ECHOCARDIOGRAPHY NOMOGRAMS IN BLACK SOUTH AFRICAN NEONATES

Submitted in the fulfilment of requirements of the

MASTERS OF HEALTH SCIENCES DEGREE (CLINICAL TECHNOLOGY)

At

Durban University of Technology

NONDUMISO MEMORY HADEBE

AUGUST 2019

Supervisor: Dr R. Prakaschandra Date: _____

Co-supervisor: Professor H. Ntsinjana Date: _____

Co-supervisor: Professor A. Cilliers Date: _____

ABSTRACT

INTRODUCTION

The subspecialty of Paediatric cardiology has adapted and refined techniques adopted from adult cardiology to manage children, (Mahle, Blaine and Silverman 2009:33). Although a quantitative evaluation of cardiac chambers, valve annulus and great vessel dimensions in paediatric echocardiography is important, nomograms for these structures are limited. Several studies have already provided normal values in the paediatric population that represent most populations of the world but there is lack of data that has been published in sub-Saharan Africans, (Lemmer et al. 2014:1 and Roge et al. 1978:285).

AIMS AND OBJECTIVES

Aim: To establish reliable echocardiography nomograms for cardiac chambers, valve annuli, great vessels and thymus dimensions in the Black South African neonatal population. **Objectives:** To determine normal values of cardiac chambers, valve annuli, great vessel and thymus dimensions. To determine inter-observer variability. To determine the effect of confounding factors such as gender and type of delivery on the measurements obtained.

METHODS

This is a descriptive, cross-sectional study evaluating cardiac chambers, valve annuli, thymus and great vessel dimensions in 386 African neonates with normal hearts using echocardiography. The study data consists of two arms, a retrospective arm utilizing echocardiographic data acquired during a previous study entitled “To assess the accuracy of pulse oximetry screening as a tool to detect critical congenital heart disease in asymptomatic newborns” and a prospective arm.

INCLUSION CRITERIA: Healthy newborns at an age of 12 hours or more before discharge. Patients without any heart disease, with a “normal heart” by echocardiography,

excluding hemodynamically non-significant patent ductus arteriosus and patent foramen ovale. Black South African patients. Full term babies delivered by caesarian section and normal vertex.

EXCLUSION CRITERIA: Patients less than 12 hours from birth. Patients with known structural heart lesion. Patients who are non- black South Africans. Pre-term neonates.

DATA COLLECTION AND ANALYSIS: Collected data was entered on excel spreadsheet and analyzed using excel, XLSTAT 2019 and STATISTICA version 13.5.0 statistical packages for analysis by the principal researcher (Nondumiso M. Hadebe). A p-value of less than 0.05 and significance level of 95% was considered statistically significant. A professional biostatistician at University of the Witwatersrand was consulted for assistance in data analysis. Homoscedasticity and heteroscedasticity were tested using Shapiro-Wilk, Kolmogorov-Smirnov, Breusch-Pagan and White tests. The inter-observer variability was tested with intraclass correlation coefficient using Pearson's correlation coefficient to detect bias. Weight was used to express measurements to body size and to predict mean values of each echocardiographic measurement that were expressed as Z-scores.

RESULTS

A total of 386 patients from both arms were enrolled with almost equal gender distribution with a slightly higher percentage of females, 191 (49%) were males and 195 (51%) were females. The study involved neonates born through normal vaginal delivery (NVD) and through caesarian section (C/S) which showed equal distribution. The assumption of normality was tested which showed most measurements being homoscedastic. Heteroscedasticity was tested which showed most measurements to be homoscedastic. Effects of confounding factors were tested which showed that body weight has a significant effect on all cardiovascular dimension measurements. Mode of Delivery (MOD) had a significant effect on the size of atrioventricular valve, pulmonary artery and pulmonary artery branch measurements. Gender and BSA had no significant effect on most measurements but with some significant effect on a few measurements. There was

no significant effects seen for body length (BL) and gestational age (GA). The echocardiography measurements of 168 patients were used to test for inter-observer variability which showed a strong correlation on most measurements. Birth weight was used to express cardiac measurements to body size. All echocardiographic measurements were grouped into 3 categories of weight and are presented as mean and +/-3 SD. Z-scores and its boundaries for all measurements is presented graphically. This study showed slightly higher dimensions to previous studies from other centres.

CONCLUSION

This study presents nomograms that are reliable because they were acquired from healthy neonates using current recommendations by the American Society of Echocardiography, (Lopez et al. 2010: 465-495). Weight showed significant potential as a confounding factor and as an independent variable for data normalization. This study showed slightly higher dimensions to previous studies emphasizing that it is important to develop and use regional nomograms because of the effects of environmental, economic and social factors of the region. This study covered the gap of knowledge on cardiac chamber, valve annuli, and arterial and thymus dimensions in the neonatal age group. Further studies are required to reinforce these findings that will also involve the right cardiac dimensions. More studies are needed based on African paediatric populations including the all paediatric age groups (0-18 years).

AUTHORS DECLARATION

I, Nondumiso Memory Hadebe declare that this research study: **Echocardiography Nomograms in Black South African Neonates** submitted to Durban University of Technology for Masters of Health Sciences (Clinical Technology) represents original work by the author. All the theoretical information and related sources that have been used or quoted have been duly acknowledged by means of complete references. It is further declared that this dissertation has not previously been submitted to any institution for degree purposes.

The research described in this dissertation was carried out at Chris Hani Baragwanath Academic Hospital and supervised by:

Dr Rosaley Prakaschandra

PhD

Head of Biomedical and Clinical Technology

Durban University of Technology

Durban, South Africa

Professor Hopewell Ntsinjana

MBBCH, FCPaeds (SA), PhD

Head of Paediatric Cardiology

Nelson Mandela Children's Hospital

Johannesburg, South Africa

Professor Antoinette Cilliers

MBBCH, FCPaeds (SA)

Head of Paediatric Cardiology

Chris Hani Baragwanath Academic Hospital

Johannesburg, South Africa

Miss Nondumiso Memory Hadebe: _____

Dr Rosaley Prakaschandra

Prof Hopewell Ntsinjana

Prof Antoinette Cilliers

DEDICATION

I dedicate this work to:

My mother- Mrs. C. D. Hadebe, for her constant love, guidance, prayers and encouragement.

My children- Miss Lesedi Mavundla and Mr Luzuko Hadebe, this work will be encouragement for them to do best in their careers when they grow up.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude and appreciation to the people who contributed towards the completion of this project, without their involvement and support this study would never have been possible.

First and foremost, my thanks are due to God Almighty for his love and guidance.

Dr Rosaley Prakaschandra, Prof Hopewell Ntsinjana and Prof Antoinette Cilliers, my project supervisors, for encouraging, guiding and advising me throughout this research project as well as in the presentation, writing and completion of this dissertation.

Chris Hani Baragwanath Academic Hospital for giving me permission to use hospital patients and equipment.

The patients who agreed to participate in this study for the sake of medical research.

My mother, Duduzile Hadebe who have educated me on the importance of studying and for her unfailing willingness, assistance, prayers and support. She is my pillar of strength and continued to urge and motivate me to complete this dissertation.

My siblings, Pumla and Sihle Hadebe and my partner, Bongani Mdawu, for their support, assistance, prayers and understanding.

Friends especially Carl Beckerling and Nolwazi Nodada for help and support.

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LIST OF ABBREVIATIONS

2-D: two dimension

AO DIA: aortic root diameter

ABD AO: abdominal aorta

ASE: American Society of Echocardiography

AV: aortic valve

AV ANN: aortic valve annulus

B: regression coefficient

BL: birth length

BSA: body surface area

CHBAH: Chris Hani Baragwanath Academic Hospital

CHD: congenital heart disease

C/S: caesarian section

DM: diabetes mellitus

ECG: electrocardiography

GA: gestational age

IAS: intra-atrial septum

IVC: inferior vena cava

IVS: interventricular septum

IVSd: interventricular septum in diastole

IVSs: interventricular septum in systole

K-S: Kolmogorov-Smirnov test

LA: left atrium

LPA: left pulmonary artery

LV: left ventricle

LVIDd: left ventricle internal diameter in diastole

LVIDs: left ventricle internal diameter in systole

LVPWDd: left ventricle posterior wall in diastole

LVPWDs: left ventricle posterior wall in systole

MOD: mode of delivery

M-mode: motion mode

MPA: main pulmonary artery

MV ANN: mitral valve annulus

N: sample size

NVD: normal vaginal delivery

PA: pulmonary artery

PLAX: parasternal long axis

PSAX: parasternal short axis

PV ANN: pulmonary valve annulus

RV: right ventricle

RPA: right pulmonary artery

SA: South Africa

SD: standard deviation

SSAX: suprasternal short-axis

SVC: superior vena cava

TGV: transposition of great vessels

TV ANN: tricuspid valve annulus

UCG: ultrasound cardiograph

W: Shapiro- Wilk W test

CHAPTER 1

1.1 INTRODUCTION

The heart is a hollow muscular cone-shaped organ whose primary function is to pump blood through pulmonary and systemic circulation, (Martin 2003:215; McPhee and Hammer 2006: 248). It is located in the mediastinum between the lungs with the apex directed downwards, forwards and to the left, (Martin 2003:215; Tortora and Derrickson 2009: 718). The cardiovascular system is one of the first systems to form in an embryo and the heart is the first functional organ, (Tortora and Derrickson 2009: 748; Iaizzo 2005:15).

1.2 PAEDIATRIC HEART

1.2.1 EMBRYOLOGY OF THE HEART

The development of the heart is a complex process and any disruptions along the way can result in congenital heart defects (CHD), figure 1.1, (Tortora and Derrickson 2009: 748-749, Iaizzo 2005:15). Heart development begins from mesoderm (develops from the ectoderm on the 15th day of life) on day 18-19 after fertilisation, (Tortora and Derrickson 2009: 748-749, Iaizzo 2005:15; Keane, Lock and Fyler 2006:13). The heart develops from mesodermal cells in the cardiogenic area of the developing embryo, (Tortora and Derrickson 2009: 748-749, Iaizzo 2005:15; Keane, Lock and Flyer 2006:13). The mesoderm in the cardiogenic area forms a pair of cardiogenic cords (elongated strands). Cords develop a hollow centre and become endocardial tubes, (Tortora and Derrickson 2009: 748-749).

On day 21 after fertilisation, the developing embryo folds laterally, (Tortora and Derrickson 2009: 748-749; Keane, Lock and Flyer 2006:13-14) and paired endocardial tubes approach each other and fuse into a single tube known as primitive heart tube, (Tortora and Derrickson 2009: 748-749, Iaizzo 2005:15).. The primitive heart tube develops into 5 distinct regions and starts to pump blood on day 22. The regions are Sinus Venosus (which later develops into part of right atrium, coronary sinus and

sinoatrial node of future heart), Atrium (develops into part of right atrium, right auricle, left atrium and left auricle), Ventricle (develops into left ventricle), Bulbus Cordis (develops into right ventricle) and Truncus Arteriosus (gives rise to ascending aorta and pulmonary trunk). The Sinus Venosus receives blood from all the veins in the embryo, contractions of the heart begins in the sinus venosus and follow sequentially in the other regions, (Tortora and Derrickson 2009: 748-749).

On day 23 the primitive heart tube elongates. The Bulbus Cordis and Ventricle grow more rapidly than other parts of the tube. The Atria and Venous ends of the tube confined by the pericardium, the tube begins to loop and fold. As a result of these movements which are completed by day 28, the atria and ventricles of the future heart are re-orientated to assume their final adult positions. The remainder of heart development consist of reconstruction of the chambers and formation of septa and valves to form a four-chambered heart, (Tortora and Derrickson 2009: 748-749; Keane, Lock and Flyer 2006:13-14).

Endocardial Cushions (thickenings of mesoderm of the inner lining of the heart wall) appear on day 28 which grow towards each other, fuse and divide the single atrioventricular canal into smaller separate left and right atrioventricular canals, (Tortora and Derrickson 2009: 249; Iaizzo 2005:15). The interatrial septum (IAS) begins its growth towards fused endocardial cushions. The IAS and endocardial cushions unite and an opening in the septum develops called foramen ovale. The IAS divides the atrial region into a right atrium (RA) and a left atrium (LA). Formation of interventricular septum (IVS) partitions the ventricular region into right ventricle (RV) and a left ventricle (LV). Partitioning of the atrioventricular canal, atrial region and ventricular region is complete by the end of the 5th and 8th week. The semilunar valves form between 5th and 9th week, (Tortora and Derrickson 2009: 249).

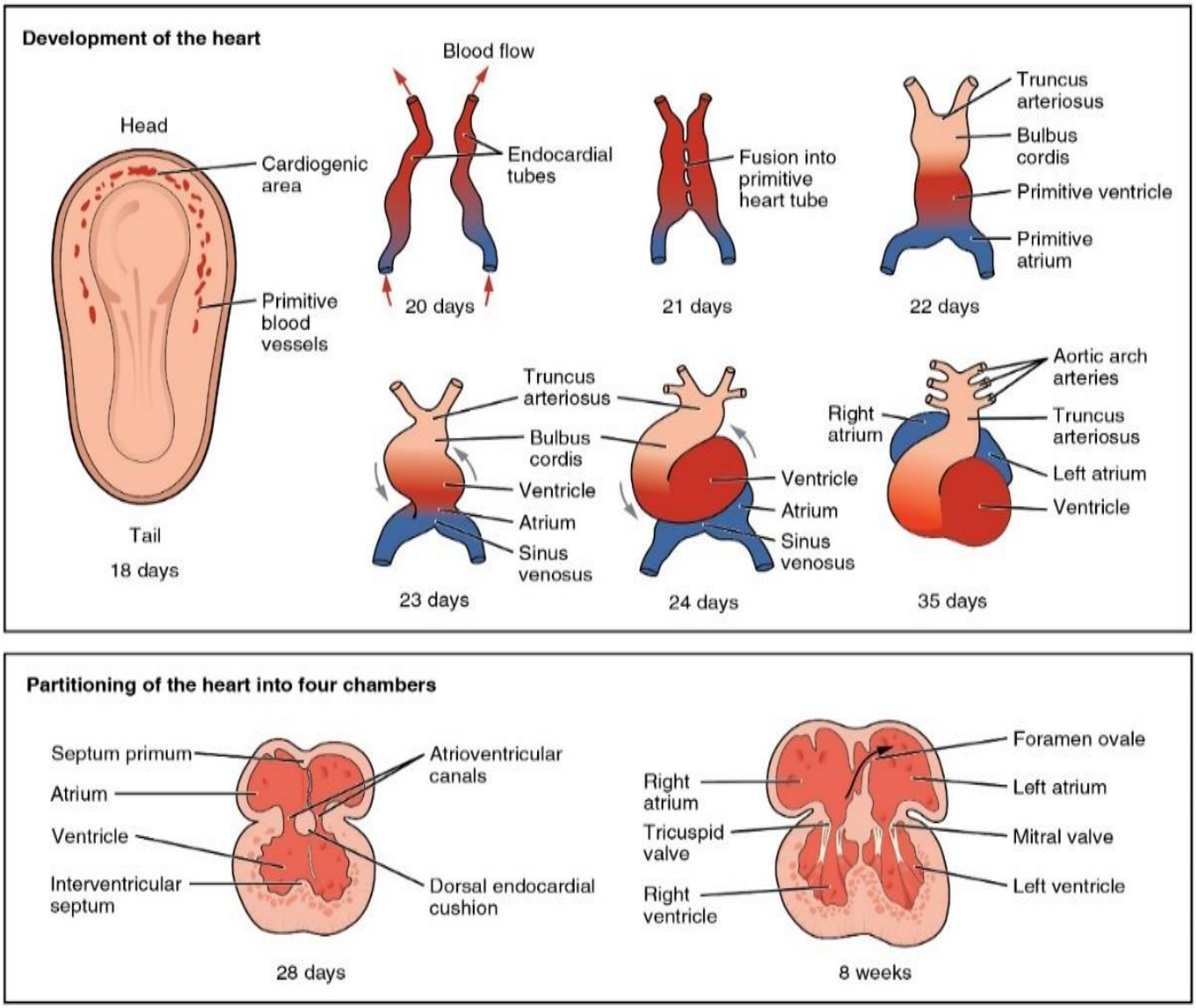

Figure 1.1 Development of the heart, (Amita 2016)

1.2.2 FOETAL CIRCULATION

The foetal circulation is arranged in parallel, rather than in a series, figure 1.2, (Keane, Lock and Flyer 2006:75). Well oxygenated blood from the placenta is delivered to the foetal heart via umbilical cord and ductus venosus, (Hillman et al. 2013:5; Pansky 1982: 128; Keane, Lock and Flyer 2006:75). The foetal blood reaches the placenta via umbilical arteries which are branches of the descending aorta. Blood from the placenta is returned by umbilical veins, about one-half passing through the liver sinusoids and the rest

bypassing the liver via ductus venosus into the inferior vena cava (IVC), (Hillman et al. 2013:5; Pansky 1982: 128; Keane, Lock and Flyer 2006:75). Then blood enters the RA. Since the IVC also receives deoxygenated blood from lower portion of the body, the blood entering the RA is not as well oxygenated as that in the umbilical veins, (Hillman et al. 2013:5; Pansky 1982: 128; Keane, Lock and Flyer 2006:75). From RA blood is directed preferentially to the LA. Here blood mixes with small amounts of deoxygenated blood returning from the lungs via pulmonary veins, (Hillman et al. 2013:5; Pansky 1982: 128; Keane, Lock and Flyer 2006:75).

Blood is preferentially delivered to the brain and coronary circulation by foetal LV. Some of the oxygenated blood from the IVC stays in the RA, mixes with deoxygenated blood from superior vena cava (SVC) and coronary sinus and passes into RV, (Hillman et al. 2013:5; Pansky 1982: 128; Keane, Lock and Flyer 2006:75). The RV is the predominant ventricle in the foetus. Most of RV output goes to the descending aorta via ductus arteriosus since very little blood enters the pulmonary circulation, (Hillman et al. 2013:5; Pansky 1982: 128; Keane, Lock and Flyer 2006:75). The blood in the descending aorta passes to the umbilical arteries and is returned to the placenta for re-oxygenation, (Hillman et al. 2013:5; Pansky 1982: 128, Keane, Lock and Flyer 2006:75).

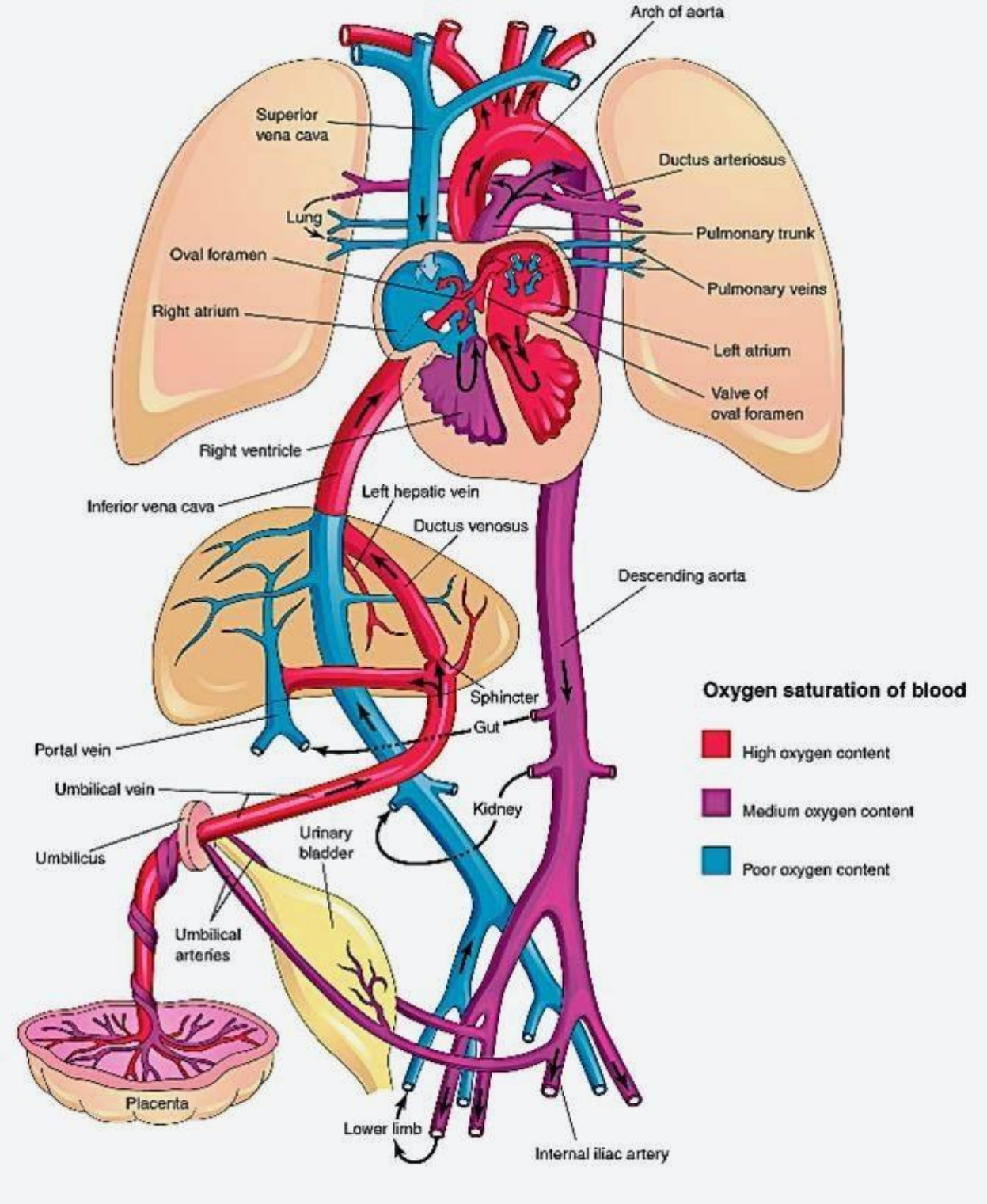

Figure 1.2 Foetal heart circulation, (American Heart Association 2019).

1.2.3 EXTRA-UTERINE ADJUSTMENT

The transition from a foetus to a newborn is the most complex physiological adaptation that occurs in human experience, (Hillman et al. 2012:1). All organ systems are involved but the major immediate adaptations are the establishment of air breathing concurrently with changes in pressures and flows within the cardiovascular system, (Hillman et al. 2012:1). At birth removal of the low resistance placenta, blood flow increases to the pulmonary circulation. The abrupt drop in intrathoracic pressure brought about the first respiration helps contribute to the initial pulmonary circulation, (Hillman et al. 2012:5-6; Pansky 1982:129; Iaizzo 2005:21). The circulation changes from parallel to series; RV and LV output are equivalent, figure 1.3, (Hillman et al. 2012: 5). Blood flow decreases in the ductus arteriosus, it walls contract and closes off completely in few days. The influx of pulmonary blood into the LA causes the septum primum to be pressed against the septum secundum and the foramen ovale is closed, (Hillman et al. 2012:5-6; Pansky 1982:129).

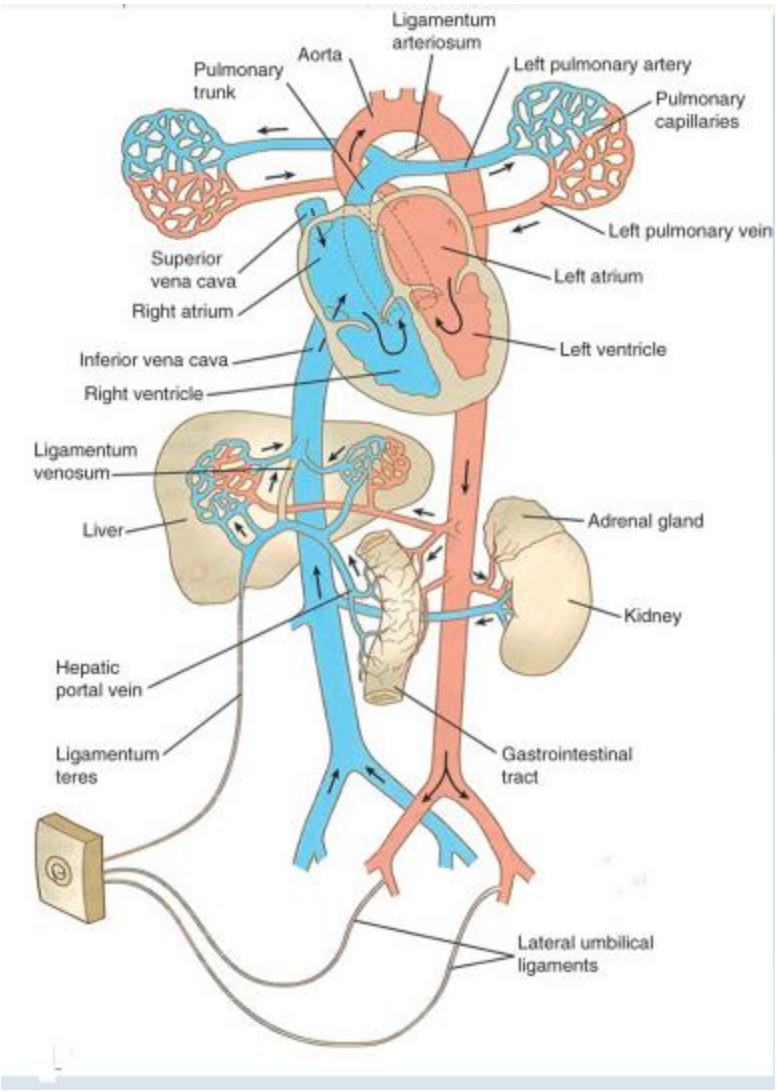

Figure 1.3 Circulation after birth, (Dees and Baldwin 2018).

1.3 PAEDIATRIC ECHOCARDIOGRAPHY

Echocardiography is the use of ultrasound waves to investigate and display the action of the heart as it beats. It is used in the diagnosis and assessment of congenital and acquired heart diseases. It is a safe, painless, reliable and reduces the need for cardiac catheterisation, (Martin 2003:215). Paediatric echocardiography developed along with the field of adult echocardiography, (Mahle, John and Silverman 2009: 26). Helmut Hertz and Inge Elder collaboration 1953 marked the birth of clinical echocardiography, (Armstrong and Ryan 2010: 5-18; Mahle, John and Silverman 2009:26; Mohamed, Arifi and Omran 2010: 71; Singh and Goyal 2007:433).

The objective of echocardiography examination should be to define as completely as possible the morphological aspects of cardiac disorders using all modalities of echocardiography, (Silverman 1993:1). The indication for paediatric echocardiography study is to identify or better define pathology that cannot be otherwise confirmed by clinical evaluation, chest X-ray and electrocardiography (ECG). Repeat studies are performed to identify the consequences of surgical or medical intervention or to monitor pertinent features of lesion progress. The examination is dictated by the need to demonstrate all aspects of normal cardiac anatomy and physiology for all regions of the heart and great vessels. The average time for paediatric echocardiography examination is 50 minutes including performance, partial or complete supervision of the full study, reporting and review of the study, (Hoosen et al. 2007:43-44).

1.4 PREVALENCE OF CONGENITAL HEART DISEASE (CHD)

Congenital heart disease is a common cause of morbidity and mortality in children. CHD is commonly missed, misdiagnosed or identified late especially in the setting of low or middle income countries. The incidence of CHD depends on how a population is studied, how early and how intensively the diagnosis is made. The incidence of CHD is similar worldwide despite the differences in environmental factors, ethnic groups and altitudes. It is estimated to be 8-12 per 1000 live births worldwide, (Hoosen et al. 2011:106; Hoffman 2013:141; Brown and Pepeta 2016:2; Olusoji and Awolola 2013:34-35). There is little reliable data concerning the prevalence of congenital or acquired heart disease in African children, but there is sufficient information about the cardiac disease burden, (Hewitson and Zilla 2010:18).

In South Africa, there are few centres in the public health care system providing paediatric cardiology services. These centres are located in: Johannesburg, Pretoria, Durban, Port Elizabeth and Cape Town. Moreover, there are provinces that still do not have dedicated paediatric cardiology services, (Brown and Pepeta 2016:2; Olusoji and Awolola 2013:34-35). Prevalence of CHD in South Africa is underestimated since a significant proportion of childhood disease remains undetected, (Brown and Pepeta 2016:3).

1.5 NEED FOR DEVELOPING NOMOGRAMS

The quantification of cardiac dimensions derived from echocardiography is important in paediatric practise. Evaluation of size and growth of cardiac chambers, valves and great vessels plays a key role in the management of CHD from initial decision making in the neonatal period to nature and timing of subsequent interventions, (Kaski and Daubeney 2008:1).

Echocardiography has played a role in the improved outcomes for children with cardiac disease. Paediatric cardiology has adapted and refined techniques from adult cardiology to manage children, (Mahale 2009:33). Although a quantitative evaluation of cardiac chambers, valve annulus and great vessels dimensions in paediatric echocardiography is important, nomograms for these structures are limited. Several studies have already provided normal values in the paediatric population that represent most populations of the world but there is lack of data originating from sub-Saharan Africans, (Lemmer et al. 2014:1 Roge et al. 1978:285; Majonga et al. 2017: 409).

The size of cardiovascular structures is influenced not only by treatments but also by confounding factors such as growth, gender, race, body composition, basal metabolic rate, haematocrit, exercise, type of delivery, gestational age and geographical factors. (Lopez et al. 2010: 466). Cardiac dimensions have shown significant racial differences with Black race children with large dimensions than White race children. It is crucial to develop regional echocardiography nomograms because environmental, social and economic factors of a region may impact standards of growth of a population, (Majonga et al. 2017: 409-410).

This study aims to establish reliable echocardiography nomograms for cardiac chambers, valve annulus, thymus and great vessels dimensions in a sub -Saharan African neonatal population. The potential impact of the study is to influence current practise for medical and surgical intervention of CHD which is currently based from first world countries that may be different from African populations. Neonatologists will also benefit from these

nomograms when doing point of care echocardiography to differentiate normal and abnormal echocardiography dimensions.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORY OF PAEDIATIC CARDIOLOGY

According to Royal College of Physicians and Surgery of Canada, paediatric cardiology is the branch of medicine concerned with the study of congenital malformations, acquired heart disease and abnormalities of the systemic and pulmonary circulations in the foetus, newborn, child and young adult, (Anon. 2010:1).

Paediatric cardiology was born when Robert Gross ligated a patent ductus in 1938. Few pediatricians who were interested in cardiology taught themselves and soon paediatric cardiology training programs developed. Pediatric cardiology became the first subspecialty board of pediatrics by 1961 in United States, (Noonan 2004:298).

Paediatric cardiac services are unique in the sense that they require specialized services and infrastructure to deal with patients. In South Africa literature on how and who started paediatric cardiology is lacking. Professor W.S. Winship established paediatrics and birth defects clinic in 1962 at King Edward VIII Hospital, in 1968 he was appointed as a principal specialist lecturer in cardiology and established paediatric cardiology service in KwaZulu Natal, (Anon 2011:1). Head of Paediatric Cardiology, Professor Cilliers (2017 pers. Comm. 28 February) and Paediatric Cardiologist Dr Ntsinjana (2017 pers. Comm. 28 February) recall that paediatric cardiology services in Johannesburg was started by Professor S. Levin.

Chris Hani Baragwanath Academic Hospital (CHBAH) was established during the 2nd world war in 1941 and was opened late in 1942, at that time it was called “The Imperial Military Hospital”. Initially, it was a military hospital which faded in 1947 and in 1948 it became a civilian hospital. It became part of Johannesburg General Hospital and University of Witwatersrand becoming one of largest teaching hospital. CHBAH is the 3rd largest hospital in the world and largest specialist hospital in the Southern Hemisphere, (Horwitz 2013: 2-5).

South Africa (SA) has a population of different ethnic backgrounds encompassing Blacks, Whites, Asians and Coloureds. CHBAH services the black African community of 1.1 million people in Soweto, SA and is a referral hospital for a large part of the country including the surrounding African states. Due to urbanization, many individuals in Soweto have adopted western lifestyles like any other urban region within Sub-Saharian Africa. Soweto is also populated by a steady number of migrants from rural regions where traditional lifestyle remains a norm, (Sliwa et al. 2009:1-2).

Head of Paediatric Cardiology, Professor Cilliers (2017 pers. Comm. 28 February) recalls that paediatric cardiology as an independent unit at CHBAH was born around 1990 or 1991 when Dr Du Plessis joined CHBAH as the first paediatric cardiologist. The 3500 bed CHBAH provides most specialist cardiac services and treatment for Soweto and surrounding communities. The cardiology unit provides gold-standard cardiology investigations for definitive diagnoses and treatment of many Sowetans and the referred patients from the surrounding regions, (Sliwa et al. 2009:1-2).

2.2 CHALLENGES OF PAEDIATRIC CARDIOLOGY IN SOUTH AFRICA

The majority of services can only be delivered by experts in the field and there are few aspects that can be delegated to juniors and support staff for example diagnosis of complex congenital heart disease on echocardiography. The vast majority of the South African population depends on the public health system for their health needs. Out of the nine SA provinces, four still have no paediatric cardiology services. In the five remaining provinces there are a few government hospitals providing paediatric cardiology services, however vast majority of paediatric cardiologists work in the public sector, (Hoosen et al. 2011: 106; Zühlke 2013: 413; Brown and Pepeta 2016:2-3).

Most patients have to travel long distances to reach major centres with paediatric cardiology services. Referral centres suffer from lack of trained personnel, infrastructure and resources, (Hoosen et al. 2011: 106; Zühlke 2013: 413; Brown and Pepeta 2016:2-3).

At the end of 2008, twenty four paediatric cardiologists were practicing in this country, half in public sector and half in private sector whereas international recommendations, (Hall et al. 2002 cited in Hoosen et al. 2011:106) suggest that South Africa needs at least 88 paediatric cardiologists. A combination of these factors have resulted in overcrowded referral hospitals, delayed hospital admissions and long surgical lists. As a result children with CHD present late, develop complications such as heart failure further increasing morbidity, mortality and burden of childhood heart disease on available resources, (Hoosen et al. 2011: 106; Zühlke 2013: 413; Brown and Pepeta 2016:2-3)

2.3 FACTORS INFLUENCING FOETAL HEART GROWTH

The cause of CHD remains unknown in most cases regardless of decades of research. Development of the foetal heart is magnificently directed by multitude of factors including maternal factors, family history, foetal factors and placental factors, (Courtney, Cnota and Jones 2018:1; Donofrio et al. 2014:2188-2191; Camm, Botting and Sferruzzi-Perri 2018:1).

2.3.1 MATERNAL FACTORS

2.3.1.1 DIABETES MELLITUS

Mothers with pre-gestational diabetes mellitus (DM) have a 3-5% increased risk of having a baby with CHD compared to the general population, (Donofrio et al. 2014: 2188). Pre-gestational DM has a high risk noted for specific cardiac defects including heterotaxy, truncus arteriosus, transposition of great vessels (TGV) and single ventricle defects, (Donofrio et al. 2014: 2188). Lack of pre-conceptional glycemic control is associated with all congenital malformations, (Donofrio et al. 2014: 2188). However, gestational DM does not appear to be associated with increased risk of CHD, (Donofrio et al. 2014: 2188). A foetus may develop ventricular hypertrophy late in gestation in the presence of poorly controlled maternal gestational or pre-gestational DM, (Donofrio et al. 2014: 2188).

2.3.1.2 PHENYLKETONURIA

Phenylketonuria is a birth defect that causes an amino acid called phenylalanine to build up in the body. Maternal phenylketonuria if untreated causes adverse pregnancy outcomes including CHD. Increased maternal serum levels of phenylalanine is associated with increased risk of CHD, (Donofrio et al. 2014: 2188-2189).

2.3.1.3 MEDICATION EXPOSURE

A number of human teratogenic medications are used clinically in women of childbearing age and exposure to these in the period of cardiogenesis increase risk of CHD, (Donofrio et al. 2014: 2188). Carbamazepine (anticonvulsant) used in pregnancy has 1.8% incidence of CHD. There is an 8% incidence of CHD in mothers using lithium as a mood stabilizer. Angiotensin-converting enzyme inhibitors exposure in 1st trimester is associated with 2.9% incidence of CHD, (Donofrio et al. 2014: 2188). Retinoic acid has an 8-20% incidence of CHD including conotruncal defects and aortic arch anomalies, (Donofrio et al. 2014: 2188). Selective serotonin reuptake inhibitors used in pregnancy has no increased risk of CHD except for paroxetine which is associated with right ventricular outflow tract obstruction lesions, (Donofrio et al. 2014: 2188). First trimester use of warfarin and other Coumadin derivatives is teratogenic and associated with skeletal abnormalities is not linked with an increased risk of CHD, (Donofrio et al. 2014: 2188). Use of non-steroidal anti-inflammatory agents in early gestation has been associated with small increased risk of CHD, (Donofrio et al. 2014: 2189-2190).

2.3.1.4 INFECTION

The effect of nonspecific maternal infection is difficult to separate definitely from effects of medication used to treat the illness. Febrile illnesses is positively associated with occurrence of CHD, (Donofrio et al. 2014: 2190). The risk of CHD is significantly increased among maternal rubella with an estimate of 20-30%, (Ye et al. 2019:3; Vaziri et al. 2011:60). Multiple and unspecified CHD is associated with congenital rubella and

includes patent ductus arteriosus, pulmonary stenosis, atrial septal defect and ventricular septal defect, (Mekonnen 2017: 200). Patent ductus arteriosus is the most common CHD seen in congenital rubella syndrome, (Vaziri et al. 2011: 60). The mechanisms whereby maternal rubella infection causes foetal damage is poorly understood but are thought to be secondary to vasculitis resulting in tissue necrosis, (Mekonnen 2017: 198; Ye et al. 2019: 10).

2.3.1.5 ASSISTED REPRODUCTION TECHNOLOGY

Use of assisted reproduction technology has increased over the past 2 decades. The increased prevalence of CHD in these pregnancies may be associated with increased risk of multiple gestations (more than one fetus develops simultaneously in the mother's womb) and singletons conceive through in vitro fertilization, (Donofrio et al. 2014: 2190).

2.3.2 FAMILY HISTORY

2.3.2.1 MATERNAL CARDIAC DISEASE

The risk of recurrence of non-syndromic and non-chromosomal CHD is 2 times higher if the mother has a congenital cardiac lesion, compared to a father or sibling. Risk differs greatly with different maternal diagnosis and is highest with heterotaxy, atrioventricular septal defect with a 10-14% incidence, 13-18% in aortic stenosis and ≤ 3% incidence in isolated tetralogy of Fallot or TGV, (Donofrio et al. 2014: 2190).

2.3.2.2 PATERNAL CARDIAC DISEASE

Risk differs according to the lesion type, there is a 2-3% risk of cardiac malformations if the father is affected with a non-syndromic CHD. Bicuspid aortic valve lesion has a higher risk than other defects, (Donofrio et al. 2014: 2191).

2.3.2.3 AFFECTED SIBLINGS

The risk of recurrence of cardiac malformations in siblings is low compared to affected parents. There is a 2-6% recurrence risk if a sibling is affected with unaffected parents, (Donofrio et al. 2014: 2191).

2.3.2.4 SECOND AND THIRD DEGREE RELATIVES

There is a less than 0.3% incidence of CHD in the 2nd and 3rd degree relatives of patients with tetralogy of Fallot, (Donofrio et al. 2014: 2191).

2.3.2.5 DISEASES, DISORDERS OR SYNDROMES WITH MENDELIAN INHERITANCE

The recurrence risk in the foetus is high in the following instances: (a) in pregnancies in which a prior child is affected by recessively inherited disease, (b) in pregnancies in which a parent is affected by an autosomal dominant genetic disorder with increased risk for cardiac malformation, (c) pregnancies with a deletion syndrome known to be associated with a significant incidence of a cardiac phenotype, (Donofrio et al. 2014: 2191).

2.3.3 FOETAL FACTORS

2.3.3.1 SUSPECTED CARDIAC ABNORMALITY ON OBSTETRIC ULTRASOUND

There is more than 40% diagnostic yield for foetal echocardiography detection of CHD when referral indication is an abnormal 4-chamber screening view on obstetric ultrasound, (Donofrio et al. 2014: 2191).

2.3.3.2 SUSPECTED ABNORMALITY OF HEART RATE OR RHYTHM

Foetal tachycardia is rarely associated with CHD. In contrast, foetal bradycardia resulting from abnormal AV conduction has a 50-55% incidence of CHD. Foetal bradycardia resulting from long QT syndrome may present with isolated sinus bradycardia or 2:1 AV block. A 0.3% incidence of CHD occurs in the presence of an irregular foetal rhythm caused by atrial extra-systoles, (Donofrio et al. 2014: 2191).

2.3.3.3 NONCARDIAC ABNORMALITIES

A 20-45% incidence of CHD is associated with one or more extra-cardiac malformations even in the presence of a normal karyotype. The risk of CHD associated with a cleft lip is very low, (Donofrio et al. 2014: 2191).

2.3.3.4 KNOWN OR SUSPECTED CHROMOSOMAL ABNORMALITIES

The risk of congenital anomalies is high if foetal chromosome testing reveals a genetic mutation, deletion, rearrangement or aneuploidy, (Donofrio et al. 2014: 2192).

2.3.3.5 INCREASED NUCHAL TRANSLUCENCY AND DUCTUS VENOSUS FLOW

Nuchal translucency is an abnormal subcutaneous collection of fluid seen on foetal ultrasound posteriorly on the neck of human foetuses at 10-14 weeks of gestation. The association of an increased nuchal translucency with CHD is 40-56%. Absence or reversal of flow with atrial contraction in the ductus venous using pulse wave Doppler has been associated with increased risk of CHD, (Donofrio et al. 2014: 2192).

2.3.3.6 MONOCHORIONIC TWINNING

Twin pregnancies have higher rates of congenital malformations than singleton gestations. Monochorionic twins, in particular, have a 2-9% incidence of CHD, (Donofrio et al. 2014: 2192).

2.3.3.7 NONIMMUNE HYDROPS FOETALIS AND EFFUSION

Foetal hydrops refers to pathological accumulation of fluid in ≥2 foetal compartments. 15-25% of foetuses with nonimmune hydrops have associated cardiac abnormalities or arrhythmias. 10% of foetuses with hydrops have high cardiac output state caused by foetal anemia, acardia twinning, sacrococcygeal teratomas and foetal or placental malformations, (Donofrio et al. 2014: 2192).

2.3.4 PLACENTAL FACTORS

The heart and placenta develop concurrently. Growth and remodeling of both the foetal heart and placenta may be impacted by signaling between the placenta and foetal organs via foetal-placental circulations, (Courtney, Cnota and Jones 2018:1-2).

Thin or large placenta area relative to birth weight is associated with the sudden cardiac death. Abnormal cord insertion is associated with CHD, (Camm, Botting and Sferruzzi-Perri 2018:2).

3.9- >50% incidence of CHD is associated with single umbilical artery. Anomalies of foetal venous system occur occasionally and have been associated with CHD in particular agenesis of ductus venosus. Absence of ductus venosus results in unimpeded placental return which can lead to volume overload and heart failure, (Donofrio et al. 2014: 2188).

2.4 FORMATION OF ECHOCARDIOGRAPHY NOMOGRAMS

2.4.1 ECHOCARDIOGRAPHY

Echocardiography refers to the evaluation of cardiac structure and function using ultrasound, (Shah et al. 2013:54). Vitruvius was the first person to use the word echo whereas Abbe Lazzaroi Spallanzani is regarded as the father of ultrasound. Christian Johann Doppler, in 1842, noted that the pitch of sound waves varied if the source was moving. Karl Dussik was the first to apply ultrasound for medical diagnosis in 1941. He attempted to outline the ventricles of the brain by using transmission ultrasound rather than reflected ultrasound. Keidel used ultrasound to examine the heart in 1950 with the purpose of determining cardiac volumes. Helmut Hertz and Inge Elder, at about the same time, established a commercial ultrasonoscope which was used to examine the heart. Their collaboration marked the beginning of clinical echocardiography as it is known today, (Armstrong and Ryan 2010: 5-18).

The development of echocardiography is an outstanding example of collaboration among physicians, engineers and clinicians. Cardiac ultrasound was initially named by Elder and Hertz as Ultrasound Cardiograph (UCG). Echocardiography has evolved over time exponentially with development of its many modalities such as A-mode, M-mode, contrast, Doppler, transoesophageal and intravascular ultrasound which are an integral part of cardiac assessment in the modern era, (Armstrong and Ryan 2010: 5-18).

Medical and surgical management is determined by accurate anatomical diagnosis from echocardiography. In order to diagnose cardiac disease echocardiographically, any deviation from normality can only be appreciated if normal values for chamber size for different age groups are available, (Shah et al. 2013:54).

2.4.1.1 ECHOCARDIOGRAPHY ADVANTAGES AND DISADVANTAGES

Echocardiography is a highly portable battery-powered instrument, (Bulwer and Rivero 2011:7). Echocardiography is very cost-effective compared with competing technologies,

(Armstrong and Ryan 2010:5-18; Shah et al. 2013:54). Echocardiography is easy to understand as many features are based on simple physical and physiological factors, (Kaddura 2008:1). Echocardiography is a versatile technique that provides a wide range of clinically useful information in a variety of settings, (Bulwer and Rivero 2011:7; Armstrong and Ryan 2010:153). Echocardiography provides more rapid results compared to other cardiac imaging techniques, (Bulwer and Rivero 2011:7). It is safe in pregnancy and childhood with minimal patient discomfort, (Bulwer and Rivero 2011:7). Echocardiography requires skill and is operator dependent, (Kaddura 2008; Armstrong and Ryan 2010: 153).

2.4.1.2 APPLICATION OF ECHOCARDIOGRAPHY

Echocardiography acquires rapid images in real time. An echocardiogram is recorded by placing a transducer in an interspace adjacent to the left sternal border and other locations on the chest and in the epigastrium. The heart is scanned from top to bottom, from front to back and from side to side from all transducer locations. There are 3 methods that are commonly used in echocardiography for clinical usage, namely, Two- dimensional (2-D) or cross-sectional, Motion or M-mode and Doppler (continuous wave, pulsed wave and color flow), (Silverman 1993: 1-2; Kaddura 2008: 10; Johnson and Moller 2014:56). Cardiac dimension measurements are derived using mainly 2-D and M-mode.

2.4.1.2.1 TWO- DIMENSIONAL OR CROSS-SECTIONAL ECHOCARDIOGRAPHY

An echocardiography study currently begins with real time 2-D echo which produces high resolution tomographic images of cardiac structures with their movement and vascular structures leaving and entering the heart. The 2-D format shows images in cross-section using real time moving images in various shades of grey depending on their echo reflection as shown in figure 2.1, (Park 2016: 65; Park 2014: 51; Shah 2013:54; Kaddura 2008: 10).

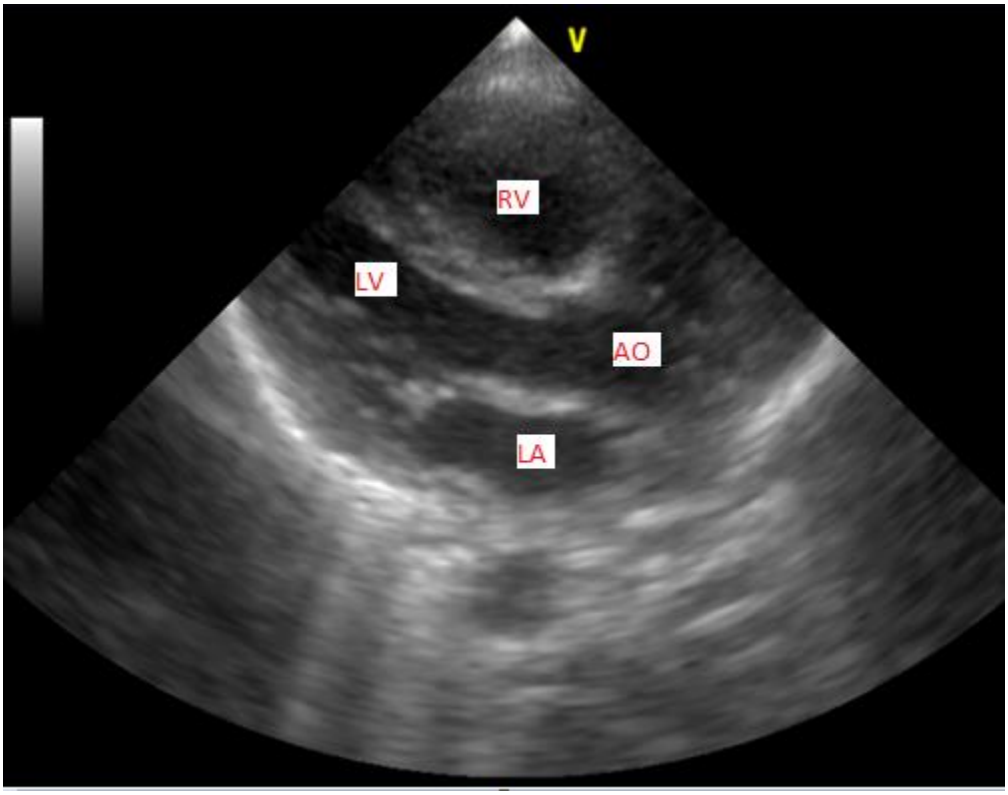

Figure 2.1 2-D image. Image from study cohort. **RV:** right ventricle, **LV:** left ventricle, **LA:** left atrium, **AO:** aorta.

Routine 2-D echocardiography is obtained from 4 transducer locations namely: parasternal, apical, subcostal and suprasternal notch positions. 2-D echocardiography is used to identify cardiac anatomy, ventricular and valve movements and is also used to assist to position for M-mode and Doppler echocardiography. Measurements of important cardiac structures can be made on the freeze frame of 2-D echo studies, (Park 2016: 65; Park 2014: 51; Shah 2013:54; Kaddura 2008: 10).

2.4.1.2.2 MOTION MODE OR M-MODE

M-mode refers to recording of the movement of cardiac structures in a single dimension plane over several cardiac cycles. M-mode is produced by transmission and reception of an ultrasound signal along one line, while the signal is aligned perpendicular to the

structure being measured as shown in figure 2.2, (Park 2016:74; Johnson and Moller 2014:55-57; Shah 2013:55; Kaddura 2008: 11-12; Silverman 1993: 35-37).

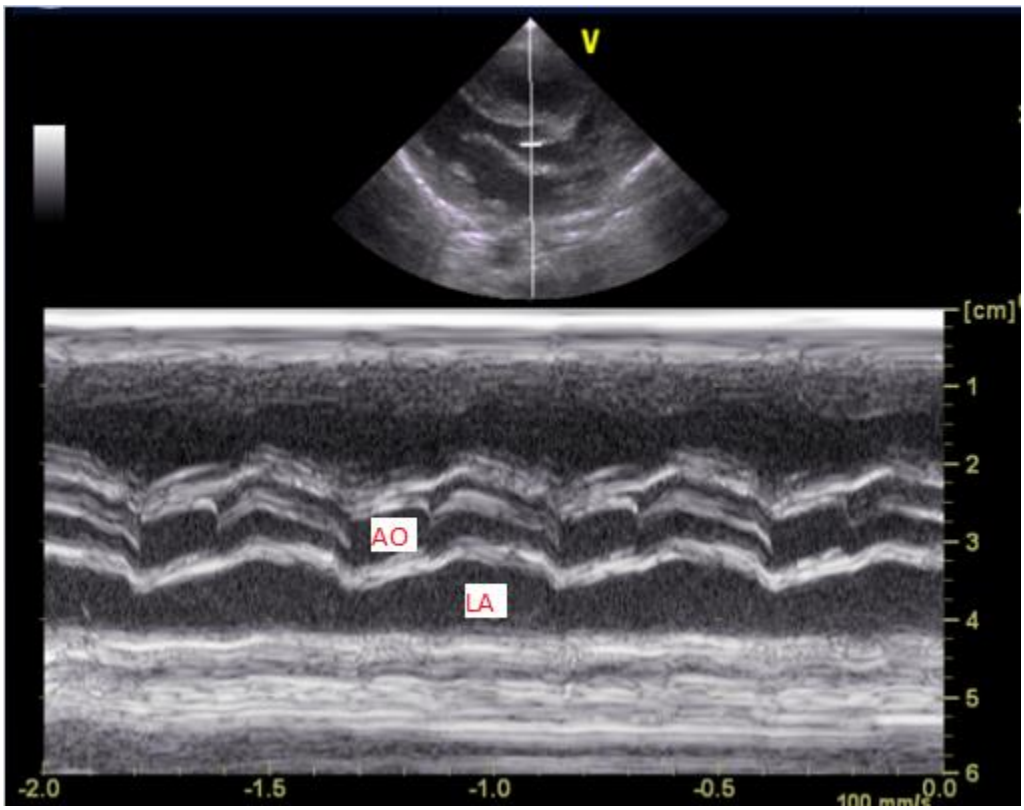

Figure 2.2 M-mode image. Image taken from study cohort. **AO:** aorta. **LA:** left atrium.

M-mode echocardiography provides an ice-peak view of the heart. It has limited capability in demonstrating the spatial relationship of the cardiac structures, but remains an important tool in the evaluation of cardiac conditions and function, by providing a means to accurately measure cardiac dimensions such as size and thickness of cardiac chambers. M-mode is usually performed as part of the 2-D study, although it is the least commonly performed mode in paediatric cardiology, it does facilitate an appropriate display from standardized acquisition positions, (Park 2016:74; Johnson and Moller 2014:55-57; Shah 2013:55; Kaddura 2008: 11-12; Silverman 1993: 35-37).

The particular advantage of M-mode is its simplicity of measurement. A high frame rate results in superior time resolution when compared with 2-D. Essentially, M-mode provides a rapid, accurate and repeatable method for assessing and measuring the changes that occur with growth and development, (Silverman 1993: 35-37).

2.4.2 PATHOLOGIES THAT CAN BE DETECTED WHEN MEASURING CARDIAC DIMENSIONS

Identifying abnormal measurements provides a way of assessing the effects of a disease, cardiac dimensions helps to determine when intervention is necessary and to monitor the effects of intervention, (Lopez et al. 2010: 466). Pathologies that can be detected by deviations in normal cardiac dimensions which are the focus of this research are presented in table 2.1.

Table 2.1 Pathologies detectable by abnormal cardiac dimension measurements, (Jacobs 2012: 21-24).

CARDIAC STRUCTURE	DETECTABLE PATHOLOGY
Cardiac chambers:	
LA	Dilatation which suggest presence of diastolic dysfunction. Thrombus. Myxomas. Cortriatriatum.
LV cavity	Dilatation which is a common feature of dilated cardiomyopathy. Systolic dysfunction. Thrombus. Intra-cavity masses.
LV wall	Hypertrophy a common feature of hypertrophic cardiomyopathy and left outflow tract obstructions.
Valve annulus (aortic, mitral, tricuspid and pulmonary valves)	Dilatation and narrowing which are underlying cause of regurgitation and stenosis commonly seen paediatrics.
Great vessels:	
Pulmonary artery and its branches	Dilatation which may suggest aneurysm. Supravalvular stenosis. Branch pulmonary stenosis.
Aorta (ascending and abdominal)	Dilatation which can suggest aneurysm. Narrowing a feature of coarctation of the aorta. Aortic dissection flaps.
Thymus	Hypoplastic or absent thymus.

2.4.3 METHODS OF OBTAINING CARDIOVASCULAR DIMENSION MEASUREMENTS

Quantification in paediatric echocardiography requires a consensus on what and how measurements should be made in a standard protocol. Previous authors have presented a comprehensive list of guidelines when performing a paediatric echocardiogram (Lopez et al. 2010:490).

Current American guidelines for chamber, annular and vessel quantification involve measurements of intraluminal dimensions from one inner edge to the opposite inner edge. Published paediatric normative databases based on Two-dimensional (2-D) echocardiography have used inner edge to inner edge of vessel diameters. Vascular diameters should be perpendicular to the long axis of the vessel. Valvular diameters should be measured at the moment of maximum expansion. Mitral valve (MV) and tricuspid valve (TV) annular diameters must be measured in diastole. Aortic valve (AV) and pulmonary valve (PV) annular diameters as well as arterial diameters must be measured in systole. (Lopez et al. 2010: 466; Cantinotti et al. 2014b: 1279-1280).

These recommendations are based on hemodynamic considerations and correspond to methodologies used in published paediatric normative databases and differs from adult approaches. Care must be taken in measurements done in apical views not to foreshorten the heart. MV and TV size helps characterize valvar pathology and diagnose ventricular hypoplasia. Annular diameters can be measured using 2-D imaging and may be measured in apical 4-chamber and parasternal long-axis (PLAX) views. (Lopez et al. 2010: 466; Cantinotti et al. 2014b: 1280).

Aortic valve (AV) and pulmonary valve (PV) diameters are best measured with magnification in PLAX views from the inner edge of proximal valve insertion hinge point within the arterial root to the inner edge of the opposite hinge point. PV annular diameter can be measured in PSAX view. However these measurements are often underestimated because they rely on lateral imaging planes which are associated with relatively low resolution and an oblique orientation is often the only one available in these view. Systolic annular diameters have correlated well with intra-operative measurements. AV and PV

annular measurements during mid-systole are recommended in children. AV annular diameter is best measured in PLAX view and PV annular diameter in PSAX view, both during early to mid-systole, (Lopez et al. 2010: 486; Cantinotti et al. 2014a: 184).

Measurements of arterial vessels helps to identify patients with various vascular abnormalities. The timing of the measurement during cardiac cycle has been discussed extensively. Some authors have recommended performing the measurements during either diastole or systole or using the average of both measurements, (Lopez et al. 2010: 487-489; Cantinotti 2012:146-148; Cantinotti et al. 2014a: 181). Systolic diameters are significantly large than diastolic diameters. The maximum effect of vascular size on vessel function occurs during peak flow and peak wall stress at the time of peak systolic pressure which is the primary determinant of dissection or rupture, (Lopez et al. 2010: 487-489; Cantinotti 2012:146-148; Cantinotti et al. 2014a: 181).

All measurements of arterial diameters in children should be made at the moment of maximal expansion, typically during mid-systole. Assessment of pulmonary artery (PA) size is important in children with various forms of CHD. When PA flow is diminished, the branch pulmonary arteries are small. PA can be evaluated in PSAX or suprasternal short-axis (SSAX) views presumably because of less cardiac motion present in the SSAX view. Main pulmonary artery (MPA) and branch pulmonary artery (right pulmonary artery- RPA and left pulmonary artery- LPA) diameters can be measured in the PSAX view. The RPA can also be measured as it crosses behind the ascending aorta in the SSAX view, whereas LPA can also be measured at its origin from MPA in a left anterior oblique or sagittal plane in a suprasternal or high left parasternal view. The descending aorta diameter is best measured during mid-systole in the subxiphoid short axis view at the level of the diaphragm. (Lopez et al. 2010: 487-489; Cantinotti 2012:146-148; Cantinotti et al. 2014a: 181).

Altered left atrial dimensions (LA) are often an indicator of left heart pathology. The LA fulfills 3 major physiologic roles that have an impact on LV filling and performance, (Lang 2005 et al.: 468-470; Lang et al. 2006: 96-97; Cantinotti et al. 2014a: 184). It acts as a contractile pump that delivers 15-30% of LV filling, it is a reservoir that collects pulmonary venous return during ventricular systole and it acts as a conduit for passage of stored

blood from LA to LV during early ventricular diastole. An increase in LA size is often associated with adverse cardiovascular outcomes, (Lang 2005:468-470; Lang et al. 2006: 96-97; Cantinotti et al. 2014a: 184). LA size is measured at the end-ventricular systole when the LA chamber is at its greatest dimension. The LA can be visualized from multiple echocardiographic views from which several LA dimensions can be measured. The LA diameter is often measured from PSAX view at the level of aorta, (Lang 2005 et al.: 468-470; Lang et al. 2006: 96-97; Cantinotti et al. 2014a: 184).

Measurements of LV size and function are essential in the assessment of patients with congenital and acquired heart disease. A qualitative visual inspection might be adequate but can be misleading and is prone to inter-observer and inter-study variability and relies on the skill of the interpreter. Published recommendations for chamber quantifications in adults have been extensively used in children because data on accuracy and reproducibility of the measurements in paediatrics are scarce, (Lang 2005 et al.: 1443-1444; Lang 2006 et al.: 82-83; Lai et al. 2006:1425; Lopez et al. 2010: 466). Several linear and volumetric methods to assess LV size should be measured both during systole and diastole, defining end-diastole as the frame with maximum chamber intraluminal area and end-systole as the frame of minimal area. These definitions are problematic because they rely on visual estimates rather than quantitative frame by frame analysis and the minimum area occurs at different times in the parasternal short-axis (PSAX) and PLAX views. Given these limitations, end-diastole can be defined as the frame at which MV closes and end-systole as the frame preceding MV opening. (Lang 2005 et al.: 1443-1444; Lang 2006 et al.: 82-83; Lai et al. 2006:1425; Lopez et al. 2010: 466).

Short-axis or minor axis measurements of left ventricular internal diameter (LVID), septal wall and left ventricle posterior wall (LVPW) thickness can be obtained in the parasternal views. The maximum short-axis dimension is often located at MV leaflets tips or chordae in young patients. PSAX LV size should be considered a surrogate for LV size only when the LV short axis geometry is circular. Linear measurements can be obtained from PLAX or PSAX views and from M-mode or 2-D images. It is recommended that to obtain accurate linear measurements of LV dimensions recordings using M-mode should be made in PSAX acoustic window because in PLAX it is difficult to get the M-mode cursor

perpendicular to long-axis of the LV which is mandatory to obtain true minor axis dimension. It further allows one to choose the diameter with the best blood-endocardium interface. This is a definite advantage when dealing with LV trabeculations but it may lead to overestimation of measurements. M-mode echocardiography provides better temporal and spatial resolution than 2-D imaging. M-mode measurements in PLAX view can overestimate LV minor-axis diameters compared to 2-D measurements. Therefore, 2-D PSAX measurements that are averaged over 3 consecutive cardiac cycles can be employed. Ideally, a combination of both PLAX and PSAX views should be used. (Lang 2005 et al.:1443-1444; Lang et al. 2006: 82-83; Lopez et al. 2010: 475; Cantinotti et al. 2014a: 185).

The thymus is measured in the PSAX 2-D view that demonstrates the bifurcation of the MPA. The distance from the anterior chest to the most anterior great vessel is determined as thymus. (Yeager and Sanders 1995:838)

2.4.4 EXPRESSION OF ECHOCARDIOGRAPHIC MEASUREMENTS TO BODY SIZE

All cardiovascular structures increase in size relative to somatic growth, thus body size is the most powerful determinant of the size of cardiovascular structures. Expressing measurements relative to body size allows meaningful distinction between normal and abnormal values in children, (Lopez et al. 2010: 466). Cardiac dimensions are significantly determined by body weight, body surface area (BSA) and gender. It is well known that body weight and BSA correlate well with cardiac structures, however body weight is preferred in newborns because BSA changes minimally in neonates, (Overbeek et al. 2005: 114; Guzeltas and Eroglu 2011: 155; Cantinotti et al. 2012: 143; Mawad et al. 2013: 30). Various formulas exist to calculate BSA (table 2.2) but there is little consensus as to which formula should be used in children, particularly in neonates and infants, (Cantinotti et al. 2014a: 184).

Table 2.2 Different formulas to calculate BSA, (Cantinotti et al. 2014a: 184).

AUTHOR	FORMULA
Du Bois and Du Bois	$Height^{0.75} \times weight^{0.425} \times 0.007184$
Haycock	$Weight^{0.5378} \times height^{0.3964} \times 0.024265$
Dreyer and Ray	$Weight^{0.6666} \times 0.1$
Boyd	$0.0003207 \times (1{,}000 \times weight)^{[0.7285 \times 0.018 \times \log(1{,}000 \times weight)]} \times height^{0.3}$
Mosteller	$\sqrt{[height \times weight)/3{,}600]}$
Gehan and George	$0.0235 \times height^{0.42246} \times weight^{0.51456}$
Meban	$6.4954 \times (1.000 \times weight)^{0.562} \times height^{0.320}$

The controversy of which body size measurement should be used for normalization and how normalized data should be expressed prevails in the scientific community, (Cantinotti 2014a: 179-180). Cantinotti (2014a: 184) has endorsed the Haycock formula as the best fit for most cardiac structures in neonates, infants and toddlers.

Normalized data may be expressed in different ways including the percentage of the mean normal value, percentile charts and Z-scores. Percentage of mean value does not consider normal variation. Centile charts are useful for determining where a given value lies within the population. However, values that lie above or below the uppermost or lowermost centile lines cannot be quantified, (Cantinotti et al. 2012: 143; Cantinotti et al. 2014a: 181; Colan 2013: 18).

The Z-score is a standardized value that indicates how many standard deviations (SD) a value is above or below the mean in a normal distributed population, (Cantinotti et al. 2012: 143; Cantinotti et al. 2014a: 181; Colan 2013: 18). A measurement that is 2 SDs above the mean has a Z-score of +2 whereas a measurement that is 2 SDs below the mean has a Z-score of -2. Z-scores provide a very simple means for comparing echocardiographic measurements with normal values, (Colan 2013: 38).

The use of Z-scores enables a more precise assessment of cardiac dimensions that are important in conditions associated with very small chamber dimensions or very large

measurements. Z-scores are easily utilized mathematically to allow statistical differentiation to be made between subgroups of patients and provide a simple means for comparing echocardiographic measurements with normal values. Z-scores can be expressed as nomograms or be used to create computer spreadsheets that generate a Z-score when body size and cardiac dimension variables are entered, (Kaski and Daubeney 2008: 1-2).

The American Society of Echocardiography and other authors recommend that when parametric normalization is done, reference values should be expressed as Z-scores, (Mawad et al. 2013: 35; Cantinotti 2014a: 181; Cantinotti 2014b: 1282). Z-scores estimates are now part of daily decision making in clinical and surgical management in paediatric cardiology since they provide a means of assessing magnitude of normality, (Mawad et al. 2013:35).

2.4.5 EFFECTS OF CONFOUNDING FACTORS ON CARDIOVASCULAR DIMENSIONS

The size of cardiovascular structures is influenced by numerous factors including growth, gender, race, body composition, basal metabolic rate, haematocrit, exercise, type of delivery, gestational age and geographical factors. (Lopez et al. 2010: 466)

Cantinotti et al. (2012: 144) reported a significant correlation between cardiac dimensions and body weight in the neonatal age group. Kizer et al. (2004 cited in Lang et al. 2006:83) indicated that no significant differences exist between different ethnic groups.

Guzeltas and Eroglu (2012: 155) found no significant differences when measurements were evaluated according to gender but measurements increased linearly in relation to body weight. Cantinotti et al. (2014a: 184) reported a minor effect of gender and type of delivery on cardiac dimensions.

Zilberman, Khoury and Kimball (2005 cited in Cantinotti et al. 2012:148); Guiter et al. (2010 cited in Cantinotti et al. 2012:148); Kaldararova et al. (2007 cited in Cantinotti et al. 2012:148) reported sex-related differences with boys showing larger annular diameters

at any age even after adjustments for differences in body sizes. Tacy, Vermilion and Ludomirsky (1995 cited in Cantinotti et al. 2012:148) demonstrated that weight was a good predictor of annular value dimension and no significant gender-related differences were noted.

Human body and geographical environment are always in a dynamic balance through material and energy exchange, (Han 2015:1557-1565). The geographical environment is a system which is composed of air, water, mineral, humidity and temperature and which varies between regions and countries, (Han 2015:1557-1565). Humans make adaptive changes for temperature, humidity and oxygen to regulate physiological mechanisms in order to maintain the stability of the body's internal environment, (Han 2015:1557-1565). The relationship between geographic environment and body function is very close especially with regard to heart function, (Han 2015:1557-1565) and an example is the significant correlation between LV performance index and latitude, (Han 2015:1557-1565).

Observer variability allows for the quantification of precision but can be a source of both error and accuracy. The minimum requirement to acquire variability assessment is to repeat the initial measurement once. For intra-observer variability an observer performs two measurements on each of the series of samples. Inter-observer variability represents the sum of repeatability and reproducibility. For inter-observer variability the first measurement of the first observer is paired to a single measurement of the second observer, (Popovic and Thomas 2017: 318).

Cantinotti et al. (2014b: 1282) found no significant effect on inter-observer and intra-observer variability. Yeager and Sanders (1995:838) reported that measurement of the thymus has excellent and reproducible inter-observer variability.

2.4.6 IMPORTANCE OF NOMOGRAMS

Body size and cardiac dimensions change dramatically during normal somatic growth and development, (Roge et al. 1978: 285-290; Kaski and Daubeney 2008: 1; Neilan et al.

2009: 50). Nomograms may increase the accuracy of echocardiographic estimation of a defect severity mostly in borderline conditions, (Cantinotti 2014b:1284). Nomograms also provide a quantitative assessment of cardiovascular structures and is essential in the evaluation of CHD in order to plan the most appropriate medical or surgical intervention, (Cantinotti 2012: 142). Nomograms play a role in the assessment and risk stratification of children with acquired heart diseases such as hypertrophic cardiomyopathy or coronary artery involvement in Kawasaki disease, (Kaski and Daubeney 2008:1). The follow-up of children with repaired and unrepaired defects, depends on the identification of structural growth that deviates from normal. Echocardiography nomograms plays an important role to avoid misinterpretations of cardiac structure sizes not based on proper referencing which could lead to inappropriate clinical and surgical management, (Mawad et al. 2013: 33).

2.4.7 PUBLISHED NOMOGRAMS

Published nomograms were sourced from various centres mainly derived from European populations namely from Italy (Cantinotti et al. 2014a, 2014b, 2017), Turkey (Guzeltas and Eroglu 2011; Yekeler et al. 2004), New York (Yeager and Sanders 1995) and Dutch (Overbeek 2006), (Table 2.3).

Table 2.3 Published nomograms, (Cantinotti et al. 2014a: 179-191; Cantinotti et al. 2014b: 1279-1292; Cantinotti et al. 2017: 208-215; Guzeltas and Eroglu 2011: 152-157; Yekeler et al. 2014: 1321-1326; Yeager and Sanders 1995: 837-839).

AUTHOR	MEASUREMENTS FOCUSED ON
Cantinotti et al. 2014a	LV, Valvular annulus (MV & TV) and arterial (AO, MPA, RPA & LPA) dimensions
Cantinotti et al. 2014b	Chamber diameters (LA & LV)
Cantinotti et al. 2017	Valvular annulus (MV, AV, PV & TV) and arterial (ABD AO, MPA, RPA & LPA) dimensions
Guzeltas & Eroglu 2011	LV, LA & AO dimensions
Yekeler et al. 2004	Thymus diameter
Yeager & Sanders 1995	Thymus diameter

2.4.8 LIMITATIONS OF PUBLISHED NOMOGRAMS

Paediatric normative data is limited by various factors. Reported studies usually have a small number of subjects enrolled (<200 patients) and are conducted predominantly in North American and European population with different studies using different techniques and there is lack of technical standardization of measurements, (Kaski and Daubeney 2008:2; Limmer et al. 2014:5; Cantinotti et al. 2014a: 190; Cantinotti et al. 2012: 142; Cantinotti et al. 2014b: 1284). In addition, there have been difficulties in translating adult knowledge to the paediatric age group, poor differentiation of age groups, and a limited amount of data in neonates. Not all studies provide normal values for all cardiac chambers, valves and great vessels, (Cantinotti et al. 2012: 144). A paucity of differentiation between age, gender and race subgroups and complexity of adjusting

values according to body size and their normalization has also been reported, (Cantinotti et al. 2012: 144). In addition, homogenous studies lack data from other ethnic groups, with some authors believe it is a strength because different races combined may present bias when interpreting results, (Kaski and Daubeney 2008:2; Limmer et al. 2014:5; Cantinotti et al. 2014a; 190; Cantinotti et al. 2012: 142; Cantinotti et al. 2014b: 1284).

2.5 RATIONALE OF THIS STUDY

Normally, diagnosis of any cardiac disease is based on cross-referencing the size and shape of the cardiac structure being evaluated to that of the normal population. This is based on published monograms of population based studies dating as far back to 1980s, (Cantinotti et al. 2012:142).

Echocardiographic nomograms are essential for guiding decision-making in the management of neonates born with critical CHD. There is lack of valid nomograms based on a robust set of healthy children using standardized approaches and formulas, which substantially affects the accuracy of assessing the severity of cardiac defects especially in neonates. Despite advances in standardization and in the sample sizes of more recent studies, the process of normalization of paediatric echocardiographic measures remains incomplete. The current available nomogram data is extrapolated from population-based studies carried on American and European neonates and infants (Roge et al. 1978:285; Lemmer et al. 2014:1; Cantinotti et al. 2014b:1279).

Reference values are significantly affected by errors made by observers, (Mawad et al. 2013: 35). Numerous publications have provided normal values for paediatric populations from various parts of the world but there is lack of data from sub-Saharan Africa, (Lemmer et al. 2014:1 and Roge et al. 1978:285).

According to Kaski and Daubeney (2008: 2) new studies are required to create more reliable, accurate nomograms and reproducible results. These should consider larger populations of healthy children with sufficient number of neonates, address the issues related to data normalization according to BSA and consider potential confounders. Further studies are required to reinforce known data, evaluate other parameters of clinical

interest and the role of different ethnicities. (Kaski and Daubeney 2008: 2; Cantinotti et al. 2012: 150; Cantinotti 2014b:1286).

2.6 **AIMS AND OBJECTIVES**

2.6.1 **AIM**

To establish reliable echocardiography nomograms for cardiac chambers, valve annuli, great vessels and thymic dimensions in an African neonatal population.

2.6.2 **OBJECTIVES**

1. To determine normal values of cardiac chambers, valve annuli, great vessel and thymus dimensions.
2. To determine interobserver variability.
3. To determine the effect of confounding factors such as gender and type of delivery on the measurements obtained.

2.6.3 **HYPOTHESIS**

- Echocardiography nomograms of the African population differs from the published nomograms based on North American and European populations.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 STUDY DESIGN

This is a descriptive, cross-sectional study evaluating cardiac chambers, valve annuli, the thymus and great vessel dimensions in 386 African neonates with normal hearts using echocardiography.

3.2 SUBJECT ENROLLMENT

A total of 386 patients with echocardiographically obtained cardiac measurements were enrolled on the study as shown in Figure 3.1. A sample size calculated with a power of 80% and alpha level 5% yielded a minimum of 164 as adequate to test the hypothesis (Appendix E).

The study data consists of two arms, a retrospective arm utilizing echocardiographic data acquired during a previous study entitled “To assess the accuracy of pulse oximetry screening as a tool to detect critical congenital heart disease in asymptomatic newborns” and a prospective arm. The retrospective arm of the study had a separate ethical approval granted by the “Human Research Ethics Committee (Medical)” – Ethics Clearance Certificate no. M150721. A separate approval to use the study echocardiographic data of neonatal nomograms for this study was granted from the same ethics committee and the Medical Advisory committee of the Chris Hani Baragwanath Academic Hospital- Ethic Clearance Certificate number M170536. After excluding patients with congenital heart disease from the retrospective arm, a total of 200 neonates with structurally normal hearts on echocardiography were selected from acquired echocardiography images. The dimensions were prospectively measured and analyzed using post-date data analysis in conjunction with the prospectively acquired images from the prospective arm of the study consisting of 186 neonates (See Figure 3.1). Overall, the study was granted an overarching ethics approval from Durban University of Technology where I, the student is registered for a senior degree (Appendix B).

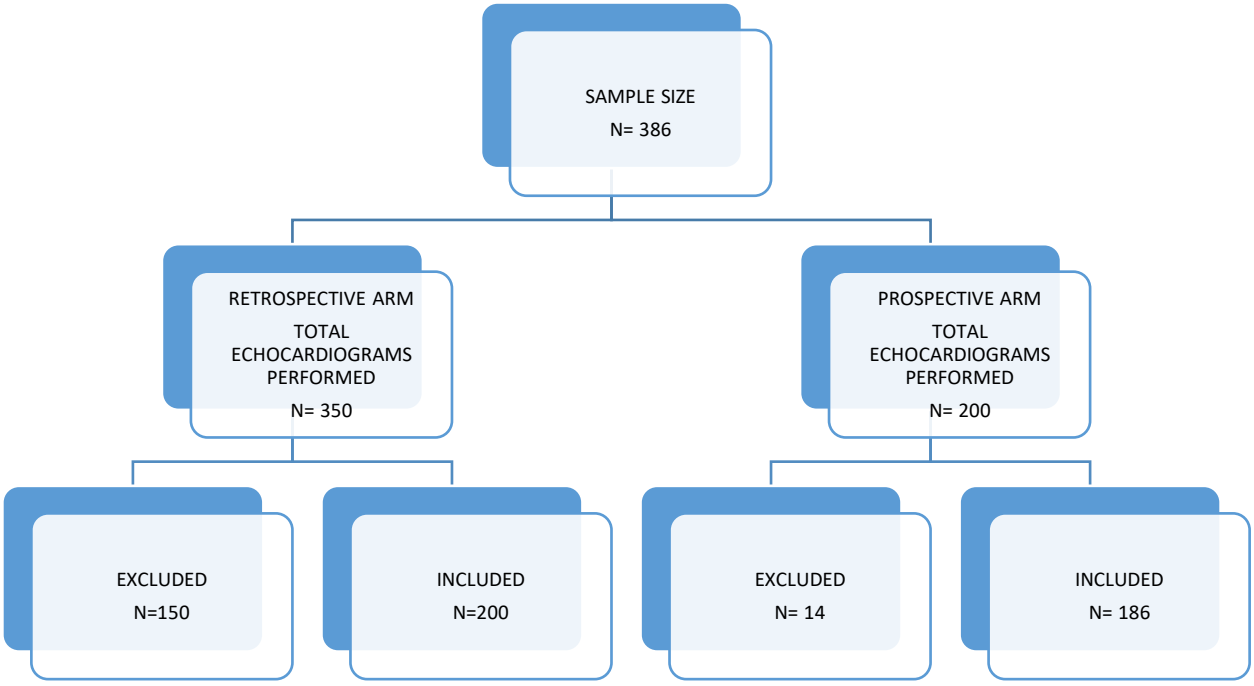

Figure 3.1 Sample size.

Prior to image acquisition, consent was obtained from mothers of all neonates in the postnatal wards before discharge (Appendix C). Echocardiographic studies were performed within 12-24 hours after birth. Neonates with structurally normal hearts had their thymus, cardiac valve annulus, vessel and chamber diameters analyzed for inclusion in the construction of nomograms. On the retrospective arm, the 150 patients who were excluded were patients who either had significant structural heart disease on echocardiography or images for post processing were not stored. On the prospective arm, the 14 patients who were excluded either had a significant structural heart disease, the mother refused to give consent, 1 of the patient's echocardiography exam was not completed because the child was crying and the mother said she does not want her baby to continue to participate in the study and 1 patient had albinism so there was no certainty that he fell under the black population or not. All patient identifiers were eliminated and

each patient received a study number in order to maintain patient anonymity.

3.2.1 SELECTION CRITERIA

3.2.1.1 INCLUSION CRITERIA

- Healthy newborns at an age of 12 hours or more before discharge.
- Patients with a “normal heart” by echocardiography.
- Presence of hemodynamically non-significant patent ductus arteriosus and patent foramen ovale were considered normal.
- Black South African patients.
- Full term babies delivered by caesarian section and normal vertex.

3.2.1.2 EXCLUSION CRITERIA

- Patients less than 12 hours from birth.
- Patients with a known structural heart lesion.
- Patients who are non- black South Africans.
- Pre-term neonates.

3.3 IMAGE ACQUISITION AND POST PROCESSING

An echocardiogram is a non-invasive test and not harmful or painful to infants in any way. Measurements of cardiovascular structures were based upon current American Society of Echocardiography (ASE) guidelines (Lopez et al. 2010:465-495). Echocardiographic measurements were performed using MV13-0034 Rev2: GE Healthcare Vivid e Compact Digital Ultrasound system. Scanning was done using 7.5 MHz transducer (S6). All cardiovascular dimension measurements were measured in millimeters (mm). For all structures measured, measurements were done only if good views were available. The abdominal aorta (ABD AO) was not measured in 2 patients.

The participants were not sedated while the echocardiogram was being performed. The echocardiogram was performed at the bedside with the patient lying comfortably in a baby crib in the supine position. Echocardiography was initiated by applying echocardiography gel on the neonate's chest which provides a conductive medium that creates a bond between the skin and the echocardiography probe. Standard thoracic echocardiography views (parasternal long and short axis, apical 4-chamber and subcoastal view) were acquired and images used to measure the cardiac dimensions were obtained, (figure 3.2). The following cardiac dimensions were measured using 2-D and M-mode echocardiography modalities.

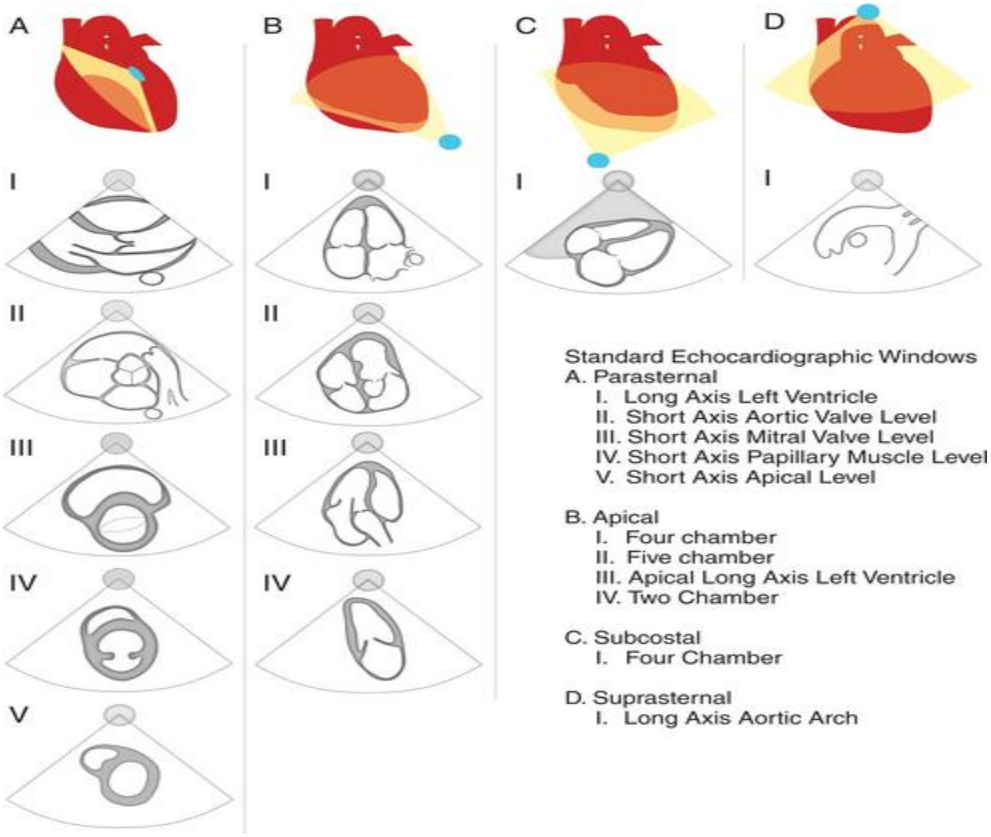

Figure 3.2 Standard thoracic echocardiographic views, (Lam and King 2015).

3.3.1 M-MODE MEASUREMENTS

- **AORTA AND LEFT ATRIUM DIMENSION:** Aortic (AO) and LA diameter was measured using M-mode in the parasternal long axis (PLAX) or parasternal short axis (PSAX) views depending on which view had the better image. The aortic diameter was measured during peak systole using outer edge to inner edge technique. The LA diameter was measured during end-ventricular systole at its greatest dimension. The measurement was taken from the leading edge of the posterior aortic wall to the leading edge of the posterior LA wall, figure 3.2. After the AO and LA dimensions were obtained, the LA to AO ratio was calculated.

- **LEFT VENTRICULAR MEASUREMENTS:** LV dimensions [left ventricle internal diameter (LVID), left ventricular posterior wall (LVPW) and interventricular septum (IVS)] were measured on M-mode during end-diastole and during end-systole on PSAX view at the level of LV (Figure 3.3).

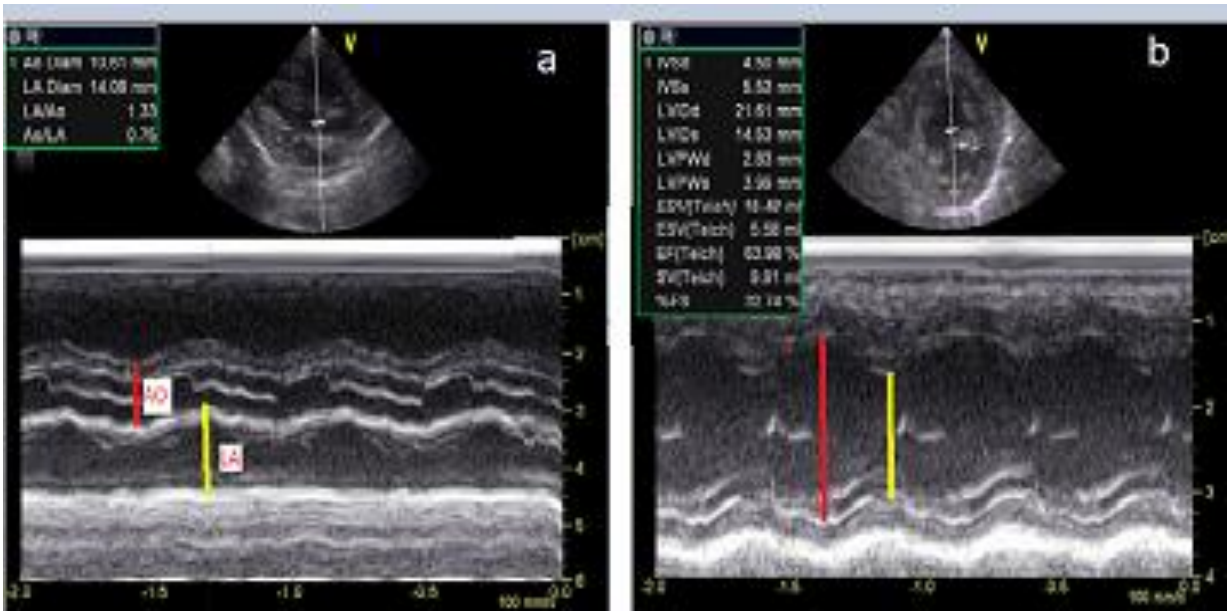

Figure 3.3 M-mode dimensions measurements. (a) AO and LA measurements. Red line highlights AO root dimension and yellow line highlights LA dimension. (b)

LV dimensions. Red line highlights diastole and yellow line highlights diastole. Image taken from the study cohort.

3.3.2 2-D DIMENSION MEASUREMENTS

VALVE ANNULUS DIMENSIONS: Semilunar valve annulus (aortic and pulmonary valve) diameters were measured on 2-D during peak systole, from hinge point to hinge point (Figure 3.3). Aortic valve annulus (AV ANN) was measured on PLAX view and pulmonary valve annulus (PV ANN) on PSAX view at the level of the aorta. Atrioventricular valve annulus (MV and TV) diameters were measured in diastole at the point of maximal valve excursion. Valve dimensions were measured from hinge point to hinge point on the apical 4 chamber view (Figure 3.4).

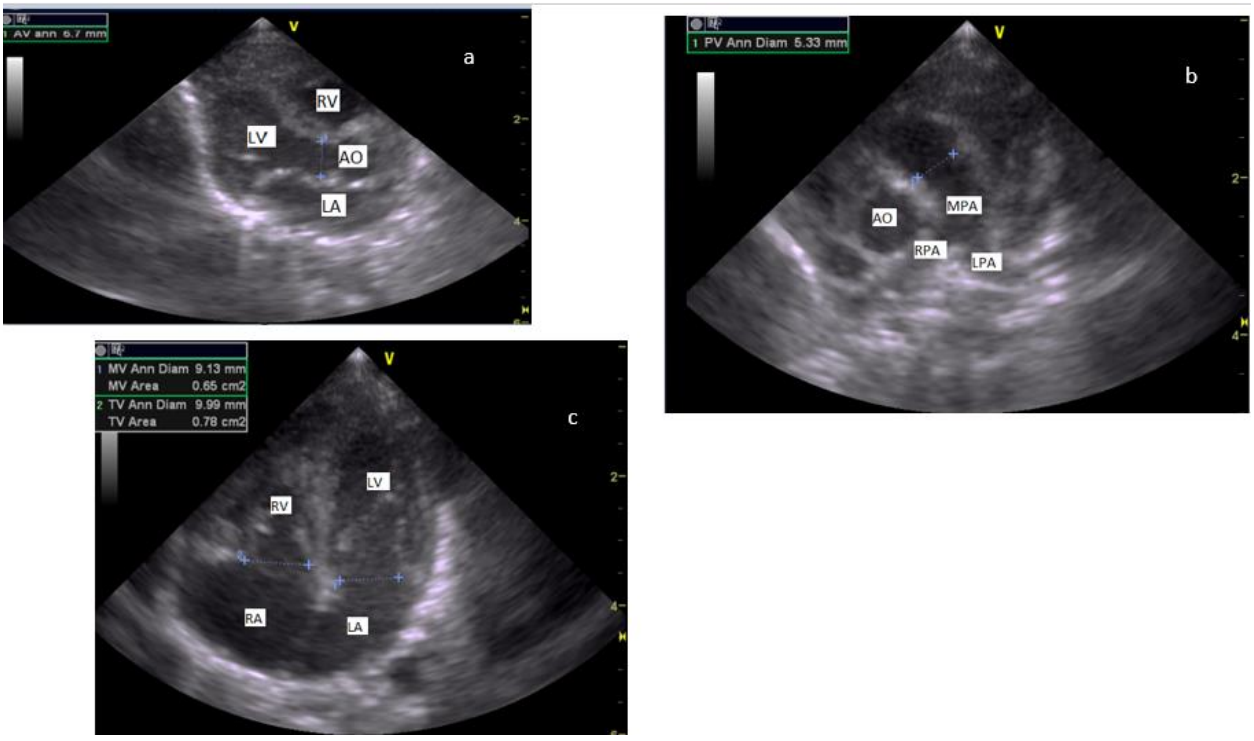

Figure 3.4 Valve annuli dimensions. (a) aortic valve annulus (AV ANN), (b) pulmonary valve annulus (PV ANN), (c) MV annulus (MV ANN) and TV annulus (TV ANN). Images taken from the study cohort.

ARTERIAL DIMENSIONS: Abdominal aorta (ABD AO), main pulmonary artery (MPA) and pulmonary branches (left and right pulmonary artery) were measured on 2-D during systole. MPA, left pulmonary artery (LPA) and right pulmonary artery (RPA) were measured on PSAX view at the level of the aorta, figure 3.5. ABD AO was measured on 2-D in subcoastal view, figure 3.5. ABD AO was measured as maximal systolic dimension at the level of the diaphragm.

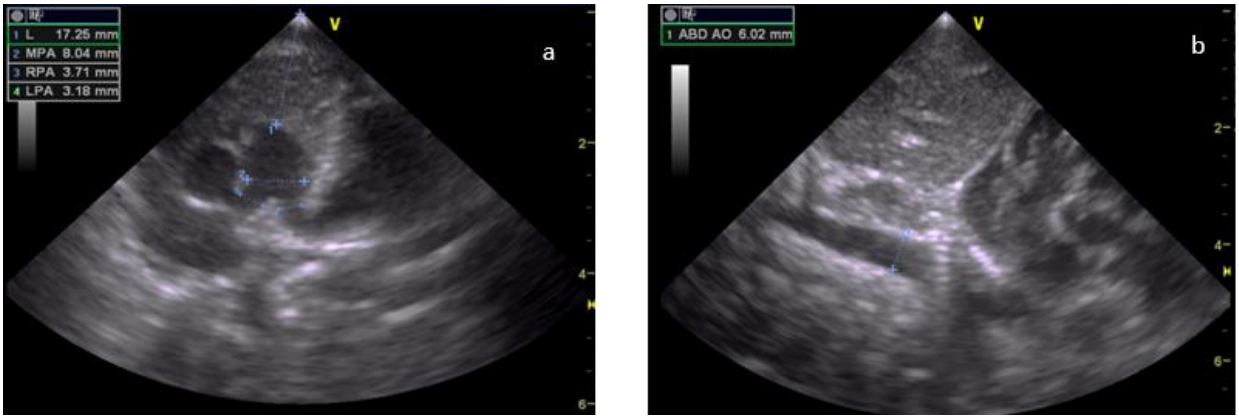

Figure 3.5 2-D arterial and thymus dimensions. (a) Main pulmonary artery (MPA), right pulmonary artery (RPA), left pulmonary artery (LPA), thymus dimensions. (b) Abdominal aorta (ABD AO) dimension measurement. Image taken from study cohort.

ADDITIONAL MEASUREMENT: The Thymus was measured in the PSAX view at level of aorta on 2-D image. Thymus was measured as the distance from anterior chest wall to the most anterior great artery (Figure 3.5a).

3.4 DATA COLLECTION AND ANALYSIS

Demographic data was collected from the clinical notes. Image post processing was carried out on the echocardiographic images saved on GE Healthcare Vivid e Compact Digital Ultrasound system. The following parameters were measured: cardiac chambers (left atrium and left ventricle) dimensions, wall thickness of the left ventricle (interventricular septum and posterior wall), thymus dimension, valve annulus (aortic,

pulmonary, mitral and tricuspid valve) and great vessel dimensions (pulmonary artery and its branches, abdominal aorta). Other non-parameter variables that were collected include birth length (BL), weight, BSA, mode of delivery (MOD) and gender.

Collected data was entered on excel spreadsheet and analyzed using excel, XLSTAT 2019 and STATISTICA version 13.5.0 statistical packages for analysis by the principal researcher (Nondumiso M. Hadebe). A p-value of less than 0.05 was considered statistically significant. A professional biostatistician (Dr Maphosa) at University of the Witwatersrand was consulted for assistance in data analysis.

Height, weight, BL, BSA, MOD and gender were used as independent variables in a regression analyses to predict the effects of confounding factors. Weight was used to express measurements to body size and to predict mean values of each echocardiographic measurement that were expressed as Z-scores.

Homoscedasticity (meaning same variance) and heteroscedasticity (a statistical term that describe residual variance) were tested. To test for homoscedasticity, Shapiro-Wilk and Kolmogorov-Smimov tests were performed and for heteroscedasticity, Breusch-Pagan and White tests were used.

The inter-observer variability was tested using intraclass correlation coefficient to detect bias. The intraclass correlation coefficient ranges from 0-1, a high coefficient close to 1 indicates high similarity and a low coefficient close to zero indicates no similarity as shown in table 3.1.

Table 3.1 shows correlation coefficient interpretation, (Wilson 2009: 2).

Correlation coefficient	Strength of correlation
-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

CHAPTER 4

RESULTS

4.1 DEMOGRAPHICS DATA

A total of 386 patients (See Patient enrollment- Chapter 3.2) were examined and there was almost equal gender distribution but with a slightly higher percentage of females [195 (51%)], than males [191 (49%)], (Table 4.1). The study cohort consisted of neonates born both by normal vaginal delivery (NVD) and by caesarian section (C/S) which showed equal distribution. Patient demographics which included weight, birth length (BL), body surface area (BSA) and gestational age (GA) are presented as mean, standard deviation, minimum and maximum in table 4.1. Birth weight was used to express cardiac measurements to according to body size. Birth weight was divided into 3 groups and its distribution is shown in figure 4.1. Birth weight groups were done to present Z-scores. The cardiovascular measurement distributions are presented in table 4.2 as mean, standard deviation (SD) and range.

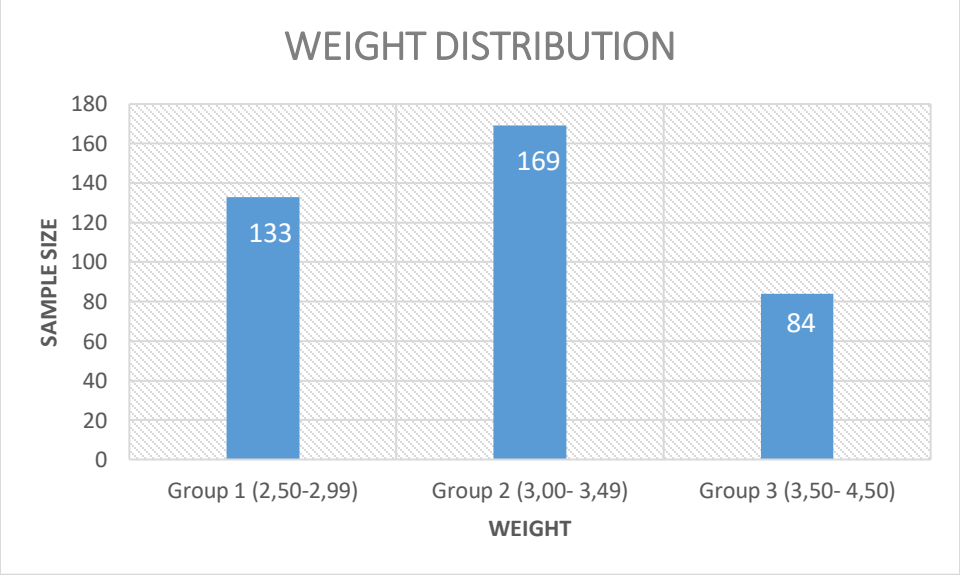

Figure 4.1 Weight distribution according to 3 groups.

Table 4.1 Patient demographics. **BL:** birth length in centimeters, **BSA:** body surface area in square meters, **GA:** gestational age in weeks, **N:** sample size, **SD:** standard deviation.

VARIABLE	MEAN (SD)	MINIMUM	MAXIMUM
WEIGHT (kilograms)	**3.180 (0.38)**	**2.50**	**4.43**
BIRTH LENGTH (centimeters)	**50.6 (3.8)**	**53**	**62**
BODY SURFACE AREA (meters^2)	**0.20 (0.01)**	**0.17**	**0.24**
GESTATIONAL AGE (weeks)	**39.0 (1.4)**	**37**	**42**
GENDER (N: %)	**FEMALES: 191 (49%)**	**MALES: 195 (51%)**	
MODE OF DELIVERY (N: %)	**NVD: 195 (50%)**	**C/S: 195 (50%)**	

Table 4.2 Cardiovascular measurement distribution, in millimeters**. LA:** left atrium. **AO DIA:** aortic diameter. **IVSd:** interventricular septum in diastole. **IVSs:** interventricular septum in systole. **LVIDd:** left ventricular internal diameter in diastole. **LVIDs:** left ventricular internal diameter in systole. **LVPWDs:** left ventricular posterior wall diameter in systole **LVPWDs:** left ventricular posterior wall diameter in diastole. **MV ANN:** mitral valve annulus. **TV ANN:** tricuspid valve annulus. **AV ANN:** aortic valve annulus. **PV ANN:** pulmonary valve annulus. **MPA:** main pulmonary artery. **RPA:** right pulmonary artery. **LPA:** left pulmonary artery. **ABD AO:** abdominal aorta.

MEASUREMENT	MEAN (SD)	MINIMUM	MAXIMUM
LA	11.29 (1.55)	7.40	16.00
AO DIA	9.29 (1.13)	6.40	12.50
LA/AO	1.22 (0.01)	0.80	1.50
IVSd	4.64 (1.06)	2.00	8.00
IVSs	5.37 (1.23)	2.10	9.30
LVIDd	16.51 (0.13)	9.50	24.10
LVIDs	9.97 (0.11)	4.20	16.60
LVPWDd	3.28 (0.04)	1.10	6.10
LVPWDs	4.63 (0.05)	2.10	7.50
MV ANN	8.70 (0.07)	4.70	12.60
TV ANN	9.55 (1.74)	5.10	14.20
AV ANN	5.80 (0.72)	4.00	7.80
PV ANN	7.20 (0.07)	3.80	11.30
MPA	7.26 (0.07)	3.60	11.00
RPA	3.17(0.03)	1.80	4.90
LPA	3.60 (0.04)	2.00	5.70
THYMUS	17.16 (2.85)	10.60	25.40
ABD AO	5.78 (0.04)	3.60	8.30

4.2 NORMALITY TESTS

The assumption of normality was tested using Kolmogorov-Smirnov (K-S) and Shapiro-Wilk W (W) tests (Table 4.4). According to statistica help (2017), the Shapiro-Wilk W test has superior power properties and was therefore selected as the preferred test for normality. The K-S test showed most measurements to be homoscedastic (meaning

same variance) with left atrial/aorta (LA/AO) ratio and LVPWDd being the exception. The W test showed most measurements to be homoscedastic with an increased number of measurements that are heteroscedastic (a statistical term that describe residual variance). Heteroscedasticity was tested using the Breusch-Pagan and White tests (table 4.3) which showed most measurements to be homoscedastic with LA/AO ratio and LVPWDd as exceptions.

Table 4.3 Normality and heteroscedasticity tests. **K-S:** Kolmogorov-Smirnov. **W:** Shapiro-Wilk W test. **LA:** left atrium. **AO DIA:** aortic diameter. **IVSd:** interventricular septum in diastole. **IVSs:** interventricular septum in systole. **LVIDd:** left ventricular internal diameter in diastole. **LVIDs:** left ventricular internal diameter in systole. **LVPWDd:** left ventricular posterior wall diameter in diastole **LVPWDs:** left ventricular posterior wall diameter in systole. **MV ANN:** mitral valve annulus. **TV ANN:** tricuspid valve annulus. **AV ANN:** aortic valve annulus. **PV ANN:** pulmonary valve annulus. **MPA:** main pulmonary artery. **RPA:** right pulmonary artery. **LPA:** left pulmonary artery. **ABD AO:** abdominal aorta. ***:** present significant values.

VARIABLES	K-S	K-S p-value	W	W p-value	BREUSCH-PAGAN	WHITE TEST
LA	0.039	p > .20	0.996	0.483	0.484	0.482
AO	0.058	p < .20	**0.992***	**0.041***	0.676	0.587
LA/AO	0.395	p < .01	**0.139***	**0.000***	**0.031***	**0.066***
IVSd	0.050	p > .20	0.993	0.075	0.139	0.129
IVSs	0.050	p > .20	0.993	0.092	0.739	0.82
LVIDd	0.039	p > .20	0.996	0.386	0.083	0.12
LVIDs	0.043	p > .20	0.996	0.408	0.055	0.14
LVPWDd	0.073	p < .05	**0.985***	**0.001***	**0.011***	**0.034***
LVPWDs	0.055	p < .20	**0.992***	**0.043***	0.126	0.299
MV ANN	0.060	p < .15	0.993	0.073	0.848	0.592
TV ANN	0.042	p > .20	0.993	0.093	0.309	0.246
AV ANN	0.051	p > .20	0.994	0.177	0.359	0.354
PV ANN	0.033	p > .20	0.995	0.312	0.05	0.016
MPA	0.044	p > .20	0.996	0.367	0.879	0.808
RPA	0.055	p < .20	**0.984***	**0.000***	0.684	0.697
LPA	0.063	p < .10	**0.981***	**0.000***	0.782	0.94
THYMUS	0.042	p > .20	0.993	0.052	0.437	0.102
ABD AO	0.048	p > .20	**0.992***	**0.036***	0.427	0.565

4.3 EFFECTS OF CONFOUNDING FACTORS

Multiple linear regression analysis was used to test the effects of confounding factors on all cardiovascular measurements (Table 4.4). Body weight showed a significant relationship with all cardiovascular dimension measurements. Mode of delivery (MOD) had significant association with atrioventricular valves, main pulmonary artery, and branch pulmonary artery measurements. Gender and BSA had no significant effect on the majority of measurements. There was also no significant relationship between all cardiac dimension measurements and body length (BL) or gestational age (GA).

Table 4.4 Effects of confounding factors: weight, MOD, gender, BL, GA and BSA on chamber measurements**. MOD:** mode of delivery. **BSA:** body surface area. **BL:** birth length. **LA:** left atrium. **AO DIA:** aortic diameter **LVIDd:** left ventricular internal diameter in diastole. **LVIDd:** left ventricular internal diameter in diastole. **LVIDs:** left ventricular internal diameter in systole. **MV ANN:** mitral valve annulus. **TV ANN:** tricuspid valve annulus. **AV ANN:** aortic valve annulus. **PV ANN:** pulmonary valve annulus. **MPA:** main pulmonary artery. **RPA:** right pulmonary artery. **LPA:** left pulmonary artery. **B:** regression coefficient. ***:** represent significant values.

VARIABLE	WEIEGHT		MOD		GENDER		BSA		GA		BL	
	b	p-value	b	p-value	b	p-value	b	p-value	b	p-value	b	p-value
LA	*1,06	*0,000	0,11	0,499	-0,04	0,807	-7,38	0,257	0,08	0,296	-0,01	0,621
AO DIA	*1,11	*0,000	0,07	0,512	-0,14	0,219	-4,42	0,331	-0,01	0,903	-0,01	0,704
LVIDd	*1,94	*0,000	-0,37	0,143	-0,19	0,444	-6,97	0,507	-0,10	0,296	0,02	0,644
LVIDs	*1,19	*0,001	-0,32	0,152	-0,19	0,393	-8,70	0,344	-0,06	0,449	0,03	0,526
MPA	*1,00	*0,000	*-0,48	*0,000	*-0,30	*0,027	1,16	0,836	0,01	0,894	-0,02	0,529
RPA	*0,31	*0,002	*-0,17	*0,008	*-0,18	*0,005	2,42	0,360	-0,03	0,180	0,01	0,632
LPA	*0,37	*0,001	*-0,31	*0,000	-0,04	0,572	-1,15	0,708	-0,02	0,512	0,01	0,639
MV ANN	*0,74	*0,001	*-0,44	*0,002	-0,11	0,455	*12,84	*0,028	0,05	0,398	-0,04	0,122
TV ANN	*1,28	*0,000	*-0,50	*0,004	0,10	0,578	-5,65	0,434	0,02	0,816	0,00	0,909
AV ANN	*0,50	*0,000	-0,10	0,178	-0,01	0,901	-4,35	0,149	0,00	0,969	0,00	0,765
PV ANN	*0,95	*0,000	-0,12	0,372	0,13	0,335	4,92	0,391	-0,01	0,848	-0,03	0,222

4.4 INTER-OBSERVER VARIABILITY

The retrospective arm of the study enrolled 200 patients where post-processing analysis of data that was saved on the echocardiographic machine was carried out. Of the 200 patients, 168 echocardiography studies were intentionally duplicated by the echocardiographer, one study with measurements and one study without measurements. The second group of duplicated studies (n=168) without measurements were re-measured online by the author and another experienced echocardiographer. In this way inter-observer variability was tested using intraclass correlation coefficient which showed a strong correlation in the majority of measurements. The exceptions which showed a weak correlation included LVPWS, PV annulus with moderate correlation and the LVPWDd (table 4.5).

Table 4.5 Interobserver variability. ***:** represents moderate correlation. ****:** represents weak correlation. **LA:** left atrium. **AO DIA:** aortic diameter. **IVSd:** interventricular septum in diastole. **IVSs:** interventricular septum in systole. **LVIDd:** left ventricular internal diameter in diastole. **LVIDs:** left ventricular internal diameter in systole. **LVPWDd:** left ventricular posterior wall diameter in diastole **LVPWDs:** left ventricular posterior wall diameter in systole. **MV ANN:** mitral valve annulus. **TV ANN:** tricuspid valve annulus. **AV ANN:** aortic valve annulus. **PV ANN:** pulmonary valve annulus. **MPA:** main pulmonary artery. **RPA:** right pulmonary artery. **LPA:** left pulmonary artery. **ABD AO:** abdominal aorta.

VARIABLE	ICC	ICC (%)
LA	0.62	62
AO	0.60	60
LA/AO	0.50	50
IVSd	0.56	56
IVSs	0.52	52
LVIDd	0.82	82
LVIDs	0.80	80
LVPWDd	***0.30**	***30**
LVPWDs	***0.35**	***35**
MV ANN	0.64	64
TV ANN	****0.49**	****49**
AV ANN	0.53	53
PV ANN	****0.44**	****44**
MPA	0.69	69
RPA	0.64	64
LPA	0.57	57
THYMUS	0.70	70
ABD AO	0.70	70

4.5 ECHOCARDIOGRAPHY MEASUREMENTS

All cardiac dimension correlated well with body weight. All echocardiographic measurements were grouped into 3 groups by weight and are presented as mean (shown as bold number) and ±3 SD (Table 4.6- 4.9). All cardiac dimensions were within the ±2 standard deviation with few exceptions thus ±3 standard deviation was calculated to

accommodate dimensions above +2 and below -2 SD. Z-score boundaries are presented as straight lines with actual values as dots in between the boundary lines. Z-scores for each cardiac dimension is shown as dots against weight. Z-scores and Z-score boundaries for all measurements are presented graphically in figure 4.2-4.19.

Table 4.6 LA and AO diameter M-mode measurements in millimeters according to weight groups: 1-3. **LA:** left atrium. **AO:** aorta.

CARDIAC DIMENSIONS MEASUREMENTS	STANDARD DEVIATION	GROUP 1: 2.50-2.99	GROUP 2: 3.00-3.49	GROUP 3: 3.50-4.50
LA	3+	15.42	15.88	16.34
	2+	13.91	14.37	14.83
	MEAN	**10.89**	**11.35**	**11.81**
	2-	7.87	8.33	8.79
	3-	6.37	6.82	7.28
AO DIAMETER	3+	12.04	12.52	13.01
	2+	10.98	11.47	11.95
	MEAN	**8.87**	**9.36**	**9.84**
	2-	6.76	7.25	7.73
	3-	5.71	6.19	6.68
LA/AO ratio	3+	1.96	1.97	1.99
	2+	1.76	1.77	1.78
	MEAN	**1.36**	**1.37**	**1.38**
	2-	0.95	0.96	0.97
	3-	0.75	0.76	0.77

Table 4.7 LV M-mode measurements in millimeters according to weight groups 1-3. **IVSd:** interventricular septum in diastole. **IVSs:** interventricular septum in systole. **LVIDd:** left ventricle internal diameter in diastole. **LVIDs:** left ventricle internal diameter in systole. **LVPWDd:** left ventricle posterior wall diameter in diastole. **LVPWDs**: left ventricle posterior wall diameter in systole.

CARDIAC DIMENSION MEASUREMENTS	STANDARD DEVIATION	GROUP 1: 2.50-2.99	GROUP 2: 3.00-3.49	GROUP 3: 3.50-4.50
IVSd	3+	7.64	7.80	7.97
	2+	6.59	6.76	6.92
	MEAN	**4.50**	**4.66**	**4.83**
	2-	2.41	2.57	2.73
	3-	1.36	1.52	1.68
IVSs	3+	8.61	8.82	9.02
	2+	7.42	7.62	7.82
	MEAN	**5.02**	**5.22**	**5.43**
	2-	2.63	2.83	3.03
	3-	1.43	1.63	1.83
LVIDd	3+	22.79	23.66	24.53
	2+	20.43	21.30	22.17
	MEAN	**15.73**	**16.59**	**17.46**
	2-	11.02	11.88	12.75
	3-	8.66	9.53	10.40
LVIDs	3+	15.88	16.43	16.98
	2+	13.75	14.30	14.86
	MEAN	**9.50**	**10.05**	**10.60**
	2-	5.24	5.79	6.34
	3-	3.11	3.66	4.21
LVPWDd	3+	5.74	5.93	6.11
	2+	4.88	5.06	5.24
	MEAN	**3.14**	**3.33**	**3.51**
	2-	1.41	1.59	1.78
	3-	0.54	0.73	0.91
LVPWDs	3+	7.52	7.62	7.73
	2+	6.53	6.63	6.74
	MEAN	**4.55**	**4.65**	**4.76**
	2-	2.56	2.67	2.77
	3-	1.57	1.68	1.78

Table 4.8 Valve 2-D measurements in millimeters according to weight groups: 1-3. **MV ANN:** mitral valve annulus. **TV ANN:** tricuspid valve annulus. **PV ANN:** pulmonary valve annulus. **AV ANN:** aortic valve annulus.

CARDIAC DIMENSION MEASUREMENTS	STANDARD DEVIATION	GROUP 1: 2.50-2.99	GROUP 2: 3.00-3.49	GROUP 3: 3.50-4.50
MV ANN	3+	12.38	12.80	13.21
	2+	11.03	11.45	11.87
	MEAN	**8.34**	**8.75**	**9.17**
	2-	5.64	6.06	6.47
	3-	4.29	4.71	5.12
TV ANN	3+	14.06	14.64	15.23
	2+	12.39	12.97	13.55
	MEAN	**9.04**	**9.62**	**10.20**
	2-	5.69	6.27	6.86
	3-	4.02	4.60	5.18
PV ANN	3+	10.78	11.19	11.61
	2+	9.47	9.88	10.30
	MEAN	**6.85**	**7.26**	**7.68**
	2-	4.22	4.64	5.05
	3-	2.91	3.33	3.74
AV ANN	3+	7.69	7.91	8.14
	2+	6.99	7.22	7.44
	MEAN	**5.60**	**5.82**	**6.05**
	2-	4.21	4.43	4.66
	3-	3.51	3.73	3.96

Table 4.9: Arterial and thymus 2-D echocardiography measurements in millimeters according to weight groups: 1-3. **MPA:** main pulmonary artery. **RPA:** right pulmonary artery. **LPA:** left pulmonary artery. **ABD AO:** abdominal aorta.

CARDIAC DIMENSION MEASUREMENT	STANDARD DEVIATION	GROUP 1: 2.50-2.99	GROUP 2: 3.00-3.49	GROUP 3: 3.50-4.50
MPA	3+	10.76	11.25	11.75
	2+	9.45	9.94	10.44
	MEAN	**6.83**	**7.32**	**7.82**
	2-	4.21	4.71	5.21
	3-	2.90	3.40	3.90
RPA	3+	4.81	4.99	5.17
	2+	4.22	4.39	4.57
	MEAN	**3.03**	**3.21**	**3.38**
	2-	1.84	2.02	2.20
	3-	1.25	1.42	1.60
LPA	3+	5.53	5.70	5.88
	2+	4.84	5.01	5.18
	MEAN	**3.45**	**3.62**	**3.79**
	2-	2.06	2.23	2.40
	3-	1.36	1.54	1.71
THYMUS	3+	24.90	25.63	26.36
	2+	22.11	22.84	23.57
	MEAN	**16.53**	**17.25**	**17.98**
	2-	10.94	11.67	12.40
	3-	8.15	8.88	9.61
ABD AO	3+	7.93	8.21	8.50
	2+	7.13	7.42	7.70
	MEAN	**5.53**	**5.82**	**6.11**
	2-	3.94	4.22	4.51
	3-	3.14	3.42	3.71

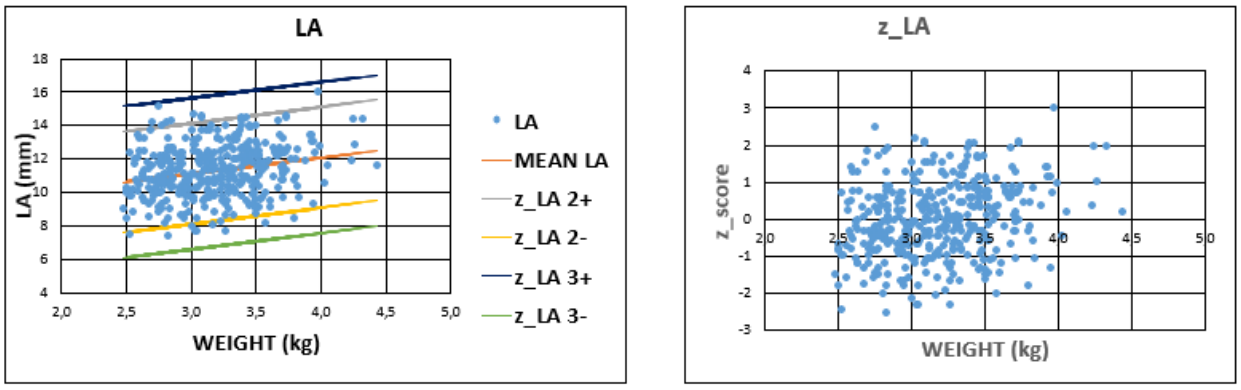

Figure 4.2 Left atrium dimension Z-scores and Z-score boundaries by body weight. **LA:** left atrium dimension

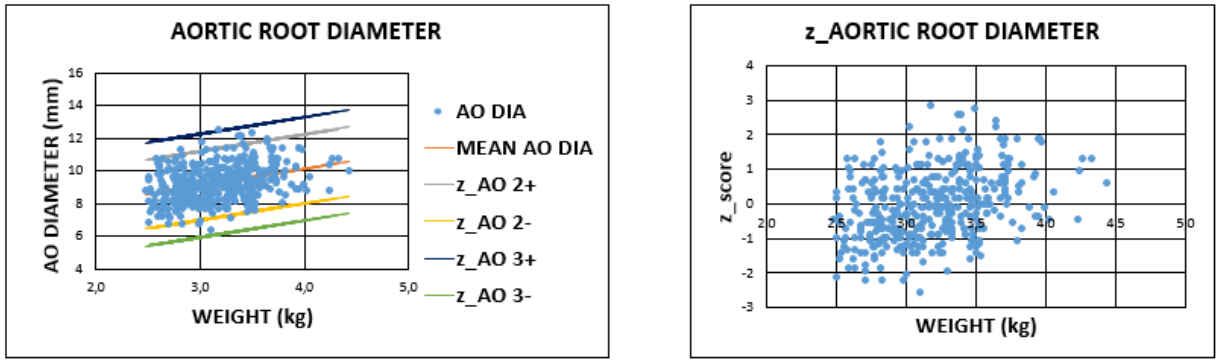

Figure 4.3 Aortic root diameter Z-scores and Z-score boundaries by body weight.

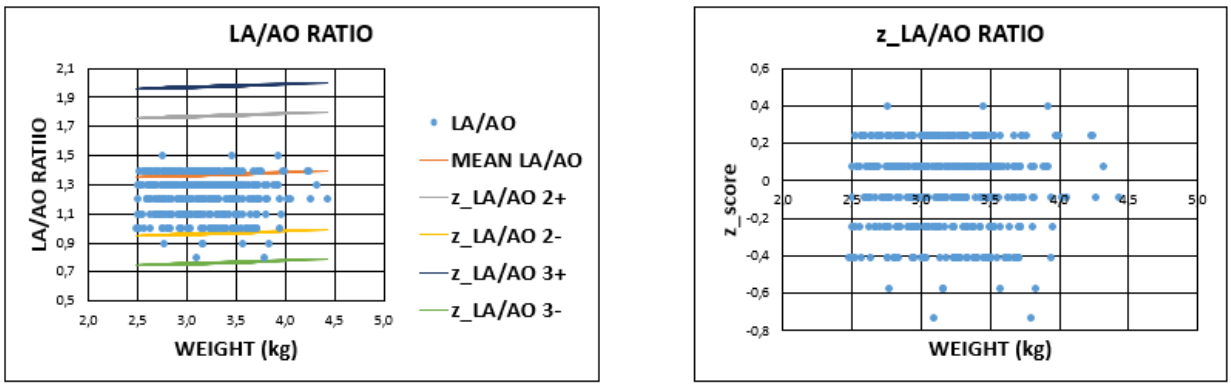

Figure 4.4 LA/AO ratio Z-scores and Z-score boundaries by weight. **LA:** left atrium dimension, **AO:** aortic root diameter.

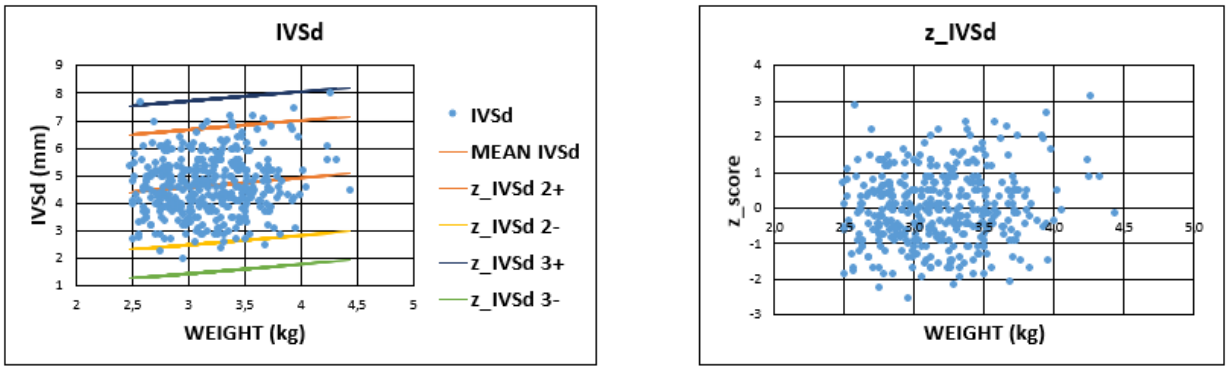

Figure 4.5 IVSd Z-scores and Z-score boundaries by weight. **IVSd:** interventricular septum in diastole.

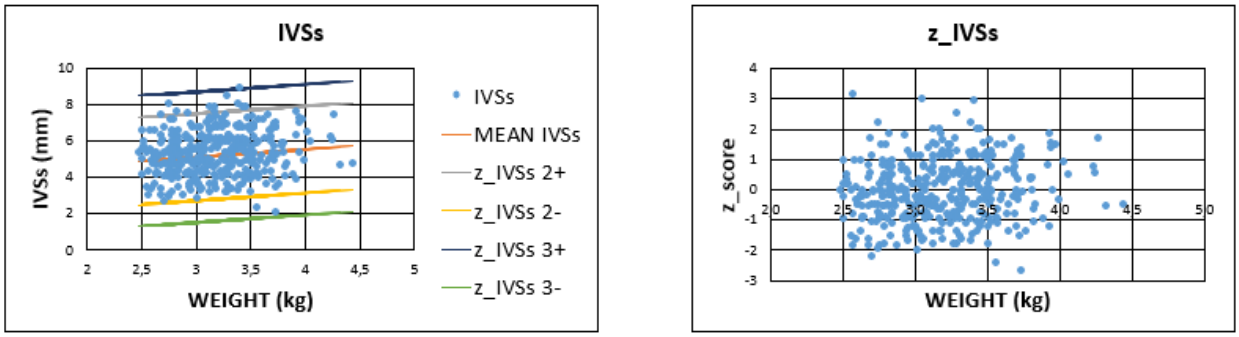

Figure 4.6 IVSs Z-scores and Z-score boundaries by weight. **IVSs:** interventricular septum in systole.

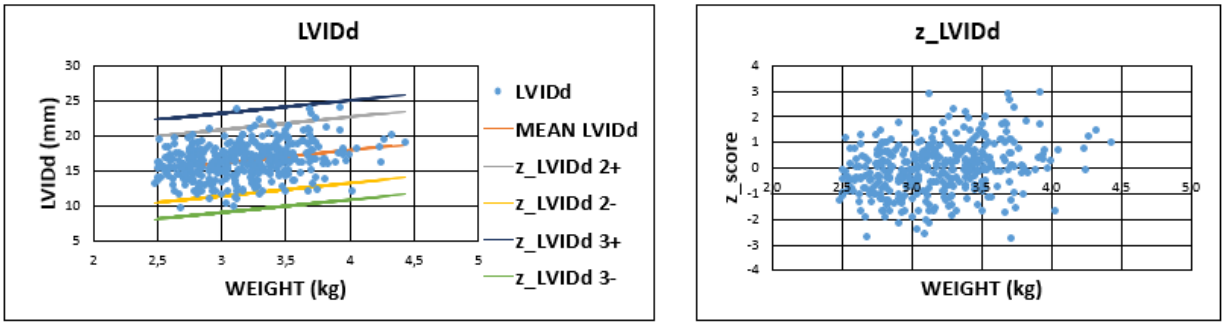

Figure 4.7 LVIDd Z-scores and Z-score boundaries by weight. **LVIDd:** left ventricular internal diameter in diastole.

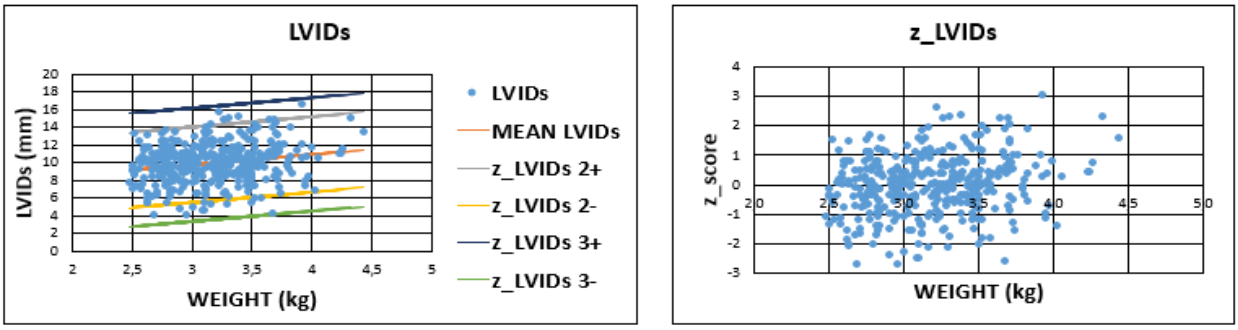

Figure 4.8 LVIDs Z-scores and Z-score boundaries by weight. **LVIDs:** left ventricular internal diameter in systole.

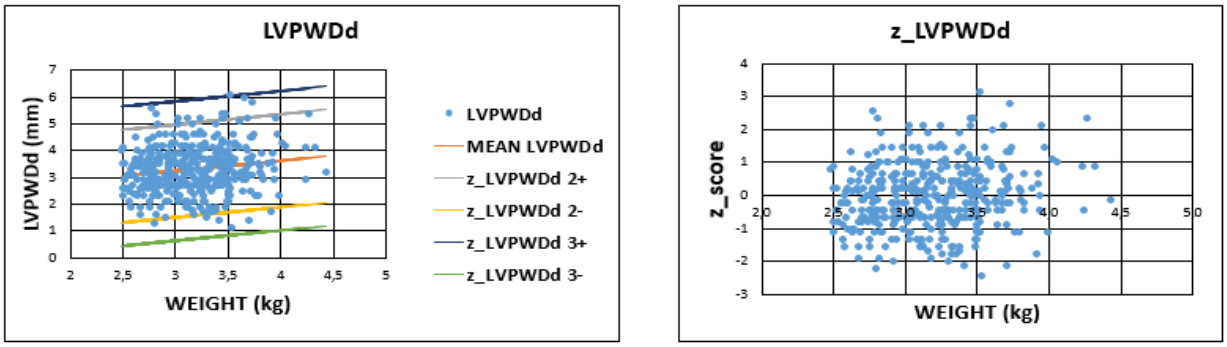

Figure 4.9 LVPWDd Z-scores and Z-score boundaries by weight. **LVPWDd:** left ventricular posterior wall diameter in diastole.

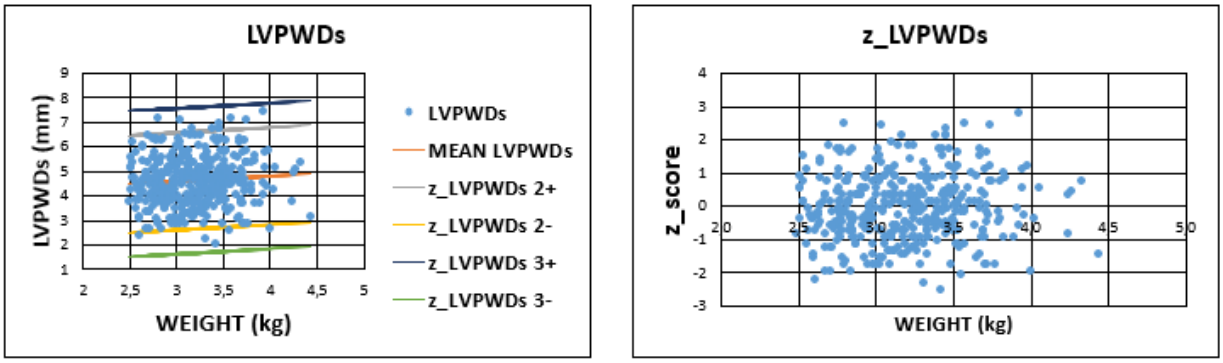

Figure 4.10 LVPWDs Z-scores and Z-score boundaries by weight. **LVPWDs:** left ventricular posterior wall diameter in systole.

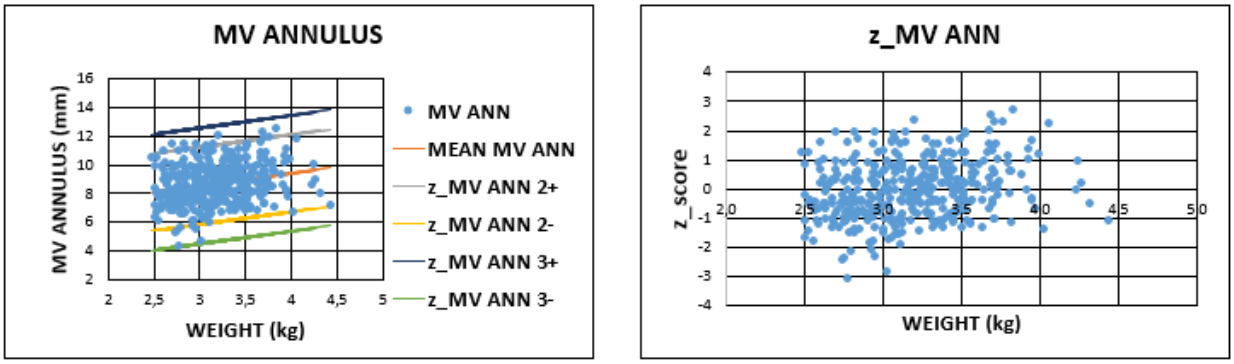

Figure 4.11 MV annulus Z-scores and MV annulus Z-score boundaries by weight. **MV:** mitral valve.

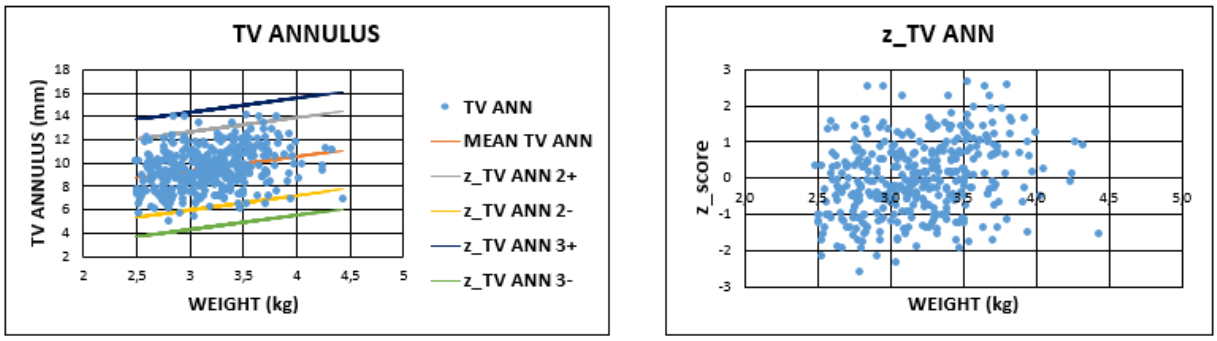

Figure 4.12 TV annulus Z-scores and Z-score boundaries by weight. **TV:** tricuspid valve.

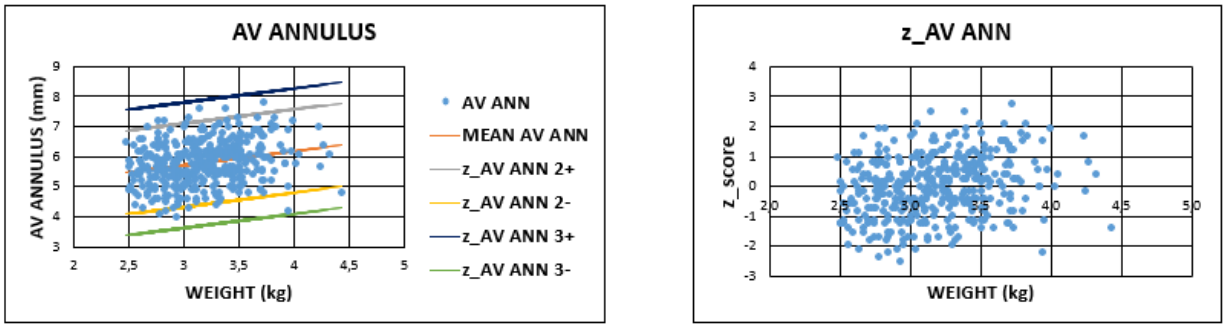

Figure 4.13 AV annulus Z-scores and Z-score boundaries by weight. **AV ANN:** aortic valve annulus.

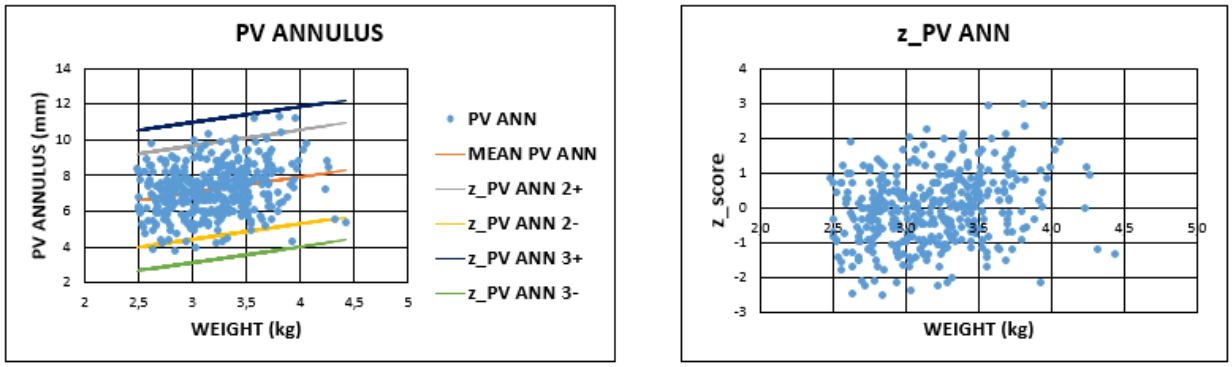

Figure 4.14 PV annulus Z-scores and Z-score boundaries by weight. **PV ANN:** pulmonary valve annulus.

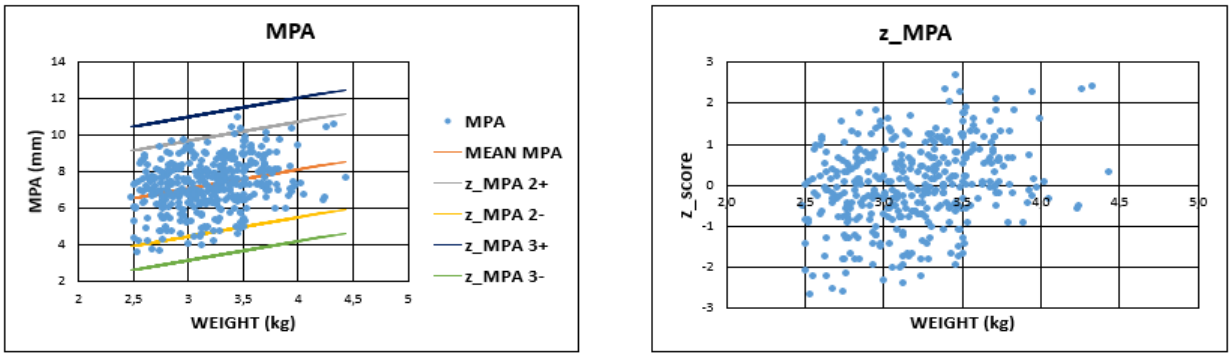

Figure 4.15 MPA Z-scores and Z-score boundaries by weight. **MPA:** main pulmonary artery.

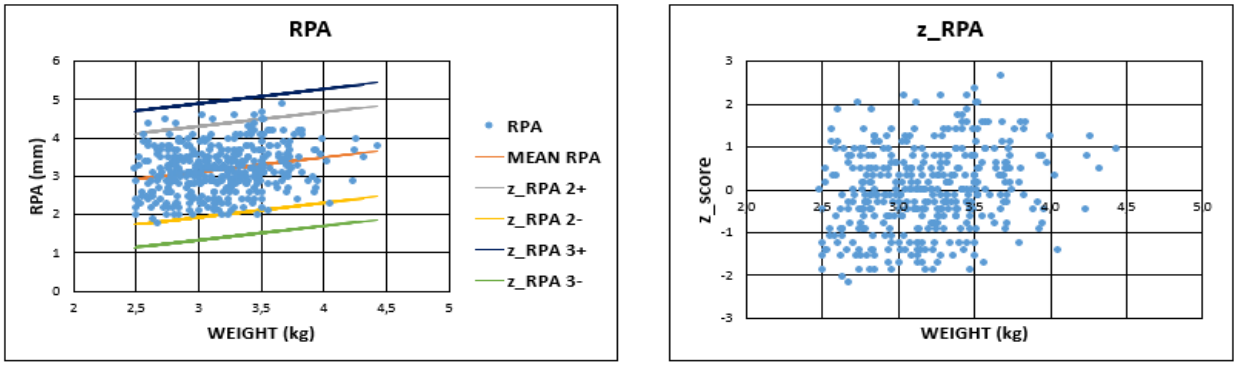

Figure 4.16 RPA Z-scores and Z-score boundaries by weight. **RPA:** right pulmonary artery.

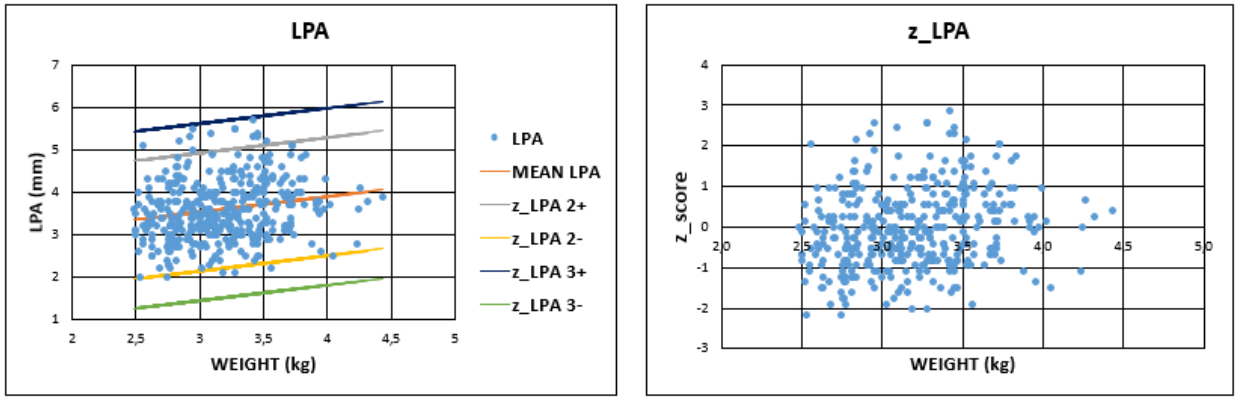

Figure 4.17 LPA Z-scores by weight and Z-score boundaries by weight. **LPA:** left pulmonary artery.

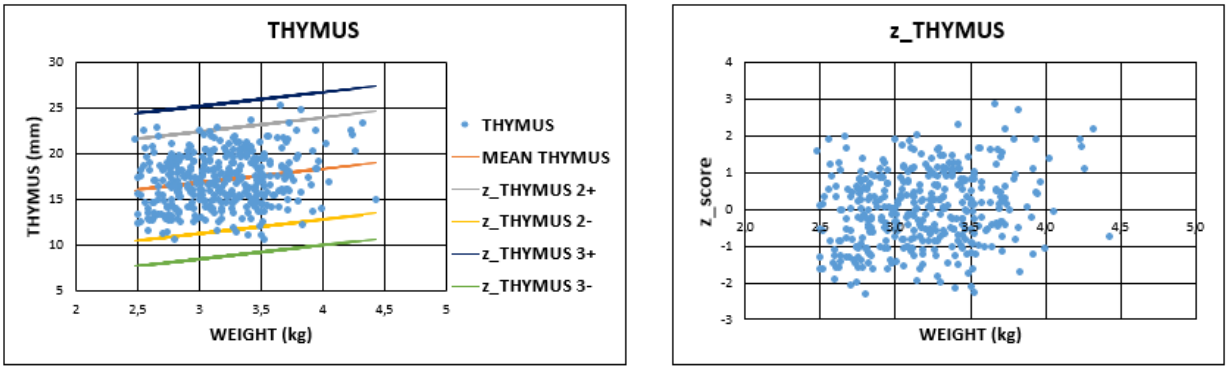

Figure 4.18 Thymus Z-scores and Z-score boundaries by weight.

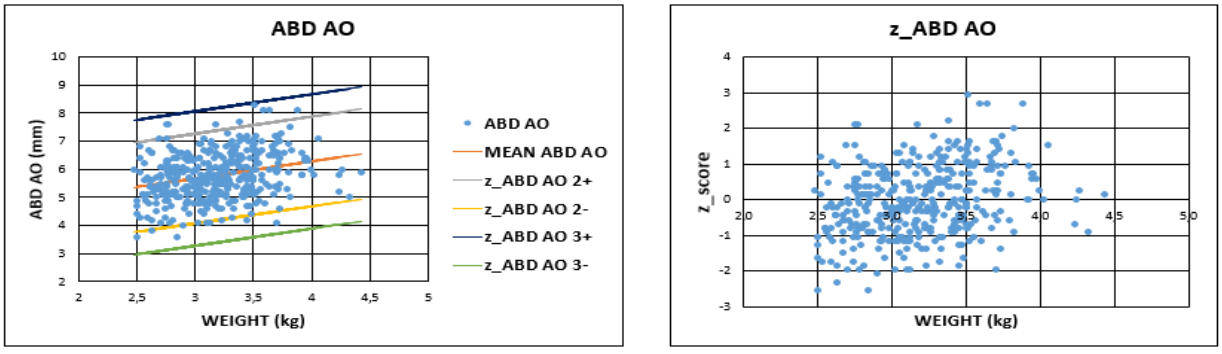

Figure 4.19 ABD AO Z-scores and Z-score boundaries by weight. **ABD AO:** abdominal aorta.

4.6 CURRENT STUDY VERSUS PREVIOUS STUDIES

M-mode measurements (LA, AO diameter, IVSd, IVSs, LVIDd, LVIDs, LVPWDd and LVPWDs) and 2-D measurement (MPA) were compared with previous studies that focused on neonates and whose results were expressed in relation to birth weight which was used as a substitute to represent body size (Table 4.10-4.12). Birth weight was grouped into 4 groups to allow comparison as follows: group 1 (2.50- 2.99), group 2 (3.00-3.49), group 3 (3.50- 3.99) and group 4 (4.00- 4.50). This study showed slightly higher dimensions compared to previous studies.

Table 4.10 LA and AO diameter measurements of previous published studies compares with this study. **LA:** left atrium. **AO DIA:** aortic diameter.

REFERENCE	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.
WEIGHT GROUPS	**GROUP 1**			**GROUP 2**			**GROUP 3**			**GROUP 4**		
2+	13.91	13.6	15.6	14.4	13.8	15.8	14.8	14.7	13.2	15.3	16.4	16.9
LA	**10.89**	**8**	**8.5**	**11.4**	**8.6**	**9.4**	**11.8**	**8.3**	**10.2**	**12.3**	**9.9**	**10.5**
2-	7.87	10.8	12.1	8.3	10.8	12.6	8.8	11.5	13.2	9.3	13.2	13.7
2+	10.98	9.8	10.2	11.5	10	10.7	11.9	10.0	11.1	12.5	11.7	11.6
AO DIA	**8.87**	**6.2**	**7.4**	**9.4**	**6.6**	**7.5**	**9.8**	**7.1**	**7.5**	**10.4**	**6.8**	**7.6**
2-	6.76	8	8.8	7.2	8.3	9.1	7.7	8.6	9.3	8.3	9.3	9.6

Table 4.11 LV dimension measurements in millimeters of previous published studies compared with this study. **IVSd:** interventricular septum in diastole. **IVSs:** interventricular septum in systole. **LVIDd:** left ventricle internal diameter in diastole. **LVIDs:** left ventricle

internal diameter in diastole. **LVPWDd:** left ventricle posterior wall diameter in diastole. **LVPWDs**: left ventricle posterior wall diameter in systole.

REFERENCE	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.	THIS STUDY	Güzeltas & Eroğlu	Kampmann et al.
WEIGHT GROUPS	**GROUP 1**			**GROUP 2**			**GROUP 3**			**GROUP 4**		
2+	6.59	4.5	4.7	6.8	4.7	4.9	6.9	4.6	5.1	7.1	5.2	5.2
IVSd	**4.50**	**2.2**	**2.1**	**4.7**	**2.7**	**2.3**	**4.8**	**2.6**	**3.7**	**5.0**	**2.7**	**2.4**
2-	2.41	3.7	3.5	2.6	3.7	3.6	2.7	3.6	2.3	2.9	3.8	3.8
2+	7.42	6	7.6	7.6	6	7.7	7.8	5.2	8.1	8.0	6.3	8.2
IVSs	**5.02**	**2.4**	**2.4**	**5.2**	**2.6**	**2.5**	**5.4**	**3.3**	**2.5**	**5.6**	**3.7**	**2.6**
2-	2.63	4.2	5	2.8	4.4	5.1	3.0	4.6	5.3	3.2	5	5.4
2+	20.43	19.1	21.1	21.3	20.3	21.3	22.1	20.8	22.2	23.1	22.1	23.3
LVIDd	**15.73**	**12.9**	**15**	**16.6**	**12**	**15.1**	**17.4**	**13.0**	**15.4**	**18.4**	**14.2**	**16.5**
2-	11.02	16	18.1	11.9	16.1	18.2	12.7	16.9	18.8	13.7	18.2	19.9
2+	13.75	12.6	14.2	14.3	11	14.2	14.8	14.9	14.3	15.4	15.7	15.2
LVIDs	**9.50**	**7.1**	**9.2**	**10.0**	**7.8**	**9.2**	**10.6**	**8.5**	**9.5**	**11.2**	**8.6**	**10.2**
2-	5.24	9.9	11.7	5.8	14.2	11.7	6.3	11.7	11.9	6.9	12.1	12.7
2+	4.88	4.3	4.2	5.1	3.5	4.6	5.2	4.7	4.7	5.4	5.2	4.8
LVPWDd	**3.14**	**2.2**	**2.2**	**3.3**	**2.5**	**2.4**	**3.5**	**2.4**	**2.5**	**3.7**	**2.5**	**2.6**
2-	1.41	3.3	3.2	1.6	4.4	3.5	1.8	3.5	3.6	2.0	3.6	3.7
2+	6.53	5.2	7.1	6.6	3.9	7.1	6.7	5.3	7.5	6.8	5.7	7.9
LVPWDs	**4.55**	**2.7**	**2.9**	**4.7**	**2.8**	**3.1**	**4.7**	**2.9**	**3.3**	**4.9**	**3.7**	**3.5**
2-	2.56	3.9	5	2.7	5.1	5.1	2.8	4.1	5.4	2.9	4.7	5.7

Table 4.12 MPA dimension measurements in millimeters of previous published studies compared with this study.

REFERENCE	THIS STUDY	Kampmann et al.	THIS STUDY	Kampmann et al.3	THIS STUDY	Kampmann et al.4	THIS STUDY	Kampmann et al.7
WEIGHT GROUPS	**GROUP 1**		**GROUP 2**		**GROUP 3**		**GROUP 4**	
2+	9.45	15.20	4.39	15	10.40	14.4	10.97	12.5
MPA	**6.83**	**6.80**	**3.21**	**7**	**7.78**	**8**	**8.36**	**9.3**
2-	4.21	11.00	2.02	11	5.16	11.2	5.74	12.5

CHAPTER 5

5.1 DISCUSSION

Echocardiography remains as the gold-standard modality for diagnosis, it is a guide in the management of congenital and acquired heart disease in children, (Lai et al. 2006: 1413-1415; Cantinotti et al. 2017:208). Transthoracic echocardiography provides comprehensive anatomic, hemodynamic and physiological data about the heart. It is vital for monitoring of cardiovascular structural growth, valve and ventricular function and for the timing of medical and surgical management, (Lai et al. 2006: 1413-1415; Cantinotti et al. 2017:208).

Quantitative assessment of the heart is critical for assessments of deviation from the norm but can only be done if there are normative values available to make comparisons with and to identify abnormalities. Paediatric 2-D and M-mode echocardiography nomograms of good quality are available for chamber size, cardiac valve annulus and great vessel dimensions which are mainly derived from European and American populations, (Kampmann et al. 2000: 667-672; Overbeek et al. 2006:113-121; Neilan 2009:50-55; Guzeltas and Eroglu 2012:152-157; Cantinotti 2014a:179-191; Cantinotti 2014b:1279-1292; Cantinotti 2017:208-215). Most normative echocardiographical data has been devised for older children and adults, but is essential in the diagnosis of abnormalities associated with CHD in the neonatal population, (Cantinotti et al. 2012:142-143).

This study aimed at establishing reliable echocardiography nomograms of Black South African neonates. After a comprehensive search on the internet, only 2 studies from South Africa and Sub-Saharan Africa were found (Jacobs 2012:1-85; Majonga et al. 2017:409-413). Majonga et al. (2017: 409-413) focused on children and adolescents without any neonates with a sample size of 282. Jacobs (2012:1-85) focused on preterm neonates with a sample size of 290.

This study, therefore, represents the largest full term neonatal echocardiography nomograms from sub-Saharian Africa to date. Environmental, social and economic factors may influence growth and development thus it is important to establish echocardiography nomograms derived from the local population, (Majonga et al. 2017:

409-410). This study is unique as it represents a homogenous South African population and focuses on an understudied neonatal age group. In addition, measurements and structures that have been poorly studied, such as the left atrium to aortic root ratio, thymus and abdominal aorta have been included.

Cardiac structures along with other aspects of the body enlarge with growth, therefore it is essential to standardize cardiac structure dimensions according to body size, (Cantinotti 2012:142). Various regression equations using variables such as height, weight, BSA and cubed weight as the denominator against which dimensions are compared is regarded as an acceptable practical approach, (Roge et al. 1978: 287). Although BSA is the parameter used to standardize normality over a range of body weights and heights, body weight is more practical and is the preferred method used to express neonatal data, (Overbeek 2005:114; Guiltas and Eroglu 2011:135; Cantinotti et al. 2012:143; Mawad et al. 2013:30). Our study included children in the neonatal period, therefore birth weight was used to gauge the normality of the measurements obtained.

All parameters satisfied the homogeneity of variance measured by the Shapiro- Wilk W test except LA/AO ratio, AO diameter, LVPWDd, LVPWDs, RPA, LPA and ABD AO, (Table 4.3).

To test for effects of confounding factors, 11 commonly measured cardiac structures were analyzed. Body weight had a significant temporal relationship with all measurements, showing that cardiovascular dimensions increase with an increase in body weight, (Table 4.4).

We found a small but significant effect of mode of delivery on 45% of the cardiac dimension measurements namely MPA, RPA, LPA, MV ANN and TV ANN which was similar to a previous study by Cantinotti et al. (2014b: 1279-1292). Smaller dimensions were found in neonates born by C/S. To support this findings, thorough search on the internet was done but no data was found on physiological effects of mode of delivery on cardiac structure dimensions. Gender showed small significant differences for a few measurements (MPA and RPA) and most measurements were non-significant. Female neonates had smaller dimensions than male neonates. Some authors (Kampmann et al. 2000:671; Guizeltas and Eroglu 2012:155) found no significant differences between male

and female dimensions which is similar to this study cohort, however Cantinotti et al. (2014a:184); Cantinotti et al. (2014b: 1284); Majonga et al. (2017: 412) found small significant differences. Boys are known to have larger dimensions, (Zilberman, Khoury and Kimball 2005:359; Cantinotti et al. 2012: 144-146). A positive correlation was found between BSA and the mitral valve annulus (MVA) size but there was no association shown with body length (BL) and gestational age (GA).

Echocardiography is an operator dependent imaging technique that requires a high level of technical and interpretive skills to maximize its diagnostic accuracy, (Lai et al. 2006: 1413). Its main limitation lies in the operator skill and technique, but may be minimized if the interobserver and intraobserver variability is within acceptable ranges. Inter-observer variability was tested which showed strong correlation for most measurements with few exceptions (LVPWDd, LVPWDs and PV annulus).

LV dimension and wall thickness echocardiography measurements are widely used in clinical practice and research. M-mode ventricular diameter measurements in the paediatric age group, is the preferred method for LV quantification, but which can lead to overestimation of measurements, (Cantinotti et al. 2014b: 1284). This deficiency in measurement may be the reason for the poor inter-observer variability seen for the LVPWd and LVPWDs measurements in our study. Similarly, the reason for the PV annulus measurements having a moderate inter-observer variability correlation, may be that measurements rely on lateral imaging plane with relatively low resolution and an oblique orientation which is often the only one available on PSAX resulting in suboptimal measurement accuracy, (Lopez et al. 2010:486).

The use of Z-scores has been recommended to express reference values by the American Society of Echocardiography and other authors, (Mawad et al. 2013:35; Cantinotti et al. 2014a: 181; Cantinotti et al. 2014b:1280). We present normal cardiovascular dimension reference values that are expressed as Z-scores which have a mean of 0 and a normal range of -2 to +2 which helps to simplify clinical interpretation, (Colan and Massachusetts 2013:38). Some of the study measurements exceeded Z-scores above +2 and below -2. In order to accommodate these extremes, Z-scores of +3 and -3 Z-scores were added.

There are several published paediatric echocardiography nomograms that were reviewed, (Yeager and Sanders 1995: 837-839; Kampmann et al. 2000: 667-672; Yekeler et al. 2004: 1321-1326; Overbeek et al. 2006:113-121; Neilan 2009:50-55; Guzeltas and Eroglu 2012:152-157; Jacobs 2012: 1-85; Cantinotti 2014a:179-191; Cantinotti 2014b:1279-1292; Cantinotti 2014c: 1-16; Cantinotti 2017:208-215, Majonga et al. 2017: 409-410). These studies focused on different age groups ranging from 0-18 years of age, very few of them (Kampmann et al. 2000: 667-672; Guzeltas and Eroglu 2012:152-157; Jacobs 2012: 1-85) focused only on neonates.

The majority of the studies that focused on the paediatric age group (0-18 years) presented their data normalized to BSA. Neonatal data was not clearly specified or separated from the other age groups. For example, a BSA of $0.2m^2$ is acceptable for the neonatal age group but it is also possible for an older non-neonate with failure to thrive to have a similar BSA, hence there was a reluctance to attempt a direct comparison between these publications and the study cohort.

Only 3 studies focused on neonatal subjects, one study (Jacobs 2012:1-85) concentrated on premature neonates and was therefore excluded from the comparison. The other 2 studies that focused on full term neonates were more suitable for comparisons and focused on M-mode measurements of the LA, AO root, LV and RV. Our study, although it did not include RV measurements, did include measurements that were unique such as valve annulus diameters, arterial dimensions and the thymus.

Two studies (Yekeler et al. 2004: 1321-1326; Yeager and Sanders 1995: 837-839) concentrated on measurements of the thymus in infants. Yeager and Sanders (1995: 837-839) evaluated 21 infants with interrupted aortic arch type B and truncus arteriosus and presented their data as Thymic index (a measure of thymus change over time) thus we could not compare our results to their data. Yekeler et al. (2004: 1321-1326) focused on healthy infants aged 2 days to 2 years of age. Yekeler et al. (2004:1322) examined the thymus in longitudinal and transverse planes using trans-sternal, parasternal and intercostal approaches. They measured the maximal transverse diameter, right lobe anteroposterior dimension and left lobe anteroposterior dimension of the thymus. The longest craniocaudal dimension was measured by parasternal and suprasternal

approaches. A comparison with this data and the study data was not possible because of the different method used to measure the thymus whereby thymus was measured as the distance from anterior chest wall to the most anterior great artery on PSAX (Figure 3.4).

In literature there was only 3 studies focusing on valves and arterial dimensions, (Cantinotti et al. 2014a: 179-191; Cantinotti et al. 2017:208-215; Zilberman, Khoury and Kimbal 2005: 356-360). These studies covered a wide age range, from 0-18 years, which included the neonatal group however, could not be separated out adequately to compare to our study cohort. In addition, their results were expressed according to BSA, while this study used birth weight.

All M-mode measurements were compared with those by Kampmann et al. (2000: 667-672) and Guzeltas and Eroglu (2012:152-157). Our data was grouped into 4 similar weight groups (group 1: 2.50-2.99kg, group 2: 3.00-3.49, group 3: 3.50- 4.00, group 4.00-4.50) to allow comparisons with these studies. Our study cohort showed slightly higher dimensional measurements for all weight groups. These findings suggest that other possible factors such as environmental, social, economic, racial and ethnic factors of the population may influence growth or development and thus account for these minor differences. Similarly, Majonga et al. (2017: 412) from Zimbabwe who studied older children (6-16 years of age) showed that the interventricular septum and left posterior wall dimensions have higher means when compared to published non-African references.

It has been previously reported that paediatric normative data has a number of limitations. The main limitation being very small sample sizes (<200 patients) which was overcome in this study by having a much larger cohort with a sample size of 386 subjects. Also, this study focused only on neonates which historically was a previously understudied paediatric age group.

5.1 STUDY STRENGTHS AND LIMITATIONS

This study presents a group of novel nomograms in a unique African neonatal population which includes measurements that have been understudied previously such as arterial dimensions, valve annuli and the thymus. The study population comprises a large homogenous Southern African neonatal group with normal hearts which can be used to make comparisons with other racial and ethnic groups.

One study limitation was that we did not include right ventricle, right atrium and inferior vena cava dimensions which are poorly studied, with little published in this regard. Another limitation was that only 43% of the sample measurements were evaluated by an independent senior cardiologist. However, interobserver variability was good for all measurements.

CHAPTER 6

CONCLUSION

Echocardiography is the foremost diagnostic modality in the management of congenital and acquired heart disease in both children and adults, (Cantinotti et al. 2012:142). Echocardiography is safe, painless, assessed by utilizing portable devices, reduces the need for more invasive diagnostic methods such as cardiac catheterization. The imaging is based on simple physiological factors, and is very easy to understand and interpret, (Kaddura 2008:1; Bulwer and Rivero 2011:7).

Echocardiography nomograms are vital because they are a source of normal ranges which assist in the identification of abnormal cardiac structures and dimensions. Several published nomograms are based on European and American populations with very scarce data from African and South African population.

This study has developed echocardiography nomograms from a large sample of healthy Black South African neonates. The measurements are based on current recommendations by the American Society of Echocardiography, (Lopez et al. 2010: 465-495). Furthermore, the measurements have been shown to be reproducible as there was strong inter-observer variability correlations.

Birth weight was used as an independent variable for data normalization. All M-mode cardiac measurements (LA, AO root, IVSd, IVSs, LVIDd, LVIDs, LVPWDd, and LVPWDs) were slightly higher than European population based nomograms. In contrast, the MPA measurement in 2-D was slightly smaller than previously published normative data. This data, therefore confirms the importance of developing and using regional nomograms that conform to the population being studied.

This study has provided a template against which cardiac chamber, valve annulus, arterial and thymus dimensions in an African Neonatal age group can be measured and compared for normality.

Future studies can be expanded to include right heart dimensions and the whole range of the African paediatric population from 0-18 years. Further studies are required to confirm and reinforce these findings.

CHAPTER 7

REFERENCES

American Heart Association. 2019. Foetal Circulation. America: American Heart Association. Available at https://www.heart.org/en/health-topics/congenital-heart-defects/symptoms--diagnosis-of-congenital-heart-defects/foetal-circulation (Accessed on 13/08/2019).

Amita R. 2016. Heart General Embryology. Available at www.pathologyoutlines.com/topic/heartembryology.html. (Accessed on 20/02/2017).

Anon. 2010. Objectives of Training in the Subspecialty of Paediatric Cardiology. Available: www.royalcollege.ca (Accessed on 16/03/2017)

Anon 2011. William Sinclair Winship. Available at http://www.sahistory.org.za/people/williamsinclairwinship (Accessed on 23/05/2017)

Armstrong W.F. and Ryan T. 2010. Feigenbaum's Echocardiography. 7th edition. Lippincott William and Wilkins.

Brown S. and Pepeta L. 2016. Paediatric Cardiology in South Africa 2016: Quo Vadis? SA Heart Journal (online), 13(1): 2-4. Available at: https://www.researchgate.net>publication (Accessed on 23/02/2017)

Bulwer B.E and Rivero J.M. 2011. Echocardiography Pocket Guide: The Transthoracic Examination. Jones and Bartlett Publishers. Canada

Camm E. J., Botting K. J. and Sferruzzi-Perri A. N. 2018. Near to One's Heart: The Intimate Relationship between the Placenta and Foetal Heart. Frontiers in Physiology (online), June: 1-32. Available at https://www.ncbi.nlm.nih.gov/pubmed/29997513 (Accessed on 25/03/2019)

Cantinotti M., Scalese M., Molinaro S., Murzi B. and Passino C. 2012. Limitations of Current Echocardiographic Nomograms for Left Ventricular, Valvular and Arterial Dimensions in Children: A Critical Review. American Society of Echocardiography (online), 25:142-152. Available: http://www.onlinejase.com/article/S0894-7317(11)00790-5/pdf (Accessed on 10/09/2015)

Cantinotti M., Scalese M., Murzi B., Assanta N., Spadoni I., Festa P., De Lucia V., Crocetti M., Marotta M., Molinaro S. Lopez L. and Lervasi G. 2014a. Echocardiographic Nomograms for Ventricular, Valvular and Arterial Dimensions in Caucasian Children with a Special Focus on Neonates, Infants and Toddlers. American Society of Echocardiography (online), 27:179-191. Available: http://www.onlinejase.com/article/S0894-7317(13)00770-0/pdf (Accessed on 10/09/2015)

Cantinotti M., Scalese M., Murzi B., Assanta N., Spadoni I., Festa P., De Lucia V., Crocetti M., Marotta M., Molinaro S. Lopez L. and Iervasi G. 2014b. Echocardiographic Nomograms for Chamber Diameters and Areas in Caucasian Children. Journal of American Society of Echocardiography (online) 27(12): 1279-1292. http://www.onlinejase.com/article/S0894-7317(14)00586-0/pdf (Accessed on 10/09/2015)

Cantinotti M., Giordano R., Scalese M., Murzi B., Assanta N., Spadoni I., Maura C., Macro M., Molinaro S., Kutty S. and Iervasi G. 2017. Nomograms for Two-

Dimensional Echocardiography Derived Valvular and Arterial Dimensions in Caucasian Children. Journal of Cardiology (online), 69:208-215. Available at https://www.ncbi.nlm.nih.gov/pubmed/27118699 (Accessed 30/06/2016).

Colan S.D. and Massachusetts B. 2013. The Why and How of Z-scores. Journal of the American Society of Echocardiography (online), 26(1): 38-40. Available at https//: www.onlinejase.com>fulltext (Accessed on 09/04/2016).

Courtney J. A., Cnota J.F. and Jonas H.N. 2018. The Role of Abnormal Placentation in Congenital Heart Disease; Cause, Correlate, or Consequence? Fontiers in Physiology (online), 9: 1-9. Available at https://www.ncbi.nlm.gov>articles (Accessed 25/03/2019).

Dees E. and Baldwin H.S. 2018. Developmental Biology of the Heart. Available at https://www.sciencedirect.com/topics/medicine-and-dentistry/foetal-heart (Accessed on 13/08/2019)

Donofrio M.T., Moon-Grady A.J., Hornberger L.K., Copel J.A., Skansky M.S., Abuhamad A., Cuneo B.F., Huhta J.C., Jones R.A., Krishnan A., Lacey S., Lee W., Michelfelder E.C., Rempel G., Sliverman N.H., Spray T.L., Strasburger J. F., Tworezky W. and Rychik J. 2014. Diagnosis and Treatment of Foetal Cardiac Disease: A Scientific Statement from the American Heart Association. American Heart Journal (online), 129; 2188-2242. Available: http://ahajournals.org (Accessed 23/03/2019).

Gautier M., Detaint D., Fermanian C., Aegerter P., Delorme G., Arnoult F., Milleron O., Raoux F., Stheneur C., Vahanian A. and Jondeau G. 2010. Nomograms for Aortic Root Diameters in Children using Two-Dimensional Echocardiography. The American Journal of Cardiology (online), 105 (6): 888- 894. Available: https://www.researchgate.net/publication/41824730 (Accessed on 02/06/2017).

Guzeltas A. and Eroglu A. G. 2012. References Values for Echocardiographic Measurements of Healthy Newborns. Cardiology in the Young (online), 22:152-157. Available: http://www.ncbi.nlm.nih.gov/pubmed/21933471 (Accessed on 10/09/2015)

Han X., Ge M., Dong J., Wang Z., He J. and Yang R. 2015. The Relationship between Left Ventricle Myocardial Performance Index of Healthy Women and Geographical Factors. Int J Biometeorol (online), 59: 1557-1565. Available: http://wu4wl9yj2g.search.serialssolutions.com/ (Accessed on 02/04/2016)

Hillman N., Kallapur S.G. and Jobe A. 2012. Physiology of Transition from Intrauterine to Extrauterine Life. Clinics in Perinatology (online), 39(4): 769-783. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3504352/ (Accessed on 12/022017).

Hoffman J.E.I. 2013. The Global burden of congenital heart disease. Cardiovascular Journal of Africa (online) 4(4): 141-145. Available: https://www.ncbi.nlm.nih.gov › NCBI › Literature › PubMed Central (PMC) (Accessed on 25/01/2017)

Hoosen E. C., Hug-Hanmar C., Cilliers A.M., Brown S.C., Lawrenson J., Harrisberg J. and Dansky R. 2007. Guidelines for Paediatric Echocardiography in South Africa. South African Heart Journal (online), 4(4); 42-45. Available: https://www.saheart.org/files/Guideline_PaedEcho.pdf (Accessed on 25/01/2017)

Hoosen E.G.M, Cilliers A.M., Hugo-Hamman C.T., Brown S.C., Lawrenson J.B., Zühlke L. and Hewitson J. 2011. Paediatric Cardiac Services in South Africa. South African Medical Journal (online), 101(2): 106-107. Available at: www.samj.org.za>article>download (Accessed on 01/06/2017)

Iaizzo P.A. 2005. Handbook of Cardiac Anatomy, Physiology and Devices. Totowa, New Jersey: Humana Press Inc.

Jacobs S. 2012. Referencing Echocardiographic Measurements for Premature and Low- Birth Weight Infants. M. Tech. Central University of Technology. Available: ir.cut.ac.za> handle. (Accessed 22/05/2017).

Johnson W. and Moller J. 2014. Pediatric Cardiology: The Essential Pocket Guide. 3rd edition. John Wiley and Sons, Oxford

Kaddura S. 2009. Echo Made Easy. 2nd edition. Churchill Livingstone Elsevier, London.

Kaldararova M., Balazova E., Tittel P., Stankovicova I., Brucknerova I and Masura J. 2007. Echocardiographic Measurements of the Aorta in Normal Children and Young Adults. Bratislavske Lekarske Listy (online) 108 (10-11): 437- 441. Available: https://www.ncbi.nlm.nih.gov/pubmed/18306722 (Accessed on 04/04/2017).

Kapmann C, Wiethoff C.M, Wenzel A., Stolz G., Betancor M., Wippermann C-F., Huth R-G., Habermehl P., Knuf M., Emschermann T. and Stopfkuchen H. 2000 Normal Values of M-mode Echocardiographic Measurements of More Than 2000 Healthy Infants and Children in Central Europe. Heart (online) 83: 667-672. Available at https://www.ncbi.nlm.nih.gov/pubmed/10814626 (Accessed on 09/04/2016)

Kaski J.P. and Daubeney P.E.F. 2008. Normalization Echocardiographically Derived Paediatric Cardiac Dimensions to Body Surface Area: Time for A standardized Approach. European Journal of Echocardiography, 1-2. Available at http://ehjcimaging.oxfordjournals.org/content/ejechocard/early/2008/01/01/ejechocard.jen242.full.pdf (Accessed on 10/09/2015)

Keane J.F., Lock J.E. and Flyer D.C. 2006. NADAS' Paediatric Cardiology. 2nd edition. Philadelphia: Elsevier Inc.

Kizer J. R., Arnett D.K., Bella J.N., Paranicas M., Rao D.C., Province M.A., Oberman A., Kitzman D.W., Hopkins P.N., Liu J.E. and Devereux R.B. 2004. Differences in left ventricular structure between Black and White Hypertensive Adults: The Hypertension Genetic Epidemiology Network Study. American Heart Association Journal (online) 43 (6): 1182- 1188. Available: https://www.ahajournals.org/doi/epub/10.1161/01.HYP.0000128738.94190.9f (Accessed on 04/04/2016)

Lai W.W., Geva T., Shirali G.S., Frommelt P.C., Humes R.A., Brook M.M., Pignateli R.H. and Rychik J. 2006. Guidelines and Standards for Performance of a Paediatric Echocardiogram: A Report from the Task Force of the Paediatric Council of the American Society of Echocardiography. Journal of American Society of Echocardiography (online), 19: 1413-1430. Available: http://www.sbccv.org.br/residentes/downloads/cong.julho2010.pdf (Accessed on 10/09/2015)

Lam K.M. and King M.E.E. 2015. Echocardiography Examination. Available at https://radiologykey.com/echocardiography-2/ (Accessed on 17/08/2019)

Lang R.M., Bierig M., Devereux R.B., Flachskampf F.A., Foster E., Pellikka P.A., Picard M. H., Roman M. J., Seward J., Shanewise J. S., Solomon S. D., Spencer K.T., Sutton M. S. and Stewart W.J. 2005. Recommendations for Chamber Quantification: A Report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, Developed in Conjunction with European Association of Echocardiography, A Branch of the European Society of Cardiology. Journal of the American Society

of Echocardiography (online) 18(12): 1440-1463. Available: https://www.onlinejase.com/article/ (Accessed on 22/05/2017).

Lang R.M., Bierig M., Devereux R.B., Flachskampf F.A., Foster E., Pellikka P.A., Picard M. H., Roman M. J., Seward J., Shanewise J. S., Solomon S. D., Spencer K.T., Sutton M. S. and Stewart W.J. 2006. Recommendations for Chamber Quantification. European Journal of Echocardiography 7: 79-108. Available: www.pac4.ch>Pdf>AnnECardiol (Accessed on 27/09/2015)

Lemmer C.E., Engel M.E., Stanfliet J.C and Mayosi B.M. 2014. Reference Intervals for the Echocardiographic measurements of Right Heart in Children and Adolescent: A Systemic Review. Cardiovascular Ultrasound (online), 12 (3):1-7. Available: http://www.cardiovascularultrasound.com/content/12/1/3 (Accessed on 27/09/2015)

Lopez L., Colan S.D., Frommelt P.C., Ensig G. J., Kendall K., Younoszai A. K., Lai W.W., and Geva T. 2010. Recommendations for Quantification Methods during the Performance of a Pediatric Echocardiogram: A Report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. Journal of American Society of Echocardiography (online), 23: 465-495. Available: http://www.onlinejase.com/article/S0894-7317(10)00266-X/pdf (Accessed on 27/09/2015).

LucMertens, Seri I, Jan Merek H., Arlettaz R., Barker P., McNamara, Moon-Grady A.J., Coon P.D., Noori S., Simpon J. and Lai W.W. 2011. Targeted Neonatal Echocardiography in the Neonatal Intensive Unit: Practice Guidelines and Recommendations for Training. European Journal of Echocardiography (online) 12: 715-735. Available: http://ejechocardio.oxfordjournals.org/ (Accessed on 12/02/2017).

Mahle W.T., Blaine J. J. and Silverman N. H. 2009. The History of the Development of Paediatric Echocardiography. Cardiology in the Young 19 (Suppl. 2): 26-34. Available: https://www.ncbi.nlm.nih.gov/pubmed/19857347 (Accessed on 26/01/2017).

Majonga E. D., Rehman A. M., McHugh G., Mujuru H.A., Nathoo K., Patel M. S., Munyati S., Odland J.O., Kranzer K., Kaski J.P. and Ferrand R. A. 2017. Echocardiographic Reference Ranges in Older Children and Adolescents in Sub-Saharian Africa. International Journal of Cardiology (online) 248:409-413. Available at https://www.ncbi.nlm.nih.gov/pubmed/28711335 (Accessed on 21/01/2019).

Martin E.A. 2003. Oxford Concise Medical Dictionary 6th edition. UK: Market House Books.

Mawad W., Drolet C., Dahdah N. and Dallaire F. 2013. A Review and Critique of the Statistical Methods Used to Generate Reference Values in Paediatric Echocardiography. Journal of the American Society of Echocardiography (online) 26:29-37. Available: https://www.ncbi.nlm.nih.gov>pubmed (Accessed on 14/01/2016)

Neilan T.G., Pradhan A.D, King M. E. and Weyman A. E. 2008. Derivation of a Size-Independant Variable for Scaling of Cardiac Dimensions in a Normal Paediatric Population. European Journal of Echocardiography (online) 10: 50-55. Available: https://academic.oup.com/ehjimaging/article/ (Accessed 09/04/2016)

Noonan J.A. 2004. A History of Paediatric Specialities: The Development of Paediatric Cardiology. Paediatric Research (online), 56:298-306. Available: https://www.ncbi.nlm.nih.gov>pubmed (Accessed on 26/01/2017).

Overbeek L.I.H., Kapusta L., Peer P.G.M, de Korte C.L., Thijssen J.M. and Daniels O. 2006. New Reference Values for Echocardiographic Dimensions of Healthy Dutch Children. European Journal of Echocardiography (online) 7: 113-121. Available: https://www.ncbi.nlm.gov/m/pubmed/ (Accessed on 09/04/2016)

Panda U.N. 2006. Clinical Cardiology. New Delhi: Mehta Publishers.

Pansky B. 1982. Review of Medical Embryology. 1st edition. USA: Macmillan Publishing Company.

Park M. K. 2016. Park's: The Pediatric Cardiology Handbook. 5th edition. Saunders, Philadelphia

Park M. K. 2014. Park's: The Pediatric Cardiology for Practitioners. 6th edition. Saunders, Philadelphia.

Roge C.L., Silverman N.H., Hart P.A. and Ray R.M. 1978. Cardiac Structure Growth Patterns by Echocardiography. American Heart Association (online), 57(2): 285-290. Available: http://circ.ahajournals.org/content/57/2/285 (Accessed on 10/09/2015)

Shah N.K., Kumar K., Prabhu S.S., Ahamed M.Z. and Gupta S. 2013. IAP Speciality Series on Paediatric Cardiology. 2nd edition. Jaypee Brothers Medical Publishers (P) LTD, New Delhi

Silverman N.H. 1993. Pediatric Echocardiography. Williams and Wilkins, Maryland.

Sliwa K., Carrington M., Mayosi B.M., Zigiriadis E., Mvungi R. and Stewart S. 2009. Incidence and Characteristics of Newly Diagnosed Rheumatic Heart Disease in

Urban African Adults: Insights from the Heart of Soweto Study, 2009. European Heart Journal (online), November 2009: 1-9. Available at: https://academic.oup.com/eurheartj/article (Accessed on 01/06/2017)

Statistica Help. 2017. Shapiro Wilk W Test. (statistics computer software). Available at: http://documentation.statsoft.com/STATISTICAHelp.aspx?path=Glossary/GlossaryTwo/S/ShapiroWilksWtest (Accessed on 05/02/2019)

Tacy T.A., Vermilion R.P. and Ludomirsky A. 1995. Range of Normal Valve Annulus Size in Neonates. American Journal of Cardiology 1995; 75:541-3.

Tortora G.J. and Derrickson. 2009. Principles of Anatomy and Physiology. 12th Edition. John Wiley and Sons, Asia.

Van der Linde D, Konings E.E.M, Slager M.A., Witsenburg M., Helbing W.A, Takkenberg J.J.M. and Roos-Hesselink J.W., 2011. Birth Prevalence of Congenital Heart Disease Worldwide: A Systematic Review and Meta-Analysis. Journal of American College of Cardiology, 21:2241-2247. Available: www.sciencedirect.com/science/article/pii/S0735109711030798 (Accessed on 18/05/2016).

Wilson L.T. 2009. Statistical Correlation. Available at https://explorable.com (Accessed on 08/07/2019).

Yeager S.B. and Sanders S.P. 1995. Echocardiographic Identification of Thymic Tissue in Neonates with Congenital Heart Disease. American Heart Journal (online), 129(4); 837-839. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7900643. (Accessed on 10/09/2015)

Yekeler E., Tambag A., Tunaci A., Genchellac H., Dursun M., Gokcay G. and Acunas G. 2004. Analysis of the Thymus in 151 Healthy Infants from 0-2 Years of Age. Journal Ultrasound in Medicine (online) 23: 1321-1326. Available at: https://onlinelibrary.wiley.com/doi/full/10.7863/jum.2004.23.10.1321 (Accessed on 09/04/2016)

Zilberman M.V., Khoury P.R. and Kimball R.T. 2005. Two-Dimensional Echocardiography Valve Measurements in Healthy Children: Gender-Specific Differences. Paediatric Cardiology 2005; 26:356-60.

Zühlke L. 2013. Challenges and Opportunities in Paediatric Cardiac Services: Time for Action. SA Heart Journal (online), 10(2): 431-433. Available: https://journals.co.za/content/sahear/10/2/EJC138796. (Accessed on 01/06/2017)

APPENDICES

APPENDIX A

PERMISSION LETTER

DEPARTMENT OF HEALTH

HOSPITAL MANAGER

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

GAUTENG PROVINCE

Dear Prof Pettifor

Request for permission to conduct a research study

My name is Nondumiso Hadebe clinical technologist at cardiology department, currently registered for Masters of Health Sciences Degree at Durban University of Technology. I would like to request to conduct a research project which is entailed in Maters of Health Sciences Degree syllabus.

Title of the research project: Echocardiography nomograms in Black African neonates.

The study will be a descriptive, cross-sectional cardiac chamber, thymus and vessel dimension evaluation of African neonates' with normal hearts using echocardiography. The study will be carried out in two phases.

Phase 1: this phase will be attached to a study 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, copy attached (Appendix B). Ethics approval has been granted by the "Human Research Ethics Committee (Medical)" on 31/7/2015 – Ethics Clearance certificate no. M150721. A separate approval to use the study echocardiographic data for neonatal nomograms for this study will be sought from the same ethics committee and the Medical Advisory committee of the Chris Hani Baragwanath Academic Hospital. A total of 350 neonates underwent comprehensive echocardiographical studies and images were stored. 200 neonates with structurally normal hearts on echocardiography will be selected from acquired echocardiography

images, dimensions will be prospectively measured and analyzed using post-date data analysis.

Phase 2: will be an evaluation of cardiac chambers, valve annulus, thymus and great vessels dimension in African neonates who will be prospectively enrolled to the study. A separate proposal will be submitted for this phase which will include ethics approval and consent obtained from mothers of the neonates in the postnatal wards before discharge. Echocardiographic studies will be carried out on neonates within 12-24 hours after birth. Those neonates with structurally normal hearts will have their cardiac valve, vessel and chamber diameters analyzed for inclusion in the construction of nomograms. This phase aims at enrolling 200 neonates.

The study is not structured to alter any routine procedures, treatment in any way and will not have any extra costs since I will be using hospital equipment. I plan to start data collection as soon as my proposal has been ethically approved and permission has been granted to use hospital patients.

Dr Ntsinjana (Paediatric Cardiologist) and Prof Cilliers (Head of Paediatric Cardiology Department) has offered me with their support in supervising the project.

I hereby apply for permission to conduct research using the patient's images recorded during echocardiography test from Chris Hani Baragwanath Academic Hospital and hospital equipment.

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

Regards

Miss Nondumiso Hadebe

Clinical Technologist (Cardiology)

Contact number: 072 268 8503

APPENDIX B

R14/49 Mlles NM Hadebe & A Cilliers

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M170536

NAME: (Principal Investigator)	Mlles NM Hadebe & A Cilliers
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics Division of Paediatric Cardiology CH Baragwanath Hospital
PROJECT TITLE:	Echocardiographic normograms in Black South African neonates
DATE CONSIDERED:	26/05/2017
DECISION:	Approved
CONDITIONS:	Please put the project title on the Consent Form
SUPERVISOR:	Dr H Ntsinjana
APPROVED BY:	Professor CB Penny, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	15/09/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

22 November, 2016

Student No: 20515849

Ms NM Hadebe
PO Box 322
Colenso
3360

Dear Ms Hadebe

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:

    Echocardiography nomograms in Black South African neonates.

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 - **Dr H Ntsinjana**
(iv) Co-Supervisor - **Mr MJ Mohapi**

2. Your request for funding totalling **R 15 000.00** 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 your Head of Department. 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 PhD studies is retained by you as the initiator of the project;

(b) Should you make any Drift from the results of your PhD studies, you will be required to repay pro rata, the **R 15 000.00** 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.

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 Executive Dean of the Faculty.

Yours sincerely

Mr S Reddy
FACULTY RESEARCH OFFICER

_____ _____

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

APPENDIX C

Patient information and consent

Title of the Research Study

Echocardiography nomograms in Black South African neonates.

Principal Investigator:

Miss N.M. Hadebe student in Masters of Health Sciences Degree: Clinical Technology (Cardiology) at Durban University of Technology.

Brief Introduction and Aim of the Study

When a baby is sick, the doctors examine what is the cause of the baby's sickness using different methods and when they suspect that the baby has heart disease then the baby is referred to the heart clinic where further tests are done to detect heart problems including echocardiography (ultrasound of the heart). For the doctor to interpret the results of echocardiography there must be reference values that will tell what is normal and what is abnormal. This study is aimed at investigating the normal reference values of heart measurements done on echocardiography in African neonates (newborn babies).

Outline of the procedure

Dear mother of a newborn baby. You are kindly invited to allow your baby to participate in the study aimed at investigating normal heart measurements done on echocardiography (ultrasound of the heart). As you have agreed that your baby participate on the study with a title "To assess the accuracy of pulse oximetry screening as a tool to detect critical congenital heart disease in asymptomatic newborns" by Dr Michael Platten, the investigator is requesting to use data on echocardiography machine that was done to your baby, to create normal reference values of heart measurements done on echocardiography.

Risk(s) to the participant

There will be no risks that the participant will be exposed to because the test conducted is a recommended test to detect heart disease and it does not include any injections.

Benefits

The new information gained from the study will help to improve diagnosis and treatment of patients with congenital and acquired heart disease seen in babies.

Reason(s) why the participant may be withdrawn from the study

The participant will be withdrawn from the study when they have congenital heart disease.

Remuneration

There will be no money that will be given to the participant.

Cost of the study

The participant will be responsible for normal routine medical procedures being involved in the study will not cost extra money.

Confidentiality

Participants will be assigned a study number that will be known by a researcher only and data will be kept safely at paediatric cardiac clinic, Chris Hani Baragwanath Academic Hospital.

Research related injuries

There will be no research related injuries.

Person(s) to contact in case of any queries:

Miss N Hadebe	Dr H Ntsinjana	Prof Cilliers	Dr R Prakachandra
Investigator	Unit Supervisor	Unit Supervisor	Internal supervisor
072 268 8503	073 735 6832	083 343 5803	083 446 7735

Statement of agreement to participate in the study

I _____ (participant name) have read the document and understand its contents. Where I had any queries these were explained to me by the researcher. Furthermore I fully understand that I may withdraw from participating at any stage without any adverse consequences and my future health care will not be compromised. I therefore involuntary agree to participate in this study.

Participant's name _____

Participant's signature _____

Date _____

Researcher's name _____

Researcher's signature _____

Date _____

Witness name _____

Witness signature _____

Date _____

APPENDIX D

DATA COLLECTION SHEET

Study number		**Sex**	M	F
Date of birth		**Weight**		
Gestational age		**Height**		
Type of delivery		**BSA**		

Site	**Diameter in mm**
LA	
AO diameter	
LA/AO ratio	
IVSd	
IVSs	
LVIDd	
LVIDs	
LVPWDd	
LPWs	
MV annulus	
AV annulus	
PV annulus	
TV annulus	
MPA	
RPA	
LPA	
Thymus	
ABD AO	

APPENDIX E

SAMPLE SIZE CALCULATION

Sample size = $(\text{Z-score})^2$ x SD x (1- SD)/ $(\text{Margin error})^2$

= $(1.282)^2$ x 0.5 x (1- 0.5)/ $(0.05)^2$

= 1.64 x 0.25/ 0.0025

= 164