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Cardiac biomarkers in pediatric cardiomyopathy: Study design and recruitment results from the Pediatric Cardiomyopathy Registry☆

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ABSTRACT

Background: Cardiomyopathies are a rare cause of pediatric heart disease, but they are one of the leading causes of heart failure admissions, sudden death, and need for heart transplant in childhood. Reports from the Pediatric Cardiomyopathy Registry (PCMR) have shown that almost 40% of children presenting with symptomatic cardiomyopathy either die or undergo heart transplant within 2 years of presentation. Little is known regarding circulating biomarkers as predictors of outcome in pediatric cardiomyopathy.

Study design: The Cardiac Biomarkers in Pediatric Cardiomyopathy (PCM Biomarkers) study is a multi-center prospective study conducted by the PCMR investigators to identify serum biomarkers for predicting outcome in children with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). Patients < 21 years of age with either DCM or HCM were eligible. Those with DCM were enrolled into cohorts based on time from cardiomyopathy diagnosis: categorized as new onset or chronic. Clinical endpoints included sudden death and progressive heart failure.

Results: There were 288 children diagnosed at a mean age of 7.2 ± 6.3 years who enrolled in the PCM Biomarkers Study at a median time from diagnosis to enrollment of 1.9 years. There were 80 children enrolled in the new onset DCM cohort, defined as diagnosis at or 12 months prior to enrollment. The median age at diagnosis for the new onset DCM was 1.7 years and median time from diagnosis to enrollment was 0.1 years. There were 141 children enrolled with either chronic DCM or chronic HCM, defined as children ≥2 years from

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diagnosis to enrollment. Among children with chronic cardiomyopathy, median age at diagnosis was 3.4 years and median time from diagnosis to enrollment was 4.8 years.
Conclusion: The PCM Biomarkers study is evaluating the predictive value of serum biomarkers to aid in the prognosis and management of children with DCM and HCM. The results will provide valuable information where data are lacking in children.

1. Introduction

Cardiomyopathies are a rare cause of heart disease worldwide and across all age groups when compared to ischemic heart disease, but they are one of the leading causes of heart failure (HF) admissions, sudden death, and need for heart transplant in childhood [1–3]. Reports from the Pediatric Cardiomyopathy Registry (PCMR) have shown that almost 40% of children presenting with symptomatic cardiomyopathy either die or undergo heart transplant within 2 years of presentation [2,4,5]. However, symptoms and even echocardiographic abnormalities do resolve in many children with cardiomyopathy over months to years after initial presentation [6,7].

Large registry and cohort studies in children with cardiomyopathy have focused primarily on echocardiographic characteristics of cardiac function at the time of diagnosis as predictors of outcome [4,7–13]. The North American PCMR and Cohort study collected demographic, clinical, electrocardiographic and Holter monitor data, and echocardiographic measures on > 3500 children across the United States and Canada [14]. The National Australian Childhood Cardiomyopathy Study, another large cohort of patients with pediatric cardiomyopathy, also collected clinical information and echocardiographic characteristics related to the causes and outcomes of cardiomyopathy in children [15]. The data collected from these studies provide valuable information pertaining to the presentation, clinical course, and predictors of outcomes of childhood cardiomyopathy. To date, the predictors of outcomes including clinical phenotype at presentation as well as predictive surrogate endpoints such as biomarkers, have not been prospectively validated in childhood cardiomyopathy.

1.1. Study rationale

Interest and knowledge continue to increase in the study of serum biomarkers of early heart disease in adults with cardiomyopathy and HF; in predicting hospital readmission for HF, mortality, and need for heart transplant; and as surrogate endpoints for clinical trials in adults [16–18]. However, similar large-scale studies have not been performed in children with cardiomyopathy. Given this lack of data, we designed a multi-center prospective study to identify which serum biomarkers or biomarker panels might be sensitive and specific enough to identify disease severity status in pediatric cardiomyopathy, including asymptomatic disease, acute HF, chronic and stable HF, and progressive HF resulting in mechanical circulatory support, heart transplant, or sudden cardiac death.

Here, we describe the design, implementation, and preliminary results of the National Heart, Lung, and Blood Institute (NHLBI)-funded Cardiac Biomarkers in Pediatric Cardiomyopathy (PCM Biomarkers) study. The authors are solely responsible for the design and implementation of this study, all analyses, and the preparation and approval of the manuscript.

2. Materials and methods

2.1. Study organization

The PCM Biomarkers study was funded by the NHLBI from August 6, 2012, through June 30, 2017. The study used the network of clinical investigators and infrastructure established by the NHLBI-funded PCMR

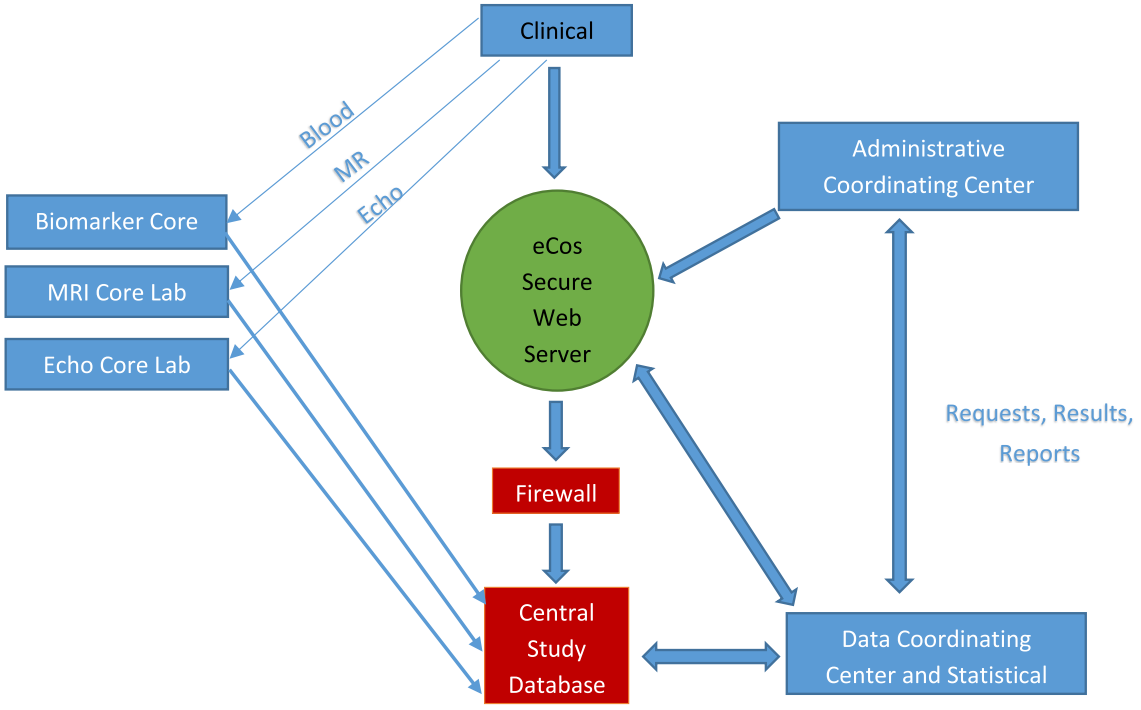

Fig. 1. The biomarker study data flow.
The diagram shows the relationship between clinical sites and data, imaging, and biospecimen transfer among core labs, the DCC, and the ACC.

Table 1
Cardiac biomarkers to be analyzed in the Cardiac Biomarkers in Pediatric Cardiomyopathy Study, by study aim and diagnosis.

Biomarker class	Biomarker	Aim 1	Aim 2	Aim 3
Neuroendocrine	NT-proBNP[a]	DCM[b]	HCM[c]	DCM/HCM
Proinflammatory	IL-6[d]	DCM		DCM
	TNF-alpha[e]	DCM		DCM
	hsCRP[f]	DCM		DCM
Cardiomyocyte protector	Protein ST2	DCM		DCM
	Growth differentiation factor 15	DCM		DCM
Beta galactoside	Galectin 3	DCM		DCM
Cardiomyocyte injury	Troponin T	DCM	HCM	DCM/HCM
Collagen metabolism	PICP[g]		HCM	HCM
	MMP-1[h]		HCM	HCM
	TIMP-1[i]		HCM	HCM
	CITP[j]		HCM	HCM

[a] NT-ProBNP, N-terminal pro brain natriuretic peptide.
[b] DCM, dilated cardiomyopathy.
[c] HCM, hypertrophic cardiomyopathy.
[d] IL-6, interleukin-6.
[e] TNF-alpha, tumor necrosis factor-alpha.
[f] hsCRP, high-sensitivity C-reactive protein.
[g] PICP, carboxyterminal propeptide type I procollagen.
[h] MMP-1, matrix metalloproteinase-1.
[i] TIMP-1, tissue inhibitor of metalloproteinase-1.
[j] CITP, carboxyl-terminal telopeptide of collagen type 1.

(funded from 1995 through 2012) [14,19]. The primary award sites for this study were the University of Miami (2012 to 2014) and Wayne State University (2014 to 2017), which served as the Administrative Coordinating Center (ACC) (Fig. 1). The ACC was responsible for implementing the study, overall regulatory and clinical compliance, protocol implementation and support, management and oversight of the core facilities that store the study biospecimens, echocardiograms and cardiac magnetic resonance imaging studies (cMRI).

The ACC served as the central storage facility for the biospecimens. The University of Miami served as the central laboratory responsible for the biomarker assays. Boston Children's Hospital served as the core laboratory for centralized review and measurement of all echocardiograms. Washington University in St. Louis, MO served as the core laboratory for centralized review and measurement of all cMRI.

In addition, the ACC managed and directed the manuscript-writing groups, coordinating activities among centers, the data collection at the participating clinical centers (Appendix A), and the study analysis planning through continued conferencing with the Steering Committee Investigators. The ACC also coordinated with The New England Research Institutes (NERI), which served as the Data and Statistical Coordinating Center (DCC) for managing data, conduct of statistical analyses, developing data collection forms, and preparing the study's operations manual and reports for the Observational Study Monitoring Board (OSMB), Fig. 1.

2.2. Study design

The PCM Biomarkers study was designed to be a 4-year prospective study of up to 480 children with either primary dilated cardiomyopathy (DCM) or hypertrophic cardiomyopathy (HCM). The study had three components: 1) clinical data collection by chart review; 2) biospecimen collection and testing; and 3) centralized review and measurement of echocardiograms and cMRI. The protocols used to acquire echocardiographic studies and cMRI studies are detailed in Appendices B and C, respectively.

The study consisted of two specific aims for DCM, two specific aims for HCM phenotypes, along with a third specific aim for both phenotypes. There were two DCM cohorts based on the acuteness of illness. The first was comprised of children who were recently diagnosed (diagnosed at enrollment or up to 12 months prior to enrollment). The purpose in studying children with DCM diagnosed between 0 and 12 months prior to enrollment was to determine the ability of serum cardiac biomarkers to predict the short-term (< 90 days) and intermediate-term (≥90 days) clinical outcomes children soon after a diagnosis of DCM. The study was designed to analyze the ability of either the concentrations of biomarkers at acute presentation or the change in concentrations over time to correlate with outcome. Serial biospecimens, echocardiograms, and clinical data were obtained at as many of the following time points as possible based on clinically indicated testing: at diagnosis and months 1, 3, 6, 12, and 24. Additionally, the study was designed to determine whether a single biomarker or a biomarker panel predicted poor outcomes (death, heart transplant, or worsening HF) in the first 2 years after diagnosis of DCM.

The second DCM cohort was comprised of children with clinically stable DCM, defined as children surviving at least 1 year after the initial diagnosis of cardiomyopathy without heart transplant and without an anticipated need for heart transplant within 12 months of enrollment. In this group, biospecimens were collected at time of enrollment and at 12 and 24 months after enrollment. The objective was to determine whether there was any association between the circulating biomarkers and symptoms, echocardiographic findings, or disease progression in children with clinically stable DCM.

With respect to HCM, we assessed the clinical utility of biomarkers of collagen metabolism in children with HCM (at any time point in their disease course) and investigated serial biomarkers in a cohort of clinically stable HCM patients (at least 1 year from initial presentation of HCM). We collected blood samples in proximity to cMRI and echocardiographic assessments. The study was designed to evaluate the relationship between biomarker concentrations and non-invasive imaging evidence of fibrosis as detected by delayed enhancement on cMRI or left ventricular (LV) diastolic dysfunction by echocardiography. The study was also planned to determine the agreement between echocardiographic and cMRI measurements of septal hypertrophy and LV mass by analyzing imaging studies acquired within 6 months of each other. In the clinically stable HCM cohort, biospecimens were obtained at the same time points as the clinically stable DCM cohort to identify an association between biomarkers and symptoms, echocardiographic findings, and disease progression in children with chronic HCM.

There were several biomarkers hypothesized to relate to cause and disease state (Table 1). These included biomarkers thought to relate to the possible cause of DCM and to acute changes in clinical condition soon after diagnosis. In addition, biomarkers of myocardial fibrosis were obtained in children with HCM to assess for a correlation between fibrosis apparent by cMRI. Biomarkers were also obtained in children with HCM to determine a biomarker profile in children with echocardiographic evidence of marked hypertrophy or diastolic dysfunction. Biomarkers thought to be associated with a clinical worsening of HF in either DCM or HCM were analyzed in the clinically stable patients to determine the profile of these biomarkers in children felt to be at low risk for clinical event.

2.3. Patient selection

Patient eligibility was based on a cardiomyopathy diagnosis that met strict echocardiographic criteria. These criteria have been consistent with prior PCMR studies with the exception of one of the HCM criteria [19]. For DCM, children eligible must have both decreased LV function and dilation as a means of excluding those with acute fulminant myocarditis and excluding those who have normal function but LV dilation for other reasons such as bradycardia or anemia. In children, an absolute septal thickness has not been used for the diagnosis of HCM, particularly because the measurements need to be interpreted in the context of the size of the child. An LV posterior wall or septal thickness

at end-diastole Z-score of 4–6 standard deviations above the normal mean for body-surface area has been suggested as possible HCM and a Z-score of 7 or more as definite HCM in children [20]. In the prior PCMR studies, a posterior wall thickness of > 2 standard deviations above the normal mean for body-surface area was used as an inclusion criteria [19]. For the current study, the investigators were more selective requiring an LV posterior wall or septal thickness Z-score > 3 to meet HCM inclusion criteria (certainly above the normal range for most children) (Table 2). Patients < 21 years old at enrollment and alive without heart transplant at the time of enrollment were eligible. The date of cardiomyopathy diagnosis was the date of the earliest available echocardiogram, cMRI, or imaging report that confirmed the diagnosis of cardiomyopathy based on the criteria in Table 2. This date of diagnosis was then used to determine eligibility for enrollment into the appropriate study cohorts. Children with secondary causes of cardiomyopathy and cardiomyopathy associated with syndromic, metabolic, or storage disease were excluded (Table 3).

2.4. Data collection

The study used the network of clinical centers and infrastructure established by the NHLBI-funded PCMR to estimate site-specific enrollment based on historic incidence and prevalence of childhood cardiomyopathy, to orient the investigators who were familiar with PCMR enrollment criteria and data collection, and to efficiently identify eligible patients.

The local coordinator at participating sites completed the electronic eligibility and enrollment forms. Data were entered into the eCOS web-based system, which was developed and maintained by the DCC. The web-based electronic data management system provided reliable and secure data entry by allowing study staff and investigators to enter and access data through the Internet using a standard browser. This system provided real-time, field-level validation, paperless data collection, automatic query management, and tracking of laboratory specimens and test results. Standardized reports were sent monthly to the ACC regarding enrollment, specimen status, and reimbursement for sites and core laboratories. Several levels of security ensured the privacy and integrity of study data. The database server allowed for archiving and backup, as well as an audit trail to identify user, date and time, and old and new values for data that were entered or changed.

All patient identifiers were removed from the data sent to the DCC. As per current NIH requirements, specific data were collected such that enrolled subjects were assigned a globally unique identifier to be used for this and future studies in which the subject might be enrolled [21]. To facilitate timely, complete, and standardized data extraction, an outreach team of full-time data collectors who traveled to participating sites to collect relevant clinical data at regular intervals was established and based at the ACC. There was an Observational Study Monitoring Board that reviewed the operations and data collection semiannually with the ACC and DCC.

Table 3
Exclusion criteria in the Cardiac Biomarkers in Pediatric Cardiomyopathy Study.

Age 21 years or older at time of enrollment
Heart transplant recipient
Endocrine disease known to cause heart muscle disease (including infants of mothers with diabetes)
History of rheumatic fever
Toxic exposures known to cause heart muscle disease (anthracyclines, mediastinal radiation, iron overload, or heavy metal exposure)
HIV-positive or born to an HIV-positive mother
Kawasaki disease
Congenital heart defects unassociated with malformation syndromes (e.g., valvular heart disease or congenital coronary artery malformations)
Immunologic disease
Invasive cardiothoracic procedures or major surgery during the preceding month, except those specifically related to cardiomyopathy, including left-ventricular assist devices, extracorporeal membrane oxygenation, and automatic internal cardiac defibrillator placement.
Active or chronic uremia
Abnormal ventricular size or function that could be attributed to intense physical training or chronic anemia
Chronic arrhythmia, unless inclusion criteria were documented before the onset of arrhythmia (not excluded were patients with chronic arrhythmia, subsequently successfully ablated, whose cardiomyopathy persists > 2 months post-ablation).
Malignancy
Systemic hypertension
Pulmonary parenchymal or vascular disease (e.g., cystic fibrosis, cor pulmonale, or pulmonary hypertension)
Ischemic coronary vascular disease
Association with drugs (e.g., growth hormone, corticosteroids, cocaine) or other diseases known to cause hypertrophy
Cardiomyopathy associated with syndromic, metabolic, or storage diseases:
▪ Alstrom syndrome
▪ Amyloidosis
▪ Beckwith–Wiedemann syndrome
▪ Carnitine deficiency
▪ Disorders of fatty acid metabolism
▪ Friedreich ataxia
▪ Glycogen storage disease (e.g. Danon, Forbes', Pompe; PRKAG2, Forbes', Danon)
▪ Lysosomal storage diseases (e.g. Anderson–Fabry, Hurler)
▪ Mitochondrial disorders
▪ Noonan syndrome and related RASopathies (Noonan syndrome with multiple lentines, cardiofaciocutaneous syndrome, Costello syndrome)
▪ Phosphorylase B kinase deficiency
▪ Swyer syndrome
▪ Syndromic hypertrophic cardiomyopathy

2.5. Biospecimen and image collection and processing

Blood was drawn either at the time of clinically indicated blood draws or at the discretion of the site study coordinator. No > 5 ml/5 lb of body weight, up to a maximum of 21.5 ml, were obtained from each patient at each sample collection. Samples were sent by express mail to the Study Biological Specimen Repository and logged in the DCC database. Blood samples were processed centrally and in batches at the Clinical Laboratory Improvement Amendments-certified Immunoassay

Table 2
Inclusion criteria for echocardiographic and cardiac magnetic resonance imaging (MRI) testing in the Cardiac Biomarkers in Pediatric Cardiomyopathy Study.

Criteria	Echocardiographic and/or cMRI diagnostic criteria
At least 2 qualifying measurements at diagnosis or enrollment	Dilated cardiomyopathy 1. LV[a] FS or EF > 2 SD *below* the normal mean for age 2. LV end-diastolic thickness-to-dimension ratio < 0.12 3. LV end-diastolic dimension or volume > 2 SD *above* the normal mean for body-surface area
Any one of 3 criteria at diagnosis or enrollment	Hypertrophic cardiomyopathy 1. If age < 18.0 years at diagnosis: LV posterior wall or septal thickness at end-diastole > 3 SD *above* the normal mean for body-surface area 2. If age ≥18.0 years at diagnosis: LV posterior wall or septal thickness at end-diastole > 1.2 cm 3. Localized LV hypertrophy, such as septal thickness > 1.5 × LV posterior wall thickness with at least normal LV posterior wall thickness

[a] LV = left ventricular; FS = fractional shortening; EF = ejection fraction.

Table 4
Baseline characteristics of eligible patients consented versus refused.

	Consented	Refused	P value
Total	288	68	
Age at diagnosis, years	6.4 (IQR = 0.8, 12.9)	4.0 (IQR = 1.0, 12.0)	0.47
Race			0.26
White	206 (71.5%)	55 (82.1%)	
Black	35 (12.2%)	6 (9.0%)	
Asian	5 (1.7%)	1 (1.5%)	
Other	42 (14.6%)	1 (1.5%)	
Ethnicity			
Hispanic ethnicity	28 (9.7%)	6 (8.8%)	0.96
Gender			
Male	163 (56.6%)	36 (52.9%)	0.59

and Chemistry Core Laboratory.

Trained sonographers at each site acquired echocardiograms for clinical purposes, as per the protocol (Appendix A) for obtaining images. The images were de-identified and sent via AG MedNet software to the Core Echocardiography Laboratory for central measurement. Each echocardiogram was assigned a unique identification number provided by the DCC. The new identification number was independent of the subject identification number and blinded the core laboratory to the study visit. Likewise, the cMRI images were for clinical purposes, as per the cMRI protocol (Appendix B). Images were de-identified, sent via a File Transfer Protocol system to the DCC, assigned a unique identification number, and then sent to the core cMRI Laboratory for central measurement. All echocardiograms and cMRI studies obtained were clinically indicated and not for the sole purpose of research.

2.6. Primary and secondary outcomes of interest

The primary outcomes to be assessed as both separate and composite endpoints for the DCM and HCM groups included death, heart transplant or transplant listing, and worsening HF. Worsening HF was defined as a worsening of the New York Heart Association or Ross HF scores or a HF-related hospital admission. Secondary outcomes were LV mechanical circulatory support with a ventricular assist device or extracorporeal membrane oxygenation or placement of an automatic internal cardioverter defibrillator.

3. Results

3.1. Study cohort

A total of 288 patients were enrolled. There were 506 patients screened, of whom 150 were ineligible. Detailed information was available to discern the reason for ineligibility in 55% of these. With respect to the DCM cohorts, 13 of 38 ineligible patients did not meet study criteria for both LV dilation and decreased function. For the HCM cohorts, 7 of 41 ineligible patients did not meet any of the LV posterior wall or septal thickness measurement criteria set forth for HCM. There were no differences between eligible patients who consented versus those who refused participation with respect to sex, age at diagnosis, race, or ethnicity (Table 4). Among screened and eligible patients, consent rate was similar between newly diagnosed DCM, HCM with cMRI, and chronic DCM or HCM (78%, 85%, and 81%, respectively).

Table 5 and Fig. 2A, B and C show the actual enrollment and rate of enrollment compared to the target enrollment for study aim. Because of low enrollment, the OSMB required adjustment of the expected enrollment beginning in September of 2014. This change is detailed in the figures.

Actual enrollment for Aim 1 (patients with newly diagnosed DCM) was lower than expected based on the PCMR historical data. It also took longer than expected to accrue newly diagnosed DCM patients, enrolling 81% of the target sample during a 30-month time frame rather than the targeted 12 months. This resulted in a truncated follow-up time for this cohort.

The enrollment of HCM patients with a recent cMRI was lower than expected due to few patients undergoing cMRI during the study period.

The number of eligible patients with chronic DCM or HCM was consistent with estimates from the PCMR Cohort Study data. The consent rate was also high which led to an actual enrollment and patient accrual time that matched the study targets.

The demographics and cardiac phenotype of the study cohort are shown in Table 6. The median age at diagnosis and age at enrollment for the entire cohort was 6.4 years and 11.6 years, respectively. The median age at diagnosis for the new onset DCM was 1.7 years and median time from diagnosis to enrollment was 0.1 years. The median age at diagnosis for the HCM group with an available cMRI was 12.1 years with median time from diagnosis to enrollment of 0.4 years. Among children with chronic cardiomyopathy, median age at diagnosis was 3.4 years and median time from diagnosis to enrollment was 4.8 years. The majority of children enrolled were of white race (71.5%) followed by black race (12.2%), Asian (1.7%), American Indian/Alaska native (1.7%), and native Hawaiian or other Pacific Islander (1.7%). The entire cohort includes 9.7% children of Hispanic or Latino ethnicity.

4. Discussion

The PCM Biomarkers study is a large multisite prospective study designed to determine any correlation between circulating biomarkers and disease state or prognosis in children with DCM and HCM. The study will examine, numerous biomarkers, including those associated with disease severity and prognosis in adults with HF. Of these, inflammatory biomarkers, tumor necrosis factor and interleukin-6 (which are associated with increased mortality and worsening HF) [22–24], protein ST2 (or interleukin-1) which predicts death or heart transplant with HF, and NT-proBNP have all been well studied in adults with HF, but few data are available from children with primary cardiomyopathy [25,26]. Some children with HCM are more susceptible to sudden cardiac death than others, and the factors predicting sudden death in adults may not necessarily be applicable to them [27]. However, cardiac fibrosis is believed to be an important substrate for arrhythmic death in both children and adults with HCM [28–30]. Thus, evaluating biomarkers of cardiomyocyte injury and collagen metabolism and correlating their concentrations with cMRI findings, echocardiographic assessments of LV diastolic dysfunction, and clinical outcome are potentially of great importance for children with HCM.

Table 5
Summary of target sample size, eligible patients, consent rate, and actual patient enrollment in the Cardiac Biomarkers in Pediatric Cardiomyopathy Study.

Population[a]	Target sample size	Actual sample size	Target accrual period (months)	Actual accrual time (months)	Screened and eligible	Consent rate (%)
New diagnosis DCM	150–180	80	18	30	103	77.7
HCM with recent cMRI	100–120	67	36	32	79	84.8
Chronic DCM or HCM	100–120	141	30	30	174	81.0

[a] DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; cMRI, cardiac magnetic resonance imaging.

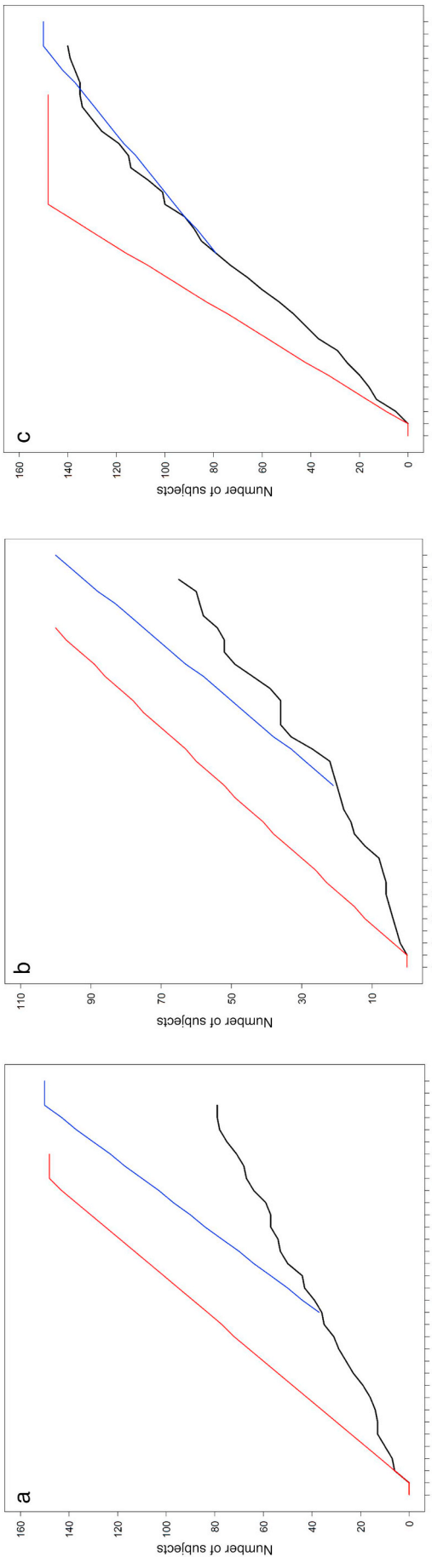

Fig. 2. A. Rate of patient accrual for Aim 1 newly diagnosed DCM cohort was lower than expected. Original Expected (shown in red line), Revised Expected (shown in blue line), and Actual (shown in black line) rate of enrollment by newly diagnosed dilated cardiomyopathy.
B. Rate of patient accrual for Aim 2 hypertrophic cardiomyopathy with recent cardiac magnetic resonance imaging was lower than expected. Original Expected (shown in red line), Revised Expected (shown in blue line), and Actual (shown in black line) rate of enrollment by hypertrophic cardiomyopathy with recent cardiac magnetic resonance imaging.
C. Rate of patient accrual for Aim 3 chronic dilated and hypertrophic cardiomyopathy was as expected. Original Expected (shown in red line), Revised Expected (shown in blue line), and Actual (shown in black line) rate of enrollment of chronic dilated or hypertrophic cardiomyopathy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 6
Patient demographics and cardiac phenotype of children enrolled in the Cardiac Biomarkers in Pediatric Cardiomyopathy Study.

	Aim1: New onset DCM	Aim 2: HCM with cMRI	Aim 3: Chronic DCM or HCM	Entire cohort
Total	80	67	141	288
Age at diagnosis, years	5.8 ± 6.6 Median = 1.7 (IQR = 0.5, 12.3)	11.0 ± 4.8 Median = 12.1 (IQR = 8.8, 14.3)	6.3 ± 6.0 Median = 3.4 (IQR = 0.7, 12.1)	7.2 ± 6.3 Median = 6.4 (IQR = 0.8, 12.9)
Age at enrollment, years	6.0 ± 6.6 Median = 2.1 (IQR = 0.7, 12.8)	13.0 ± 3.7 Median = 13.8 (IQR = 11.1, 15.3)	12.4 ± 5.6 Median = 13.2 (IQR = 8.0, 17.8)	10.7 ± 6.3 Median = 11.6 (IQR = 5.4, 16.2)
Time from diagnosis to enrollment, years	0.3 ± 0.5 Median = 0.1 (IQR = 0.02, 0.2)	2.0 ± 3.2 Median = 0.4 (IQR = 0.1, 2.7)	6.0 ± 4.4 Median = 4.8 (IQR = 2.8, 8.0)	3.5 ± 4.3 Median = 1.9 (IQR = 0.1, 5.1)
Race				
White	46 (57.5%)	55 (82.1%)	105 (74.5%)	206 (71.5%)
Black	16 (20.0%)	6 (9.0%)	13 (9.2%)	35 (12.2%)
Asian	1 (1.3%)	1 (1.5%)	3 (2.1%)	5 (1.7%)
American Indian/Alaska Native	1 (1.3%)	1 (1.5%)	3 (2.1%)	5 (1.7%)
Native Hawaiian or Other Pacific Islander	4 (5.0%)	0 (0%)	1 (0.7%)	5 (1.7%)
More than one race or unknown	12 (15.0%)	4 (6.0%)	16 (11.4%)	32 (11.1)
Ethnicity				
Hispanic or Latino	12 (15.0%)	4 (6.0%)	12 (8.5%)	28 (9.7%)
Not Hispanic or Latino	65 (81.3%)	63 (94.0%)	127 (90.1%)	255 (88.5%)
Not reported	3 (3.8%)	0 (0%)	2 (1.4%)	5 (1.7%)
Gender				
Female	44 (55.0%)	15 (22.4%)	66 (46.8%)	125 (43.4%)
Male	36 (45.0%)	52 (77.6%)	75 (53.2%)	163 (56.6%)

Mean ± standard deviation unless otherwise noted.

We acknowledge several challenges involved in conducting this research. First, enrolling children with acute DCM has proved to be more difficult than expected. To achieve sufficient follow up time, we anticipated an accrual period of 18 months would enroll 100 incident cases of DCM; however, it took 30 months to enroll 81 cases. Limitations to enrollment included the requirement that patients have both dilation and decreased LV function at presentation. Several patients had decreased LV systolic function without LV dilation (defined as LV size > 2 SD above normal). Moreover, the number of eligible patients and families who agreed to participate was also lower than expected, particularly for the children with newly diagnosed DCM. Investigators and coordinators found that the acuteness of the illness and the number of blood draws anticipated (6 total) hindered participation of eligible subjects. Moreover, this study did not afford any direct clinical benefits to the patient such as is perceived in drug trials or with other trials with an intervention arm. This lack of perceived benefit to the patient could have negatively impacted willingness of participation by either the parents or the child, particularly when multiple blood draws or excess blood volume with each draw was required.

Another challenge was the stipulation that clinical visits, echocardiography, and cMRI were to be scheduled as clinically indicated, rather than solely for research purposes. Although the planned follow-up was based on standard clinical practice, variation in follow-up intervals occurred likely due to center and provider-specific practice, the patient's clinical severity, or patient's adherence to medical follow up. The requirement for clinically indicated cMRI studies in patients with HCM impacted enrollment because cMRI was obtained by many of the enrolling centers less often than expected during the study. This was probably related to the lack of published guidelines with recommendations for timing of baseline cMRI and frequency of serial cMRI in pediatric HCM wherein the prevalence and progression of cardiac fibrosis is not known. cMRI acquisition necessitates sedation for young children, generally < 10 years old, so there is a hesitancy to perform sedation if the prognostic value of the test is not known in young children with HCM. Additionally, it was uncommon for clinicians to obtain a simultaneous echocardiogram and cMRI likely because it is unknown if there is added value in doing so. Finally, a U.S. Food and Drug Administration Drug Safety Alert issued after a recent report of gadolinium accumulation in the pituitary gland following repeated use of gadolinium-based contrast agents for MRI. Although of unknown relevance for children undergoing a single cMRI to assess for fibrosis, this may have proved an impediment to cMRI performance at some sites [31,32]. All are possible reasons that contributed to the lower-than-expected enrollment of HCM patients with a cMRI and recent echocardiogram. To mitigate the under-enrollment of HCM patients with a cMRI, the protocol was modified from an initial requirement of a cMRI within 2-months of the collected biospecimen to include patients with a cMRI acquired within the past 12 months and an echocardiogram within 6 months. The enrollment period was also extended and additional participating sites were added to increase HCM enrollment. These amendments were feasible because the Aim 2 study objectives required a one-time only biospecimen sample and did not depend upon long-term follow-up data for the analysis correlating markers of fibrosis with cMRI or echocardiography.

Despite these limitations, we anticipate that this study will answer several important questions about pediatric cardiomyopathy and biomarkers.

5. Conclusions

The PCM Biomarkers study is determining the predictive value of serum biomarkers to aid in the prognosis and management of children with DCM and HCM. The results may provide valuable information in an area where data are currently lacking in children. We anticipate that this study will impact clinical practice and facilitate additional trials in pediatric cardiomyopathy evaluating survival and quality of life by identifying those children most at risk for an adverse outcome. Additionally, knowledge from study design and subject recruitment during the conduct of the PCM Biomarkers study will help future investigators design better clinical trials with appropriate enrollment goals and recruitment strategies to have sufficient power to assess study endpoints for pediatric HF and cardiomyopathy.

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Conflicts of interest

None.

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Contributors

Drs. Lipshultz, Everitt, Wilkinson, Shi, Sleeper, Towbin, Colan, Kantor, Canter, Webber, Hsu, Pahl, Addonizio, Dodd, Jefferies, Rossano, and Ware designed the study.

Drs. Wilkinson, Shi, and Sleeper, drafted the study protocol.

All co-authors participated in protocol amendments, reports to the Observational Study Monitoring Board, patient enrollment, IRB approval, data collection and completion of the study.

Drs. Everitt, Wilkinson, Shi, Mr. Czachor, Ms. Razoky, and Ms. Westphal drafted the initial manuscript.

All co-authors provided review and several rounds of edits to the manuscript and approved the final version.

Appendix A. PCM Biomarkers study institutions

Site	Principal investigators
Wayne State University School of Medicine and Children's Hospital of Michigan, Detroit, MI -Administrative Coordinating Center	Steven Lipshultz
New England Research Institutes, Watertown, MA-Data Coordinating Center	Ling Shi, Lynn Sleeper[a]
The Heart Institute, Cincinnati Children's Hospital Medical Center, Cincinnati, OH	John Lynn Jefferies
Boston Children's Hospital, Boston, MA	Steven Colan
Children's Hospital of Philadelphia, Philadelphia, PA	Joseph Rossano
Columbia University Medical Center, New York, NY	Linda Addonizio
Ann and Robert H. Lurie Children's Hospital of Chicago and Northwestern University Feinberg School of Medicine, Chicago, IL	Elfriede Phal
Vanderbilt University School of Medicine and Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, TN	Steve Webber Debra Dodd
Albert Einstein College of Medicine, Children's Hospital at Montefiore, Bronx, NY	Daphne Hsu
Primary Children's Medical Center, Salt Lake City, UT	Kimberly Molina
University of Alberta and Stollery Children's Hospital, Edmonton, Canada	Paul Kantor
St. Louis Children's Hospital, Washington University, St. Louis, MO	Charles Canter
Miller School of Medicine, University of Miami, Miami, FL	Paolo Rusconi
University of Colorado and Children's Hospital of Colorado, Aurora, CO	Melanie Everitt
Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA	Brian Feingold
University of Tennessee Health Science Center, St. Jude Children's Research Hospital and Le Bonheur Children's Hospital, Memphis, TN	Jeffrey Towbin

[a] Affiliation at the time of study design.

Appendix B. Echocardiographic protocol: assessment of systolic and diastolic function

The echocardiogram included a complete two-dimensional echocardiogram and Doppler evaluation including a complete assessment for anatomic abnormalities, valve dysfunction and intracardiac thrombi, standard short and long axis views of the left ventricle to assess regional wall motion. The site provided age, height, weight, and blood pressure with the DICOM images uploaded using a commercial, HIPAA-compliant image transfer service (AMBRA, Inc.). Images were de-identified during import to the core lab server and all analyses were performed using custom software that enables archiving of all measurements as a non-destructive overlay and derivation of all calculated variables. All measurements were performed in triplicate and the average of the three measurements was used as the reported value. All measurements and derived variables were performed as described in the American Society of Echocardiography guidelines (Lopez L, Colan SD, Frommelt PS, Ensing GJ, Kendall K, Younoszai AK, Lai WW, Geva T. Recommendations for quantification methods during the performance of a pediatric echocardiogram: A report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. J Am Soc Echocardiogr 2010; 23: 465–95). The measured and derived variables are listed in the table below. Body surface area was calculated according to the formula of Haycock et al. (Haycock GB, Schwartz GJ and Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. J Pediatr 93: 62–66, 1978) and z-scores were obtained relative to age or BSA as previously described (Sluysmans T, Colan SD. Structural measurements and adjustment for growth. In: Lai WW, Cohen MS, Geva T, Mertens L, editors. Echocardiography in Pediatric and Congenital Heart Disease Edition 2. Wiley-Blackwell, West Sussex, UK, 2015).

Table
Measured and derived echocardiographic variables.

Name	Units	Z-score
Age	Years	
Date of birth	Date	Yes
Systolic blood pressure	mm Hg	Yes
Diastolic blood pressure	mm Hg	Yes
Height	cm	Yes
Weight	kg	Yes
Body surface area	m^2	Yes

(*continued on next page*)

Table (*continued*)

Name	Units	Z-score
Isovolumic relaxation time	s	Yes
Aortic root anteroposterior diastolic dimension	cm	Yes
Interventricular end-diastolic septal thickness	cm	Yes
Left ventricular posterior wall end-diastolic thickness	cm	Yes
Left ventricular maximal end-diastolic thickness	cm	Yes
Left ventricular posterior wall end-diastolic thickness	cm	Yes
Left ventricular end-diastolic dimension	cm	Yes
Left ventricular end-systolic dimension	cm	Yes
Left ventricular short axis end-diastolic endocardial area	cm^2	Yes
Left ventricular short axis end-diastolic epicardial area	cm^2	
Left ventricular short axis end-systolic endocardial area	cm^2	Yes
Left ventricular long axis end-diastolic endocardial length	cm	Yes
Left ventricular long axis end-diastolic epicardial length	cm	
Left ventricular long axis end-systolic endocardial length	cm	Yes
Left ventricular end-diastolic volume (5/6 × Area × Length)	ml	Yes
Left ventricular end-systolic volume (5/6 × Area × Length)	ml	Yes
Left ventricular mass (5/6 × Area × Length)	gm	Yes
Left atrial anteroposterior diameter	cm	Yes
Left atrial maximal length (apical 4-chamber view)	cm	Yes
Left atrial maximal area (apical 4-chamber view)	cm^2	Yes
Left atrial single plane volume	ml	Yes
Pulsed Doppler peak mitral E-wave velocity	cm/s	Yes
Pulsed Doppler peak mitral A-wave velocity	cm/s	Yes
Pulsed Doppler peak mitral E-wave/peak mitral A-wave	None	Yes
Pulsed Doppler E-wave Deceleration time	ms	Yes
Tissue Doppler peak e-wave velocity - lateral wall	cm/s	Yes
Tissue Doppler peak a-wave velocity - lateral wall	cm/s	Yes
Mitral E-wave/tissue Doppler e-wave velocity - lateral wall	None	Yes
Tissue Doppler peak e-wave velocity - septum	cm/s	Yes
Tissue Doppler peak a-wave velocity - septum	cm/s	Yes
Mitral E-wave/tissue Doppler e-wave velocity - septum	None	Yes
Tissue Doppler peak e wave velocity - average	cm/s	Yes
Tissue Doppler peak a wave velocity - average	cm/s	Yes
Mitral E-wave/tissue Doppler e-wave velocity - average	None	Yes

Appendix C. MRI protocol: imaging acquisition

Clinically performed cardiac MRI examinations were collected for this study. All scans were performed at 1.5 T on conventional medical scanners with clinically approved hardware and software at each clinical center. The cMRI scan included: scout scans for localization; horizontal and vertical long- and short-axis, multiphase gated cine views using steady state free precession SSFP sequences for function and anatomy at the optimally highest flip angle; phase contrast flow quantification cine sequence acquisition above the aortic valve and below the aortic annulus; and late gadolinium-enhanced phase-sensitive inversion recovery (PSIR) gradient echo imaging performed after the administration of 0.2 mmol/kg gadolinium-based MR contrast agent (gadopentetic acid (Magnevist), gadoversetamine (Optimark), gadodiamide (Omniscan) or gadoterate meglumine (Dotarem)) (all with similar relaxivities), or 0.1 mmol/kg gadobenate dimeglumine (MultiHance) (higher relaxivity), I.V. Late gadolinium enhancement (LGE) image was acquired at 8–10 min, after administering a gadolinium bolus using a T1-finder sequence to estimate inversion times. Inversion times were between 200 and 350 ms, set to null signal from the normal myocardium. All long axis images were acquired at 6 mm slice thickness. Short axis images were acquired at 6 mm slice thickness (0 gap) in children $<$ 6 years of age, and at 8 mm (0 gap) in children 6 years of age or older. Imaging examinations were performed breathhold in patients able to hold their breath. In patients unable to breathhold (i.e.; sedated patients or young children), multiaverage (3 averages) data collection was performed.

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