PEDIATRIC ECHOCARDIOGRAPHY Z-SCORE AND ELECTROCARDIOGRAM DATABASE PROJECT

PROTOCOL

VERSION DATE: October 22, 2013

Funded by
the National Heart, Lung, and Blood Institute,
NIH/DHHS

1. GENERAL INFORMATION

1.1 Protocol Signature Page

I have read the foregoing protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the conduct of the study.

I will use the informed consent form approved by the NHLBI and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol and, in particular, I agree to report any serious adverse experiences.

I further agree that the NHLBI and/or its designee has access to any source documents from which case report form information may have been generated.

I also agree to collect all echocardiograms and electrocardiograms in accordance with the protocol.

The below signed confirm herewith to have read and understood this study protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance to the protocol and Good Clinical Practice guidelines, as well as local regulations and regulatory authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Investigator		
Investigator		
Investigator		
Investigator		

1.2 Protocol Synopsis

Title	Pediatric Echocardiography Z-Score and Electrocardiogram Database Project
Grant Number	
Study Objectives	1. To establish a Z-score database for common echocardiographic (echo) measurements based on a uniformly defined and racially diverse population of normal children from multiple centers over a wide geographic area 2. To collect electrocardiograms (ECG) from the same population of normal children for future establishment of ECG reference values
Significance	Currently available normal reference values for echo measurements are heterogeneous and based on studies which are limited by small sample sizes, few neonatal data, inconsistent nomenclature, non-standardized methodology for measurement performance and normalization, and failure to account for the effects of gender and race. Hence echo Z-scores can vary for the same patient and the same measurement depending on which echo Z-score nomogram is used. Studies to establish ECG reference values are similarly limited by small sample sizes and homogeneous populations. This study will establish Z-scores for the most commonly used echo measurements using a uniformly defined group of normal children of diverse ages, racial backgrounds, and genders from multiple centers over a large geographic area. The Z-score database will then serve as a key reference for clinical and research studies in pediatric heart diseases. In addition, ECGs from the same population will be available for the development of robust reference values.
Study Design	1. Retrospective collection of demographic and clinical data and echo images from a large group of normal children from multiple centers 2. Prospective performance of echo measurements by a Core Laboratory 3. Statistical analysis of the data and establishment of echo Z-scores based on body size, age, gender, and race 4. Retrospective collection of available ECGs from the same population into a repository for future studies
Primary Aim	To calculate the mean and standard deviation (SD) for common echo measurements adjusted for body size, age, gender, and race for a large group of normal children using retrospective data from multiple centers
Secondary Aim(s)	1. To determine the best body surface area (BSA) calculation method for describing the relationship between BSA and the sizes of cardiovascular structures 2. To evaluate the effects of other potential confounders such as height, weight, body mass index (BMI), and ethnicity on echo measurements

	3. To develop a repository of ECGs obtained from normal children that would be used to establish robust pediatric ECG reference values
Accrual Objective	Total sample size of 3600 study patients in 36 study groups based on 6 age categories, 2 genders, and 3 racial categories
Study Duration	Approximately 24 months
Inclusion Criteria	1. ≤18.0 years of age 2. Echo images in DICOM (Digital Imaging and Communications in Medicine) format from studies performed after January 1, 2008 3. Documentation of height and weight 4. Documentation of gender and race
Exclusion Criteria	1. Echo images inadequate for analysis 2. Acquired or congenital heart disease (CHD) as determined by history, physical examination, chest x-ray, or echo (other than hemodynamically insignificant lesions such as a patent foramen ovale (PFO), a tiny patent ductus arteriosus (PDA), mild peripheral pulmonic stenosis (PPS), or a tiny coronary artery fistula) 3. Acquired or CHD based on the following ECG findings (if an ECG is performed): PR interval >220 ms, 2nd or 3rd degree heart block, QRS interval >120 ms, ventricular pre-excitation, non-sinus rhythm, and confirmed diagnosis of long QT syndrome 4. Corrected gestational age <37 weeks at the time of the echo 5. BMI ≥95th percentile for children ≥2 years old, or weight-for-length Z-score ≥2 based on the World Health Organization (WHO) Child Growth Standards for children <2 years old 6. Acute or systemic disorder with cardiovascular manifestations (including but not limited to Marfan syndrome, sickle cell disease, cancer, renal failure, human immunodeficiency virus infection, Kawasaki disease, rheumatic fever, autoimmune disorder, and systemic hypertension) using criteria which are not based on ECG or echo findings but rather on specific physical exam findings or abnormal blood tests 7. Documented history of a 1st degree relative with non-ischemic cardiomyopathy 8. Documented history of a 1st degree relative with the following congenital left-sided heart lesions: mitral stenosis, left ventricular outflow tract obstruction, bicuspid aortic valve, aortic coarctation, and/or hypoplastic left heart syndrome

1.3 Table of Contents

1.4 List of Abbreviations

BMI	Body Mass Index
BSA	Body Surface Area
CHD	Congenital Heart Disease
CRF	Case Report Form
CVRG	Cardiovascular Research Grid
DCC	Data Coordinating Center
DICOM	Digital Imaging and Communications in Medicine
DMS	Data Management System
DSMB	Data and Safety Monitoring Board
EC	Executive Committee
ECG	Electrocardiogram
Echo	Echocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
HTTPS	Hypertext Transfer Protocol Secure
IRB	Institutional Review Board
JHU	Johns Hopkins University
LVEDA	Left ventricular short-axis end-diastolic area
LVEDD	Left ventricular short-axis end-diastolic diameter
LVEDL	Left ventricular long-axis end-diastolic length
LVEDV	Left ventricular short-axis end-diastolic volume
LVESA	Left ventricular short-axis end-systolic area
LVESD	Left ventricular short-axis end-systolic diameter
LVESL	Left ventricular long-axis end-systolic length
LVESV	Left ventricular short-axis end-systolic volume
MOO	Manual of Operations
NHLBI	National Heart, Lung and Blood Institute
PDA	Patent Ductus Arteriosus
PFO	Patent Foramen Ovale
PHI	Personal Health Information
PHN	Pediatric Heart Network
PI	Principal Investigator
PII	Personally Identifying Information
PPS	Peripheral Pulmonic Stenosis
QA	Quality Assurance
QC	Quality Control
SC	Steering Committee
SD	Standard Deviation
SID	Subject Identification Number
SSL	Secure Socket Layer
WHO	World Health Organization

2. STUDY AIMS AND HYPOTHESES

2.1 Primary Aim

To calculate the mean and SD for common echo measurements adjusted for body size, age, gender, and race for a large group of normal children using retrospective data from multiple centers.

2.2 Secondary Aims

1. To determine the best BSA calculation method for describing the relationship between BSA and the sizes of cardiovascular structures.

2. To evaluate the effects of other potential confounders such as height, weight, BMI, and ethnicity on common echo measurements in normal children.

3. To develop a repository of ECGs obtained from normal children that would be used to establish robust pediatric ECG reference values.

3. BACKGROUND INFORMATION

3.1 Background

Echocardiography is crucial for the evaluation of children with congenital and acquired heart diseases. It is the primary imaging modality for establishing diagnoses, determining treatment options, monitoring disease progression, and assessing the effects of intervention. The sizes of cardiovascular structures are frequently affected by the abnormal hemodynamics of a disease state, particularly in children. Because treatment decisions rely on an accurate determination of cardiovascular size, quantification is an important component of the echo study (1, 2). Reliable, accurate, and generalizable normal reference values must be readily available for clinicians to distinguish a normal finding from an abnormal one and to determine if the size of a structure is adequate, too small, or too large to function effectively. In addition to their importance in the clinical setting, reference values are also used to define the population of interest and as primary or secondary outcomes in research studies designed to evaluate medical or surgical therapies (3-5).

Previous studies suggest that measurements in normal children are confounded by body size, age, gender, and race (6-11). Adjustments must be made for these factors before the effect of a disease state on the cardiovascular system can be assessed. Echo Z-scores have gained widespread acceptance in both clinical practice and research in pediatric cardiology (9, 12-18). Similar to growth charts, they allow comparisons of cardiac measurements obtained for an individual child with measurements obtained from a normal population adjusted for the effects of body size, age, gender, and/or race. The Z-score corresponding to a particular observation in a dataset is defined as

$$\mathrm{Z} = \frac{observation - mean}{standard\ deviation}$$

The Z-score tells us how many SDs the observation is from the mean, and it is positive or negative according to whether the observation lies above or below the mean. It can be calculated from various subpopulations based on a particular set of parameters (including body size, age, gender, and race). For example, a Z-score of +2 (or -2) for a given population corresponds to a measurement that is two SDs above (or below) the expected mean,

corresponding to the 95% confidence interval where 2.5% of the population is expected to have values above and 2.5% below this interval.

3.2 Prior Studies

As Z-scores have become more widely used in both clinical practice and research, the limitations of the currently available databases have become apparent. A recent paper published by Cantinotti, *et al*, evaluated at least 30 previously published pediatric echo nomograms and Z-score databases from different institutions and revealed wide variation in the specific Z-score obtained by using the different databases (19). The table below illustrates how a mitral annular diameter of 11 mm in a boy with a BSA of 0.3 m^2 can correspond to a Z-score ranging from -4.8 (17) to +2.5 (7). This wide variation limits the clinician's ability to make important treatment decisions.

Study	Center	Year	N	BSA (m^2)	MV (mm)	Z
King (6)	Houston	1985	103	0.3	11	-3.5
Hanseus (7)	Sweden	1988	120	0.3	11	***+2.5***
Daubeney (17)	Wessex	1999	125	0.3	11	***-4.8***
Sluysmans (9)	Boston	2005	496	0.3	11	-0.9
Zilberman (13)	Cincinnati	2005	434	0.3	11	-0.9
Pettersen (14)	Detroit	2008	782	0.3	11	-1.6

The variability in echo Z-scores is due to several factors:

- Single clinical sites
- Small sample sizes
- Paucity of neonatal data
- Inconsistent nomenclature of cardiovascular structures
- Non-standardized methodology for performing measurements
- Inconsistent inclusion and exclusion criteria to define the normal population
- Failure to adjust measurements for the effects of gender and race
- Non-standardized methodology for normalizing measurements
- Variable expressions of normalized data

3.3 Rationale for the Study

As a result of these limitations, the Z-score for a specific measurement depends on the specific database used. The ability to reliably determine thresholds for abnormal sizes and the effectiveness of echo standards for predicting best treatment pathways are diminished. To date, normal reference values for echo measurements obtained from multiple centers and geographic locations and adjusted for body size, age, gender, and race do not exist, and a multicenter study to determine these Z-scores for a large and racially diverse population of normal children is warranted. The Pediatric Heart Network (PHN) is the ideal environment for this study with experienced study coordinators, an established infrastructure to transfer digital media into a Bioinformatics Grid (as described in Section 7.1) where images are available for analysis by the Core Laboratory and other investigators, and extensive statistical support to develop the Z-score database.

3.4 Rationale for the Study Outcomes

The study outcomes were chosen to bridge a significant clinical knowledge gap by providing echo data from a uniformly defined group of normal children from multiple centers over a large geographic area. At present, both clinical decision-making and the conduct of research are affected by the limitations of current Z-score nomograms. The American Society of

Echocardiography Pediatric and CHD Council has recently published standards for obtaining images and performing pediatric echo measurements (1) that can be used by the Core Laboratory as well as local centers. This protocol includes the most commonly used two-dimensional measurements. Because there may also be interest and value in evaluating other measurements, all echo data will be collected and available in the PHN Bioinformatics Grid for future approved ancillary studies.

Because ECGs are often obtained along with echo studies during the cardiac evaluation, we are taking advantage of the opportunity to collect these studies in an imaging repository (PHN Bioinformatics Grid). The establishment of a database of normal ECGs for future determination of ECG reference values using the same group of normal children will overcome a long-standing limitation of current ECG reference values.

4. STUDY DESIGN

4.1 Overview

The study will involve the following components:

- Retrospective identification by the local study coordinator of a large group of normal children meeting the study inclusion criteria and without any exclusion criteria
- Retrieval and review of echo images for all eligible patients by the local principal investigator (PI) or alternate PI to ensure adequate image quality
- Collection of demographic and clinical data for all eligible subjects
- Retrieval of ECGs performed closest to the date of the echo (absence of a recent ECG will not preclude study eligibility)
- Submission of all data, echo images, and ECGs into the PHN Bioinformatics Grid
- Prospective performance of echo measurements by a PHN-approved Core Laboratory
- Statistical analysis of the data and creation of echo Z-scores based on body size, age, gender, and race
- Availability of the ECGs for future analysis

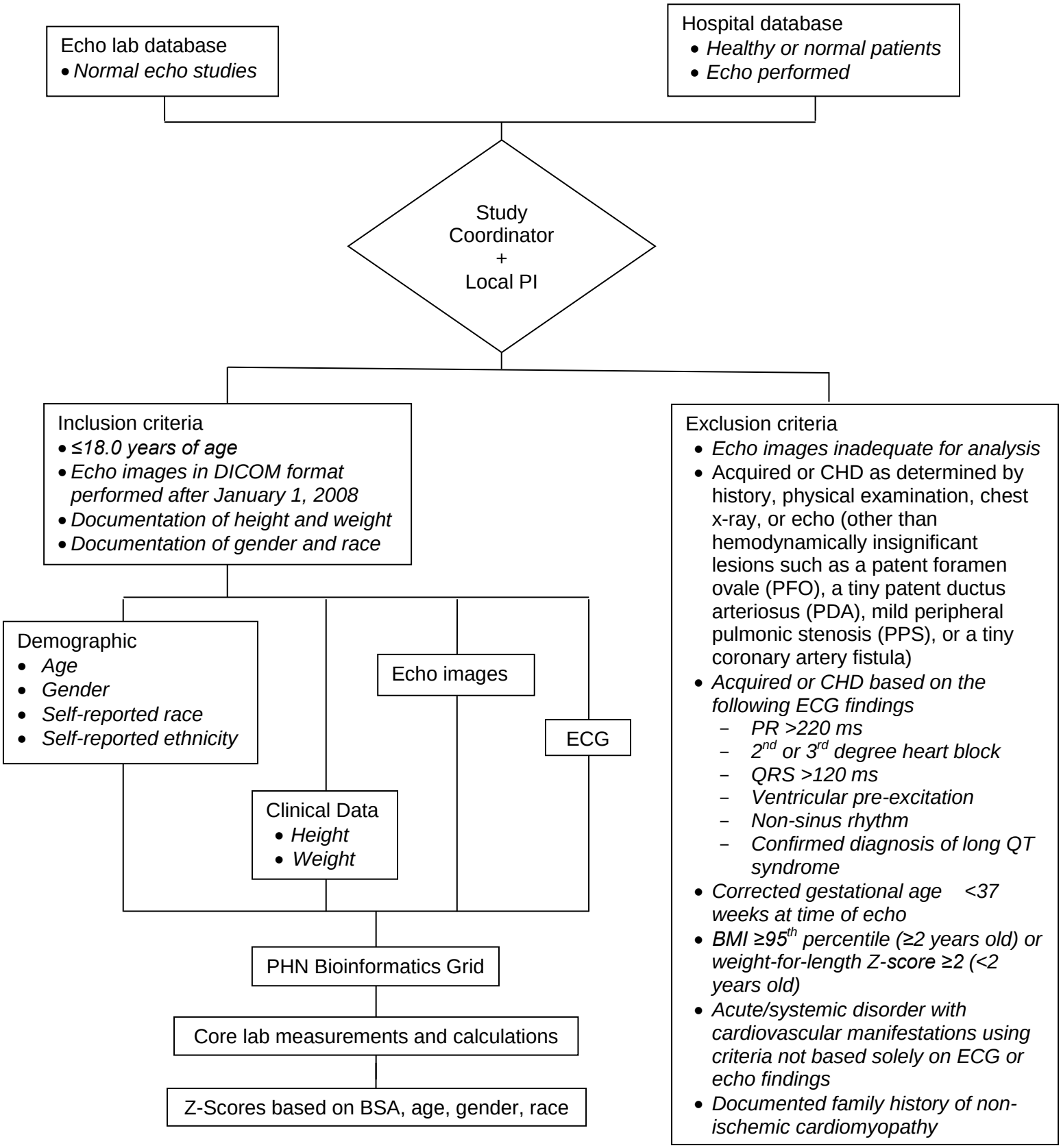
4.2 Study Institutional Review Board or Research Ethics Board

Because all data, echo images, and ECGs will be de-identified prior to submission into the PHN Bioinformatics Grid in this retrospective study, patients will be enrolled into the study for most centers under a waiver of consent after consideration and approval by the Institutional Review Board or Research Ethics Board at each participating center. As discussed in the following section, some centers will require parental consent in order to obtain race and ethnicity data prospectively, and this approach will require approval by the local Institutional Review Board or Research Ethics Board.

4.3 Measures

For each patient who fulfills the study criteria, the study coordinator will collect the following demographic and clinical data:

- Age
- Date of study
- Gender
- Self-reported race
- Self-reported ethnicity
- Height
- Weight


Echo lab database
• Normal echo studies
Hospital database
• Healthy or normal patients
• Echo performed
Study Coordinator + Local PI
Inclusion criteria
• ≤18.0 years of age
• Echo images in DICOM format performed after January 1, 2008
• Documentation of height and weight
• Documentation of gender and race
Exclusion criteria
• Echo images inadequate for analysis
• Acquired or CHD as determined by history, physical examination, chest x-ray, or echo (other than hemodynamically insignificant lesions such as a patent foramen ovale (PFO), a tiny patent ductus arteriosus (PDA), mild peripheral pulmonic stenosis (PPS), or a tiny coronary artery fistula)
• Acquired or CHD based on the following ECG findings
– PR >220 ms
– 2nd or 3rd degree heart block
– QRS >120 ms
– Ventricular pre-excitation
– Non-sinus rhythm
– Confirmed diagnosis of long QT syndrome
• Corrected gestational age <37 weeks at time of echo
• BMI ≥95th percentile (≥2 years old) or weight-for-length Z-score ≥2 (<2 years old)
• Acute/systemic disorder with cardiovascular manifestations using criteria not based solely on ECG or echo findings
• Documented family history of non-ischemic cardiomyopathy
Demographic
• Age
• Gender
• Self-reported race
• Self-reported ethnicity
Echo images
ECG
Clinical Data
• Height
• Weight
PHN Bioinformatics Grid
Core lab measurements and calculations
Z-Scores based on BSA, age, gender, race

Based on guidelines from the National Institutes of Health and the US Office of Management and Budget, three racial categories will be used in the study: Whites (defined as having origins in any of the original peoples of Europe, the Middle East, or North Africa), African-Americans (defined as having origins in any of the black racial groups in Africa), and an Other classification encompassing Asians, Pacific Islanders, Native Americans, and those from multiracial origins. Ethnicity will be designated as Hispanic or non-Hispanic.

Because race information is not routinely obtained at some centers, an alternative method to collect race and ethnicity data is available. When an eligible study patient with acceptable echo images is identified from the local echo and/or hospital databases, race and ethnicity data can be collected prospectively by sending a letter to the family requesting the information and outlining the purpose for requesting this information. This process will require approval by the local Institutional Review Board. Once the data are obtained, the prospectively collected race and ethnicity data will be submitted along with the retrospectively collected demographic and clinical data, echo images, and ECGs to the PHN Bioinformatics Grid.

4.3.1 Measures of Primary Outcome

The Core Laboratory will perform measurements and calculations on the echo images based on recent guidelines by the American Society of Echocardiography Pediatric and CHD Council (1). The measurements and calculations will be adjusted for the effects of BSA, age, gender, and race. The following two-dimensional measurements will be performed for each submitted study:

- Mitral valve annular anteroposterior diameter
- Mitral valve annular lateral diameter
- Tricuspid valve annular anteroposterior diameter
- Tricuspid valve annular lateral diameter
- Aortic valve annular diameter
- Aortic root diameter
- Aortic sinotubular junction diameter
- Ascending aortic diameter
- Proximal transverse arch diameter
- Distal transverse arch diameter
- Aortic isthmus diameter
- Left main coronary arterial diameter
- Proximal left anterior descending coronary arterial diameter
- Proximal right coronary arterial diameter
- Pulmonary valve annular diameter
- Main pulmonary arterial diameter
- Right pulmonary arterial diameter
- Left pulmonary arterial diameter
- Left ventricular short-axis end-diastolic diameter (LVEDD)
- Left ventricular short-axis end-diastolic posterior wall thickness
- Left ventricular short-axis end-diastolic septal wall thickness
- Left ventricular short-axis end-systolic diameter (LVESD)
- Left ventricular long-axis end-diastolic length (LVEDL)
- Left ventricular long-axis end-diastolic epicardial length ($LVEDL_{epi}$)
- Left ventricular long-axis end-systolic length (LVESL)
- Left ventricular short-axis end-diastolic area (LVEDA)
- Left ventricular short-axis end-diastolic epicardial area ($LVEDA_{epi}$)
- Left ventricular short-axis end-systolic area (LVESA)

The following calculations will also be performed on the same set of measurements when available for each submitted study:

- Left ventricular shortening fraction = (LVEDD – LVESD)/LVEDD
- Left ventricular end-diastolic volume (LVEDV) = (5/6)(LVEDA)(LVEDL)
- Left ventricular end-diastolic epicardial volume ($LVEDV_{epi}$) = (5/6)($LVEDA_{epi}$) ($LVEDL_{epi}$)
- Left ventricular end-systolic volume (LVESV) = (5/6)(LVESA)(LVESL)
- Left ventricular ejection fraction = (LVEDV – LVESV)/LVEDV
- Left ventricular mass = (1.05)($LVEDV_{epi}$ – LVEDV)

4.3.2 Measures of Secondary Outcome

This is a unique and timely opportunity to collect a large number of ECGs from patients with documented normal echo studies. The data may be used to define normal reference values for certain ECG parameters, or they may be combined with other data (such as those collected from patients with hypertrophy) to define clinical thresholds for specific parameters (such as voltage in lead V6 to define left ventricular hypertrophy). Similar to echo reference values, current ECG reference values are outdated and derived from a relatively small and homogenous population. The specific scientific questions and methodologies will be determined by the investigators who propose studies using the ECGs. The intent is to ensure maximal flexibility for future studies.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Subject Inclusion Criteria

1. ≤18.0 years of age
2. Echo images in DICOM (Digital Imaging and Communications in Medicine) format performed after January 1, 2008
3. Documentation of height and weight
4. Documentation of gender and race

5.2 Subject Exclusion Criteria

1. Echo images inadequate for analysis
2. Acquired or CHD as determined by history, physical examination, chest x-ray, or echo (other than hemodynamically insignificant lesions such as a patent foramen ovale (PFO), a tiny patent ductus arteriosus (PDA), mild peripheral pulmonic stenosis (PPS), or a tiny coronary artery fistula)
3. Acquired or CHD based on the following ECG findings (if an ECG is performed): PR interval >220 ms, 2nd or 3rd degree heart block, QRS interval >120 ms, ventricular pre-excitation, non-sinus rhythm, and confirmed diagnosis of long QT syndrome
4. Corrected gestational age <37 weeks at the time of the echo
5. BMI ≥95th percentile for children ≥2 years old, or weight-for-length Z-score ≥2 based on the WHO Child Growth Standards (20, 21) for children <2 years old
6. Acute or systemic disorder with cardiovascular manifestations (including but not limited to Marfan syndrome, sickle cell disease, cancer, renal failure, human immunodeficiency virus infection, Kawasaki disease, rheumatic fever, autoimmune disorder, and systemic hypertension) using criteria which are not based on ECG or echo findings but rather on specific physical exam findings or abnormal blood tests as documented in the medical record
7. Documented history of a 1st degree relative with non-ischemic cardiomyopathy

8. Documented history of a 1st degree relative with the following congenital left-sided heart lesions: mitral stenosis, left ventricular outflow tract obstruction, bicuspid aortic valve, aortic coarctation, and/or hypoplastic left heart syndrome.

5.2.1 Rationale for Exclusion Criteria

Cardiovascular pathology has been reported in association with obesity in children (22, 23). Therefore children ≥2 years old with a BMI ≥95th percentile for age and gender (24) will be excluded from the study. Because there are no BMI standards to diagnose obesity in children under 2 years old, those in this age range with a weight-for-length Z-score ≥2 based on the WHO Child Growth Standards (20, 21) will also be excluded. Similarly, premature babies with a corrected gestational age of <37 weeks at the time of the echo will be excluded because of the high prevalence of hemodynamically significant cardiovascular and respiratory pathology in this patient population. Many centers have used most or all of the following exclusion criteria in their collection of normal data (9): acquired or CHD, acute or systemic disorder, and family history of cardiomyopathy. More recently, studies have shown that subjects who carry genes associated with hypertrophic cardiomyopathy manifest abnormal parameters of diastolic function prior to measurable hypertrophy (25). In addition, investigators have presented evidence that a family history of CHD may also be important, since healthy individuals who are first-degree relatives of patients with left-sided obstructive heart lesions tend to have smaller left-sided cardiovascular structures (26). No published studies to date have accounted for family history of CHD when establishing a Z-score database. It is important to note that none of the exclusion criteria listed above is based purely on individual echo measurements unless the measurement specifically defines the disease. *Outliers who are suspected to have "disease" based on echo measurements will be evaluated, and a panel of experts will decide whether to include or exclude their data.*

5.3 Data Exclusion Criteria

Echo images deemed of poor quality by the Core Laboratory Investigator based on criteria established by the Study Submission Sub-Committee (Appendix A) will be excluded. In addition, any submitted echo image for a particular measurement deemed of poor quality and precluding accurate measurement by the Core Laboratory Investigator will be excluded from the analysis of that measurement.

5.4 Subject Availability

Several centers were polled to determine the number of eligible patients that would have been available from the 2011 hospital and/or echo database:

Site	**Eligible Patients in 2011**
Wisconsin	650
Montefiore	550
Boston	>300
CHOP	300
Columbia	300
Utah	250
Total	**>2350**

Therefore, the total sample size (as discussed in Section 6.2) can be fulfilled by having a minimum of 15 participating centers review their database since the availability of the DICOM format and submit data and echo images on 250 eligible patients. In order to optimize

geographic, racial, and ethnic diversity, it may be desirable to increase the number of sites beyond this minimum number to as many as 25.

The US Census Bureau published the following statistics regarding the racial distribution in the United States for the year 2010: 72% Whites, 13% African-Americans, and 15% in the Other category. Because of the potential problem of recruiting non-White patients for the project, several centers were polled to assess the racial distribution in their eligible patients from 2011, and the data are listed in the table below:

Site	Whites	African-Americans	Other
US Census	72%	13%	15%
Montefiore	24%	38%	39%
CHOP	50%	50%	

Based on these numbers, the target sample size and appropriate racial diversity needed for the study are both feasible as long as the racial distributions at the participating centers are determined at the outset of the project.

5.5 Recruitment / Enrollment Procedures

Eligible study patients will be identified at the participating centers by one of two ways. In some institutions, the echo database will be mined for all the normal echo studies that have been performed during the study period. The study coordinator will then review the charts of the patients to identify those who meet all inclusion criteria and do not have any of the exclusion criteria. At other centers, the hospital database will be mined for all healthy or normal patients meeting study criteria. The study coordinator will verify eligibility by reviewing the medical record and identifying the patients who have had an echo. The study coordinator will then review the echo reports to identify all those with a normal study. Other centers may use a combination of these two approaches. As discussed in Section 4.3, eligible study patients with acceptable echo images identified at centers that do not routinely collect race data will be contacted to obtain race and ethnicity data prospectively.

6. STATISTICS

6.1 Statistical Analysis Plan

BSA for each subject will be calculated using the Haycock formula (27). The distribution of BSA and each echo measurement will be assessed and tested for normality. Any variable found to be skewed or otherwise non-normal will be transformed appropriately in order to obtain a normal (or close to normal) distribution. The analysis will require classification of each subject within one of 36 study groups based on:

- 6 age categories
  - <1 month old
  - 1 month old – <3 years old
  - 3 – <6 years old
  - 6 – <12 years old
  - 12 – <16 years old
  - 16 – 18 years old

- 2 gender categories
  - Male
  - Female
- 3 race categories
  - White
  - African-American
  - Other

6.1.1 Derivation of Mean and Standard Deviation

The mean and SD of a given measurement will be derived for each of the 36 study groups described above. For a given group, the mean (*E*) and SD of an echo measurement (*Y*), given the BSA, are as follows:

$$E(Y|BSA) = \mu_y + \rho \frac{\sigma_y}{\sigma_{BSA}} (BSA - \mu_{BSA})$$

$$SD(Y|BSA) = \sigma_y \sqrt{1 - \rho^2}$$

where ρ is the correlation between the echo measurement and BSA in each group, σ_y and σ_{BSA} are the group SDs of the echo measures and BSA respectively, and μ_{BSA} is the group mean BSA. These quantities can be estimated using each group's observed data.

The equations above are equivalent to fitting a linear regression model of the form

$$Y = \alpha + \beta(BSA)^n$$

The power *n* of BSA will be determined so that the transformed BSA and echo measurement will have approximately a linear relationship. For example, the diameters of the great vessels and the semilunar valve orifices have been found to relate most closely in a linear fashion to $BSA^{0.5}$ (9).

6.1.2 Analysis of Secondary Outcomes

A secondary analysis will determine whether another method of calculating BSA is superior to the Haycock formula for describing the relationship between BSA and the cardiovascular parameters. The published formulas in most common use are:

The formula of Du Bois and Du Bois (28):

$$BSA = 0.00718 \times height^{0.725} \times weight^{0.425}$$

The method of Dreyer and Ray (29):

$$BSA = 0.1 \times weight^{0.6666}$$

The two methods published by Boyd (30):

$$BSA = 0.0004688 \times (1000 \times weight)^{0.8168} - 0.0154 \log(1000 \times weight)$$

$$BSA = 0.0003207 \times (1000 \times weight)^{0.7285} - 0.0188 \log(1000 \times weight) \times height^{0.3}$$

The formula of Haycock and colleagues (27):

$$BSA = 0.024265 \times height^{0.3964} \times weight^{0.5378}$$

The formula of Gehan and George (31) based on Boyd's data:

$$BSA = 0.02350 \times height^{0.42246} \times weight^{0.51456}$$

The formula of Mosteller (32):

$$BSA = ((height \times weight)/3600)^{0.5}$$

The data will be divided into two groups, 30% for training and 70% for testing. The training dataset will be used to determine the best method for calculating BSA, and the remaining data will be used to validate the results. For each regression using the training dataset, the coefficient of determination will be derived for each method of calculating BSA, and the method demonstrating the highest mean coefficient of determination for all variables will be selected as the optimal BSA calculation method. Alternate approaches to identify the optimal BSA calculation method will be considered and will include likelihood-based comparisons of model fit (such as the Aikake or the Bayesian information criteria). In addition, other cross-validation techniques using different sizes for the training and testing datasets or the jackknife (or "leave one out") procedure will also be utilized. If these analyses reveal a better method for calculating BSA than the Haycock formula, then the primary analysis to determine the relationship between the individual echo measurements and BSA will be performed again, this time using the BSA derived from the best method.

Once the best available method for obtaining BSA has been determined and the simplest, best-fit model has been identified for each of the variables, the next step will involve examination for non-constant variance (heteroscedasticity) for each regression. In general, all of these relationships are anticipated to manifest heteroscedasticity characterized by a progressively but not necessarily linearly increasing variance with increasing BSA. Under these circumstances, the method described by Altman (33) provides a simple and adequate approach to generate a constant variance relationship. Although more complex methods (log or other transforms of one or both variables) have been described, they lack a physiologic basis and do not generally outperform the Altman technique. If there are individual variables for which the Altman methodology fails to adequately eliminate heteroscedasticity, the Lambda-Mu-Sigma method described by Cole and colleagues (34) will be adopted for those specific variables.

The Z-scores for each of the 36 study groups (based on age, gender, and race) will be compared using a t-test and adjusted for multiple comparisons. Groups which are not statistically different may be combined. For example, if the Z-scores for a particular measurement are not statistically different between males and females, the two groups may be combined and a new estimate of the mean and SD for the combined group will be determined.

The potential relationship of each set of Z-scores to height, weight, and BMI will also be examined using linear regression, and the potential for differences related to ethnicity will be examined using analysis of variance.

6.2 Number of Subjects to be Enrolled

Sample size calculations were performed to determine the number of patients needed for each of the 36 study groups that will provide reasonable estimates of the population mean and SD for each measurement (35). There are several assumptions associated with these calculations:

- All variables should be normally distributed. Any variable that does not meet the normality assumption will need to undergo a mathematical transformation.
- At least 80% of the submitted echo studies will contain all the necessary images to perform each of the study measurements. In other words, for every group of 100 study patients, one would expect at least 80 studies in which each particular measurement can be made.

Assuming a normal distribution, a confidence interval for the SD is given by the following equation:

$$\sqrt{\frac{(n-1)s^2}{\chi^{2\left(1-\frac{\alpha}{2}\right)}_{n-1}}} \leq \sigma \leq \sqrt{\frac{(n-1)s^2}{\chi^{2\left(\frac{\alpha}{2}\right)}_{n-1}}}$$

We can minimize the width and solve for n such that

$$\sqrt{n-1}\left(\frac{1}{\sqrt{\chi^{2\left(\frac{\alpha}{2}\right)}_{n-1}}} - \frac{1}{\sqrt{\boldsymbol{\chi}^{\mathbf{2\left(1-\frac{\alpha}{2}\right)}}_{\mathbf{n-1}}}}\right) < r$$

where *r* is some proportion of the observed point estimate of the SD. For n = 80 and α = 0.05, the width of a 95% confidence interval would be 32% (r = 0.32) of the observed SD. In addition, the margin of error is calculated as

$$z_{\frac{\alpha}{2}}\frac{\sigma}{\sqrt{n}}$$

For n = 80, the margin of error for the mean would be 22% of the observed SD. Given the 80% echo quality assumption discussed above, each of the 36 study groups should have 100 patients in order to satisfy the requirements for the 95% confidence interval and margin of error for the mean. Thus the total sample size for the study is 3600 patients.

6.3 Level of Significance

The primary aim of this study will be the estimation of the mean and SD of the normal population for the measurements listed in Section 4.3.1. When hypothesis testing is conducted for some secondary aims, the type I error probability will be 0.05. No adjustment will be made to account for comparing the groups with respect to more than one outcome variable. However, multiple comparison adjustments will be made to control the type I error when comparing study groups for the purpose of collapsing group categories.

6.4 Spurious Data Procedures

Consistency checks and range checks will be built into the data management system (DMS). This will allow many errors to be identified and corrected at the time of data entry. Queries regarding any problems with data will be sent to site coordinators regularly throughout the course of the study. Sites will also be monitored during the study. Therefore, spurious data are expected to be rare. Any data which are judged by the medical monitors to be definitely incorrect, and which cannot be resolved, will be set to missing.

The study report will indicate the number of subjects who have missing data on each study endpoint. For covariate-adjusted analyses, the number of subjects who have missing data on the covariates will be reported.

Throughout the study, the rate and reasons for data exclusion will be monitored by site and by measurement, and the Core Laboratory will provide monthly quality assessment data to each site. Any site with a high frequency of unacceptable data quality will be queried. If necessary, retraining will take place or the site may be barred from further participation in the study. If any measurement is absent in >20% of studies, the Study Submission Sub-Committee will address measures to improve acquisition of these data.

6.5 Deviation Reporting Procedures
Any modifications or deviations from the statistical plan described in this protocol will be documented in a "Revised Statistical Plan" document.

6.6 Subjects to be Included in Analyses
Echo measurements from all eligible subjects will be included in the analysis.

6.7 Data and Safety Monitoring Plan
The Data and Safety Monitoring Plan for this study will follow standard PHN monitoring principles. Oversight of data and safety is provided by the PHN DSMB, appointed by NHLBI. The DSMB, which meets at least biannually, is composed of experts in pediatric cardiology, congenital heart surgery, biostatistics and study design, and ethics, as well as a lay member. For this study, the DSMB will review study accrual, data quality, and protocol violations on a regular basis and make recommendations about study conduct to the Director, NHLBI.

7. DATA MANAGEMENT
An Electronic Data Capture (EDC) system will be used for the study that is designed to support reliable and secure data entry for clinical research purposes. The system also provides seamless integration of electronic Case Report Forms (eCRF) and paper-based Case Report Forms (CRF) within a single protocol if desired; implementation of protocol amendments; and SAS and XML study data exports.

7.1 Data Entry
Data can be entered directly from multiple study sites via a fully validated and 21 CFR Part 11 compliant, secure Web application and stored centrally. A configurable sample-based double data entry system is available. Data are entered by subject study identification number; names will not be linked with subject data in the database. Study sites will maintain records in secure areas linking the subject name with the identification number assigned for the study. Study sites will have full access to their own data and be able to view these data remotely. Study staff will not be able to view subject data associated with other sites.

The PHN Bioinformatics Grid provides the software tools and secure infrastructure for clinical sites to submit image studies and other PHN study data to a central study archive (the PHN Data Hub). Study images transmitted from the clinical sites are completely de-identified and anonymized through PHN software tools to remove any patient-specific information, and these are assigned PHN study blind identifiers (numbered identifiers provided by the DCC). Studies are de-identified with removal of all personal health information (PHI) or personally identifying information (PII) by PHN software at the clinical site and are electronically transferred to the PHN Data Hub hosted at Johns Hopkins University (JHU). The PHN Grid Portal provides role-based access control to review image studies and related study data from a web browser, to ensure that they are de-identified, and to route them to the PHN DCC and/or Core Laboratory for processing and analysis. All data transfers are completed through secure protocols using Hypertext Transfer Protocol Secure (HTTPS).

At JHU, de-identified image studies are stored in a dedicated PHN Data Hub and accessed by the PHN Portal database and web services that are password protected and encrypted. The system server holding the PHN data is separated from other JHU systems and can only be accessed by Information Technology staff specifically assigned to support the PHN (members of JHU and Booz Allen Hamilton contractor team supporting this work). No PHI is kept on the PHN Grid or Image/ECG Archive servers, nor can identity be derived by any combination of data contained within the PHN Grid platform.

Later in 2012, ECG data files will also be stored at the PHN Data Hub, leveraging open-source ECG management and analysis tools (ECG Grid) developed by JHU under the Cardiovascular Research Grid (CVRG) program. The ECG data will also undergo similar de-identification as the images before the data leave the clinical site and will be stored with the corresponding image and study data. Management and storage of the Subject relationship is performed externally and is not integrated by any mechanism that can associate the identifiers (typically 10 digit numbers) to a real patient. Only the clinical sites, as custodians of the patient data and physician-patient relationship, hold the information necessary to match patient-to-PHN identifier information.

7.2 Data Validation and Monitoring

Integrated into the data entry system are real time validations, including both inter- and intra-instrument data checks. Inconsistent or questionable values are flagged during entry, and an edit report is automatically generated to the data entry client. These edit reports provide the information necessary to investigate any data entry errors or resolved questions regarding out-of-range or questionable values. Second level query tracking allows monitors and data managers real time access to unresolved queries as well as the date and time of query generation and resolution.

7.3 Data Security and Integrity

All data changes are written to an audit trail. The audit trail identifies the data item by table, column and key field. The entry includes the user, date and time, as well as the old value and new value. Both patient related data as well as trial configuration data are written to the audit trail. Data are saved at regular intervals during data entry to prevent loss of information in the event of a disruption of the Internet connection. In the unlikely event of a major disruption, a backup connection allows full access to the DMS.

Several levels of security are employed to ensure privacy and integrity of the study data, including the following: Study access requires use of assigned user names and passwords. Individual roles and access levels are assigned by the study data manager. Passwords are changed regularly. Web-based entry uses secure socket layer (SSL) data encryption. Data will not be stored on laptop computers.

8. QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA)

The DCC has primary responsibility for QC/QA activities of the phenotypic data. The DCC also requires that the sites complete certain QC activities, most of which are monitored by the DCC. The key QC/QA activities are:

- Development of a Study Manual;
- Clearly formatted and carefully constructed Data Forms with clear, up-to-date manuals of instruction;
- Sign-Off Procedures for all CRFs;
- Central protocol training and certification of all site data collection staff with the use of standardized checklists;
- Data management training and certification of site personnel completing data entry and/or data management;
- Verification of patient eligibility;
- On-going monitoring of all protocols/data collection activities;
- Completion of reliability and/or pilot studies for key measurements as appropriate;

- Inclusion of repeat measurements, as feasible, in the course of the study; and
- Monitoring visits to sites as required with pre-specified goals and/or remote monitoring activities.

The DCC may conduct site visits to the Core Laboratories to review QA and QC procedures and data transfer to the DCC. Review of central laboratory-related reports will be conducted at least monthly to identify overall or site-specific problems in data or specimen acquisition and reporting of results.

9. ETHICS AND HUMAN SUBJECTS CONSIDERATIONS

9.1 Potential Risks

Because this is a retrospective collection of de-identified demographic and clinical data, echo images, and ECGs from patients who have already undergone evaluation and testing, there are no significant procedural risks associated with the data collection.

9.2 Confidentiality, Protection against Risks

Investigators will take all reasonable measures to protect the confidentiality of subjects and their families, including the following:

Use of Subject ID numbers

Each subject is assigned a subject identification number (SID). All clinical research data are stripped of identifiers and labeled with the study number. The enrollment log with participant identifiers will be maintained at each site in a secured, locked location available only to the study staff. The subject's name and any other identifying information will not appear in any presentation or publication resulting from this study.

9.3 Potential Benefits

Because this is a retrospective collection of demographic and clinical data, echo images, and ECGs from patients who have already undergone evaluation and testing, and because prospective measurements will be performed on de-identified echo images, the study will not provide direct benefit to individual participants or families. The benefits are those to society as a whole in the improvement of knowledge of the causes or treatment of CHD, in the development of new diagnostic tests, and ultimately in the improvement of prognosis.

9.4 Risk/Benefit Ratio and Importance of Information to Be Obtained

The risk/benefit ratio is favorable for this study, and adverse events are not anticipated. The baseline risk is minimal because there are no therapeutic interventions. In addition, although an individual subject may not benefit from participation, the results of this study will make important contributions to the improvement of knowledge of the causes of CHD, in the development of new diagnostic tests, and ultimately in the improvement of treatment and prognosis.

10. STUDY LIMITATIONS

- There are inherent limitations associated with self-reported race and ethnicity within a retrospective study. Most centers collect these data when patients are evaluated and undergo testing, and the accuracy of the data is determined purely by patient response on standard hospital intake forms.
- The Other race category encompasses Asians, Pacific Islanders, Native Americans, and those from multiracial origins. The study will have enough power to determine

differences in normal echo reference values between Whites and African-Americans. There are limitations associated with combining all the other races in the third category, but creating study groups with each of the other racial categories is not feasible within the context of cost, time, and racial distribution at the participating centers.

- Although there are no reports of differences in normal echo reference values between Hispanics and non-Hispanics, a secondary analysis to see if there are differences between the two populations in the study will be performed. Although it is not likely that the study will have enough power to fully determine these differences, the project will establish the framework for future studies to evaluate this and other similar questions.
- Among the exclusion criteria are documented family history of cardiomyopathy and documented first degree relative with congenital left-sided heart disease. There will likely be patients for whom this documentation is not available, and some patients might be erroneously included in the study population. Given that most of the echocardiograms for the study will be normal, the effect of including some of these patients into the analysis will hopefully be negligible.

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