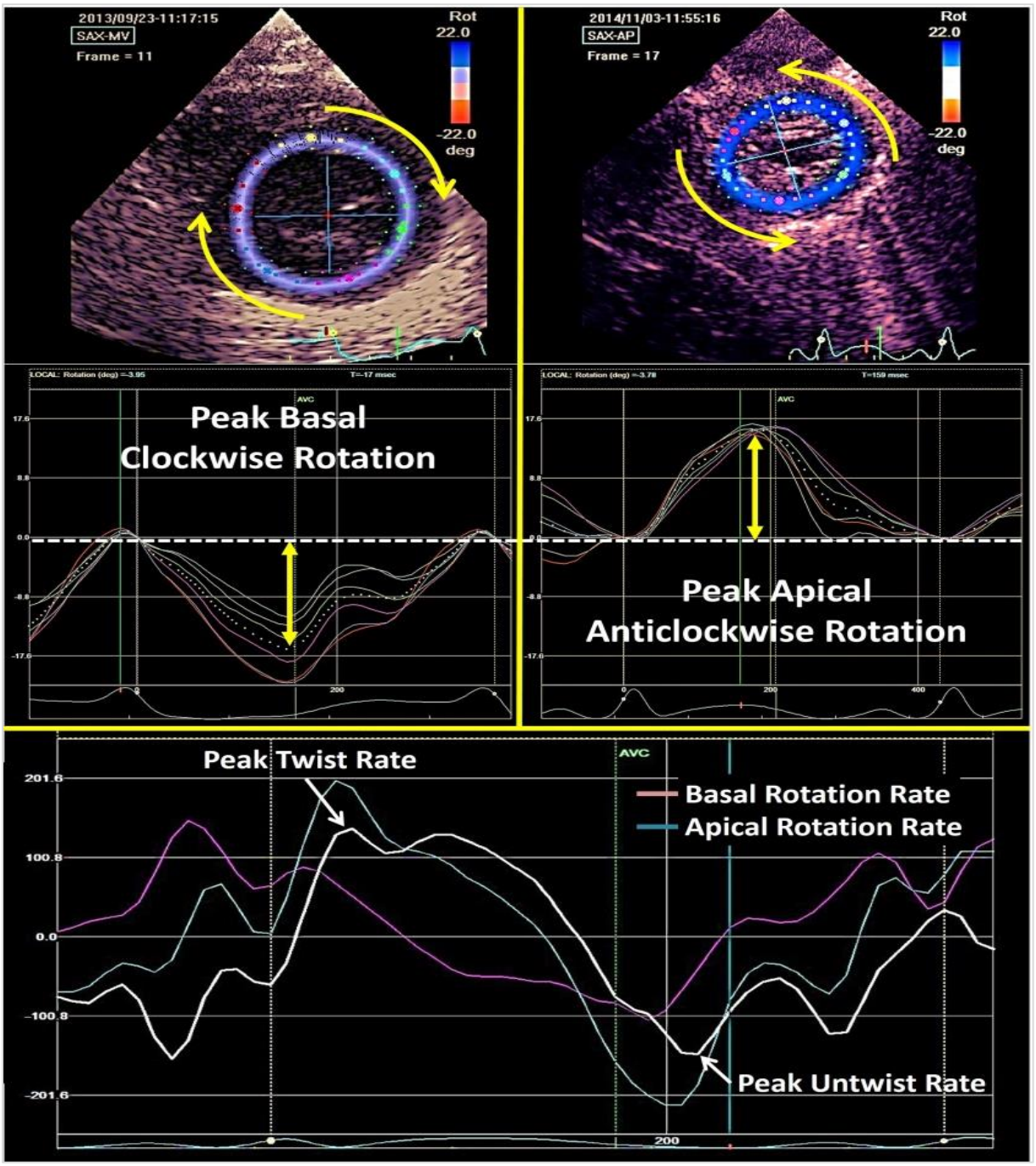


Figure 2.3: Measurement of left ventricular rotation mechanics.

This illustration depicts the clockwise, basal rotation and anti-clockwise apical rotation of the left ventricle (LV) at the level of the mitral valve and LV apex respectively. The degree of basal rotation is displayed as a negative deflection while the degree of apical rotation is displayed as a positive deflection. Peak values of basal and apical rotation may be measured from these deflections. LV twist is the net effect of basal and apical rotation and LV twist and LV untwist rates are the speed at which these events are occurring.

2.9.5 Right Ventricular Longitudinal Strain and Longitudinal Strain Rate by Speckle Tracking Echocardiography

As per deformation analysis of the left ventricle, 2D speckle tracking echocardiography may be utilised to provide longitudinal strain and longitudinal strain rate measurements of the right ventricle (RV). A focused 4 chamber view of the RV free wall is obtained for deformation analysis **(Figure 2.4).** The requirements and techniques of offline analysis to derive RV longitudinal strain and longitudinal strain rate measurements are as per the left ventricular analysis described above[41]. Normative ranges are available[33].

2.9.6 Right Ventricular Pulmonary Vascular Coupling

Right ventricular pulmonary vascular coupling (RV-PV coupling) describes the ability of the RV to increase its contractility in tandem with increasing RV afterload[130]. There are three stages of RV-PV coupling **(Figure 2.5).** Stage 1 represents normal conditions where the RV-PV axis is appropriately coupled. In stage 2 an increase in RV afterload has occurred. This is matched with a concomitant increase in RV contractility through the development of RV hypertrophy and RV-PV coupling is maintained. However, by stage 3, in the face of unrelenting increases in RV afterload the RV is no longer able to sustain normal contractility. As a result, the RV dilates, RV dysfunction ensues and now the RV is uncoupled from its afterload with an associated decrease in RV efficiency.

Assessments of RV-PV coupling may be derived from echocardiography surrogates. RV strain is utilised as a marker of RV performance and PAAT represents RV afterload. RV-PV assessments provide important insights into pulmonary haemodynamics and RV function in many populations[131, 132]. Lower values indicate diminished RV-PV coupling.

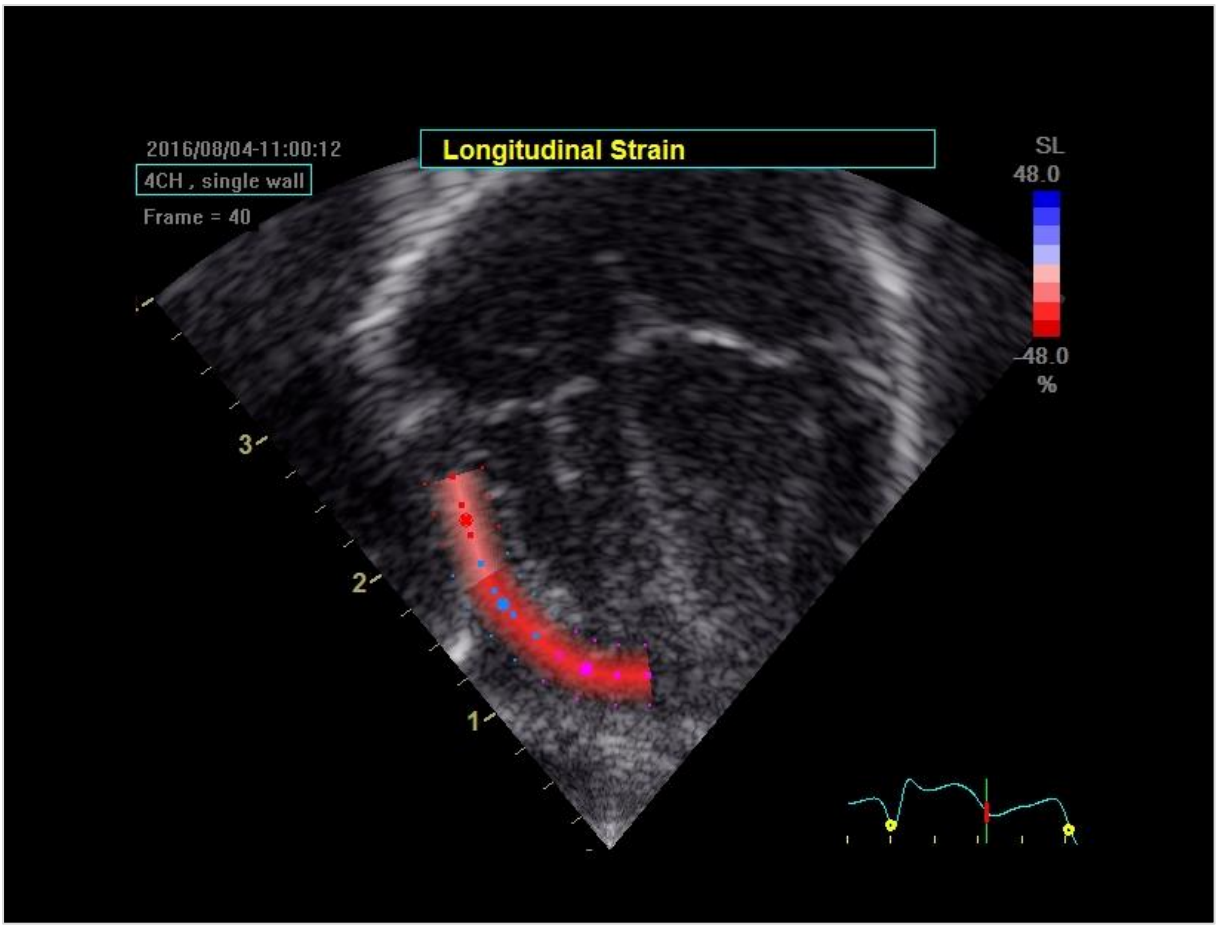


Figure 2.4: Speckle tracking echocardiography of the right ventricle.

Speckle tracking echocardiography of the right ventricle (RV) is derived from a focussed image of the RV in the 4 chamber view. The software calculates the global RV strain values as the average of the peaks from the curve for the entire region of interest.

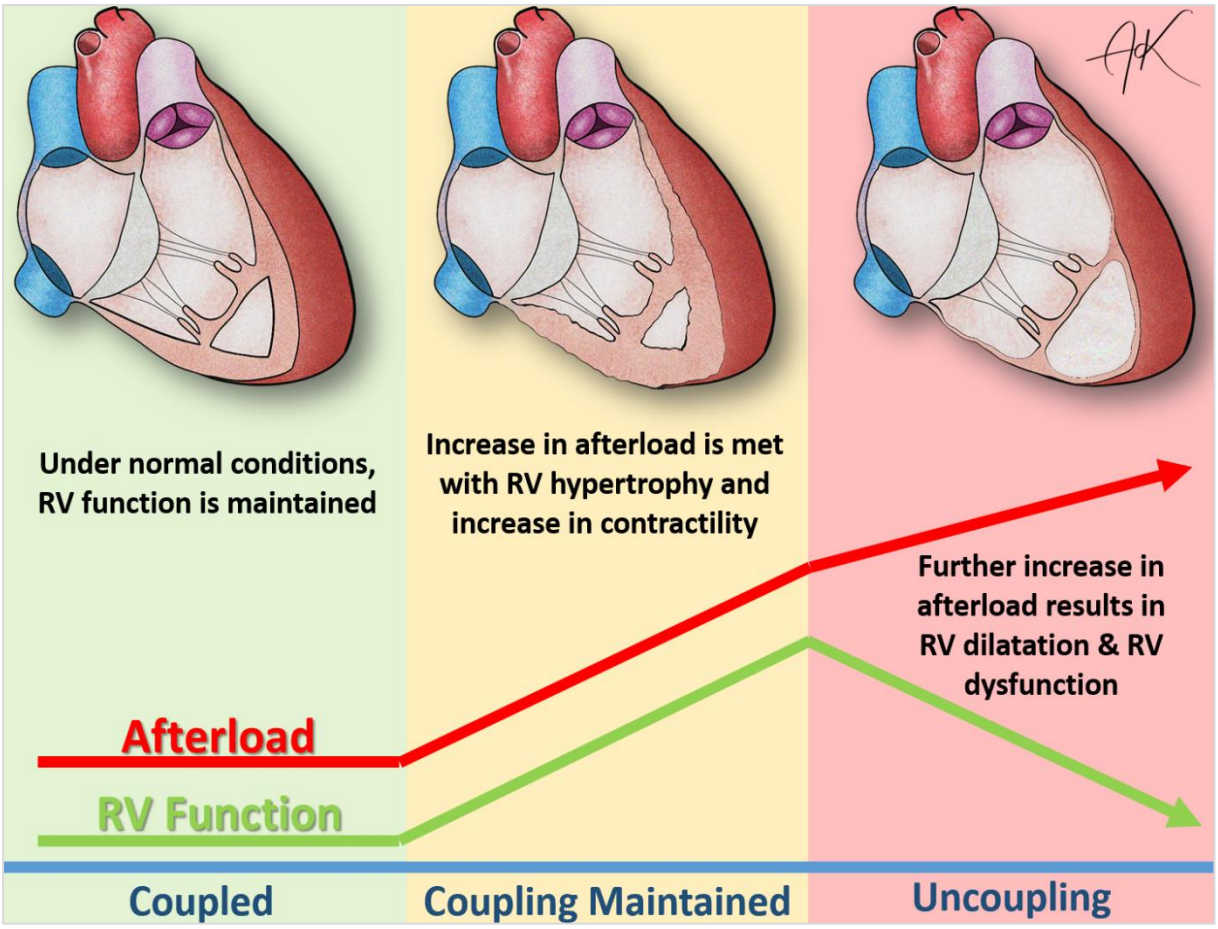


Figure 2.5: Right ventricular pulmonary vascular coupling.

There are three stages of right ventricular (RV) pulmonary vascular (PV) coupling. RV-PV coupling describes the ability of the RV to increase its contractility in tandem with increasing RV afterload.

2.10 Assessment of Pulmonary Haemodynamics

2.10.1 Pulmonary Artery Acceleration Time

PAAT is the time in milliseconds from the onset of the pulmonary artery wave to its maximum velocity as measured on the pulmonary artery velocity time integral (VTI) envelope **(Figure 2.6)**. PAAT is derived from the long axis parasternal view of the pulmonary artery. The PAAT is inversely correlated to pulmonary vascular resistance and has been validated against right heart catheter-derived measurements[44]. It is therefore a useful, non-invasive echocardiography derived marker of pulmonary hypertension. Normative ranges within the term neonatal population over the early newborn period are available[33]. Our research group defined a PAAT < 40 milliseconds as part of the diagnostic criterion for pulmonary hypertension in this study[44].

2.10.2 Right Ventricular Ejection Time

RV ejection time (RVET) is the time in milliseconds over which pulmonary artery flow occurs. This measurement is also obtained from the long axis parasternal view of the pulmonary artery from the pulmonary artery VTI envelope **(Figure 2.6)**.

2.10.3 PAAT:RVET ratio

The PAAT:RVET ratio correlates negatively with pulmonary hypertension and provides a semi-quantitative measure of the severity of pulmonary hypertension[133]. Our research group defined a PAAT:RVET ratio < 0.25 as part of the diagnostic criterion for pulmonary hypertension in this study[134].

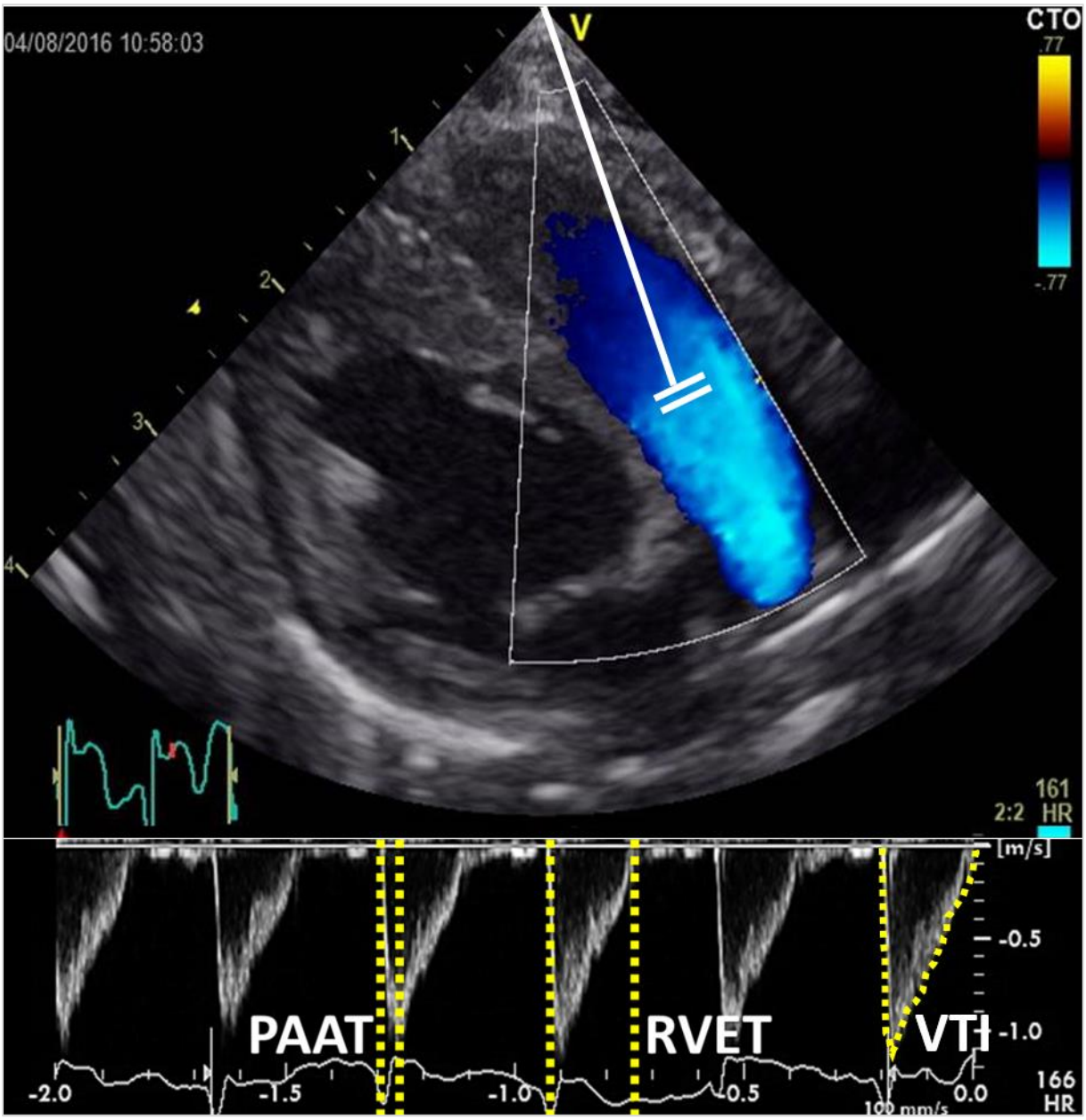


Figure 2.6: Measurement of pulmonary artery acceleration time and right ventricular ejection time.

The PAAT and right ventricular ejection time (RVET) measurements are captured from the long axis parasternal view of the pulmonary artery using pulse wave Doppler to produce the velocity time integral (VTI). PAAT is the time from the onset of the pulmonary artery wave to its maximum velocity as measured on the pulmonary artery VTI envelope. RVET is the total time over which pulmonary artery flow occurs and is also measured via the pulmonary artery VTI envelope.

2.10.4 Left Ventricular Eccentricity Index

Examination of the interventricular septum wall in systole from the short axis parasternal view can provide insight into the presence and degree of pulmonary hypertension. Typically, the septal wall curves into the right ventricle (RV) as systemic, left ventricular (LV) pressures exceed those of the RV. However, if RV pressures rise to half systemic or systemic levels the interventricular septum will become flat. If RV pressures exceed systemic pressures the interventricular septum will bow into the LV. Therefore, assessment of interventricular septum morphology can provide useful insights into the presence and severity of pulmonary hypertension.

LV eccentricity index (LV EI) is a quantitative assessment of the degree of interventricular septal flattening in systole from the short axis parasternal view **(Figure 2.7)**. The LV EI was defined as the ratio between the septal-posterolateral (D1) and anterior-inferior (D2) cavity dimensions at the mid-ventricular level of the papillary muscles (D2)[135]. The higher the ratio the higher the degree of interventricular septal flattening and associated elevation of RV pressures. Our research group defined an LV EI ratio > 1.8 as part of the diagnostic criterion for pulmonary hypertension (PH) in this study. This cut-off to define PH was ascertained from the control cohort data as an LV EI of 1.8 was the upper value of the mean + 2SD in this group. Therefore, 97.8% of the control cohort were below this cut off value of 1.8.

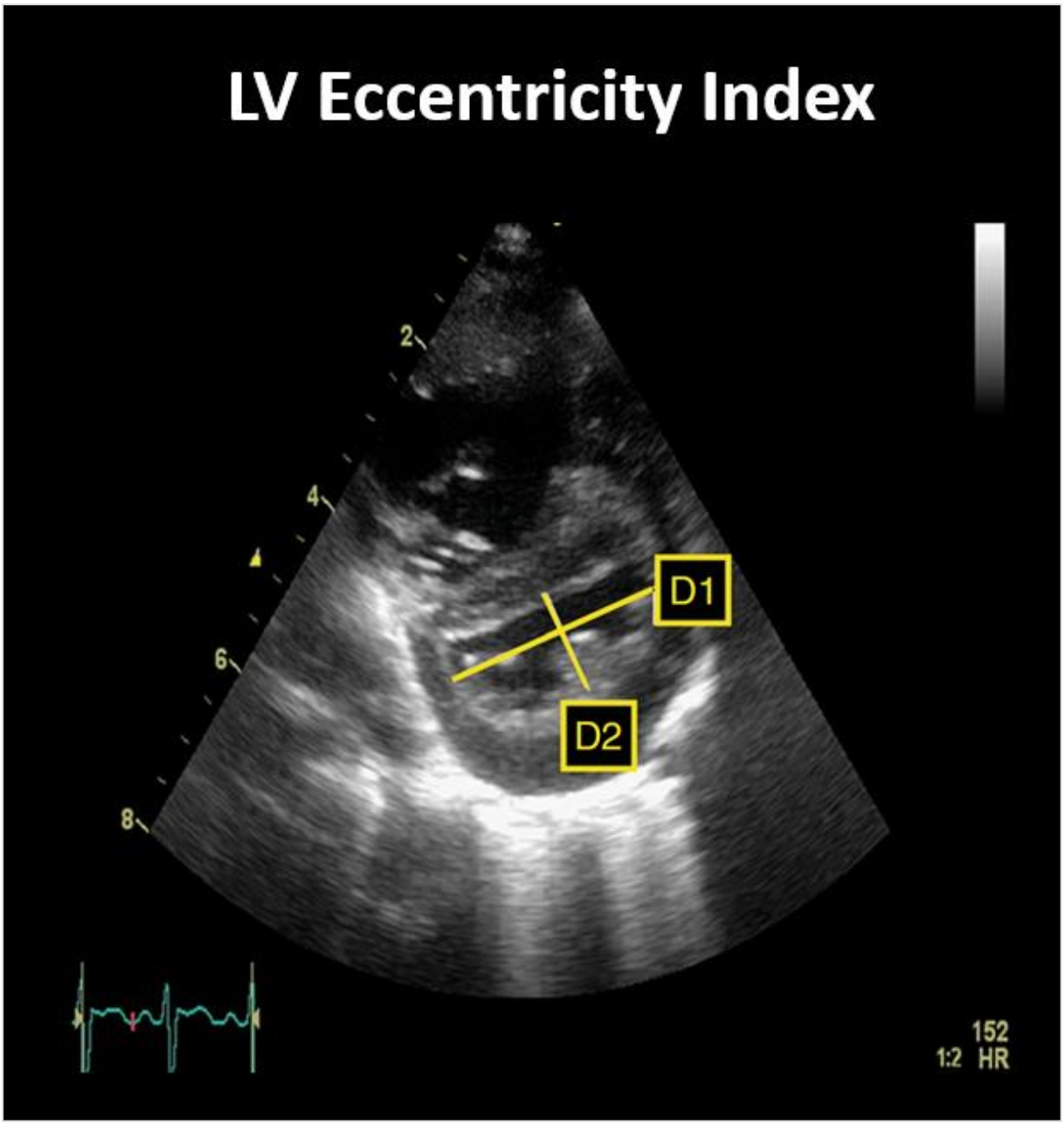


Figure 2.7: Measurement of the left ventricular eccentricity index.

Left ventricular end-systolic eccentricity index (EI) was assessed from the short axis parasternal view. EI is defined as the ratio between the septal-posterolateral (D1) and anterior-inferior (D2) cavity dimensions at the mid-ventricular level of the papillary muscles (D2)[135].

2.11 Assessment of Patent Ductus Arteriosus Parameters

2.11.1 Patent Ductus Arteriosus Diameter Size in 2D

The patent ductus arteriosus (PDA) was evaluated from the ductal view in the high parasternal window **(Figure 2.8)**. The diameter of the PDA was measured using 2D mode at the pulmonary artery end in millimetres.

2.11.2 The Direction and Peak Velocity of Flow across the Patent Ductus Arteriosus

If systemic pressures exceed pulmonary pressures the blood flow across the PDA will be aortic to pulmonary; left to right flow. If pulmonary pressures exceed systemic pressures the blood flow across the PDA will be pulmonary to aortic; right to left flow. The direction of blood flow across the PDA was assessed using colour Doppler and continuous wave Doppler imaging. Left to right flow (aortic to pulmonary flow) will be red on colour Doppler imaging and possess an upward deflection on continuous wave Doppler imaging. Right to left flow (pulmonary to aortic) will be blue on colour Doppler imaging an possess a downward deflection on continuous wave Doppler imaging.

Bidirectional blood flow across the PDA occurs when pulmonary pressures exceed systemic pressures in systole. In this case colour Doppler imaging will be blue in systole and red in diastole. The continuous wave Doppler imaging will depict a downward deflection in systole and an upward deflection in systole **(Figure 2.9)**.

2.11.3 Pressure Gradient across the Patent Ductus Arteriosus Shunt

The systolic and diastolic velocities of the PDA were measured using pulse wave and continuous wave Doppler with the cursor aligned parallel to the ductal flow **(Figure 2.8, Figure 2.9)**.

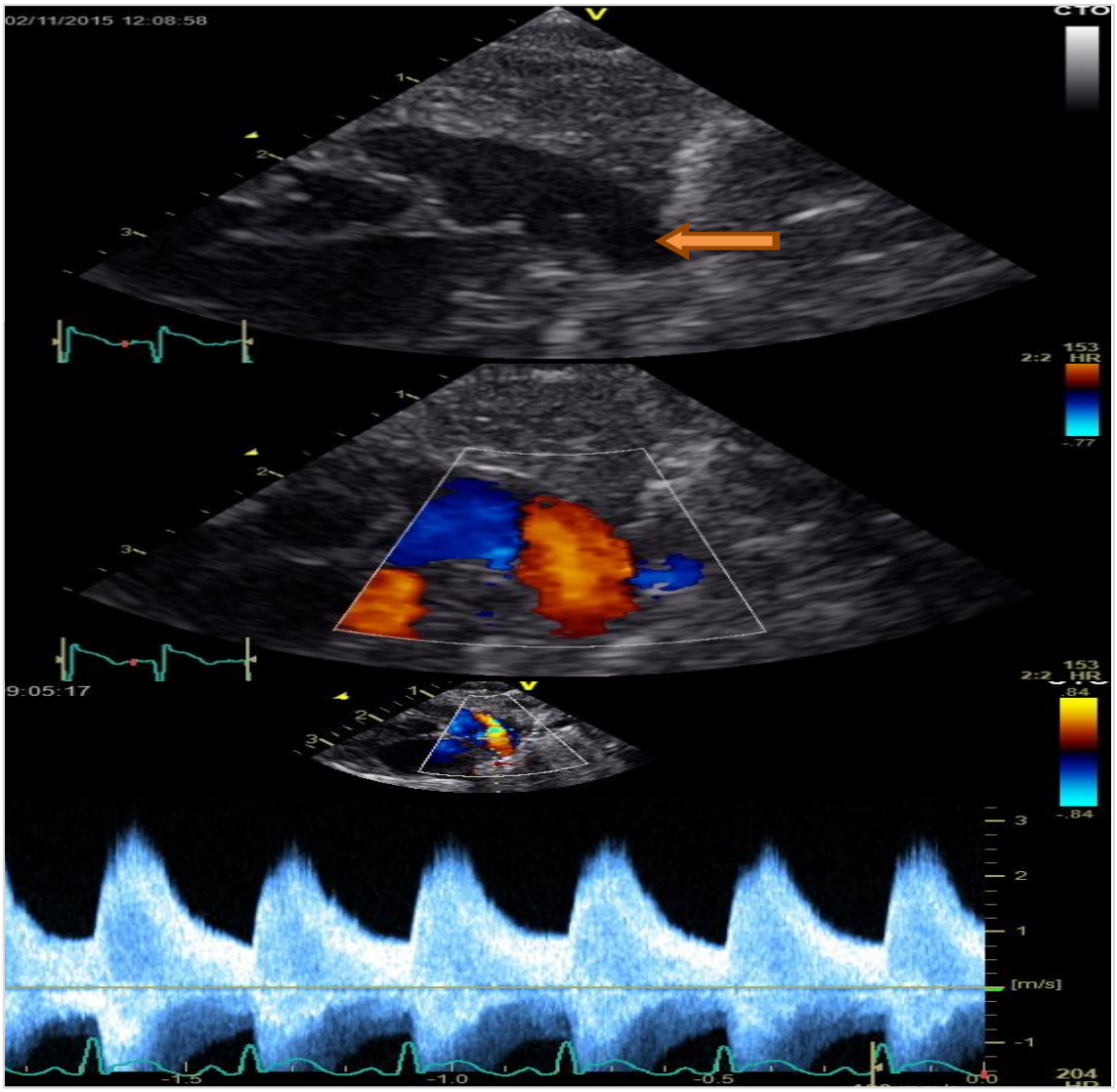
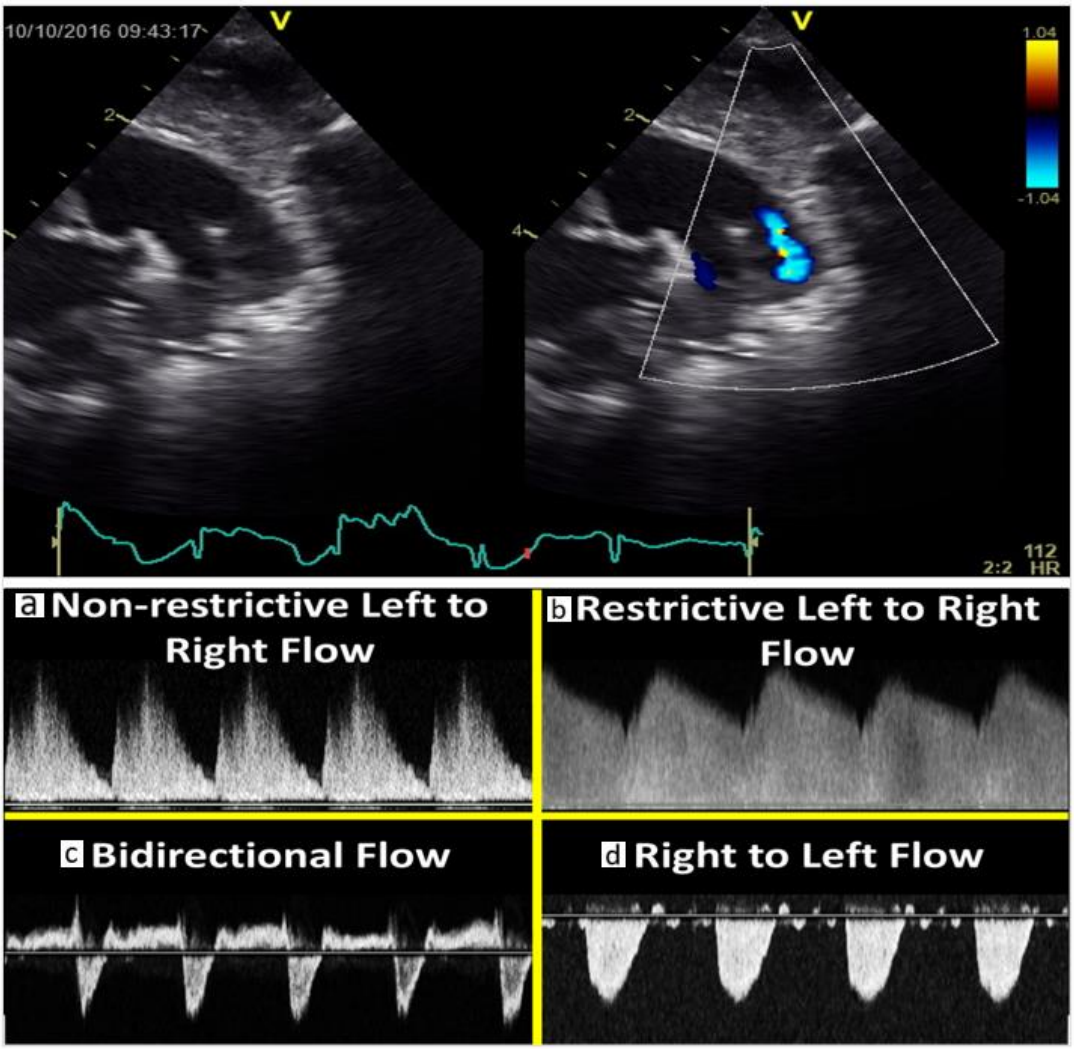


Figure 2.8: Measurement of patent ductus arteriosus diameter, colour Doppler flow and continuous wave Doppler flow.

The patent ductus arteriosus (PDA) was assessed from the ductal view in the high parasternal window. The diameter was measured in 2D mode at the pulmonary artery end (top panel). Colour Doppler imaging permitted evaluation of the direction of ductal flow (middle panel). Pulse and continuous wave Doppler imaging was utilised to assess peak systolic and diastolic flow velocities (bottom panel). The timing of the cardiac cycle is identified via the electrocardiography (ECG) trace.



van Laere D, van Overmeire B, Gupta S, El-Khuffash A, Savoia M, McNamara PJ, et al. Application of NPE in the assessment of a patent ductus arteriosus. Pediatr Res. 2018;84(Suppl 1):46-56

Figure 2.9: Measurement of patent ductus arteriosus colour Doppler flow and continuous wave Doppler flow patterns.

The top panel illustrates blue, right to left flow across the patent ductus arteriosus (PDA) in colour Doppler imaging; a marker of suprasystemic pulmonary pressures. The bottom panel illustrates the four common flow patterns across the PDA; a) non-restrictive left to right flow indicates an open PDA with aortic to pulmonary flow, b) a restrictive left to right flow indicates a PDA which is almost closed with aortic to pulmonary flow, c) bidirectional flow across the PDA indicates that the pulmonary pressures are suprasystemic in systole with pulmonary to aortic flow in systole and d) pure right to left flow indicates that pulmonary pressures exceed systemic pressures entirely with pure pulmonary to aortic flow throughout the cardiac cycle.

2.12 Outcome Measures

Our primary outcome was the assessment of indices of pulmonary hypertension and myocardial function using advanced echocardiography techniques over the first two years of age in infants born with Down syndrome.

At present there is no specific definition of PH based on echocardiography derived markers. However, the PAAT has been validated against right heart catheter derived measurements and normative ranges within the term neonatal population over the early newborn period are available[33]. It is accepted that a PAAT < 40 defines abnormally elevated pulmonary pressures[44]. Similarly, normative ranges of right ventricular ejection time (RVET) within the Day 1 neonatal population have been published[33]. A cut-off of PAAT:RVET ratio of < 0.25 is now also accepted as an echocardiography derived marker of abnormally high pulmonary pressures[134]. As 97.8% of left ventricular eccentricity index (LV EI) measurements within the control cohort were < 1.8 our research group defined an LV EI ratio > 1.8 as part of the diagnostic criterion for pulmonary hypertension (PH) in this study. Tricuspid regurgitation (TR) was not included in this criterion for several reasons; a TR jet was not present in the majority of scans in both the DS and control groups, TR jet measurements have poor agreement with right heart catheter measures of PH and TR jets in some instances may underestimate RV pressure in the context of RV functional impairment (which is the case in the DS cohort).

Therefore, in this study a diagnosis of PH was made if two or more of the PH markers defined as follows were present:

- A PAAT < 40ms
- A PAAT:RVET < 0.25[134]
- In the presence of a patent ductus arteriosus (PDA), the demonstration of bidirectional flow across the vessel or right to left flow
- A left ventricular eccentricity index > 1.8

In addition, I also assessed any associations present between the development of pulmonary hypertension and myocardial impairment and important clinical outcomes during the study period as follows:

- Respiratory morbidity
- clinically evident PH
- Hospital admissions
- Admission to a paediatric intensive care unit
- Necessity for Extracorporeal membrane oxygenation (ECMO)
- Death within the first two years of age

2.13 Statistical Analysis

The cohort was divided into two groups, infants with DS and control infants or three groups, infants with DS and congenital heart disease (CHD) (DS-CHD), infants with DS and no CHD (DS-no CHD) and controls. Continuous data was tested for normality using the Shapiro-Wilk test and a histogram representation of data and summarised as means (standard deviation) or medians [inter-quartile range] as appropriate. If the Shapiro-Wilk test indicated a skewed distribution but the histogram demonstrated normal distribution I used the histogram to assign normality. Categorical data was summarised as counts (%). Two group analyses were conducted using the student t-test, the Mann Whitney U test as appropriate, or Chi square test as appropriate. Three group analyses was conducted using one way ANOVA or the Kruskal-Wallis Test. Serial data was compared using two way ANOVA with repeated measures. Correlations were examined using Pearson's or Spearman's correlation coefficients as appropriate. Regression analysis was conducted to assess the independent effect of predictor variables on important outcomes. We used SPSS version 23 to conduct the analyses. Statistical significance is achieved with a p value < 0.05.

Chapter 3 : Clinical Outcomes of Infants with Down Syndrome over the First Two Years of Age

3.1 Background

Down syndrome (DS) has a multisystemic phenotype characterised by over 80 clinical features with significant variability in their frequency and severity[3]. Babies with DS are a vulnerable patient cohort who require increased respiratory support, intensive care and longer hospital admissions when compared to the general neonatal and paediatric population[4-7]. A 2018 study by our group identified the most common reason for neonatal unit admission of infants with DS as low oxygen saturations, the differential diagnosis for which context of DS includes sepsis, congenital heart disease (CHD) and pulmonary hypertension (PH)[8]. Approximately half of neonates born with DS will have some form of congenital heart disease (CHD) with atrioventricular septal defect (AVSD), ventricular septal defect (VSD), secundum atrial septal defect (ASD) and Tetralogy of Fallot (TOF) the most common lesions[49]. In addition, recent data indicates that between 27% and 34% of newborns with DS will have PH, a figure much higher than previous estimates[77, 78].

Our hypothesis was that babies with DS will display higher rates of prematurity, respiratory and cardiac morbidity, hospital admissions and mortality when compared to non-DS control babies. The aim of this chapter was to examine such clinical parameters in the DS and control cohorts enrolled in our study over the first two years of age.

3.2 Specific Methodology Employed

3.2.1 Study Design and Study Population

This was a prospective observational cohort study performed across the three tertiary neonatal intensive care units of Dublin: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. All infants with a diagnosis of DS made antenatally or postnatally (later confirmed with Karyotying) were eligible for inclusion. The DS cohort consisted of both babies with DS born with CHD and structurally normal hearts. In addition, a cohort of healthy term infants without a diagnosis of DS were enrolled in The Rotunda Hospital to serve as a control population. Significant antenatal, birth and neonatal characteristics and outcome data were recorded for both the DS and control cohorts at the local neonatal unit of recruitment. Clinical data was recorded as per parental response at each follow up assessments performed in The Rotunda Hospital at 6 months, 1 year and 2 years of age for both the DS and control populations. Enrolment criteria and data collection techniques are fully detailed in the methodology chapter (Chapter 2).

3.2.2 Statistical Analysis

Continuous data was tested for normality using the Shapiro-Wilk test and a histogram representation of data and summarised as means (standard deviation) or medians [inter-quartile range] as appropriate. Categorical data was summarised as counts (%). Two group analyses were conducted using the student t-test, the Mann Whitney U test as appropriate, or Chi square test as appropriate. SPSS version 23 was used to conduct the analyses. Statistical significance is achieved with a p value < 0.05.

3.3 Our Findings

Our original aim was to recruit 120 infants with DS and 60 non-DS control infants over a two year period. Many infants born with DS have acute cardiac and gastrointestinal diagnoses that necessitate prompt transfer to paediatric intensive care units in Children's Health Ireland at Temple Street or Children's Health Ireland at Crumlin. Such transfers prohibited enrolment into this study as our ethically approved recruitment sites were the three tertiary Dublin neonatal units. Furthermore, the goal of the study was to assess longitudinal myocardial function and pulmonary haemodynamics I wanted to ensure that I had the necessary time to complete 6 month, 12 month and 24 month echocardiograms on the majority of infants recruited. With the Covid-19 pandemic striking in early 2020 our ability to perform longitudinal echocardiograms on outpatient infants with DS and infants in the control cohort was significantly hindered. The current data set reflects our best efforts at performing as many longitudinal echocardiograms at 6 months, 12 months and 2 years as possible given the circumstances.

Therefore, a total of 130 infants were enrolled during the study period: 70 infants with a confirmed diagnosis of DS and 60 infants born without DS (controls). An antenatal diagnosis of DS was made for 23 (33%) infants **(Table 3.2)**.

3.3.1 Basic Characteristics of the Down Syndrome and Control Populations

Babies born with DS were delivered at an earlier gestational age, 37.7 ± 2.1 vs 39.6 ± 1.2 weeks ($p < 0.01$), with 20 (29%) of the DS cohort born before 37 weeks gestation. There were more infants born male in the DS cohort than the control cohort; 46 (66%) vs 25 (42%) ($p < 0.01$). Babies born with DS had a lower birth weight, 3.02 ± 0.68 vs 3.56 ± 0.42 Kg ($p<0.01$), were born to women with a higher maternal age, 37 ± 4 vs 34 ± 4 years ($p < 0.01$) and a lower rate of Caesarean Section (CS) delivery, 36 (51%) vs 41 (68%) ($p = 0.05$) compared to the control cohort. The control cohort were enrolled entirely within the Rotunda Hospital where the Caesarean section rate is 40%. To capture as many Day 3 echocardiograms as possible in the control population I decided to enrol more infants delivered by Caesarean section in this group. This decision was made as infants delivered by vaginal delivery are typically discharged home on day 2 of age precluding a day 3 echocardiogram **(Table 3.1)**.

3.3.2 Pre-Discharge Clinical Outcomes

Forty-eight (69%) of infants with DS were born with structural congenital heart disease (CHD). Ventricular septal defect (VSD) was the most common CHD lesion in the DS cohort detected in 34 infants followed by atrial septal defect (ASD), atrioventricular septal defect (AVSD) and Tetralogy of Fallot in 17, 7 and 1 infants respectively **(Table 3.2)**.

In the DS cohort 3 (4%) infants required inotropes to support haemodynamic stability following delivery and 4 (6%) required inhaled nitric oxide (iNO) for treatment of pulmonary hypertension (PH). Six (9%) of babies with DS were intubated and placed on invasive ventilation for a median of 6 days [4 – 17]. Nine (13%) required continuous positive airway pressure (CPAP) following delivery for a median of 3 days [1 – 3]. Twenty-eight babies (40%) with DS required supplemental oxygen for a median of 4 days [1 – 8] to maintain adequate oxygen saturation measurements. Infants with DS were established on full enteral feeds within a median of 2 days [1 – 4]. Hospital admission days pre discharge in the DS cohort were a median of 9 days [5 – 18] and 1 (1%) infant died before discharge home from their maternity hospital of birth **(Table 3.2)**.

3.3.3 Clinical Outcomes of Infants with DS over the First Two Years of Age

Thirty-one (44%) infants with DS required hospitalisation over the first two years of age. Fourteen (20%) were hospital admissions for surgical corrections of CHD and 17 (24%) hospitalisations were non-CHD related. Thirteen (18%) of babies with DS requiring surgical corrections of CHD were admitted to the paediatric intensive care unit post operatively. The non-CHD related hospitalisations were for the following reasons: pneumonia, bronchiolitis, obstructive sleep apnoea investigations, vomiting, insertion of percutaneous endoscopic gastrostomy (PEG) tube, investigation for suspected seizures and management of infantile spasms. Eleven (16%) of the DS cohort required L-thyroxine for hypothyroidism management and 10 (14%) required diuretic therapy over the first two years of age. There were no deaths in the DS cohort following discharge home from their maternity hospital of birth **(Table 3.3)**.

3.3.4 Down Syndrome Cohort

Overall, our cohort of babies with DS was a relatively healthy population **(Table 3.1)**. Although 48 (69%) of babies with DS in our study were born with CHD and 20 (29%) were premature, the mortality rate and number of babies with DS requiring mechanical ventilation, inotropic support and iNO administration were low. This particularly healthy population of enrolled infants with DS is likely multifactorial. First, infants born with DS and time critical cardiac or gastrointestinal disorders were transferred promptly from their maternity hospital of birth to an appropriate paediatric intensive care (PICU) or paediatric surgical centre and not enrolled in this study. Second, infants were not enrolled if there is a high likelihood of death over the first week of age. Third, the consultant physician was always contacted prior to approaching parents of babies with DS regarding study enrolment. If the consultant physician did not deem it appropriate to approach parents during such a sensitive time, particularly for infants with a post-natal diagnosis of DS, the baby was not enrolled. Forth, study enrolment took place across the three Dublin tertiary neonatal units who care for high numbers of babies born with DS annually and have vast expertise in the specific needs of this population. As such our DS cohort reflects an essentially healthy population of newborns with DS who did not possess time critical cardiac or gastrointestinal issues, received expert care in tertiary neonatal units and had relatively stable post-natal courses pre discharge home.

Table 3.1: Basic characteristics of the Down syndrome and control populations.

Values are presented as means (SD), medians [IQR] or absolute count (%).

	Down Syndrome n=70	Controls n=60	p
Gestation (Weeks)	37.7 ± 2.1	39.6 ± 1.2	<0.01
Birth before 37 weeks	20 (29%)	0	<0.01
Birth Weight (Kg)	3.02 ± 0.68	3.56 ± 0.42	<0.01
Maternal Age (Years)	37 ± 4	34 ± 4	<0.01
Parity	2 [2 – 3]	2 [1 – 2]	0.02
Male	46 (66%)	25 (42%)	<0.01
Caesarean Section	36 (51%)	41 (68%)	0.05

Table 3.2: Pre-discharge clinical outcomes.

Values are presented as means (SD), medians [IQR] or absolute count (%).

	Down Syndrome n=70
Antenatal Diagnosis	23 (33%)
Congenital Heart Disease	48 (69%)
Atrial Septal Defect	17
Ventricular Septal Defect	34
Atrioventricular septal defect	7
Tetralogy of Fallot	1
Inotropes	3 (4%)
Nitric Oxide	4 (6%)
Invasive ventilation	6 (9%)
CPAP	9 (13%)
Oxygen	28 (40%)
Ventilation Days	6 [4 – 17]
CPAP Days	3 [1 – 3]
Oxygen Days	4 [1 – 8]
Days to Enteral Feeds	2 [1 – 4]
Hospital Stay	9 [5 – 18]
Mortality	1 (1%)

Table 3.3: Clinical outcomes over the first two years of age.

Values are presented as absolute count (%).

	Down Syndrome n=70
Hospital Admissions	31 (44%)
CHD Surgical Correction	14 (20%)
Non-CHD Cause	17 (24%)
CHD Post OP ICU admission	13 (18%)
Hypothyroidism requiring L-thyroxine	11 (16%)
Diuretic Use	10 (14%)
Mortality	0

Chapter 4 : The Relationship between Diastolic Impairment and Pulmonary Hypertension in Infants with Down Syndrome over the First Week of Age

4.1 Background

The cardiovascular system of infants with DS is characterised by pulmonary hypertension (PH) and biventricular systolic dysfunction over the early neonatal period[9]. The DS population is particularly vulnerable to early PH due to developmental lung disorders (reduced alveolarisation, decreased vessel density, persistence of the double-capillary network and hypertensive arterial remodelling), a blunted response to nitric oxide and the presence of congenital heart disease (CHD)[49, 77, 101]. Early PH is a leading cause of morbidity for neonates with DS and is significantly associated with an increased necessity for invasive ventilation, more days of inhaled nitric oxide therapy, longer hospital admission and higher mortality before discharge[8, 79].

Recent foetal studies report that myocardial function in foetuses with DS is often abnormal, independent of CHD. Mula *et al* demonstrated evidence of foetal diastolic dysfunction via increased ductus venosus pulsatility index and increased tricuspid regurgitation in 28 foetuses with DS when compared to other anuploidal foetuses and control foetuses[56]. Clur *et al* documented both systolic and diastolic dysfunction in foetuses with DS compared to controls which manifest as reduced tricuspid valve (TV) A-wave velocity, reduced mitral valve A-wave velocity, reduced aortic valve peak velocity, reduced pulmonary valve peak velocity and reduced stroke volume[57].

However, the presence of left ventricular (LV) diastolic impairment and its relationship with PH in the neonatal DS population remains unexplored. Our hypothesis was that infants with DS exhibit early diastolic impairment that is related to the degree of PH in the early newborn period. The aim of this work was to assess myocardial performance and pulmonary haemodynamics using advanced echocardiography techniques to interrogate the

relationship between diastolic function and indices of PH in infants with DS over the first week of age, and to compare these measurements to a control cohort. Advanced echocardiography modalities provide a more objective and sensitive evaluation of cardiovascular parameters than routine measurements, allow serial assessment over time and permit detection of sub-clinical myocardial impairment prior to the presentation of overt clinical disease.

4.2 Specific Methodology Employed

4.2.1 Study Design and Study Population

This was a prospective, tri-centre, observational cohort study performed across the tertiary neonatal intensive care units of Dublin: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. All infants with an antenatal or postnatal (later confirmed with Karyotyping) diagnosis of DS were eligible for inclusion. Babies with DS with and without congenital heart disease (CHD) were enrolled. A cohort of healthy term infants without a diagnosis of DS were recruited from The Rotunda Hospital to serve as a control population. Significant antenatal, birth and neonatal characteristics and outcome data were recorded for both the DS and control cohorts at the local neonatal unit of recruitment. Enrolment criteria and data collection techniques are fully detailed in the methodology chapter (Chapter 2).

4.2.2 Echocardiography Assessment

Echocardiography scans were performed at three time points over the first week of age for both the DS and control cohorts: Day 1 (Echo 1), Day 2 (Echo 2) and Day 3-5 (Echo 3). The first echocardiogram of each infant included formal evaluation for any CHD. Echocardiography imaging assessed myocardial structure and function, pulmonary haemodynamics and patent ductus arteriosus (PDA) shunt characteristics. Novel echocardiography techniques were employed to measure myocardial performance. Novel echocardiography techniques included tissue Doppler imaging (TDI) and deformation

analysis via 2D STE. A detailed explanation of all advanced techniques utilised in this study is available within Chapter 2.

4.2.3 Statistical Analysis

Continuous data was tested for normality using the Shapiro-Wilk test and a histogram representation of data and summarised as means (standard deviation) or medians [inter-quartile range] as appropriate. If the Shapiro-Wilk test indicated a skewed distribution but the histogram demonstrated normal distribution I used the histogram to assign normality. Categorical data was summarised as counts (%). Two group analyses were conducted using the student t-test, the Mann Whitney U test as appropriate, or Chi square test as appropriate. SPSS version 23 was used to conduct the analyses. Statistical significance is achieved with a p value < 0.05. Correlations were examined using Pearson's or Spearman's correlation coefficients as appropriate. We used SPSS version 23 to conduct the analyses. Statistical significance was achieved with a p value < 0.05.

4.3 Our Findings

Seventy infants with DS and 60 control infants were enrolled into this study. Three echocardiograms were performed in both the DS and control cohorts over the first week of age. Utilising novel echocardiography techniques significant differences in left ventricular (LV) and right ventricular (RV) morphology, indices of pulmonary hypertension and biventricular performance were detected between the DS and control cohorts.

4.3.1 Timing and Number of Echocardiograms in the Down Syndrome and Control Cohorts over the First Week of Age

For the DS cohort 58 Day 1, 66 Day 2, and 65 Day 3-5 echocardiograms were performed. For the control cohort 60 Day 1, 58 Day 2 and 52 Day 3-5 echocardiograms were performed **(Table 4.1)**.

4.3.2 Anthropometric Measurements over the First Week of Age

Day 1 echocardiography assessment revealed that infants with DS had significantly smaller left and right ventricular dimensions compared to control infants. Babies with DS had smaller mitral valve annuli (mm), 7.4 ± 1.1 vs 9.7 ± 1.1 ($p < 0.01$), smaller internal left ventricular (LV) diastolic diameters (mm), 14 ± 2 vs 17 ± 2 ($p < 0.01$) and shorter LV lengths (mm) 27 ± 4 vs 31 ± 2 ($p < 0.01$) and compared to control infants. The right ventricle (RV) of infants with DS was similarly affected. The DS cohort displayed smaller tricuspid valve diameters (mm), 9.8 ± 1.8 vs 10.7 ± 1.2 ($p < 0.01$), smaller RV mid cavity diameters (mm), 14 ± 2 vs 13 ± 1 ($p < 0.01$) and shorter RV lengths (mm), 28 ± 3 vs 31 ± 2 ($p < 0.01$). There were no differences detected between the DS and control cohorts in aortic root diameter of pulmonary artery root diameter measurements **(Table 4.2)**.

4.3.3 Patent Ductus Arteriosus Characteristics over the First Week of Age

Patent ductus arteriosus (PDA) characteristics were evaluated on Day 1 and Day 3 of age in both cohorts. On Day 1 echocardiography assessment more infants with DS had a PDA compared to controls, 52 (74%) vs 30 (50%) ($p < 0.01$), and the vast majority of PDAs in the DS population were bidirectional, 47/52 (90%) vs 10/30 (33%) ($p < 0.01$). Importantly, these findings persisted on Day 3-5 evaluation. At Day 3-5 assessment 38 (54%) of infants with DS still had a PDA compared to 5 (8%) of the control group ($p < 0.01$) and notably 14 (37%) of the PDAs in the DS cohort were still bidirectional compared to 0 (0%) of the PDAs in the control cohort. Therefore, PDA characteristics reveal continued elevation of pulmonary vascular resistance in the DS group that persists at discharge **(Table 4.3)**.

4.3.4 Pulmonary Vascular Resistance Measurements over the First Week of Age

On Day 1 evaluation babies with DS had significantly shorter PAAT (ms) indicating higher degrees of PVR compared to control babies; 43 ± 10 vs 62 ± 14 ($p < 0.01$) **(Table 4.4)**. In keeping with the PAAT findings, PAAT:RVET ratios were lower in the DS group compared to the control group at 0.21 ± 0.04 vs 0.29 ± 0.06 ($p < 0.01$) **(Table 4.4)**.Infants with DS had higher LV eccentricity measurements of 1.8 ± 0.3 vs 1.4 ± 0.2 ($p < 0.01$) compared to the control population indicating flatter interventricular septum configurations on day 1 echocardiography assessment **(Table 4.4)**. Notably each of the aforementioned echocardiography surrogates of PH, namely the PAAT, PAAT:RVET ratio and LV eccentricity index, remained persistently and significantly abnormal in the DS group over the first week of age **(Figure 4.1)**.

4.3.5 Infants in Down Syndrome and Control Cohorts Meeting Diagnostic Criteria for Pulmonary Hypertension on Day 1 and Day 3 Echocardiography Evaluation.

As explained in Chapter 2 there is no specifically delineated criterion currently available to diagnose pulmonary hypertension (PH) based on echocardiography derived markers. However, it is accepted that a PAAT < 40ms and a PAAT:RVET ratio of < 0.25 are markers of abnormally high pulmonary pressures [44, 134]. Within our own dataset, as 97.8% of left ventricular eccentricity index (LV EI) measurements in the control population were < 1.8 I defined an LV EI ratio > 1.8 as part of the diagnostic criterion for pulmonary hypertension (PH) in this study. Therefore, in this study a diagnosis of PH was made if two or more of the PH markers defined as follows were present with each marker possessing equal weight:

- A PAAT < 40ms
- A PAAT:RVET < 0.25
- In the presence of a patent ductus arteriosus (PDA), the demonstration of bidirectional flow across the vessel or right to left flow
- A left ventricular eccentricity index > 1.8

This definition of PH was applied to the DS and control cohorts echocardiography measurements of pulmonary vascular resistance on Day 1 and Day 3 of age. On Day 1 of age 84% of babies with DS met this criterion for PH compared to 15% of controls. By Day 3 28% of babies with DS still met this criterion for PH compared with 0% of the controls. **(Table 4.5)**.

4.3.6 Tissue Doppler Imaging Event Timings over the First Week of Age

There was no difference in Day 1 heart rate or diastolic time measurements between the DS and control groups. However, babies with DS had longer systolic times (ms) and systolic: diastolic ratios (S:D) than control babies; 201 ± 22 vs 188 ± 20 ($p < 0.01$) and 1.2 ± 0.3 vs 1.0 ± 0.2 ($p < 0.01$) respectively. There were no differences detected in isovolumic relaxation time or isovolumic contraction time between the DS and control populations **(Table 4.6)**.

4.3.7 Tissue Doppler Imaging Measurements over the First Week of Age

At the LV lateral wall TDI s', e' and ea' measurements were lower in the DS cohort compared to the control cohort; 4.2 ± 1.0 vs 4.8 ± 1.1, 5.4 ± 1.8 vs 6.3 ± 1.6 and 1.0 ± 0.4 vs 1.2 ± 0.3, all $p < 0.01$. In addition, the Ee' ratio was higher in babies with DS at 14 ± 5 vs 10 ± 3 ($p < 0.01$) indicating elevated left atrial pressures. There was no difference detected in LV late diastolic TDI velocities (a') between the groups **(Table 4.7)**.

The interventricular septum displayed decreased s', 3.0 ± 0.8 vs 3.5 ± 0.7 ($p <0.01$) and e', 3.9 ± 1.4 vs 4.4 ± 1.3 ($p = 0.04$), velocities in the DS group on Day 1 evaluation. However, there were no differences identified between the groups on septal late diastolic TDI velocities (a') or septal ea' ratio. The isovolumic contraction velocity was lower in the DS group compared to the control group, 3.0 ± 1.4 vs 3.6 ± 1.4 ($p < 0.01$), however there were no differences identified in isovolumic relaxion velocity **(Table 4.7)**.

Regarding Day 1 RV function at the RV free wall, TDI assessment found no differences in s' or e' velocities between the groups. However, RV a' velocities were significantly higher in the DS group on Day 1 assessment than controls, 10.4 ± 3.6 vs 8.8 ± 2.1 ($p < 0.01$), and the RV ea' ratio was significantly lower in the DS group than controls, 0.9 ± 0.2 vs 1.0 ± 0.2 ($p < 0.01$), on Day 1 evaluation **(Table 4.7)**.

TDI measurements were further evaluated over the first week of age on Day 2 and Day 3-5 echocardiography scans. LV TDI s' velocities remained impaired on Day 2 but had

normalised by Day 3 assessment. Equally, LV e' and LV ea' ratio measurements were comparable to control values beyond Day 1 **(Figure 4.2)**. Septal s' and e' velocities in the DS cohort normalised by Day 2 and Day 3 imaging respectively. **(Figure 4.3)**. Regarding RV TDI assessment RV systolic velocity (s') was only significantly different in the DS cohort from controls on Day 2 assessment. Day 2 and Day 3 echocardiography evaluation demonstrated higher RV e' and a' diastolic velocities in the DS cohort compared to controls. The RV ea' ratio was significantly reduced in the DS group on Day 3 of age **(Figure 4.4)**.

4.3.8 Deformation Measurements over the First Week of Age

Day 1 deformation analysis in the DS population revealed biventricular systolic and LV diastolic impairment. LV and RV longitudinal strain was lower in the DS group compared to controls with measurements 19.0 ± 2.8 vs 21.8 ± 1.9 and 18.8 ± 3.9 vs 21.7 ± 3.5 (both $p < 0.01$) respectively. Longitudinal systolic strain rate, a surrogate of myocardial contractility, was also impaired on both LV and RV assessment in the DS cohort compared to controls; 1.6 ± 0.3 vs 1.9 ± 0.2 and 1.4 ± 0.3 vs 1.8 ± 0.4 (both $p < 0.01$). Both LV early and LV late diastolic function was reduced in the DS group when compared to controls, with low early and late diastolic strain rate values of 2.5 ± 0.6 vs 3.4 ± 0.7 ($p < 0.01$) and 1.5 ± 0.4 vs 1.7 ± 0.5 ($p = 0.02$). In addition, the LV early to late diastolic strain rate ratio further highlighted impaired diastolic performance in infants with DS; 1.8 ± 0.7 vs 2.1 ± 0.6 ($p = 0.02$). Deformation analysis of the RV revealed impaired early diastolic strain rate measurements compared to the control population of 2.0 ± 0.8 vs 2.5 ± 0.8 ($p < 0.01$) with no difference in late diastolic strain rate or RV early to late diastolic strain rate ratio measurements between the groups **(Table 4.8).**

Day 2 and Day 3-5 echocardiography imaging examined the evolution of deformation measurements over time. LV global longitudinal strain, LV longitudinal systolic strain rate, and both LV early and late diastolic strain rate measurements remained significantly impaired on Day 3-5 evaluation in the DS group **(Figure 4.5)**. Similarly, RV deformation analysis revealed continuing diminished RV free wall longitudinal strain, RV longitudinal systolic strain rate and RV early diastolic strain rate measurements on Echo 3 assessment of the DS cohort **(Figure 4.6).**

Focusing specifically on diastolic function, there is persistent biventricular early diastolic impairment in the DS cohort as exhibited by significantly lower LV and RV early diastolic strain rates over the first week of age compared to controls **(Figure 4.5, Figure 4.6).**

4.3.9 The Relationship between Left Ventricular Diastolic Impairment and Pulmonary Hypertension in Infants with Down Syndrome over the First Week of Age

Upon evaluation of the relationship between LV diastolic impairment and pulmonary hypertension a direct correlation was visualised across several echocardiography derived markers.

There was a significant positive correlation between LV early diastolic strain rate and PAAT measurements and the PAAT:RVET ratio, $r = 0.46$ ($p < 0.01$) and $r = 0.41$ ($p < 0.01$) respectively. This data demonstrates that with worse diastolic function there is a direct correlation with worse indices of PH in the DS population. Importantly, there was also a significant negative correlation between Ee' ratio and PAAT measurements and the PAAT:RVET ratio, $r = -0.37$ ($p < 0.01$) and $r = -0.32$ ($p < 0.01$). **(Figure 4.7)**.

4.3.10 The Relationship between Pulmonary Hypertension and Right Ventricular Systolic Function in Infants with Down Syndrome over the First Week of Age

Following assessment of the relationship between PH and RV systolic function a direct correlation was visualised across several echocardiography derived markers. There was a significant positive correlation between PAAT measurements and RV free wall strain and RV systolic strain rate, $r = 0.34$ ($p < 0.01$) and $r = 0.32$ ($p< 0.01$). In addition, there was a significant negative correlation with LV eccentricity index and RV free wall strain and RV systolic strain rate, $r = -0.33$ ($p < 0.01$) and $r = -0.43$ ($p < 0.01$). **(Figure 4.8)**.

4.3.11 The Relationship between Left Ventricular Diastolic Impairment and Right Ventricular – Pulmonary Vascular Coupling in Infants with Down Syndrome over the First Week of Age

RV-PV coupling describes the ability of the RV to increase its contractility in tandem with increasing RV afterload. The RV can adapt to increasing degrees of RV afterload by increasing RV contractility via RV hypertrophy. However, in the face of unrelenting increases in RV afterload the challenge of maintaining normal RV performance becomes insurmountable. Ultimately the RV uncouples from its afterload with an associated decrease in RV efficiency. A significant positive correlation between LV early diastolic strain rate and RV-PV coupling, $r = 0.55$ ($p < 0.01$) was identified. **(Figure 4.9)**.

4.3.12 Day 3 Clinical characteristics, pulmonary vascular resistance measurements and myocardial functional measurements within the control cohort of infants born via Caesarean section and vaginal delivery

Day 3 clinical and echocardiographic characteristics were evaluated within the control cohort to assess the potential impact of Caesarean section delivery versus vaginal delivery. Infants in the control group delivered via Caesarean section were born at an earlier gestational age, 39.3 ± 1.1 vs 40.2 ± 1.0 ($p < 0.01$), and with a higher maternal age, 35 ±4 vs 32 ± 4 ($p = 0.05$), than those delivered via vaginal delivery. There were no other detectable differences between the Caesarean section and vaginal delivery groups with regards to PVR measurements of PAAT, PAATi or LV EI, RV or LV length or LV global longitudinal strain or RV free wall longitudinal strain values on Day 3 assessment. As such mode of delivery did not have a significant impact on PVR, LV or RV functional parameters in this control cohort (**Table 4.9**).

Table 4.1: Timing and number of echocardiograms in the Down Syndrome and control cohorts over the first week of age.

Values are presented as medians [IQR] and absolute counts.

	Echo 1	**Echo 2**	**Echo 3**
Down Syndrome	*Hours*	*Hours*	*Hours*
Time	19 [9 – 24]	46 [39 – 52]	95 [71 – 122]
Number of Scans	58	66	65
Controls			
Time	22 [17 – 25]	44 [38 – 47]	70 [67 – 81]
Number of Scans	60	58	52

Table 4.2: Day 1 Anthropometric measurements.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Down Syndrome n=70	**Controls n=60**	**p**
Left Ventricle			
Mitral Valve Annulus (mm)	7.4 ± 1.1	9.7 ± 1.1	<0.01
Length(mm)	27 ± 4	31 ± 2	<0.01
Internal Diastolic Diameter (mm)	14 ± 2	17 ± 2	<0.01
Posterior Wall Thickness (mm)	3.4 ± 0.8	3.5 ± 1.0	0.71
Septal Wall Thickness (mm)	3.7 ± 0.8	3.6 ± 0.8	0.45
Aortic Root Diameter (mm)	6.4 ± 0.5	6.5 ± 0.4	0.51
Right Ventricle			
Tricuspid Valve Diameter (mm)	9.8 ± 1.8	10.7 ± 1.2	<0.01
Length (mm)	28 ± 3	31 ± 2	<0.01
Basal Diameter (mm)	16 ± 3	16 ± 1	0.06
Mid Cavity Diameter (mm)	14 ± 2	13 ± 1	<0.01
Pulmonary Artery Root diameter (mm)	8.6 ± 1.4	8.8 ± 0.9	0.29

Table 4.3: Patent ductus arteriosus characteristics.

Values are presented as means (SD), medians [IQR] and absolute counts (%). A p value < 0.05 was considered significant.

	Down Syndrome n=70	**Controls n=60**	**p**
Day 1			
PDA Present	52 (74%)	30 (50%)	<0.01
PDA Diameter (mm)	3.3 [2.7 – 4.2]	1.8 [1.5 – 2.4]	<0.01
Systolic Velocity (m/s)	-1.1 [-1.4 – -0.8]	1.4 [-0.9 – 2.2]	<0.01
Diastolic Velocity (m/s)	0.9 [0.6 – 1.3]	1.2 [0.9 – 1.6]	0.05
Bidirectional Shunting	47/52 (90%)	10/30 (33%)	<0.01
Day 3-5			
PDA Present	38 (54%)	5 (8%)	<0.01
PDA Diameter (mm)	2.1 [1.8 – 3.1]	1.7 [1.2 – 1.8]	0.03
Systolic Velocity (m/s)	-0.9 [-1.4 – 0.7]	1.8 [1.5 – 2.2]	0.03
Diastolic Velocity (m/s)	1.3 [0.7 – 1.7]	0.8 [0.8 – 0.9]	0.26
Bidirectional Shunting	14/38 (37%)	0/5 (0%)	0.16

Table 4.4: Day 1 Pulmonary vascular resistance measurements.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Down Syndrome n=70	**Controls n=60**	**p**
PAAT (ms)	43 ± 10	62 ± 14	<0.01
RVET (ms)	206 ± 25	210 ± 22	0.30
PAAT:RVET	0.21 ± 0.04	0.29 ± 0.06	<0.01
LV Eccentricity index	1.8 ± 0.3	1.4 ± 0.2	<0.01

Table 4.5: Infants in DS and control cohorts meeting criteria for diagnosis of PH on Day 1 and Day 3 echocardiography evaluation.

Values are presented as absolute counts (%). A p value < 0.05 was considered significant.

	Down Syndrome n=70	**Controls n=60**	**p**
Day 1			
PAAT < 40 ms	24/58 (41%)	4/60 (7%)	<0.01
PAAT:RVET ≤ 0.25	52/58 (90%)	17/60 (28%)	<0.01
Bidirectional PDA Shunt	47/52 (90%)	10/30 (33%)	<0.01
LV EI ≥ 1.8	25/58 (43%)	3/60 (5%)	<0.01
Two or more PH Markers	49/58 (84%)	9/60 (15%)	<0.01
Day 3-5			
PAAT < 40 ms	5/65 (8%)	0/51 (0%)	0.04
PAAT:RVET ≤ 0.25	27/65 (42%)	4/51 (8%)	<0.01
Bidirectional PDA Shunt	14/38 (37%)	0/5 (0%)	0.10
LV EI ≥ 1.8	17/65 (26%)	1/52 (2%)	<0.01
Two or more PH Markers	18/65 (28%)	0	<0.01

Table 4.6: Day 1 Tissue Doppler imaging cardiac cycle event timings.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Down Syndrome n=70	Controls n=60	p
Heart Rate (bpm)	126 ± 17	127 ± 14	0.58
Systolic Time (ms)	201 ± 22	188 ± 20	<0.01
Diastolic Time (ms)	176 ± 44	189 ± 44	0.12
S:D ratio	1.2 ± 0.3	1.0 ± 0.2	<0.01
Isovolumic Relaxation Time (ms)	62 ± 32	52 ± 16	0.04
Isovolumic Contraction Time (ms)	57 ± 19	54 ± 12	0.23

Table 4.7: Day 1 tissue Doppler imaging measurements.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Down Syndrome n=70	Controls n=60	p
LV Lateral Wall (centimetres/second)			
s`	4.2 ± 1.0	4.8 ± 1.1	<0.01
e`	5.4 ± 1.8	6.3 ± 1.6	<0.01
a`	5.3 ± 1.8	5.5 ± 1.7	0.60
ea`	1.0 ± 0.4	1.2 ± 0.3	<0.01
Ee`	14 ± 5	10 ± 3	<0.01
Septum (centimetres/second)			
s`	3.0 ± 0.8	3.5 ± 0.7	<0.01
e`	3.9 ± 1.4	4.4 ± 1.3	0.04
a`	4.8 ± 1.8	4.7 ± 1.0	0.89
ea`	0.9 ± 0.3	1.0 ± 0.3	0.11
RV Free Wall (centimetres/second)			
s`	6.1 ± 1.4	6.3 ± 1.4	0.44
e`	8.1 ± 2.0	8.4 ± 1.7	0.40
a`	10.4 ± 3.6	8.8 ± 2.1	<0.01
ea`	0.9 ± 0.2	1.0 ± 0.2	<0.01

Table 4.8: Day 1 Deformation measurements.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Down Syndrome n=70	**Controls n=60**	**p**
LV Global Longitudinal Deformation Measurements			
Systolic Strain (%)	19.0 ± 2.8	21.8 ± 1.9	<0.01
Systolic Strain Rate (1/s)	1.6 ± 0.3	1.9 ± 0.2	<0.01
Early Diastolic Strain Rate (1/s)	2.5 ± 0.6	3.4 ± 0.7	<0.01
Late Diastolic Strain Rate (1/s)	1.5 ± 0.4	1.7 ± 0.5	0.02
Early to Late Diastolic Strain Rate Ratio	1.8 ± 0.7	2.1 ± 0.6	0.02
RV Free Wall Longitudinal Deformation Measurements			
Systolic Strain (%)	18.8 ± 3.9	21.7 ± 3.5	<0.01
Systolic Strain Rate (1/s)	1.4 ± 0.3	1.8 ± 0.4	<0.01
Early Diastolic Strain Rate (1/s)	2.3 ± 1.1	2.5 ± 0.8	<0.01
Late Diastolic Strain Rate (1/s)	2.0 ± 0.82	2.1 ± 0.8	0.28
Early to Late Diastolic Strain Rate Ratio	1.5 ± 1.6	1.7 ± 1.5	0.59

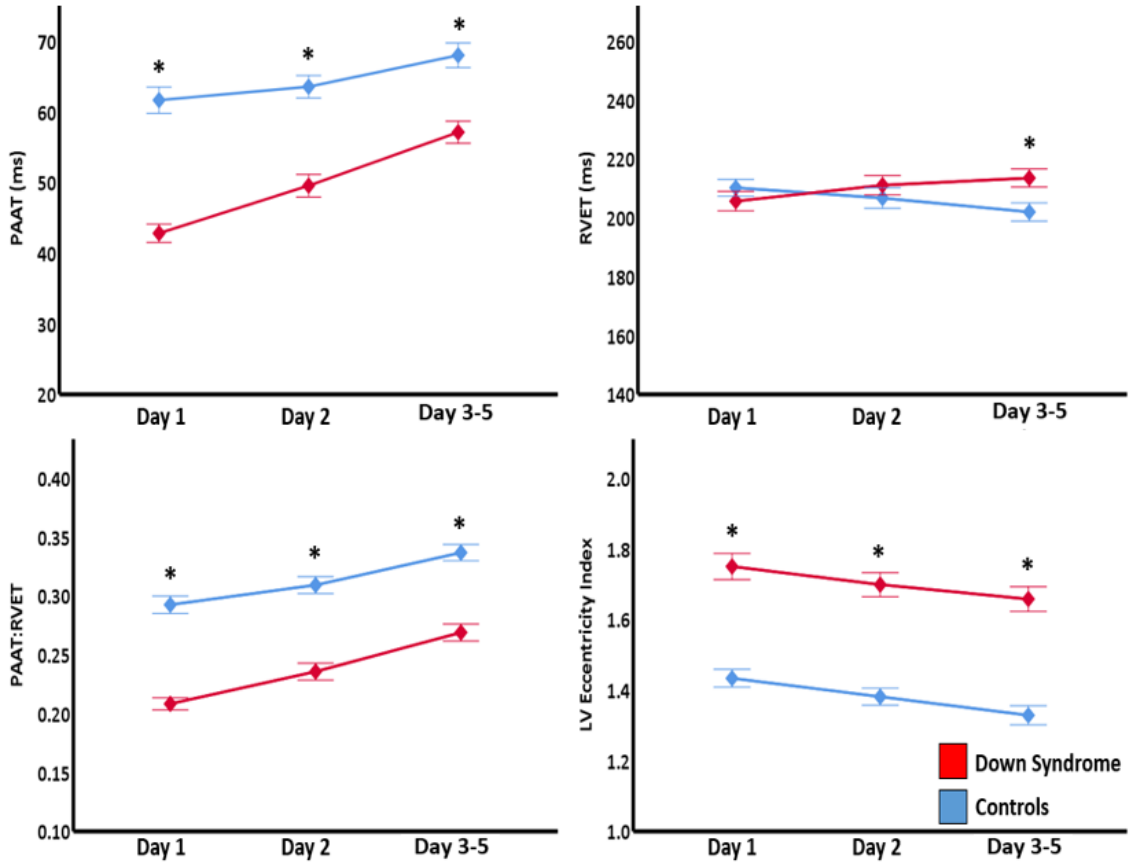


Figure 4.1: Measurement of echocardiography surrogates of pulmonary hypertension over the first week of age.

Values are presented as mean and standard error.

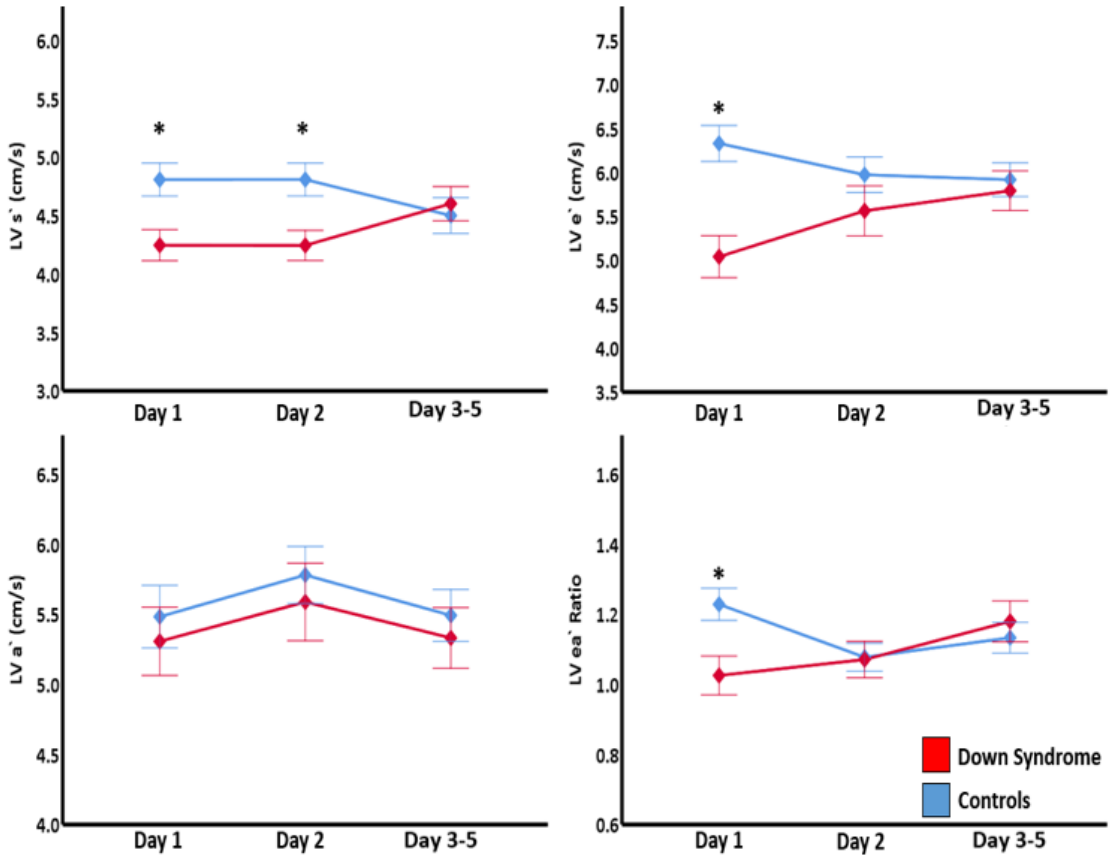


Figure 4.2 Measurement of left ventricular function by left ventricular tissue Doppler velocities over the first week of age.

Values are presented as mean and standard error.

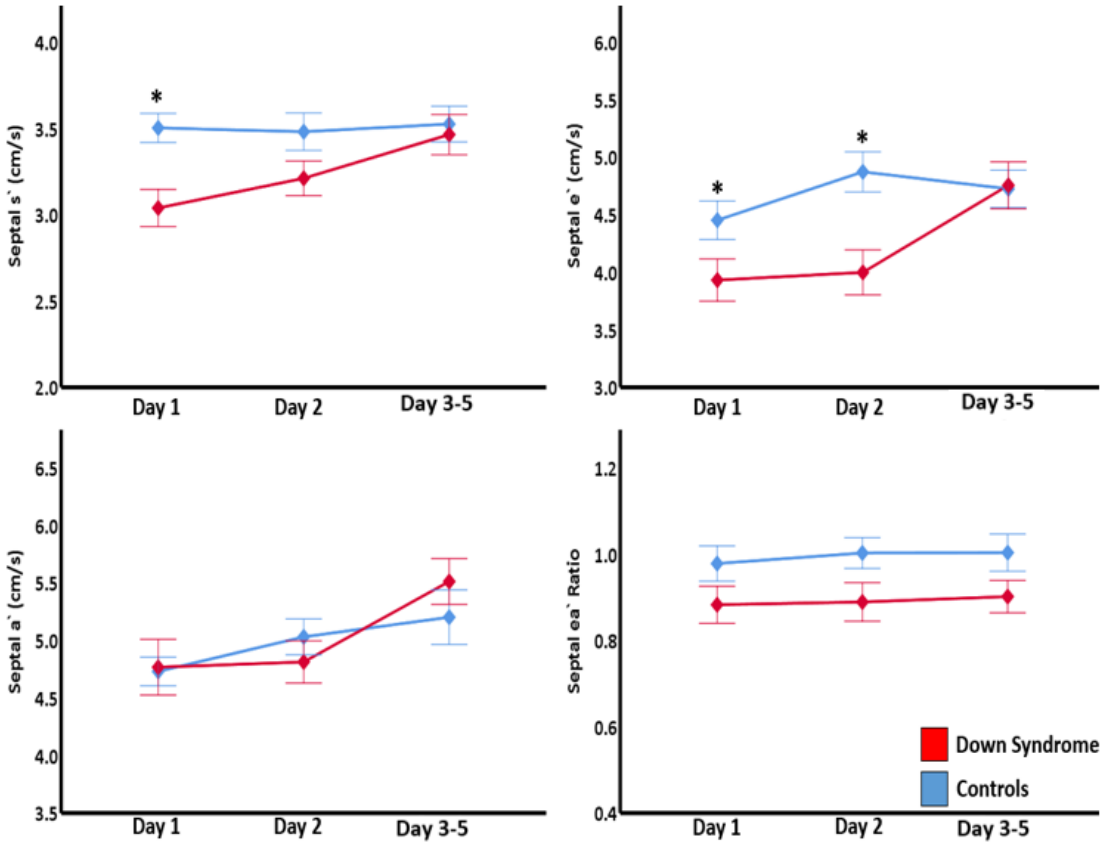


Figure 4.3: Measurement of septal function by septal tissue Doppler velocities over the first week of age.

Values are presented as mean and standard error.

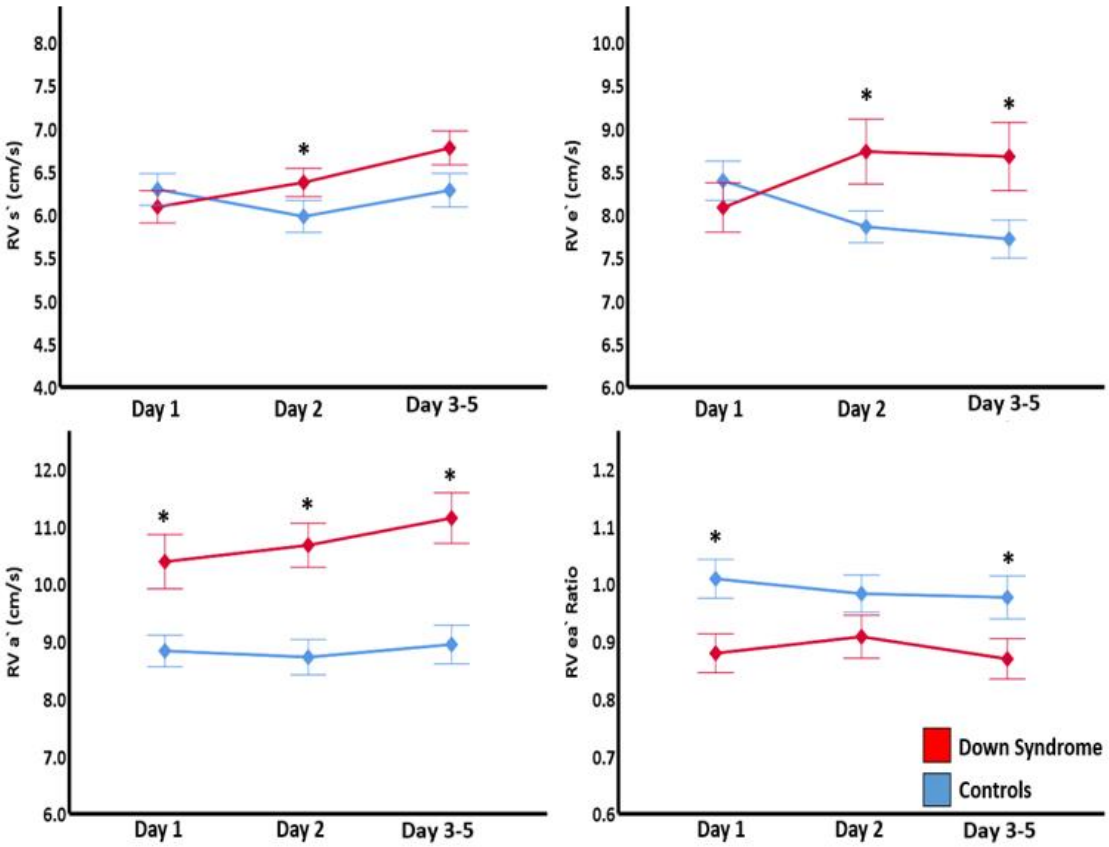


Figure 4.4: Measurement of right ventricular function by right ventricular tissue Doppler velocities over the first week of age.

Values are presented as mean and standard error.

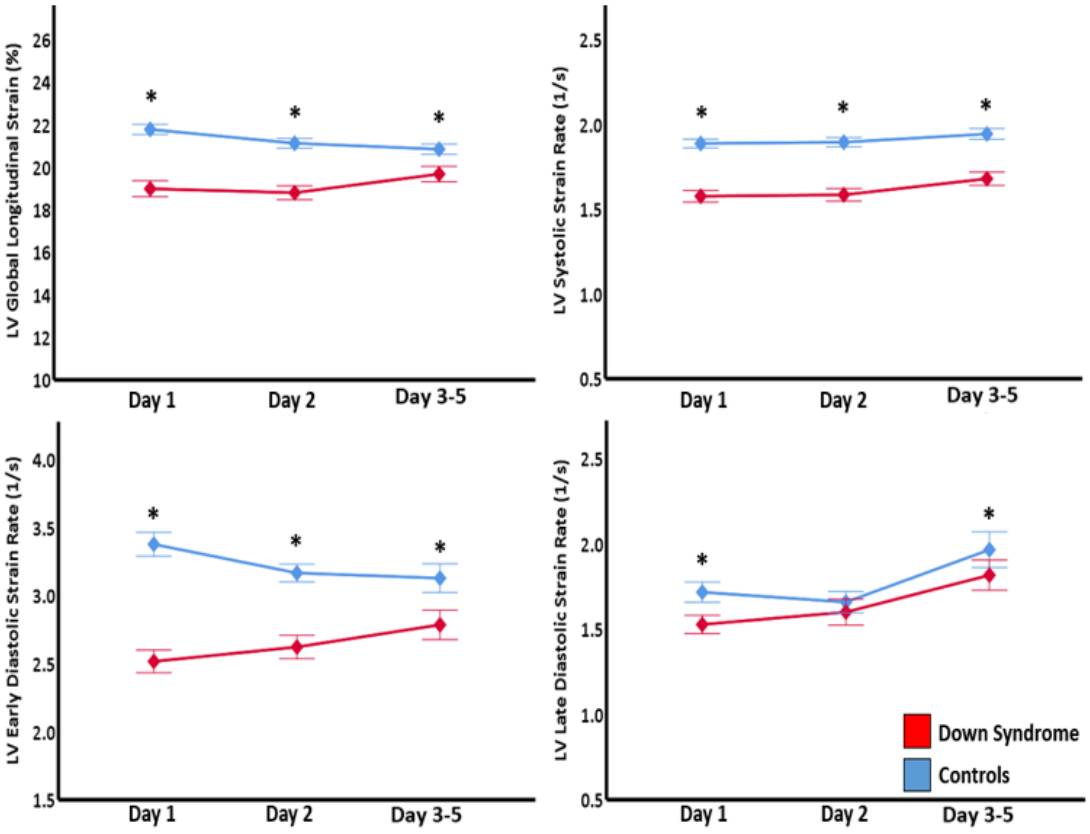


Figure 4.5: Measurement of left ventricular function by deformation analysis over the first week of age.

Values are presented as mean and standard error.

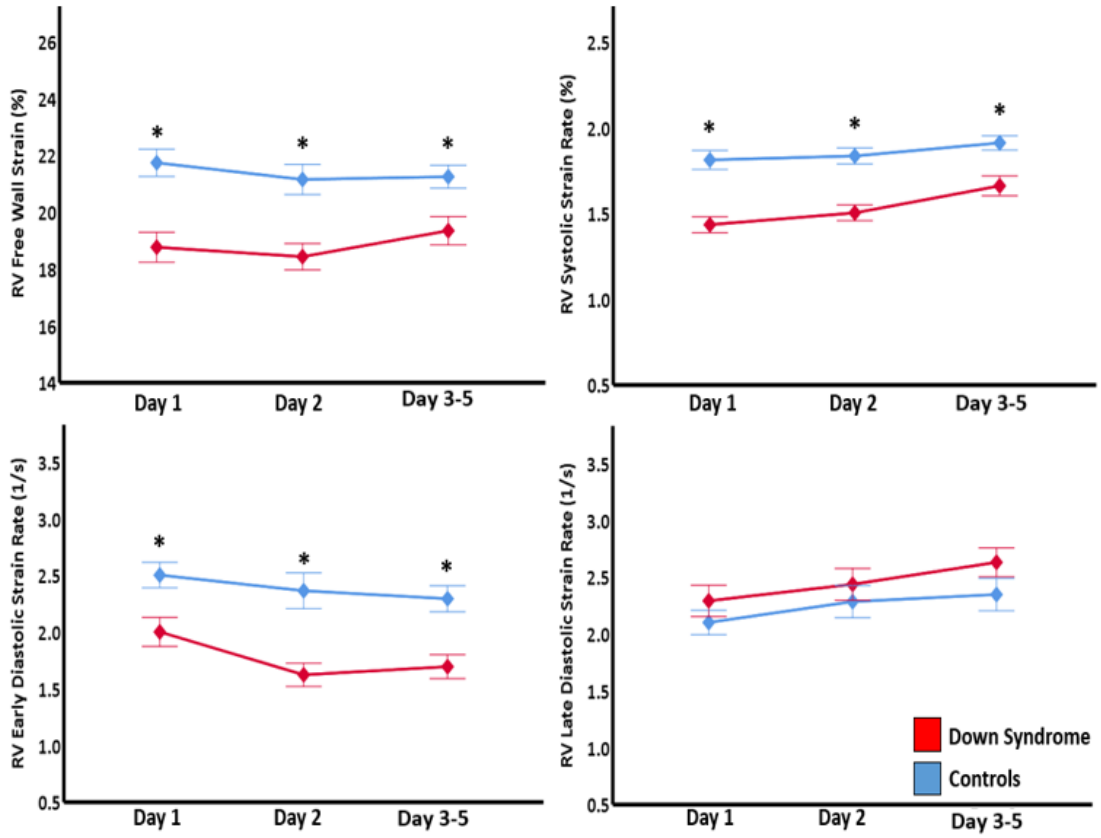


Figure 4.6: Measurement of right ventricular function by deformation analysis over the first week of age.

Values are presented as mean and standard error.

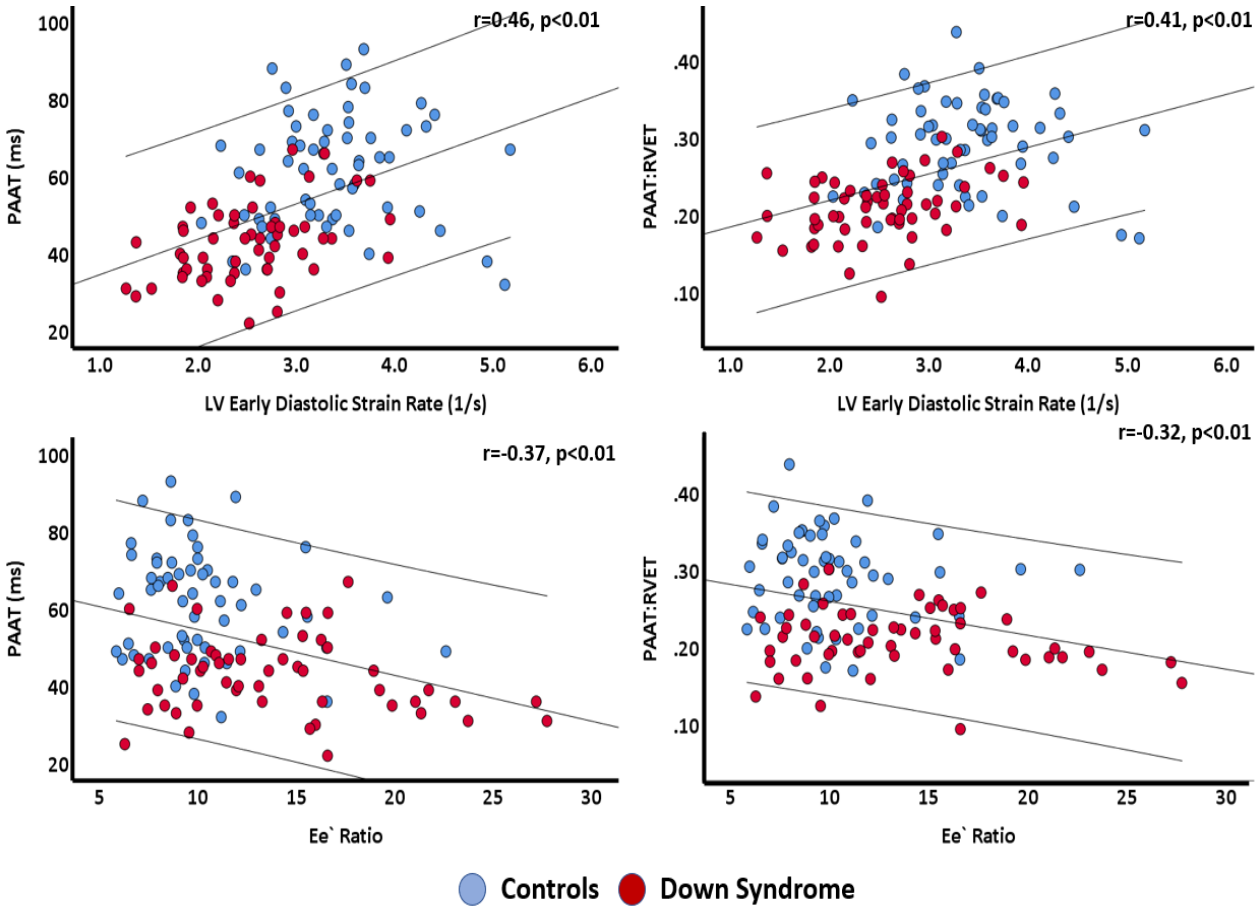


Figure 4.7: Correlation between left ventricular diastolic impairment and pulmonary hypertension in infants with Down Syndrome over the first week of age.

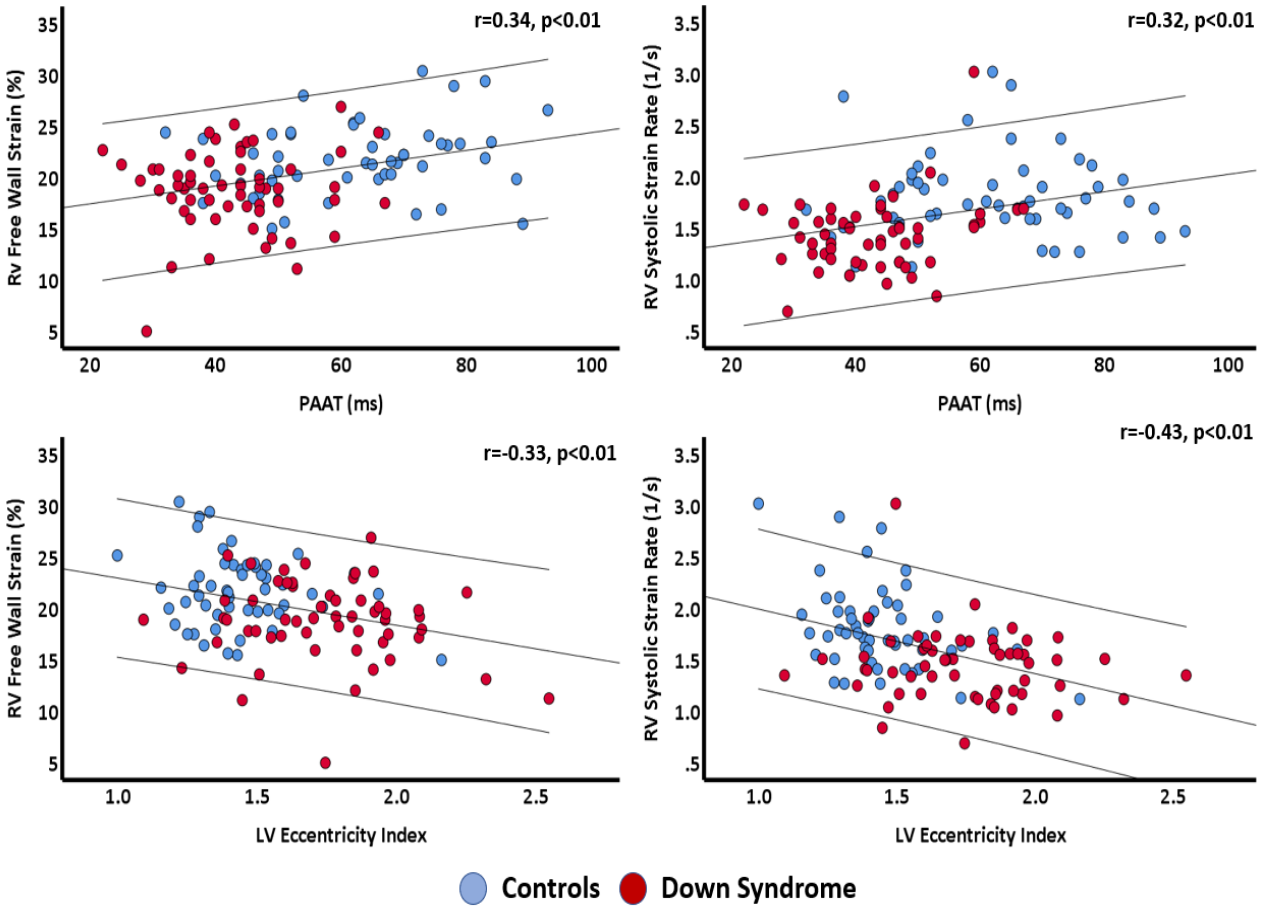


Figure 4.8: Correlation between pulmonary hypertension and right ventricular systolic impairment in infants with Down Syndrome over the first week of age.

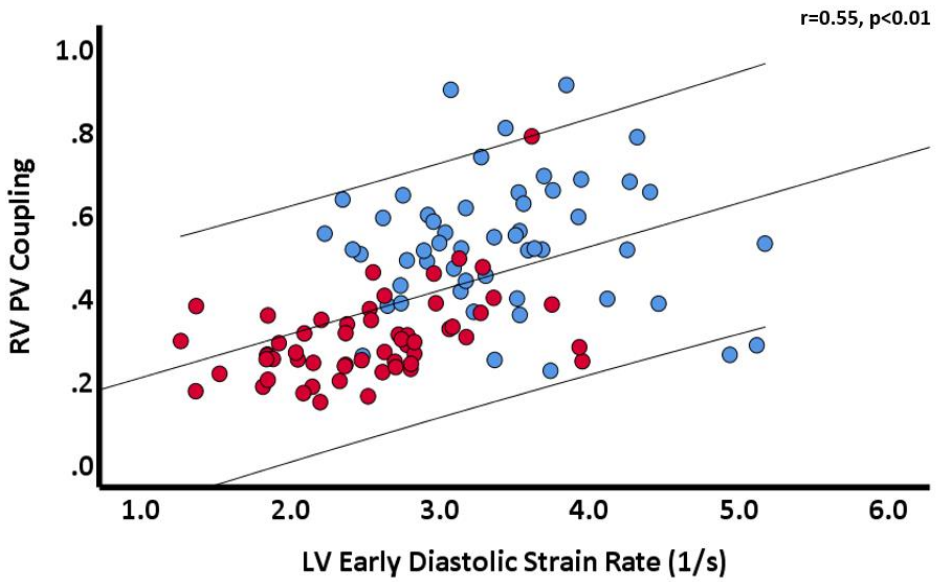


Figure 4.9: Correlation between left ventricular diastolic impairment and right ventricular – pulmonary vascular (RV-PV) coupling in infants with Down Syndrome over the first week of age.

Table 4.9: Day 3 Clinical characteristics, pulmonary vascular resistance measurements and myocardial functional measurements within the control cohort of infants born via Caesarean section and vaginal delivery.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Caesarean Section (n=41)	Vaginal Delivery (n=19)	p
Gestation (weeks)	39.3 ± 1.1	40.2 ± 1.0	<0.01
Birthweight (Kg)	3.52 ± 0.40	3.63 ± 0.46	0.32
Maternal age (Years)	35 ±4	32 ± 4	0.05
PAAT (ms)	68 ± 13	66 ± 11	0.61
PAATi (ms)	0.34 ± 0.05	0.33 ± 0.04	0.71
LV Eccentricity Index	1.3 ± 0.2	1.4 ± 0.3	0.36
LV Length (mm)	31 ± 2	31 ± 2	0.59
LV GLS (%)	21 ± 2	21 ± 2	0.98
RV Length (mm)	30 ± 2	31 ± 2	0.52
RV FW LS (%)	21 ± 2	22 ± 3	0.15

Chapter 5 : Longitudinal Assessment of Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome using Novel Echocardiography Techniques over the First Two Years of Age

5.1 Background

As explored in chapter 1, infants with Down syndrome (DS) are at an increased risk for both early and late pulmonary hypertension (PH)[136]. Biventricular myocardial dysfunction has also been documented from the foetal stage to adulthood in individuals with DS, irrespective of the presence of CHD[9-11, 56, 57]. With growing appreciation in the paediatric and neonatal communities of the high prevalence of PH in the DS population the relationship between PH and myocardial function warranted further elucidation.

There is a dearth of longitudinal data describing the evolution of myocardial performance and pulmonary haemodynamics in infants with DS, both with and without CHD, over the first two years of age. The interplay between myocardial performance and development of PH over time was unclear and I queried if impaired myocardial function could be an unexplored contributing factor to the development of PH with age in the DS cohort.

To thoroughly assess these phenomena, I utilised advanced echocardiography techniques including tissue Doppler imaging (TDI) and STE, full explanations for which are detailed in Chapter 2. Such novel modalities are superior to conventional echocardiography techniques as they provide a more objective and sensitive appraisal of myocardial performance.

My hypothesis was that babies with DS, independent of the presence of CHD, will demonstrate biventricular myocardial systolic and diastolic impairment and sustained elevation of pulmonary pressures over the first two years of age. I sought to expand on the previous work by our group with a particular focus on the assessment of diastolic function in

the DS group and to evaluate if the discrepancies between the DS and control cohorts regarding RV performance, markers of PH and anthropometric measurements persisted or dissipated over time in a larger study cohort. The aim of this prospective, tri-centre, observational cohort study was to serially assess left ventricular (LV) and right ventricular (RV) anthropometry, biventricular performance, and measurements of pulmonary haemodynamics in babies with DS with and without CHD using advanced echocardiography techniques over the first two years of age and to compare those measurements to a cohort of non-DS healthy controls.

5.2 Specific Methodology Employed

5.2.1 Study Design and Study Population

This was a prospective, observational cohort study performed across the three tertiary neonatal intensive care units of Dublin: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. All babies with a diagnosis of DS made antenatally or postnatally (later confirmed with Karyotyping) were eligible for inclusion. Babies with DS with and without congenital heart disease (CHD) were enrolled. A cohort of healthy term infants without a diagnosis of DS were recruited from The Rotunda Hospital to act as a control population. Pertinent antenatal, birth and neonatal characteristics and outcome data were documented for both the DS and control cohorts at the local neonatal unit of recruitment. Clinical data was recorded as per parental response at each follow up assessments performed in The Rotunda Hospital at 6 months, 1 year and 2 years of age for both the DS and control populations. Enrolment criteria and data collection techniques are fully detailed in the methodology chapter (Chapter 2).

5.2.2 Echocardiography Assessment

Echocardiography scans were performed at six time points over the first two years of age for both the DS and control cohorts. Three echocardiograms were carried out over the first week of age: Day 1 (Echo 1), Day 2 (Echo 2) and Day 3-5 (Echo 3) and at three further time points over the first two years of age: 6 months (Echo 4); 12 months (Echo 5); and 2 years (Echo 6). The first echocardiogram of each infant included formal evaluation for any congenital heart disease. Echocardiography assessed myocardial structure and function and pulmonary haemodynamics. Novel echocardiography techniques were utilised to evaluate myocardial performance. Novel echocardiography techniques included tissue Doppler imaging (TDI) and deformation analysis via 2D STE. A detailed explanation of all advanced techniques utilised in this study is available within Chapter 2.

5.2.3 Statistical Analysis

The cohort was be divided into three groups: infants with DS and congenital heart disease (CHD) (DS-CHD), infants with DS and no CHD (DS-no CHD) and controls. Continuous data was tested for normality using the Shapiro-Wilk test and a histogram representation of data and summarised as means (standard deviation) or medians [inter-quartile range] as appropriate. Categorical data was summarised as counts (%). Three group analyses was conducted using one way ANOVA or the Kruskal-Wallis Test. Serial data was compared using two way ANOVA with repeated measures. We used SPSS version 23 to conduct the analyses. Statistical significance is achieved with a p value < 0.05.

5.3 Our Findings

Seventy infants with DS and 60 control infants were enrolled into this prospective, tri-centre cohort study. Echocardiography was performed over the first week of age and at 6 months, 12 months and 2 years of age. Advanced echocardiography modalities were employed to evaluate anthropometric measurements, biventricular systolic and diastolic performance and pulmonary haemodynamics between the DS and control cohorts.

5.3.1 Timing and Number of Echocardiograms in the Down Syndrome and Control Cohorts over the First Two Years of Age

A total of 626 echocardiograms were performed between the DS and control cohorts over the study period. For the DS cohort 58 Day 1, 66 Day 2, and 65 Day 3-5, 41 6-month, 52 12-month and 40 24-month echocardiograms were performed. For the control cohort 60 Day 1, 58 Day 2 and 52 Day 3-5, 50 6 month, 45 12 months and 39 24 month echocardiograms were performed **(Table 5.1)**. There was a degree of loss to follow up for the longitudinal echocardiograms. However, there were no statistically significant differences in important clinical parameters between those infants with missing versus complete data in the DS cohort at 2 years of age **(Table 5.2).**

5.3.2 Anthropometric Measurements over the First Two Years of Age

There were significant differences in birth weight between the three cohorts: namely the control, the DS-CHD and the DS-no CHD cohort. Although weight measurements in both DS cohorts had normalised by 6 months of age there were significant differences between the control and DS-CHD cohort at both 12 and 24 months of age and between the control- and no CHD cohort at 24 months of age. There was no difference in weight detected at any time point between the DS-CHD and DS-no CHD groups **(Figure 5.1).**

Mitral valve annuli measurements were smaller in diameter on Day 1 of age between the control and DS-CHD and the control and DS-no CHD infants, a finding which is consistent with previous work by our group[2]. These differences were also evident at the 6 and 24 month time points. There were no differences in mitral valve annuli diameter measurements between the DS-CHD and DS-no CHD babies at any stage **(Figure 5.2)**.

Babies with DS, both those with and without CHD, had significantly shorter LV lengths throughout the entire study period. There was no difference in LV length between the DS-CHD and DS-no CHD groups at any timepoint indicating that structural heart disease does not appear to impact LV length **(Figure 5.3)**.

Infants with DS and structurally normal hearts had smaller tricuspid valve annulus diameters on Day 1 of age compared to controls, however their measurements normalised over time. No differences in tricuspid valve annuli values between the groups at any other time point were identified **(Figure 5.4)**.

Similar to LV length measurements, infants with DS, both those with and without CHD, had significantly shorter RV lengths consistently throughout the first two years of age. The DS -no CHD cohort had shorter RV length measurements than the DS-CHD cohort on Day 1 of age, however this difference had dissipated by the 6 month echocardiogram **(Figure 5.5)**.

5.3.3 Pulmonary Vascular Resistance Measurements over the First Two Years of Age

Pulmonary vascular resistance (PVR) remained abnormally elevated throughout the first two years of age in infants with DS regardless of the presence of structural heart disease. The PAAT, an echocardiography marker inversely related to PVR, remained significantly lower in both the DS-CHD and the DS-no CHD groups compared to controls from birth to 2 years of age **(Figure 5.6)**. The PAAT to right ventricular ejection time ratio (PAAT:RVET), a surrogate for PH, also remained persistently low in babies with DS, both with and without CHD, compared to controls at each echocardiography assessment **(Figure 5.7)**. Similarly, the significantly increased LV eccentricity index measurements in both the DS-CHD and DS-no CHD cohorts compared to controls from birth to two years of age document

persistent elevation of right ventricular pressures **(Figure 5.8)**. There was no difference detected between the DS-CHD and DS-no CHD groups across any of the three echocardiography derived surrogates of PH; PAAT, PAAT:RVET or LV eccentricity index at any timepoint throughout the study **(Figure 5.6, Figure 5.7, Figure 5.8)**.

5.3.4 Tissue Doppler Imaging Event Timings over the First Two Years of Age

There was no difference in heart rate detected between the groups at any point in the study period **(Figure 5.9)**. On Day 1 assessment the systolic: diastolic time ratio was significantly elevated in both DS-CHD and DS-no CHD groups compared to controls however this normalised on follow up assessment at 6 month, 1 year and 2 years. There was no difference in systolic: diastolic time ratio between the DS-CHD and DS-no CHD groups at any time point **(Figure 5.10)**. The isovolumic relaxation time was significantly prolonged in babies with DS, both with and without CHD, on Day 1 of age compared to controls but these differences were not observed on longitudinal assessment **(Figure 5.11).**

5.3.5 Tissue Doppler Imaging Measurements over the First Two Years of Age

TDI assessment demonstrated significantly reduced LV peak systolic velocities (s') in the DS-CHD group on Day 1 of age, 6 months, 12 months and 24 months of age compared to the control group. Regarding the DS-no CHD cohort; their LV s' velocities were also significantly reduced compared to controls at the 6 and 12 month assessments. There was no difference in LV s' measurements between the DS-CHD and DS-no CHD babies at any time point over the first two years of age **(Figure 5.12)**. LV early diastolic velocities (e') were similarly affected in the DS-CHD group over the first two years of age with impaired velocities compared to controls at each time point. LV e' velocities were also significantly diminished in the DS-no CHD group compared to controls at the 6 month, 12 month and 24 month echocardiograms. No difference was detected in LV e' velocities between the DS-CHD and DS-no CHD groups at on any stage **(Figure 5.13)**. LV late diastolic velocities (a') were significantly lower in the DS-no CHD cohort at 12 months of age and significantly higher than both the control and DS-CHD cohort at 24 months of age. No other differences

in LV a' velocities were identified over the study period **(Figure 5.14)**. The LV ea' ratio demonstrated persistent diastolic impairment in the DS-CHD group from birth to 2 years of age. There was no difference in LV ea' values between the two DS groups at any stage **(Figure 5.15)**.

Septal peak systolic velocities (s') were significantly lower in the DS-CHD group compared to the control group throughout the first two years of age. In addition, the DS-CHD cohort had significantly lower septal s' velocities compared to the DS-no CHD cohort at both 6 and 12 months of age. Babies with DS and structurally normal hearts demonstrated impaired septal s' velocities compared to controls at 12 months of age only **(Figure 5.16)**. Ongoing early diastolic impairment in the DS-CHD group was identified in abnormal septal e' velocities on Day 1, 6 month, 12 month and 24 month evaluation. Babies with DS and structurally normal hearts also had reduced septal e' velocities at 6 month and 24 month assessment with comparable results to controls on Day 1 and 12 month assessment. There was no difference in septal e' measurements between the DS-CHD and DS-no CHD groups at any time point over the first two years of age **(Figure 5.17)**. Regarding septal late diastolic function (a') infants with DS and CHD had significantly lower a' velocities at 12 months of age compared to controls which had normalised by 24 months of age. Septal a' values were otherwise comparable between the three groups over the study period **(Figure 5.18)**. A significant difference in septal ea' ratio was identified between the control and DS-no CHD cohort at 6 months of age which normalised on longitudinal assessment. Septal ea' values were comparable between the three groups at all other echocardiography assessments **(Figure 5.19)**.

On TDI assessment of RV systolic performance the peak systolic velocity (s') revealed evolving RV systolic impairment with age in the DS-CHD cohort. While normal on Day 1 assessment, RV s' velocities were significantly decreased in infants with DS and CHD at 6 months, 12 months and 2 years of age compared to controls. Although no difference in RV s' was detected between the control group and DS-no CHD at any time point, the DS-no CHD group had significantly greater RV s' velocities at 12 months of age compared to the DS-CHD group **(Figure 5.20)**. RV early diastolic velocities (e') show a similar trajectory to RV s' evaluation; with impaired RV e' measurements from 6 months to 2 years of age in the DS-

CHD cohort compared to controls. Significant differences were also identified in RV e' values between the DS-CHD and DS-no CHD infants at 6 months, 12 months and 2 years of age with DS-CHD infants displaying RV early diastolic impairment at each of these time points **(Figure 5.21)**. RV late diastolic velocities (a') were abnormally high in the DS-no CHD group compared to controls on Day 1 evaluation but this finding had dissipated by 6 month assessment. There were no other significant differences in RV a' velocities detected between the three groups over the two year study period **(Figure 5.22)**. On RV ea' assessment babies with DS and CHD had significantly diminished ratios on Day 1 assessment and 6 months of age which had normalised at 12 month and 2 year evaluation. The DS-no CHD group was equivalent to controls in RV ea' measurements at all time points **(Figure 5.23)**.

5.3.6 Deformation Measurements over the First Two Years of Age

LV global longitudinal strain was significantly reduced in both the DS-CHD and DS-no CHD groups compared to control infants at Day 1, 6 months, 12 months and 2 years of age indicating sustained systolic impairment in infants with DS over the study period. There was no difference in LV global longitudinal strain measurements between infants with and without structural cardiac defects and DS over the first two years of age **(Figure 5.24)**. Babies in the DS-CHD group also displayed persistently, and significantly low LV longitudinal systolic strain rate measurements compared to the control group from birth to 2 years of age at each study time point. Babies in the DS-no CHD group recorded abnormally low LV systolic strain rate values on Day 1, 6 months and 12 months of age which had normalised by 2 years of age. There was no difference in LV systolic strain rate detected between the DS-CHD and DS-no CHD groups over the two year period **(Figure 5.25)**. Akin to TDI LV early diastolic velocities, the DS-CHD cohort displayed significant, sustained early diastolic impairment on LV early diastolic strain rate measurements at each assessment over the two year period. LV early diastolic strain rate was also significantly low on Day 1 and 6 months for babies with DS-no CHD, however this finding had normalised by the 12 month and 2 year assessments. There were no differences in LV early diastolic strain rate values between the DS-CHD and DS-no CHD cohorts at any time point over the first two years of age **(Figure**

5.26). On evaluation of LV late diastolic strain rate measurements no difference was detected between any of the groups throughout the study period **(Figure 5.27)**.

RV free wall longitudinal systolic strain was significantly reduced in infants with DS-CHD compared to controls from birth to 2 years of age at each echocardiography assessment; data which is consistent with RVI TDI s' findings. The DS-no CHD group also displayed decreased RV free wall systolic strain in comparison to the control group on Day 1, 6 months at 12 months of age which normalised by 2 years of age. No difference was identified in RV free wall systolic strain between DS-CHD and DS-no CHD infants over the first two years of age **(Figure 5.28)**. RV systolic longitudinal strain rate was also significantly depressed in both the DS-CHD and DS-no CHD groups compared to controls on Day 1, 6 months and 12 months of age. By 2 years of age RV systolic strain rate was comparable across the three groups and no difference was ever detected between the DS-CHD and DS-no CHD groups **(Figure 5.29)**. RV early diastolic strain rate was significantly lower in the DS-no CHD cohort compared to the DS-CHD cohort on Day 1 of age. Additionally, RV early diastolic strain rate was lower in the DS-no CHD group compared to the control group on Day 1. Both the DS-CHD and DS-no CHD groups had significantly reduced RV early diastolic strain rate values at 12 months of age, however measurements were equivalent across the three groups at 6 months and 2 years of age **(Figure 5.30)**. There were no differences identified in RV late diastolic strain rate values over the first two years of age between the control, DS-CHD and DS-no CHD babies **(Figure 5.31)**.

Table 5.1: Timing and number of echocardiograms in the Down syndrome and control cohorts over the first two years of age

	Echo 1	**Echo 2**	**Echo 3**	**Echo 4**	**Echo 5**	**Echo 6**
Down Syndrome	*Hours*	*Hours*	*Hours*	*Months*	*Months*	*Months*
Time [IQR]	19 [9 – 24]	46 [39 – 52]	95 [71 – 122]	6 [6 – 6]	12 [12 – 13]	24 [20 – 24]
Number of Scans	58	66	65	41	52	40
Controls						
Time [IQR]	22 [17 – 25]	44 [38 – 47]	70 [67 – 81]	6 [6 – 6]	12 [12 – 13]	23 [21 – 24]
Number of Scans	60	58	52	50	45	39

Table 5.2: Clinical characteristics of missing versus complete data in the Down syndrome cohort at two years of age

Values are presented as means (SD) or absolute count (%). A p value < 0.05 was considered significant.

	Missing n=30	**Complete n=40**	**p**
Gestation (Weeks)	37.2 ± 2.4	38.2 ± 1.72	0.05
Birth Weight (Kg)	2.9 ± 0.7	3.2 ± 0.7	0.08
Maternal Age (Years)	37 ± 5	37 ± 4	0.71
Male	23 (79%)	23 (58%)	0.45
Caesarean Section	17 (57%)	19 (48%)	0.82

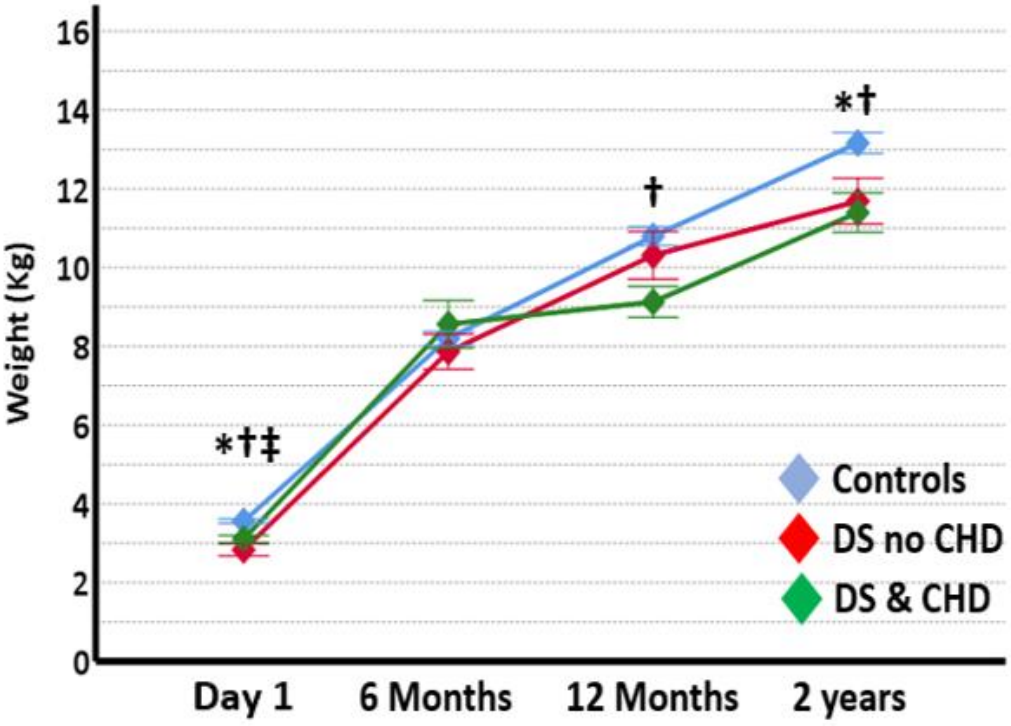
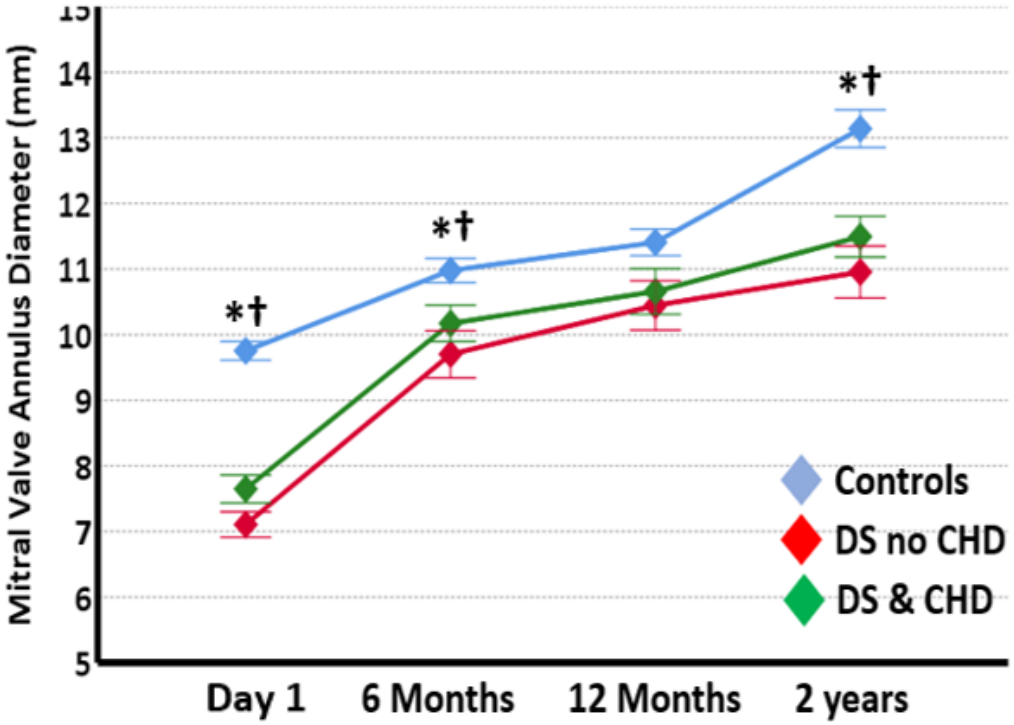


Figure 5.1: Measurement of weight over the first two years of age.

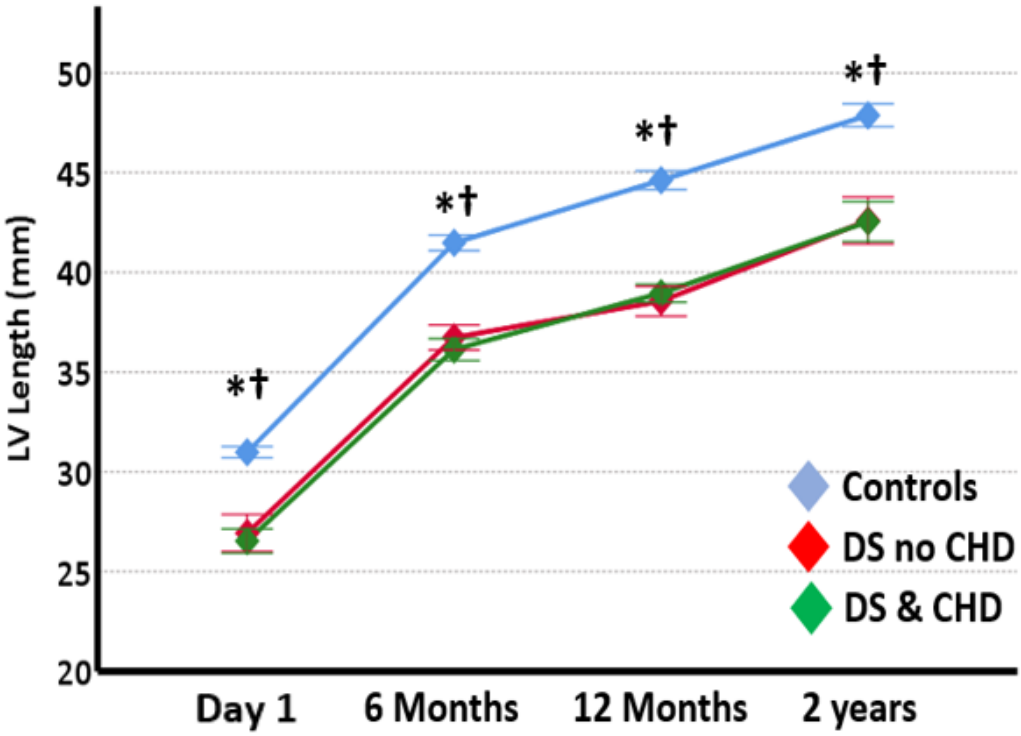
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; kg: kilograms.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.2: Measurement of mitral valve annulus diameter over the first two years of age.

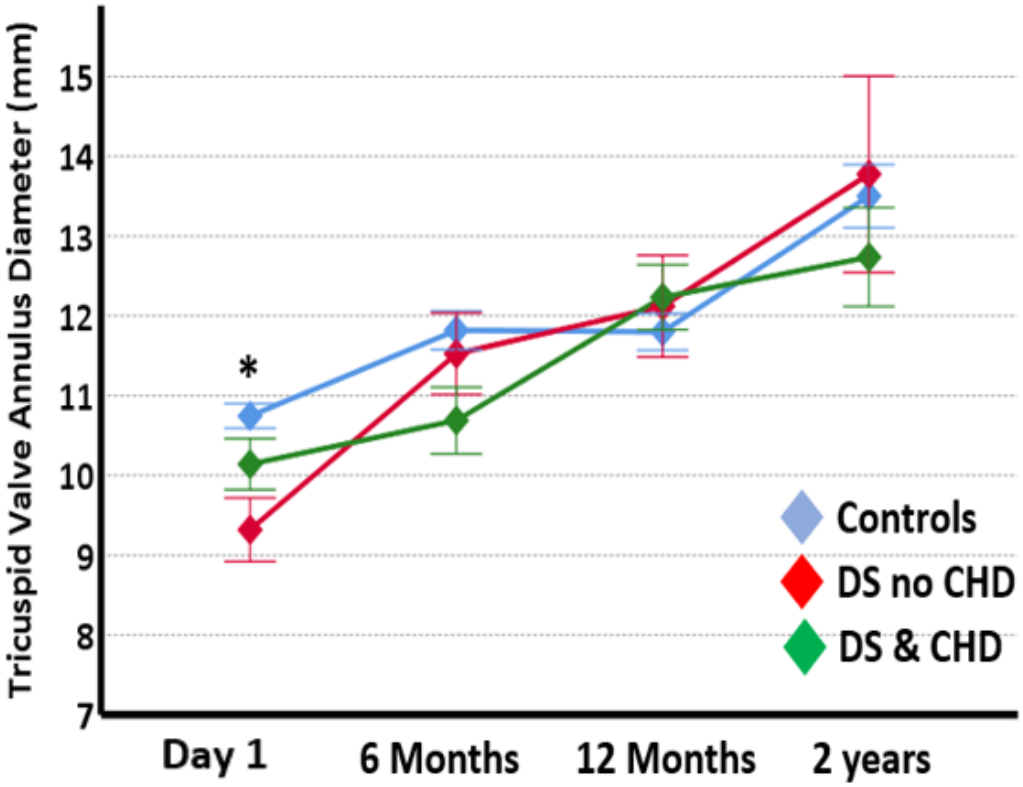
Values are presented as mean and standard error.DS: Down syndrome; CHD: congenital heart disease; mm: millimetres.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.3: Measurement of left ventricular length over the first two years of age.

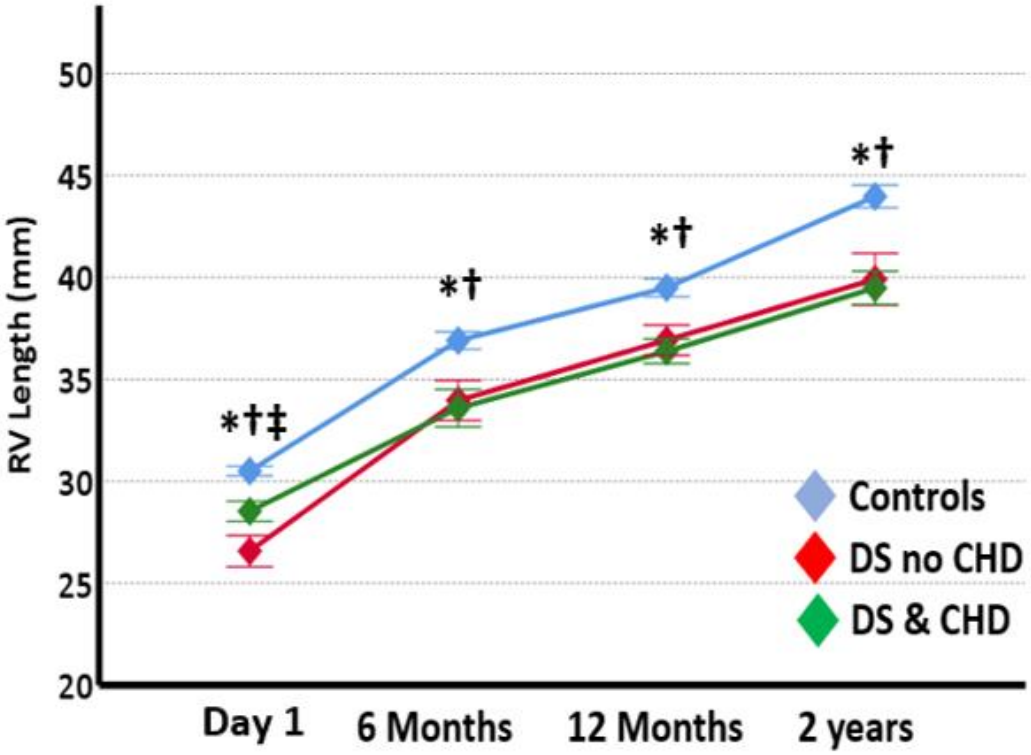
Values are presented as mean and standard error.DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle; mm: millimetres.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.4: Measurement of tricuspid valve annulus diameter over the first two years of age.

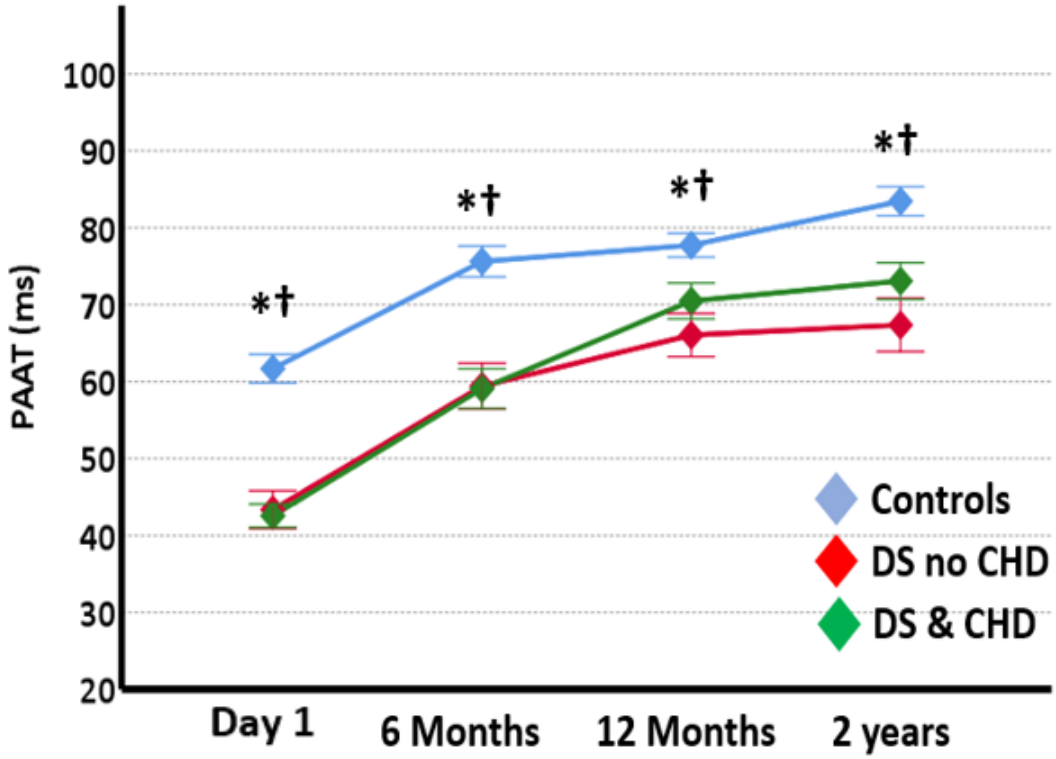
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; mm: millimetres.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.5: Measurement of right ventricular length over the first two years of age.

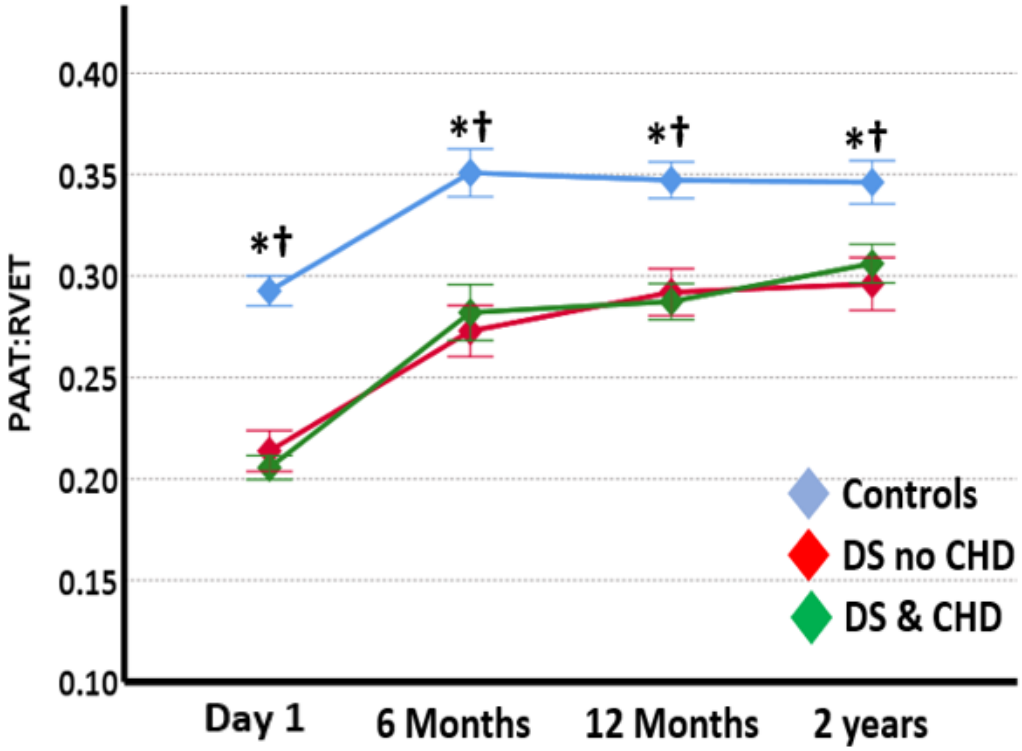
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle; mm: millimetres.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.6: Measurement of pulmonary artery acceleration time over the first two years of age.

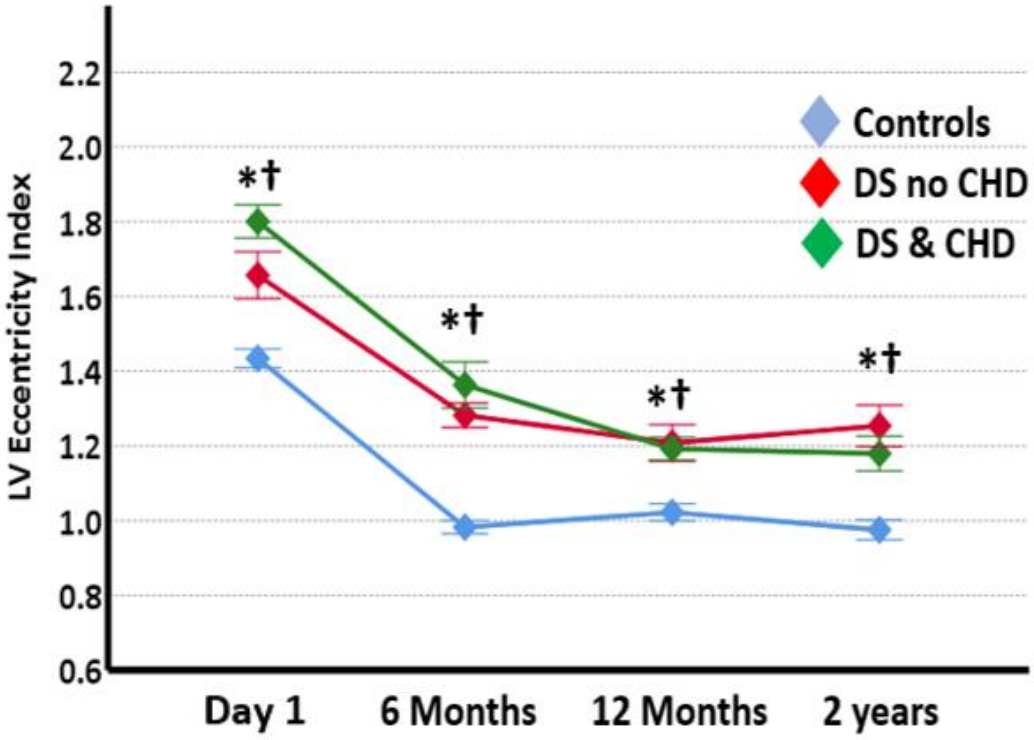
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; PAAT: pulmonary artery acceleration time; ms: milliseconds.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.7: Measurement of pulmonary artery acceleration time to right ventricular ejection time ratio over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; PAAT: pulmonary artery acceleration time; RVET: right ventricular ejection time.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.8: Measurement of left ventricular eccentricity index over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.

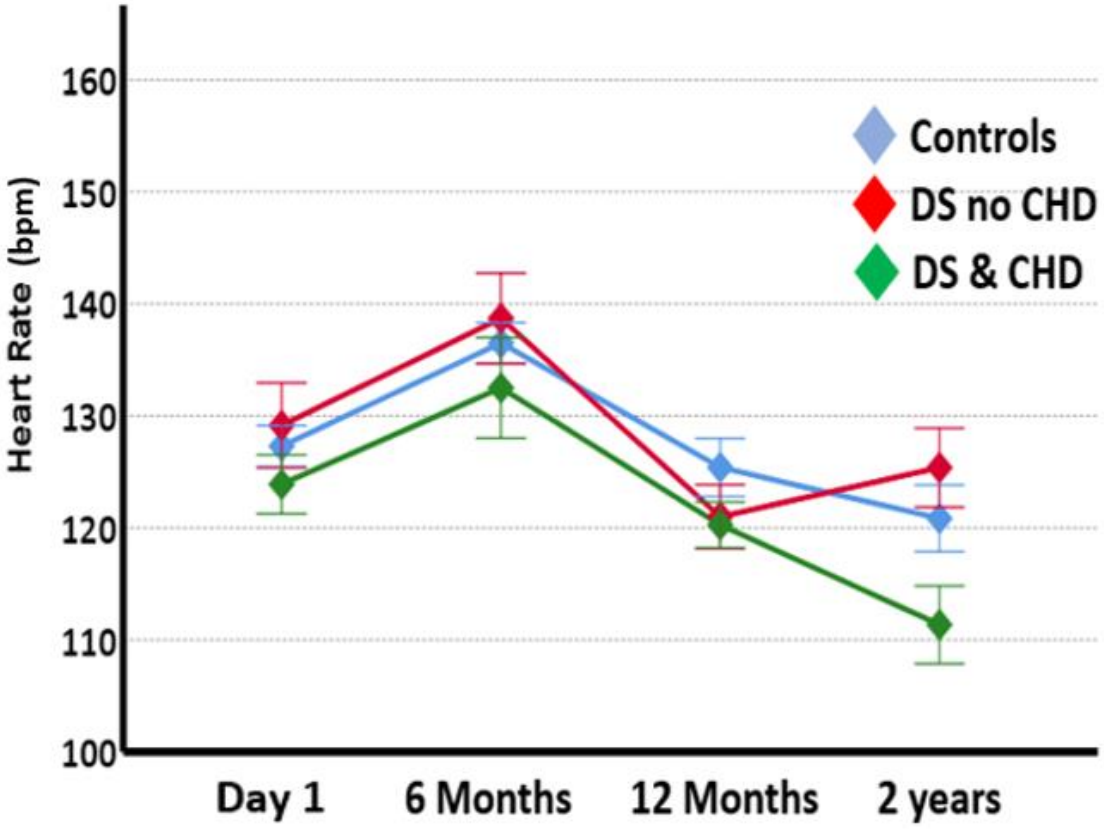
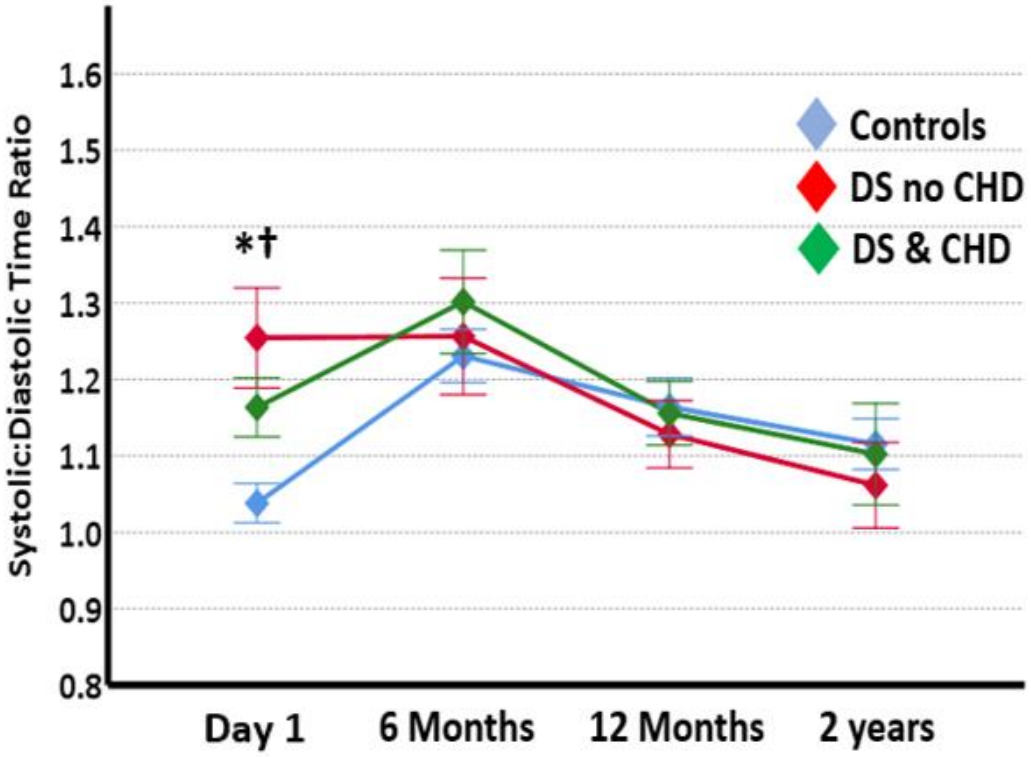


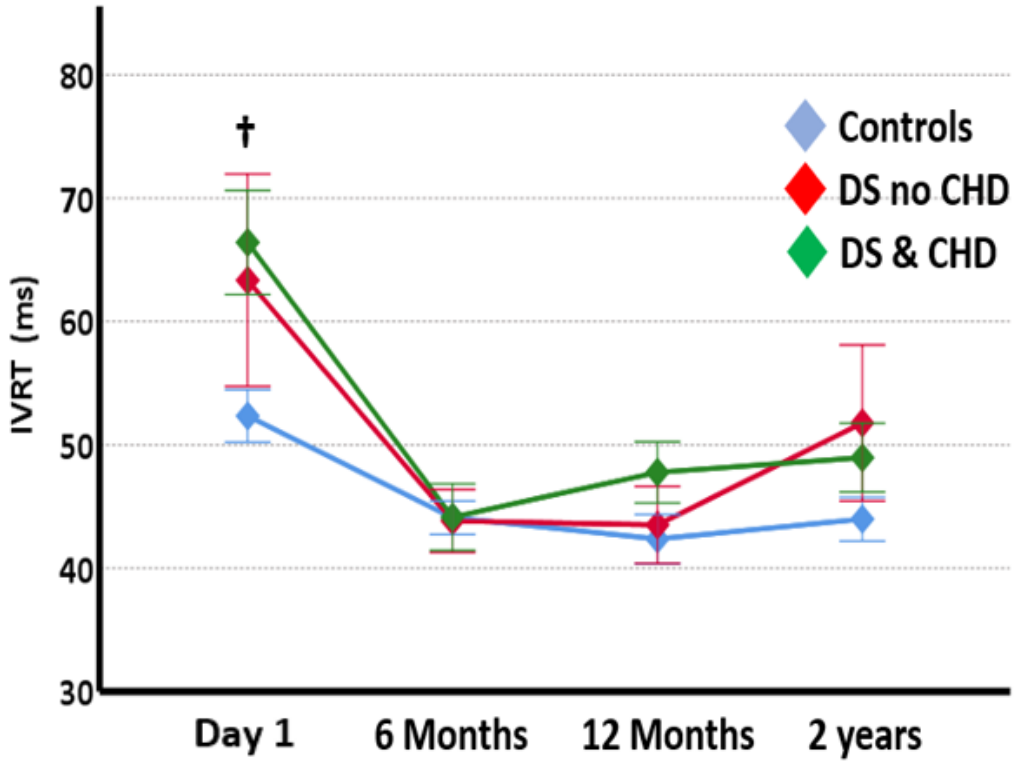
Figure 5.9: Measurement of heart rate (bpm) over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease.



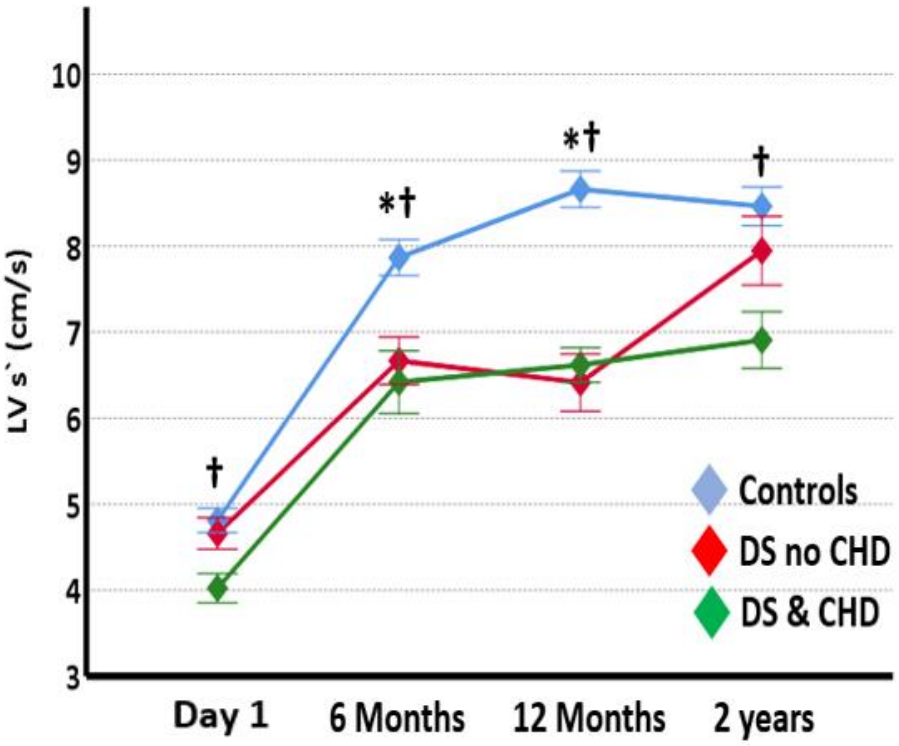
*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.10: Measurement of systolic to diastolic time ratio over the first two years of age. Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

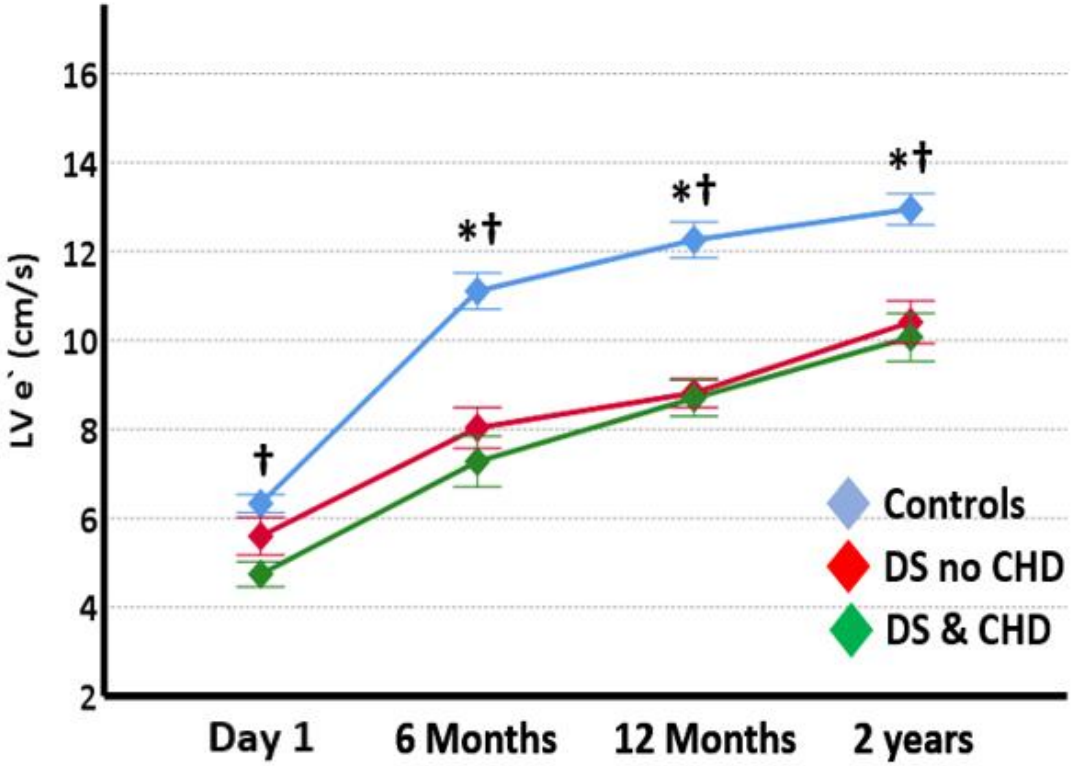
Figure 5.11: Measurement of isovolumic relaxation time over the first two years of age. Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; IVRT: isovolumic relaxation time; mm: millimetres.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.12: Measurement of left ventricular systolic function by tissue Doppler imaging over the first two years of age.

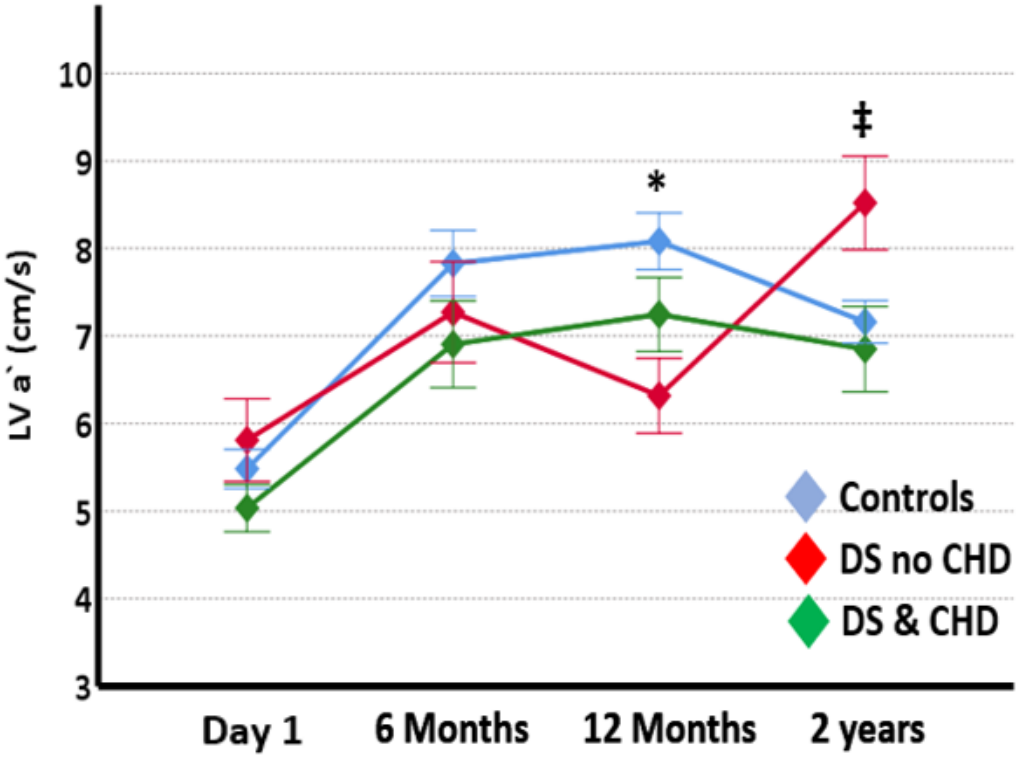
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle; s': peak systolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.13: Measurement of left ventricular early diastolic function by tissue Doppler imaging over the first two years of age.

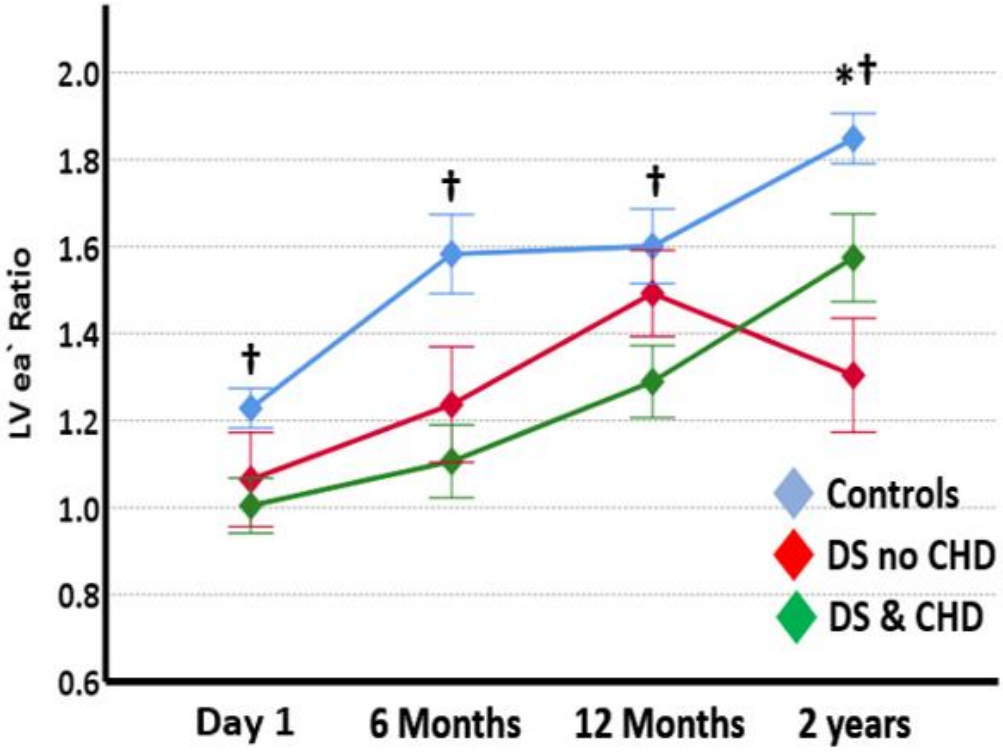
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle; e': early diastolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.14: Measurement of left ventricular late diastolic function by tissue Doppler imaging over the first two years of age.

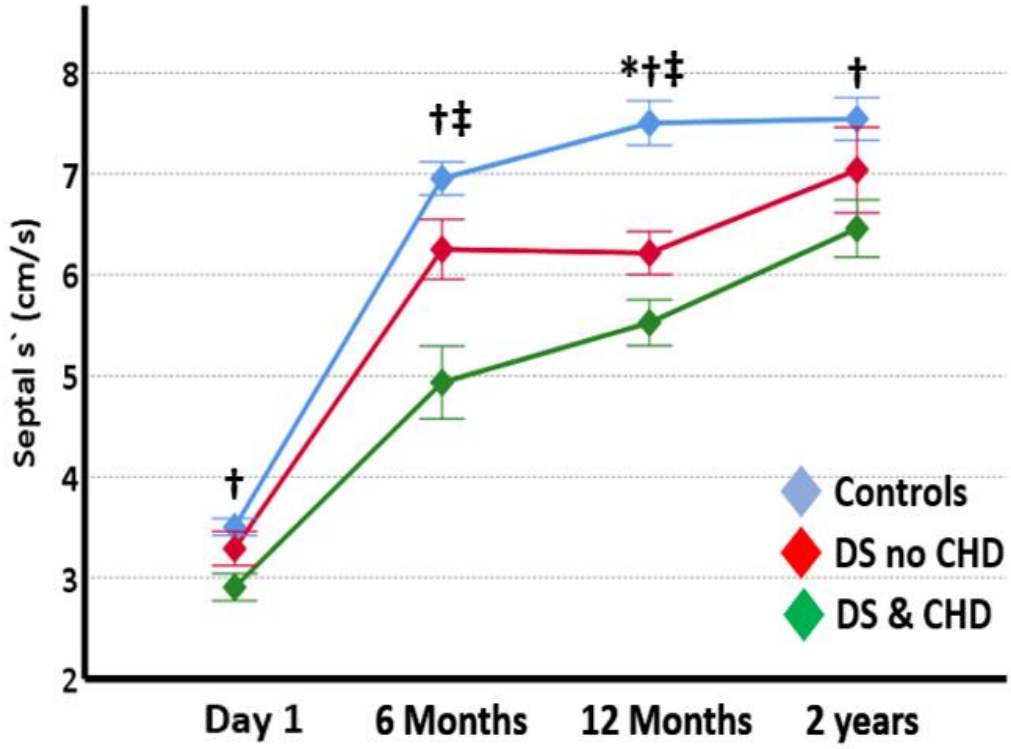
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle; a' late diastolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.15: Measurement of left ventricular early diastolic to late diastolic velocity ratio by tissue Doppler imaging over the first two years of age.

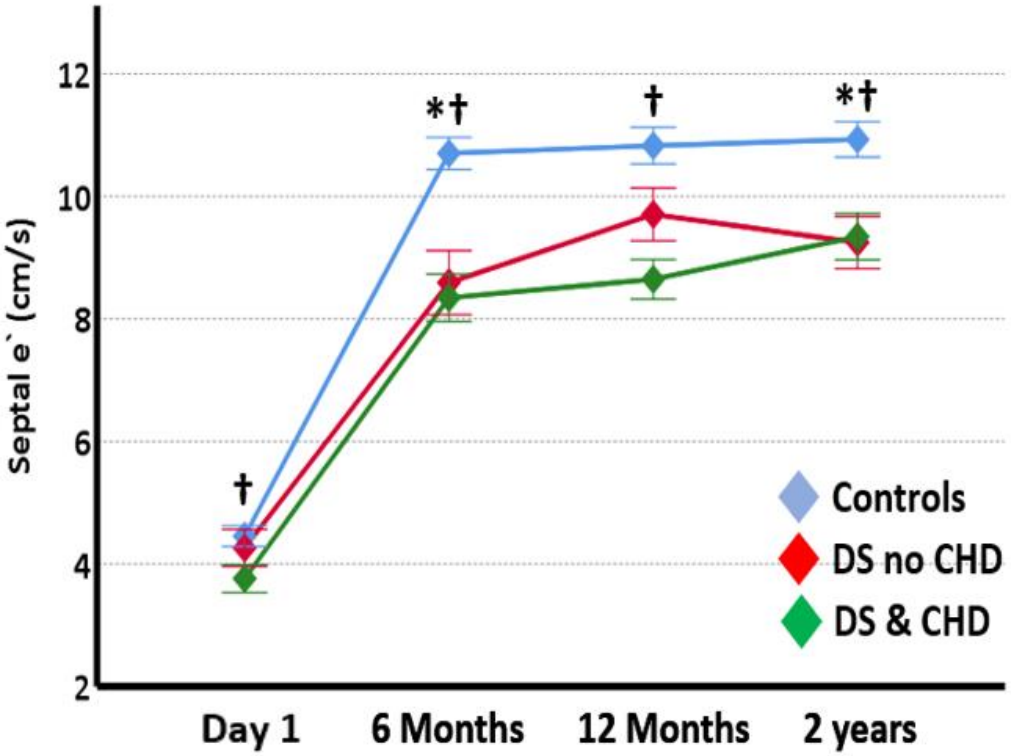
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle; ea': early diastolic to late diastolic velocity ratio.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.16: Measurement of septal ventricular systolic function by tissue Doppler imaging over the first two years of age.

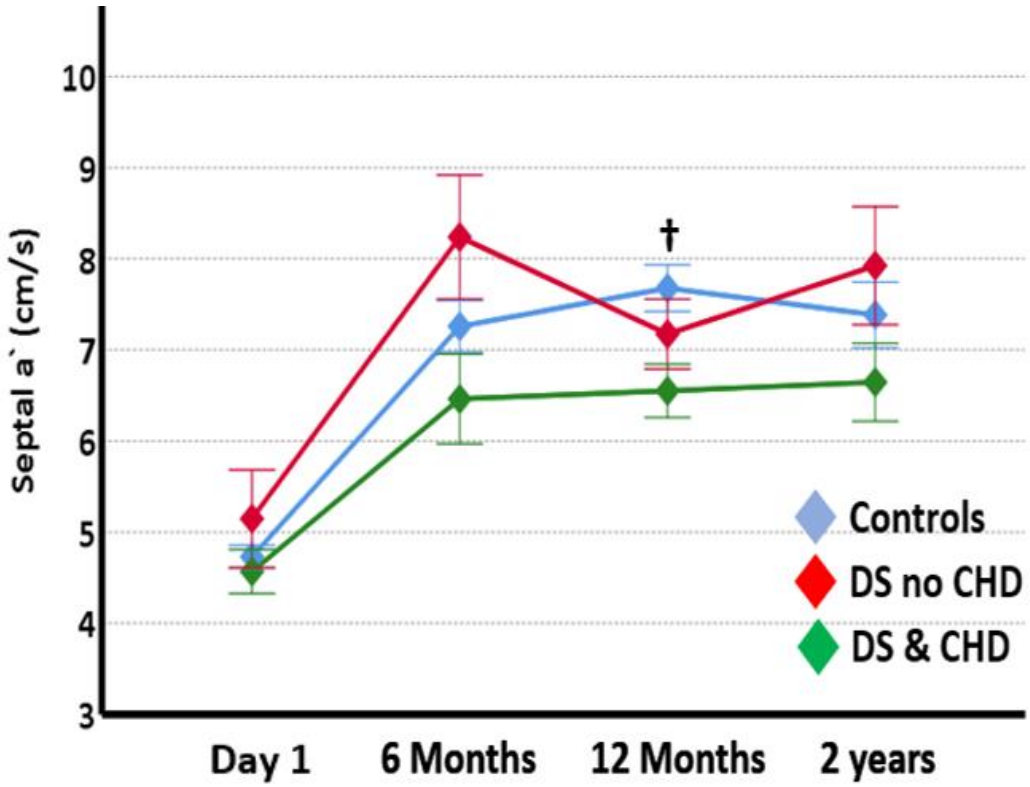
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; s': peak systolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.17: Measurement of septal early diastolic function by tissue Doppler imaging over the first two years of age.

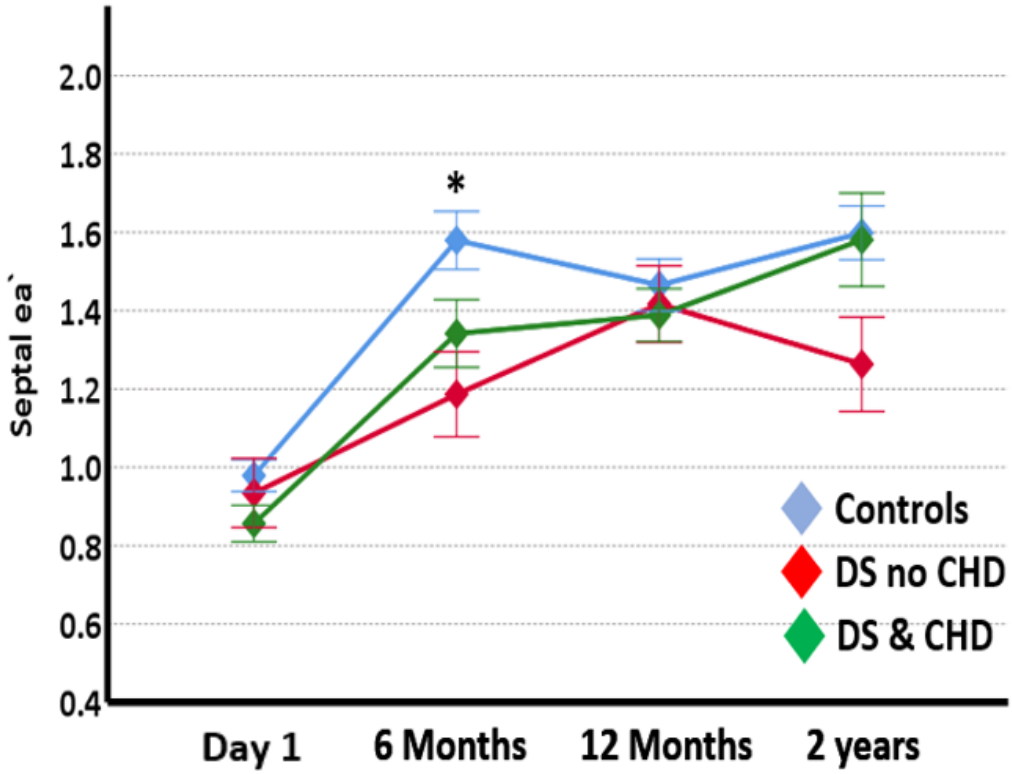
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; e': early diastolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.18: Measurement of septal late diastolic function by tissue Doppler imaging over the first two years of age.

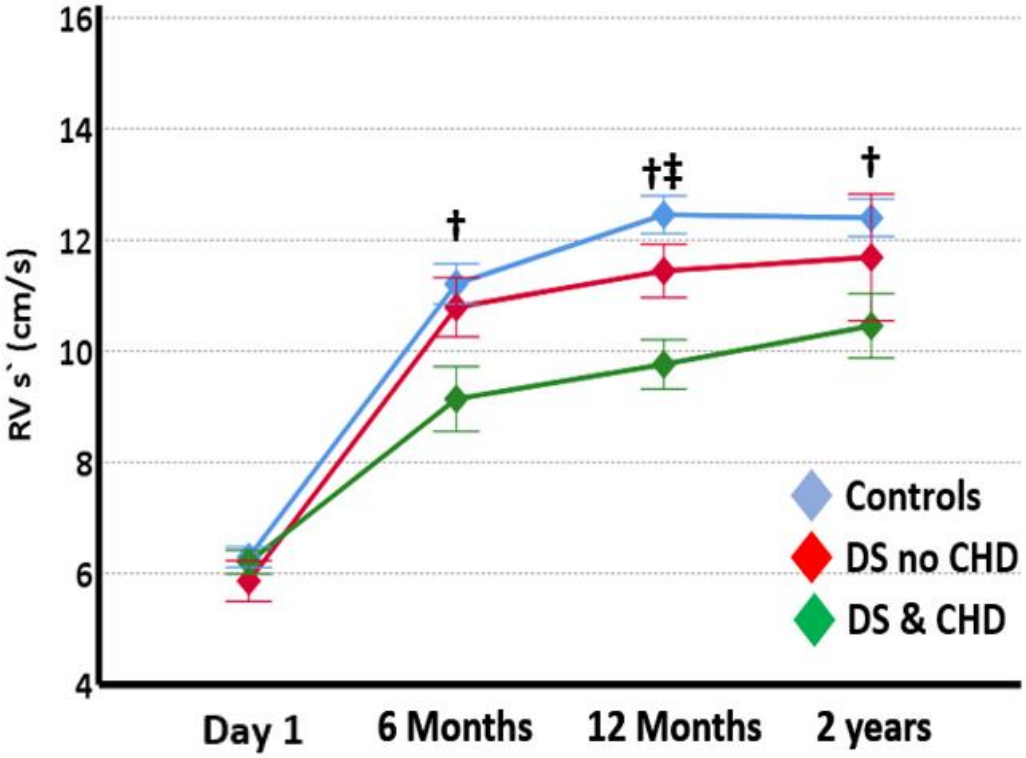
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; a' late diastolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.19: Measurement of septal early diastolic to late diastolic velocity ratio by tissue Doppler imaging over the first two years of age.

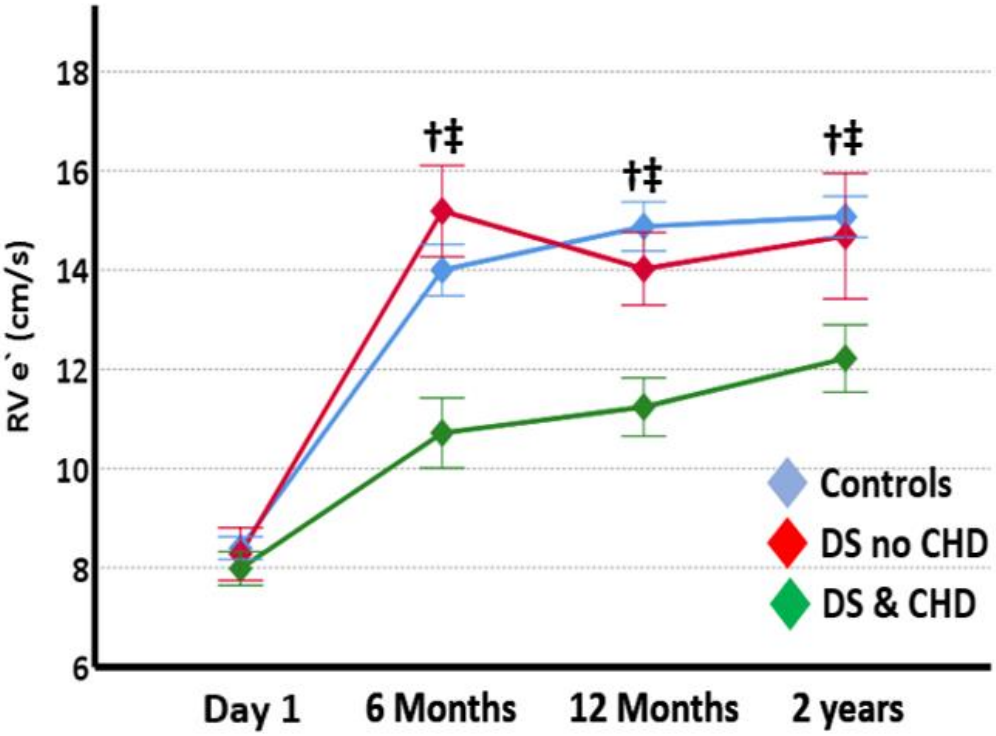
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; ea': early diastolic to late diastolic velocity ratio.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.20: Measurement of right ventricular systolic function by tissue Doppler imaging over the first two years of age.

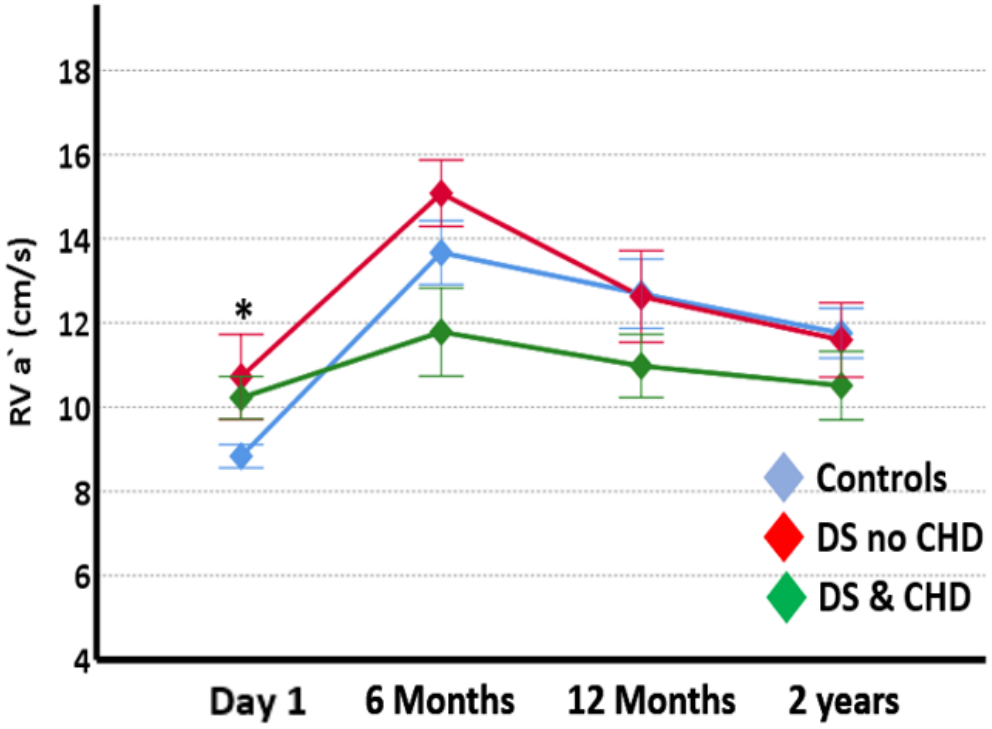
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle; s': peak systolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.21: Measurement of right ventricular early diastolic function by tissue Doppler imaging over the first two years of age.

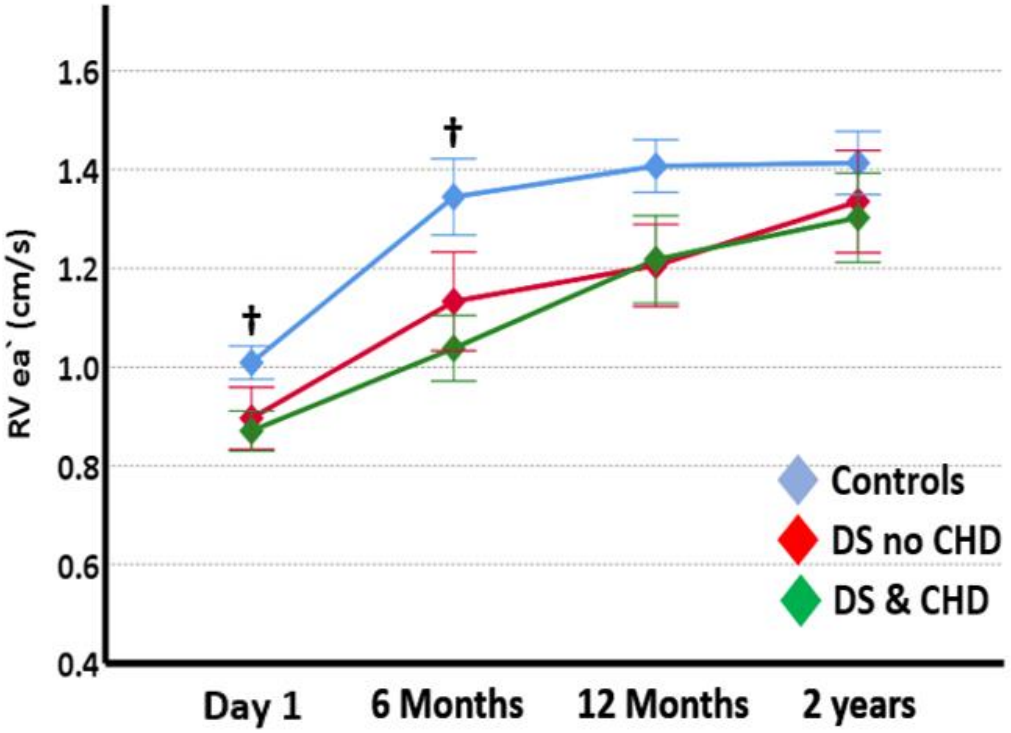
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle; e': early diastolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.22: Measurement of right ventricular late diastolic function by tissue Doppler imaging over the first two years of age.

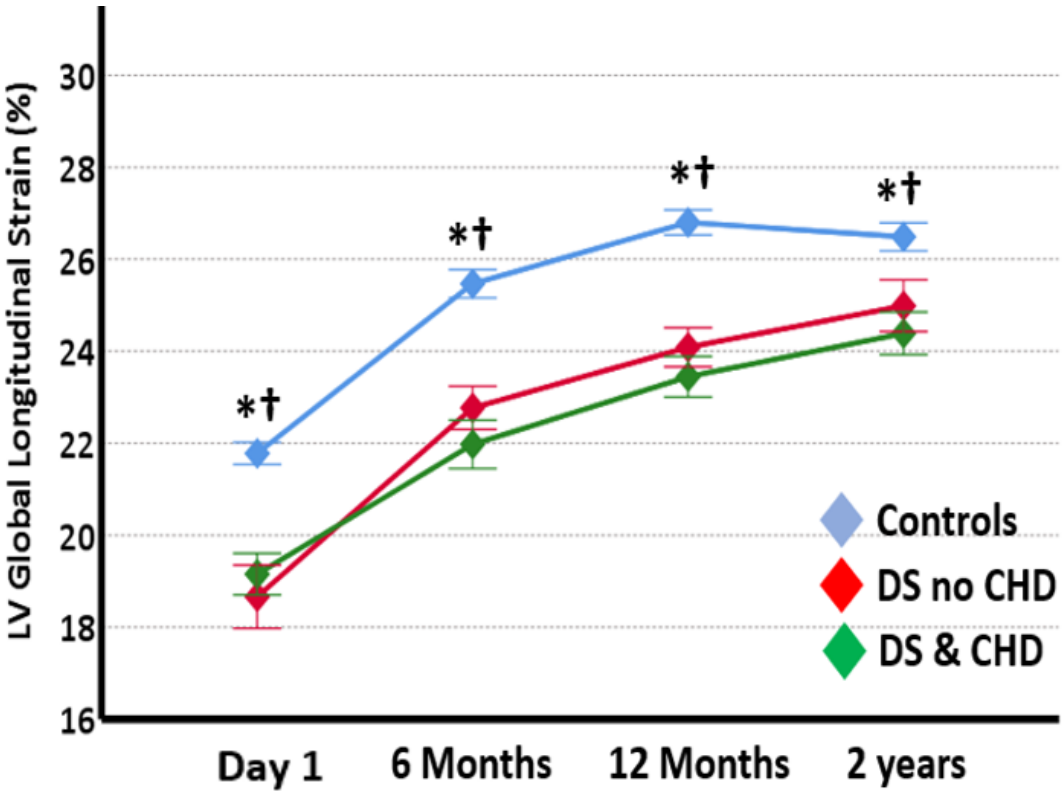
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle; a' late diastolic velocity; cm/s: centimetres per second.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.23: Measurement of right ventricular early diastolic to late diastolic velocity ratio by tissue Doppler imaging over the first two years of age.

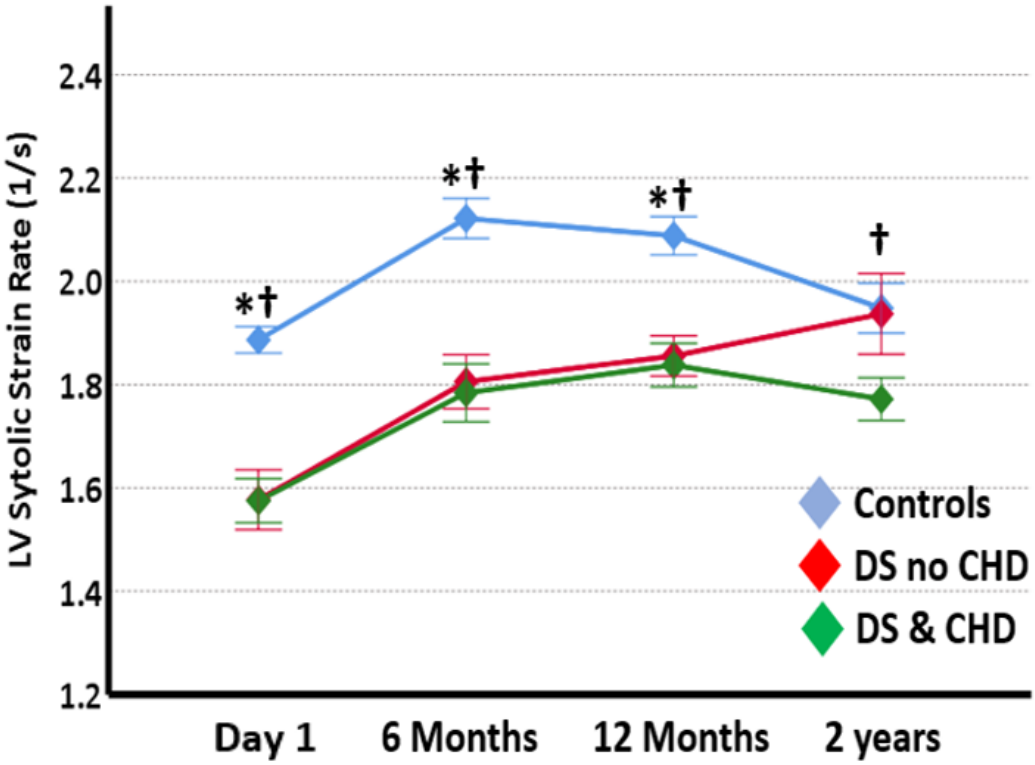
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle; ea': early diastolic to late diastolic velocity ratio.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.24: Measurement left ventricular global longitudinal strain by deformation analysis over the first two years of age.

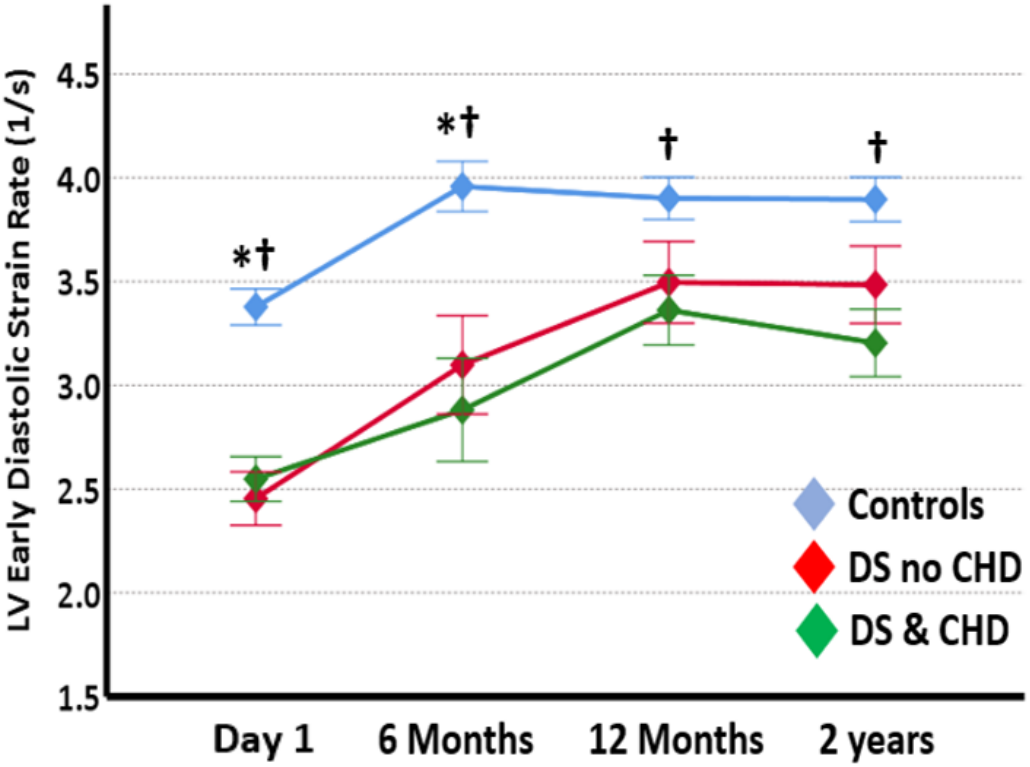
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.25: Measurement left ventricular longitudinal systolic strain rate by deformation analysis over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.26: Measurement left ventricular longitudinal early diastolic strain rate by deformation analysis over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.

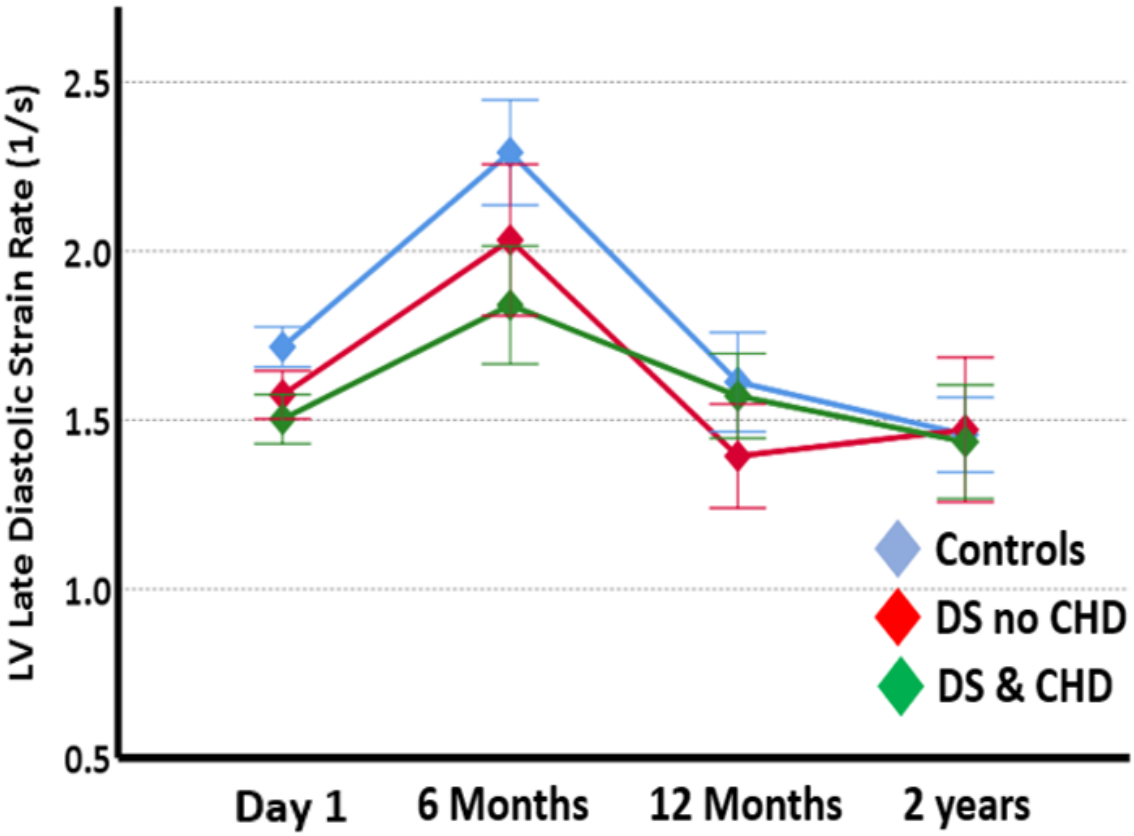
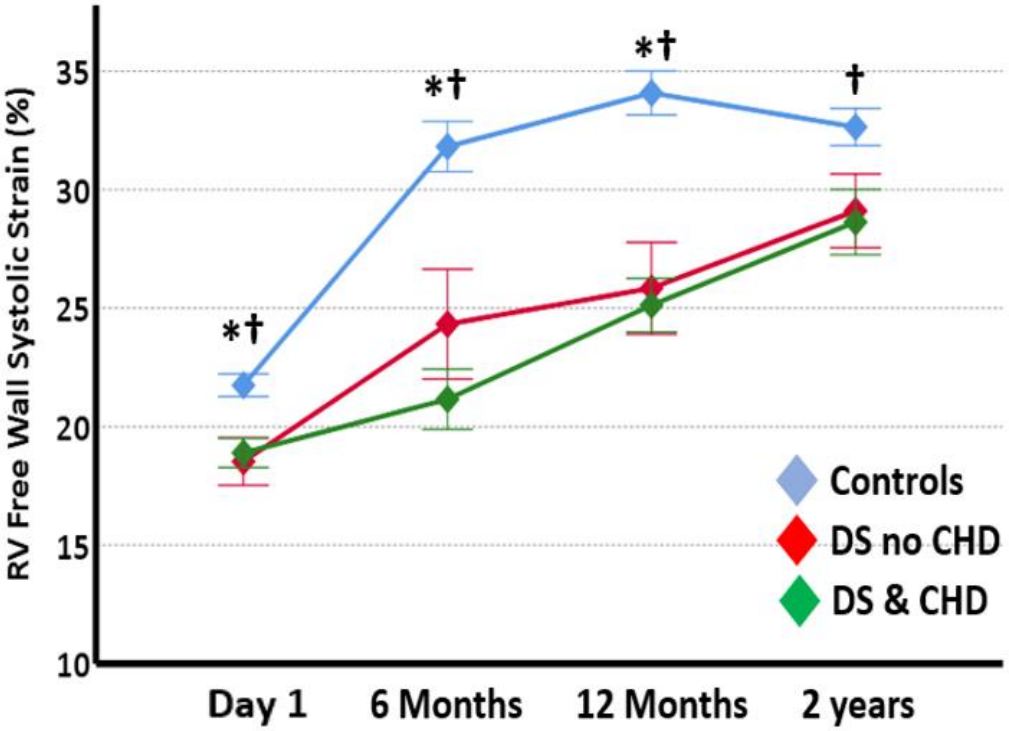


Figure 5.27: Measurement left ventricular longitudinal late diastolic strain rate by deformation analysis over the first two years of age.

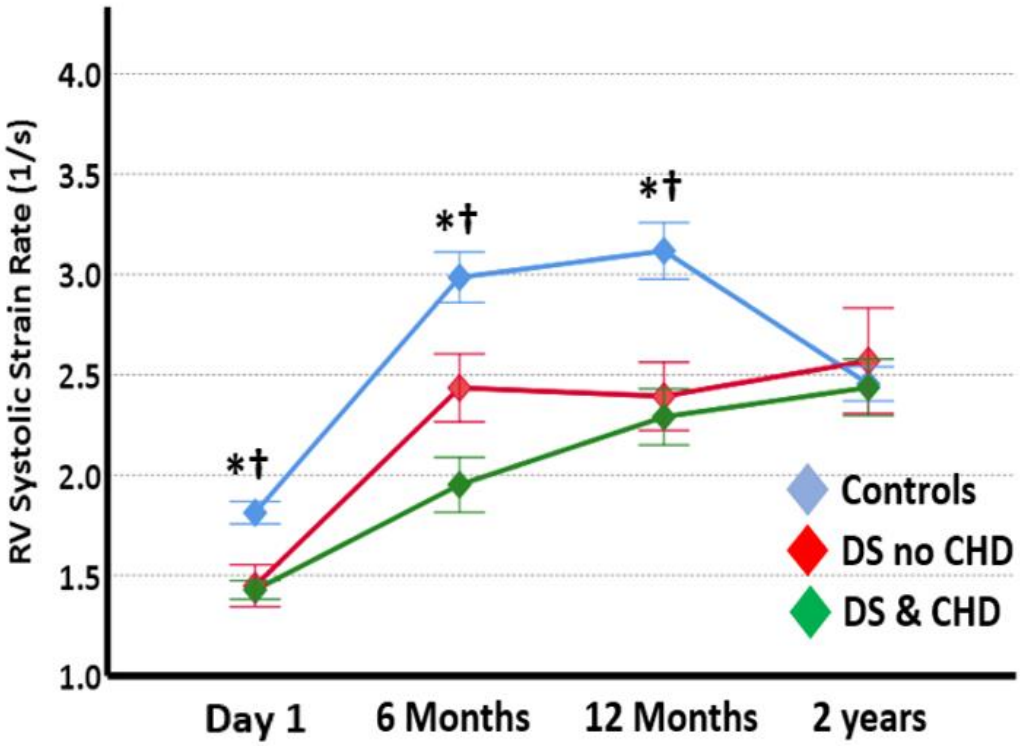
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.28: Measurement right ventricular free wall strain by deformation analysis over the first two years of age.

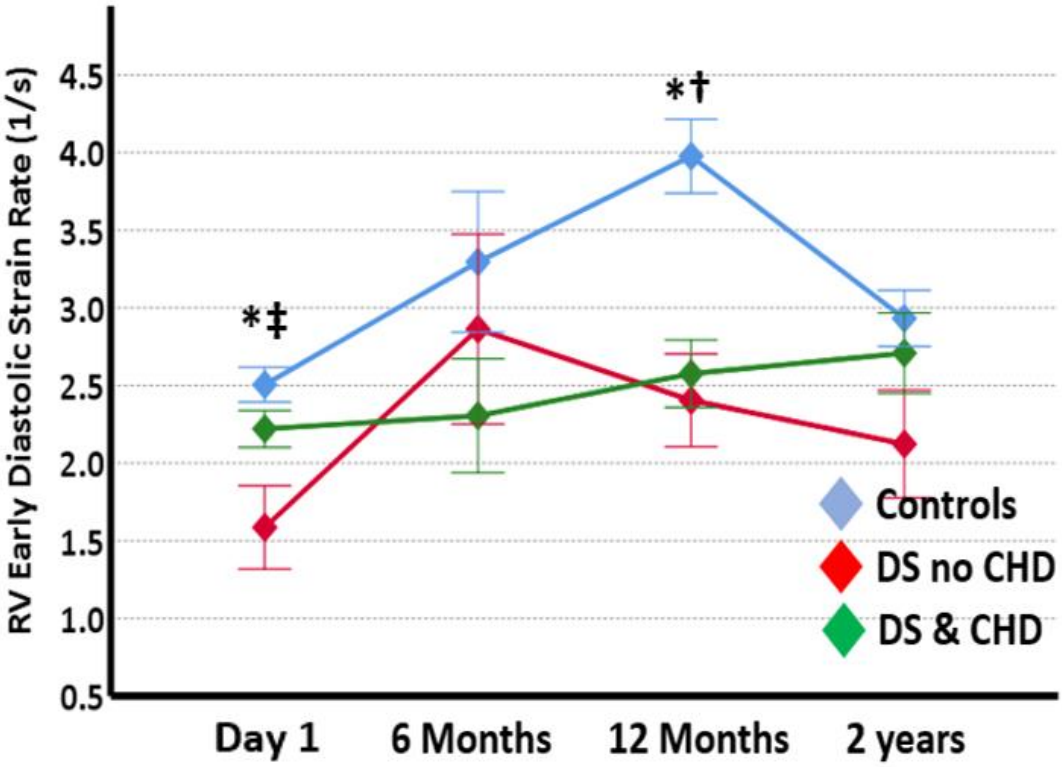
Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.29: Measurement right ventricular longitudinal systolic strain rate by deformation analysis over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 5.30: Measurement right ventricular longitudinal early diastolic strain rate by deformation analysis over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle.

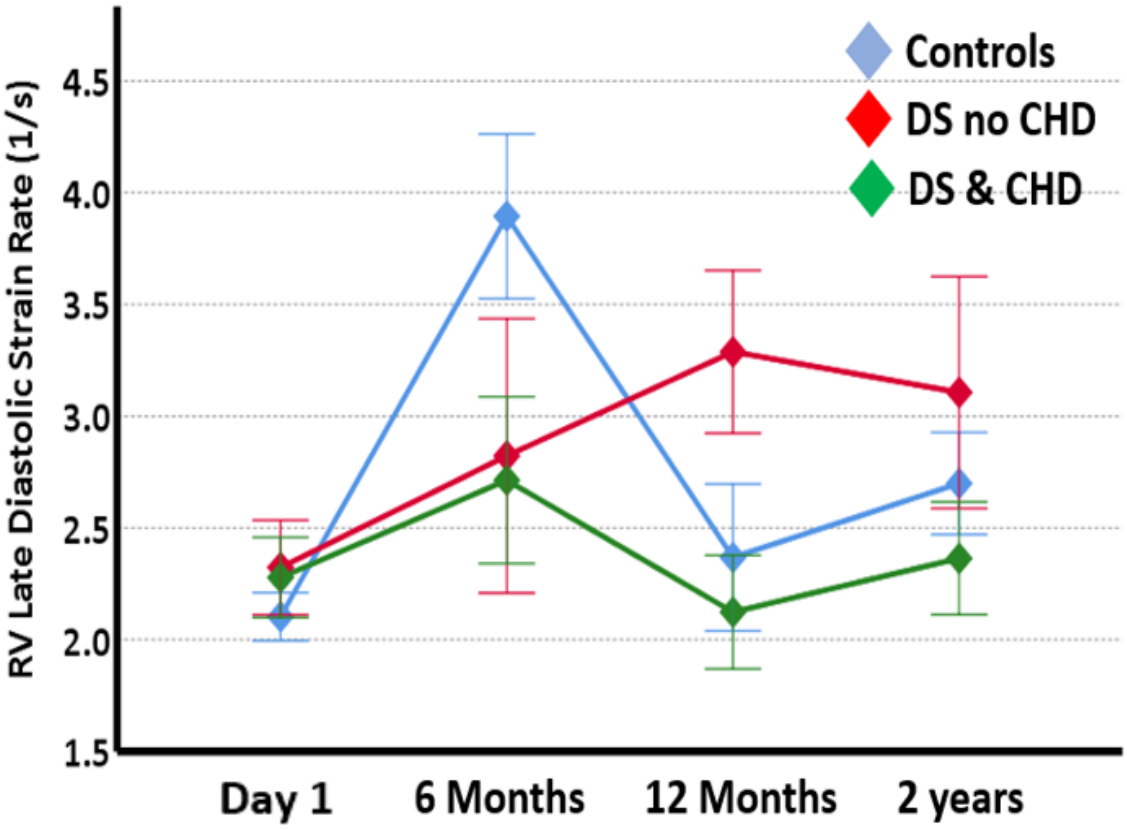


Figure 5.31: Measurement right ventricular longitudinal late diastolic strain rate by deformation analysis over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; RV: right ventricle.

Chapter 6 : Left Ventricular Rotational Mechanics in Infants with Down Syndrome over the First Two Years of Age

6.1 Background

A summary of left ventricular (LV) rotational mechanics is available in Chapter 2. Left ventricular rotation mechanics are an emerging echocardiography technique for detailed assessment of LV performance. Specialised echocardiography software measures the degree of clockwise, basal rotation at the level of the mitral valve and the degree of anti-clockwise, apical rotation at the LV apex. The net effect of these two opposing rotation forces is called LV twist. The speed at which the LV twists is termed the LV twist rate and the speed at which the LV untwists at is termed the LV untwist rate.

LV twist supports ejection of blood during systole. LV untwist augments early diastolic filling by generating a negative pressure gradient and a 'suction' effect which promotes early diastolic LV filling. Work performed by our group in premature infants born < 29 weeks gestation and term infants born to mothers with gestational diabetes mellitus suggests that both cohorts evoke LV twist as compensatory mechanism to maintain normal myocardial performance in the face of biventricular dysfunction[137, 138]. However, a 2019 study with 20 infants with DS and 17 non-DS controls carried out by our group also documented that LV rotation mechanics were impaired in infants with DS over the first week of age via reduced LV basal rotation compared to non-DS controls. Consequently, babies with DS in this 2019 cohort demonstrated reduced LV twist, torsion and twist rate[9].

My hypothesis was that infants with DS may have persistently altered LV rotational mechanics compared to controls over the first two years of age. I sought to expand upon the previous work carried out by our group with a larger cohort of infants with DS. Through this prospective, tri-centre, observational cohort study my aim was to establish if differences in LV rotational mechanics between DS and control cohorts over the first week of age were preserved at two years of age.

Specific Methodology Employed

6.1.1 Study Design and Study Population

This was a prospective, tri-centre, observational cohort study performed across the tertiary neonatal intensive care units of Dublin: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. All infants born with DS, either antenatally or postnatally (later confirmed with Karyotyping) were eligible for inclusion. Babies with DS with and without congenital heart disease (CHD) were enrolled. A cohort of healthy term infants without a diagnosis of DS were enrolled from The Rotunda Hospital to serve as a control population. Enrolment criteria are fully explained in the methodology chapter (Chapter 2).

6.1.2 Echocardiography Assessment

Six echocardiograms were performed over the first two years of age for both the DS and control cohorts. Three echocardiograms were carried out over the first week of age: Day 1 (Echo 1), Day 2 (Echo 2) and Day 3-5 (Echo 3) and at three further time points over the first two years of age: 6 months (Echo 4); 12 months (Echo 5); and 24 months (Echo 6). Echocardiography assessed LV rotation mechanics using advanced echocardiography techniques. A detailed explanation of all novel techniques utilised in this study is available within Chapter 2.

6.1.3 Statistical Analysis

The cohort was be divided into three groups: infants with DS and congenital heart disease (CHD) (DS-CHD), infants with DS and no CHD (DS-no CHD) and controls. Serial data was compared using two way ANOVA with repeated measures. We used SPSS version 23 to conduct the analyses. Statistical significance is achieved with a p value < 0.05.

6.2 Our Findings

Sixty infants with DS and 70 control infants were enrolled into this prospective, tri-centre cohort study. Advanced echocardiography techniques were employed to assess rotation mechanics in the DS and control groups over the first two years of age.

6.2.1 Left Ventricular Rotation Mechanics in Infants with Down Syndrome over the First Two Years of Age

There were no differences in basal rotation, apical rotation, LV twist or LV torsion detected between the three groups at any point over the first two years of age **(Figure 6.1, Figure 6.2, Figure 6.3, Figure 6.4, Figure 6.5)**. There were significant differences in LV twist rate between control and DS-no CHD and control and DS-CHD infants on Day 1 assessment and in LV untwist rate between control and DS-CHD infants on Day 1 , however this had dissipated on the longitudinal echocardiograms at 6 months, 12 months and 2 years of age **(Figure 6.6)**.

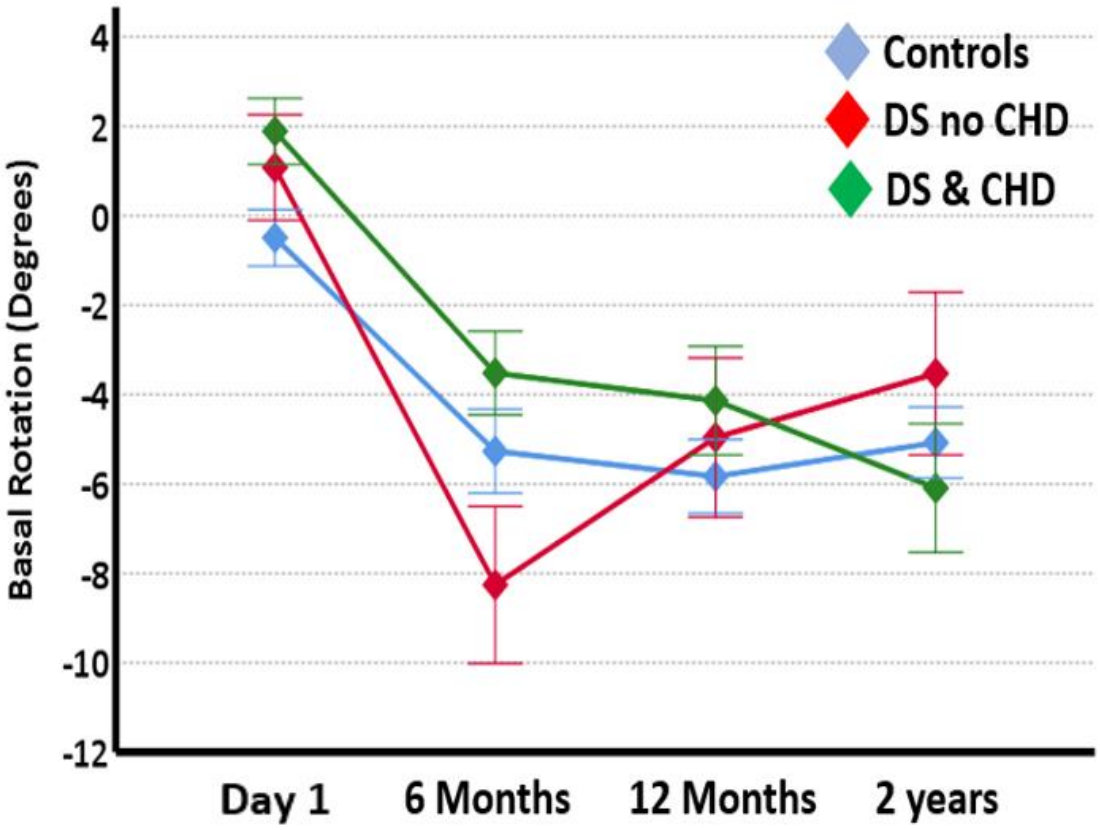


Figure 6.1: Measurement of basal rotation over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease.

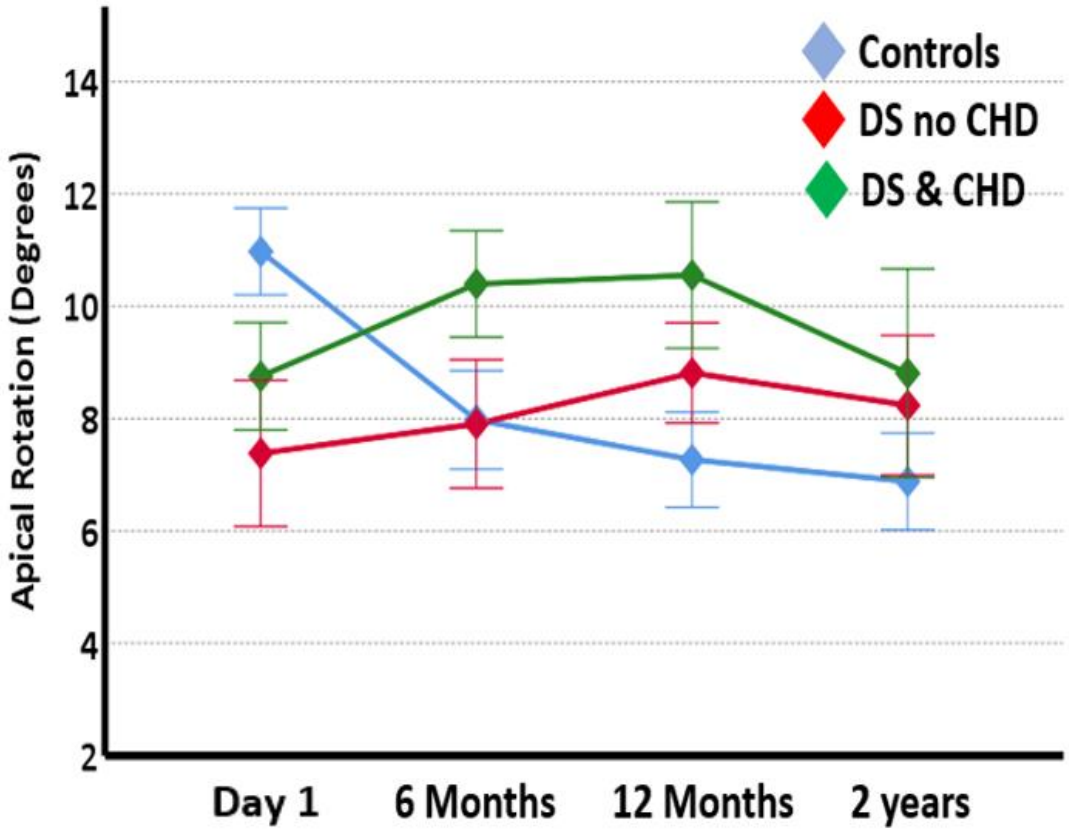


Figure 6.2: Measurement of apical rotation over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease.

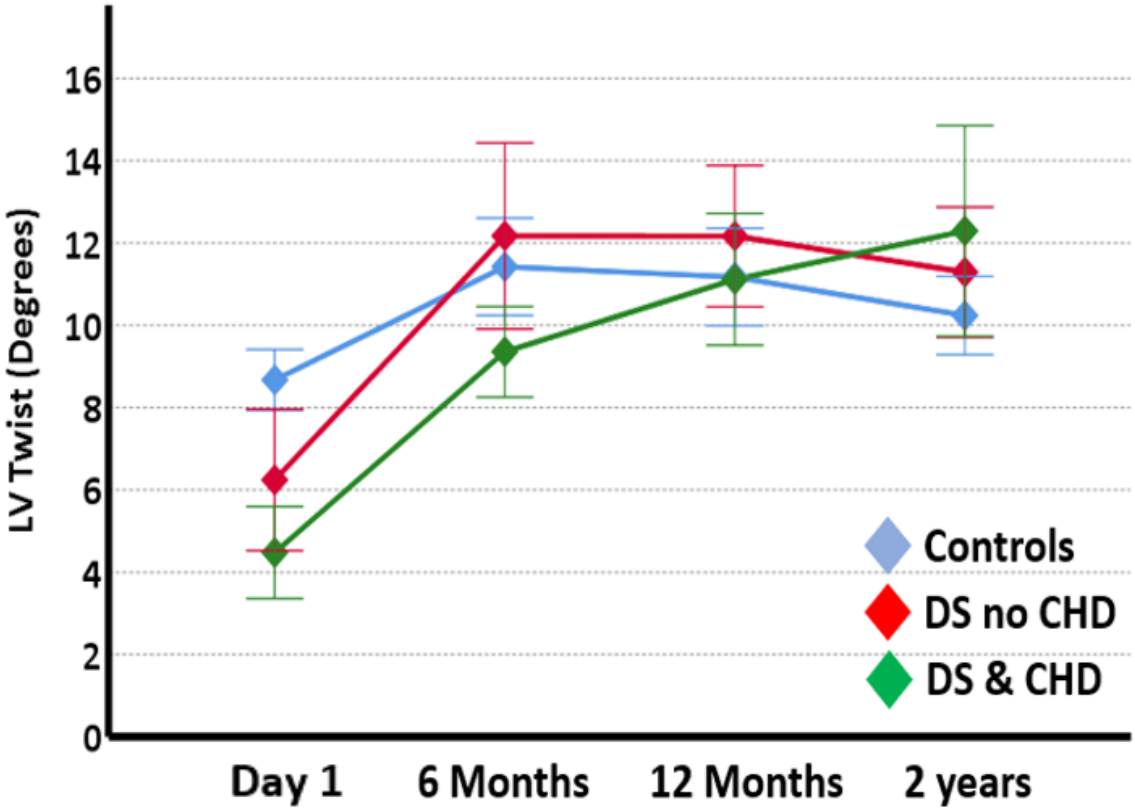


Figure 6.3: Measurement of left ventricular twist over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.

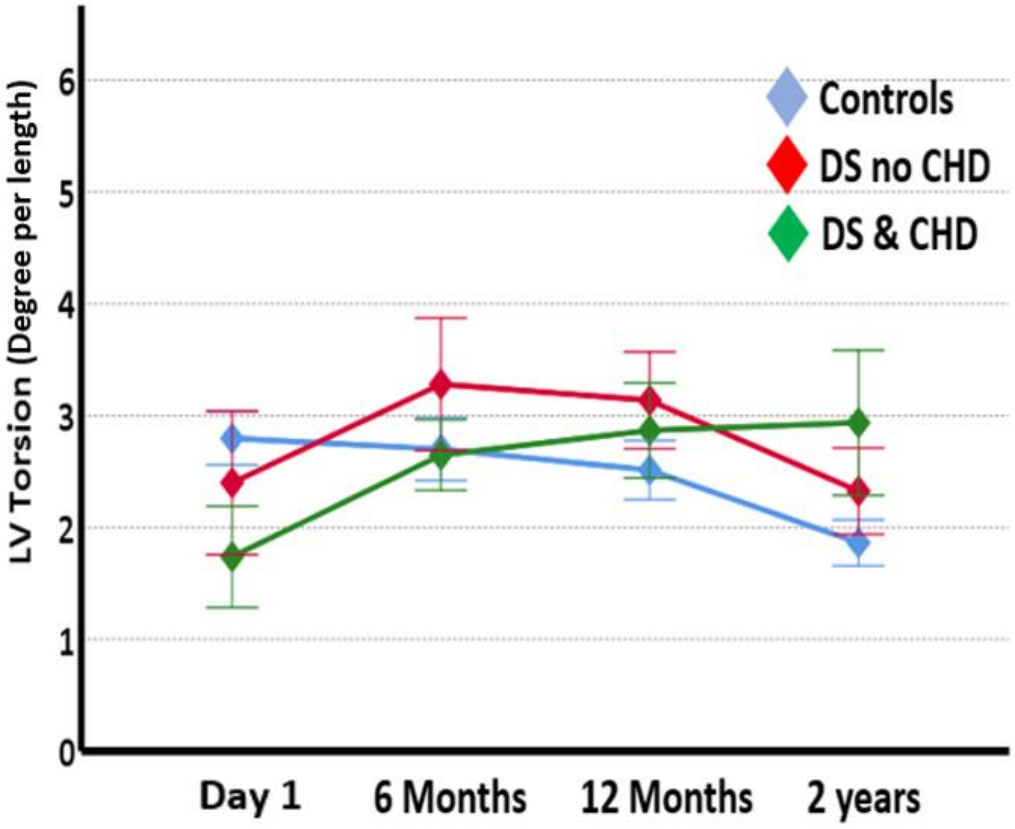
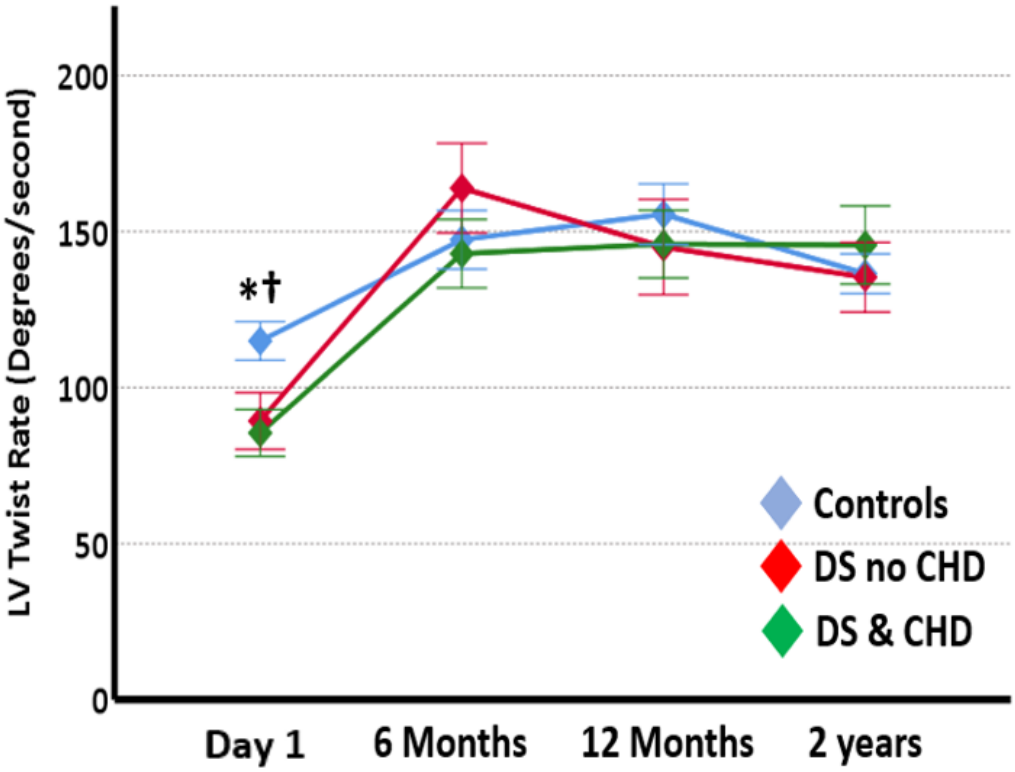


Figure 6.4: Measurement of left ventricular torsion over the first two years of age.

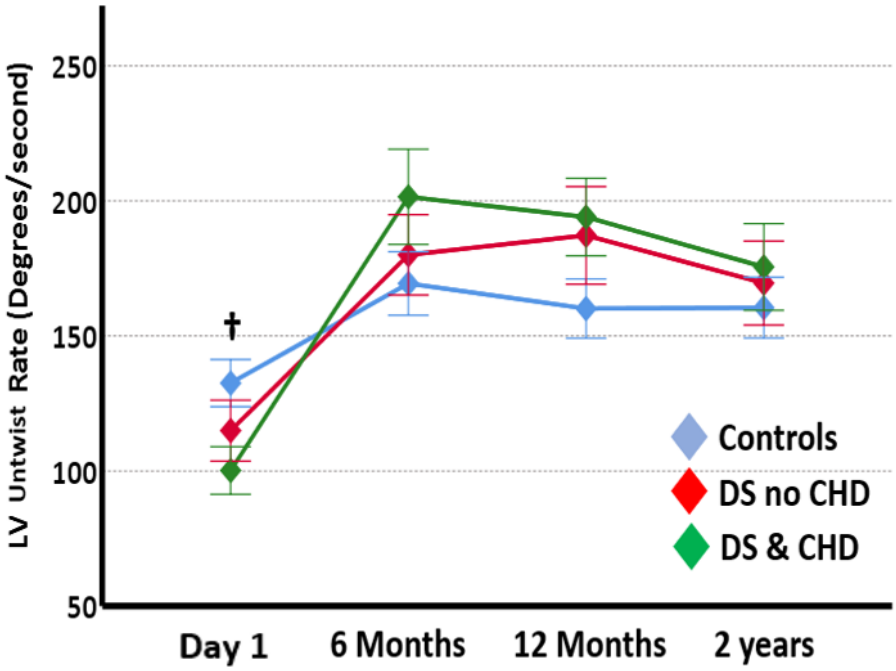
Values are presented as mean and standard error. LV torsion is LV twist indexed to LV length and permits assessment of twist across varying ventricular lengths. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 6.5: Measurement of left ventricular twist rate over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.



*p<0.05 Control vs. DS no CHD; †p<0.05 Control vs. DS & CHD; ‡p<0.05 DS no CHD vs. DS & CHD

Figure 6.6: Measurement of left ventricular untwist rate over the first two years of age. Values are presented as mean and standard error. DS: Down syndrome; CHD: congenital heart disease; LV: left ventricle.

Chapter 7 : Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome requiring Surgical Correction for Congenital Heart Disease over the First Two Years of Age

7.1 Background

The diagnosis of Down syndrome (DS) confers a high risk of congenital heart disease (CHD) with approximately half of all babies with DS affected[49]. The most common CHD lesions in the DS population are atrioventricular septal defects (AVSD) (~45%) , ventricular septal defects (VSD)(~35%), secundum atrial septal defects (ASD) (~8%) and Tetralogy of Fallot (TOF) (4%) respectively[50]. CHD carries a high risk of morbidity and mortality and is the most common cause of death in children with DS over the first two years of age[51, 52]. Despite surgical correction, structural heart disease confers long term health implications including the risk of re-operation and bacterial endocarditis[53]. CHD also impacts on neurodevelopmental outcomes with lower scores for receptive, expressive and composite language in infants and toddlers with DS who required corrective surgery in the first year of age compared to those children with DS and no CHD[54].

My hypothesis was that babies with DS and CHD necessitating surgical correction may display reduced biventricular myocardial performance compared to babies with DS not requiring surgical correction over the first two years of age. The aim of this prospective, observational cohort study was to serially assess left ventricular (LV) and right ventricular (RV) function and pulmonary haemodynamics in babies with DS who underwent surgical correction for CHD employing advanced echocardiography modalities and compare these findings to babies with DS who did not undergo surgical correction for CHD over the first two years of age.

7.2 Specific Methodology Employed

7.2.1 Study Design and Study Population

This was a prospective, observational cohort study carried out across the three tertiary neonatal intensive care units of Dublin: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. All infants born with DS, either antenatally or postnatally (later confirmed with Karyotyping) were eligible for inclusion. Babies with DS with and without congenital heart disease (CHD) were enrolled. A cohort of non-DS, healthy term infants were enrolled from The Rotunda Hospital to serve as a control population. Pertinent antenatal, birth and neonatal characteristics and outcome data were documented for both the DS and control cohorts at the local neonatal unit of recruitment. Clinical data was recorded as per parental response at each follow up assessments performed in The Rotunda Hospital at 6 months, 1 year and 2 years of age for both the DS and control populations. Enrolment criteria and data collection techniques are fully detailed in the methodology chapter (Chapter 2).

7.2.2 Echocardiography Assessment

Echocardiography scans were performed at six time points over the first two years of age for both the DS and control cohorts. Three echocardiograms were carried out over the first week of age: Day 1 (Echo 1), Day 2 (Echo 2) and Day 3-5 (Echo 3) and at three further time points over the first two years of age: 6 months (Echo 4); 12 months (Echo 5); and 2 years (Echo 6). The first echocardiogram of each infant included formal evaluation for any congenital heart disease. Echocardiography at each of the six echocardiograms performed over the first two years of age evaluated myocardial performance and pulmonary haemodynamics. Novel echocardiography techniques were utilised to evaluate myocardial function. Novel echocardiography techniques included tissue Doppler imaging (TDI) and deformation analysis via 2D STE. A detailed explanation of all advanced techniques utilised in this study is available within Chapter 2.

7.2.3 Statistical Analysis

The cohort was divided into two groups, infants with DS who required surgical correction for congenital heart disease (DS-Surg) and infants with DS who did not require surgical correction for CHD over the first two years of age (DS-no Surg). Continuous data was tested for normality using the Shapiro-Wilk test and a histogram representation of data and summarised as means (standard deviation) or medians [inter-quartile range] as appropriate. Categorical data was summarised as counts (%). Two group analyses were conducted using the student t-test, the Mann Whitney U test as appropriate, or Chi square test as appropriate. Three group analyses was conducted using one way ANOVA or the Kruskal-Wallis Test. Serial data was compared using two way ANOVA with repeated measures. Regression analysis was conducted to assess the independent effect of predictor variables on important outcomes. We used SPSS version 23 to conduct the analyses. Statistical significance is achieved with a p value < 0.05.

7.3 Our Findings

Fourteen infants with DS required surgical correction for congenital heart disease (CHD) over the first two years of age compared with 57 infants with DS who did not **(Table 7.1)**. One infant with DS had an ASD catheter device closure procedure which did not necessitate post-operative paediatric intensive care (PICU) admission. Thirteen of the 14 infants with DS who underwent surgical correction for CHD required post-operative admission to paediatric intensive care, and this is the group we evaluated in detail. All 13 infants would have undergone cardiopulmonary bypass during CHD surgical correction.

7.3.1 Clinical Characteristics and Outcomes of Infants with Down Syndrome who required Surgical Correction for Congenital Heart Disease over the First Two Years of Age.

There were no differences evident in gestational age, birth weight, male sex, Caesarean section rate, likelihood of intubation, oxygen requirement or duration, time to full enteral feeds or duration of hospital stay post-delivery between infants with DS who required surgical correction of CHD (DS-Surg) versus those that did not over the first two years of age (DS-no Surg). In addition, there was no differences in feeding type between the two groups over the study period ($p = 0.72$). As expected, surgical corrections were more likely to be performed for atrioventricular septal defects ($p < 0.01$) and Tetralogy of Fallot ($p = 0.04$) cases. There was no difference in hypothyroidism necessitating L-thyroxine administration between the DS-Surg and DS-no Surg groups, 1 (8%) vs 10 (18%), $p = 0.38$. However, diuretic use was higher in infants with DS who required surgical closure of CHDs, 7 (54%) vs 3 (5%) ($p< 0.01$). Weight at 2 years of age was comparable between babies in the DS-Surg and DS-no Surg cohorts, 10.4Kg [9.0 – 14.3] vs 11.7Kg [10.6 – 12.8] ($p = 0.62$) **(Table 7.1)**.

7.3.2 Day 1 Echocardiography Measurements in Infants with Down Syndrome who required Surgical Correction for Congenital Heart Disease over the First Two Years of Age.

Several Day 1 echocardiography derived left ventricular measurements were significantly impaired in the DS-Surg cohort. LV global longitudinal strain (%), LV longitudinal systolic strain rate (1/s) and LV early diastolic strain rate (1/s) were all significantly lower on Day 1 evaluation in the DS-Surg group compared to the DS-no Surg group with 16.3 ± 2.8 vs 19.6 ± 2.5 ($p < 0.01$), 1.4 ± 0.2 vs 1.6 ± 0.2 ($p = 0.02$) and 2.1 ± 0.8 vs 2.6 ± 0.6 ($p = 0.04$) respectively. This data indicates that from Day 1 of age the babies with DS requiring surgical correction of CHDs were a markedly different sub-cohort of babies in terms of LV systolic and early diastolic performance. There were no differences detected between the DS-Surg and DS-no Surg groups with regards to indices of pulmonary hypertension or RV function **(Table 7.2)**.

7.3.3 Longitudinal assessment of pulmonary haemodynamics in infants with Down syndrome who required surgical correction for congenital heart disease over the first two years of age.

Assessment of the pulmonary artery acceleration time to right ventricular ejection time ratio (PAAT:RVET) found no differences between the DS-Surg versus DS-no Surg groups over the first two years of age. In addition, the LV eccentricity index measurements from birth to 2 years of age were also comparable between the two groups **(Figure 7.1, Figure 7.2)**.

7.3.4 Longitudinal assessment of cardiac function in infants with Down syndrome who required surgical correction for congenital heart disease over the first two years of age.

On evaluation of LV longitudinal data the LV global longitudinal strain was significantly lower in the DS-Surg group compared to the DS-no Surg group at Day 1 and 6 months of age. These differences in LV global longitudinal strain between the groups had normalised by the 12 month and 2 years evaluations **(Figure 7.3)**. However, LV longitudinal

systolic strain rate measurements remained significantly lower in the DS-Surg vs DS-no Surg cohort throughout the first two years of age at all study time points **(Figure 7.4)**. There were no differences identified between the DS-Surg and DS-no Surg infants in LV early diastolic strain rate measurements over the two year period **(Figure 7.3)**. LV late diastolic strain rate values were equivalent between infants with DS who required surgical CHD correction and those that did not on Day 1 of age but were significantly lower in the DS-Surg group by 6 months of age. Longitudinal evaluation revealed this disparity between the groups in LV late diastolic strain rate had resolved by 12 months and 2 years. **(Figure 7.6)**.

Longitudinal assessment of RV performance identified significantly compromised RV free wall longitudinal strain in the DS-Surg group at 12 months and 2 years of age compared to the DS-no Surg group. This impairment in RV free wall longitudinal strain had developed over the first two years of age from comparable values to the DS-no Surg group at Day 1 and 6 months of age **(Figure 7.7)**. This data is further substantiated by significantly diminishing RV longitudinal systolic strain rate measurements in the DS-Surg group also at 12 months and 2 years of age compared to the DS-no Surg group and points to the evolution of intrinsic RV contractility impairment in the DS-Surg group over time despite surgical intervention for CHD **(Figure 7.8)**. RV early diastolic strain rate values were significantly decreased in the DS-Surg group compared to the DS-no Surg group at 12 months of age but were otherwise equivalent at all other study time points **(Figure 7.9)**. RV late diastolic strain rate values were significantly lower in the DS-Surg group compared to the DS-no Surg group at 6 months, 12 months and 2 years of age. **(Figure 7.10)**.

Table 7.1: Clinical characteristics and outcomes of infants with Down syndrome who required surgical correction for congenital heart disease over the first two years of age. Values are presented as means (SD), medians [IQR] and absolute counts (%). A p value < 0.05 was considered significant.

	Surgical Correction n=13	**No Surgical Correction n=57**	**p**
Gestation at birth (weeks)	37.2 ± 2.1	37.9 ± 2.1	0.32
Birthweight (Kg)	2.8 ± 0.5	3.1 ± 0.7	0.26
Male	10 (77%)	36 (63%)	0.35
Caesarean Section	9 (69%)	27 (47%)	0.16
Intubation After Delivery	2 (15%)	4 (7%)	0.33
Any Oxygen Pre-Hospital Discharge	7 (54%)	35 (61%)	0.62
Oxygen Days	2 [2 – 11]	4 [1 – 8]	0.64
Time to Full Enteral Feeds (Days)	3 [1 – 8]	2 [1 – 4]	0.56
Hospital Stay (Days)	8 [6 – 28]	12 [11 – 13]	0.59
Congenital Heart Disease			
Atrial Septal Defect	3 (23%)	14 (25%)	0.91
Ventricular Septal Defect	6 (46%)	28 (49%)	0.85
Atrioventricular Septal Defect	7 (54%)	0 (0)	<0.01
Tetralogy of Fallot	1 (8%)	0 (0)	0.04
Patent Ductus Arteriosus	1 (8%)	6 (11%)	0.76
L-thyroxine Over First 2 Years	1 (8%)	10 (18%)	0.38
Diuretic Use	7 (54%)	3 (5%)	<0.01
Weight at 2 Years (Kg)	10.4 [9.0 – 14.3]	11.7 [10.6 – 12.8]	0.62

Table 7.2: Day 1 echocardiography values in infants with Down syndrome who required surgical correction for congenital heart disease over the first two years of age.

Values are presented as means (SD). A p value < 0.05 was considered significant.

	Surgical Correction n=13	No Surgical Correction n=57	p
PAAT (ms)	38 ± 10	44 ± 10	0.08
PAAT:RVET	0.20 ± 0.04	0.21 ± 0.04	0.25
LV EI	1.7 ± 0.2	1.8 ±0.3	0.53
LV GLS (%)	16.3 ± 2.8	19.6 ± 2.5	<0.01
LV SRs (1/s)	1.4 ± 0.2	1.6 ±0.2	0.02
LV SRe (1/s)	2.1 ± 0.8	2.6 ± 0.6	0.04
LV SRa (1/s)	1.5 ± 0.4	1.5 ± 0.5	0.94
RV FW LS (%)	17.3 ± 5.1	19.7 ± 3.6	0.19
RV SRs (1/s)	1.3 ± 0.3	1.5 ± 0.4	0.33
RV SRe (1/s)	2.2 ± 0.5	2.0 ± 0.8	0.55
RV SRa (1/s)	2.4 ± 1.5	2.3 ± 0.9	0.68

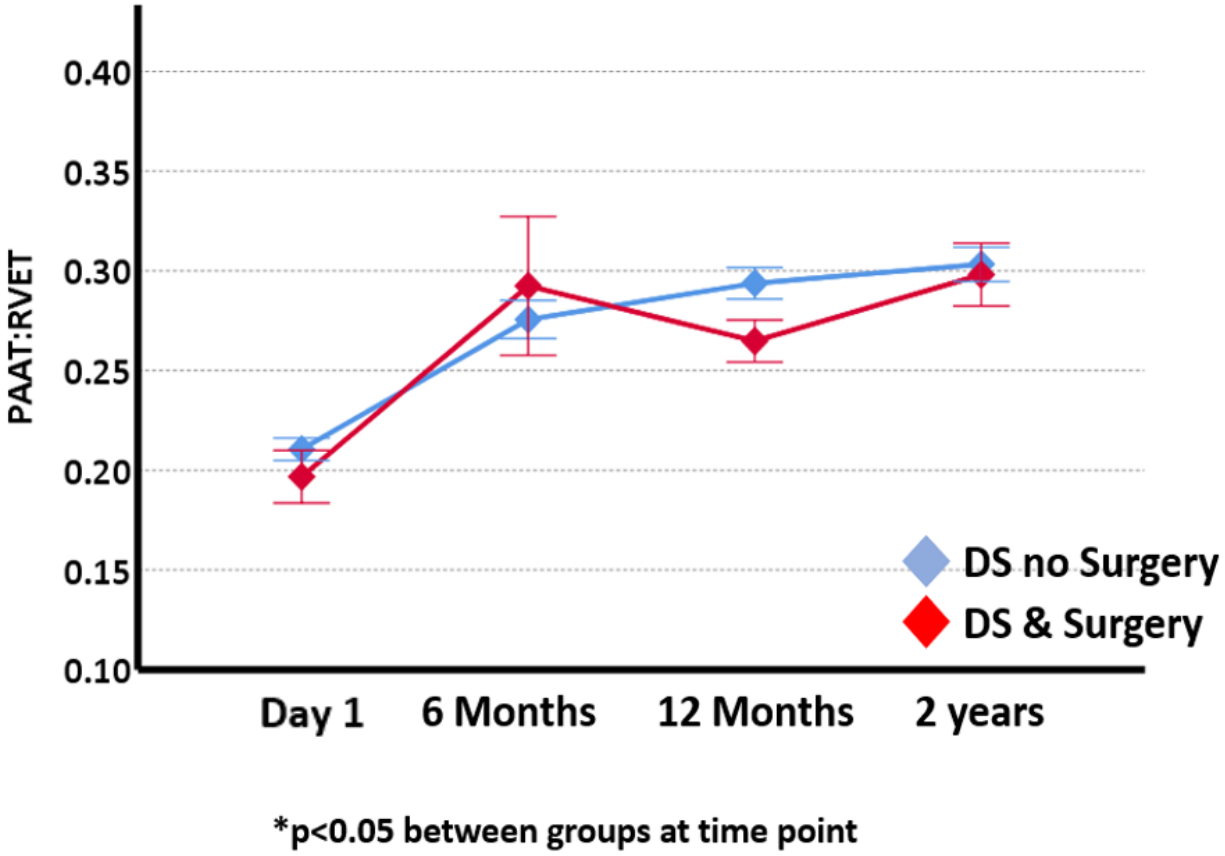


Figure 7.1: Measurement of pulmonary artery acceleration time to right ventricular ejection time ratio in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; PAAT: pulmonary artery acceleration time; RVET: right ventricular ejection time.

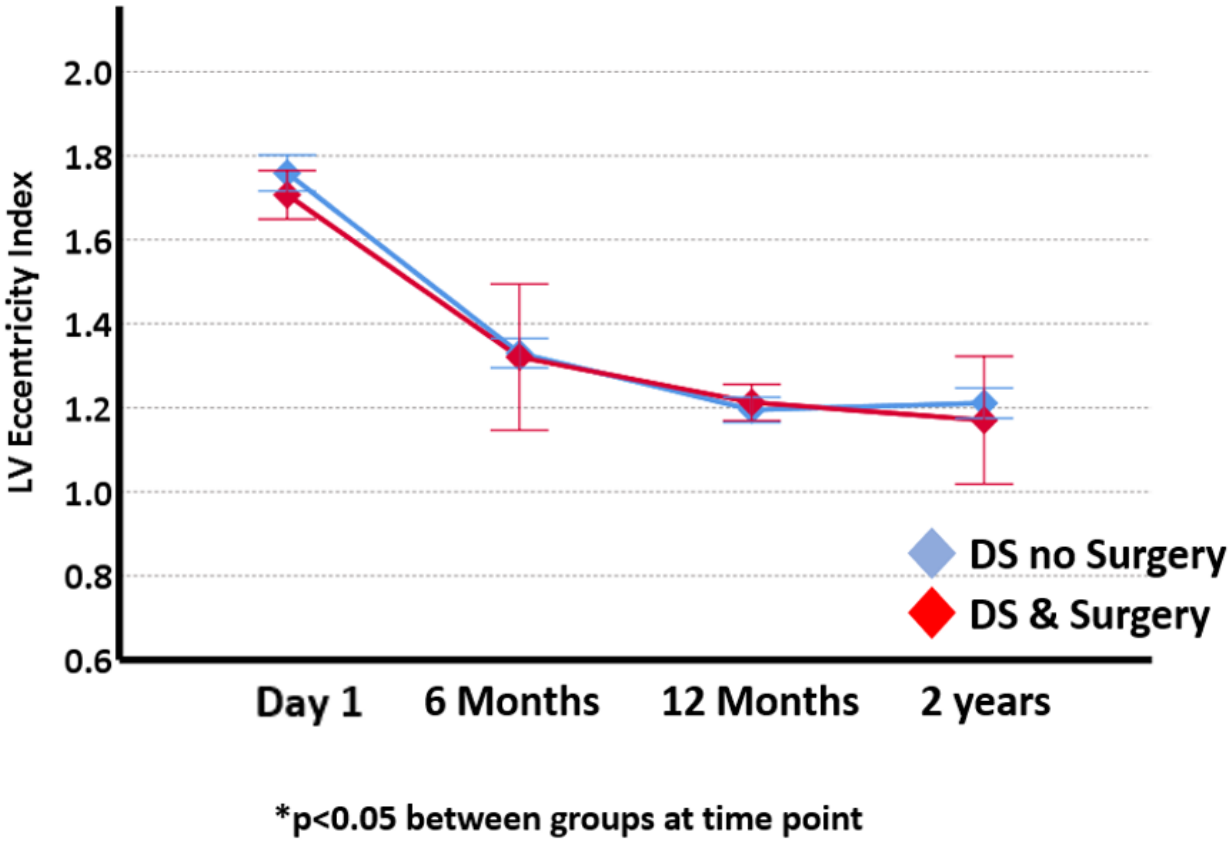


Figure 7.2: Measurement of left ventricular eccentricity index in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; LV: left ventricle.

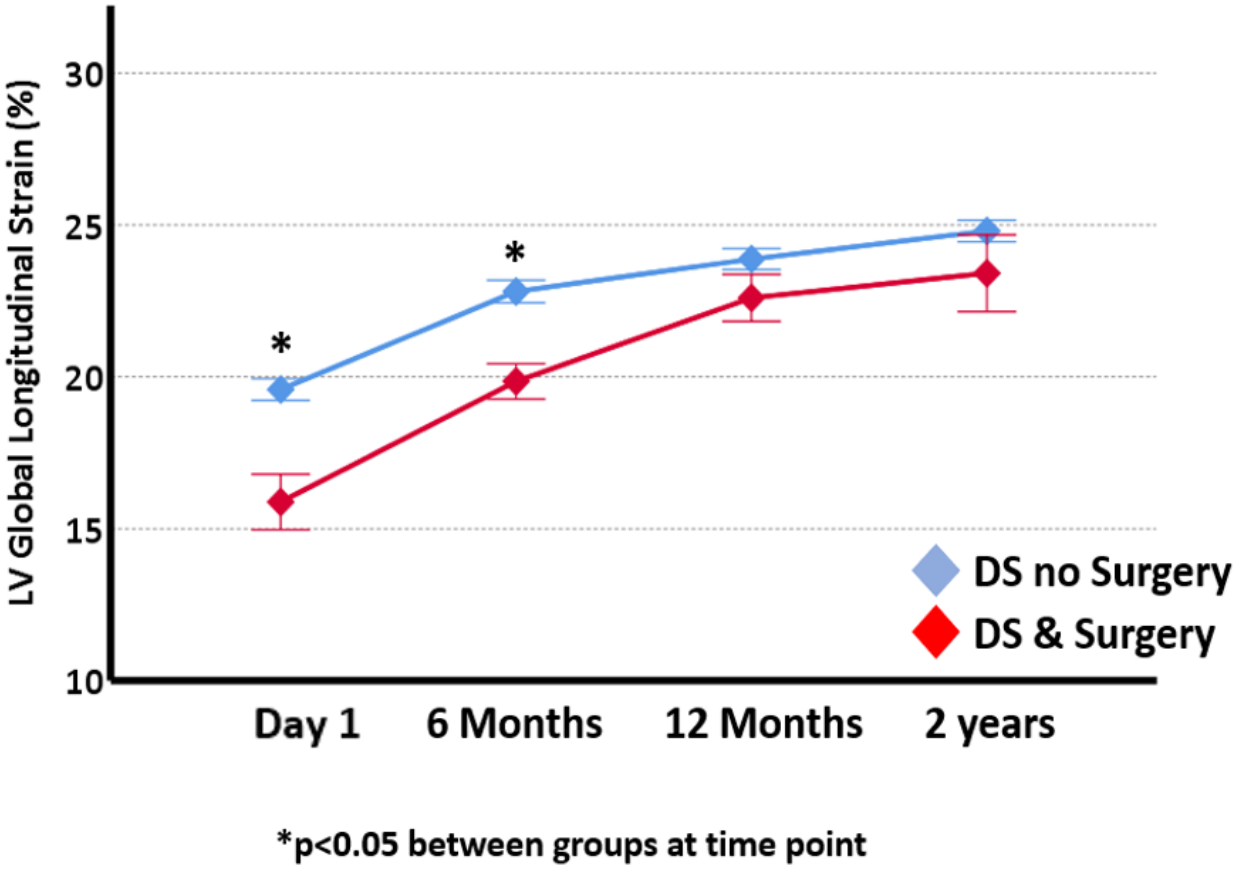


Figure 7.3: Measurement of left ventricular longitudinal strain by deformation analysis in in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; LV: left ventricle.

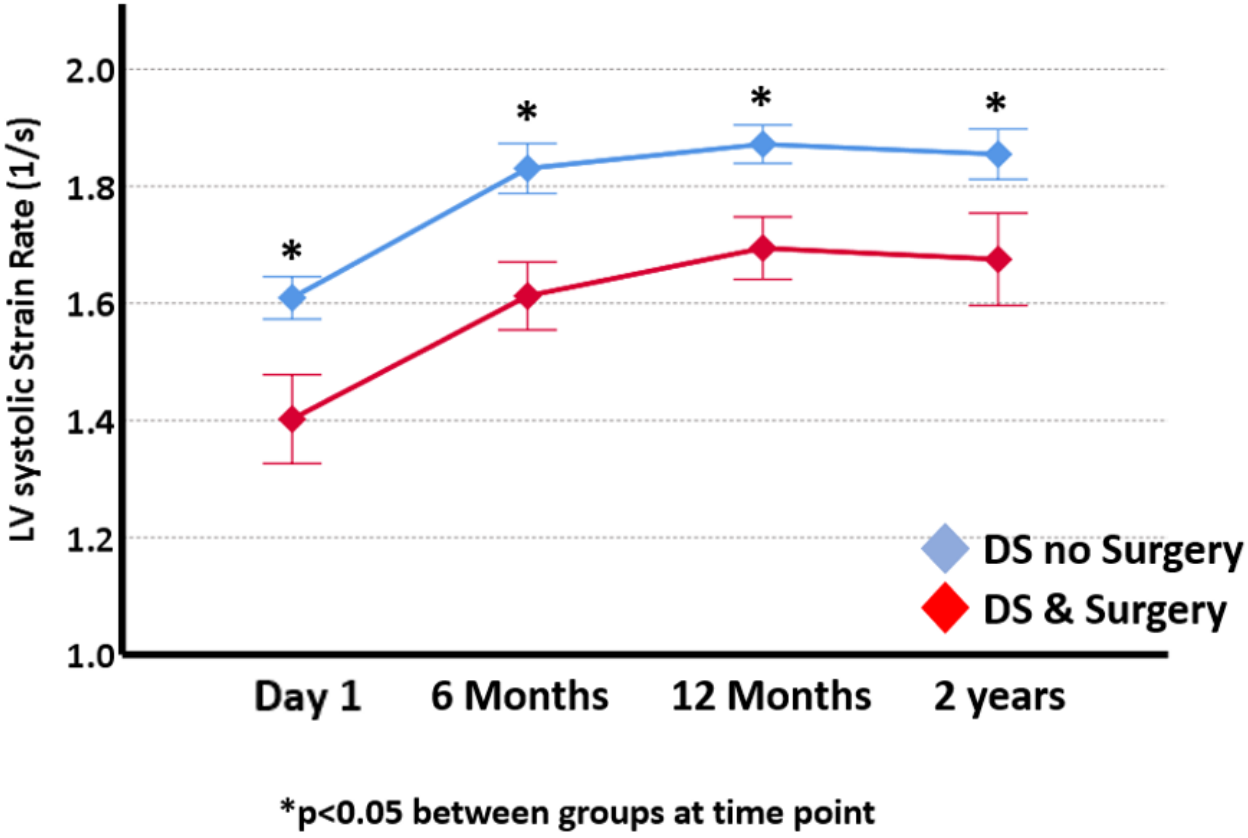


Figure 7.4: Measurement of left ventricular longitudinal systolic strain rate by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; LV: left ventricle.

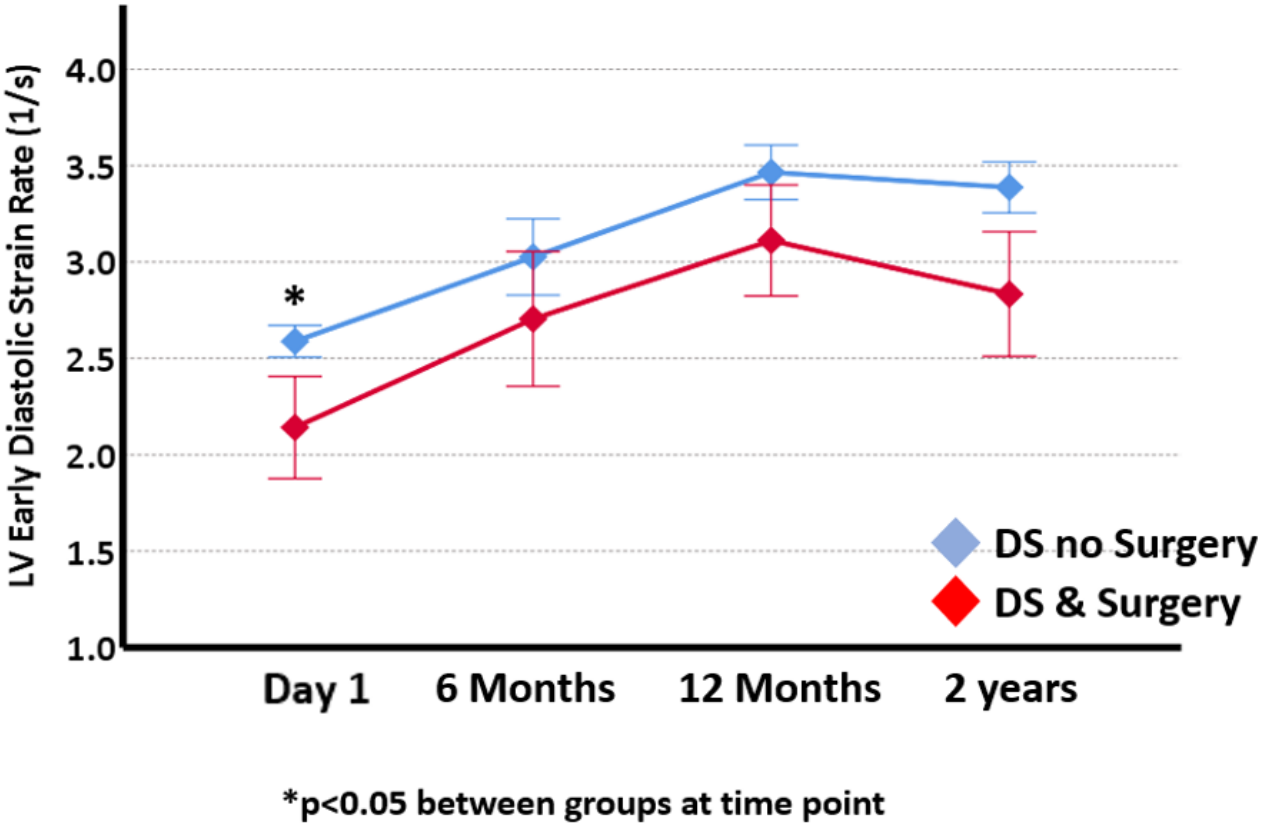


Figure 7.5: Measurement of left ventricular longitudinal early diastolic strain rate by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; LV: left ventricle.

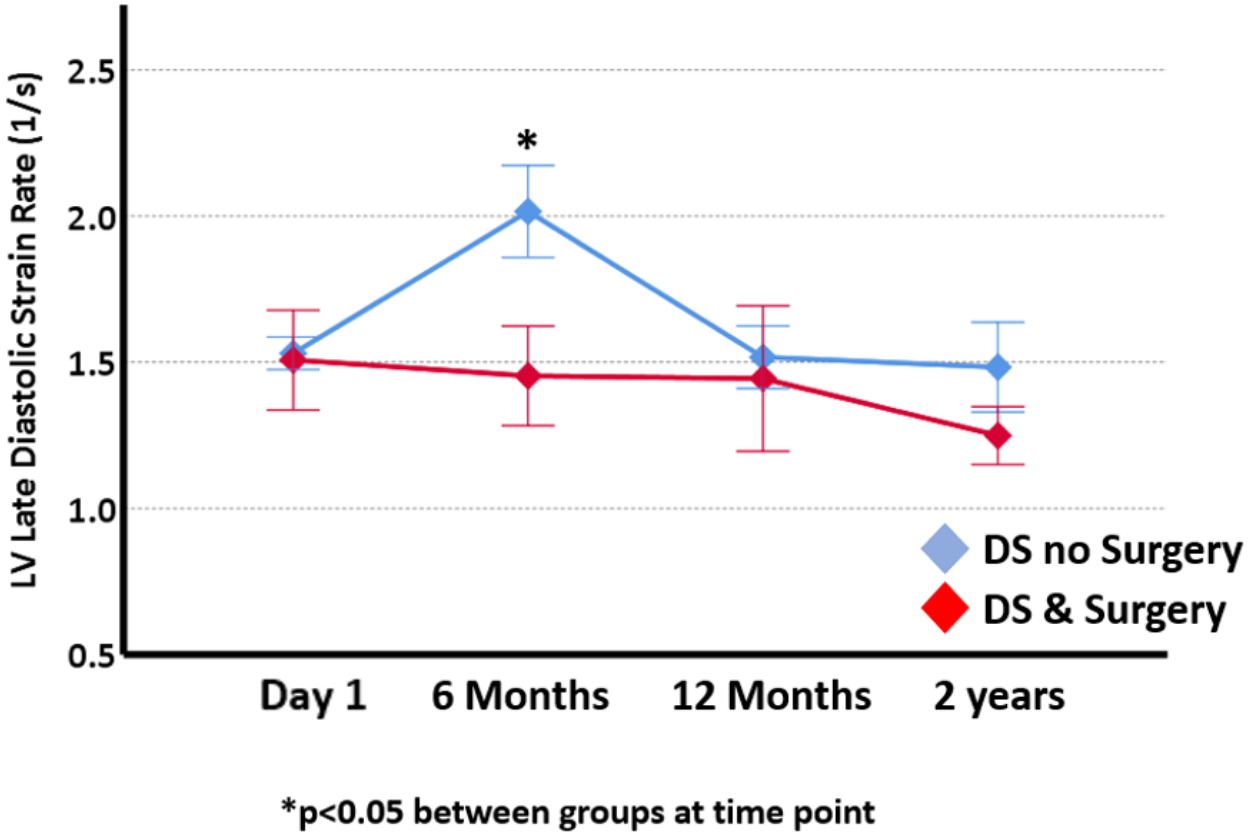


Figure 7.6: Measurement of left ventricular longitudinal late diastolic strain rate by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; LV: left ventricle.

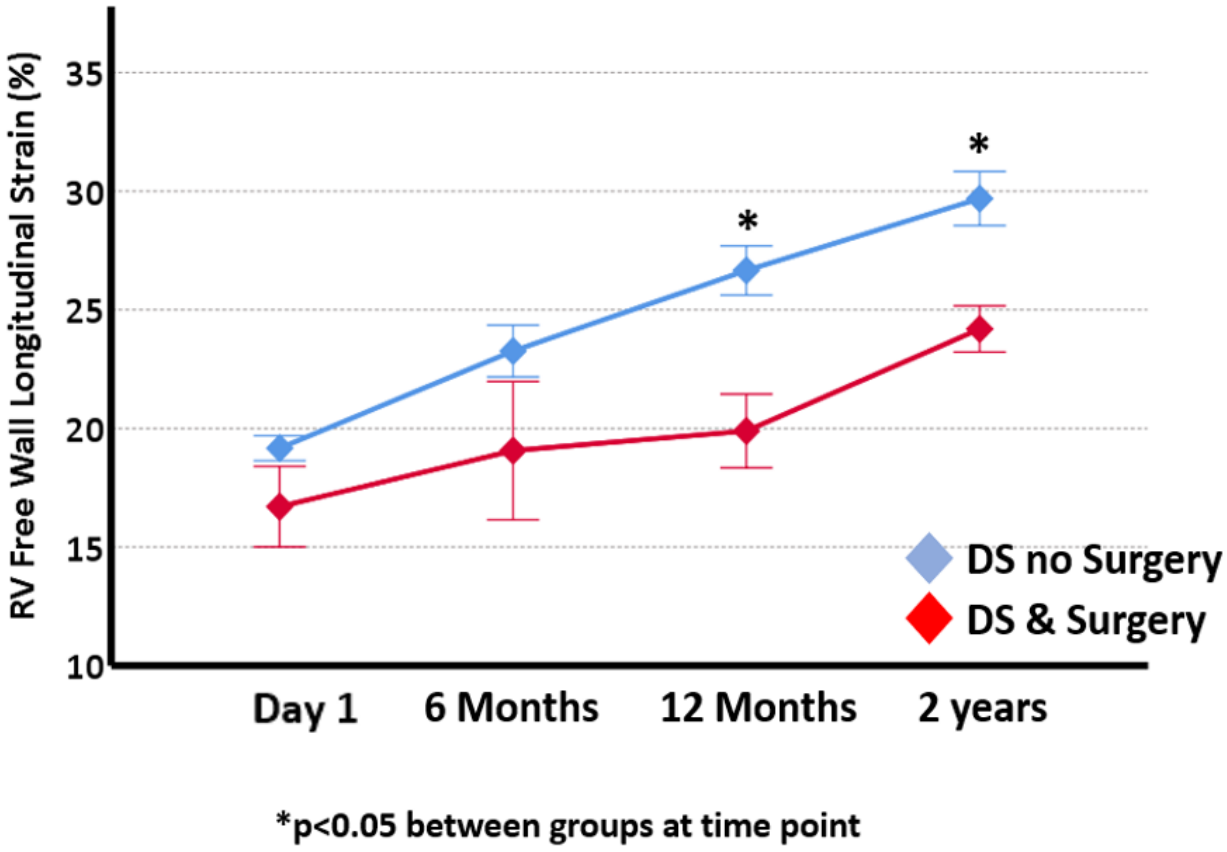


Figure 7.7: Measurement of right ventricular free wall longitudinal strain by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; RV: right ventricle.

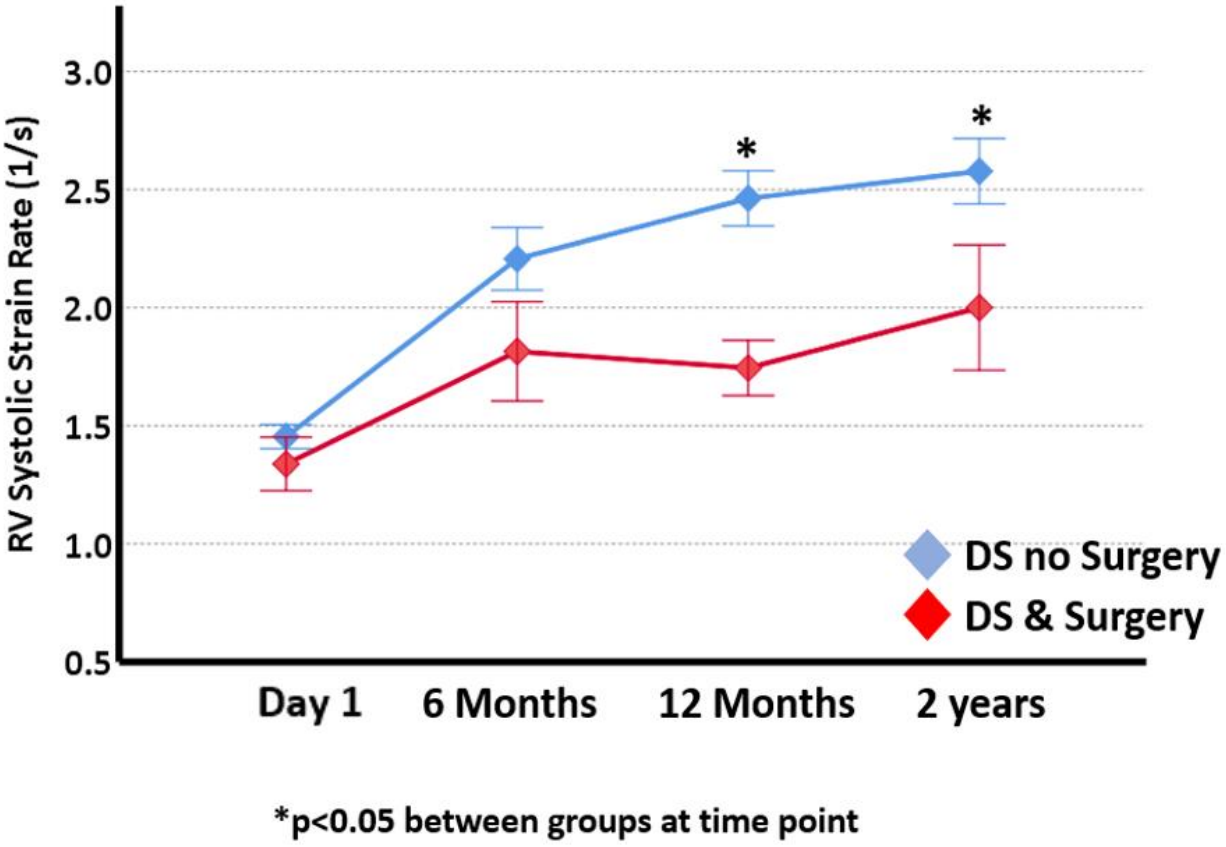


Figure 7.8: Measurement of right ventricular longitudinal systolic strain rate by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; RV: right ventricle.

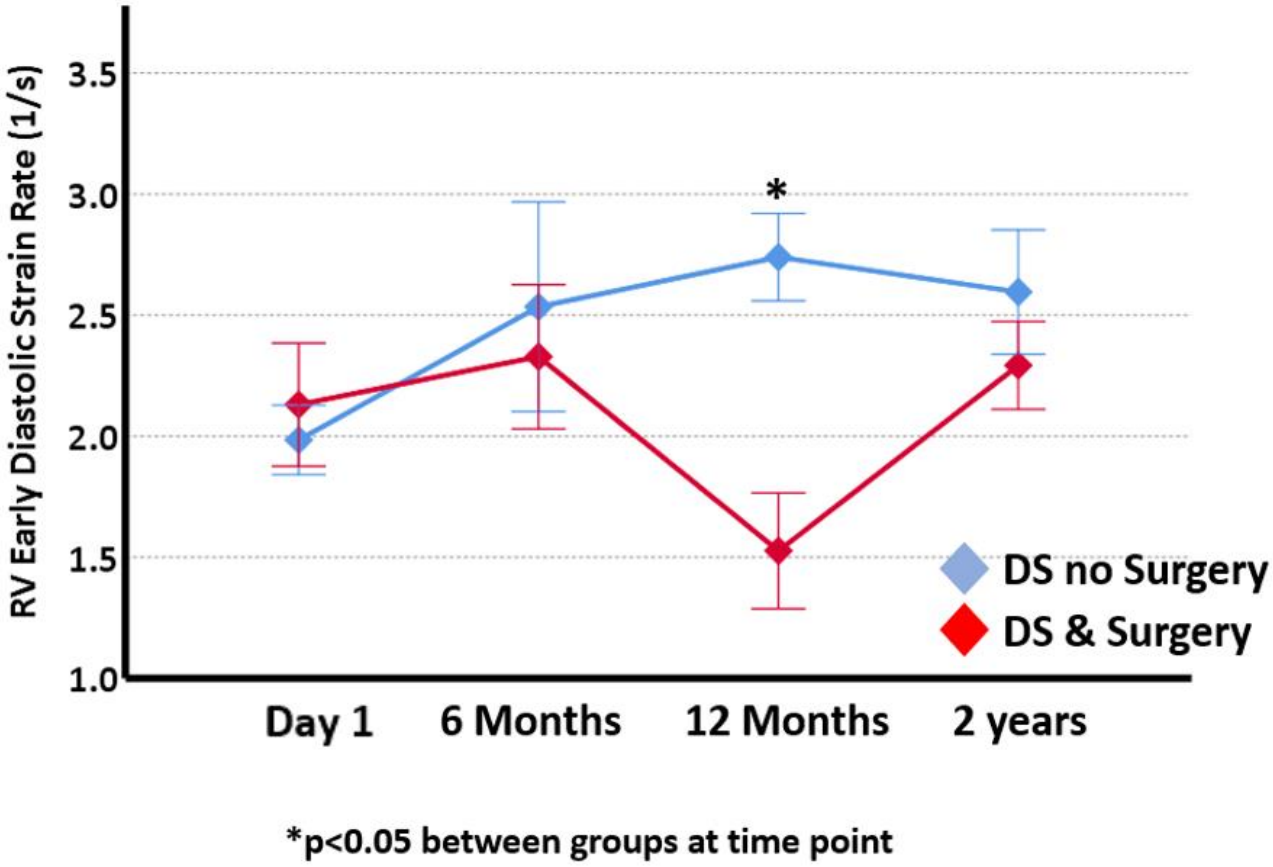


Figure 7.9: Measurement of right ventricular longitudinal early diastolic strain rate by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; RV: right ventricle.

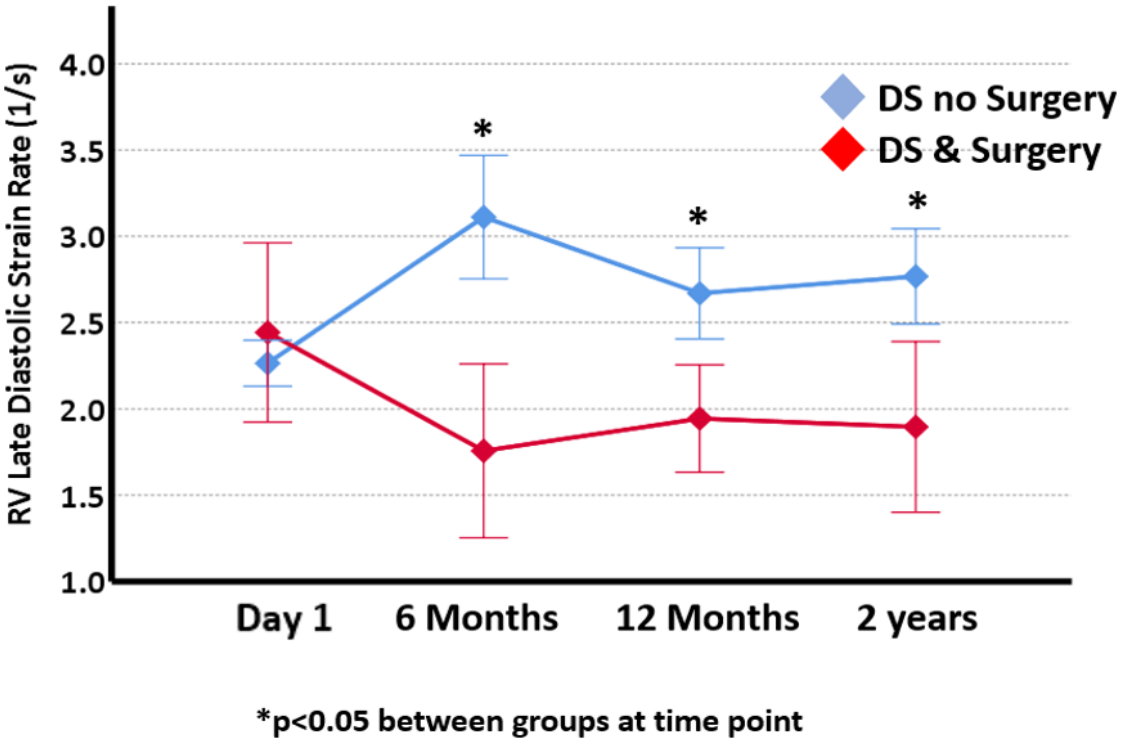


Figure 7.10: Measurement of right ventricular longitudinal late diastolic strain rate by deformation analysis in infants with Down syndrome requiring surgical correction for congenital heart disease over the first two years of age.

Values are presented as mean and standard error. DS: Down syndrome; RV: right ventricle.

Chapter 8 : Discussion

8.1 Our Findings

Ireland is uniquely situated to study the cardiovascular implications of Down syndrome (DS) with the highest incidence of DS in Europe. This prospective, observational study was conducted across the three tertiary neonatal intensive care units of Dublin with a combined delivery rate of 25,000 babies per year: The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. I enrolled 70 infants with Down syndrome (DS) over the three sites and 60 non-DS controls were recruited in the Rotunda Hospital. The DS cohort consisted of both babies with DS born with congenital heart disease (CHD) and structurally normal hearts. The aim of the study was to utilise advanced echocardiography techniques to serially assess biventricular myocardial performance and pulmonary haemodynamics at six time points over the first two years of age in infants with DS. Specifically, I wished to interrogate the relationship between myocardial function and pulmonary hypertension (PH). I also sought to evaluate the ability of echocardiography derived markers to predict important clinical outcomes including respiratory morbidity, clinically evident PH, hospital admissions, admission to a paediatric intensive care unit, necessity for ECMO and death. This study intended to build upon earlier work performed by our research group and to assess if previous findings were confirmed or dispelled with a larger study cohort of infants with DS[8, 9]. To the best of my knowledge, this work is the largest prospective study performed to date focusing on myocardial function and pulmonary haemodynamics in infants with DS from birth to two years of age

This study has succeeded in a thorough assessment of the evolution of myocardial function and pulmonary haemodynamics from birth to two years of age in infants with DS. In addition, novel physiological mechanisms in the development of PH were revealed with correlations identified between both reduced indices of both left ventricular systolic and diastolic function as well as left ventricular dimensions and higher degrees of PH in the DS

cohort. Our data also supported the functional interdependence of the right and left ventricles.

8.2 Clinical Outcomes of Infants with Down Syndrome over the First Two Years of Age

8.2.1 Clinical Outcomes of Infants with DS in the Early Newborn period

The antenatal detection rate of DS in this study cohort was 33%. The DS cohort were born at an earlier gestational age, had a lower birthweight, were more likely to be male, were born to women with a higher maternal age and were less likely to be delivered by CS compared to the control cohort in this study. Forty-eight (69%) of infants with DS were born with CHD of which VSDs were the most common lesion followed by ASD, AVSD and TOF respectively. Although the number of babies with DS requiring inotropic support, intubation, iNO, CPAP or supplemental oxygen were low in this study cohort their requirement for such interventions was clearly abnormal when such outcomes were non-existent in the control cohort. Our findings are consistent with known data documenting the DS population as a vulnerable group during the early newborn period due to the presence of congenital anomalies and cardiorespiratory morbidity[4-7]. One baby with DS died before discharge home from their maternity hospital of birth indicating a relatively low mortality rate in this study.

8.2.2 Clinical Outcomes of infants with DS over the First Two Years of Age

Thirty-one (44%) infants with DS required hospitalisation over the first two years of age. Fourteen (20%) infants required hospital admission for surgical corrections of CHD and 17 required (24%) hospitalisation for non-CHD related issues. The non-CHD related hospitalisations were for the following reasons: pneumonia, bronchiolitis, obstructive sleep apnoea investigations, vomiting, insertion of percutaneous endoscopic gastrostomy (PEG) tube, investigation for suspected seizures and management of infantile spasms. Such data is in keeping with previously published literature documenting that childhood is the period of life during which most hospitalisations occur in DS populations and that the rate of hospitalisation in children <2 years of age with DS is significantly greater that for infants with a normal chromosome complement [110]. Eleven (16%) of the DS cohort required L-thyroxine for hypothyroidism management and 10 (14%) required diuretic therapy over the

first two years of age. There were no deaths in the DS cohort following discharge home from their maternity hospital of birth.

One of the study aims was to assess the ability of echocardiography derived markers to predict important clinical outcomes including respiratory morbidity, clinically evident PH, hospital admissions, admission to a paediatric intensive care unit, necessity for ECMO and death. Statistical analysis did not reveal any single echocardiography derived marker which could predict the clinical outcomes listed above. I would postulate that the primary reason for this was that our study cohort represented a relatively healthy population of babies with DS. The mortality rate and number of infants with DS requiring intensive care interventions in the neonatal period were low. The reasons for this have been outlined previously and include the fact that infants born with DS and time critical cardiac or gastrointestinal disorders were transferred promptly out from their maternity hospital of birth for ongoing management which precluded study enrolment, babies with a high likelihood of death over the first week of age were not enrolled and the three sites involved in this study have extensive expertise in the specific needs of babies with DS. In addition, the only admissions to PICU over the first two years of age in the DS cohort were those infants who required surgical correction of CHDs. There was no PICU admission in our group consequent to acute respiratory disease. As such our DS cohort reflects an essentially healthy population children with DS who did not possess time critical cardiac or gastrointestinal issues at birth, received expert neonatal care in tertiary units and had relatively stable clinical courses over their first two years of age. Although this situation precluded the ability of echocardiography derived markers to predict clinically important outcomes in the DS cohort it renders my findings of sustained myocardial impairment and abnormal indices of PH compared to the control cohort over the first two years of age especially striking. My data emphasises that despite the absence of clinically overt cardiorespiratory illness in early childhood infants and children with DS are a distinct and significantly vulnerable patient cohort with sustained, sub-clinical cardiovascular morbidity that is only detectable with advanced functional echocardiographic assessment.

8.2.3 Conclusion

In conclusion, my work reiterated that infants with DS are a unique patient cohort from birth who require more frequent medical interventions including specialist functional cardiac assessment over the first two years of age compared to non-DS infants.

8.3 The Relationship between Diastolic Impairment and Pulmonary Hypertension in Infants with Down Syndrome over the First Week of Age

Early literature estimated the rate of pulmonary hypertension (PH) in children with DS between 1% and 5%. However, more recent data indicates that this was a significant underestimation with the true figure more likely to range between 27% and 34%[77, 78]. Left and right ventricular dysfunction is evident from infancy to adulthood in patients with DS, irrespective of the presence of CHD[9-11]. While PH and myocardial impairment are well recognised entities for cardiorespiratory morbidity in the DS population the exact relationship between the two remains to be fully characterised. In particular, how left ventricular (LV) diastolic impairment may negatively impact indices of PH. The aim of this work was to assess anthropometric measurements, myocardial performance and pulmonary haemodynamics using advanced echocardiography techniques to interrogate the relationship between left ventricular diastolic function and indices of PH in infants with DS over the first week of age and to compare these measurements to a control cohort.

8.3.1 Evaluation of Anthropometric Measurements, Myocardial Performance and Pulmonary Haemodynamics in Infants with Down Syndrome over the First Week of Age

A total of 189 and 179 echocardiograms in the DS and control cohorts were performed over the first week of age respectively. Echocardiography assessment on Day 1 of age revealed that infants with DS had significantly smaller mitral valve annuli, internal left ventricular (LV) diastolic diameters, shorter LV lengths, tricuspid valve diameters, RV mid cavity diameters and shorter RV lengths. My findings of significantly smaller left and right ventricular dimensions in babies with DS compared to control infants is consistent with previously reported data[9].

Serial assessment of pulmonary haemodynamics over the first week of age utilising several echocardiography derived markers identified sustained elevation of pulmonary pressures in the DS group compared to controls. This was evidenced by each of the echocardiography surrogates of PH evaluated remaining persistently and significantly abnormal in the DS group over the first week of age compared to controls; namely

significantly shorter PAAT times, lower PAAT:RVET ratios, higher LV EIs and ongoing bidirectional PDAs in over half of the DS cohort on Day 3-5 evaluation.

Currently, there are no specifically delineated, internationally accepted criteria with which to diagnose pulmonary hypertension (PH) based on echocardiography derived markers. However, it is accepted that a PAAT < 40ms and a PAAT:RVET ratio of < 0.25 are markers of abnormally high pulmonary pressures [44, 134]. Within my own dataset, as 97.8% of left ventricular eccentricity index (LV EI) measurements in the control population were < 1.8 I defined an LV EI ratio > 1.8 as part of the diagnostic criterion for pulmonary hypertension (PH) in this study. Therefore, in this study a diagnosis of PH was made if two or more of the PH markers defined as follows were present:

- A PAAT < 40ms
- A PAAT:RVET < 0.25
- In the presence of a patent ductus arteriosus (PDA), the demonstration of bidirectional flow across the vessel or right to left flow
- A left ventricular eccentricity index > 1.8

This definition of PH was applied to echocardiography measurements in the DS and control groups on Day 1 and Day 3 of age. On Day 1 of age 84% of babies with DS met this criterion for PH compared to 15% of controls. By Day 3 28% of babies with DS still met this criterion for PH compared with no controls. Such findings indicate sustained elevation of pulmonary pressures in newborns with DS which persists at discharge. Such persistent elevation of pulmonary pressures will expose both the right and left ventricular myocardium to abnormal loading conditions.

Babies with DS had longer systolic times (ms) and systolic: diastolic ratios (S:D) than control babies; a marker of diastolic impairment. On Day 1 TDI assessment LV s', LV e', LV ea', septal s', septal e' and RV ea' velocities were significantly lower and RV a' velocities were significantly higher in the DS cohort compared to the control cohort. In addition, the Ee' ratio was higher in babies with DS signalling elevated left atrial pressures. However, all

LV and septal TDI measurements in the DS cohort had normalised by Day 3 of age. RV TDI evaluation on Day 3-5 demonstrated significantly higher RV e' and a' diastolic velocities and reduced RV ea' ratio in infants with DS versus controls. Day 1 deformation analysis in the DS population revealed biventricular systolic and diastolic impairment with reduced LV and RV longitudinal strain, LV and RV longitudinal systolic strain rate, early and late LV diastolic strain rate and RV early diastolic strain rate measurements in the DS group compared to the control group. LV global longitudinal strain, LV longitudinal systolic strain rate, and both LV early and late diastolic strain rate measurements remained significantly impaired on Day 3-5 evaluation in the DS group Similarly, RV deformation analysis revealed continuing diminished RV longitudinal free wall strain, RV longitudinal systolic strain rate and RV early diastolic strain rate values on Echo 3 assessment of the DS cohort. TDI measures myocardial wall velocity while STE analysis measures ventricular deformation; STE appears to be a more sensitive tool for serial assessment of myocardial performance[41]. In summary, TDI and STE measurements identified abnormal biventricular systolic and diastolic function in the DS cohort on Day 1 with continuing impairment evident on STE measurements on Day 3-5 of age compared to controls.

This data establishes that neither pulmonary pressures nor systolic or diastolic myocardial performance have normalised by discharge home for infants with DS, independent of CHD. Focusing specifically on diastolic function; there was persistent biventricular early diastolic impairment in the DS cohort as exhibited by significantly lower LV and RV early diastolic strain rates over the first week of age compared to controls. As strain rate measurements are reflective of inherent myocardial contractility such impaired diastolic values are indicative of stiff, non-compliant ventricles. This data informs us that the diastolic impairment apparent in babies with DS may be attributed to the combination of a stiff, non-compliant ventricular myocardium (as per abnormal strain rate values) and the abnormal loading conditions (as per abnormal strain values) to which that myocardium is exposed. It is likely that LV myocardial pathology as evidenced by reduced LV volumes, reduced LV GLS values and impaired LV diastolic function are significant drivers of PH in the DS population. These adverse findings combine to generate PH in the DS cohort independent of the co-existence of CHD.

8.3.2 Biventricular Impairment and its Relationship with Pulmonary Hypertension demonstrating Ventricular Interdependence in Infants with Down Syndrome over the First Week of Age

Upon evaluation of the relationship between LV diastolic impairment and indices of PH, a direct correlation was visualised. To reiterate, strain rate is relatively load independent and therefore a closer surrogate of inherent myocardial contractility. PAAT is inversely related to PVR and the Ee' ratio provides an estimate of left atrial filling pressure with higher values indicating elevated left atrial pressures[126, 127]. A significant positive correlation was observed between LV early diastolic strain rate and PAAT measurements and LV early diastolic strain rate and the PAAT:RVET ratio. This data demonstrates that with worse diastolic function, there is a direct correlation with more concerning indices of PH in the DS population. Importantly, there was also a significant negative correlation between Ee' ratio and PAAT measurements and the PAAT:RVET ratio. This emphasises that with increasing left atrial (LA) pressure there is a direct correlation with poorer indices of PH. I propose that the physiological mechanism to explain these findings is that, in the presence of a stiff, non-compliant LV, there is abnormally elevated LA pressure and subsequent elevated pulmonary venous pressure. This elevated pulmonary venous pressure can lead to an increase in pulmonary vascular resistance with a consequent negative impact on PAAT and PAAT:RVET measurements. As such the diastolic impairment demonstrated in babies with DS is directly contributing to their PH phenotype. A 2019 study of 154 adolescents with DS compared to 102 non-DS controls documented reduced LV mass and higher Ee' values in the DS group despite adjustment for age, height, heart rate, systolic blood pressure and body mass index ($\beta = 2.6$, $p < 0.0001$) and inferred that DS is associated with a lifelong impairment of left ventricular diastolic function[139]. Such findings suggest that the smaller ventricular size and diastolic impairment identified in our study cohort compared to controls may persist into early adulthood.

I next sought to assess the impact of an increased PVR environment on RV function in infants with DS. Significant positive correlations between PAAT measurements and RV longitudinal free wall strain and RV longitudinal systolic strain rate measurements and a significant negative correlation between LV eccentricity index and RV free wall strain and RV

systolic strain rate measurements were identified. Overall, these data clearly demonstrate that with higher degrees of PVR there is a direct association with diminishing RV systolic function. Since both RV strain and RV systolic strain rate were significantly abnormal in the DS cohort, the correlations imply that their elevated PVR is driving a high RV afterload environment which actively suppresses adequate RV systolic function; a phenomenon which is occurring in the context of pre-existing impaired RV ventricular contractility.

With elevated pulmonary pressures exerting a negative influence on RV systolic function I also examined the ability of the RV in infants with DS to respond to this high afterload state. This was evaluated via RV-PV coupling. RV-PV coupling expresses the ability of the RV to increase its contractility in tandem with increasing RV afterload. The RV can adapt to increasing degrees of RV afterload by increasing RV contractility via RV hypertrophy. However, in the face of unrelenting increases in RV afterload the challenge of maintaining normal RV performance becomes insurmountable and ultimately the RV uncouples from its afterload, with an associated decrease in RV efficiency. Following evaluation of RV-PV coupling, a significant positive correlation between LV early diastolic strain rate and RV-PV coupling was identified. This demonstrates that worse early LV diastolic function is associated with poorer RV-PV coupling. Physiologically, this implies that infants with DS have a diminished capacity to mount RV adaptations to high afterload conditions because of inherent myocardial diastolic impairment.

8.3.3 Conclusion

Over recent years, the concept of ventricular interdependence is coming to the fore as clinicians gain further insights into how vulnerabilities of one ventricle may impose negative effects on its co-ventricle, especially in the context of PH[140-143]. This work has confirmed that elevated pulmonary pressures and impaired biventricular systolic and diastolic function persist at hospital discharge home in babies with DS compared to non-DS controls. The small, stiff LVs of the DS group exhibit inherent diastolic impairment that increases LA pressure and pulmonary venous pressure. This is a novel explanation for an additional physiological mechanism contributing to the early PH phenotype frequently observed in the DS population. This intrinsic LV diastolic impairment is directly associated

with worsening indices of PH, which in turn depress RV systolic performance. In addition, the ability of the RV in babies with DS to respond efficiently to such conditions is hindered by diminished RV-PV coupling. Such findings highlight ventricular interdependence and the importance of biventricular appraisal when evaluating the relationship between PH and myocardial performance. These findings have significant implications for the ICU treatment of DS infants with PH as it demands therapies directed at RV and LV dysfunction as well as the traditional therapies directed at the pulmonary vascular bed. In conclusion, this work emphasises the abnormal pulmonary vascular state and sustained impairment of biventricular myocardial function in babies with DS over the first week of age and, specifically, how impaired LV diastolic performance imposes detrimental effects on pulmonary haemodynamics and RV systolic performance through ventricular interdependence.

8.4 Longitudinal Assessment of Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome using Novel Echocardiography Techniques over the First Two Years of Age

There is a growing evidence base purporting the presence of biventricular myocardial dysfunction in people with Down syndrome (DS) from foetal to adult life, independent of structural cardiac disease[9-11, 56, 57]. There is also increasing recognition in the medical profession of the high risk of pulmonary hypertension (PH) at any age in the DS population which may accelerate cardiorespiratory morbidity[136]. In a study of 20 infants with DS and structurally normal hearts and 17 non-DS controls, our group previously reported that this study cohort with DS had smaller LV dimensions, significantly lower RV strain values on day 2 and days 5-7 of age and higher indices of PH when compared to non-DS controls over the first week of age[9]. However, there is a paucity of longitudinal data describing the evolution of myocardial performance and pulmonary haemodynamics in infants with DS with and without congenital heart disease (CHD) over the first two years of age. My hypothesis was that babies with DS, independent of the presence of CHD, would demonstrate biventricular myocardial systolic and diastolic impairment, and sustained elevation of pulmonary pressures, over the first two years of age. I sought to expand on earlier work by our group by evaluating if the differences between the DS and control cohorts in anthropometric measurements, PH indices and markers of biventricular performance observed over the first week of age were sustained or dissipated over time in a larger study cohort.

8.4.1 Evaluation of Anthropometric Measurements, Myocardial Performance and Pulmonary Haemodynamics in Infants with Down Syndrome over the First Two Years of Age

A total of 626 echocardiograms were performed over the study period: 322 for the DS group and 304 for the control group. Significant differences in birth weight were observed between the control, infants with DS and CHD (DS-CHD) and infants with DS and no CHD (DS-no CHD) cohorts. Although weight measurements in both DS cohorts had normalised by 6 months of age there were significant differences between the control and DS-CHD cohort at both 12 and 24 months of age and between the control-and no CHD

cohort at 24 months of age. This is consistent with well-established knowledge confirming slower growth trajectories of infants and children with DS[144].

Regarding anthropometric assessment, infants with DS had significant differences in LV and RV dimensions compared to controls over the first two years of age. Mitral valve annuli measurements were smaller in diameter on Day 1 of age between the control and DS-CHD and the control and DS-no CHD infants. These differences were also evident at the 6 and 24 month echocardiograms. Babies with DS, both those with and without CHD, had significantly shorter LV lengths throughout the entire study period. Our group had previously reported that babies with DS had smaller mitral valve annuli and shorter LV lengths at birth[2]. These data now confirms that this phenotype persists over the first two years of age. Infants with DS and structurally normal hearts had smaller tricuspid valve annulus diameters on Day 1 of age compared to controls, however their measurements normalised over time. Similar to LV length measurements, infants with DS, both those with and without CHD, had significantly shorter RV lengths consistently throughout the first two years of age. There was no significant difference in LV or RV length between the DS-CHD and DS-no CHD groups from 6 months to 2 years of age indicating that structural heart disease does not appear to impact ventricular length over 6 months of age.

Pulmonary vascular resistance (PVR) indices were significantly worse throughout the first two years of age in infants with DS independent of the presence of structural heart disease. This was identified through evaluation echocardiography surrogates of PH revealing ongoing and significantly abnormal measurements in the DS group; namely significantly shorter PAAT times in both the DS-CHD and the DS-no CHD groups, lower PAAT:RVET ratios in both the DS-CHD and DS-no CHD groups, and significantly increased LV eccentricity index measurements in both the DS-CHD and DS-no CHD groups compared to controls over the first two years of age. There was no difference detected between the DS-CHD and DS-no CHD groups across any of the three echocardiography derived surrogates of PH; PAAT, PAAT:RVET or LV eccentricity index at any timepoint throughout the study. This corroborates the theory that infants with DS, regardless of the presence or absence of structural cardiac disease, have persistent, abnormal elevation of PVR over the first two years of age.

Tissue Doppler imaging demonstrated significantly impaired LV s', LV e' and LV ea' ratio values in the DS-CHD cohort at each evaluation from birth to two years of age compared to the control cohort. In comparison to the control group the infants in the DS-no CHD group displayed significantly lower LV s' velocities at 6 months and 12 months, significantly lower LV e' velocities at the 6 month, 12 month and 24 month timepoints and significantly lower LV ea' ratio at 2 years of age. No differences were detected in LV s' or LV e' values between the DS-CHD and DS-no CHD groups at any stage. Septal s' and e' measurements were significantly reduced in the DS-CHD group compared to the control group at each evaluation throughout the first two years of age. Septal e' values were also significantly reduced in the DS-no CHD group at 6 months and 2 years of age compared to controls. RV s' and RV e' velocities in infants with DS and CHD were significantly impaired at 6 months, 12 months and 2 years of age in comparison to infants in the control group. In addition, RV e' values were significantly lower from birth to 2 years of age in the DS-CHD group in comparison to the DS-no CHD group.

On deformation analysis, LV global longitudinal strain, LV systolic strain rate and LV early diastolic strain rate measurements were significantly impaired at each evaluation from birth to two years of age in the DS-CHD group compared to controls. In the DS-no CHD group LV global longitudinal strain was also significantly reduced at each assessment from birth to two years of age, LV systolic strain rate was significantly lower from birth to 12 months of age and LV early diastolic strain rate was significantly impaired at birth and 6 months of age in comparison to control infants. There was no difference detected at any point over the study period in LV global longitudinal strain, LV systolic strain rate or LV early diastolic strain rate measurements between the DS-CHD and DS-no CHD cohorts. Regarding RV functional assessment, RV free wall strain values were significantly impaired at each echocardiogram performed over the first two years of age in the DS-CHD cohort and at birth, 6 months and 12 months of age in the DS-no-CHD cohort compared to controls. RV systolic strain rate measurements were significantly abnormal in comparison to control infants for both the DS-CHD and DS-no CHD groups from birth to 12 months of age. RV early diastolic strain rate measurements were significantly lower for both the DS-CHD and DS-no CHD groups at the 12 month assessment.

This longitudinal assessment of cardiac function and pulmonary haemodynamics has produced noteworthy results. Babies with DS had significantly smaller LV and RV measurements, sustained abnormal elevation of pulmonary pressures and biventricular systolic and diastolic impairments in comparison to non-DS controls over the first two years of age. Persistently high pulmonary pressures were not influenced by the presence of structural heart disease in the DS group. Both TDI and STE appraisal of myocardial function identified persistent systolic and early diastolic impairment over the first two years of age in infants with DS. Across evaluation of TDI derived LV s' and LV e' and STE derived LV global longitudinal strain, LV systolic strain rate and LV early diastolic strain rate measurements there was no difference between the DS-CHD and DS-no CHD groups detected, indicating that the sustained impairment in LV systolic and early diastolic performance over the first two years in comparison to controls is occurring irrespective of structural cardiac disease. TDI evaluation of RV function detected systolic and early diastolic impairment in the DS-CHD cohort only, However, STE evaluation of RV function detected reduced RV free wall systolic strain and RV systolic strain rate values in both the DS-CHD and DS-no CHD cohorts, suggesting STE is capable of more sensitive appraisal of myocardial impairment.

Notably, both LV and RV strain and systolic strain rate values were negatively affected in the DS cohort in comparison to controls, suggesting a combination of abnormal loading conditions and impaired inherent contractility conspire to cause impairment of biventricular systolic performance in infants with DS over the first two years of age. Sustained elevation of pulmonary pressures may partially account for such findings; increased PVR will create a high RV afterload environment with adverse effects on RV strain. Reduced RV performance may decrease pulmonary blood flow and consequently LV preload[145]. Impairments of biventricular myocardial contractility, indicated by reduced LV and RV strain rates in the DS cohort, will also negatively impact on LV and RV strain. With LV early diastolic strain rate measurements reduced in the DS-no CHD and DS-CHD groups at birth and 6 months and persistently reduced in the DS-CHD group at 12 months at 2 years of age, in comparison to controls, the early LV diastolic impairment identified during assessment over the first week of age clearly persists with time. Such findings indicate the persistence of a stiff, non-compliant LV during early childhood, particularly in babies with DS and CHD, which may also negatively impact LV preload.

8.4.2 Conclusion

This work has demonstrated sustained abnormal elevation of pulmonary pressures and impaired systolic and diastolic function in infants with DS compared to controls over the first two years of age. The observation that indices of PH and myocardial performance are negatively impacted in babies with DS irrespective of structural cardiac disease is nuance which is missing in currently available literature. As such this work highlights that the DS infant population have elevated baseline pulmonary pressures as well as persistent reduced indices of both RV and LV systolic and diastolic function. These findings would indicate that this vulnerable population has a reduced capacity to increase cardiac output and are at risk of further elevation in pulmonary pressures at times of intercurrent illness. Such novel findings provide valuable insights into the pathophysiology affecting cardiorespiratory morbidity in this population.

8.5 Left Ventricular Rotational Mechanics in Infants with Down Syndrome over the First Two Years of Age

A previous study performed by our group which enrolled 20 infants with Down syndrome (DS) and structurally normal hearts, and 17 non-DS control infants investigated LV rotational mechanics over the first week of age. This work documented a reduction in LV twist, torsion, and twist rate suggesting impaired rotational mechanisms in the DS cohort[9]. Adult studies have shown that pulmonary hypertension negatively affects basal twist measurements[146]. I aimed to further examine these findings in a larger DS cohort with longitudinal assessments over the first two years of age.

In this cohort of 70 infants with DS, with and without congenital heart disease (CHD), and 60 non-DS controls I did not identify any differences in basal rotation, apical rotation, LV twist, LV torsion or LV twist rate between the DS-CHD, DS-no CHD and control infants at any point over the first two years of age. Although there was a significant difference in LV untwist rate between control and DS-CHD infants on Day 1 of age, this finding dissipated on the longitudinal assessment at 6 months, 12 months and 2 years of age.

These results may differ from the previous work performed by our group for several potential reasons. The group of babies with DS enrolled in the 2019 study were infants with DS who were considerably sicker than our cohort with 10% requiring invasive ventilation and 75% requiring supplemental oxygen. Potentially, these impairments in LV rotational mechanics are only present in babies with DS and significant neonatal cardiorespiratory morbidity. In addition, this was pilot work with a relatively small sample size of twenty infants. This point was fully appreciated at the time and the necessity of recruiting a larger cohort of infants with DS was stressed to fully explore these physiological mechanisms.

Other studies conducted by our group in premature infants born < 29 weeks gestation and term infants born to mothers with gestational diabetes mellitus suggests that both cohorts evoke LV twist as compensatory mechanism to maintain normal myocardial performance in the face of biventricular longitudinal dysfunction[137, 138]. However, this current investigation of LV rotational mechanics in infants with DS over the first two years of

age demonstrates that this is not the case in this specific population. Potentially the longitudinal function of infants with DS is sufficient to not require evoking LV twist to support their cardiac output. It may be that the helical configuration of subendocardial and subepicardial fibres which bestow the LV with unique rotational properties are somehow disrupted in babies and children with DS precluding LV twist altogether.

8.5.1 Conclusion

These results document that measurements of LV rotational mechanics in infants with DS over the first two years of age are comparable to their non-DS counterparts.

8.6 Cardiac Function and Pulmonary Haemodynamics in Infants with Down Syndrome requiring Surgical Correction for Congenital Heart Disease over the First Two Years of Age

Thirteen infants with DS required surgical correction for congenital heart disease (CHD) and post-operative paediatric intensive care (PICU) admission over the first two years of age compared with 57 infants with DS who did not require surgical correction.

8.6.1 Clinical Outcomes of Infants with Down Syndrome who required Surgical Correction for Congenital Heart Disease over the First Two Years of Age.

There were no differences evident in gestational age, birth weight, male sex, Caesarean section rate, likelihood of intubation, oxygen requirement or duration, time to full enteral feeds, hospital stay, feeding type over the first 6 months of age, hypothyroidism necessitating L-thyroxine administration and weight at two years of age between infants with DS who required surgical correction of CHD (DS-Surg) versus those that did not over the first two years of age (DS-no Surg). The findings regarding L-thyroxine therapy are consistent with a study of 16 children with DS performed by Toscano *et al* which documented no discrepancy in myocardial function between infants with DS requiring L-thyroxine administration and those that did not[147]. Diuretic use was higher in infants with DS who required surgical closure of CHDs, in keeping with their expected clinical course. In this cohort, surgical corrections were more likely to be performed for atrioventricular septal defects and Tetralogy of Fallot cases, which is also consistent with previous publications[148].

8.6.2 Evaluation of Myocardial Performance and Pulmonary Haemodynamics in Infants with Down Syndrome who required Surgical Correction for Congenital Heart Disease over the First Two Years of Age.

LV global longitudinal strain, LV systolic strain rate and LV early diastolic strain rate were all significantly lower on Day 1 evaluation in the DS-Surg group compared to the DS-no Surg group, indicating that from Day 1 of age the babies with DS requiring surgical

correction of CHDs were a significantly different sub-cohort of babies in terms of LV systolic and early diastolic performance.

With longitudinal evaluation over the first two years of age, no differences in pulmonary artery acceleration time to right ventricular ejection time ratio (PAAT:RVET) of LV eccentricity index were detected between the DS-Surg versus DS-no Surg groups. This data corroborates our previous findings that CHD, even CHD requiring surgical closure, is not the primary pathology driving the evolution and persistence of PH in babies with DS over the first two years of age.

On evaluation of longitudinal echocardiograms, LV global longitudinal strain was significantly lower in the DS-Surg group compared to the DS-no Surg group at Day 1 and 6 months of age. However, LV systolic strain rate measurements remained significantly worse in the DS-Surg vs DS-no Surg cohort throughout the first two years of age, at all study time points. Such findings indicate persistent, inherent impairment of LV systolic myocardial contractility in the DS-Surg babies which is not ameliorated by surgical repair of structural CHD and does not normalise by two years of age. Longitudinal assessment of RV performance identified significantly compromised RV free wall longitudinal strain in the DS-Surg group at 12 months and 2 years of age compared to the DS-no Surg group. This impairment in RV free wall longitudinal strain had developed over the first two years of age from values that were comparable to the DS-no Surg group at Day 1 and 6 months of age. This data is further substantiated by concomitant diminishing RV systolic strain rate measurements in the DS-Surg group also at 12 months and 2 years of age compared to the DS-no Surg group and points to the evolution of intrinsic RV contractility impairment in the DS-Surg group over time despite surgical intervention for CHD. Of note, RV late diastolic strain rate values were significantly lower in the DS-Surg group compared to the DS-no Surg group at 6 months, 12 months and 2years of age implying evolving late diastolic function in the DS-Surg group with age. Such findings indicate that with time the DS-Surg cohort are relying to a greater extent on LA contraction during late diastole to complete LV ventricular filling.

8.6.3 Conclusion

To conclude, our work has provided a greater understanding of abnormalities of RV and LV systolic and diastolic myocardial performance and pulmonary haemodynamics in the sub-cohort of babies with DS who require surgical closure of CHDs. The analysis has documented previously unrecognised LV systolic and LV early diastolic impairment on Day 1 echocardiography in this group in comparison to infants with DS who do not require surgical intervention. Significantly, elevated pulmonary pressures persist over the first two years of age in babies with DS and do not appear to be ameliorated by CHD surgical correction. Likewise, longitudinal evaluation has confirmed sustained impairments of inherent LV systolic contractility from birth to two years of age and evolving inherent RV systolic contractility impairments in the DS-Surg cohort compared to the DS-no-Surg cohorts. Such data flags babies requiring surgical closure as a particularly vulnerable group within the overall DS population necessitating long term advance functional imaging.

8.7 Current Literature and Summary of Our Research

To the best of our knowledge, this work is the largest prospective study performed evaluating longitudinal myocardial function and pulmonary haemodynamics in infants with Down syndrome (DS) from birth to two years of age. In 2015 there were an estimated 417,000 people with DS living in Europe [149]. While life expectancy has increased in recent decades cardiovascular and pulmonary disease still account for an estimated 75% of mortality in individuals with DS. The majority of deaths occur in young adults, however neonatal and infant mortality rates are higher in DS than the general population [110-113]. Many people with DS are now living into their 6th decade so thoroughly optimising health from birth is essential to safeguard optimal clinical and developmental outcomes[150].

8.7.1 Current Literature

There is a growing appreciation within the neonatal and paediatric communities that the incidence of pulmonary hypertension (PH) in the DS population is considerably higher than once thought[8, 75]. Early PH in babies with DS is significantly associated with an increased necessity for invasive ventilation, more days of inhaled nitric oxide therapy, longer hospital admission and higher mortality before discharge[8, 79]. In a French study of 24 children from 1 month to 16 years of age, pulmonary arterial hypertension was a leading cause of ICU admission[6]. The PH phenotype of the DS paediatric population is complex, multifactorial and often particularly challenging to manage as children with DS have a blunted response to iNO and require ECMO more frequently than the adult population with DS[151]. In light of the current covid 19 pandemic, there is also growing concern that individuals with DS are a particularly vulnerable group due to immunocompromise and an increased risk of viral respiratory illness[152-156]. In a 2021 study of 18 people with DS requiring hospitalisation for Covid 19 infection Emami *et al* reported that people in the DS cohort were more significantly more likely to be intubated ($p = 0.002$) and to die than controls ($p = 0.007$)[157]. Twelve adults with DS and covid-19 infection in an American study were hospitalized at a younger age and had a more severe disease course than their non-DS counterparts with a higher incidence of sepsis and mechanical ventilation[158]. It is a distinct

possibility that PH is contributing the morbidity and mortality associated with covid-19 in the DS population.

It is also well established that structural cardiac disease confers a high risk of morbidity and mortality with congenital heart defects (CHD) being the most frequent cause of death in children with DS over the first two years of age[51, 52]. In addition, CHD also adversely affects neurodevelopmental outcome with lower scores for receptive, expressive and composite language in infants and toddlers with DS who required corrective surgery in the first year of age compared to those children with DS and no CHD[54]. However, several studies in older children have highlighted that biventricular dysfunction is not an issue exclusive to those babies with DS and structural cardiac disease. A 2013 echocardiography study of 85 children aged between 7 and 13 years with DS and structurally normal hearts reported sub-clinical LV diastolic dysfunction and RV systolic and diastolic dysfunction, and Balli *et al* assessed 115 children with DS and structurally normal hearts aged between 6 and 13 years old and documented significantly reduced diastolic performance compared to age and sex matched controls [10, 11]. This myocardial dysfunction may impose longstanding impacts into later life with an Italian study of adults with DS, mean age 36.1 +/-9.7 years, reporting that individuals with LV diastolic dysfunction demonstrated lower cognitive scores compared to those with preserved diastolic function[58].

With a growing body of literature declaring the clinical and neurodevelopmental sequelae of PH and myocardial impairment in the DS population, great efforts have been made by basic science community in recent years to scrutinize the genetic underpinnings of such phenomena, establish more accurate diagnostic biomarkers and develop potential therapeutic strategies[159]. Particularly impressive advancements have been achieved in elucidating the genetic basis for the DS phenotype. Alharbi *et al* suggested mutations in GATA3, KCNH2, ENG, FLNA, and GUSB increase the risk of CHD in people with DS, Wu *et al* reported specific genetic pathways postulated to play a role in the development of endocardial cushion defects in the DS population and Quinones-Lombrana *et al* discussed the presence of inter-individual variation in genetic expression of the myocardial DYRK1A-SRSF6-TNNT2 pathway in the context of DS-CHD in their 2019 publication [63] [160, 161]. A 2021 study found 93 highly differentially methylated CpG sites and 16 differentially methylated

regions in myocardial DNA from individuals with DS and a 2015 study documented hypermethylation of the GATA4 gene in foetuses with DS with and without CHDs and as well as in foetuses with isolated heart malformations. Importantly aberrant genetic expression of the abnormally methylated genes was also detected[67, 162].Laufer *et al* have also identified epigenetic alterations in brain tissue from individuals with DS and non-DS controls in pathways involved in membrane transport, glutamatergic synaptic signalling, glial immune response and apoptosis[163]. Detailed genetic and biochemical analysis is also underway to identify specific mechanisms involved in the development of PH[164]. Salvolini *et al* reported that both VEGF and eNOS expression was significantly reduced in amniotic fluid mesenchymal stem cells from DS pregnancies compared to controls and suggested binvolvement of nitric oxide and VEGF in the pathophysiological pathways involved in DS pregnancies[165]. Indeed, due to the high prevalence of PH in people with DS this population have been proposed as an ideal patient cohort to study in detail the underlying pathophysiology of PH and potentially identify new therapeutic targets[166, 167]. Some preliminary work has been performed to examine if biochemical markers may stratify people with DS with and without PH[168, 169]. A 2019 study reported that reduced angiogenin or reduced angiogenin with elevated angiopoietin levels distinguished PH in those with DS from DS-no PH, non-DS with PH and non-DS-no PH and supports speculation that dysregulated angiogenesis may be a causative factor for the development of PH in children with DS[168].

There is limited data evaluating differences at myocyte level between people with DS and non-DS cohorts. A 1986 study examined the size and number of cardiac muscle fibres in 15 individuals with DS without CHD against 15 age and sex matched controls. This study determined that the mean ratio of muscle cells per unit area in the DS cohort was 84.9%, the mean cross sectional area of cardiac muscle fibres was 117% and the mean volume of cardiac cells in DS cohort was 127% compared to controls[170]. Of note the average weight of hearts in the DS group compared to age matched controls was 79% of the control values[170]. Murine studies have revealed that quadriceps femoris skeletal fibres display structural alterations of their mitochondria and myonuclei similar to those documented in age related sarcopenia[171]. Such findings illustrate that the trisomy 21 phenotype exerts morphologic alterations at myocyte level which may have functional impacts.

Concomitant to a deeper understanding of the biological processes at play regarding PH and CHD in people with DS there are emerging therapeutic strategies under investigation. The first drug to be approved for the treatment of pulmonary arterial hypertension was the synthetic prostacyclin (epoprostenol) then followed by prostaglandin analogs including iloprost, treprostinil, and beraprost which act on prostaglandin receptors[172]. Aerosolized iloprost and treprostinil have been approved by the United States Food and Drug Administration to treat pulmonary arterial hypertension and inhaled iloprost has been demonstrated to be a beneficial treatment for adult patients with severe PH[173, 174]. A retrospective study of non-DS neonates with PH on ECMO assessing the use of intravenous treprostinil demonstrated improved right ventricular function, reversed right-to-left shunting through the ductus arteriosus, and stable or decreasing need for vasopressor support[175]. Within the DS population specifically, long term oral administration of the dual endothelin receptor antagonist, bosentan was shown to significantly improve oxygen saturation following exercise and exercise capacity in adults with DS and Eisenmenger syndrome[176]. These results were mirrored in a 2013 adult study of PH with 18 adults with DS and CHD that documented beneficial haemodynamic effects following 12 months of bosantan treatment with reduced pulmonary to systemic flow ratio and reduced pulmonary vascular resistance measurements[177]. Sildenafil is a phosphodiesterase-5 inhibitor which has demonstrated efficacy in the treatment of neonatal PH in non-DS infants[178]. However, a post-hoc analysis of children with Down syndrome and pulmonary arterial hypertension enrolled in the Sildenafil in Treatment-Naive Children, Aged 1-17 Years, With Pulmonary Arterial Hypertension (STARTS-1) trial reported that treatment with sildenafil for 4 months had no effect on mean pulmonary arterial pressure in children with DS and suggested that the DS population may be less responsive to sildenafil than the non-DS population[179]. Selexipag is another oral medication undergoing assessment for treatment of paediatric PH which has been approved for use in adult PH[180]. Selexipag is a newer, selective prostacyclin receptor agonist for which there is promising pilot study data available in non-DS patients with CHD related PH whereby selexipag administration enabled parenteral prostacyclins to be weaned following CHD surgical repair[181]. A 2019 Cochrane review evaluated the efficacy and safety of prostacyclin, prostacyclin analogues or prostacyclin receptor agonists for pulmonary arterial hypertension in adults and children and concluded that intravenous prostacyclin demonstrates clinical and statistical benefit with improved mortality, symptoms

scores, and cardiopulmonary haemodynamics, but at a cost of adverse events; that inhaled prostacyclin demonstrated a small clinical benefit in function and haemodynamics but had uncertain effects on mortality; that the effects of oral prostacyclins were overall less certain and that selexipag demonstrated less clinical deterioration without an obvious impact on survival[182]. Other therapies under investigation for DS-related morbidities include melatonin and medications that enhance mitochondrial biogenesis[72, 183]. The most advanced and controversial therapeutic strategy currently under consideration is experimental research of various in vitro technologies to remove or silence the extra chromosome 21 material in cells at single gene, whole chromosome or epigenetic level[184, 185].

With such considerable advancements in our collective understanding of the frequency, pathophysiology and potential therapies to ameliorate the multitude morbidities associated with DS active health surveillance services for people with DS have developed over time. There is a growing appreciation that a holistic, multidisciplinary approach which tackles the complexity of medical issues facing infants and children with DS is essential[186, 187]. Some countries offer DS specific clinics to centralise expertise, and many have written national guidelines to ensure consistency in service provision[188-191]. The aim of such endeavours is to diagnose and instigate appropriate therapy early for any DS associated morbidity to maximize the medical, cognitive and neurodevelopmental potential of every person with DS[192].

8.7.2 Summary and Implications of Our Research

This study adds new insights to the canon of knowledge on myocardial function and pulmonary haemodynamics in infants and children with Down syndrome (DS). Utilising advanced echocardiography techniques our prospective, tri-centre observational study of 70 babies with DS and 60 non-DS controls has documented sustained abnormal elevation of pulmonary pressures and impaired systolic and diastolic function in infants with DS compared to controls over the first two years of age. The results emphasise the abnormal pulmonary vascular state and ongoing impairment of biventricular myocardial performance in babies with DS over the first week of age and, specifically, how impaired left ventricular (LV) diastolic function imposes detrimental effects on pulmonary haemodynamics and right

ventricular (RV) systolic performance through ventricular interdependence. This is a novel physiological mechanism which is contributing to the early pulmonary hypertension (PH) phenotype frequently observed in the DS population. In addition, the ability of the RV in babies with DS to respond efficiently to such conditions was demonstrably hindered by diminished RV-PV coupling. Longitudinal echocardiography identified sustained abnormalities in indices of PH and biventricular systolic and diastolic performance in babies with DS compared to controls irrespective of structural cardiac disease that do not normalise by two years of age. This is nuance which is currently missing in available literature. The findings that infants with DS who require surgical correction of congenital heart disease (CHD) display sustained LV systolic contractility impairment from birth and evolving RV systolic contractility impairment from one year of age to two years of age also further distinguish this group as a particularly vulnerable one within the overall DS population.

The potential for evolving PH consequent to chronic left to right shunting via CHD is well recognised[193, 194]. In recent years greater attention has been paid to indices of PH in babies with DS and structurally intact hearts[195]. Earlier work by our group demonstrated elevated pulmonary pressures in babies with DS without CHD, however the degree of myocardial impairment and evolution of PH with age had not been explored beyond the neonatal period [9]. This current work expands on our 2019 study, and it is now clear that babies with DS have sustained biventricular impairment and continuing PH over the first two years of age independent of CHD. The implications of this research are that a greater awareness of such cardiorespiratory vulnerabilities in the neonatal and paediatric DS populations should be promoted and may prompt clinicians to monitor all babies and children with DS more closely, not only those with CHD. Efforts to understand the pathophysiological basis of the DS phenotype and identify potential therapies have made great strides. As such it is vital that clinicians accurately detect myocardial impairment and PH early before the appearance of clinically overt disease to afford babies and children with DS the opportunity to benefit from effective management strategies.

8.8 Study Limitations

There are a number of important limitations of this study. The original aim was to recruit 120 infants with DS and 60 non-DS control infants over a two year period. In hindsight the aim of enrolling 120 infants with DS over the study period was overly ambitious as many infants born with DS have acute cardiac and gastrointestinal diagnoses that necessitate prompt transfer to paediatric intensive care units in Children's Health Ireland at Temple Street or Children's Health Ireland at Crumlin. Such transfers prohibited enrolment into this study as our ethically approved recruitment sites were the three Dublin neonatal units in The Rotunda Hospital, The National Maternity Hospital, Holles Street and The Coombe Women & Infants University Hospital. Furthermore, as the goal of the study was to assess longitudinal myocardial function and pulmonary haemodynamics I wanted to ensure that I had the necessary time to complete 6 month, 12 month and 24 month echocardiograms on the majority of infants recruited. The findings of this study cannot be directly applied to the general infant DS population as my study cohort did not include the aforementioned infants with DS and acute cardiac or gastrointestinal diagnoses that necessitated immediate transfer to paediatric ICU. In addition, I did not record the total number of infants born with DS in Dublin over the study period.

The differences in gestational age, sex and mode of delivery between the two groups may play a potential role in the differences in echocardiography findings over the first week of age between the DS and control groups. In addition, the timing of the third echocardiogram was different between the DS and control cohorts which may have also had an impact on the results over the first week of age. It is possible that there are other confounding variables and biases which may have influenced the results.

I did not record weight or length centiles or blood pressure measurements for the DS or control cohorts during the study. I did not perform Z scores for weight in the DS or control cohorts.

There was a high rate of Caesarean section in the control cohort. The control cohort were enrolled entirely within the Rotunda Hospital where the Caesarean section rate is 40%.

In order to capture as many Day 3 echocardiograms as possible in the control population I decided to enrol more infants delivered by Caesarean section in this group. This decision was made as infants delivered by vaginal delivery are typically discharged home on day 2 of age precluding a day 3 echocardiogram.

The incidence of CHD in the DS cohort in this study was 69%, higher than what is usually reported in the literature. This may have had a potential impact on the measurements of pulmonary haemodynamics and myocardial function in this study.

The study was underpowered to detect the ability of echocardiography parameters to predict clinically relevant outcomes in the DS population due to the low incidence of such outcomes in our cohort. I did not collect detailed information on the number of infants in the DS cohort who were admitted specifically for respiratory syncytial virus bronchiolitis, the number receiving nasogastric feeds following hospital discharge or the total number of infants in the DS cohort who were referred for investigation of obstructive sleep apnoea. It is possible that the Covid-19 pandemic related restrictions favourably impacted on the incidence of intercurrent illness in the DS cohort due to reduced direct contact with other children and adults.

In some echocardiography measurements the lack of statistically significant differences between the DS and control cohorts may be due inadequate study participants in the DS cohort and type 2 error. For example, LV twist in the control cohort was approximately twice that of the DS-CHD group and although this was not statistically significant it may be clinically relevant. However, given the wide standard deviation of this measurement the study was underpowered to detect a statistically significant difference.

Off-line echocardiography analysis was unblinded to the grouping of the baby and was performed by one operator (Aisling Smith). If there was fusion of e' and a' waves on TDI assessment the single wave was documented as an a' wave. If this occurred measurements such as E/e', E/A and e'/a' were not assessed and this method could have had a potential impact on the study findings and external validity. TDI and STE techniques have not been

specifically validated in the DS population and future studies are required to perform this work.

There is no international consensus on the cut off value of LV EI that defines abnormal PVR. The definition of an abnormal LV EI of 1.8 designated in this study could not have been set a priori as this has not been previously defined in a healthy, term infant cohort. The gold standard for diagnosing PH is right heart catheterisation. At present there is also no international consensus regarding the definition of PH as diagnosed by echocardiography surrogate markers. Until such echocardiography markers are validated against right heart catheterisation measurements deriving a definition of PH based on those surrogate markers will remain a challenge. The echocardiography defined diagnosis of PH utilised in this study was not validated against right heart catheter measurements and its individual components carried equal weight. This echocardiography defined diagnosis of PH should be validated against right heart catheter measurements of pulmonary arterial pressure and assessed for its ability to predict clinically important outcomes both in a larger cohort of infants with DS and other neonatal cohorts of interest.

With the Covid-19 pandemic striking in early 2020, the ability to perform longitudinal echocardiograms on outpatient infants with DS and infants in the control cohort was significantly hindered. In order to comply with hospital policies and to ensure parental comfort these scans were delayed or cancelled at various time points over the 12 month period from March 2020 to March 2021. The current data set reflects our best efforts at performing as many longitudinal echocardiograms at 6 months, 12 months and 2 years as possible given the circumstances.

8.9 Future Directions

Impaired myocardial function and pulmonary haemodynamics may be evident from foetal to adult life in the Down syndrome (DS) population. Neonates with DS represent a particularly vulnerable group with a heterogeneous, multifactorial pulmonary hypertension (PH) phenotype that confers a significant risk of morbidity and mortality. I hope a greater understanding of myocardial performance and pulmonary haemodynamics in babies and children with DS, both with and without congenital heart disease (CHD), over the first two years of age will aid in the ability to monitor patients and ameliorate adverse outcomes.

Regarding future directions for this work, our findings should be further investigated in a larger cohort of infants with DS with a higher incidence of clinical outcomes including surgical morbidity. Ideally a study examining myocardial function and pulmonary haemodynamics in a DS cohort over a much longer period of time into childhood and their adolescent years could assess if the sub-clinical echocardiography findings observed in this study manifest as clinically relevant findings with increasing age.

As previously discussed, there is no international consensus available at present regarding the definition of PH as diagnosed by echocardiography surrogate markers. Further work is needed to validate echocardiography derived markers of PH, such as LV EI, against the gold standard of right heart catheter measurements of pulmonary arterial pressure. Until then the diagnosis of PH based off echocardiography surrogate markers will remain a challenge. In time studies should also assess the ability of such echocardiography surrogates of PH to predict clinically important outcomes in neonatal and paediatric populations of interest.

Following the results from this study I would suggest that increased surveillance of infants and children with DS, particularly those with structurally normal hearts, is warranted. At present our national guideline states 'It is highly desirable to establish the cardiac status of every child with Down syndrome by age 6 weeks' however there is no suggested echocardiography schedule or standardised approach for longitudinal monitoring of myocardial function or PH in children with DS in Ireland[196]. I would suggest a national,

consensus guideline for cardiovascular monitoring of infants and children with DS is devised with yearly echocardiographic assessment. Such an approach could provide an essential window of opportunity for detection of sub-clinical myocardial impairment or evolving PH prior to the development of clinically overt disease. In addition, any infant with DS admitted to hospital with an intercurrent illness should be screened for both RV and LV myocardial impairment as well as having an echocardiography-based assessment of pulmonary pressures.

The importance of continued basic science examining the biological underpinnings of DS associated morbidity and well designed, adequately powered studies investigating the safety and efficacy of emerging medications for the treatment of PH in the DS population cannot be overstressed[197-199]. To this end our research group is currently planning a prospective cohort study assessing the effect of early sildenafil treatment on PVR measurements in a DS cohort. The aim of the study is to evaluate if early intervention with sildenafil can mitigate or reverse elevated PVR in a DS cohort over the first six months of age.

In conclusion, this work has gleaned new insights into the evolution of left and right ventricular function and pulmonary haemodynamics in infants with DS over the first two years of age. The results demonstrate sustained impairment of biventricular systolic and diastolic myocardial performance and elevated pulmonary pressures in babies with DS from birth to two years of age regardless of structural heart disease. I hope this data will help improve cardiovascular monitoring, clinical and neurodevelopmental outcomes for babies and children with DS in Ireland and beyond.

References

1. Mégarbané A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethoré MO, et al. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genet Med. 2009;11(9):611-6.

2. Ni She R, Filan PM. Trisomy 21--incidence and outcomes in the first year, in Ireland today. Ir Med J. 2014;107(8):248-9.

3. Akhtar F, Bokhari SRA. Down Syndrome. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.

4. Mann JP, Statnikov E, Modi N, Johnson N, Springett A, Morris JK. Management and outcomes of neonates with down syndrome admitted to neonatal units. Birth Defects Res A Clin Mol Teratol. 2016;106(6):468-74.

5. McAndrew S, Acharya K, Nghiem-Rao TH, Leuthner S, Clark R, Lagatta J. NICU management and outcomes of infants with trisomy 21 without major anomalies. J Perinatol. 2018;38(8):1068-73.

6. Joffre C, Lesage F, Bustarret O, Hubert P, Oualha M. Children with Down syndrome: Clinical course and mortality-associated factors in a French medical paediatric intensive care unit. J Paediatr Child Health. 2016;52(6):595-9.

7. Seither K, Tabbah S, Tadesse DG, Suhrie KR. Neonatal complications of Down syndrome and factors necessitating intensive care. Am J Med Genet A. 2021;185(2):336-43.

8. Martin T, Smith A, Breatnach CR, Kent E, Shanahan I, Boyle M, et al. Infants Born with Down Syndrome: Burden of Disease in the Early Neonatal Period. J Pediatr. 2018;193:21-6.

9. Breatnach CR, Bussmann N, Smith A, Levy P, McCallion N, Franklin O, et al. Cardiac mechanics in infants with Down syndrome in the early neonatal period. J Perinatol. 2019;39(5):626-33.

10. Balli S, Yucel IK, Kibar AE, Ece I, Dalkiran ES, Candan S. Assessment of cardiac function in absence of congenital and acquired heart disease in patients with Down syndrome. World J Pediatr. 2016;12(4):463-9.

11. Al-Biltagi M, Serag AR, Hefidah MM, Mabrouk MM. Evaluation of cardiac functions with Doppler echocardiography in children with Down syndrome and anatomically normal heart. Cardiol Young. 2013;23(2):174-80.
12. Kwiatkowska J, Zuk M, Migdal A, Kusa J, Skiba E, Zygielo K, et al. Children and Adolescents with Pulmonary Arterial Hypertension: Baseline and Follow-Up Data from the Polish Registry of Pulmonary Hypertension (BNP-PL). J Clin Med. 2020;9(6).
13. Vincent JL. Understanding cardiac output. Crit Care. 2008;12(4):174.
14. O'Keefe E, Singh P. Physiology, Cardiac Preload. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.
15. Delicce AV, Makaryus AN. Physiology, Frank Starling Law. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.
16. DeMers D, Wachs D. Physiology, Mean Arterial Pressure. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.
17. Mielke G, Benda N. Cardiac output and central distribution of blood flow in the human fetus. Circulation. 2001;103(12):1662-8.
18. Friedman AH, Fahey JT. The transition from fetal to neonatal circulation: normal responses and implications for infants with heart disease. Semin Perinatol. 1993;17(2):106-21.
19. Kamlin CO, O'Donnell CP, Davis PG, Morley CJ. Oxygen saturation in healthy infants immediately after birth. J Pediatr. 2006;148(5):585-9.
20. Widrich J, Shetty M. Physiology, Pulmonary Vascular Resistance. StatPearls. Treasure Island (FL): StatPearls Publishing
Copyright © 2021, StatPearls Publishing LLC.; 2021.
21. Haworth SG. Pulmonary vascular remodeling in neonatal pulmonary hypertension. State of the art. Chest. 1988;93(3 Suppl):133s-8s.
22. Jain A, Mohamed A, Kavanagh B, Shah PS, Kuipers BCW, El-Khuffash A, et al. Cardiopulmonary Adaptation During First Day of Life in Human Neonates. J Pediatr. 2018;200:50-7.e2.

23. Joyce JJ, Dickson PI, Qi N, Noble JE, Raj JU, Baylen BG. Normal right and left ventricular mass development during early infancy. Am J Cardiol. 2004;93(6):797-801.
24. Clark SJ, Yoxall CW, Subhedar NV. Measurement of right ventricular volume in healthy term and preterm neonates. Arch Dis Child Fetal Neonatal Ed. 2002;87(2):F89-93; discussion F-4.
25. Clarke WR. The transitional circulation: physiology and anesthetic implications. J Clin Anesth. 1990;2(3):192-211.
26. Noori S, Seri I. Pathophysiology of newborn hypotension outside the transitional period. Early Hum Dev. 2005;81(5):399-404.
27. Alagarsamy S, Chhabra M, Gudavalli M, Nadroo AM, Sutija VG, Yugrakh D. Comparison of clinical criteria with echocardiographic findings in diagnosing PDA in preterm infants. J Perinat Med. 2005;33(2):161-4.
28. McElhinney DB, Straka M, Goldmuntz E, Zackai EH. Correlation between abnormal cardiac physical examination and echocardiographic findings in neonates with Down syndrome. Am J Med Genet. 2002;113(3):238-41.
29. El-Khuffash AF, Jain A, Weisz D, Mertens L, McNamara PJ. Assessment and treatment of post patent ductus arteriosus ligation syndrome. J Pediatr. 2014;165(1):46-52.e1.
30. Breatnach CR, Levy PT, James AT, Franklin O, El-Khuffash A. Novel Echocardiography Methods in the Functional Assessment of the Newborn Heart. Neonatology. 2016;110(4):248-60.
31. Jain A, El-Khuffash AF, Kuipers BCW, Mohamed A, Connelly KA, McNamara PJ, et al. Left Ventricular Function in Healthy Term Neonates During the Transitional Period. J Pediatr. 2017;182:197-203.e2.
32. Karlsen S, Dahlslett T, Grenne B, Sjøli B, Smiseth O, Edvardsen T, et al. Global longitudinal strain is a more reproducible measure of left ventricular function than ejection fraction regardless of echocardiographic training. Cardiovasc Ultrasound. 2019;17(1):18.
33. Jain A, Mohamed A, El-Khuffash A, Connelly KA, Dallaire F, Jankov RP, et al. A comprehensive echocardiographic protocol for assessing neonatal right ventricular dimensions and function in the transitional period: normative data and z scores. J Am Soc Echocardiogr. 2014;27(12):1293-304.

34. Levy PT, Dioneda B, Holland MR, Sekarski TJ, Lee CK, Mathur A, et al. Right ventricular function in preterm and term neonates: reference values for right ventricle areas and fractional area of change. J Am Soc Echocardiogr. 2015;28(5):559-69.
35. James AT, Corcoran JD, Franklin O, El-Khuffash AF. Clinical utility of right ventricular fractional area change in preterm infants. Early Hum Dev. 2016;92:19-23.
36. Koestenberger M, Nagel B, Ravekes W, Urlesberger B, Raith W, Avian A, et al. Systolic right ventricular function in preterm and term neonates: reference values of the tricuspid annular plane systolic excursion (TAPSE) in 258 patients and calculation of Z-score values. Neonatology. 2011;100(1):85-92.
37. Dallaire F, Slorach C, Bradley T, Hui W, Sarkola T, Friedberg MK, et al. Pediatric Reference Values and Z Score Equations for Left Ventricular Systolic Strain Measured by Two-Dimensional Speckle-Tracking Echocardiography. J Am Soc Echocardiogr. 2016;29(8):786-93.e8.
38. Levy PT, Sanchez Mejia AA, Machefsky A, Fowler S, Holland MR, Singh GK. Normal ranges of right ventricular systolic and diastolic strain measures in children: a systematic review and meta-analysis. J Am Soc Echocardiogr. 2014;27(5):549-60, e3.
39. Marcus KA, Mavinkurve-Groothuis AM, Barends M, van Dijk A, Feuth T, de Korte C, et al. Reference values for myocardial two-dimensional strain echocardiography in a healthy pediatric and young adult cohort. J Am Soc Echocardiogr. 2011;24(6):625-36.
40. Nestaas E, Schubert U, de Boode WP, El-Khuffash A. Tissue Doppler velocity imaging and event timings in neonates: a guide to image acquisition, measurement, interpretation, and reference values. Pediatr Res. 2018;84(Suppl 1):18-29.
41. El-Khuffash A, Schubert U, Levy PT, Nestaas E, de Boode WP. Deformation imaging and rotational mechanics in neonates: a guide to image acquisition, measurement, interpretation, and reference values. Pediatr Res. 2018;84(Suppl 1):30-45.
42. Levy PT, El-Khuffash A, Patel MD, Breatnach CR, James AT, Sanchez AA, et al. Maturational Patterns of Systolic Ventricular Deformation Mechanics by Two-Dimensional Speckle-Tracking Echocardiography in Preterm Infants over the First Year of Age. J Am Soc Echocardiogr. 2017;30(7):685-98.e1.
43. Levy PT, Machefsky A, Sanchez AA, Patel MD, Rogal S, Fowler S, et al. Reference Ranges of Left Ventricular Strain Measures by Two-Dimensional Speckle-Tracking

Echocardiography in Children: A Systematic Review and Meta-Analysis. J Am Soc Echocardiogr. 2016;29(3):209-25.e6.

44. Levy PT, Patel MD, Groh G, Choudhry S, Murphy J, Holland MR, et al. Pulmonary Artery Acceleration Time Provides a Reliable Estimate of Invasive Pulmonary Hemodynamics in Children. J Am Soc Echocardiogr. 2016;29(11):1056-65.

45. Asanuma T, Nakayama T, Masuda K, Takeda S, Nakatani S. Regional heterogeneity of afterload sensitivity in myocardial strain. J Med Ultrason (2001). 2020;47(4):501-6.

46. Alvarez SV, Fortin-Pellerin E, Alhabdan M, Lomelin JS, Kantoch M, Atallah J, et al. Strain Rate in Children and Young Piglets Mirrors Changes in Contractility and Demonstrates a Force-Frequency Relationship. J Am Soc Echocardiogr. 2017;30(8):797-806.

47. Breatnach CR, Levy PT, Franklin O, El-Khuffash A. Strain Rate and Its Positive Force-Frequency Relationship: Further Evidence from a Premature Infant Cohort. J Am Soc Echocardiogr. 2017;30(10):1045-6.

48. Ferferieva V, Van den Bergh A, Claus P, Jasaityte R, Veulemans P, Pellens M, et al. The relative value of strain and strain rate for defining intrinsic myocardial function. Am J Physiol Heart Circ Physiol. 2012;302(1):H188-95.

49. Bull MJ. Health supervision for children with Down syndrome. Pediatrics. 2011;128(2):393-406.

50. Hosokawa S, Vanderpool RR, Ishii T, Nishiyama M, Doi S. What Causes Pulmonary Arterial Hypertension in Down Syndrome With Congenital Heart Disease? Circ J. 2018;82(6):1513-4.

51. Al-Biltagi MA. Echocardiography in children with Down syndrome. World J Clin Pediatr. 2013;2(4):36-45.

52. Peterson JK, Kochilas LK, Knight J, McCracken C, Thomas AS, Moller JH, et al. Long-Term Survival and Causes of Death in Children with Trisomy 21 After Congenital Heart Surgery. J Pediatr. 2021;231:246-53.e3.

53. Martínez-Quintana E, Rodríguez-González F, Medina-Gil JM, Agredo-Muñoz J, Nieto-Lago V. Clinical outcome in Down syndrome patients with congenital heart disease. Cir Cir. 2010;78(3):245-50.

54. Alsaied T, Marino BS, Esbensen AJ, Anixt JS, Epstein JN, Cnota JF. Does Congenital Heart Disease Affect Neurodevelopmental Outcomes in Children with Down Syndrome? Congenit Heart Dis. 2016;11(1):26-33.

55. Calkoen E, Adriaanse B, Haak M, Bartelings M, Kolesnik A, Niszczota C, et al. How Normal is a 'Normal' Heart in Fetuses and Infants with Down Syndrome? Fetal Diagn Ther. 2016;39(1):13-20.
56. Mula R, Grande M, Bennasar M, Crispi F, Borobio V, Martinez JM, et al. Further insights into diastolic dysfunction in first-trimester trisomy-21 fetuses. Ultrasound Obstet Gynecol. 2015;45(2):205-10.
57. Clur SA, Oude Rengerink K, Ottenkamp J, Bilardo CM. Cardiac function in trisomy 21 fetuses. Ultrasound Obstet Gynecol. 2011;37(2):163-71.
58. Vetrano DL, Carfì A, Brandi V, L'Angiocola PD, Di Tella S, Cipriani MC, et al. Left ventricle diastolic function and cognitive performance in adults with Down syndrome. Int J Cardiol. 2016;203:816-8.
59. Hamerton JL, Briggs SM, Giannelli F, Carter CO. Chromosome studies in detection of parents with high risk of second child with Down's syndrome. Lancet. 1961;2(7206):788-91.
60. Li H, Cherry S, Klinedinst D, DeLeon V, Redig J, Reshey B, et al. Genetic modifiers predisposing to congenital heart disease in the sensitized Down syndrome population. Circ Cardiovasc Genet. 2012;5(3):301-8.
61. Lana-Elola E, Watson-Scales SD, Fisher EM, Tybulewicz VL. Down syndrome: searching for the genetic culprits. Dis Model Mech. 2011;4(5):586-95.
62. Antonarakis SE. Down syndrome and the complexity of genome dosage imbalance. Nat Rev Genet. 2017;18(3):147-63.
63. Alharbi KM, Al-Mazroea AH, Abdallah AM, Almohammadi Y, Carlus SJ, Basit S. Targeted Next-Generation Sequencing of 406 Genes Identified Genetic Defects Underlying Congenital Heart Disease in Down Syndrome Patients. Pediatr Cardiol. 2018;39(8):1676-80.
64. Barlow GM, Chen XN, Shi ZY, Lyons GE, Kurnit DM, Celle L, et al. Down syndrome congenital heart disease: a narrowed region and a candidate gene. Genet Med. 2001;3(2):91-101.
65. Okur M, Eroz R, Bektas MS, Gulsen S, Bahadir A, Turker Y, et al. THE RELATIONSHIP BETWEEN CONGENITAL HEART DEFECTS AND e-NOS GENE IN DOWN SYNDROME. Genet Couns. 2016;27(3):285-93.
66. Sánchez O, Domínguez C, Ruiz A, Ribera I, Alijotas J, Cabero L, et al. Angiogenic Gene Expression in Down Syndrome Fetal Hearts. Fetal Diagn Ther. 2016;40(1):21-7.

67. Serra-Juhé C, Cuscó I, Homs A, Flores R, Torán N, Pérez-Jurado LA. DNA methylation abnormalities in congenital heart disease. Epigenetics. 2015;10(2):167-77.
68. Bean LJ, Allen EG, Tinker SW, Hollis ND, Locke AE, Druschel C, et al. Lack of maternal folic acid supplementation is associated with heart defects in Down syndrome: a report from the National Down Syndrome Project. Birth Defects Res A Clin Mol Teratol. 2011;91(10):885-93.
69. Torfs CP, Christianson RE. Maternal risk factors and major associated defects in infants with Down syndrome. Epidemiology. 1999;10(3):264-70.
70. Izzo A, Mollo N, Nitti M, Paladino S, Calì G, Genesio R, et al. Mitochondrial dysfunction in down syndrome: molecular mechanisms and therapeutic targets. Mol Med. 2018;24(1):2.
71. Conti A, Fabbrini F, D'Agostino P, Negri R, Greco D, Genesio R, et al. Altered expression of mitochondrial and extracellular matrix genes in the heart of human fetuses with chromosome 21 trisomy. BMC Genomics. 2007;8:268.
72. Bayona-Bafaluy MP, Garrido-Pérez N, Meade P, Iglesias E, Jiménez-Salvador I, Montoya J, et al. Down syndrome is an oxidative phosphorylation disorder. Redox Biol. 2021;41:101871.
73. Smith AM, Levy PT, Franklin O, Molloy E, El-Khuffash A. Pulmonary hypertension and myocardial function in infants and children with Down syndrome. Arch Dis Child. 2020;105(11):1031-4.
74. Rosenzweig EB, Abman SH, Adatia I, Beghetti M, Bonnet D, Haworth S, et al. Paediatric pulmonary arterial hypertension: updates on definition, classification, diagnostics and management. Eur Respir J. 2019;53(1).
75. Steurer MA, Jelliffe-Pawlowski LL, Baer RJ, Partridge JC, Rogers EE, Keller RL. Persistent Pulmonary Hypertension of the Newborn in Late Preterm and Term Infants in California. Pediatrics. 2017;139(1).
76. Cua CL, Blankenship A, North AL, Hayes J, Nelin LD. Increased incidence of idiopathic persistent pulmonary hypertension in Down syndrome neonates. Pediatr Cardiol. 2007;28(4):250-4.
77. Bush D, Galambos C, Ivy DD, Abman SH, Wolter-Warmerdam K, Hickey F. Clinical Characteristics and Risk Factors for Developing Pulmonary Hypertension in Children with Down Syndrome. J Pediatr. 2018;202:212-9.e2.

78. Weijerman ME, van Furth AM, van der Mooren MD, van Weissenbruch MM, Rammeloo L, Broers CJ, et al. Prevalence of congenital heart defects and persistent pulmonary hypertension of the neonate with Down syndrome. Eur J Pediatr. 2010;169(10):1195-9.
79. Fitzgerald P, Leonard H, Pikora TJ, Bourke J, Hammond G. Hospital admissions in children with down syndrome: experience of a population-based cohort followed from birth. PLoS One. 2013;8(8):e70401.
80. Southgate WM, Annibale DJ, Hulsey TC, Purohit DM. International experience with trisomy 21 infants placed on extracorporeal membrane oxygenation. Pediatrics. 2001;107(3):549-52.
81. Cashen K, Thiagarajan RR, Collins JW, Jr., Rycus PT, Backer CL, Reynolds M, et al. Extracorporeal Membrane Oxygenation in Pediatric Trisomy 21: 30 Years of Experience from the Extracorporeal Life Support Organization Registry. J Pediatr. 2015;167(2):403-8.
82. Backes CH, Nicholson L, Rivera BK, Swier N, Marshall W, Cua CL. Extracorporeal Membrane Oxygenation Incidence, Characteristics, and Outcomes in Neonatal Down Syndrome Patients. Asaio j. 2016;62(4):477-81.
83. Bush D, Abman SH, Galambos C. Prominent Intrapulmonary Bronchopulmonary Anastomoses and Abnormal Lung Development in Infants and Children with Down Syndrome. J Pediatr. 2017;180:156-62.e1.
84. Biko DM, Schwartz M, Anupindi SA, Altes TA. Subpleural lung cysts in Down syndrome: prevalence and association with coexisting diagnoses. Pediatr Radiol. 2008;38(3):280-4.
85. Cooney TP, Thurlbeck WM. Pulmonary hypoplasia in Down's syndrome. N Engl J Med. 1982;307(19):1170-3.
86. Galambos C, Minic AD, Bush D, Nguyen D, Dodson B, Seedorf G, et al. Increased Lung Expression of Anti-Angiogenic Factors in Down Syndrome: Potential Role in Abnormal Lung Vascular Growth and the Risk for Pulmonary Hypertension. PLoS One. 2016;11(8):e0159005.
87. Yaylali YT, Kilic-Toprak E, Ozdemir Y, Senol H, Bor-Kucukatay M. Impaired Blood Rheology in Pulmonary Arterial Hypertension. Heart Lung Circ. 2019;28(7):1067-73.
88. Nasser BA, Mesned AR, Mohamad T, Kabbani MS. Incidence and causes of prolonged mechanical ventilation in children with Down syndrome undergoing cardiac surgery. J Saudi Heart Assoc. 2018;30(3):247-53.

89. Iwaya Y, Muneuchi J, Inoue Y, Watanabe M, Okada S, Ochiai Y. Relationship Between Pulmonary Arterial Resistance and Compliance in Patients with Down Syndrome. Pediatr Cardiol. 2019;40(4):841-7.
90. Ploegstra MJ, Ivy DD, Wheeler JG, Brand M, Beghetti M, Rosenzweig EB, et al. Growth in children with pulmonary arterial hypertension: a longitudinal retrospective multiregistry study. Lancet Respir Med. 2016;4(4):281-90.
91. Santoro SL, Chicoine B, Jasien JM, Kim JL, Stephens M, Bulova P, et al. Pneumonia and respiratory infections in Down syndrome: A scoping review of the literature. Am J Med Genet A. 2021;185(1):286-99.
92. Guimaraes CV, Donnelly LF, Shott SR, Amin RS, Kalra M. Relative rather than absolute macroglossia in patients with Down syndrome: implications for treatment of obstructive sleep apnea. Pediatr Radiol. 2008;38(10):1062-7.
93. Diskin C, McVeigh TP, Cox DW. Sleep disordered breathing in children with Down syndrome in the Republic of Ireland. Am J Med Genet A. 2020;182(12):2847-56.
94. Fockens MM, Hölscher M, Limpens J, Dikkers FG. Tracheal anomalies associated with Down syndrome: A systematic review. Pediatr Pulmonol. 2021;56(5):814-22.
95. Siriwardhana LS, Nixon GM, Davey MJ, Mann DL, Landry SA, Edwards BA, et al. Children with down syndrome and sleep disordered breathing display impairments in ventilatory control. Sleep Med. 2021;77:161-9.
96. McDowell KM, Craven DI. Pulmonary complications of Down syndrome during childhood. J Pediatr. 2011;158(2):319-25.
97. O'Driscoll DM, Horne RS, Davey MJ, Hope SA, Anderson V, Trinder J, et al. Cardiac and sympathetic activation are reduced in children with Down syndrome and sleep disordered breathing. Sleep. 2012;35(9):1269-75.
98. Brun H, Holmstrøm H, Thaulow E, Damås JK, Yndestad A, Aukrust P, et al. Patients with pulmonary hypertension related to congenital systemic-to-pulmonary shunts are characterized by inflammation involving endothelial cell activation and platelet-mediated inflammation. Congenit Heart Dis. 2009;4(3):153-9.
99. Yamaki S, Horiuchi T, Sekino Y. Quantitative analysis of pulmonary vascular disease in simple cardiac anomalies with the Down syndrome. Am J Cardiol. 1983;51(9):1502-6.
100. Saji T. Clinical characteristics of pulmonary arterial hypertension associated with Down syndrome. Pediatr Int. 2014;56(3):297-303.

101. Cannon BC, Feltes TF, Fraley JK, Grifka RG, Riddle EM, Kovalchin JP. Nitric oxide in the evaluation of congenital heart disease with pulmonary hypertension: factors related to nitric oxide response. Pediatr Cardiol. 2005;26(5):565-9.
102. Bloemers BL, van Bleek GM, Kimpen JL, Bont L. Distinct abnormalities in the innate immune system of children with Down syndrome. J Pediatr. 2010;156(5):804-9, 9.e1-9.e5.
103. Guazzarotti L, Trabattoni D, Castelletti E, Boldrighini B, Piacentini L, Duca P, et al. T lymphocyte maturation is impaired in healthy young individuals carrying trisomy 21 (Down syndrome). Am J Intellect Dev Disabil. 2009;114(2):100-9.
104. Huggard D, Doherty DG, Molloy EJ. Immune Dysregulation in Children With Down Syndrome. Front Pediatr. 2020;8:73.
105. Chaushu S, Yefenof E, Becker A, Shapira J, Chaushu G. A link between parotid salivary Ig level and recurrent respiratory infections in young Down's syndrome patients. Oral Microbiol Immunol. 2002;17(3):172-6.
106. Jackson A, Maybee J, Moran MK, Wolter-Warmerdam K, Hickey F. Clinical Characteristics of Dysphagia in Children with Down Syndrome. Dysphagia. 2016;31(5):663-71.
107. Alimi A, Taytard J, Abou Taam R, Houdouin V, Forgeron A, Lubrano Lavadera M, et al. Pulmonary hemosiderosis in children with Down syndrome: a national experience. Orphanet J Rare Dis. 2018;13(1):60.
108. Bhattacharya S, Sen S, Levy PT, Rios DR. Comprehensive Evaluation of Right Heart Performance and Pulmonary Hemodynamics in Neonatal Pulmonary Hypertension : Evaluation of cardiopulmonary performance in neonatal pulmonary hypertension. Curr Treat Options Cardiovasc Med. 2019;21(2):10.
109. Yang Q, Rasmussen SA, Friedman JM. Mortality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study. Lancet. 2002;359(9311):1019-25.
110. Miodrag N, Silverberg SE, Urbano RC, Hodapp RM. Deaths among children, adolescents, and young adults with Down syndrome. J Appl Res Intellect Disabil. 2013;26(3):207-14.
111. Weijerman ME, van Furth AM, Vonk Noordegraaf A, van Wouwe JP, Broers CJ, Gemke RJ. Prevalence, neonatal characteristics, and first-year mortality of Down syndrome: a national study. J Pediatr. 2008;152(1):15-9.

112. Cua CL, Haque U, Santoro S, Nicholson L, Backes CH. Differences in mortality characteristics in neonates with Down's syndrome. J Perinatol. 2017;37(4):427-31.
113. Frid C, Drott P, Lundell B, Rasmussen F, Annerén G. Mortality in Down's syndrome in relation to congenital malformations. J Intellect Disabil Res. 1999;43 (Pt 3):234-41.
114. Mertens L, Ganame J, Claus P, Goemans N, Thijs D, Eyskens B, et al. Early regional myocardial dysfunction in young patients with Duchenne muscular dystrophy. J Am Soc Echocardiogr. 2008;21(9):1049-54.
115. Knirsch W, Dodge-Khatami A, Kadner A, Kretschmar O, Steiner J, Böttler P, et al. Assessment of myocardial function in pediatric patients with operated tetralogy of Fallot: preliminary results with 2D strain echocardiography. Pediatr Cardiol. 2008;29(4):718-25.
116. Mertens L, Seri I, Marek J, Arlettaz R, Barker P, McNamara P, et al. Targeted Neonatal Echocardiography in the Neonatal Intensive Care Unit: practice guidelines and recommendations for training. Writing Group of the American Society of Echocardiography (ASE) in collaboration with the European Association of Echocardiography (EAE) and the Association for European Pediatric Cardiologists (AEPC). J Am Soc Echocardiogr. 2011;24(10):1057-78.
117. de Boode WP, Roehr CC, El-Khuffash A. Comprehensive state-of-the-art overview of neonatologist performed echocardiography: Steps towards standardization of the use of echocardiography in neonatal intensive care. Pediatr Res. 2018;84(4):472-3.
118. de Boode WP, Singh Y, Gupta S, Austin T, Bohlin K, Dempsey E, et al. Recommendations for neonatologist performed echocardiography in Europe: Consensus Statement endorsed by European Society for Paediatric Research (ESPR) and European Society for Neonatology (ESN). Pediatr Res. 2016;80(4):465-71.
119. Groves AM, Singh Y, Dempsey E, Molnar Z, Austin T, El-Khuffash A, et al. Introduction to neonatologist-performed echocardiography. Pediatr Res. 2018;84(Suppl 1):1-12.
120. van Laere D, van Overmeire B, Gupta S, El-Khuffash A, Savoia M, McNamara PJ, et al. Application of NPE in the assessment of a patent ductus arteriosus. Pediatr Res. 2018;84(Suppl 1):46-56.
121. Singh Y, Roehr CC, Tissot C, Rogerson S, Gupta S, Bohlin K, et al. Education, training, and accreditation of Neonatologist Performed Echocardiography in Europe-framework for practice. Pediatr Res. 2018;84(Suppl 1):13-7.

122. de Boode WP, Singh Y, Molnar Z, Schubert U, Savoia M, Sehgal A, et al. Application of Neonatologist Performed Echocardiography in the assessment and management of persistent pulmonary hypertension of the newborn. Pediatr Res. 2018;84(Suppl 1):68-77.
123. de Boode WP, van der Lee R, Horsberg Eriksen B, Nestaas E, Dempsey E, Singh Y, et al. The role of Neonatologist Performed Echocardiography in the assessment and management of neonatal shock. Pediatr Res. 2018;84(Suppl 1):57-67.
124. Levy PT, Tissot C, Horsberg Eriksen B, Nestaas E, Rogerson S, McNamara PJ, et al. Application of Neonatologist Performed Echocardiography in the Assessment and Management of Neonatal Heart Failure unrelated to Congenital Heart Disease. Pediatr Res. 2018;84(Suppl 1):78-88.
125. El-Khuffash A. Neonatologist Performed Echocardiography: Teaching Manual. 2019 ed2019.
126. Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, et al. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. Circulation. 2000;102(15):1788-94.
127. Tissot C, Singh Y, Sekarski N. Echocardiographic Evaluation of Ventricular Function-For the Neonatologist and Pediatric Intensivist. Front Pediatr. 2018;6:79.
128. Mondal T, Slorach C, Manlhiot C, Hui W, Kantor PF, McCrindle BW, et al. Prognostic implications of the systolic to diastolic duration ratio in children with idiopathic or familial dilated cardiomyopathy. Circ Cardiovasc Imaging. 2014;7(5):773-80.
129. Levy PT, Holland MR, Sekarski TJ, Hamvas A, Singh GK. Feasibility and reproducibility of systolic right ventricular strain measurement by speckle-tracking echocardiography in premature infants. J Am Soc Echocardiogr. 2013;26(10):1201-13.
130. Levy PT, El Khuffash A, Woo KV, Hauck A, Hamvas A, Singh GK. A Novel Noninvasive Index to Characterize Right Ventricle Pulmonary Arterial Vascular Coupling in Children. JACC Cardiovasc Imaging. 2019;12(4):761-3.
131. Bussmann N, El-Khuffash A, Breatnach CR, McCallion N, Franklin O, Singh GK, et al. Left ventricular diastolic function influences right ventricular - Pulmonary vascular coupling in premature infants. Early Hum Dev. 2019;128:35-40.

132. Jone PN, Schäfer M, Pan Z, Ivy DD. Right Ventricular-Arterial Coupling Ratio Derived From 3-Dimensional Echocardiography Predicts Outcomes in Pediatric Pulmonary Hypertension. Circ Cardiovasc Imaging. 2019;12(1):e008176.
133. Habash S, Laser KT, Moosmann J, Reif R, Adler W, Glöckler M, et al. Normal values of the pulmonary artery acceleration time (PAAT) and the right ventricular ejection time (RVET) in children and adolescents and the impact of the PAAT/RVET-index in the assessment of pulmonary hypertension. Int J Cardiovasc Imaging. 2019;35(2):295-306.
134. Jain A, El-Khuffash AF, van Herpen CH, Resende MHF, Giesinger RE, Weisz D, et al. Cardiac Function and Ventricular Interactions in Persistent Pulmonary Hypertension of the Newborn. Pediatr Crit Care Med. 2021;22(2):e145-e57.
135. Abraham S, Weismann CG. Left Ventricular End-Systolic Eccentricity Index for Assessment of Pulmonary Hypertension in Infants. Echocardiography. 2016;33(6):910-5.
136. Bush D, Galambos C, Dunbar Ivy D. Pulmonary hypertension in children with Down syndrome. Pediatr Pulmonol. 2021;56(3):621-9.
137. Smith A, Bussmann N, Levy P, Franklin O, McCallion N, El-Khuffash A. Comparison of left ventricular rotational mechanics between term and extremely premature infants over the first week of age. Open Heart. 2021;8(1).
138. Smith A, Franklin O, McCallion N, Breatnach F, El-Khuffash A. Effect of Gestational Diabetes Mellitus on Neonatal Myocardial Function. Neonatology. 2021;118(1):64-72.
139. Kelly A, Gidding SS, Walega R, Cochrane C, Clauss S, Townsend RR, et al. Relationships of Body Composition to Cardiac Structure and Function in Adolescents With Down Syndrome are Different than in Adolescents Without Down Syndrome. Pediatr Cardiol. 2019;40(2):421-30.
140. Koestenberger M, Sallmon H, Avian A, Cantinotti M, Gamillscheg A, Kurath-Koller S, et al. Ventricular-ventricular interaction variables correlate with surrogate variables of clinical outcome in children with pulmonary hypertension. Pulm Circ. 2019;9(2):2045894019854074.
141. Haeck ML, Höke U, Marsan NA, Holman ER, Wolterbeek R, Bax JJ, et al. Impact of right ventricular dyssynchrony on left ventricular performance in patients with pulmonary hypertension. Int J Cardiovasc Imaging. 2014;30(4):713-20.

142. Hardegree EL, Sachdev A, Fenstad ER, Villarraga HR, Frantz RP, McGoon MD, et al. Impaired left ventricular mechanics in pulmonary arterial hypertension: identification of a cohort at high risk. Circ Heart Fail. 2013;6(4):748-55.
143. Burkett DA, Slorach C, Patel SS, Redington AN, Ivy DD, Mertens L, et al. Left Ventricular Myocardial Function in Children With Pulmonary Hypertension: Relation to Right Ventricular Performance and Hemodynamics. Circ Cardiovasc Imaging. 2015;8(8).
144. Zemel BS, Pipan M, Stallings VA, Hall W, Schadt K, Freedman DS, et al. Growth Charts for Children With Down Syndrome in the United States. Pediatrics. 2015;136(5):e1204-11.
145. Motoji Y, Tanaka H, Fukuda Y, Sano H, Ryo K, Imanishi J, et al. Interdependence of right ventricular systolic function and left ventricular filling and its association with outcome for patients with pulmonary hypertension. Int J Cardiovasc Imaging. 2015;31(4):691-8.
146. Ramani GV, Bazaz R, Edelman K, López-Candales A. Pulmonary hypertension affects left ventricular basal twist: a novel use for speckle-tracking imaging. Echocardiography. 2009;26(1):44-51.
147. Toscano E, Pacileo G, Limongelli G, Verrengia M, Di Mita O, Di Maio S, et al. Subclinical hypothyroidism and Down's syndrome; studies on myocardial structure and function. Arch Dis Child. 2003;88(11):1005-8.
148. Dias FM, Cordeiro S, Menezes I, Nogueira G, Teixeira A, Marques M, et al. [Congenital Heart Disease in Children with Down Syndrome: What Has Changed in the Last Three Decades?]. Acta Med Port. 2016;29(10):613-20.
149. de Graaf G, Buckley F, Skotko BG. Estimation of the number of people with Down syndrome in Europe. Eur J Hum Genet. 2021;29(3):402-10.
150. Hartley D, Blumenthal T, Carrillo M, DiPaolo G, Esralew L, Gardiner K, et al. Down syndrome and Alzheimer's disease: Common pathways, common goals. Alzheimers Dement. 2015;11(6):700-9.
151. Duffy V, Gomez D, Rycus P, Rivera B, Santoro SL, Backes CH, et al. Extracorporeal Membrane Oxygenation Characteristics and Outcomes in Adult Patients With Down Syndrome. Artif Organs. 2018;42(9):921-5.
152. De Cauwer H, Spaepen A. Are patients with Down syndrome vulnerable to life-threatening COVID-19? Acta Neurol Belg. 2020:1-3.

153. Hüls A, Costa ACS, Dierssen M, Baksh RA, Bargagna S, Baumer NT, et al. Medical vulnerability of individuals with Down syndrome to severe COVID-19-data from the Trisomy 21 Research Society and the UK ISARIC4C survey. EClinicalMedicine. 2021;33:100769.
154. Krishnan US, Krishnan SS, Jain S, Chavolla-Calderon MB, Lewis M, Chung WK, et al. SARS-CoV-2 Infection in Patients with Down Syndrome, Congenital Heart Disease, and Pulmonary Hypertension: Is Down Syndrome a Risk Factor? J Pediatr. 2020;225:246-8.
155. Vita S, Di Bari V, Corpolongo A, Goletti D, Espinosa J, Petracca S, et al. Down Syndrome patients with COVID-19 pneumonia: A high-risk category for unfavourable outcome. Int J Infect Dis. 2021;103:607-10.
156. Newman AM, Jhaveri R, Patel AB, Tan TQ, Toia JM, Arshad M. Trisomy 21 and Coronavirus Disease 2019 in Pediatric Patients. J Pediatr. 2021;228:294-6.
157. Emami A, Javanmardi F, Akbari A, Asadi-Pooya AA. COVID-19 in patients with Down syndrome. Neurol Sci. 2021;42(5):1649-52.
158. Malle L, Gao C, Hur C, Truong HQ, Bouvier NM, Percha B, et al. Individuals with Down syndrome hospitalized with COVID-19 have more severe disease. Genet Med. 2021;23(3):576-80.
159. Pierpont ME, Brueckner M, Chung WK, Garg V, Lacro RV, McGuire AL, et al. Genetic Basis for Congenital Heart Disease: Revisited: A Scientific Statement From the American Heart Association. Circulation. 2018;138(21):e653-e711.
160. Wu H, Kao SC, Barrientos T, Baldwin SH, Olson EN, Crabtree GR, et al. Down syndrome critical region-1 is a transcriptional target of nuclear factor of activated T cells-c1 within the endocardium during heart development. J Biol Chem. 2007;282(42):30673-9.
161. Quiñones-Lombraña A, Blanco JG. Comparative analysis of the DYRK1A-SRSF6-TNNT2 pathway in myocardial tissue from individuals with and without Down syndrome. Exp Mol Pathol. 2019;110:104268.
162. Cejas RB, Wang J, Hageman-Blair R, Liu S, Blanco JG. Comparative genome-wide DNA methylation analysis in myocardial tissue from donors with and without Down syndrome. Gene. 2021;764:145099.
163. Laufer BI, Hwang H, Vogel Ciernia A, Mordaunt CE, LaSalle JM. Whole genome bisulfite sequencing of Down syndrome brain reveals regional DNA hypermethylation and novel disorder insights. Epigenetics. 2019;14(7):672-84.

164. Haarman MG, Kerstjens-Frederikse WS, Vissia-Kazemier TR, Breeman KTN, Timens W, Vos YJ, et al. The Genetic Epidemiology of Pediatric Pulmonary Arterial Hypertension. J Pediatr. 2020;225:65-73.e5.

165. Salvolini E, Orciani M, Lucarini G, Vignini A, Tranquilli AL, Di Primio R. VEGF and nitric oxide synthase immunoexpression in Down's syndrome amniotic fluid stem cells. Eur J Clin Invest. 2011;41(1):23-9.

166. Colvin KL, Yeager ME. What people with Down Syndrome can teach us about cardiopulmonary disease. Eur Respir Rev. 2017;26(143).

167. Colvin KL, Yeager ME. Proteomics of pulmonary hypertension: could personalized profiles lead to personalized medicine? Proteomics Clin Appl. 2015;9(1-2):111-20.

168. Bush D, Wolter-Warmerdam K, Wagner BD, Galambos C, Ivy DD, Abman S, et al. EXPRESS: Angiogenic Profile Identifies Pulmonary Hypertension in Children with Down Syndrome. Pulm Circ. 2019;9(3):2045894019866549.

169. Fukushima H, Kosaki K, Sato R, Yagihashi T, Gatayama R, Kodo K, et al. Mechanisms underlying early development of pulmonary vascular obstructive disease in Down syndrome: An imbalance in biosynthesis of thromboxane A2 and prostacyclin. Am J Med Genet A. 2010;152a(8):1919-24.

170. Recalde AL, Landing BH, Lipsey AI. Increased cardiac muscle fiber size and reduced cell number in Down syndrome: heart muscle cell number in Down syndrome. Pediatr Pathol. 1986;6(1):47-53.

171. Cisterna B, Costanzo M, Scherini E, Zancanaro C, Malatesta M. Ultrastructural features of skeletal muscle in adult and aging Ts65Dn mice, a murine model of Down syndrome. Muscles Ligaments Tendons J. 2013;3(4):287-94.

172. Mubarak KK. A review of prostaglandin analogs in the management of patients with pulmonary arterial hypertension. Respir Med. 2010;104(1):9-21.

173. Olschewski H, Simonneau G, Galiè N, Higenbottam T, Naeije R, Rubin LJ, et al. Inhaled iloprost for severe pulmonary hypertension. N Engl J Med. 2002;347(5):322-9.

174. Hill NS, Preston IR, Roberts KE. Inhaled Therapies for Pulmonary Hypertension. Respir Care. 2015;60(6):794-802; discussion -5.

175. De Bie FR, Allegaert K, Hedrick HL, Rintoul NE, Davidson A. Treprostinil Attains Clinically Therapeutic Concentrations in Neonates with Pulmonary Hypertension on Extracorporeal Membrane Oxygenation Support. Pharmacotherapy. 2020;40(10):1054-60.

176. Crepaz R, Romeo C, Montanaro D, De Santis S. Long-term results of treatment with bosentan in adult Eisenmenger's syndrome patients with Down's syndrome related to congenital heart disease. BMC Cardiovasc Disord. 2013;13:74.
177. D'Alto M, Romeo E, Argiento P, D'Andrea A, Sarubbi B, Correra A, et al. Therapy for pulmonary arterial hypertension due to congenital heart disease and Down's syndrome. Int J Cardiol. 2013;164(3):323-6.
178. Kamran A, Rafiq N, Khalid A, Amin F, Kumari V, Shaikh AS, et al. Effectiveness of oral sildenafil for neonates with persistent pulmonary hypertension of newborn (PPHN): a prospective study in a tertiary care hospital. J Matern Fetal Neonatal Med. 2021:1-7.
179. Beghetti M, Rudzinski A, Zhang M. Efficacy and safety of oral sildenafil in children with Down syndrome and pulmonary hypertension. BMC Cardiovasc Disord. 2017;17(1):177.
180. Koo R, Lo J, Bock MJ. Transition from intravenous treprostinil to enteral selexipag in an infant with pulmonary arterial hypertension. Cardiol Young. 2019;29(6):849-51.
181. Blissett S, Blusztein D, Mahadevan VS. Contemporary use of Selexipag in pulmonary arterial hypertension associated with congenital heart disease: a case series. Eur Heart J Case Rep. 2020;4(6):1-7.
182. Barnes H, Yeoh HL, Fothergill T, Burns A, Humbert M, Williams T. Prostacyclin for pulmonary arterial hypertension. Cochrane Database Syst Rev. 2019;5(5):Cd012785.
183. Figueroa EG, Gonzaléz-Candia A, Villanueva CA, Ebensperger G, Reyes RV, Llanos AJ, et al. Beneficial effects of melatonin on prostanoids pathways in pulmonary hypertensive neonates. Vascul Pharmacol. 2021:106853.
184. Gough G, O'Brien NL, Alic I, Goh PA, Yeap YJ, Groet J, et al. Modeling Down syndrome in cells: From stem cells to organoids. Prog Brain Res. 2020;251:55-90.
185. Rondal JA. From the lab to the people: major challenges in the biological treatment of Down syndrome. AIMS Neurosci. 2021;8(2):284-94.
186. Hawkins A, Langton-Hewer S, Henderson J, Tulloh RM. Management of pulmonary hypertension in Down syndrome. Eur J Pediatr. 2011;170(7):915-21.
187. Valentini D, Di Camillo C, Mirante N, Vallogini G, Olivini N, Baban A, et al. Medical conditions of children and young people with Down syndrome. J Intellect Disabil Res. 2021;65(2):199-209.
188. Tsou AY, Bulova P, Capone G, Chicoine B, Gelaro B, Harville TO, et al. Medical Care of Adults With Down Syndrome: A Clinical Guideline. Jama. 2020;324(15):1543-56.

189. Santoro SL, Campbell A, Balasubramanian A, Haugen K, Schafer K, Mobley W. Specialty clinics for adults with Down syndrome: A clinic survey. Am J Med Genet A. 2021.
190. Lagan N, Huggard D, Mc Grane F, Leahy TR, Franklin O, Roche E, et al. Multiorgan involvement and management in children with Down syndrome. Acta Paediatr. 2020;109(6):1096-111.
191. Lagan NC, Mc Grane F, Huggard D, Sharkey J, Purcell C, Balfe J, et al. Implementation of a health surveillance clinic for children with Down syndrome. Arch Dis Child Educ Pract Ed. 2021;106(1):60-2.
192. Gandy KC, Castillo HA, Ouellette L, Castillo J, Lupo PJ, Jacola LM, et al. The relationship between chronic health conditions and cognitive deficits in children, adolescents, and young adults with down syndrome: A systematic review. PLoS One. 2020;15(9):e0239040.
193. Clapp S, Perry BL, Farooki ZQ, Jackson WL, Karpawich PP, Hakimi M, et al. Down's syndrome, complete atrioventricular canal, and pulmonary vascular obstructive disease. J Thorac Cardiovasc Surg. 1990;100(1):115-21.
194. Alhuzaimi AN, Alotaibi NM, Alsuhaibani GI, Alanazi RK, Temsah MH. Congenital Heart Defect and Pulmonary Hypertension in Children With Down Syndrome: Clinical Profile Over Two Decades. Cureus. 2021;13(2):e13212.
195. Breatnach CR, El-Khuffash A, James A, McCallion N, Franklin O. Serial measures of cardiac performance using tissue Doppler imaging velocity in preterm infants <29weeks gestations. Early Hum Dev. 2017;108:33-9.
196. Roche E, Hoey H, Murphy J. Medical Management of Children & Adolescents with Down Syndrome in Ireland. Down's Syndrome Medical Interest Group UK & Ireland; 2015.
197. Adams AD, Hoffmann V, Koehly L, Guedj F, Bianchi DW. Novel Insights from Fetal and Placental Phenotyping in Three Mouse Models of Down Syndrome. Am J Obstet Gynecol. 2021.
198. Antonarakis SE, Skotko BG, Rafii MS, Strydom A, Pape SE, Bianchi DW, et al. Down syndrome. Nat Rev Dis Primers. 2020;6(1):9.
199. Shivanna B, Gowda S, Welty SE, Barrington KJ, Pammi M. Prostanoids and their analogues for the treatment of pulmonary hypertension in neonates. Cochrane Database Syst Rev. 2019;10(10):Cd012963.

Appendices

Publications

1. **Smith AM**, Levy PT, Franklin O, Molloy E, El-Khuffash A. Pulmonary hypertension and myocardial function in infants and children with Down syndrome. Arch Dis Child. 2020;105(11):1031-4.
2. **Smith A**, Molloy E, Miletin J, Curley A, Balfe J, Franklin and EL-Khuffash A. Longitudinal assessment of cardiac function in infants with Down's syndrome using novel echocardiography techniques – project protocol. Health Research Board Open Research. 2020.

International Presentations

1. **Smith A**, Levy P, Molloy E, McCallion N, Franklin O and EL-Khuffash A (2021) *Left Ventricular Diastolic Dysfunction is associated with Pulmonary Hypertension in Infants with Down Syndrome.* Paediatric Academic Societies Meeting [Oral Presentation – Virtual Meeting]

Research Awards

1. Health Research Board (HRB) Ones to Watch Prize Winner, 2019

Funding Awards

Award	**Funding Body**	**Project**	**Research Supervisor**	**Role**	**Date**	**Amount**
Research Grant	European Society for Paediatric Research (ESPR) Young Investigator START-UP Award 2019	The Impact of Blood Transfusion on the Haemodynamics of Preterm Infants with and Without a Patent Ductus Arteriosus	Prof. Afif EL-Khuffash	PhD Student	September 2019	€7,000
Research Grant	Health Research Board/National Children's Hospital Foundation	Longitudinal Assessment of Cardiac Function in Infants with Down Syndrome using Novel Echocardiography Techniques	Prof. Afif EL-Khuffash	PhD Student	June 2017	€298,381
Research Education Support Grant, October 2017 – July 2021	National Children's Research Centre, Our Lady's Children's Hospital, Crumlin, Dublin,	Longitudinal Assessment of Cardiac Function in Infants with Down Syndrome using Novel Echocardiography Techniques	Prof. Afif EL-Khuffash	PhD Student	October 2017	€20,970