THE DIASTOLIC INFLOW AND LONGITUDINAL MOVEMENT OF THE HEART IN THE AFRICAN FULL-TERM NEWBORN INFANT.

Submitted in fulfilment of the requirements of the degree of Masters of Health Sciences (Clinical Technology)
In the Department of Clinical and Biomedical Technology
Faculty of Health Sciences at the Durban University of Technology

Bongiwe Jessie Beckerling

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Supervisor: Dr. R Prakaschandra Date: 13 September 2019

Co-Supervisor: Prof A Cilliers Date 13 September 2019

ABSTRACT

Introduction: Echocardiography has been used mainly to assess the contractility of the left where do you find the staff that ventricle (LV) and its systolic function. The diastolic component of the cardiac cycle is an important component of cardiac output but has been under-explored, especially in neonates.

Methodology: The aim of this study was to determine normal echocardiographic references for diastolic inflow and longitudinal movement of both the left and right heart in healthy full-term black African neonates. A descriptive, bidirectional study design was employed. Healthy African (Black) full-term infants who met inclusion criteria were recruited at the Chris Hani Baragwanath Academic Hospital. The study consisted of 2 series: a retrospective post processing of data and a prospective data collection. Left and right ventricular (RV) systolic and diastolic function were assessed using multiple echocardiographic methods Myocardial performance indexes of the RV and LV were calculated using mitral and tricuspid valve inflow patterns, and the aortic valve outflow Doppler envelope in the case of the LV myocardial performance index (MPI) and the pulmonary valve outflow measurement in the case of RV MPI. Statistical analysis was performed using Excel and Statistica version 13.1. Normal ranges were calculated using means ± standard deviations.

Results: Two hundred and ninety-two neonates (142 males, 152 females; median gestational age 39 weeks, range 37-42 weeks) were included in the study. Most of the subjects (175/292;60%) were born by caesarean section. Median body surface area was 0.20 m^2 (range, 0.16-0.25m^2). Median weight was 3.12 kg (range, 2.5 kg 4.43kg). Median post-delivery age at echocardiography was 31 hours (range, 12-216 hours).The following measurements (means ± SD) were observed: LVEF and LVFS were 73.56% (±8.93) and 40.34% (±7.91) respectively. Mitral valve (MV) peak E= 0.58 m/s (±0.113), MV peak A = 0.59 m/s (±0.123), MV peak E/A ratio = 1.01 (±0.21), MV E' = 0.058 m/s (±0.012), MV E/E' ratio = 10.38 (±2.65), MV S' = 0.052 m/s (±0.009) and LV Tei = 0.306 (±0.139). Right ventricular (RV) function measurements were: TAPSE = 7.51mm (±1.304), tricuspid valve (TV) peak E = 0.512 m/s (±0.126), TV peak A = 0.616 m/s (±0.127), TV E/A = 0.845 (±0.199), TV E' = 0.079 m/s (±0.021), TV E/E' ratio = 6.78 (±2.02), TV S' = 0.071 m/s (±0.045) and RV Tei = 0.283 (±0.132).

Conclusion: This large study established normal reference values for diastolic function and longitudinal systolic and diastolic movement of the heart in healthy full-term African neonates on echocardiography.

AUTHOR'S DECLARATION

This study represents original work by the author. It has not been submitted to any other Tertiary Institution. Where use of the work of others were made, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Department of Biomedical and Clinical Technology, Faculty of Health Sciences, Durban University of Technology under the supervision of Dr R Prakaschandra (Head of the Clinical Technology programme) and the Department of Paediatric Cardiology, Chris Hani Baragwanath Academic Hospital (CHBAH), Johannesburg,
South Africa
under the supervision of Prof, A Cilliers (Head of Paediatric Cardiology in Chris Hani Baragwanath Academic Hospital) and Prof H Ntsinjana (Head of Paediatric Cardiology in Nelson Mandela Children's Hospital)

SIGNED

Mrs BJ Beckerling
B. TECH: Clinical Technology
I hereby certify that the above statement is correct.

SIGNED:

Dr. R Prakaschandra
PhD in (Cardiology)

SIGNED:

Prof. A Cilliers
MBBCh, DCH, FCPaeds (SA)

SIGNED:

Prof. H Ntsinjana

MBBCH, FCPaed (SA), PhD

DEDICATION

I dedicate this work to:

My family, my son, Nathan Beckerling, my husband, Carl Beckerling who has been my pillar of strength, his constant love, guidance, support and encouragement and my Mother who never stops praying for me.

ACKNOWLEDGEMENTS

This very long journey I chose to embark would have not been possible without the never-ending support and assistance of the following people.
Thus, I would like to take this opportunity to pass my genuine appreciation
To my loving God, I would not be here today if it were not for your will.

To my family, my dear husband: I would like to thank you from the bottom of my heart. You have been my strength through it all, you gave me hope when I thought this was never going to end.

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LIST OF ABBREVIATIONS

2D	Two dimensional
AS	Aortic stenosis
AVSD	Atrioventricular septal defects
BL	Birth length
BSA	Body surface area
BW	Birthweight
C/S	Caesarean section
CHBAH	Chris Hani Baragwanath Academic Hospital
CHD	Congenital Heart Defects
C-MRI	Cardiac magnetic resonance imaging
CO2	Carbon dioxide
CT	Cardiac computerized tomography
DA	Ductus arteriosus
DV	Ductus venosus
EF	Ejection fraction
ET	Ejection time
F	Females
FO	Foramen ovale
FS	Fractional shortening
GA	Gestational age
HCU	Hand-carried ultrasound
ICC	Intraclass correlation coefficient
IVC	Inferior vena cava
IVC	Inferior vena cava
IVCT	Isovolumetric contraction time

IVRT	Isovolumetric relaxation time
LA	Left atrium
LV LVOT	Left ventricle Left ventricular outflow tract
M	Males
MOD	Mode of delivery
MPI	Myocardial performance index
MV	Mitral valve
NSAIDS	Nonsteroidal Anti-Inflammatory Agents
NVD	Normal vertex delivery
PDA	Patent ductus arteriosus
PFO	Patent foramen ovale
PO2	Partial pressure of oxygen
PVR	Pulmonary vascular resistance
RA	Right atrium
RV	Right Ventricle
SD	Standard deviation
SSRIs	Selective serotonin reuptake inhibitors
SVC	Superior vena cava
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler imaging
TTE	Transthoracic echocardiography
TV	Tricuspid valve
UV	Umbilical Vein
PLAX	Parasternal long axis
PSAX	Parasternal short axis

LIST OF APPENDICES

CHAPTER ONE: INTRODUCTION

Explanations of the growing cardiovascular system originated centuries ago when Aristotle first explained the beating heart in a chicken egg (Lai et al. 2006) The understanding of the cardiovascular circulation in the modern era stems from the work done by William Harvey in the 17th Century (Lai et al. 2006).

Measurement techniques of cardiovascular function were established in the 19th century followed by the development of skills to treat heart defects which had its origins in the mid-20th century. More precise methods of diagnosis followed (Lai et al. 2006).

Accurate diagnosis of congenital heart disease (CHD) using echocardiography and their outcomes depends on establishing references of normal values of cardiac and vascular dimensions (Lai et al. 2006).

Transducers in the current era have allowed for a better anatomic definition of pathology in neonates which is equivalent to and in some instances better than angiography (Williams 1985). Echocardiography has become a cost-effective approach in detecting cardiac abnormalities in neonates with CHD. It provides a comprehensive and conclusive anatomic diagnosis, in some circumstances eliminating the requirement for additional procedures such as cardiac catheterization (Williams 1985).

Measurement of systolic function and diastolic function have become increasingly important in the assessment of myocardial function in all age groups and can be undertaken using two-dimensional (2D) echocardiography (Solinger, Elble and Minhas 1973). However, there is little information about the neonatal heart and even less information regarding the African newborn infant.

Up to now the focus has been on contractility of the LV systolic function. It has become apparent that adequate filling of the heart is an important component of the eventual cardiac output but has been a very under-explored component of myocardial function (Panesar and Burch 2017). However, the assessment of ventricular diastolic function is of great clinical importance, particularly in the paediatric population since it assists in differentiating diastolic dysfunction from other underlying cardiac pathology so that optimal clinical treatment can be instituted.

Approximately thirty-three published studies evaluating diastolic function in normal children have been published to date (Cantinotti and Lopez 2013), but several of these studies are

hampered by a small sample size with few neonatal subjects. Various statistical methodologies were used to present normal measurements including z scores, percentiles, and mean values according to age and body size (Cantinotti and Lopez 2013).

Some of the studies do show reproducible patterns in diastolic function in older children, but these patterns cannot be extrapolated to neonates who have much smaller cardiac chambers, a faster heart rate and a rapidly changing physiological pattern in the early neonatal period (Cantinotti and Lopez 2013). The lack of diastolic function data in the healthy neonate offers an opportunity to establish normal values that can be used in the evaluation of myocardial function of neonates with heart disease, particularly in the African population.

The importance of this study lies in the fact that a number of studies have established that ethnicity is a significant determinant of cardiac chamber sizes and hence, it is highly recommended to use ethnic-specific reference values for echocardiographic interpretations (The EchoNoRMAL study 2015; Lang et al. 2015; Badano 2014; Bansal, Mohan and Sengupta 2016 and Majonga et al. 2017).

The consequences of not accounting for these ethnic differences may result in serious repercussions since numerous vital therapeutic decisions hinge entirely on precise assessment of cardiac chamber size and function (Yancy et al. 2013 and Bansal, Mohan and Sengupta 2016).

Majonga et al. (Majonga et al 2017) findings suggested that differences in reference ranges amongst the different racial groups do exist but may remain unnoticed because of scarcity of data e.g. African children. The use of inappropriate reference ranges may result in the misdiagnosis of cardiac abnormalities (Badano 2014). According to the researcher's knowledge, there is no research that has been done thus far to assess systolic and diastolic function parameters in newborn infants from an African tertiary care center and further, no studies have been done to compare the findings to published studies with other ethnic groups. This study is therefore the first to report normal values for systolic and diastolic function in healthy black African neonates.

CHAPTER TWO: LITERATURE REVIEW

2.1 History of Paediatric Cardiology and the CHBAH Paediatric Cardiology Service

Helen Brooke Taussig (1898–1986) is regarded as the mother of paediatric cardiology and played a leading role in establishing the specialty. Her thoughts and views were chronicled in her book titled "Congenital Malformations of the Heart" published in 1947, two years after the successful surgical palliation of the "blue baby", also known as Tetralogy of Fallot. The surgical procedure in this groundbreaking development in the treatment of these patients was conceptualized in collaboration with Dr Alfred Blalock (a surgeon) and Mr. Vivien Thomas (a laboratory technician) (Engle 2005).

Internationally, the field of Paediatric Cardiology came into its own in 1967 after the establishment of the Association of European Paediatric Cardiology with 57 members from Europe and 17 from the United States. The first Paediatric Cardiology World Congress was held in London in 1980, (Engle 2005).

The South African connection with Paediatric Cardiology began in 1962, when Prof William Sinclair Winship established the first Paediatric Cardiology and Birth Defects Clinic at King Edward VIII Hospital in Durban, which was the first Paediatric Cardiac Service in Kwa Zulu Natal. He also served as a corresponding member for South Africa of the Association of European Paediatric Cardiologists for 15 years, (Anon 2011).

Cardiac Surgery in Gauteng was established at the Chris Hani Baragwanath Academic Hospital (CHBAH) in the 1970s to treat the scourge of rampant and severe Rheumatic Heart Disease. The unit was discontinued and transferred to the JG Strydom Hospital in the 1980s. Paediatric Cardiology was not an independent entity at CHBAH until the early 1990's when Dr Jan du Plessis joined the Department of Paediatrics and undertook to serve the patients on a full-time basis. Prior to this, patients were being assessed by Private Paediatric Cardiologists at a cardiac clinic held once a week.

The Cardiology department is currently run by Prof A Cilliers and 11 Paediatric Cardiologist have trained and completed at CHBAH to date. The department sees 1700 inpatients and

outpatients per year, 2700 cardiac clinic patients per year and does approximately 3400 echocardiography studies per year.

2.2 The Human foetal circulatory system

The neonatal circulation (fig 1) differs from the foetal circulation (fig 2), mainly because the lungs do not play a role in oxygenation. The placenta plays a vital role in ensuring the adequate oxygenation of the foetus. There are also a number of intrauterine shunts which assist in deviating most of the blood away from the lungs (Morton and Brodsky 2016). Oxygen from the richly oxygenated blood of the maternal uterine artery is exchanged across a concentration gradient to the umbilical vein which has the oxygen saturations of between 70% and 80%. A portion of the blood in the umbilical vein perfuses the hepatic circulation while some of the blood will enter the ductus venosus (DV) which enters the inferior vena cava (IVC) (Morton and Brodsky 2016).

The Eustachian valve which lies between the IVC and right atrium (RA) is designed to redirect oxygenated blood through the foramen ovale (FO) into the left atrium (LA) which is followed by entry of blood into the LV which circulates the blood to the coronary arteries and vital organs including the brain (Murphy 2005).

Blood which is poor in oxygen (SvO2 25-40%) derived mainly from the superior vena cava (SVC), coronary sinus and lower extremities passes through the tricuspid valve (TV) into the right ventricle (RV) followed by the pulmonary artery (PA). Only 12% of blood from the RV enters the pulmonary circulation due to the high pulmonary vascular resistance (PVR) while 88% crosses into the ductus arteriosus (DA) and straight into the descending aorta. The high PVR results from the lungs that are collapsed in utero and where there is a low resting oxygen tension (Murphy 2005).

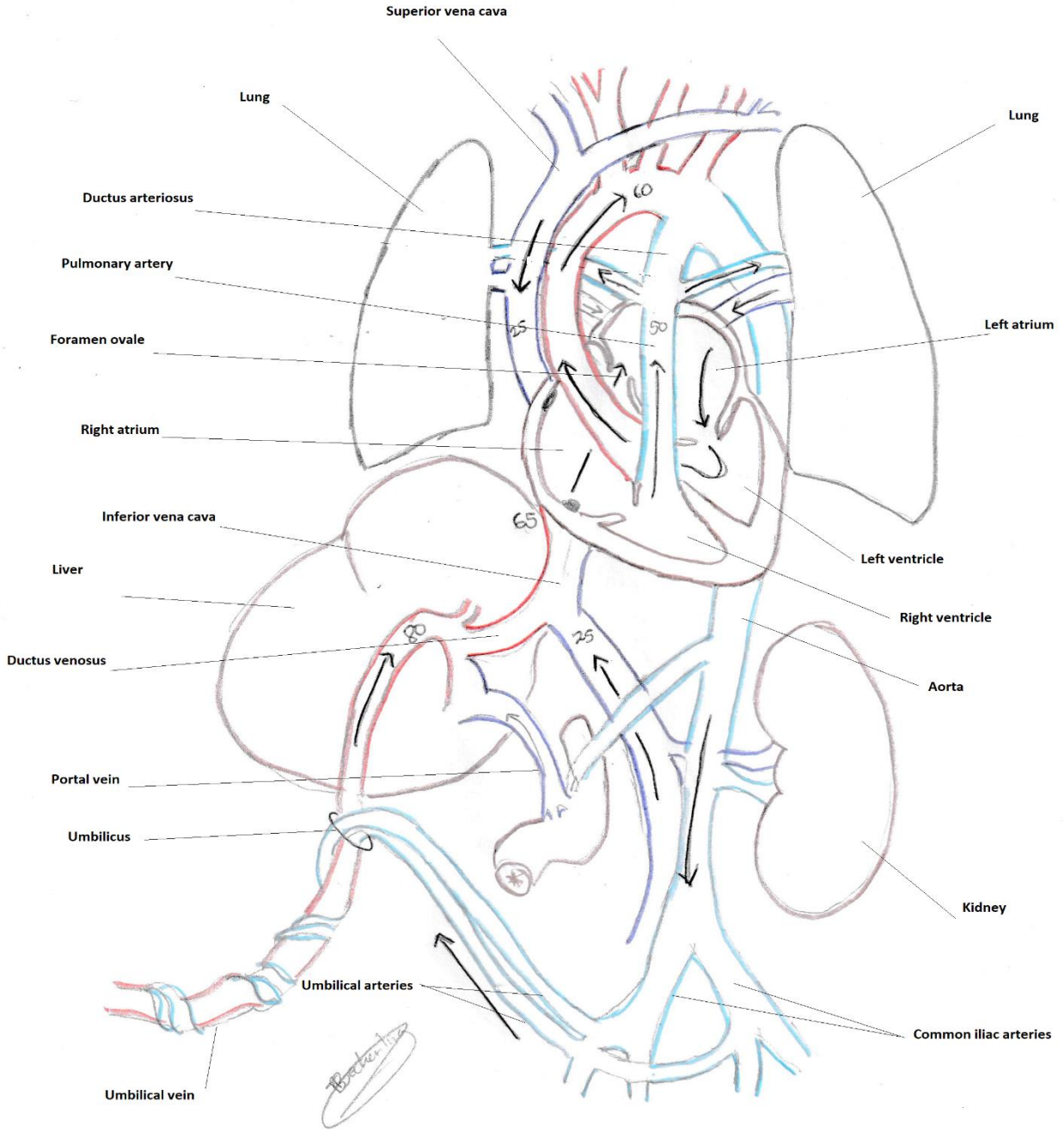


Figure 2. 1 The foetal circulation

2.2.1 The normal transition from foetal to neonatal circulation

The transition from foetal to neonatal circulation is intricate and is accompanied by numerous physiological changes in the cardiopulmonary system immediately after birth. Gas exchange is transferred from the placenta to the lungs, foetal circulatory shunts must close and left ventricular output needs to increase. When a baby is born there are numerous factors involved in the termination of placental circulation (Singh and Tissot 2018; Moss and Adams 2013; Murphy 2005).

The umbilical vessels constrict in response to longitudinal stretch and the increase in partial pressure of oxygen (PO2). The external clamping of the umbilical cord enhances this process. There is usually a significant decrease in the flow through the ductus venosus and venous return through the IVC after the placental circulation has been removed. The ductus venosus closes passively within 3 to 10 days after birth (Singh and Tissot 2018; Moss and Adams 2013; Murphy 2005).

Soon after birth, and immediately after the expansion of the lungs, there is a drastic drop in PVR and an 8-10-fold increase in pulmonary blood flow. This physiological change has also been demonstrated in foetal lambs when mechanical expansion of lungs with non-oxygenated gas results in a huge fall in PVR. Apart from lung expansion, opening of pulmonary vasculature may also contribute to a drop in PVR (Singh and Tissot 2018; Moss and Adams 2013; Murphy 2005). A further drop in PVR occurs after the first month of life.

The mechanism of reduction in PVR is believed to be facilitated in part, by the stimulation of the pulmonary stretch receptors which results in reflex vasodilation. In addition, improved oxygenation of the neonatal blood reverses the pulmonary vasoconstriction caused by hypoxia. The rise in pulmonary blood flow results in a huge rise in pulmonary venous return to the LA (Singh and Tissot 2018; Moss and Adams 2013; Murphy 2005).

The reduction in the IVC flow, as described above, results in a drop in venous return to the RA. These two factors allow the pressures in the LA and RA to equalize. This in turn results in the flap of the foramen ovale (FO) being pushed against the atrial septum and the atrial shunt is then effectively closed. This process occurs in the first minutes to hours after birth. The anatomical closure occurs later via tissue proliferation and adherence of the septum primum to the septum secundum. Once the drop in PVR occurs, the shunting across the DA becomes bi-

directional. The specific ductal closure mechanism is not really known but it is known that the increase in PO2 in neonatal blood causes smooth muscle constriction within the duct.

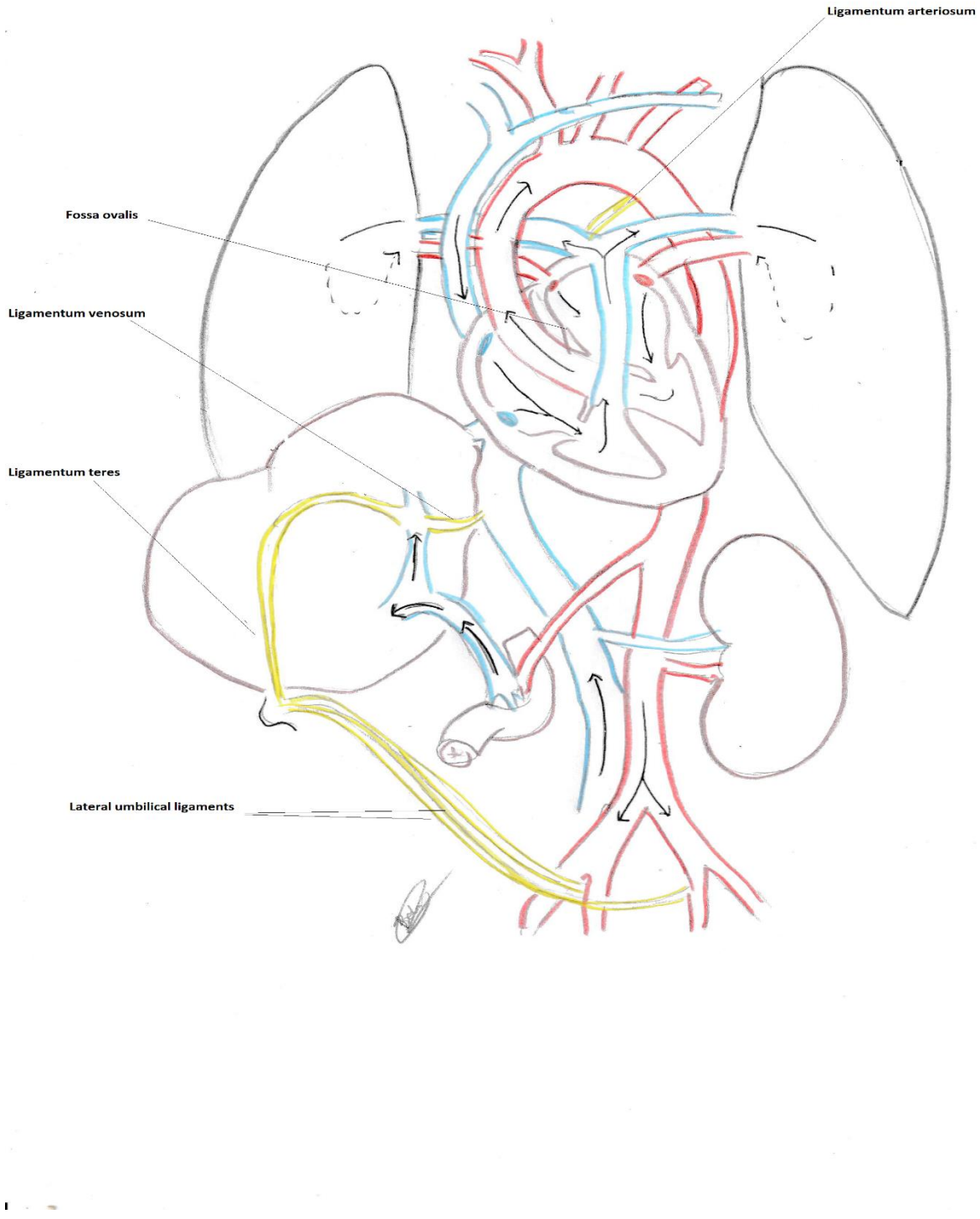


Figure 2. 2 The neonatal circulation

2.3 Factors affecting foetal heart development

For every 100 live births one is likely to have a congenital heart defect (CHD) (Maslen 2018:1) while approximately 10% of foetal loss is due to severe forms of CHD.

CHD consequently are the most frequently occurring birth defect and the single most common cause of in utero fatality in humans (Maslen 2018). Substantial evidence shows that CHD is heritable, which indicates a strong involvement from genetic risk factors (Maslen 2018). External environment exposures which are significantly linked with the risk of CHD have also been recognised (Maslen 2018).The cause therefore of most cases of CHD cases are multifactorial and are caused by the convergence of several hypothetical risk factors which include genetic, epigenetic and environmental sources (Maslen 2018).

As a result, a specific cause can be very difficult to determine and therefore hard to prevent. Even though the foetal environment is a highly protective, the foetus remains very vulnerable to insults (Maslen 2018). A few important factors are discussed below.

2.3.1 Maternal factors

(a) Diabetes Mellitus

One of the most common maternal conditions that complicates pregnancies is diabetes mellitus (DM) which affects about 3% to 10% expectant mothers (Donofrio et al.2014). Amongst these, 20% have DM prior to conception and are considered to have pregestational DM. There is an approximately 3-5% increase in the incidence of CHD when compared to the general population in women with pregestational DM (Donofrio et al.2014). A higher relative risk was noted for certain cardiac defects, which included heterotaxy, truncus arteriosus, transposition of the great arteries single-ventricle defects (Donofrio et al.2014). While the risk may be highest in mothers with blood glucose levels >8.5%, all pregnancies of pregestational diabetic women are at some increased risk. Foetuses may develop ventricular hypertrophy in late gestation (Donofrio et al.2014).

(b) Phenylketonuria

Maternal phenylketonuria when left untreated can have negative outcomes such as mental retardation, microcephaly, growth restriction and CHD in offspring. Maternal

serum phenylalanine levels of >15mg/dL are associated with 10 to 15-fold increased risk of CHD. (Donofrio et al.2014).

(c) Medication exposure

There are numerous cardiac teratogens in the form of medications that women of childbearing age are exposed to in the first trimester. These include anticonvulsants, lithium, angiotensin-converting enzyme inhibitors, retinoic acid, selective serotonin reuptake inhibitors (SSRIs) and nonsteroidal anti-inflammatory agents (NSAIDS). (Donofrio et al.2014).

I. Anticonvulsants

The anticonvulsants that are used in pregnancy include carbamazepine, diphenylhydantoin, and valproate. 1.8% of 1208 carbamazepine -exposed foetuses exhibited cardiac malformations (Donofrio et al.2014).

II. Lithium

The use of lithium has been associated with cardiac malformations in up to 8% of offspring in a registry study (Donofrio et al.2014).

III. Angiotensin-converting enzyme inhibitors

Exposure of these medications, in the first trimester is occasionally associated with increased risk for CHD. Most of the reported defects are atrial septal defects and patent ductus arteriosus (Donofrio et al.2014).

IV. Retinoic Acid

Retinoic acid, a vitamin A analogue, has been found to be teratogenic in laboratory animals. In a retrospective series of human subjects, cardiac malformations (conotruncal defects and aortic arch anomalies predominating) were reported in 8% of foetuses exposed unintentionally to retinoic acid in utero (Donofrio et al.2014).

V. Nonsteroidal Anti-Inflammatory Agents (NSAIDS)

NSAIDS are occasionally used for tocolysis but there is doppler evidence of ductal constriction reported in 25% to 50% foetuses exposed to indomethacin in late second and third trimesters. However, the effect is usually mild and usually resolves with drug termination.

(d) Family history

I. *Maternal cardiac disease*

The recurrence risk of nonsyndromic, nonchromosomal CHD is reported to be greater than 2 times as high if the mother is affected as opposed to the father or a sibling. The risk is highest with heterotaxy and atrioventricular septal defects (AVSD) (Donofrio et al.2014).

II. *Paternal cardiac disease*

A 2% to 3% risk of cardiac malformation has been documented if the father was affected with non-syndromic CHD. The recurrence risk aortic stenosis (AS) is much higher in children born to fathers with the same defect (Donofrio et al.2014).

III. *Diseases, disorders, or syndromes with mendelian inheritance*

A higher risk of CHD occurs in pregnancies where the parent is affected by an autosomal-dominant genetic disorder associated with an increased risk for cardiac malformation, as well as parents who have a deletion syndrome such as 22q11 deletion, Alagille syndrome and Williams syndrome (Donofrio et al.2014).

2.3.2 Foetal factors

(a) *Suspected cardiac abnormality on obstetric ultrasound*

The percentage diagnosis of CHD on foetal echocardiography after detection of an abnormal four-chamber view during screening is greater than 40% (Donofrio et al.2014).

(b) *Suspected abnormality of heart rate or rhythm*

Foetal bradycardia as a result of abnormal atrioventricular (AV) conduction, has been reported to be associated with CHD in 50% to 55% of cases (Donofrio et al.2014).

(c) *Non cardiac abnormalities*

The percentage of CHD associated with extracardiac malformations is estimated to be about 20% to 45% (Donofrio et al.2014).

2.3.3 Placental factors The placenta plays a crucial role in maintaining foetal health and is the major connection between the mother and foetus. Because it is not a completely formed organ at the start of pregnancy, its development needs to keep pace with the growth of the foetus for it to accomplish its critical role during pregnancy (Maslen 2018).

(a) *The placenta-heart axis*

A recently recognised phenomenon is the placenta-heart axis, where the foetal heart and the placenta develop in parallel (Maslen 2018:1). Thus, the developing heart can be affected by early placental insufficiency. Both these organs share numerous developmental pathways, which makes them susceptible to genetic defects.

(b) Placental insufficiency

Recent studies have showed that placental insufficiency may contribute to the development of CHD (Maslen 2018). One study reported that the number of terminal villi, as well as paucity of vascular structures and small size of the placenta was associated with the presence of a hypoplastic left heart in the foetus. In another study, 13% of foetuses with a single umbilical artery had cardiac anomalies, including hypoplastic left heart syndrome, coarctation of the aorta, tetralogy of Fallot, hypoplastic right heart, pulmonary atresia/stenosis, absent ductus venosus with cardiomegaly, left isomerism, right isomerism and transposition of the great vessels (Maslen 2018).

(c) Hypoxia

Foetal hypoxia does not only change placental development, but it also tends to change gene expression in the developing heart. This can have extreme effects on cardiac morphogenesis resulting in CHD (Maslen 2018:3). Hypoxia results in ischaemia-induced premature induction of vascular endothelial growth factor expression which prevents formation of the endocardial cushions, which are the endocardium-derived analogues of the heart septa and valves (Maslen 2018:3). This phenomenon may be an explanation for the development of cardiac septal defects which are the most common type of CHD. There may be an important role for localized low oxygen microenvironments in the developing heart which could be contributing to normal heart development (Maslen 2018).

2.4 Echocardiography

2.4.1 Definition and utility of Echocardiography

Echocardiography or cardiac ultrasound is a diagnostic test that uses standard ultrasound waves in order to generate two-dimensional image slices of the heart muscle (Silverman 1993:1). The ultrasound waves that rebound or echo off cardiac structures can be converted into images that show the size of cardiac chambers, the valvular structures, and the function of the myocardium (Silverman 1993).

Although the use of the clinical examination and other imaging modalities such as the chest X-ray contributes enormously to the diagnosis of cardiac disease, echocardiography is definitive in making an accurate anatomical diagnosis that aids in the management of the disease (Kannan and Kumar 2013).

One of the most significant features of echocardiographic imaging is its capacity to acquire rapid images in real time. Approximately 1800 images per minute can be acquired which allows for the production of a three-dimensional perspective of cardiac morphology (Silverman 1993).

Echocardiography has turned out to be the key imaging tool when it comes to diagnosing and assessing congenital and acquired heart disease in infants, children, and adolescents (Lai et al. 2006). Transthoracic echocardiography (TTE) is the ultimate tool for cardiac assessment, since it is non-invasive, transportable, and effective in providing comprehensive anatomic, haemodynamic, and physiologic information about the paediatric heart (Lai et al. 2006).
Paediatric echocardiography is an inimitable radiological modality with emphasis mainly on anatomy. There is an extensive spectrum of different CHDs. There are special views that are of added importance for paediatric examinations: namely the subxiphoid (or subcostal), suprasternal notch, and right parasternal views (Lai et al. 2006).
The attainment and proper display of images from these views are characteristic of paediatric TTE. Furthermore, special methods are vital for imaging restless infants and young children, which includes sedation and distraction tools that will allow performance of a complete echocardiography examination (Lai et al. 2006).
Sequential studies to assess the progress of heart disease may be performed at routine intervals for monitoring of valve function, growth of cardiovascular structures, ventricular

function, and potential sequelae of medical or surgical interventions (Gutgesell and Rembold 1990 and Lai et al. 2006).

The general categories of paediatric echocardiography indications are as follows:

- *CHD:* 2D - Two-dimensional echocardiography offers crucial structural information in all forms of cardiac and great vessel disease in the paediatric population (Musewe et al 1987 and Cheitlin et al 1997). Doppler echocardiography offers important physiological information which, when linked with anatomical data, provides a guide in the therapeutic management of cardiac disease (Musewe et al 1987 and Cheitlin et al 1997). The re-assessment of examinations allows the tracking of haemodynamic changes that usually occur during the transition phase from foetal to newborn and infancy periods (Musewe et al 1987 and Cheitlin et al 1997).

  Perinatal physiological changes usually disguise or obscure the existence of haemodynamically significant cardiac lesions. Echocardiography permits the early recognition of lesions in the neonate with alleged sepsis or pulmonary disease in which either the pulmonary or the systemic circulation depends highly on the patency of the DA (Bash et al 1986; Leung, Mok and Hi 1988 and Huhta et al. 1984). A conclusive diagnosis in these lesions before the ductal closure prevents severe morbidity or death (Bash et al 1986; Leung, Mok and Hi 1988 and Huhta et al. 1984). Usually newborns with a loud murmur, features of congestive heart failure, cyanosis, or failure to thrive have a high likelihood of significant heart lesions and should undergo an immediate echocardiographic evaluation (Cheitlin et al 1997).

The following are the common categories of **structural congenital cardiac diseases** that can be diagnosed in the neonatal population using echocardiography:

- **Intracardiac shunts**: The location, morphology and size of the defect, direction of flow and gradient across the defect, pulmonary/systemic flow profiles, ventricular function and the exclusion of associated lesions (Bierman and Williams 1979; Bierman, Fellows and Williams 1980 and Cheitlin et al 1997).
- **Obstructive lesions**: The location, morphology, pressure gradients, ventricular compensation, and associated lesions (Huhta et al. 1984; Lima et al 1983; Simpson et al. 1988; Murphy, Ludomirsky and Huhta 1986; Huhta et al. 1984 and Cheitlin et al 1997).

- **Regurgitant lesions**: The valve morphology, assessment of severity, atrial/ventricular dilation, ventricular compensation, and associated lesions (Meijboom et al. 1986; Roberson and Silverman 1989; Silverman and Hudson 1983 and Colan, Borow and Neumann 1984).
- **Anomalous venous connections**: The location and connections of proximal systemic and pulmonary veins, assessment of left-to-right and right-to-left shunts, presence of venous obstruction, and associated lesions (Smallhorn et al. 1987; Van der Velde 1991; Van Hare et al. 1988 and Huhta, Gutgesell and Nihill 1985).
- **Conotruncal abnormalities**: The position of great arteries, ventriculoarterial connections, spatial and haemodynamic relation of the great arteries to coexisting ventricular septal defect, the nature of sub arterial obstruction, great vessel anatomy and ventricular compensation (Pasquini et al. 1993; Sanders, Bierman and Williams 1982; Trowitzsch, Colan and Sanders 1985 and Borow et al. 1981).
- **Coronary anomalies**: The origin, size and the flow in coronary arteries, presence of abnormal coronary artery/ventricular fistulae, and ventricular compensation (Pasquini et al. 1993 and Day, Laks and Drinkwater 1992)
- **Complex lesions**: cardiac segmental analysis of situs and connections, size and location of all cardiac chambers, atrioventricular valve morphology and function, sub arterial and arterial obstruction, interatrial and interventricular communications, venous and great artery anatomy, and ventricular compensation (Cheitlin et al 1997).

<u>Cardiopulmonary diseases</u>

The changeover from foetal to extrauterine haemodynamic impacts the clinical expression of cardiovascular and pulmonary disease in the neonate (Martin et al. 1988). Echocardiography can be used to assess patency of the DA, the direction, the degree of shunting at the ductal level, and approximation of pulmonary artery pressure measurements (Martin et al. 1988). Echocardiography also identifies coexisting ductal-dependent cardiovascular lesions before therapeutic pharmacological or surgical closure of a patent DA is undertaken.

Neonates with primary pulmonary hypertension (persistent foetal circulation) can present with or without perinatally acquired pulmonary parenchymal disease (Martin et al 1988). To exclude structural abnormalities, Doppler echocardiography can be applied to assess the degree of atrial and ductal shunting, pulmonary artery pressures, and ventricular function.

Myocardial diseases

Myocardial abnormalities in the neonate are not uncommon and are related to transplacental acquired pathogens, metabolic abnormalities, structural CHD, maternal systemic disease, or peripartum injury (Reller et al. 1985 and Ino et al. 1988). Echocardiography can be used to detect reversible structural anomalies contributing to myocardial dysfunction. It can also be used to monitor the response of the neonatal myocardium to therapeutic interventions, and to assess recovery of the myocardium from peripartum injury (Cheitlin et al 1997).

Diastolic function

The foetal and neonatal heart show different physiological responses to the adult heart. Invasive animal studies have established that the myocardium of the neonatal heart is more susceptible to a rise in afterload and it is less compliant than that of the adult heart (Romero and Friedman 1979; and Reimenshneider et al. 1981). Systolic and diastolic function properties of the infant heart change with maturation of the myocardium (Shiota et al. 2002).

Pulmonary blood flow, which is the preload for the LV, is elevated throughout the early neonatal period due to additional blood supply provided by left-to-right shunting from the aorta via the DA (Shiota et al. 2002). Studies on newborn lambs show a reduced LV response to increased preload (Klopfenstein and Rodolph 1978). Other researchers have shown that the neonatal LV copes adequately with the increased volume provided by shunting through the DA to the pulmonary arteries followed by pulmonary venous return to the left atrium and LV (Clyman et al. 1987). The LV systolic and diastolic filling state in the setting of DA patency in neonates has not been fully evaluated (Shiota et al. 2002).

The significant role that ventricular diastolic function and diastolic filling plays in paediatric heart disease has not been well documented (O'Leary 1999 Moskowitz et al. 1990; Gidding et al. 1986; Frommelt et al. 1992; Cullen, Shore and Redington 1995 and Schmitt et al. 1995). Until the advent of Doppler echocardiography, the evaluation of diastolic function was undertaken invasively by means of cardiac catheterization where direct measurements of atrial and ventricular pressures were done. Diastolic function of the adult heart has been studied extensively (Oh et al. 1997), but the availability of normal diastolic Doppler data in the neonatal and paediatric age groups has limited the interpretation of Doppler findings in younger patients.

Numerous congenital and acquired cardiac disorders result in impaired diastolic ventricular function (Klein et al 1991; Rihal et al.1994 and Belardinelli et al. 1995), and in many instances diastolic dysfunction may precede the onset of systolic dysfunction (Shen et al. 1992 and Fragola et al.1997). Sequential assessment of diastolic indices may provide insights into disease progression as well as responses to treatment (O'Leary 1999).

There has been an explosion of studies in recent times that have published normal data for Doppler flow patterns, Tissue Doppler, myocardial performance index (Tei index) measurements for the left and right ventricle including tricuspid annular plane excursion (TAPSE) for right ventricle in the neonatal population (Riggs et al 1989; Harada et al 1994; Shiota, Harada and Takada 2002; Mori et al. 2004; Iwashima, Seguchi and Ohzeki 2005; Koestenberger et al 2012; Bokiniec et al. 2016 and Taksande 2018). However, none of these studies reported neonatal data from sub-Saharan Africa.

2.4.2 Application of Echocardiography

Echocardiography is non-invasive but extremely accurate and provides a quick assessment of the overall health of the heart (Silverman 1993; Armstrong and Ryan 2010:2). During a routine echocardiography examination, a transducer is positioned on the chest wall over the heart to acquire images.

When appropriately applied, the diagnostic and prognostic effectiveness of echocardiography are unmatched. Images are shown on a monitor and are recorded for future reference (Silverman 1993; Armstrong and Ryan 2010).

2.4.3 Advantages of Echocardiography

Echocardiography is a versatile cardiac imaging method. It can provide an extensive range of clinically valuable information rapidly in a variety of settings, and at a much lower cost compared to other cardiac imaging techniques (Bulwer and Rivero 2009).

Portable battery-powered instruments, also known as hand-carried ultrasound (HCU), can now be used outside the hospital setting in locations such as community clinics, resource-poor environments, ambulances, and aircrafts (Bulwer and Rivero 2009).

Echocardiography also provides a cost-effective means for identifying the neonate with a life-threatening cardiovascular disease. It provides a complete and definitive anatomical diagnosis, and in most cases eliminates the need for further procedures, such as cardiac catheterization. It also exhibits the following advantages:

- *Excellent diagnostic utility*: Prognosis in patients with suspected or established cardiovascular disease is guaranteed. Safety is also well established in adults, pregnancy, and children (Bulwer and Rivero 2009).
- *Safe, highly portable, and versatile:* Battery-powered forms can be used in community and remote settings; Transoesophageal echocardiography can be used in real time during cardiac surgery without the interruption of surgical procedures (Bulwer and Rivero 2009:7).
- *No radiation involved:* Unlike Cardiac Computed tomography (CT) and nuclear cardiology imaging (Bulwer and Rivero 2009).

2.4.4 Limitations of Echocardiography

It is imperative to recognize that there may be circumstances where echocardiography has major limitations. In these cases, alternative imaging modalities may be required, and ultrasound tends to be scattered by air, consequently it cannot be used to see vascular structures inside lungs (Kannan and Kumar 2013).

In addition, the consistency of echocardiogram is extremely reliant on the presence of a suitable "window". Solid tissue in proximity to the heart permits good ultrasound transmission and provides an excellent window. The liver and thymus are examples of organs that provide acoustic windows and enable acquisition of good images (Kannan and Kumar 2013).

2.4.5 Measurements and modalities obtainable using Echocardiography

2.4.5.1 Two-dimensional (2-D) Echocardiography: Provides a snapshot in time of a cross-section of tissue. For a 2-D image to be created the ultrasound beam must be swept across the area of interest. If these sections are formed in a quick sequence and demonstrated on a viewing monitor, they can show 'real-time imaging' of the heart chambers (Kaddoura 2009).

2.4.5.2 Motion or M-Mode echo: Is produced by the transmission and reception of an ultrasound signal along only one line, producing high sensitivity which is greater than 2-D echo, for recording moving structures. It produces a graph of depth and strength of reflection with time. Changes in movement (e.g. valve opening and closing or ventricular wall movement) can be displayed (Kaddoura 2009).

2.4.5.3 Doppler echocardiography: Doppler uses the reflection of ultrasound by moving red blood cells and is used to measure velocity information and to assess the direction of the blood pool. The reflected ultrasound has a frequency shift relative to the transmitted ultrasound which is determined by the velocity and direction of blood flow. In essence, Doppler echocardiography is used to detect and quantify normal and disturbed blood and tissue velocities which translates into information about haemodynamic changes within the heart and blood vessels. This attribute enables the assessment of regurgitant or stenotic valves, narrowed vessels and abnormal tissue velocities secondary to cardiac disease (Kaddoura 2009).

2.5 Methods of assessing diastolic function

2.5.1 Pulsed Doppler

Pulsed Doppler is traditionally used to assess diastolic function by measuring early (E) and late (A) diastolic filling velocities across the mitral valve which reflects the pressure gradient between the left atrium and the LV (Mottram and Marwick 2005). Doppler assessment of ventricular inflow is best performed with the help of colour mapping in apical views where the transducer position and angulation changes are often needed to optimize alignment (Lopez et al 2010).

It is best to take the sample volume in the LV at the tips of the valve leaflets (distal to the annulus) since both the peak early diastolic velocity E-wave and the A-wave (peak velocity during atrial contraction) decrease in value as the sample volume is moved towards the atrium (figure 2.3). The of peak E wave can be used as another parameter of diastolic function since it is easily influenced by the ventricle's relaxation and compliance, these diastolic measurements are usually fused in neonates due to their fast heart rates. (Lopez et al 2010). The diastolic indices based on the E and A waves are usually restricted by their dependence on loading conditions, and the fusion of the E and A waves resulting from rapid heart rates usually adds to this hinderance (Lopez et al 2010).

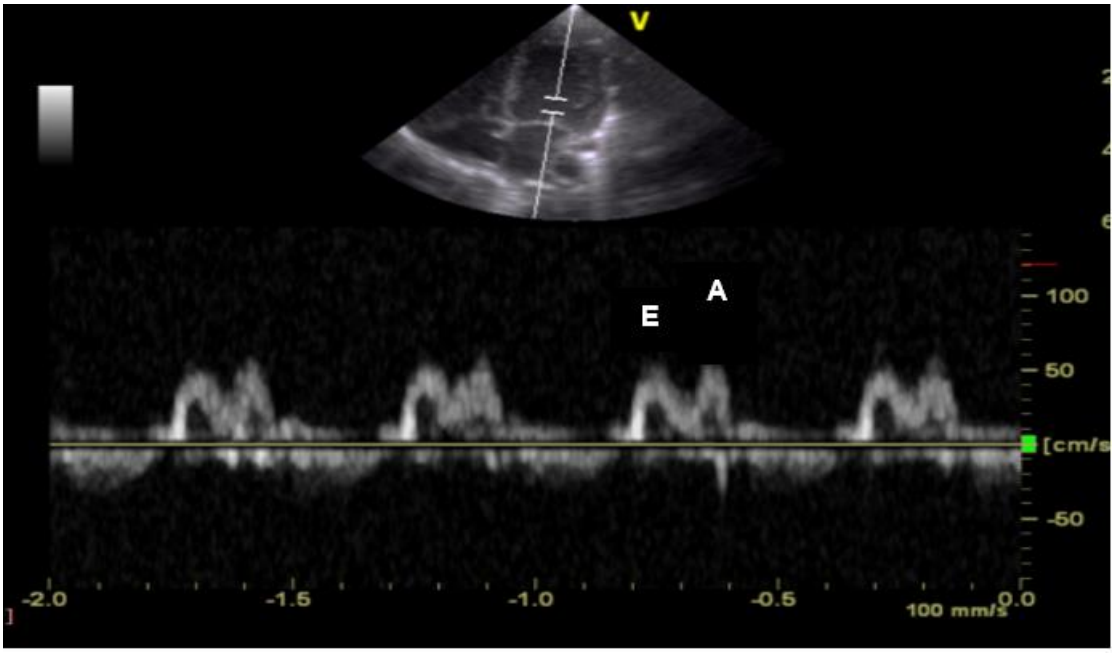


Figure 2. 3 Pulsed-wave Doppler pattern of mitral inflow

(Image taken by main author at CHBAH 2017)

2.5.2 Tissue Doppler imaging (TDI)

Physical principles of tissue Doppler are similar to those of normal Doppler flow imaging except that TDI filters out the high velocity low amplitude signal of the blood pool, thus displaying only the slow-moving high amplitude signal of myocardium (Ho and Solomon 2006). TDI only measures the direction of motion which is parallel to the direction of the ultrasound beam (Ho and Solomon 2006). Furthermore, it measures absolute tissue velocity (Ho and Solomon 2006). The advantage of using TDI in the assessment of diastolic function is that it is less load dependent when compared to the standard Doppler techniques. TDI has been validated against invasive haemodynamic measures, hence it can be correlated with the time constant of isovolumetric relaxation (Sohn et al. 1997).

Tissue Doppler imaging is assessed by a sample volume (2–5 mm) placed at the septal or lateral border of the mitral annulus and tricuspid annulus in an apical four chamber view (see A, B and C in figure 2.4) which reflects the longitudinal velocities at the mitral annulus (Garcia, Thomas and Klein 1998 cited in Mottram and Marwick 2005). The systolic velocity (S') mirrors ventricular ejection as the ventricle shortens along its length while the early (E') and late (A') diastolic velocities correspond to the trans-mitral Doppler flow (figure 2.5) (Nagueh, Sun and Kopelen 2001 cited in Mottram and Marwick 2005). These wave forms are affected by both

ventricular diastolic and atrial systolic function (Lopez et al 2010:474). Isovolumetric relaxation time (IVRT') may be measured from the end of the S' wave to the onset of the E' wave, and isovolumetric contraction time (IVCT') may be measured from the end of the A' wave to the onset of the S' wave. It is imperative to distinguish that IVRT' measured by atrio-ventricular (AV) valve annular motion may not correlate with IVRT assessed by blood flow Doppler interrogation, particularly when diastolic dysfunction is present, because IVRT' appears to be less influenced by filling pressures (Lopez et al 2010:474).

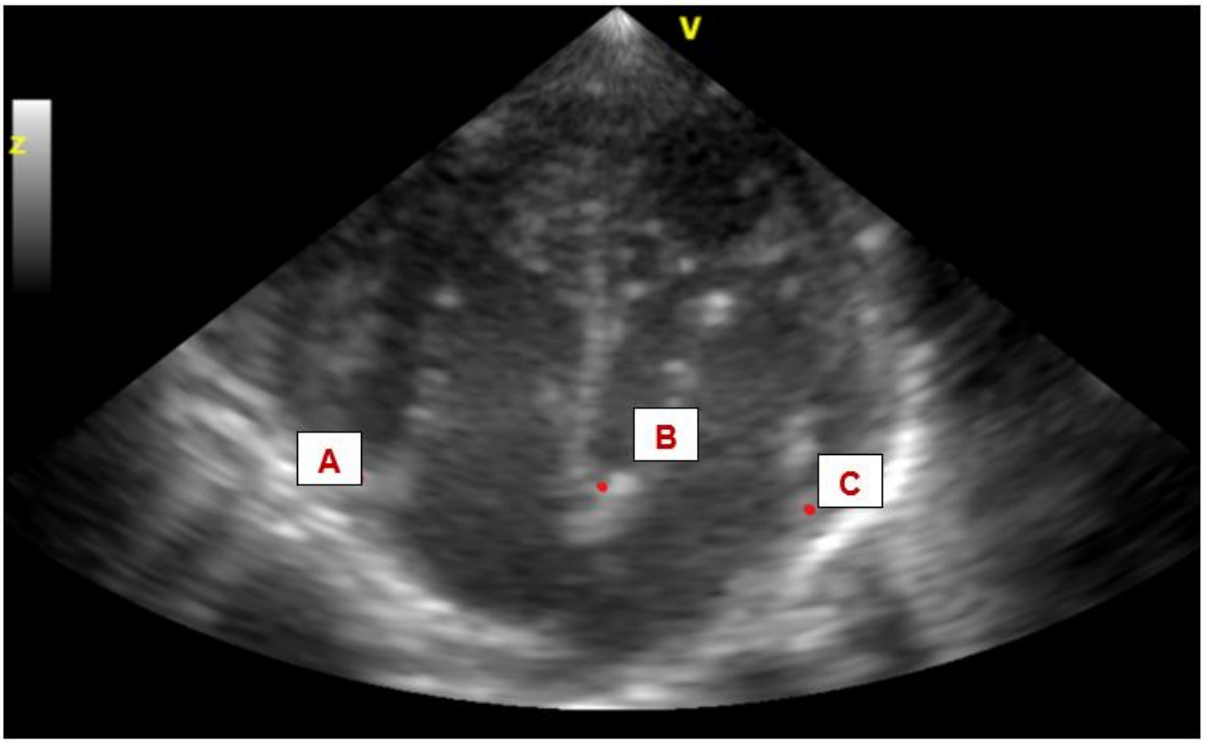


Figure 2. 4 Apical 4-chamber view with tissue Doppler evaluation points, A: Tricuspid valve lateral annulus, B: Mitral valve Septal annulus and C: Mitral valve lateral annulus.

(Image taken by the main author at CHBAH 2017)

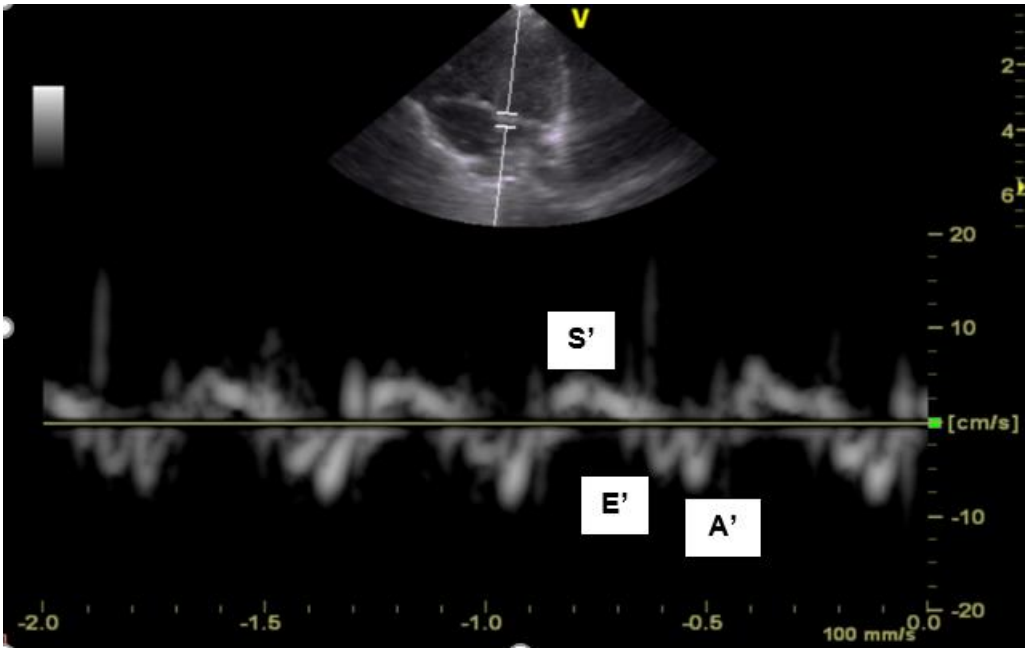


Figure 2. 5 Septal mitral annular tissue Doppler (Image taken by main author at CHBAH 2017).

2.5.3 Myocardial performance index (MPI or Tei index)

MPI was first described by Tei et al. 1995 and has been shown to be a predictor of global left ventricular (Tei et al. 1997) and right ventricular function in various clinical settings (Weissler, Harris and Schoenfeld 1968). MPI is a Doppler derived method which combines the intervals of systolic and diastolic function to assess global cardiac function and the advantage of using MPI (Tei index) is that it has been reported to be independent of heart rate (Grossman, McLaurin and Rollet et al. 1979 cited in Tei et al. 1995 and Lakoumentas et al. 2005) and ventricular geometry. Systolic dysfunction is gauged by a prolonged isovolumetric contraction time (IVCT) and a shortened ejection time (ET), whereas diastolic dysfunction results in prolongation of the isovolumetric relaxation time (IRT) (Lakoumentas et al 2005). MPI is a ratio which is derived from the sum of isovolumetric contraction time and isovolumetric relaxation time divided by left ventricular ET (Tei et al 1995).

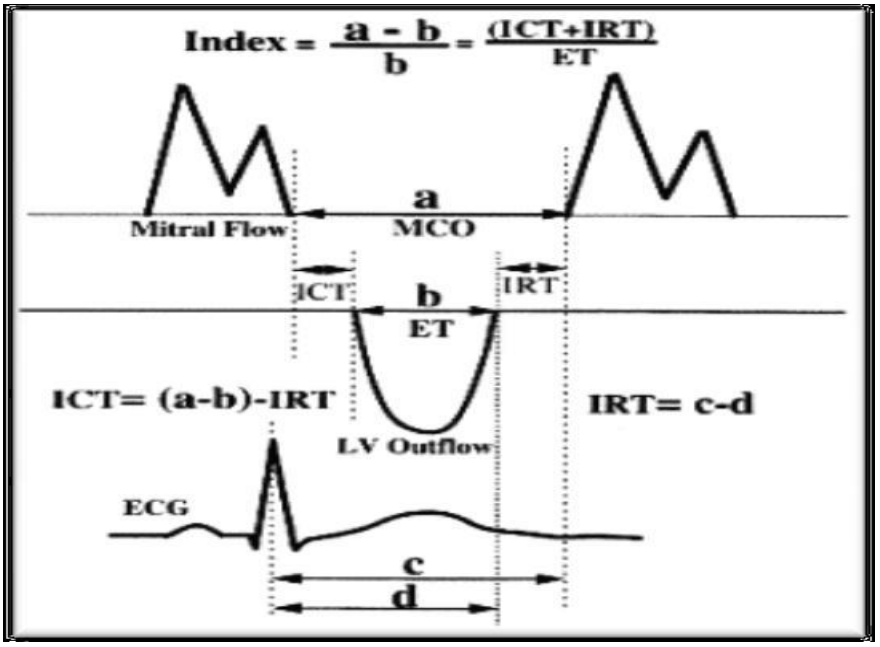


Figure 2. 6 Method of calculating MPI (Tei et al. 1995)

2.5.3.1 The significance of MPI

The calculation of the left ventricular index of myocardial performance, described as (a-b)/b, where a, is the interval between the termination and commencement of mitral inflow, and b is the ET of left ventricular outflow (Tei et la 1995).

The isovolumetric relaxation time (IRT) is measured by subtracting interval c between R wave and onset of mitral inflow. The Isovolumetric contraction time (ICT) is measured by subtracting IRT from (a-b) see figure 2.6 (Tei *et al* 1995).

Global ventricular performance is a function of both ventricular filling and ejection.

Several metrics have been used to measure either systolic or diastolic cardiac function. The evaluation of cardiac function in dilated cardiomyopathy has mostly been focused on systolic dysfunction or has been separated into systolic and diastolic phases (Tei *et al.* 1995). Since diastolic dysfunction is generally associated with primary systolic dysfunction, a measure which combines the assessment of systolic and diastolic function may better reflect 'global' function than the isolated evaluation of either ejection or relaxation (Tei *et al* 1995).

2.5.3.2 Tricuspid annular plane excursion (TAPSE)

The lengthwise shortening of the interventricular septum together with the RV free wall tend to cause the tricuspid valve (TV) to move toward the apex of the RV during ventricular systole, the simple measurement of TAPSE reproduces this motion as a measure of the integrity of both walls of the RV (Kaul et al 1984).

TAPSE represents the measure of RV longitudinal function**.** It is measured using M-mode where the cursor is aligned perpendicular to the tricuspid lateral annulus in the apical four chamber view and is the distance between end-diastolic (B in figure 2.7) and end-systolic (A in figure 2.7) positions of the lateral TV annulus (figure 2.7) (Lang et al 2015). While this index mainly reflects the RV longitudinal function, a good correlation has been demonstrated with parameters estimating RV global systolic function, such as the radionuclide-derived RV EF (Lang et al 2015). Since TAPSE is a one-dimensional measurement depending on the placement of the transducer it lends itself to both over and underestimation. While there may be slight variations in TAPSE values according to gender and body surface area (BSA), Adult TAPSE values of <20mm are usually regarded as having reduced right ventricle function (Forfia et al. 2006 and Frommelt et al. 2002).

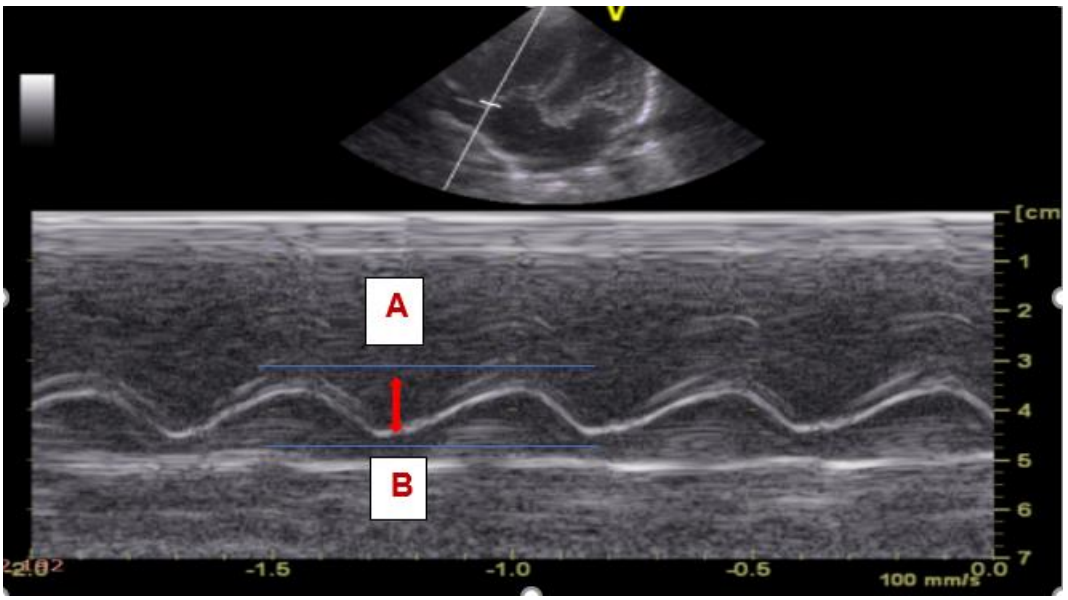


Figure 2. 7 TAPSE acquisition A: end-systolic and B: end-diastolic components

(Image taken by main author at CHBAH 2017).

2.6 Published echocardiography reference values

There are approximately thirty-five published studies conducted in either Europe or North America evaluating diastolic and systolic function in normal children that have been published to date. To the best of the author's knowledge there are only two studies that have been done in Sub Saharan Africa which includes Jacobs et al 2012, from the Free State in South Africa, who established reference values for preterm and low-birth weight infants only, and Majonga et al 2017, from Zimbabwe who created reference values in children aged 6 to 16 years.

There are quite a few studies that have shown ethnicity to be a significant determinant of cardiac chamber size and therefore, it has been recommended to use ethnic-specific reference values for echocardiographic interpretations (The EchoNoRMAL study 2015; Lang et al. 2015; Badano 2014; Bansal, Mohan and Sengupta 2016 and Majonga et al. 2017). Furthermore, the use of inappropriate reference ranges may result in the misdiagnosis of cardiac abnormalities (Badano 2014).

Some therapeutic interventions may depend on the precise assessment of cardiac chamber size and function, which may have detrimental consequences if the ethnic differences are not accounted for (Yancy et al. 2013 and Bansal, Mohan and Sengupta 2016). Majonga et al. (2017) reported differences in reference ranges amongst different racial groups but these were largely ignored because of the scarcity of data available particularly from African children. These reasons therefore necessitated a study of this nature.

2.6.1 Pulsed Doppler

Previous studies have shown echocardiographic differences between neonates and older children. Both (Ekici et al 2007) and (Mori et al 2004), found that the mean peak E velocity and the mean value of E/A in neonates were significantly lower than those in children, and was thought to be due to the decreased relaxation ability of the neonatal myocardium. Trans-mitral peak E and A velocities have been reported to increase significantly from preterm ($p=0.02$) to term ($p=0.04$) neonates (Elkiran et al 2014).

2.6.2 Tissue Doppler imaging (TDI)

(Ekici et al 2007), noted that the mean E', S' and ratio of E'/A' obtained from both the lateral and medial annuli of the mitral valve in neonates were found to be significantly lower than those of children, which was thought to be due to the immaturity of the neonatal myocardium.
Myocardial E' and A' velocities have also been shown to increase significantly after the neonatal period up to 1 month of age (p=0.001) (Elkiran et al 2014) and (Mori et al 2004).

There is also a difference between the two ventricles. (Mori et al 2004), showed the S' of the tricuspid annulus to be higher than that of the mitral annulus which is thought to be due to the lower afterload in the right ventricle. This finding is evident in both children and neonates.
Unlike the LV, the peak E and E' velocities of the tricuspid annulus do not change much after birth most likely due to a delay in diastolic alterations of the RV post-delivery (Mori et al 2004).

(Alp et al 2012), showed a higher E' wave velocity in infants compared to neonates as well as an increase in TDI velocities with age. Similarly, (Eidem et al 2004), demonstrated that parameters of cardiac growth, namely LV end diastolic diameter and LV mass had significant correlations with TDI velocities. This was especially apparent in neonates and infants since significant cardiac growth occurred during the first year of life, suggesting that TDI velocities increased with cardiac size and growth rather than age.

There is little information on the E/E' ratio in the paediatric population. The normal adult E/E' ratio of the LV is reported to be 7.7+-3.0, (Mori et al 2004). The E/E' ratio during childhood is reported to be lower and may be due to lower left ventricular filling pressures, (Mori et al 2004). In contrast, the E/E' ratio is reported to be higher in newborn babies indicating a relatively higher LV filling pressure possibly caused by the presence of a patent DA or due to the immaturity of the diastolic properties of the neonatal myocardium. Closure of the patent DA has been shown to be associated with a decrease in the E/E' ratio over the first week of life (Mori et al 2004).

2.6.3 Myocardial Performance Index (MPI)/Tei Index in neonates and children

Values for MPI in paediatrics and adults have been established. It is a doppler derived non-geometric measure of ventricular function, is independent of heart rate (Borzoee and Kheirandish 2004:85) and it is affected by age (Lakoumentas et al 2005). There is a progressive reduction in the value over the first 3 years of life. The age dependent changes in the index

may portray changes during the maturation of the myocardial structure and function of the ventricles in neonates and children (Lakoumentas et al 2005).

2.6.4 Tricuspid annular plane systolic excursion (TAPSE)

The physiological significance of the right ventricle is often undervalued; it is crucial to assess its function in various diseases to predict prognosis (Ghio et al. 2000). The right ventricular dimensions and functions in patients with pulmonary hypertension determine severity of the disease and act as an important indicator of survival (Uysal, Boston and Çil 2016).

Normal ranges of TAPSE have been determined for children (Nunez-Gil et al. 2011; Koestenberger et al. 2009 and Hashimoto et al. 2015). However, there are no established reference values for neonates and children in the sub-Saharan Africa.

Adult TAPSE values of <20mm are usually regarded as indicating reduced right ventricular function (Forfia et al. 2006 and Frommelt et al. 2002). Unfortunately, adults' nomograms cannot be applied to the paediatric population because of developmental changes and anthropometric variables that are related to changes in patient and heart size (Lipshultz and Miller 2005).

2.6.5 Confounding factors and their effect on cardiac function

The neonatal myocardium changes over time as the cardiac myocytes increase in number and size during somatic growth. Additional changes occurring with aging include development of ion channels, the density as well as the anatomical arrangement of myocardial fibrils. All of these factors contribute to the characteristics of the neonatal myocardial systolic and diastolic function (Harada et al 1999; Mori et al 2004 and Taksande 2018).

A mitral inflow doppler and tissue Doppler (TDI) study in Caucasian neonates, infants and children conducted by (Cantinotti et al 2016) demonstrated that age, BSA and heart rate (HR) had a temporal correlation with echocardiographic parameters except E' measured using TDI and mitral valve PW Doppler E velocity (E). The same study also found a positive correlation between gender and mode of delivery, and mitral A waves as well as the E/E' ratio (Taksande 2018).

2.7 Rationale

(Ciccone et al 2011), stated that we have in the current era, a better understanding of the heart evolution dynamics, and the various alterations which occur during the transitional period after birth. The advent of echocardiography, which is a non-invasive imaging modality, has provided a means to study the effects of these physiological changes and their effect on the diastolic function of the newborn heart.

There are a number of significant haemodynamic changes that occur during the transitional period from the foetal to the neonatal environment, as a consequence of the DA closing and the decrease in the pulmonary flow resistance (Shiota, Harada and Takada 2002, cited in Alp et al 2012). The preload in the LV, the systolic arterial blood pressure and systemic afterload increases after birth after separation from the placenta which during foetal life is a low-pressure system (Shiota, Harada and Takada 2002, cited in Alp et al 2012). In contrast, the high pulmonary pressure and pulmonary vascular resistance during foetal life decreases substantially after birth which results in a reduction in right ventricular afterload. Both the right and left ventricular myocardial diastolic functions change after birth due to the influence of these haemodynamic alterations (Shiota, Harada and Takada 2002, cited in Alp et al 2012).

Left ventricular longitudinal myocardial motion differs between neonates and older children (Ekici et al 2007). Pulsed Doppler and tissue Doppler measurements of myocardial annular velocities are widely used to assess right and left ventricular longitudinal motion as well as diastolic myocardial performance (Ekici et al 2007).

The realization of the importance of diastolic function and longitudinal movement as a means of diagnosing myocardial pathology in neonates and paediatrics, has increased in recent times. However, although there are published paediatric nomograms for echocardiographic diastolic measurements there is paucity of data representing the neonatal population, especially from the African continent. Majonga et al (2017) published echocardiographic reference ranges for older children and adolescents from sub-Saharan Africa, but neonates were not included. Numerous studies which have recognised that ethnicity is a significant determinant of cardiac chamber sizes and the use of ethnic-specific reference values for echocardiographic interpretations are recommended (The EchoNoRMAL study 2015; Lang et al. 2015; Badano 2014; Bansal, Mohan and Sengupta 2016 and Majonga et al. 2017).

2.8 Aims and Objectives

The aim of this study, therefore, was to determine normal echocardiographic references for diastolic inflow and longitudinal movement of the heart in healthy African neonates.

The objectives were:

1. To measure diastolic function using tissue Doppler in the left and right heart of the neonate;
2. To measure diastolic function using pulsed Doppler echocardiography in the left and right heart;
3. To measure Tricuspid annular plane systolic excursion;
4. To determine the Tei index in the left and right heart.

CHAPTER THREE: METHODOLOGY

3.1 Study design

This study was a descriptive, bidirectional design using echocardiography to analyse the diastolic function and longitudinal movement of the right and left ventricles of the heart in African (Black) full term neonates at the Chris Hani Baragwanath Academic Hospital in Johannesburg.

3.1.1 Social demographics

Chris Hani Baragwanath Hospital is the 3rd largest hospital in the world, occupying approximately 0.70 km^2, with an estimate of 3200 beds and 6760 staff members. Approximately 60000 patients per year are admitted in the maternity Hospital, while there are 2000 to 2400 newborns delivered on a monthly basis at the facility (Platten 2015).

3.2 Subject enrolment

Healthy neonates aged 12 hours, or more were recruited in the post-natal wards of Chris Hani Baragwanath Academic Hospital as soon as ethical approval and permission was obtained. The study recruited a total of 325 infants, with a final sample size of 292, due to some participants not meeting the inclusion criteria. The sample size was calculated and verified by a biostatistician from the University of Witwatersrand using the Cochran's formula, with an 80% confidence level, and a 5% margin of error. This was deemed to be adequate to show statistical significance (see calculation below). Participant identifiers were omitted and were replaced with a study number to maintain participant anonymity.

Sample size calculation

$$= \frac{(\text{Z-Score})^2 \times SD \times (1-SD)}{(\text{Margin of error})^2}$$

$$= \frac{(1.282)^2 \times 0.5 \times (1-0.5)}{(0.05)^2}$$

$$= \frac{1.64 \times 0.5 \times .05}{0.0025}$$

$$= \frac{1.64 \times 0.25}{0.0025}$$

$$= \frac{0.41}{0.0025}$$

$$= 164$$

3.3 This study was conducted in two parts:

3.3.1 Part one of the study (Retrospective Post Processing of data)

The echocardiographic data had been acquired with consent from each mother during a study for a MMed thesis entitled “To assess the accuracy of pulse oximetry screening as a tool to detect critical congenital heart disease in asymptomatic newborns” by Dr. Michael Platten. Ethics approval was granted by the “Human Research Ethics Committee (Medical)” on 31/7/2015 – Ethics Clearance certificate no. M150721. A separate approval to use the study’s echocardiographic data for neonatal diastolic function analysis for this study was sought from the same ethics committee and the Medical Advisory committee of the Chris Hani Baragwanath Academic Hospital. 150 healthy African (Black) newborn infants underwent a comprehensive echocardiographic examination looking at aspects of diastolic function and all data was stored for later post processing. 131 of the 150 patients were recruited into this study after passing the inclusion criteria. In this study, the echocardiographic data collected during Dr Platten’s study were analysed retrospectively. In particular, the diastolic function and longitudinal movement of the left and right ventricles was measured and calculated for the purposes of the study.

3.3.2 Part two of the study (Prospective data collection)

An additional 161 healthy neonates with structurally normal hearts were prospectively recruited after ethical approval was granted by the institution (Witwatersrand University) following which

echocardiographic acquisition of their diastolic function and longitudinal movement of their ventricles was undertaken and analysed. The echocardiographic exam was performed 12 or more hours after birth.

3.4 Selection criteria:

3.4.1 Inclusion criteria:

- Healthy African neonates/newborns;
- Full term Newborns aged 12 hours and older before discharge, and with birth weight ≥2.5kg;
- Participants with structurally normal hearts who had no detectable congenital heart defects on echocardiography;
- Newborns with a small haemodynamically insignificant patent ductus arteriosus (PDA) and patent foramen ovale (PFO);
- Newborns delivered by normal vertex and caesarean section (C/S).

3.4.2 Exclusion criteria:

- Participants older than 30 days;
- Non-Africans were not included in the study (non-Black);
- Participants with CHD/ structurally abnormal heart;
- Newborns with a PFO and PDA > 2mm with a dilated left or right atrium.

3.5 Sample characteristics

A total of 292 healthy full-term neonates, delivered both by normal vertex and caesarean section at Chris Hani Baragwanath Hospital were enrolled after consent was obtained to perform an echocardiographic examination from their mothers at the bedside after delivery, see (figure 3.1). 19 retrospective and 14 prospective neonates were excluded from the study due to the exclusion criteria.

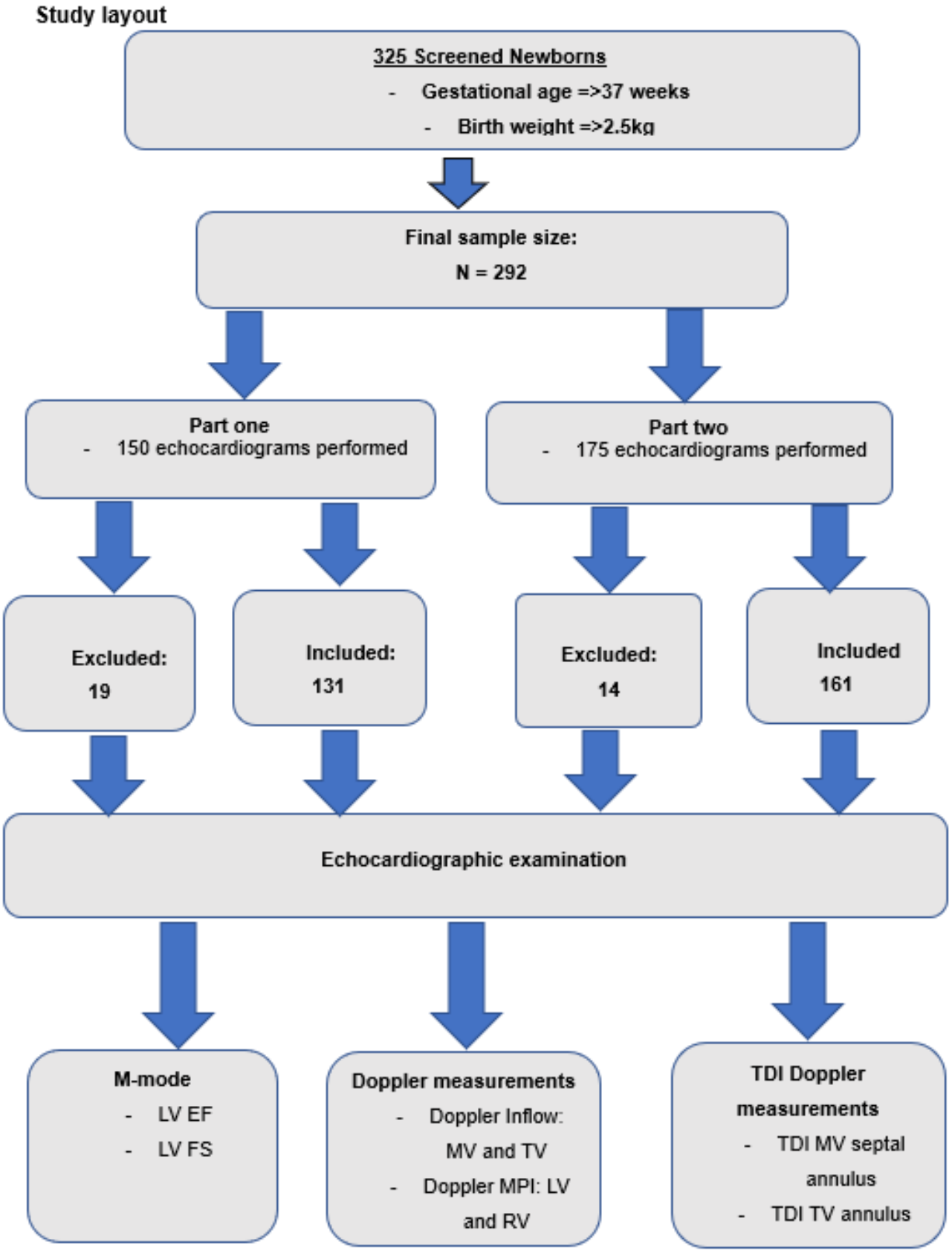


Figure 3. 1 Study layout

3.5.1 Demographic data

The following demographic data were recorded from patient files:

- Date of birth;
- Weight (kg);
- Length (cm);
- Gender (M/F);
- Gestational age in weeks.

Recorded echocardiographic measurements

- ✓ Ejection Fraction (EF%);
- ✓ Fraction Shortening (FS%);
- ✓ Inflow patterns, left and right ventricle: Peak E (m/s), Peak A (m/s), E/A ratio;
- ✓ Myocardial performance index (MPI/Tei index): Left and right ventricle;
- ✓ Tissue Doppler imaging (TDI) to assess the longitudinal movement of the right ventricle (lateral annulus) and LV (medial annulus): E', A' and S';
- ✓ TAPSE.

3.6 Echocardiographic assessment

The echocardiograms were performed in the post-natal wards at Chris Hani Baragwanath hospital, at the mother's bedside in a cot bed or in their mother's arms. The participants were not sedated during the echocardiographic study. Echocardiographic parameters were acquired according to the American Society of Echocardiography Paediatric and Congenital Heart Disease Council guidelines 2010 (Lopez et al 2010).

The echocardiographers that acquired the images for analyses included the study author, a second Cardiac Technologist and two Paediatric Cardiologists skilled in echocardiography.

All images (Part one and Two) were recorded by the MV13-0034 Rev2: GE Healthcare Vivid E compact digital ultrasound console BT12 machine (General Electric, Milwaukee, United States) using a 5-6 MHz transducer. Statistical analyses were done to assess the presence of inter-observer and intra-observer bias.

Reproducibility

The inter-observer variability was calculated using data of 131 echocardiographical studies that was re-analysed in a blinded fashion by a second observer. Each echocardiographical study included second stored blank images where re-measurement was undertaken by the second observer without them knowing the result of the recorded measurements. The variability was calculated by intraclass correlation coefficient (ICC).

3.6.1 Calculating Ejection fraction (EF) and Fraction shortening (FS) on M-Mode

The LV function was measured using M-mode. The standard leading-edge to leading-edge technique was performed. The left ventricular M-mode tracing was obtained from the parasternal short-axis (PSAX) view (Figure 3.2) the cursor in M-mode was placed perpendicular to the intraventricular septum (IVS) and posterior wall of the LV at the level of the papillary muscles. Measurements of the intraventricular septum, end-diastole and end-systole diameter and posterior wall measurements were taken; the EF and FS were calculated by the automated calculation package installed on the echocardiographic machine.

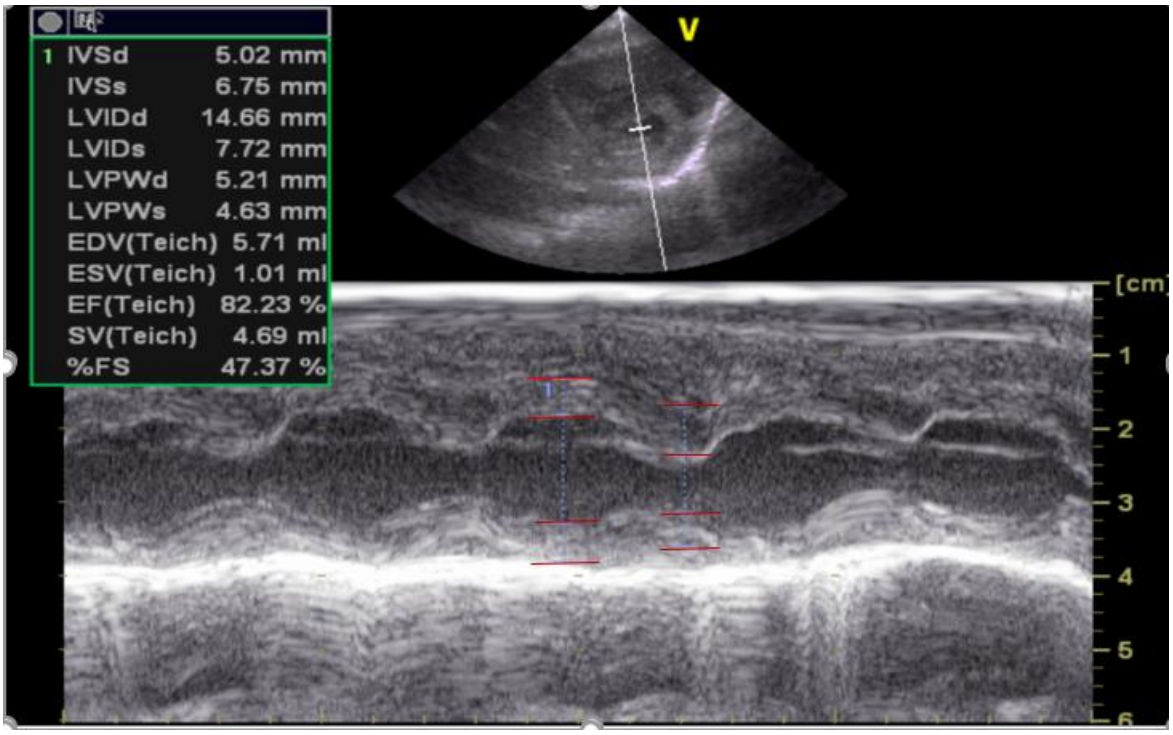


Figure 3. 2 LV function measurements

(Image taken by the main author at CHBAH 2017)

3.6.2 Measuring Tricuspid annular plane systolic excursion (TAPSE)

TAPSE was measured using M-mode where the cursor was aligned perpendicular to the tricuspid lateral annulus in the apical four chamber view. It measures the movement of the tricuspid annulus between end-diastolic and end-systolic and represents the measure of RV longitudinal function**.**

3.6.3 Calculating the LV and RV inflow patterns

The sample volume in the LV was measured at the tips of the valve leaflets (distal to the annulus) where early diastole/passive filling represented by the peak of the E wave and late filling was represented by the peak of the A wave. The right ventricle measurements were done in the same fashion.

3.6.4 Tissue Doppler imaging measurements

The pulsed wave TDI was measured in the apical four chamber view by taking a sample volume from the septal border of the mitral annulus and RV free wall of the tricuspid annulus. The velocities measured reflect the longitudinal movements of the mitral and tricuspid annuli and include the mitral valve septal E', A' and S' waves, and tricuspid valve lateral E', A' and S' waves.

3.6.5 Measuring myocardial performance index (MPI)

The MPI of the RV and LV were calculated using measurements taken in the apical four chamber view for mitral and tricuspid valve inflow patterns, and the apical five chamber view for the aortic valve outflow Doppler envelope in the case of the LV MPI and the parasternal short axis view for the pulmonary valve outflow measurement in the case of RV MPI. The pulsed wave Doppler sample volume was set at 4mm width. The sample volume was taken at the tips of the mitral valve/tricuspid valve leaflets in diastole, while the second sample volume was taken at the left ventricular outflow tract (LVOT), right below the aortic valve cusps (Figure 3.3). No angle correction was used.

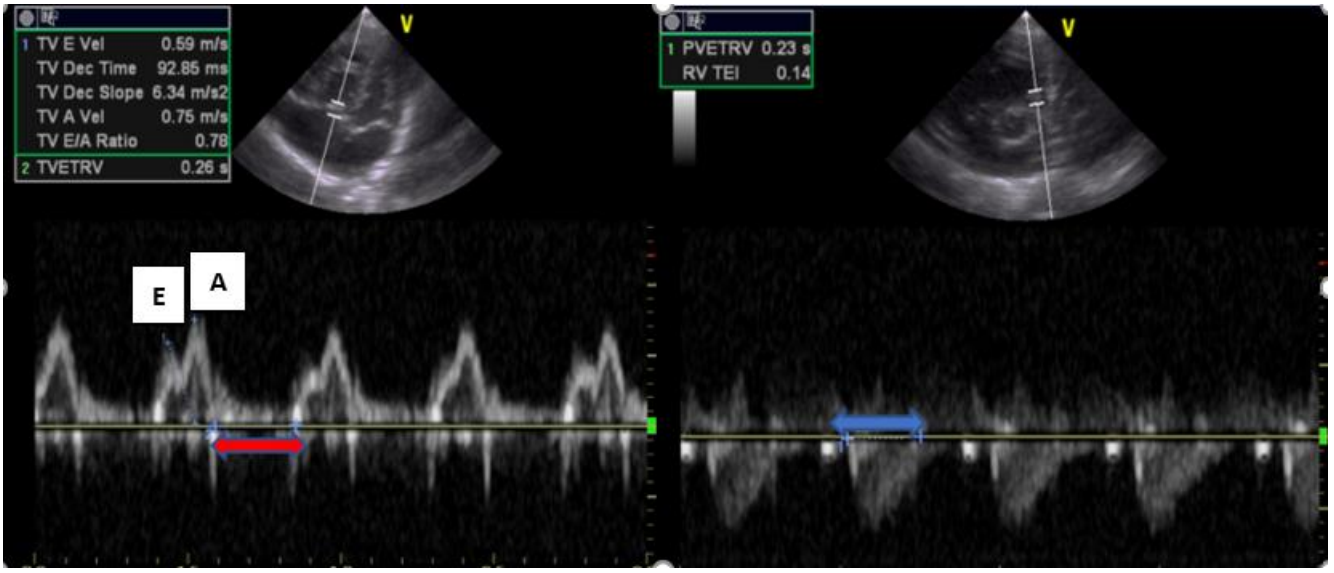


Figure 3. 3 RV Tei measurements

(Image taken by the main author 2017)

3.7 Statistical analysis

All statistical analysis was performed using Excel and Statistica version 13.1 with the guidance of the department of biostatistics at the University of Witwatersrand, Dr Kebashni Thandreyan (Chris Hani Baragwanath Academic Hospital, University of the Witwatersrand) and Prof Hopewell Ntsinjana (Nelson Mandela Children's Hospital). Raw data were captured on Excel. Normal ranges were calculated using means ± standard deviations. Categorical data was expressed in frequencies and percentages. Confounding factors were fitted to determine the effects on the recorded variables using multiple regression analysis. P-values < 0.05 was considered statistically significant. Z-scores were also calculated for the variables. The Shapiro-Wilk test was used in order to assess the normality of distribution, a probability value of < 0.05 was considered to not be normally distributed. The Shapiro-Wilk test is a way of telling if a random sample comes from a normal distribution or not (Statistica how to 2014). Exploratory statistical testing for normality of the measured data was done using the Breusch-Pagan and White test to check for the presence of heteroscedasticity which is a statistical term used to describe the behaviour of variance and normality of the residuals (Cantinotti et al. 2014).

Before any measurement instruments or assessment tools may be utilised for research or clinical applications, it is recommended that its reliability be established. Reliability is defined as the degree to which measurements can be replicated (Daly and Bourke 2000 cited in Koo and Li 2016). Reliability value or correlation coefficient (r) is said to range between 0 and 1, where the values closer to 1 represent a stronger reliability. Intraclass correlation coefficient

(ICC) reflect both degree of correlation and agreement between measurements. Currently, ICC has been broadly used in conventional care medicine in order to evaluate interrater, test-retest, and intra-rater reliability (see Table 3.1 for their definitions) (Cramer et al. 2010; Owens et al. 2004 and Koo, Cohen and Zheng 2011 cited in Koo and Li 2016). These assessments are essential to clinical assessment because, without them, we have no confidence in our measurements, nor can we reach any rational conclusions from our measurements (Koo and Li 2016). Therefore, in this study ICC was used to analyse the reliability between the inter-raters. Table 3.2 shows the r value and its strength.

Table 3. 1 Types of reliability and their definitions

Types	Definitions
Interrater reliability	It reflects the variation between 2 or more raters who measure the same group of subjects.
Test-retest reliability	It reflects the variation in measurements taken by an instrument on the same subject under the same conditions. It is generally indicative of reliability in situations when raters are not involved or rater effect is neglectable, such as self-report survey instrument.
Intrarater reliability	It reflects the variation of data measured by 1 rater across 2 or more trials.

(Image taken from Koo and Li 2016)

Table 3. 2 r value and its significance

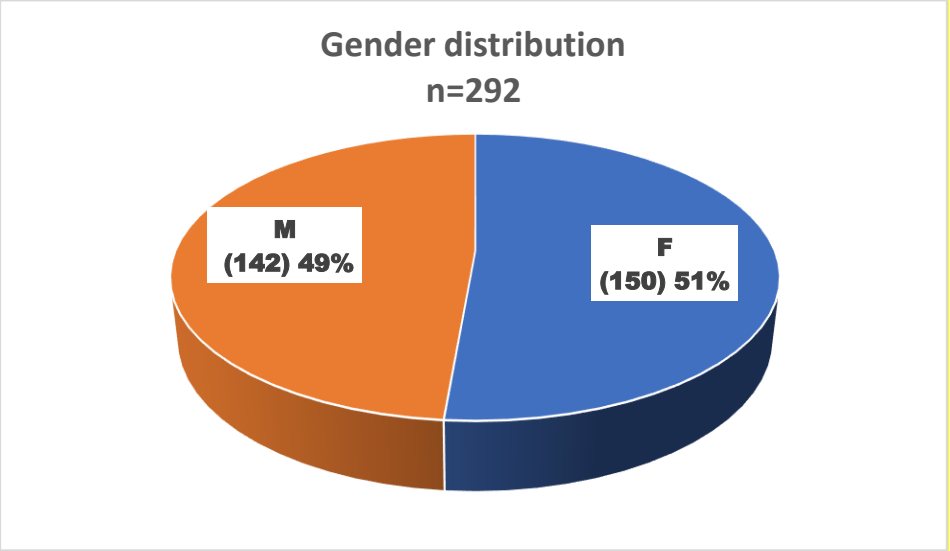
Value of r	Strength of relationship
-1.0 to -0.5 or 1.0 to 0.5	Strong
-0.5 to -0.3 or 0.3 to 0.5	Moderate
-0.3 to -0.1 or 0.1 to 0.3	Weak
-0.1 to 0.1	None or very weak

(Wilson 2009)

CHAPTER FOUR: RESULTS

4.1 Demographic data

Two hundred and ninety-two (n = 292) neonates met the inclusion criteria and participated in the research. The study was conducted in Chris Hani Baragwanath Academic hospital. The gender distribution was almost equal with a slight female majority (Figure 4.1). There were 142 (49%) male and 152 (51%) female neonates that participated in the study (Figure 4.1). The gestational age ranged between 37 to 42 weeks, with a median of 39 weeks (Figure 4.2). The neonatal age at the time of cardiac echo ranged between 12 hours to 216 hours with a Median of 31 hours. According to mode of delivery, 175 (59.9%) of the sample was born via C/S, 86 (29.5%) of which were females and 89 (30.5%) were males. There were 117 (40.1%) neonates who was born via normal vertex delivery (NVD) of which 64 (21.9%) were females and 53 (18.1%) were males (Figure 4.3).



M: Male, F: Female

Figure 4. 1 Gender distribution

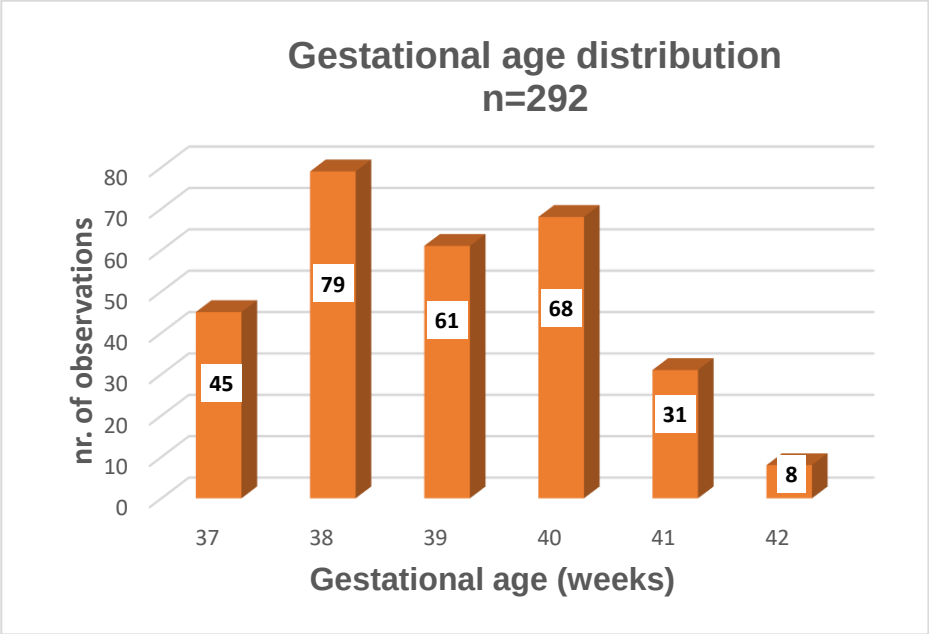
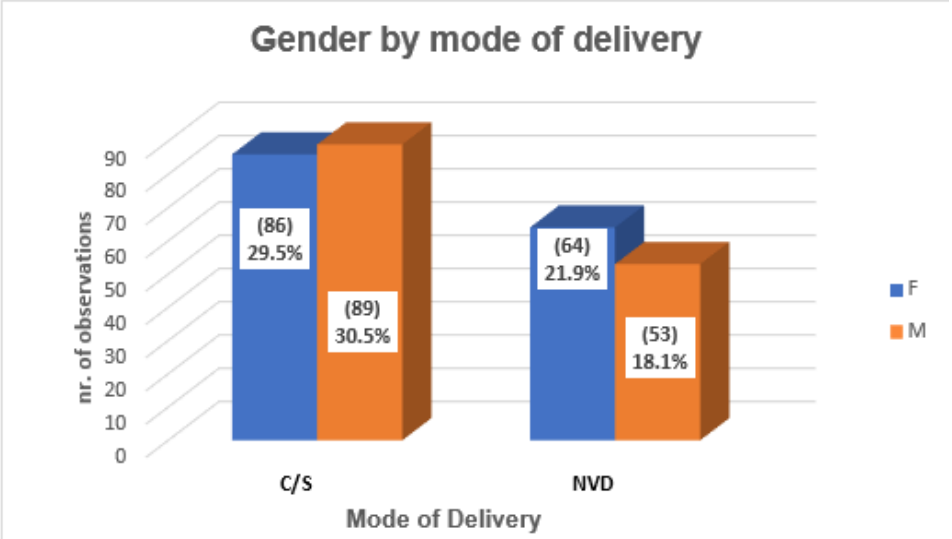


Figure 4. 2 Gestational age distribution.



Variable	Mean	Median	Minimum	Maximum	Std.Dev.
GESTATIONAL AGE (WEEKS)	38,9	39,0	37,0	42,0	1,3

M: Male, **F:** Female, **C/S:** Caesarean, **NVD**: Normal vertex delivery

Figure 4. 3 Gender by mode of delivery

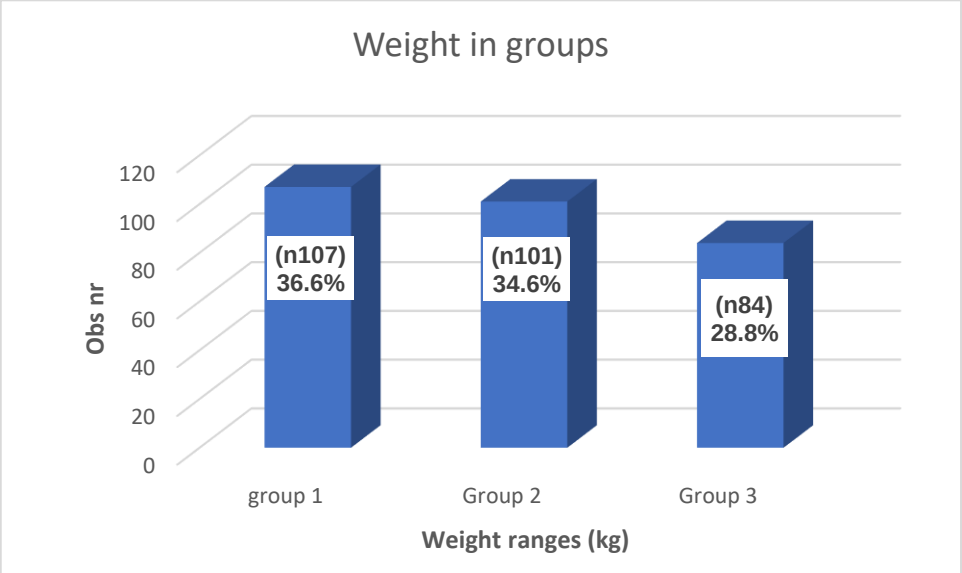
4.2 Population characteristics

The BSA for the study cohort ranged between a minimum of 0.16m² and a maximum of 0.25m², with a median of 0.2 m². The body weight (BW) ranged between a minimum of 2.5 kg and a maximum of 4.43kg, with a median of 3.12 kg. (Table 4.1). One hundred and seven (36.6%) of the population weighed between 2.5kg to 3kg, 101 (34.6%) weighed between 3.1 to 3.4kg and 84 (28.8%) weighed 3.5kg and more (Figure 4.4).

Table 4. 1 Population characteristics (n=292)

Variable	Mean	Median	Minimum	Maximum	Std.Dev
Birth weight (Kg)	3,2	3,1	2,5	4,4	0,4
Birth length (cm)	50,5	50,0	40,0	60,0	3,1
BSA m²	0,2	0,2	0,2	0,3	0,0
Age (hrs)	40,1	31,0	12,0	216,0	29,2

KG: *kilograms,* **CM:** *centimeter,* **BSA:** *Body surface area*



n: number of patients in the group/range, **1,0**: 2.5-3kg, **2,0**: 3.1-3.4kg, **3,0**:>=3.5kg

Figure 4. 4 Weight ranges

4.3 Effects of confounding factors

In the univariate regression analysis, birth weight (BW), birth length (BL), body surface area (BSA), gestational age (GA), mode of delivery (MOD), gender and age in hours were independent variables (predictor variables) as shown in Table 4.3. MV peak A (p=0.014), MV S' (p=0.040) and TAPSE (p=0.001) showed a significant increase when there was a corresponding increase in birth weight, whereas MV E/A ratio (p=0.011), showed a significant decrease when there was an increase in weight. TAPSE (p=0.009) also showed a significant increase with an increase in BL. MV peak E (p=0.035) and TAPSE (p=0.001) showed a significant increase with an increase in BSA. MV S' showed a significant increase in velocity with an increase in gestational age. TV peak E (p=0.023) showed an increase in the C-section group, whereas RV Tei (p=0.031) showed a significant decrease in the C-section group. Gender did not affect any of the variables. MV S' (p=0.027), TV peak A (p=0.017) and RV Tei (p=0.007) showed a significant increase with an increase in age in hours post-delivery (Table 4.3)

Table 4. 2 Univariate regression analysis of confounding factors

Co-founding factors														
Variables	BW (kg)		BH (cm)		BSA		GA		MOD		Gender		AGE	
	b	p-value	b	p-value	b	p-value	b	p-value	b	p-value	b	p-value	b	p-value
LV EF%	-2,985	0,401	0,124	0,408	129,337	0,266	-0,047	0,912	1,693	0,115	0,407	0,698	0,020	0,275
LV FS%	-2,021	0,521	-0,275	0,383	97,879	0,342	-0,037	0,923	1,401	0,141	0,215	0,817	0,018	0,245
MV PEAK E	-0,023	0,610	-0,001	0,888	0,836	0.035	0,007	0,197	0,006	0,663	-0,011	0,420	0,000	0,421
MV PEAK A	0,045	0,014	0,000	0,964	-0,283	0,858	-0,005	0,436	0,019	0,206	-0,023	0,110	0,000	0,489
MV E/A RATIO	-0,210	0,011	-0,008	0,342	5,274	0,052	0,020	0,049	-0,038	0,125	0,029	0,250	0,000	0,728
MV E'	-0,001	0,764	0,000	0,616	0,081	0,619	0,000	0,653	0,000	0,893	-0,002	0,290	0,000	0,675
MV E/E'	-0,034	0,974	0,025	0,812	6,849	0,843	0,067	0,599	-0,107	0,740	-0,105	0,739	0,005	0,339
MV S'	0,002	0,040	0,000	0,537	0,030	0,783	0,001	0,004	0,000	0,745	0,000	0,648	0,000	0,027
LV Tei	0,040	0,474	-0,002	0,722	-0,708	0,697	0,005	0,462	-0,004	0,826	-0,003	0,846	0,000	0,136
TAPSE	0,875	0,001	0,087	0,009	20,879	0,001	0,003	0,973	-0,020	0,930	-0,175	0,414	0,000	0,960
TV PEAK E	-0,037	0,492	-0,010	0,075	2,291	0,194	0,000	0,956	0,040	0,023	0,003	0,835	0,000	0,099
TV PEAK A	-0,005	0,918	-0,010	0,087	1,279	0,471	-0,011	0,096	0,033	0,064	0,003	0,844	0,001	0,017
TV E/A RATIO	-0,065	0,440	-0,002	0,856	2,293	0,417	0,018	0,099	0,022	0,442	0,013	0,636	0,000	0,683
TV E'	0,005	0,593	0,000	0,741	0,142	0,632	-0,002	0,109	-0,003	0,322	-0,003	0,360	0,000	0,405
TV E/E'	-0,912	0,292	-0,116	0,199	27,793	0,331	0,098	0,380	0,481	0,098	0,111	0,687	0,007	0,089
TV S'	-0,013	0,498	-0,001	0,657	0,377	0,562	-0,004	0,159	0,008	0,241	0,004	0,468	0,000	0,821
RV Tei	0,014	0,841	-0,009	0,226	2,540	0,277	0,004	0,706	-0,053	0,031	-0,031	0,178	-0,001	0,007

b: beta coefficient**, BW:** body weight, **BH**: birth length, **BSA:** body surface area, **GA:** gestational age, **MOD:** mode of delivery, **AGE:** age in hours. **LV EF**: Left ventricular ejection fraction, **LV FS**: Left ventricular fraction shortening, **MV peak E:** Mitral valve - early diastolic filling measured by pulsed Doppler, **MV Peak A**: Mitral valve - late diastolic filling measured by pulsed Doppler, **MV E/A:** Mitral valve- early diastolic filling/late diastolic filling ratio, **TDI MV E'**: Mitral valve - peak myocardial velocity in early diastole measured by TDI, **MV E/E':** Mitral valve- early diastolic filling/Peak myocardial velocity in early diastole ratio, **TDI MV S'**: Mitral valve - Systolic wave representing peak myocardial systolic velocity at the septal MV annulus measured by TDI, **LV Tei**: Myocardial performance index, **TAPSE**: Tricuspid annular plane excursion,**TV peak E:** Tricuspid valve - early diastolic filling measured by pulsed Doppler, **TV Peak A**: Tricuspid valve - late diastolic filling measured by pulsed Doppler, **TV E/A**: Tricuspid valve – early diastolic filling/ late diastolic filling ratio, **TDI TV E'**: Tricuspid valve – Peak myocardial velocity in early diastole measured by TDI, **TV E/E':** Tricuspid valve – early diastolic filling/ Peak myocardial velocity in early diastole ratio, **TDI TV S'**: Tricuspid valve - Systolic wave representing peak myocardial systolic velocity at the lateral TV annulus measured by TDI, **RV Tei:** Myocardial performance index, **Std.Dev**: Standard deviation.

4.4 Inter-observer variability

Inter-observer variability was calculated using intraclass correlation coefficient. Measurements were performed by an independent experienced paediatric cardiologist and a senior cardiac technologist (myself) and compared, (Table 4.3) depicts the differences between the first set of inter-observer analysis versus the second set. Most of the measurements between the observers showed a significant strong similarity/relationship except for MV E/A ratio, LV Tei and TV S' which had an r value of less than moderate similarity/relationship.

Table 4. 3 Inter observer variability

Measurements	ICC
LV EF%	0,678
LV FS%	0,657
LA/AO RATIO	0,600
MV PEAK E	0,913
MV PEAK A	0,900
MV E/A RATIO	0,316
MV E'	0,756
MV E/E'	0,763
MV S'	0,711
LV Tei	0,410
TV PEAK E	0,953
TV PEAK A	0,944
TV E/A RATIO	0,857
TV E'	0,922
TV E/E'	0,876
TV S'	0,325
RV Tei	0,635

ICC: Intraclass correlation coefficient, **LV EF**: Left ventricular ejection fraction, **LV FS**: Left ventricular fraction shortening, **LA/AO ratio**: Left atrium/aorta ratio, **MV peak E:** Mitral valve - early diastolic filling measured by pulsed Doppler, **MV Peak A**: Mitral valve - late diastolic filling measured by pulsed Doppler, **MV E/A:** Mitral valve- early diastolic filling/late diastolic filling ratio, **TDI MV E'**: Mitral valve - peak myocardial velocity in early diastole measured by TDI, **MV E/E':** Mitral valve- early diastolic filling/Peak myocardial velocity in early diastole ratio, **TDI MV S'**: Mitral valve - Systolic wave representing peak myocardial systolic velocity at the septal MV annulus measured by TDI, **LV Tei**: Myocardial performance index, **TV peak E:** Tricuspid valve - early diastolic filling measured by pulsed Doppler, **TV Peak A**: Tricuspid valve - late diastolic filling measured by pulsed Doppler, **TV E/A**: Tricuspid valve – early diastolic filling/ late diastolic filling ratio, **TDI TV E'**: Tricuspid valve – Peak myocardial velocity in early diastole measured by TDI, **TV E/E':** Tricuspid valve – early diastolic filling/ Peak myocardial velocity in early diastole ratio, **TDI TV S'**: Tricuspid valve - Systolic wave representing peak myocardial systolic velocity at the lateral TV annulus measured by TDI, **RV Tei:** Myocardial performance index

4.5 Echocardiographic measurements

4.5.1 M-mode measurements

Left ventricular function measurements included systolic assessment using ejection and shortening fraction which was derived from M-mode measurements. Out of the 292 participants 291 had LV EF% and LV FS% measurements with LV EF% ranging from 51% to 95% with a mean of 73.56% and a median of 74%. LV FS% ranged from 24% to 65% with a mean of 40.3% and a median of 40% (Table 4.4).

Table 4. 4 Left heart M-mode measurements

	Left heart measurements					
Variable	**Valid N**	**Mean**	**Median**	**Minimum**	**Maximum**	**Std.Dev.**
LV EF%	291	73,6	74,0	51,0	95,0	8,9
LV FS%	291	40,2	40,0	24,0	65,0	7,9

LV EF: Left ventricular ejection fraction, **LV FS**: Left ventricular fraction shortening. left heart, **Std.Dev**: Standard deviation

There was no correlation between LV EF (r = 0.029), LV FS (r = 0.027) and birth weight. Scatter plots and predicted Z score boundaries are shown in (Figure 4.5 and Figure 4.6). Most (LV EF 96.6%, LV FS 95.5%) of the population clustered within the -2 and +2 Z-Score.

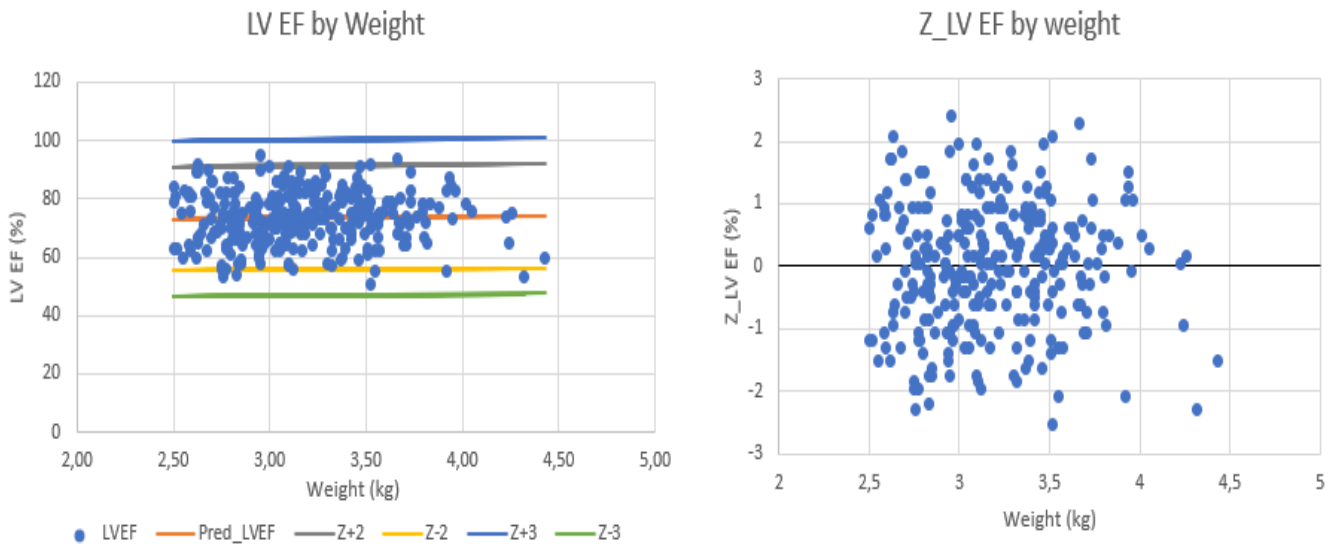


Figure 4. 5 LV EF scatterplots and Z-score boundaries.

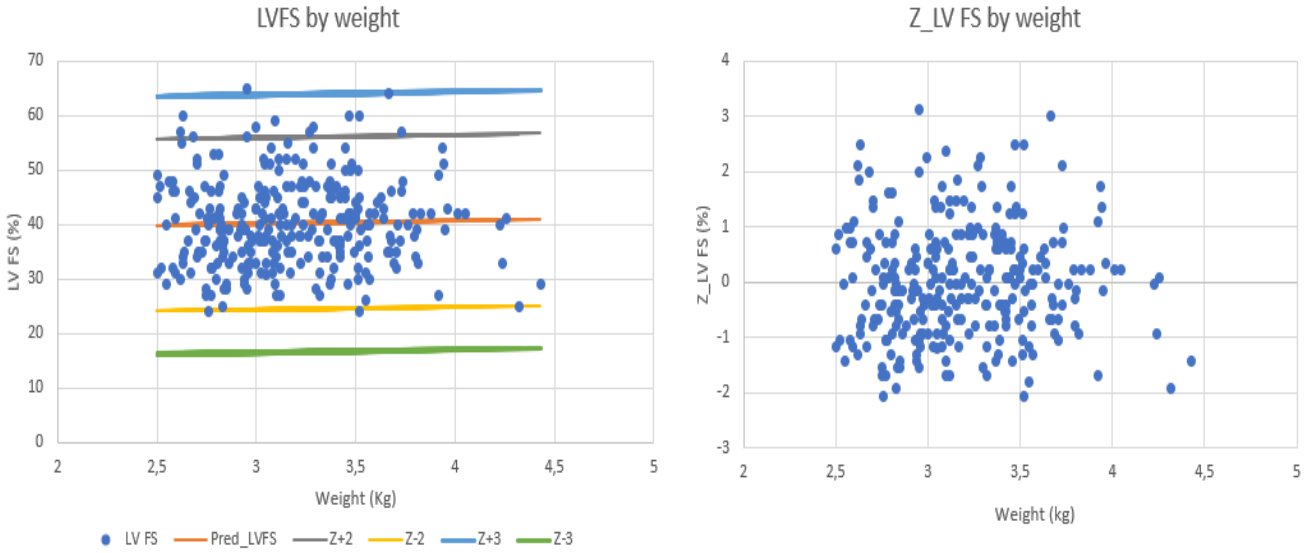


Figure 4. 6 LV FS scatterplots and Z-score boundaries

TAPSE was used as a measure of right ventricular longitudinal systolic function. The TAPSE was determined in 152 participants, ranging from 4.8mm to 11.4mm with a mean of 7.5mm and a median of 7.5mm (Table 4.5).

Table 4. 5 Right heart M-mode measurements

	Right Heart measurements					
Variable	**Valid N**	**Mean**	**Median**	**Minimum**	**Maximum**	**Std.Dev.**
TAPSE (mm)	152	7,5	7,5	4,8	11,4	1,3

TAPSE: Tricuspid annular plane excursion, **Std.Dev**: Standard deviation

TAPSE displayed a weak to moderate positive correlation (r = 0,277), with birthweight. A scatterplot and predicted Z score boundaries are shown in (Figure 4.7), with most (96.1%) observations plotting between the -2 and +2 Z-scores.

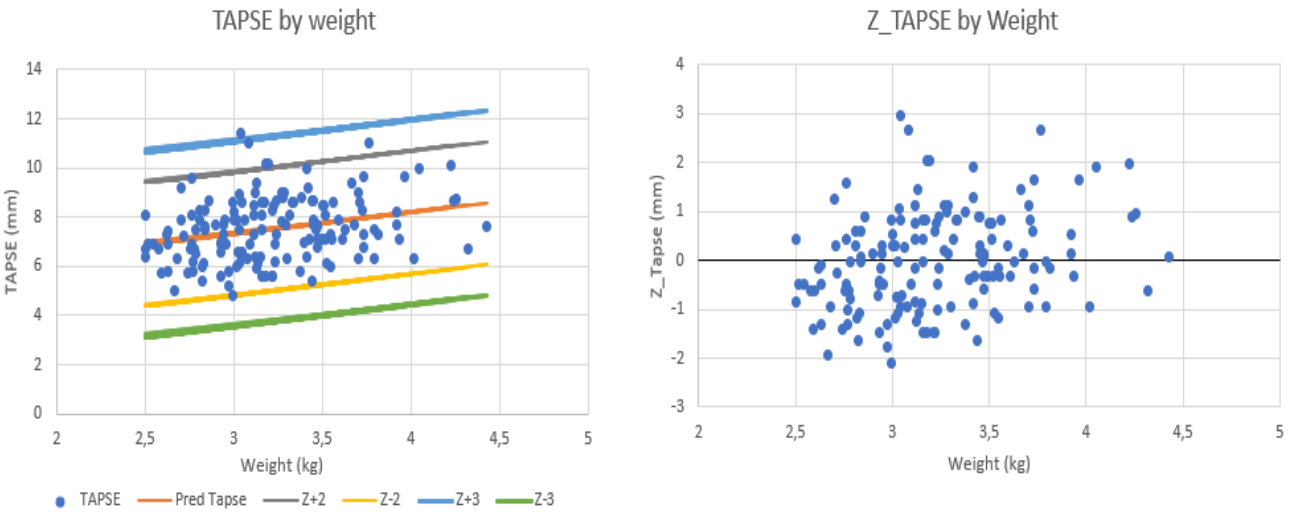


Figure 4. 7 TAPSE scatterplots and Z-score boundaries.

4.5.2 Doppler measurements

4.5.2.1 The left heart

Doppler echocardiography was used to determine left ventricular diastolic function, Table 4.6 displays the measurements' characteristics. The majority of the LV measurements showed no correlation with birth weight.

Table 4. 6 Left heart Doppler measurements

	Left heart measurements					
Variable	**Valid N**	**Mean**	**Median**	**Minimum**	**Maximum**	**Std.Dev.**
MV PEAK E (m/s)	292	0,583	0,585	0,290	0,900	0,113
MV PEAK A (m/s)	292	0,592	0,580	0,340	1,100	0,123
MV E/A RATIO	292	1,011	1,000	0,455	1,686	0,210
MV E' (m/s)	289	0,058	0,060	0,040	0,110	0,012
MV E/E' ratio	289	10,375	10,000	4,833	18,250	2,653
MV S' (m/s)	289	0,052	0,050	0,030	0,090	0,009
LV Tei	283	0,306	0,300	0,040	0,930	0,139

MV peak E: Mitral valve - early diastolic filling measured by pulsed Doppler, **MV Peak A**: Mitral valve - late diastolic filling measured by pulsed Doppler, **MV E/A:** Mitral valve- early diastolic filling/late diastolic filling ratio, **TDI MV E'**: Mitral valve - peak myocardial velocity in early diastole measured by TDI, **MV E/E':** Mitral valve- early diastolic filling/Peak myocardial velocity in early diastole ratio, **TDI MV S'**: Mitral valve - Systolic wave representing peak myocardial systolic velocity at the septal MV annulus measured by TDI, **LV Tei**: Myocardial performance index.

There was no correlation between MV peak E (r = 0.09) and birth weight. Scatter plots and predicted Z score boundaries are shown in (Figure 4.8). Most (96%) of the population plotted between the -2 and +2 Z-score.

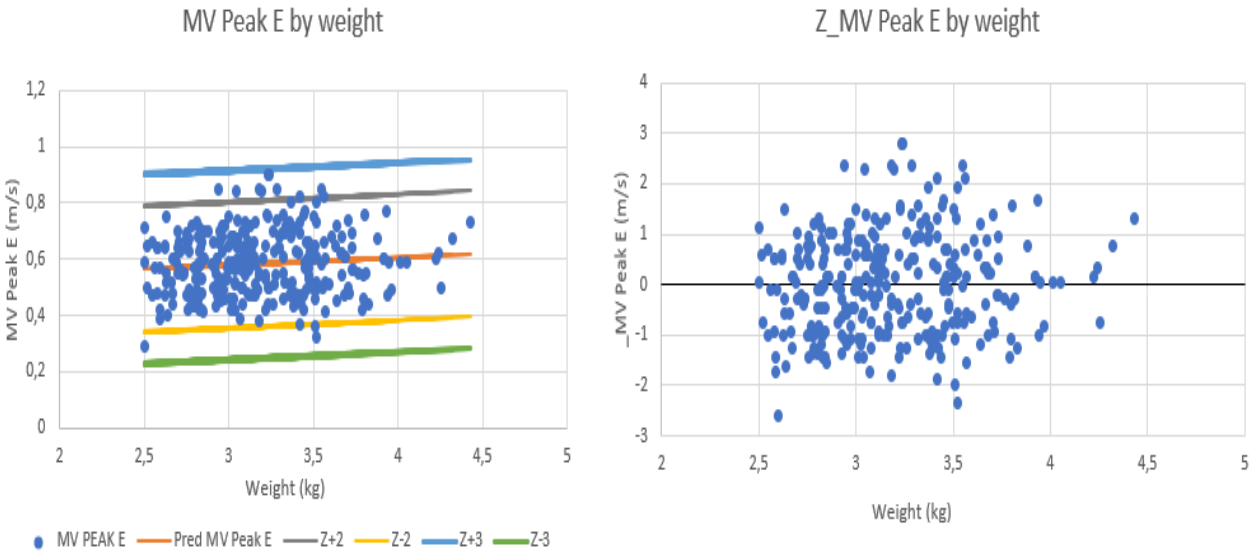


Figure 4. 8 MV Peak E scatterplot and Z-score boundaries

The MV peak A (r=0.142) showed a very weak positive correlation with weight. Scatter plots and predicted Z score boundaries are shown in (Figure 4.9). Most (95%) of the population plotted between the -2 and +2 Z-scores.

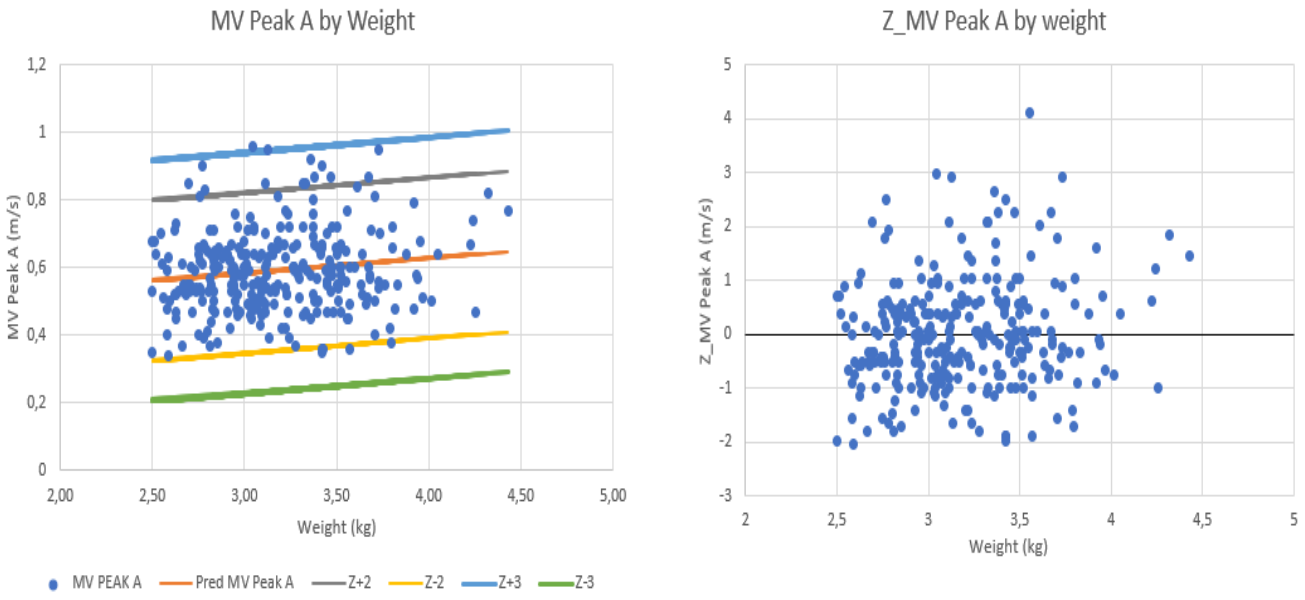


Figure 4. 9 MV Peak A scatterplot and Z-score boundaries

Scatter plots and predicted Z-score boundaries are shown in figure 4.10 to 4.12 for MV E/A, MV E' and MV E/E'. Most (MV E/A: 95%, MV E' 96% and MV E/E' 95%) of the population plotted between -2 and +2 Z-scores. The MV E' (figure 4.11) and MV S' (figure 4.13) was captured into 2 decimal places, only 7 to 8 distinct MV E' and MV S' values were captured therefore resulting in a very linear outcome of the scatterplots unlike the other variables which had a very wide range between them.

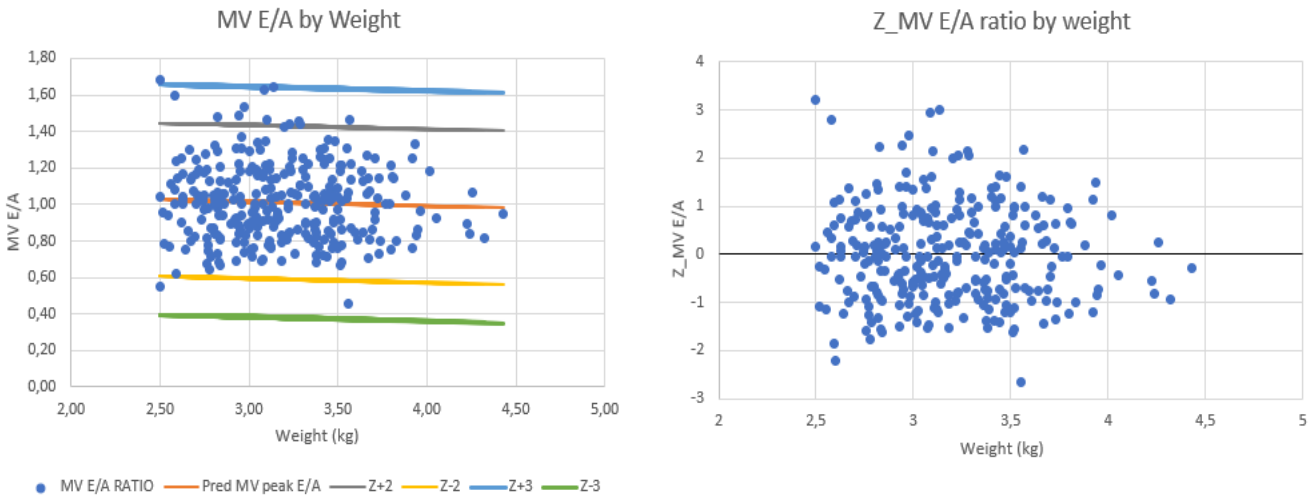


Figure 4. 10 MV E/A scatterplot and Z-score boundaries

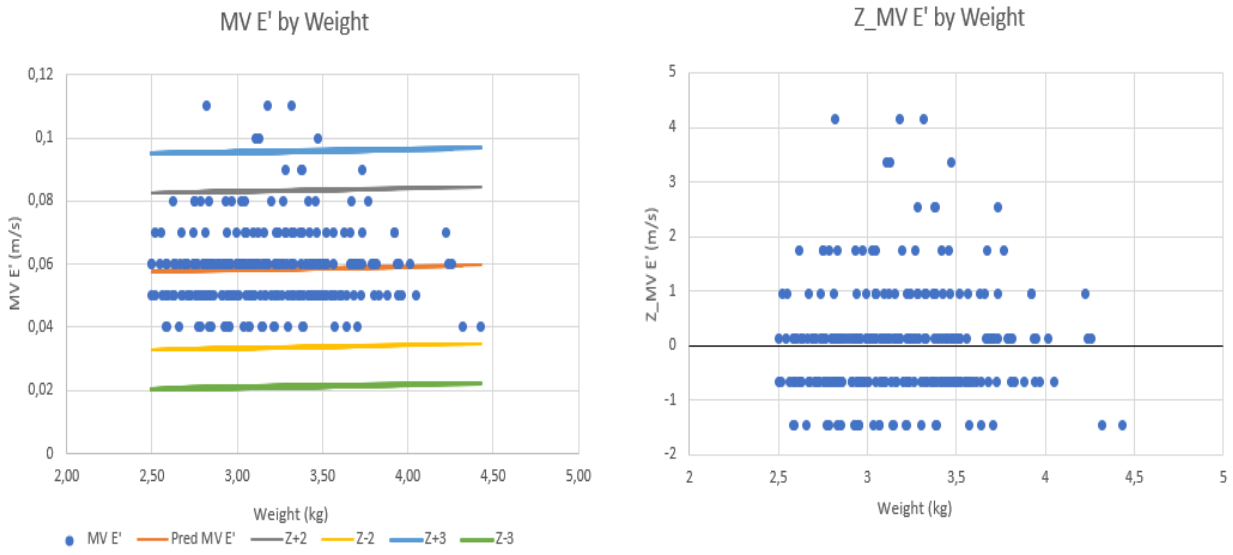


Figure 4. 11 MV E' scatterplot and Z-score boundaries

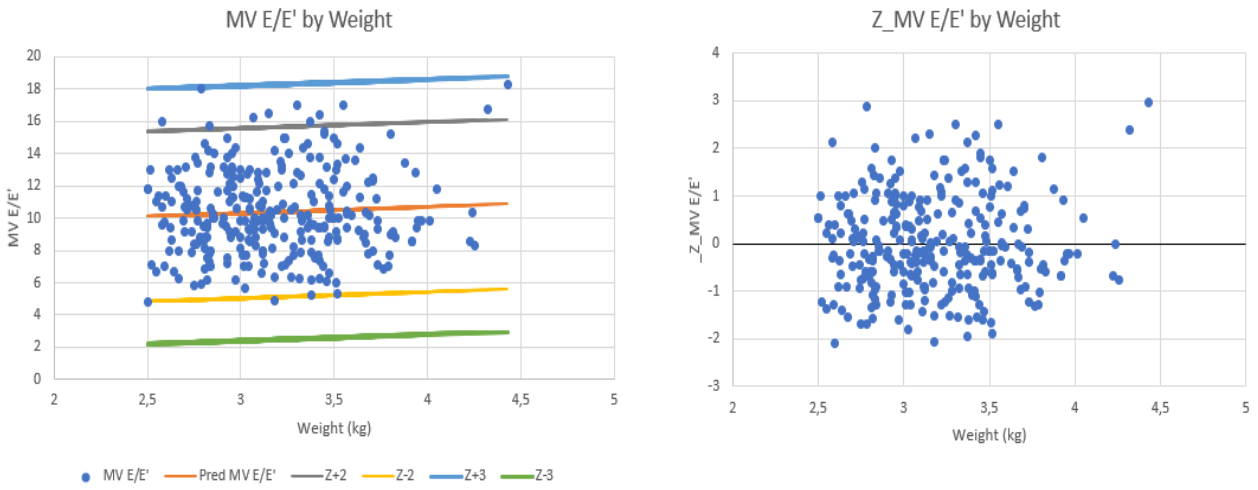


Figure 4. 12 MV E/E' scatterplot and Z-score boundaries

MV S' (r=0.119) showed a very weak positive correlation with weight. Scatter plots and predicted Z score boundaries are shown in (Figure 4.13). Most (95%) of the population plotted between the -2 and +2 Z-scores.

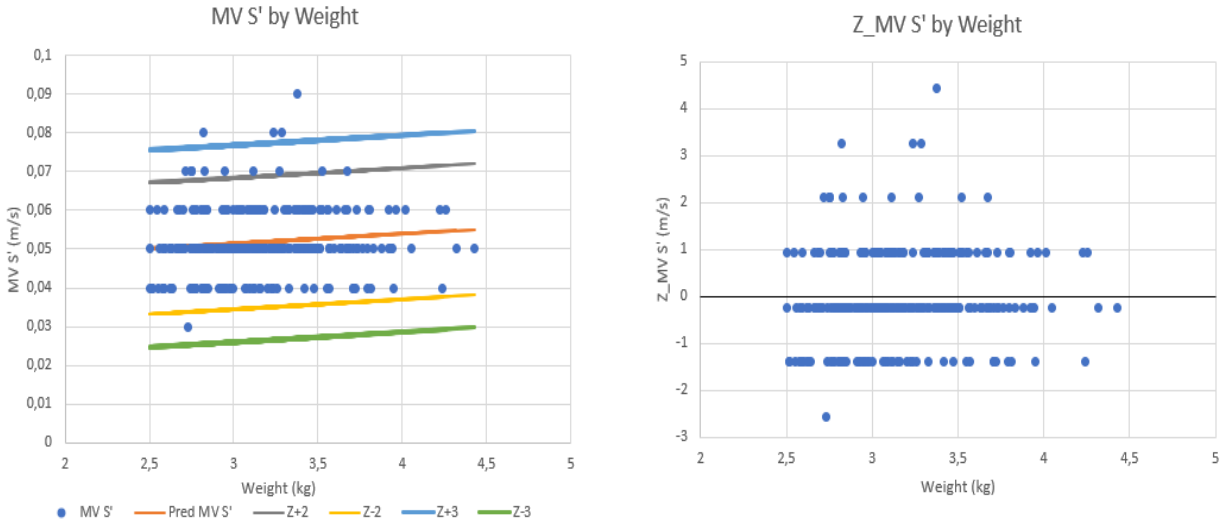


Figure 4. 13 MV S' scatterplot and Z-score boundaries

LV Tei (r=0.035) showed no correlation with weight. Scatter plots and predicted Z score boundaries are shown in (Figure 4.14). Most (93%) of the population plotted between -2 and +2 Z-scores.

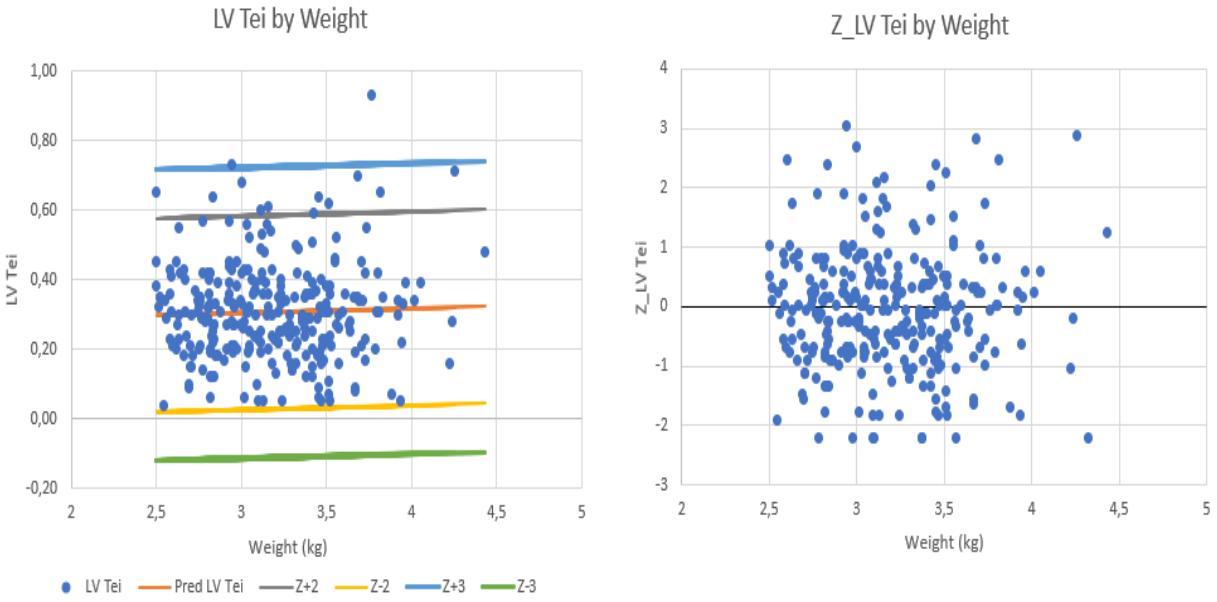


Figure 4. 14 LV Tei scatterplot and Z-score boundaries

4.5.2.2 The right heart

Doppler and tissue Doppler echocardiography were used to assess right ventricular diastolic function (Table 4.8) The majority of the parameters did not show any correlation with birth weight except for TV E' (r=0.12) and RV Tei (r=0.113) which showed weak correlations. Scatter plots and predicted Z score boundaries are shown in Figures 4.15 to Figure 4.21. The TV E' (figure 4.18) and TV S' (figure 4.20) was captured into 2 decimal places, only 6 to 7 distinct TV E' and TV S' values were captured therefore resulting in a very linear outcome of the scatterplots unlike the other variables which had a very wide range between them.

Table 4. 7 Right heart Doppler measurements

Variable	Right Heart measurements Valid N	Mean	Median	Minimum	Maximum	Std.Dev.
TV PEAK E (m/s?)	226	0,512	0,495	0,260	1,060	0,126
TV PEAK A (m/s?)	226	0,616	0,615	0,360	1,170	0,127
TV E/A RATIO	226	0,845	0,807	0,480	1,980	0,199
TV E' (m/s?)	214	0,079	0,080	0,040	0,190	0,021
TV E/E' RATIO	214	6,783	6,424	2,786	15,800	2,016
TV S' (m/s)	215	0,071	0,070	0,030	0,700	0,045
RV Tei	205	0,283	0,260	0,020	0,730	0,132

Tricuspid annular plane excursion,**TV peak E:** Tricuspid valve - early diastolic filling measured by pulsed Doppler, **TV Peak A**: Tricuspid valve - late diastolic filling measured by pulsed Doppler, **TV E/A**: Tricuspid valve – early diastolic filling/ late diastolic filling ratio, **TDI TV E'**: Tricuspid valve – Peak myocardial velocity in early diastole measured by TDI, **TV E/E':** Tricuspid valve – early diastolic filling/ Peak myocardial velocity in early diastole ratio, **TDI TV S'**: Tricuspid valve - Systolic wave representing peak myocardial systolic velocity at the lateral TV annulus measured by TDI, **RV Tei:** Myocardial performance index, **Std.Dev**: Standard deviation.

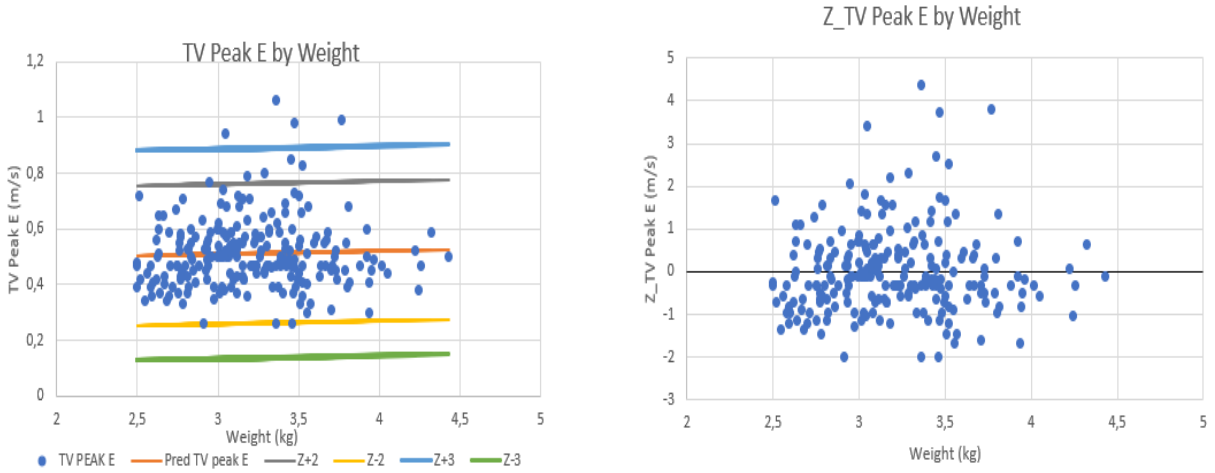


Figure 4. 15 TV Peak E scatterplot and Z-score boundaries

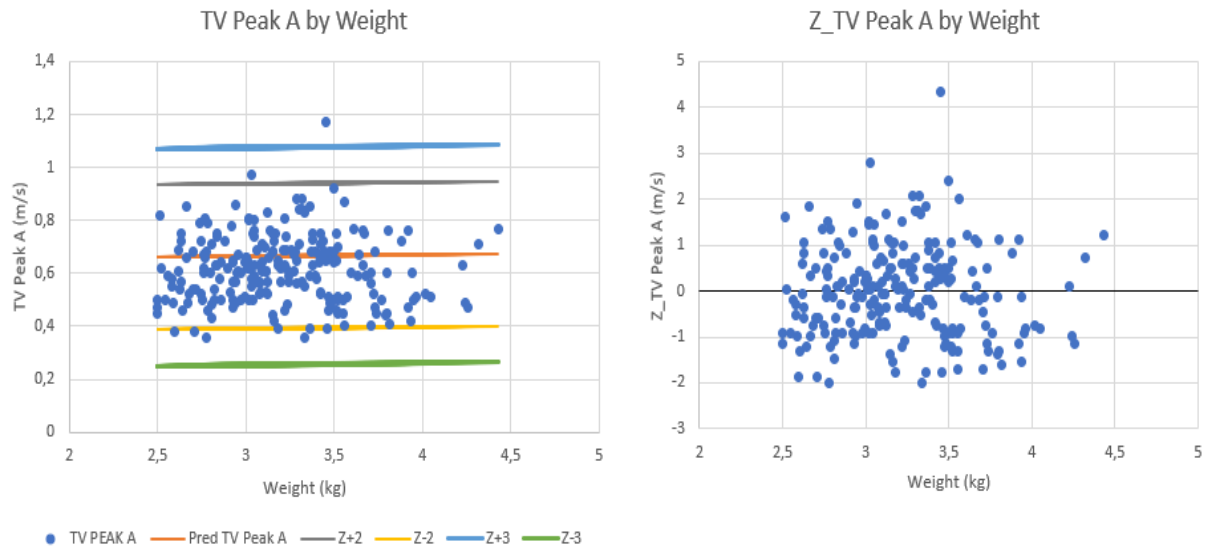


Figure 4. 16 TV Peak A scatterplot and Z-score boundaries

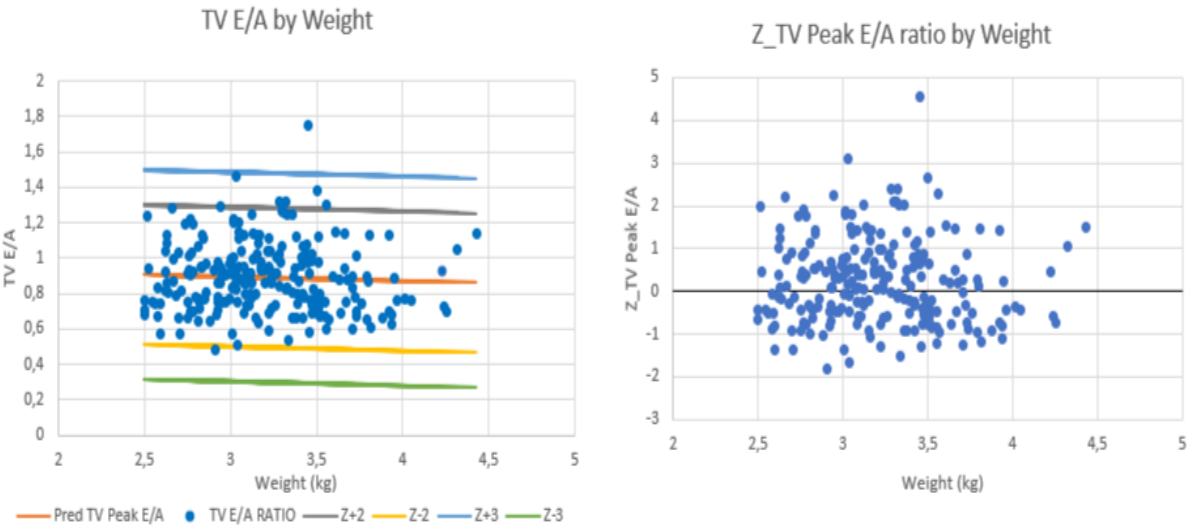


Figure 4. 17 TV E/A scatterplot and Z-score boundaries

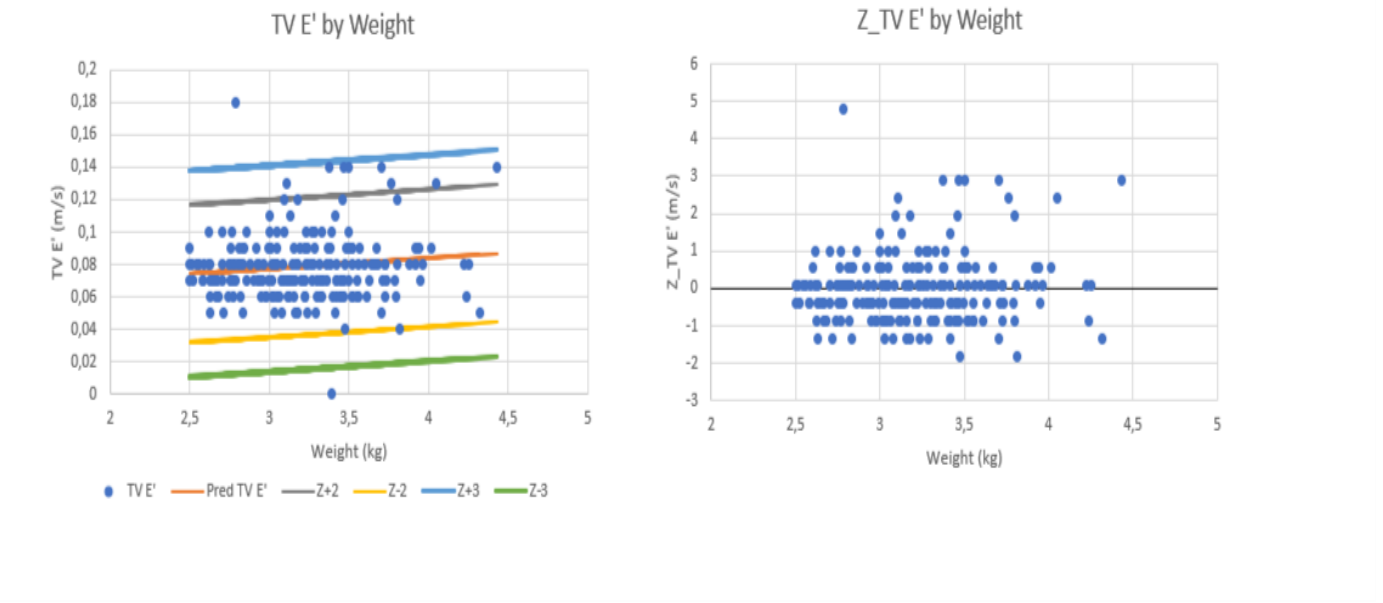


Figure 4. 18 TV E' scatterplot and Z-score boundaries

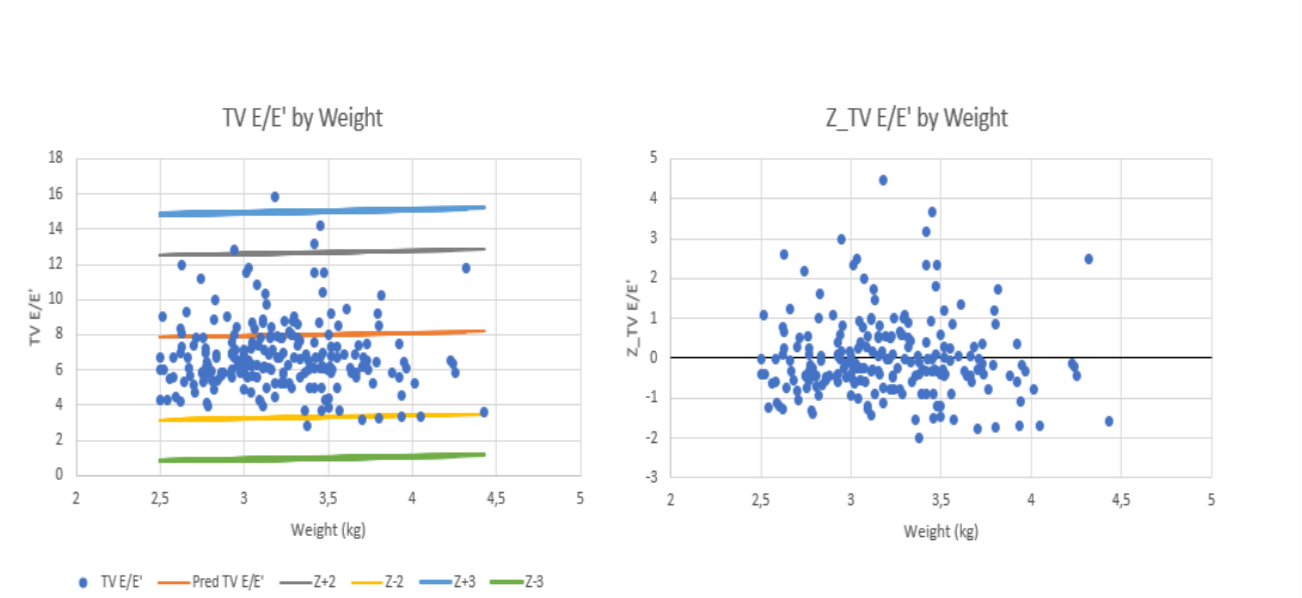


Figure 4. 19 TV E/E' scatterplots and Z-score boundaries

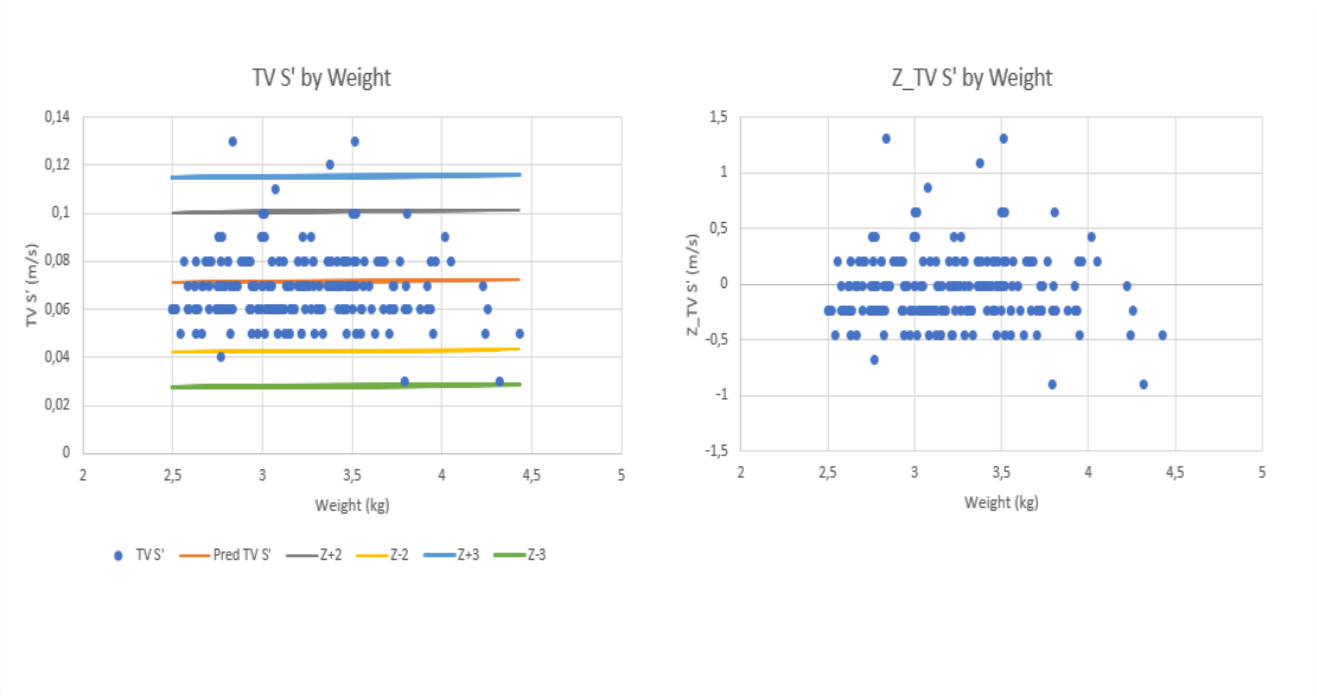


Figure 4. 20 TV S' Scatterplot and Z-score boundaries

RV Tei (r= 0.113) showed a very weak but positive correlation with birth weight. Scatter plots and predicted Z score boundaries are shown in (4.21). Most (99%) of the population plotted between -2 and +2 Z-scores.

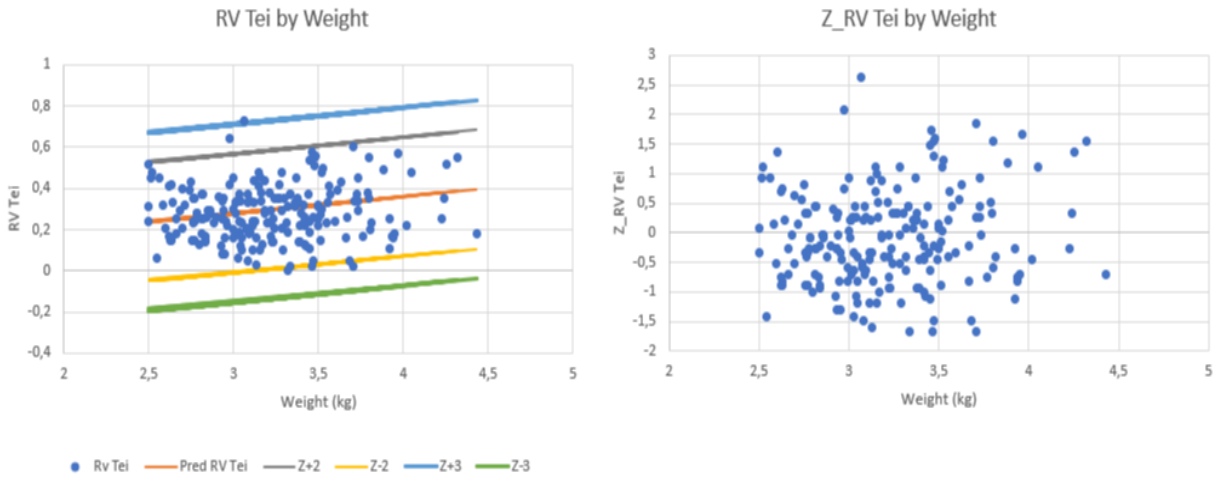


Figure 4. 21 RV Tei scatterplot and Z-score boundaries

4.6 Cardiac findings

A large number of neonates were found to have clinically insignificant expected cardiac lesions (Figure 4.22). The most common findings consisted of the PDA and PFO (n=111). There were 38% (n=111) with PDA and PFO, 23% (n=66) with PDA only, 20% (n=58) with PFO only, and 20% (n=57) with no cardiac lesion.

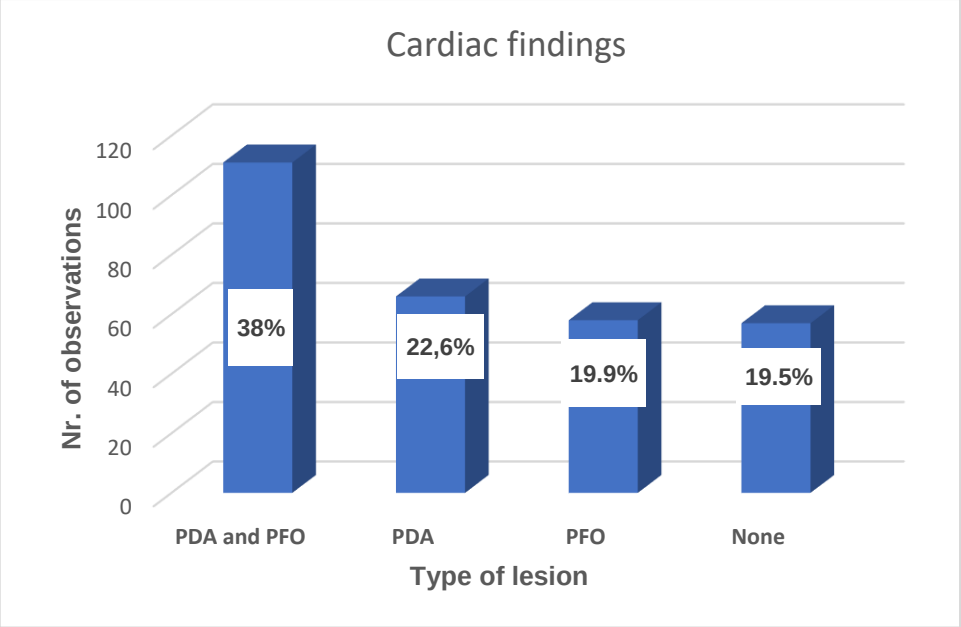


Figure 4. 22 Cardiac findings

4.7 Left and right heart systolic and diastolic parameters displayed for use in the clinical setting

Left heart and right heart measurements for clinical use are displayed in Table 4.9 and Table 4.10. Standard deviation/z- score of -3 and +3 is included.

Table 4. 8 Left heart systolic and diastolic measurements for clinical use

Predicted values (mean +- SD) variables expressed by weight			
Variables	**Weight group 1 2,5-3kg**	**Weight group 2 3,1-3,4kg**	**Weight group 3 =>3,5kg**
+3 SD	101,881	99,103	100,133
+2 SD	92,354	90,592	91,384
LV EF%	**73,300**	**73,572**	**73,886**
-2 SD	54,246	56,551	56,387
-3 SD	44,720	48,040	47,638
+3 SD	65,099	62,911	63,971
+2 SD	56,742	55,358	56,152
LV FS%	**40,028**	**40,253**	40,514
-2 SD	23,314	25,148	24,875
-3 SD	14,957	17,596	17,056
+3 SD	1,994	**2,118**	1,972
+2 SD	1,781	1,867	1,772
LA/AO RATIO	**1,356**	**1,364**	**1,373**
-2 SD	0,931	0,861	0,974
-3 SD	0,718	0,610	0,775
+3 SD	0,876	**0,949**	0,940
+2 SD	0,775	0,827	0,826
MV PEAK E (m/s)	**0,572**	**0,583**	0,597
-2 SD	0,370	0,340	0,367
-3 SD	0,268	0,218	0,253
+3 SD	0,889	0,970	1,026
+2 SD	0,784	0,844	0,889
MV PEAK A (m/s)	**0,574**	**0,592**	**0,614**
-2 SD	0,364	0,341	0,339
-3 SD	0,259	0,215	0,201

LV EF: Left ventricular ejection fraction (%), **LV FS**: Left ventricular fraction shortening (%), **LA/AO ratio**: Left Atrium/Aorta ratio **MV peak E:** Mitral valve - early diastolic filling, measured by pulsed Doppler (m/s), **MV Peak A**: Mitral valve - late diastolic filling, measured by pulsed Doppler (m/s).

Table 4. 8 Left heart measurements for clinical use continued

Predicted values (mean +- SD) variables expressed by weight			
Variables	**Weight group 1 2,5-3kg**	**Weight group 2 3,1-3,4kg**	**Weight group 3 =>3,5kg**
+3 SD	**1,672**	**1,655**	1,593
+2 SD	1,455	1,440	1,396
MV PEAK E/A ratio	**1,021**	**1,011**	**1,000**
-2 SD	0,587	0,582	0,604
-3 SD	0,370	0,367	0,406
+3 SD	0,092	0,109	0,098
+2 SD	0,081	0,092	0,085
MV E' (m/s)	**0,058**	**0,058**	**0,059**
-2 SD	0,035	0,025	0,033
-3 SD	0,024	0,008	0,020
+3 SD	**17,898**	18,373	18,888
+2 SD	15,340	15,709	16,114
MV E/E' ratio	**10,222**	**10,381**	**10,567**
-2 SD	5,104	5,052	5,019
-3 SD	2,546	2,388	2,245
+3 SD	0,079	**0,078**	**0,075**
+2 SD	0,069	0,069	0,068
MV S' (m/s)	**0,051**	**0,052**	**0,053**
-2 SD	0,032	0,034	0,039
-3 SD	0,023	0,026	0,031
+3SD	0,697	**0,703**	0,827
+2 SD	0,565	0,571	0,656
LV Tei	**0,301**	**0,306**	**0,313**
-2 SD	0,037	0,042	-0,031
-3 SD	-0,094	-0,090	-0,202

MV E/A: Mitral valve- early diastolic filling/late diastolic filling (ratio), **TDI MV E'**: Mitral valve - peak myocardial velocity in early diastole measured by TDI (m/s), **MV E/E':** Mitral valve- early diastolic filling/Peak myocardial velocity in early diastole ratio, **TDI MV S'**: Mitral valve - Systolic wave representing peak myocardial systolic velocity at the septal MV annulus, measured by TDI (m/s), **LV Tei**: Myocardial performance index**.**

Table 4. 9 Right heart measurements for clinical use

Predicted values (mean +- SD) variables expressed by weight			
Variables	**Weight group 1 2,5-3kg**	**Weight group 2 3,1-3,4kg**	**Weight group 3 =>3,5kg**
+3 SD	10,431	11,870	11,492
+2 SD	9,333	10,405	10,304
TAPSE	**7,136**	**7,475**	**7,928**
-2 SD	4,940	4,544	5,552
-3 SD	3,842	3,079	4,364
+3 SD	0,813	0,903	0,947
+2 SD	0,711	0,772	0,804
TV PEAK E	**0,507**	**0,512**	**0,518**
-2 SD	0,302	0,251	0,232
-3 SD	0,200	0,121	0,089
+3 SD	1,052	**1,035**	1,147
+2 SD	0,922	0,912	0,988
TV PEAK A	**0,663**	**0,666**	**0,670**
-2 SD	0,403	0,420	0,351
-3 SD	0,273	0,296	0,192
+3 SD	**1,435**	1,503	1,510
+2 SD	1,257	1,299	1,300
TV PEAK E/A	**0,901**	**0,891**	**0,879**
-2 SD	0,545	0,483	0,459
-3 SD	0,367	0,279	0,248

TAPSE: Tricuspid annular plane excursion (mm), **TV peak E:** Tricuspid valve - early diastolic filling measured by pulsed Doppler (m/s), **TV Peak A**: Tricuspid valve - late diastolic filling, measured by pulsed Doppler (m/s), **TV E/A**: Tricuspid valve – early diastolic filling/ late diastolic filling ratio.

Table 4.9 Right heart measurements for clinical use continued

Predicted values (mean +- SD) variables expressed by weight			
Variables	**Weight group 1 2,5-3kg**	**Weight group 2 3,1-3,4kg**	**Weight group 3 =>3,5kg**
+3SD	0,136	0,134	0,166
+2 SD	0,116	0,116	0,138
TV E' (m/s)	**0,076**	**0,079**	**0,082**
-2 SD	0,036	0,042	0,026
-3 SD	0,016	0,023	-0,002
+3 SD	**14,260**	14,667	**16,052**
+2 SD	12,133	12,430	13,383
TV E/E' ratio	**7,880**	**7,955**	**8,044**
-2 SD	3,626	3,480	2,706
-3 SD	1,499	1,242	0,036
+3 SD	0,112	0,114	0,120
+2 SD	0,285	0,224	0,234
TV S' (m/s)	**0,115**	**0,118**	**0,122**
-2 SD	-0,054	0,012	0,009
-3 SD	0,030	0,029	0,024
+3 SD	**0,647**	0,789	**0,994**
+2 SD	0,518	0,624	**0,774**
RV Tei	**0,261**	**0,294**	**0,334**
-2 SD	0,003	-0,036	-0,106
-3 SD	-0,126	-0,201	-0,326

TDI TV E': Tricuspid valve – Peak myocardial velocity in early diastole, measured by TDI (m/s), **TV E/E':** Tricuspid valve – early diastolic filling/ Peak myocardial velocity in early diastole (ratio) **TDI TV S':** Tricuspid valve - Systolic wave representing peak myocardial systolic velocity at the lateral TV annulus, measured by TDI (m/s), **RV Tei:** Myocardial performance index.

CHAPTER FIVE: DISCUSSION

The aim of the study was to establish reference values for systolic and diastolic function in the term newborn infants. Normal values are required in order to interpret the presence of deviations that may indicate pathology or changes that may be positive or negative responses to treatment (Lai et al. 2006).

There has been an increased interest in the assessment of ventricular systolic and diastolic function in neonates of which pulsed Doppler and tissue Doppler imaging are an integral component of this appraisal. However, the strength and limitations of published paediatric nomograms for echocardiographic functional parameters have not been critically evaluated, especially in the neonatal population (Cantinotti and Lopez 2013) and in particular, the African neonatal population. Regardless of the importance of these measurements in practice, an all-inclusive set of normative data to assist in the evaluation of myocardial function of neonates in the sub Saharan region has not been established up to now (Majonga et al 2018 and Lopez et al 2017).

The definition of what is “normal” varies widely according to age, body surface area (BSA), gender and race. These changes may be more apparent in paediatrics as cardiac chamber dimensions change with somatic growth (The EchoNoRMAL study 2018 and Majonga 2018). Therefore, it is imperative to normalize echocardiographic measurements according to body size.

There are several echocardiographic references that have been published, but the majority are derived from North America and the European populations. The study cohorts from which most of the current data is obtained is mostly derived from Caucasian and some from Asian populations. The information derived from these studies may not be applicable to other populations since a few studies established that ethnicity is a significant determinant of cardiac chamber sizes and hence, it is highly recommended to use ethnic-specific reference values for echocardiographic interpretations (The EchoNoRMAL study 2015; Lang et al. 2015; Badano 2014; Bansal, Mohan and Sengupta 2016; Majonga et al. 2017; Majonga 2018 and Cantinotti and Lopez 2013). Furthermore, study methodology varies widely, for example many studies had small sample sizes, heterogenous methodologies and the use of variable body size

parameters and regression equations which have resulted in a wide range of Z-scores for a single measurement (Lopez: 2017 and Majonga et al. 2018).

This study aimed to cover the gap in the lack of global cardiac function nomograms and in particular the sub-Saharan African region neonate. To our knowledge, the study represents the first such study from the Sub Saharan region showing normal values for diastolic in-flow and longitudinal movement of the full-term neonatal heart. The study population included 292 African (Black) neonatal participants. This cohort represents the largest studied neonatal population recorded thus far. Most other publications include a wide age range of children up to 17 years in some studies, with only a small number of neonates In the present study, of the total of 292 neonates, n=142(49%) were males and n=150 (51%) were females (Figure 4.1 in chapter 4). The neonatal age at cardiac echo ranged between 12 hours to 216 hours, and the gestational age ranged from 37 weeks to 42 weeks. The mode of delivery was split between 175 neonates who were born via caesarean section (C/S), with 86 females and 89 males, and 117 neonates who were born via normal vertex delivery (NVD), with 64 females and 53 males (Figure 4.3 in chapter 4).

Few previous studies included their neonatal gender and mode of delivery findings, see (Table 5.1). Male neonates were predominant in the other studies whereas in our study, females were dominant. Our study also showed a high number of caesarean section deliveries than normal vertex delivery than the other studies. This phenomenon may depend on the need for a caesarian section delivery following on complications experienced by the mother during normal vertex delivery, parent's choice, or the Obstetricians experience. Another reason could also be that Chris Hani Baragwanath hospital is a tertiary hospital where mothers of the subjects were admitted following referral from other centres following complications for which a C/S may be required.

The neonatal ages varied amongst the studies; four studies enrolled neonates who were 24 hours of age post-delivery, (Harada et al. 1994; Shiota, Harada and Takada 2002; Bokiniec et al. 2016 and Riggs et al. 1989). Two studies enrolled neonates who were between 1-7 days of age post-delivery (Mori et al. 2004 and Iwashima, Seguchi and Ohzeki 2005). Four studies were undertaken when the neonates were aged between zero days, to just less than a month post-delivery, the number of hours post-delivery were not specified (Taksande 2018; Uysal, Boston and Çil 2016 and Nu´n˜ez-Gil et al 2011) while our study enrolled neonates with a mean age of

40.1 hours and a median of 31 hours post-delivery. Most of the neonatal studies indicated that a study limitation was the small sample size of the study population.

Table 5. 1 Gender and mode of delivery (Local vs International values)

	This study 2019 (South Africa) n = 292	**Riggs et al. 1989 (Northern America) n = 22**	**Iwashima, Seguchi and Ohzeki 2005 (Asia) n = 55**	**Bokiniec at al. 2016 (Europe) n = 29**
Gender - M/F	142M/150F	**-**	31M/24F	18M/11F
Mode of delivery C/S: NVD	175:117	05:17	18/37	11:18

M: male, **F**: female, **C/S**: caesarean section, **NVD**: Normal vertex delivery

Overbeek et al. 2006; Abushaban et al. 2014. and Cantinotti et al. 2012 demonstrated that BSA, body weight and gestational age correlated well with the cardiac measurements but concluded that it was better to present neonatal measurements in relation to body/birth weight since the neonatal BSA range is small and varies slightly when compared to older and bigger subjects.

In our study an increase in birthweight was associated with a significant increase in certain left heart measurements (MV peak A, MV S'), and a significant increase in TAPSE. Only the MV S' velocity showed a very small but significant increase with gestational age. In this study there was little to no correlation of cardiac function measurements with body size(weight, length and BSA), perhaps this could be because cardiac function is mostly affected by changes in cardiac hemodynamics than body size (weight, length and BSA) (Ramachanrappa and Jain 2008). The C/S group showed a significant increase in the TV peak E velocity whereas the RV Tei showed a decrease in the C/S group.

M- Mode

Ejection Fraction (EF) and Fractional shortening (FS)

It is claimed that the assessment of ventricular contractility in the newborn infant with the use of standard echocardiographic indexes used to assess patients beyond the neonatal period may result in error due to the unique physiologic state that exists in the neonatal period (Rowland and Gutgesell 1995). Normal values for fractional shortening (FS) in infants and children have been established and are reported to be between 28 and 46%, ejection fraction (EF) being 56-78% (Rowland and Gutgesell 1995 and Tissot, Singh and Sekarski 2018). This study showed that the M-mode fractional shortening (FS:40.25%) and ejection fraction (EF:73.56%) were similar to previously published normal values (Table 5.2 in chapter 5).

The disadvantage of M-Mode is that one assumes a cylindrical shape of the LV, and if the LV function is reduced the estimation of FS can be under- or overestimated (Tissot, Singh and Sekarski 2018). In addition, if the M-mode cursor is not placed correctly over the myocardium, errors in measurement may also result (Tissot, Singh and Sekarski 2018). The LV shape and consequently the calculation of M-mode derived parameters may be skewed in the presence of congenital heart defects (CHD), change in loading conditions (preload and afterload), and may also be affected by RV dysfunction which causes a change in the shape of the LV because of ventricular interdependence (Tissot, Singh and Sekarski 2018).

Tricuspid annular plane systolic excursion (TAPSE)

While the physiological significance of the right ventricle is often undervalued, it is crucial to assess its function in various diseases to predict prognosis (Ghio et al. 2000). The right ventricular dimensions and functions in patients with pulmonary hypertension determine severity of the disease and act as an important indicator of survival (Uysal, Boston and Çil 2016).

TAPSE is an important measurement of the longitudinal function of the right ventricle, since the majority of the right ventricle ejection depends on the longitudinal contraction of the right ventricular myocardium (Nunez-Gil et al. 2011; Koestenberger et al. 2009 and Hashimoto et al. 2015). Normal ranges of TAPSE have been determined for children (Nunez-Gil et al. 2011; Koestenberger et al. 2009 and Hashimoto et al. 2015). However, there are no established reference values for neonates and children in the sub-Saharan Africa. The TAPSE values in our

study ranged from 4.8 to 11.4mm with a mean of 7.5 mm (Table 4.5 in chapter 4); lower than those described in previous studies (Table 5.2 in chapter 5). There was a significant positive correlation between TAPSE and weight, length and body surface area, most likely due to an increased excursion of the tricuspid valve annulus in a bigger heart in a bigger neonate (Nu´n˜ez-Gil et al 2011 and Uysal, Boston and Çil 2016). The BSA however, was lower in our study (Table 5.2 in chapter 5).

Although adult TAPSE values of <20mm are associated with reduced right ventricle function (Forfia et al. 2006 and Frommelt et al. 2002), adult nomograms cannot be applied to the paediatric population because of developmental changes and anthropometric variables that are related to changes in patient and heart size (Lipshultz and Miller 2005). However, the limited information on TAPSE in neonates indicate it as a valuable prognostic marker for RV function assessment (Nu´n˜ez-Gil et al. 2011 and Uysal, Boston and Çil 2016).

Pulsed Doppler

During the neonatal period, non-invasive pulse Doppler measurements of trans-mitral flow have been widely used for assessment of left ventricular relaxation abnormalities (Iwashima, Seguchi and Ohzeki 2005). In this study the mean mitral valve (MV) peak E velocity (0.583m/s) was slightly less than the mean MV peak A velocity (0.592m/s) with a MV E/A ratio of 1.01. The mean tricuspid Valve (TV) peak E was 0.51m/s, the TV peak A was 0.61 m/s, with a TV E/A ratio of 0.845. MV peak E velocity increased with an increase in the body surface area, while the MV peak A velocity seemed to decrease with increasing BSA, although not significant. MV peak E and MV peak A velocities were measured to be marginally higher than other publications (Table 4.12 in chapter 4) (Riggs et al. 1989; Harada 1994; Iwashima, Seguchi and Ohzeki 2005 and Taksande 2018).

There was some variability with regard to the mitral valve E and A velocities across the various studies included in Table 4.12 which may be explained by the heterogeneity amongst the studies such as the smaller patient numbers in some of the studies as well as the differences in the age of the neonates when the parameters where acquired (Riggs et al. 1989; Shiota, Harada and Takada 2002; Mori et al. 2004; Iwashima, Seguchi and Ohzeki 2005 and Taksande 2018). The cardiovascular system of the neonate undergoes dramatic changes within the first few hours of birth from a high pulmonary vascular resistant dominance to a systemic vascular resistance dominance, which may explain why in the study by Taksande 2018, the mitral valve

E velocity was the dominant velocity compared to the other studies. This is reflective of a higher systemic vascular resistance state of the neonate at an older age (Ramachanrappa and Jain 2008).

The tricuspid valve (TV) peak E velocity as part of the assessment of the right ventricle showed a significant positive relationship with delivery by caesarean section. On the contrary, Riggs et al. 1989, found no differences between C/S and NVD variables whereas Tao et al. 2019 demonstrated that the TV and MV E/E' ratios were higher in neonates born via C/S compared to those born via NVD. The higher diastolic function values in patients born by C/S suggest the presence of an element of diastolic dysfunction associated with elevated ventricular filling pressures. The risk of respiratory distress secondary to transient tachypnea of the newborn, surfactant deficiency and pulmonary hypertension are increased in neonates after delivery by C/S and may explain some of the differences according to the mode of delivery (Ramachanrappa and Jain 2008). TV peak A velocity showed a positive significant relationship with age in hours post-delivery.

Tissue Doppler imaging

Tissue Doppler imaging (TDI) is used to document load independent myocardial contraction and relaxation velocities which is then applied to the analysis of the longitudinal axis function of both ventricles (Mori et la 2004). Tissue Doppler imaging measurements of diastolic function and the longitudinal motion of the heart is preload independent as opposed to pulse Doppler evaluation of the TV and MV inflow (Vignon et al. 2007; Eidem et al. 2004 and Tissot, Singh and Sekarski 2018). The study showed MV S' did have, but the MV E' velocity and MV E/E' ratios did not have any correlation with birthweight, gestational age and age after delivery. The implication is that the bigger the baby, the bigger the heart and the bigger the excursion of the mitral valve annulus during systole. There are clearly differences between the neonate and adult tissue Doppler parameters. For example, the normal value for an early mitral annular or septal E' wave velocity of <0.08m/s (Ichihashi et al. 2010 and Mori et al. 2004) in an adult differs from the mean E' wave velocity of 0.06m/s +/- 0.01 m/s found in the study neonatal population. Similarly, the E/E' ratio of a normal adult LV which correlates with the pulmonary capillary wedge pressure (Nagueh at al 1997 and Tissot, Singh and Sekarski 2018) is reported to be 7.7 (±3.0) (Sohn et al. 1997 and Mori et al. 2004) compared to a much higher value of E/E' 10.38 in the neonatal study population. This higher value may be reflective of a sudden higher left

ventricular filling pressure of the newborn infant following separation from the placenta which is associated with an abrupt increase in systemic afterload (Ramachanrappa and Jain 2008).

In our study the mean MV septal S' velocity which represents the mitral annular movement towards the left ventricular apex during systole, was found to be 0.05m/s, which was slightly higher than Mori et al. 2004 and Taksande 2018 who both reported a MV S' of 0.04m/s. The differences may again represent heterogeneity amongst the studies where measurements may have been done at different times after delivery when the systemic vascular resistance which goes up after delivery (Ramachanrappa and Jain 2008) may have been at a lower point for the current study and at a higher value for the Mori et al and Taksande et al publications.

The TV E' velocities and E/E' ratio showed no correlation with any of the independent variables unlike Tao et al. 2019, who demonstrated the TV E/E' ratio to be significantly higher in the C/S group when compared with the NVD group.

Our study showed the mean MV E/E' ratio of 10.38m/s, to be higher than the mean TV E/E' 6.78m/s which agrees with Mori et al. 2004. This may be attributed to the relatively higher left ventricular filling pressures so soon after birth representing an adaptation of the LV myocardium to the sudden increase in post-natal systemic afterload (Ramachanrappa and Jain 2008).

The mean TV S' velocity in the study population was noted higher than the mean MV S' velocity but was not statistically significant after being tested with the Z-test, but is similar to findings by Mori et al. 2004 and Taksande 2018 (Table 4.12 in chapter 4). The higher TV S' velocity is normal and signifies a much bigger movement of the TV annulus during right ventricular systole compared to the MV annulus because the right ventricle is intrinsically dependent on longitudinal movement against a lower afterload during systole whereas the left ventricular movement is more complex and also incorporates twisting and circumferential shortening components (Kukulski et al. 2000 and Mori et al. 2004).

Myocardial performance index (Tei index)

The Tei index may be used as an alternative means of measuring the myocardial function when using the ejection fraction becomes less accurate in abnormally shaped ventricles or diastolic inflow velocity waves are fused due to intrinsically fast neonatal heart rates (Borzoee and Kheirandish 2004). The Tei index reflects systolic and diastolic function and can be applied to

both the left and the right ventricle. It correlates well with invasive measurements of systolic and diastolic function. (Nagueh, Sun and Kopelen 2001 cited in Mottram and Marwick 2005, Taksande et al. 2018 and Borzoee and Kheirandish 2004).

Myocardial performance index (MPI) or Tei index can be used to assess myocardial function in both adults and children. Its value is independent of chamber geometry, heart rate (Borzoee and Kheirandish 2004) or age (Moller et al. 2000; Sato et al. 2001; Borzoee and Kheirandish 2004).

In our study the RV Tei index showed a moderate, but not significant, positive correlation with birthweight. The RV Tei mean was 0.28 and LV Tei mean was 0.31 at a mean age of 40.13 hours. In the study by Bokoniec et al 2016, the mean ventricular Tei indexes were slightly higher, with RV Tei index calculated to be 0.42 and the LV Tei index 0.37 but the neonatal cohort were much younger at 24 hours and less post-delivery (Table 4.12 in chapter 4). The Bokiniec et al 2016 study also showed a decrease in the mean RV Tei index towards the end of the neonatal period, from 0.42 to 0.29 which may reflect the physiological drop in pulmonary artery pressures with age. The LV Tei remained unchanged (Ramachanrappa and Jain 2008 and Bokiniec et al 2016).

Clinical use table

A unique clinical use table has been compiled with predicted values and Z-scores to be used in an African setting to assist in decision making regarding the longitudinal systolic function of both ventricles as well as diastolic function of the newborn infant (Table 4.9 and 4.10)

Normality and Homoscedastic Testing

The only variables that were normally distributed were left ventricular ejection fraction (LV EF) and TAPSE (Table 4.2 in chapter 4). The Breusch-Pagan and White test showed that most parameters/measurements were homoscedastic with the random disturbance in the relationship between all the dependent and independent variables being the same across all values of the independent variables. The exceptions were the LV Tei index, the TV E/E' ratio and the RV Tei index (Table 4.2) and could be explained by the presence of a few outliers.

Reproducibility and inter observer variability

To test for reproducibility, intraclass correlation (ICC) was conducted between the 1st and the 2nd observer. The overall ICC average was 71.86% which shows a strong correlation between the two observer measurements. Nu´n˜ez-Gil et al 2011 and Iwashima, Seguchi and Ohzeki 2005 had ICCs of 0.74 and 0.91 respectively which is similar to our ICC results. See (Table 4.4) for the correlation strength.

Local versus International values

The findings of this study were compared to obtainable international values. The sample size, mean weight, mean and standard deviations of the echocardiographical measurements were compared, all measurement are reported in percentage (%), millimetres (mm) and meters per second (m/s). Similar measurements are highlighted in green (Table 5.2 and Table 5.3).

Table 5. 2 M-mode measurements (Local vs international values)

	This study 2019 (Africa)	Mori et al 2004 (Asia)	Iwashima, Seguchi and Ohzeki 2005 (Asia)	Nu´n˜ez-Gil et al 2011 (Europe)	Uysal, Boston and Çil 2015 (Europe)	Taksande 2018 (Asia)
	n = 292	n = 135	n = 55	n = 30	n = 22	n = 15
Mean weight (kg)	3,18	2.95	2.46	-	-	2.56
Mean BSA (m^2)	0,202	-	-	0.23	<0.25	-
LV EF (%)	**73,56**(±8,93)	-	**71.20**(±6.5)	-	-	-
LV FS (%)	**40,25**(±7,91)	**31**(±0.06)	-	-	-	**39.06**(±3.72)
TAPSE (mm)	**7,516**(±1,30)	-	-	**10.56**(±7.26)	**9.09**(±5.91)	-

n: number of participants in study**, BSA:** Body surface area**, m^2:** meter squared**, LV EF** left ventricular ejection fraction**, LV FS:** left ventricular fraction shortening**, TAPSE:** tricuspid annular plane systolic excursion. Data is expressed as mean ± standard deviation.

This study's mean weight showed to be similar with the two Asian studies, Harada et al 1994 and Shiota, Harada and Takada 2002. The mean age at cardiac echo was 40 hours and the Asian's study measurements were taken at 24 hours of age post-delivery.

Table 5. 3 Doppler measurements (Local vs International values)

	This study 2019 (Africa) n = 292	Riggs et al. 1989 (Northern America) n = 22	Harada et al. 1994 (Asia) n = 16	Shiota, Harada and Takada 2002 (Asia) n = 45	Mori et al. 2004 (Asia) n = 135	Iwashima, Seguchi and Ohzeki 2005 (Asia) n = 55	Koestenb-erger et al. 2012 (Europe) n = 83	Bokiniec et al. 2016 (Europe) n = 29	Taksande 2018 (Asia) n = 15
Mean weight (kg)	3,18	3,44	3,09	3,06	2,95	2,46	-	3,443	2,56
Mean BSA (m^2)	0,20	-	-	-	-	-	0,22	-	-
MV PEAK E (m/s)	0,58(±0,11)	0,50(±7,9)	-	0,53(±9)	0,52(±9,5)	0,54(±13,6)	-	-	0,46(±0,88)
MV PEAK A (m/s)	0,59(±0,12)	0,49(±8,3)	-	0,44(±5)	0,48(±8,0)	0,48(±8,5)	-	-	0,54(±0,85)
MV E/A Ratio	1,01(±0,21)	1,00(±0,25)	-	1,19(±0,15)	-	1,14(±0,15)	-	-	0,86(±0,18)
MV E' (m/s)	0,06(±0,01)	-	-	-	0,05(±0,9)	-	-	-	0,03(±0,88)
MV E/E' Ratio	10,38(±2,65)	-	-	-	7,0(±1,6)	-	-	-	-
MV S' (m/s)	0,05(±0,01)	-	-	-	0,04(±0,7)	-	-	-	0,04(±0,75)
LV Tei	0,31(±0,14)	-	-	-	-	-	-	0,37(±0,10)	0,30(±4,77)
TV PEAK E (m/s)	0,51(±0,13)	0,47(±8,5)	0,42(±7,3)	-	0,38(±7,1)	-	-	-	-
TV PEAK A (m/s)	0,62(±0,13)	0,53(±9,9)	0,49(±7,9)	-	0,49(±7,5)	-	-	-	-
TV E/A Ratio	0,84(±0,20)	0,85(±0,23)	0,87(±0,17)	-	-	-	-	-	-
TV E' (m/s)	0,08(±0,02)	-	-	-	0,08(±1,3)	-	-	-	0,09(±0,14)
TV E/E' Ratio	6,78(±2,01)	-	-	-	5,2(±1,2)	-	-	-	-
TV S' (m/s)	0,07(±0,05)	-	-	-	0,07(±1,2)	-	0,07(±4,75)	-	0,09(±0,14)
RV Tei	0,28(±0,13)	-	-	-	-	-	-	0,42(±0,14)	0,40(±5,24)

n: number of participants in study, **BSA:** body surface area, **MV peak E:** Mitral valve - early diastolic filling measured by pulsed Doppler, **MV Peak A**: Mitral valve - late diastolic filling measured by pulsed Doppler, **MV E/A:** Mitral valve- early diastolic filling/late diastolic filling ratio, **TDI MV E'**: Mitral valve - peak myocardial velocity in early diastole measured by TDI, **MV E/E':** Mitral valve- early diastolic filling/Peak myocardial velocity in early diastole ratio, **TDI MV S'**: Mitral valve - Systolic wave representing peak myocardial systolic velocity at the septal MV annulus measured by TDI, **LV Tei**: Myocardial performance index, ,**TV peak E:** Tricuspid valve - early diastolic filling measured by pulsed Doppler, **TV Peak A**: Tricuspid valve - late diastolic filling measured by pulsed Doppler, **TV E/A**: Tricuspid valve – early diastolic filling/ late diastolic filling ratio, **TDI TV E'**: Tricuspid valve – Peak myocardial velocity in early diastole measured by TDI, **TV E/E':** Tricuspid valve – early diastolic filling/ Peak myocardial velocity in early diastole ratio, **TDI TV S'**: Tricuspid valve - Systolic wave representing peak myocardial systolic velocity at the lateral TV annulus measured by TDI, **RV Tei:** Myocardial performance index.

The study sample size (n=292) was much larger than other studies performed on healthy full-term neonates. It is therefore highly powered and provides a high level of confidence in the variation of normality in term African neonatal cardiac function. The next biggest study is the one conducted by Mori et al. 2004, in which 135 Asian neonates were interrogated (Table 5.3). Other studies from Africa include Jacobs et al 2012 who had a total number of 290 preterm newborns, from the Free State in South Africa, who established reference values for preterm and low-birth weight infants only, and Majonga et al 2017, from Zimbabwe who created reference values in 282 children aged 6 to 16 years.

Study strengths and limitations

This study is one of the largest and most recent undertaken in the neonatal population worldwide. Our study is also strongly powered in terms of our sample size, a strong ICC, and the fact that the study incorporated more cardiac function parameters compared to the other studies.

The intention was to recruit 350 healthy neonates but due to time constraints, only 292 were recruited. Another deficiency, due to the lack of availability, is cardiac speckle tracking or strain which is a measure of tissue deformation and offers an alternative and more global assessment of ventricular systolic function occurs (Khuffash et al. 2018).

CHAPTER SIX: CONCLUSION

This study has established normal reference values for diastolic function and longitudinal systolic and diastolic movement of the heart in full-term African neonates and is the first such study to be done in the Sub Saharan Africa. It is also one of the largest and most recent studies undertaken in the neonatal population worldwide. The study assessed normal cardiac function measurements using M-Mode, Doppler, tissue Doppler and Myocardial performance index (Tei index). These normal values provide a means to assess deviations from normal systolic and diastolic function values in the African neonate.

An increase in body size was associated with a small but significant increase in the left heart in-flow patterns. The right ventricle Tei index was found to be less than the LV Tei index which is a normal finding across all age groups.

In conclusion, this study incorporated multiple measurement techniques for the assessment of normal values for diastolic and systolic function of both ventricles in the African neonatal population from which a table has been compiled, which can be used in the clinical setting.

A study establishing normal cardiac reference values using echocardiography extended to other ethnic groups may be useful in assessing the differences or similarities between neonates from the various population groups of the vastly diverse inhabitants of Southern Africa.

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APPENDIX A

PERMISSION LETTER

DEPARTMENT OF HEALTH

HOSPITAL MANAGER

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

GAUTENG PROVINCE

Dear Prof Pettifor

Request for permission to conduct the study

My Bongiwe Jessie Beckerling, I am a clinical technologist in the Cardiology department at the C.H. Baragwanath Academic Hospital in Johannesburg and I am currently registered for Masters of Health Sciences Degree at the Durban University of Technology.
I would like to request to conduct a research project which is required in Masters of Health Sciences Degree syllabus.

Title of project: The diastolic inflow and longitudinal movement of the heart in the African full-term new-born infant.

This study will be a descriptive, bidirectional design, which will be supervised by Prof Antoinette Cilliers and Dr Hopewell Ntsinjana at the Chris Hani Baragwanath Academic Hospital in Johannesburg**.**
This study will be carried out in two part:

Part one of the study (Retrospective Post Processing of data)

This will include prospective post processing data analysis measuring the diastolic function of healthy African neonates using echocardiography as the tool of acquiring the data.
The echocardiographic data has been acquired with consent from each mother during a study for a MMed thesis entitled “To assess the accuracy of pulse oximetry screening as a tool to detect critical congenital heart disease in asymptomatic newborns” by Dr. Michael Platten (see appendix B). 150 newborn infants underwent an intensive echocardiographic examination and all data was stored for later post processing.

Part two of the study (Prospective data collection)

Part two will be prospective data collection and analysis of diastolic function of healthy African neonates using echocardiography as an assessment tool. Additional ethical approval will be requested for this part of the study and a consent /information form will be sorted for the parents.

The study is not structured to alter any routine procedures and treatment in any way. I wish to recruit Participants for part two as soon as approval has been granted.

Prof Cilliers and Dr Ntsinjana (Pediatric cardiologists) have offered me with their support in supervising my project. There will be no additional cost to the Participant or hospital.

I hereby apply for permission to conduct research by using and post processing the data/images acquired by echocardiography in Pediatric cardiology from Chris Hani Baragwanath Hospital.

My research proposal is attached for your perusal. Your support and permission to perform this project will be highly appreciated.

Regards

Miss Bongiwe Jessie Beckerling Clinical Technologist (Cardiology)
Contact: 08364253623

APPENDIX B

Letter of information

Study title: Diastolic inflow and longitudinal movement of the heart in the African term new-born infant

Greeting: Dear Mother, Dad/ guardian

Introduction:
We, BJ Msibi, and cardiology department, are doing research aimed at looking at your baby's heart to look at how it relaxes when the blood fills the rooms of the heart.
Research is just the process where we try and solve problems and finding facts in an organized way to learn the answer to a question.

There are different ways of checking a neonate/new-born's health in order to make sure that you, the mother take home a healthy baby.
The heart is divided into four rooms with four doors separating these rooms. The tricuspid valve separates the right atrium from the right ventricle (the big room). The mitral valve separates the left atrium from the left ventricle (the big room).

In order for the blood to enter the big rooms: the left and right ventricle, the heart must be able to relax properly so that the doors can open. If the heart cannot relax properly the blood struggles to enter these rooms. These two big rooms are very important since they are the ones who pump blood to the lungs to get more oxygen and the other to the body and brain.

This study will be looking at your baby's heart to see how the heart relaxes so that blood from the body and lungs can enter the heart. I will look at this using a heart ultrasound machine.
The aim is to find the normal relaxation patterns of healthy babies in the African population. This will help us find normal measurements for healthy babies in the African population and this will help in being able to notice abnormal heart behaviour.

Invitation to participate: We are asking for your permission to include your baby in this research study.

What is involved in the study – This research will take place in the ward before you are discharged, we will be looking at your baby's heart to see how the heart relaxes so that blood from the body and lungs can enter the heart. I will look at this using a heart ultrasound machine. We will try our best not to delay you and your baby from being discharged in time if no heart problems are found. We are looking at testing about 200 babies. The ultrasound (echocardiography) will not take more than 30 minutes.

Risks There will be no risk to the newborn. Heart ultrasound is a painless procedure and does not involve the introduction of instruments into the body. Just like the one you had when you were pregnant, this ultrasound exam will look at your baby's heart function.

Benefits The new information gained from the study will help in making things easier in finding heart that are failing to relax properly in African neonates/new-borns.

The participant will be given pertinent information on the study while involved in the project and after the results are available.

Participation is voluntary, if you decide to take part in the research, please know that we are not forcing you. You can decide not to take part if you do not feel comfortable. This will not change or affect your baby's medical care.

Reimbursements The mother will be liable for normal routine procedures e.g. baby delivery costs and being admitted at Chris Hani Baragwanath Hospital. There will be no remuneration given to the participant.

Confidentiality: Participants will be allocated with numbers so that Participant stays anonymous. Details will be kept confidential in a subject file, which will be stored in a locked office in the Paediatrics' department.

Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee and the Medicines Control Council (where appropriate).
If results are published, may lead to individual / cohort identification.

Contact details of researcher/s – for further information / reporting of study related adverse events.
Miss B.J Msibi, Prof A Cilliers, Dr H Ntsinjana (Unit supervisors) and Prof R Prakaschandra (Internal supervisor)
Principal Investigator Supervisor
0836425362, 0833435803, 0737356832, 0834467735

Contact details of REC administrator and chair
If you have any problems or complaints that you feel have not been adequately resolved by me, you can contact the Research Ethics Committee to lodge a complaint

HSRC REC Administrator
[Tel] 012-302-2012
[Fax] 012-302-2005

The following must be included in the consent form when applicable:

a. A statement that the particular treatment or procedure may involve risks to the participant (or to the embryo or foetus, if the subject is or may become pregnant) that are currently unforeseeable.
b. Anticipated circumstances under which participation may be terminated by the investigator without regard to the participant's consent.
c. Any additional costs to the participant that may result from participation in the research.
d. The consequences of a participant's decision to withdraw from the research and procedures for orderly termination of participation by the subject.
e. A statement that significant new findings developed during the course of the research which may relate to the participant's willingness to continue participation will be provided to the subject.
f. Where genetic tests are to be done, a separated information sheet and consent form will be made available.
g. A statement that specimens would be stored for future research pertaining to the specific research question being studied and a separate information sheet and consent form will be made available. Specify how long specimens will be stored for, where they will be stored, and whether these will be anonymised. If stored for future genetic testing, a further consent form will be signed.

Consent

Statement of Agreement to Participate in the Research Study:

- I hereby confirm that I have been informed by the researcher, _____ (Miss BJ Msibi), about the nature, conduct, benefits, and risks of this study-Research Ethics Clearance Number: _____
- I have also received read and understood the above written information (Participant Letter of Information) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during this research which may Relate to my participation will be made available to me.

_____	_____	_____	_____
Full Name of Participant	**Date**	**Time**	**Signature / Right Thumbprint**

I, _____ (Name of researcher) herewith confirm that the above participant has been fully

Informed about the nature, conduct and risks of the above study.

Full Name of Researcher	**Date**	**Signature**
Full Name of Witness (If applicable)	**Date**	**Signature**
Full Name of Legal Guardian (If applicable)	**Date**	**Signature**

Please note the following:

Research details must be provided in a clear, simple, and culturally appropriate manner and prospective participants should be helped to arrive at an informed decision by use of appropriate language (grade10level
-use Flesch Reading Ease Scores on Microsoft Word), selecting of anon-threatening environment for interaction and the availability of peer counseling (Department of Health, 2004)

If the potential participant is unable to read/illiterate, the right thumb print is required and an impartial witness, who is literate and knows the participant e.g. parent, sibling, friend, pastor, etc. should verify in writing, duly signed that in formed verbal consent was obtained (Department of Health, 2004).

If anyone makes a mistake completing this document e.g. a wrong date or spelling mistake a new document must be completed. The incomplete original document must be kept in the participant's file and not thrown away, and copies thereof must be issued to the participant.

Department of Health: 2004.*Ethics in Health Research: Principles, Structures and Processes*
http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/

Department ofHealth.2006.*SouthAfricanGood Clinical Practice Guidelines*. 2ndEd.Available at: http://www.nhrec.org.za/?page_id=14

APPENDIX C

Data collection sheets

Data collection sheet for part one

Study No.		**Gender**		**Race**		**Nationality**	
Date of birth		**Age in hours**		**Gestational age**			
Date of echo		**Type of birth**					
Birth screening	**Birth weight**		**Birth length**		**BMI**		
Apgars	**1**		**2**		**Family history of Cardiac disease**		**Province/ Country**	

Echo collection sheet: Part one

Study no.		**Date**											
LV function	**IVSd**		**LVIDd**		**LVPWd**		**LVIDs**		**EF**		**FS**		
Ao		**LA**		**Ratio**		**PDA size if present**							
Diastolic function	**MV-Peak E**		**MV-Peak A**		***TV-Peak E***		**TV-Peak A**						
Tissue Doppler	**MV- E'**		**MV- A'**		**TV- E'**		**MV-E/E'**		**TV-E/E'**				
	MV- S'		**TV-S'**										
Tei index	**LV Tei**		**RV Tei**										

APPENDIX D

Data collection sheets

Data collection sheet for part two

Study No.		**Gender**		**Race**		**Nationality**	
Date of birth		**Age in hours**		**Gestational age**			
Date of echo			**Type of birth**				
Birth screening	**Birth weight**			**Birth length**		**BMI**	
Apgars	**1**		**2**	**Family history of Cardiac disease**		**Province/ Country**	

Echo collection sheet: Part two6

Study no.		**Date**									
LV function	**IVSd**		**LVIDd**		**LVPWd**		**LVIDs**		**EF**		**FS**	
Ao		**LA**		**Ratio**		**PDA size if present**					
Diastolic function	**MV-Peak E**		**MV-Peak A**		***TV-Peak E***		**TV-Peak A**				
Tissue Doppler	**MV- E'**		**MV- A'**		**TV- E'**		**MV-E/E'**		**TV-E/E'**		
	MV- S'		**TV-S'**								
Tei index	**LV Tei**		**RV Tei**								

APPENDIX E

Sample size calculation

$$= \frac{(\text{Z-Score})^2 \times SD \times (1-SD)}{(\text{Margin of error})^2}$$

$$= \frac{(1.282)^2 \times 0.5 \times (1-0.5)}{(0.05)^2}$$

$$= \frac{1.64 \times 0.5 \times .05}{0.0025}$$

$$= \frac{1.64 \times 0.25}{0.0025}$$

$$= \frac{0.41}{0.0025}$$

$$= 164$$

APPENDIX F

9 March, 2017

Student No: 20803722

Ms BJ Msibi
78 Falcons Nest
Kelly Avenue
Bromhof
Randburg
2188

Dear Ms Msibi

MASTER OF HEALTH SCIENCES IN CLINICAL TECHNOLOGY

I am pleased to advise that:

1. The Research and Higher Degrees Committee approved the following:

    (i) Your research proposal and dissertation title, being:

The diastolic inflow and longitudinal movement of the heart in the African full term new-born infant.

Please note: ANY PROPOSED CHANGES in the DISSERTATION TITLE require the approval of your supervisor and the Faculty Research Committee.

(ii) Supervisor – **Dr DR Prakaschandra**
(iii) Co-Supervisor – **Professor A Cilliers**
(iv) Co-Supervisor – **Mr ME Memela**

2. Your request for funding totalling **R 10 000.00** subject to any literature referred to in Section A of the PG 4a form being accessioned by this University, and any equipment purchased shall become the property of the department.

NOTE: - This funding is not paid directly to you but is controlled by Faulty Research Officer. Any proposed changes to this funding allocation needs the approval of your supervisor, and Faculty Research Committee

The University Research Committee has stipulated that:

(a) Ownership of any patent registered in respect of the results of your Master's Degree in Technology studies is retained by you as the initiator of the project;

(b) Should you make any drift from the results of your Master's Degree in Technology studies, you will be required to repay pro rata, the **R 10 000.00** investment which the University Research Committee has made in approving your request for funding;

(c) If the Durban University of Technology provided the equipment/materials for the creation of artefacts, this cost would be refunded to the University if such artefacts were sold and

(d) Durban University of Technology is given first refusal in respect of any possible future sale by you of any patent that may be registered in respect of your said project.

(e) All journal articles, referenced in your dissertation, are to accompany your ring-bound copies when submitting for examination purposes.

May I remind you that notwithstanding Rule LX.CM2, if a student fails to obtain the Masters Degree within two years of first registering for the fifth year, re-registration may be denied. The Academic Board may refuse to renew such registration or may impose any conditions it deems fit.

Should you experience any problems relating to your research studies, your supervisor must be informed as soon as possible. If the difficulty persists, you must then approach your Head of Department and thereafter the Dean of the Faculty.

Yours sincerely

Mr S Reddy
FACULTY RESEARCH OFFICER

_____ _____

Student's signature in acceptance of the conditions contained herein. **Date:**