Cardiac Biomarkers in HIV-Exposed Uninfected Children: The Pediatric HIV/AIDS Cohort Study (PHACS)

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Abstract

Objectives—To evaluate associations of cardiac biomarkers with *in utero* antiretroviral (ARV) drug exposures and cardiac function/structure measured by echocardiograms in HIV-exposed but uninfected (HEU) children.

Design and methods—We analyzed the association of three cardiac biomarkers (cardiac troponin T, cTnT; high sensitivity C-reactive protein, hsCRP; and N-terminal pro-brain natriuretic peptide, NT-proBNP) with prenatal ARV exposures, maternal-child characteristics, and echocardiographic parameters.

Results—Among 338 HEU children (mean age=4.3 years), 51% had at least 1 elevated cardiac biomarker. Maternal tobacco use was associated with elevated NT-proBNP (adjusted odds ratio [aOR]=2.28, *P*=0.02). Maternal alcohol and abacavir use were associated with elevated cTnT levels (aOR=3.56, *P*=0.01 and aOR=2.33, *P*=0.04, respectively). Among 94 children with paired echocardiogram-biomarker measurements, cTnT measurements were correlated with increased left ventricular (LV) thickness-to-dimension ratio (r=0.21, *P*=0.04); and elevated cTnT was associated with higher mean LV end-diastolic (ED) posterior wall thickness (*P*=0.04). hsCRP measurements were negatively correlated with septal thickness (r=-0.22, *P*=0.03) and elevated hsCRP was

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associated with lower mean LV contractility *Z*-scores (*P*=0.04). NT-proBNP measurements were correlated with increased LVED dimension (r=0.20, *P*=0.05) and elevated NT-proBNP was associated with lower mean end-systolic septal thickness (*P*=0.03).

Conclusion—Our findings suggest that cardiac biomarkers may help identify HEU children who require further cardiac evaluation including echocardiography. Potential cardiac effects of prenatal abacavir exposure in this population need further investigation.

Keywords

cardiac biomarkers; echocardiography; HIV; antiretroviral drugs; pediatric

Introduction

Nearly 9000 HIV-exposed but uninfected (HEU) infants are born in the US each year, and from 85% to nearly 97% were exposed *in utero* and/or in early infancy to antiretroviral drugs (ARVs) [1,2]. *In utero* ARV exposure has been associated with significant changes in echocardiographic parameters of left ventricular (LV) structure and function in HEU children within the first two years of life in the HAART era [3]. ARV-exposed HEU infants need to be followed to monitor the long-term safety of these exposures including cardiac health [4].

Cardiac biomarkers which have proven useful in evaluating cardiac status in children with a variety of conditions may be similarly useful in HIV-infected or HEU children [5,6]. In children, serum measurements of cardiac troponins T (cTnT) and I (cTnI) are sensitive and specific for detecting myocardial injury in a variety of settings [7-12]. Elevated NT-proBNP levels are sensitive markers of asymptomatic LV systolic and diastolic dysfunction, as determined by echocardiography [13,14]. Serum high-sensitivity C-reactive protein (hsCRP) is a general inflammatory marker produced in response to infection or inflammation associated with a variety of cardiovascular conditions [15-17].

Echocardiographic screening and monitoring of the thousands of HEU children born annually in the US is not affordable or practical. Cardiac biomarkers could potentially identify which HEU patients might benefit from a more comprehensive clinical assessment including echocardiography. Using data from a cohort of HEU children, we tested two hypotheses: first, that there would be associations between specific maternal ARV regimens and abnormal cardiac biomarker levels and, second, that there would be a significant association between levels of cardiac biomarkers and echocardiographic measurements.

Methods

Study Population

The Surveillance Monitoring for ART Toxicities Study (SMARTT) of the Pediatric HIV/AIDS Cohort Study (PHACS) is a prospective study of the potential adverse outcomes of maternal ARV use on HEU infants and children using longitudinal demographic, anthropomorphic, clinical and laboratory data collected at 22 U.S. pediatric centers. The study protocol was reviewed and approved by the institutional review boards at all sites.

Written informed consent was obtained from the children's parent or legal guardian and assent from child participants as appropriate. Details of the SMARTT study design have been described previously [18].

Children underwent a single echocardiogram and blood draw between the ages of 3 and 5 years. Cardiac biomarker evaluation was initiated prior to performing an echocardiogram on all children, and only a subset of children had biomarker assays performed within 3 months of the echocardiogram. Biomarker assays were performed using the repository specimen closest in time to the echocardiogram for participants who had an echocardiogram; the most recently obtained specimen was used for participants who did not have an echocardiogram.

Cardiac Biomarker Assays

For consistency, all specimens were analyzed for cardiac biomarkers at the Clinical Chemistry Laboratory at Boston Children's Hospital: high sensitivity cTnT, with a detection limit of 3 pg/ml and normal value defined as 3 pg/ml; hsCRP, with normal defined as 1 mg/L); and NT-proBNP, with normal defined as 100 pg/ml [10,19,20]. cTnT and NT-proBNP were measured by a quantitative sandwich enzyme immunoassay technique on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN). The concentration of hsCRP was determined using an immunoturbidimetric assay on the Roche P Modular system (Roche Diagnostics, Indianapolis, IN). These biomarkers are stable in cryopreserved specimens [7,21-25].

Child and Maternal Factors and Maternal Antiretroviral Use

Child demographic, anthropometric factors, and birth characteristics (preterm birth [<37 weeks], and low birth weight [<2500 grams]) were considered. Maternal factors included young age at delivery (<25 years), low household income (<$20,000), less than a high school education, viral load >1000 copies/mL, CD4+ T-lymphocyte (CD4) count <250 cells/mm^3, and illicit substance use during pregnancy. Intrauterine exposures to individual ARV drugs, ARV drug classes, and highly active antiretroviral therapy (HAART) were examined overall and by trimester. ARV drug classes included nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). HAART was defined as at least 3 ARV drugs from at least 2 ARV classes.

Echocardiographic Assessment of Cardiac Structure and Function

An M-mode echocardiogram including 2-D and tissue Doppler imaging was performed by PHACS trained sonographers. Echocardiographic measures were centrally re-measured at the echocardiographic core lab at Boston Children's Hospital to improve measurement reliability. Using normative data from a reference cohort of healthy children from Boston Children's Hospital Echocardiographic Laboratory, echocardiographic parameter Z-scores were calculated. This population was not known to be HIV-infected or ARV-exposed and was not matched to the study cohort for race, ethnicity, or socioeconomic status [26]. Reporting the data as Z-scores adjusts for the effects of variation in age and body surface area.

Statistical Analysis

We calculated the percent of children with elevated levels of each cardiac biomarker, along with exact 95% confidence intervals (CIs) based on a binomial distribution. Logistic regression models were used to assess the association of maternal and infant characteristics and of *in utero* ARV exposure with abnormal biomarker levels. Child characteristics considered included age at biomarker measurement, sex, race, ethnicity, prematurity, low birth weight, and body mass index (BMI) Z-score. Maternal characteristics included age at delivery of child, household income, educational level, maternal CD4 count and viral load, and use of illicit drugs, alcohol or tobacco during pregnancy. Univariate logistic regression models were first fit for each covariate and those with $P<0.20$ were considered for inclusion in a multivariate model. The multivariate model was reduced to those covariates with $P<0.15$ to develop a separate core model for each biomarker. Adjusted logistic regression models were then fit for intrauterine exposure to HAART and for each drug class overall and by trimester, adjusting for potential confounders. Similar analyses evaluating associations between individual ARV agents and elevated cardiac biomarkers were also conducted, restricting to children with intrauterine exposure to HAART to reduce residual confounding. Because prematurity and/or low birth weight may be on the causal pathway between *in utero* ARV exposure and abnormalities in cardiac biomarkers, sensitivity analyses were also conducted excluding these birth characteristics from models.

The association between cardiac biomarkers with echocardiographic Z-scores was based on a subset of participants (the echocardiogram group) aged 3-5 years who also had an echocardiogram conducted within 3 months of their blood draw and was evaluated in two ways. First, Spearman rank-based correlation coefficients were calculated between cardiac biomarker measurements and each targeted echocardiographic parameter. Secondly, the mean echocardiographic Z-scores were compared between those with elevated versus those with normal biomarker levels in linear regression models adjusting for potential confounders, including age sex, ethnicity, and BMI Z-score. Two sided *P* values <0.05 were considered statistically significant. Because SMARTT is a safety study, we did not adjust for multiple comparisons in order to minimize the Type II error rate (e.g., the probability of failing to detect true associations). SAS version 9.2 (SAS Institute, Cary, NC) was used to conduct all analyses, based on data submitted as of October 1, 2010.

Results

Study Group

We evaluated 338 children enrolled between March 2007 and June 2009 who had cardiac biomarker measurements obtained at a mean age of 4.3 years (Table 1). Ninety-four of these patients also had an echocardiogram within 3 months of the biomarker measurements. The group was 50% female, 66% non-White, and 43% Hispanic. Almost all subjects (96%) received neonatal prophylaxis with zidovudine for the first six weeks of life, while a small number (N=10, 3%) received zidovudine in combination with at least one other ARV drug as prophylaxis. There were no significant differences between those with versus those without an echocardiogram for any child or maternal characteristic.

Prevalence of Elevated Cardiac Biomarkers

For the 338 children with biomarker measurements, the median level of NT-proBNP was 57.6 pg/ml (IQR=29.8, 94.8), 87% of cTnT results were at or below the limit of detection of 3.0 pg/ml, and the median level of hsCRP was 0.22 mg/L (IQR=0.11, 0.97). No child had an NT-proBNP level in the cardiomyopathy (>100 pg/ml) or heart failure range (>400 pg/ml) [9]. Two children had cTnT measurements which exceeded the 10 pg/ml level (10.9 and 72.4 pg/ml, respectively), the limit of detection of the older, less-sensitive cTnT assay; no child had cTnT measurements in the unstable angina range of 100-200 pg/ml or the acute myocardial infarction range of >200 pg/ml. However, 42/338 children (12%) had a cTnT between 3 and 10 pg/ml, a low-level elevation of unclear clinical significance. Elevated biomarker levels ranged from 13% to 24% (Fig. 1). At least one elevated biomarker value was found in 51% of children.

Association of Cardiac Biomarkers with Child Demographic and Maternal Factors

Elevated levels of hsCRP were associated with older age at biomarker draw (adjusted Odds Ratio [aOR]=1.42, 95% CI: 1.03, 1.94, *P*=0.03), while children born prematurely had reduced odds of elevated hsCRP (aOR=0.46, 95% CI: 0.22, 0.97, *P*=0.04). Elevated NT-proBNP levels were associated with maternal tobacco use (aOR=2.28, 95% CI: 1.13, 4.58, *P*=0.02); female sex (aOR=1.73, 95% CI: 0.94, 3.18, *P*=0.08), Hispanic ethnicity (aOR=1.83, 95% CI: 0.99, 3.40, *P*=0.05), maternal viral load >1000 copies/mL (aOR=2.10, 95% CI: 0.99, 4.46, *P*=0.05), and marginally with low birth weight (aOR=1.86, 95% CI: 0.92, 3.76, *P*=0.08). Increased odds of elevated cTnT were associated with younger age of the child (aOR=0.56, 95% CI: 0.38, 0.85, *P*=0.01), and marginally associated with younger age of the mother at delivery (aOR=0.46, 95% CI: 0.20, 1.07, *P*=0.07). Maternal alcohol use during pregnancy was associated with more than 3-fold odds of an elevated level of cTnT (aOR=3.56, 95% CI: 1.30, 9.74, *P*=0.01).

Association of Maternal ARV Exposures and Cardiac Biomarkers

Most (84%) participants had intrauterine exposure to HAART, 97% had exposure to NRTIs (primarily zidovudine and lamivudine), 23% had exposure to NNRTIs (primarily nevirapine), and 69% had exposure to PIs (primarily nelfinavir) (Table 2). With one exception, no significant associations were found between *in utero* HAART exposures or specific drug classes, either overall or during the 1st trimester, and elevated cardiac biomarkers. Overall exposure to NRTIs was associated with decreased odds of elevated NT-proBNP levels (aOR=0.08, 95% CI: 0.01, 0.85; *P*=0.04); however, only 3% of participants were unexposed to NRTIs. The only individual ARV drugs for which intrauterine exposure showed associations with elevated cardiac biomarkers after adjustment for maternal and child covariates were abacavir and stavudine. Abacavir exposure was associated with increased odds of elevated cTnT levels (aOR=2.33, 95% CI: 1.03, 5.26; *P*=0.04). Stavudine was significantly associated with decreased odds of elevated NT-proBNP (aOR=0.13, 95% CI: 0.02, 0.99; *P*=0.05). In sensitivity analyses excluding birth characteristics (prematurity and low birth weight) from models for NT-proBNP and hsCRP due to the possibility of these measures being part of the causal pathway between *in utero* ART exposure and elevated cardiac biomarkers, the above findings remained unchanged.

Association between Cardiac Biomarkers and Echocardiographic Parameters

For the 94 children with an echocardiogram within 3 months of a biomarker assay, the mean echocardiographic Z-scores were 0.00 for LV fractional shortening (a load-dependent measure of LV systolic performance), and 0.09 for LV contractility (a load-independent measure of LV systolic performance that reflects the health of the cardiomyocytes), consistent with cardiac functioning in the reference population. However, mean Z-scores for LV thickness-to-dimension ratio (a measure of potential LV remodeling which is LV structural changes of the LV wall thickness and dimension in response to a cardiac injury or insult) (−0.52), and ED septal thickness (a measure of ventricular structure) (−0.23) were significantly below 0 ($P<0.0001$ and $P=0.011$, respectively).

Table 3 summarizes the association of cardiac biomarkers with echocardiographic parameter Z-scores, based on both linear regression models comparing mean echocardiographic Z-scores between children with abnormal versus normal biomarker levels and on Spearman correlation coefficients for biomarker levels with echocardiographic Z-scores. The adjusted mean Z-scores for children with abnormal cTnT levels were significantly higher than those for children with normal cTnT levels for LVED posterior wall thickness (a measure of ventricular structure) (mean $Z=0.33$ versus −0.21, respectively, $P=0.04$) and LV thickness-to-dimension ratio (mean $Z=−0.10$ versus -0.61, respectively, $P=0.04$). Mean end-systolic septal thickness Z-scores were significantly lower in children with elevated NT pro-BNP than children with normal NT pro-BNP (mean $Z=−0.89$ versus -0.39, $P=0.03$). In addition, LV contractility Z-scores for children with abnormal hsCRP levels were significantly lower than for those with normal hsCRP (mean $Z=−0.37$ versus 0.21, $P=0.04$).

Using continuous measures of biomarker levels, hsCRP was inversely associated with ED septal thickness (correlation coefficient $r=−0.22$, $P=0.03$; NT-proBNP was positively associated with LVED dimension (a measure of ventricular structure) ($r=0.20$, $P=0.05$); and cTnT was positively associated with LVED thickness-to-dimension ratio ($r=0.21$, $P=0.04$).

Discussion

The first study aim was to determine if maternal ARV regimens or specific ARV agents were associated with cardiac biomarkers in HEU infants and children exposed *in utero* to these agents. No association between elevated cardiac biomarkers and maternal HAART or ARV drug class was found. Analysis of individual ARV agents revealed that abacavir was associated with significantly increased odds of elevated cTnT, suggesting injury or death of cardiomyocytes. Abacavir has been associated with cardiac toxicity, including increased risk of myocardial infarction in HIV-infected adults, although recent reports have challenged this association [27-30]. Although these results are not definitive, they highlight the need for further evaluation of the potential cardiotoxicity of *in utero* exposure to abacavir. Using cardiac biomarkers, we found no evidence of cardiotoxicity after *in utero* exposure to other ARVs using observations at a single time point.

We found an association of maternal alcohol use during pregnancy with elevated cTnT levels. Maternal alcohol use during pregnancy has been associated with structural heart defects, long QT syndrome, and heart muscle disease in human, animal, and *in vitro* studies

[31-36]. Maternal tobacco use was associated with significantly increased NT-proBNP levels. Prenatal tobacco exposure is associated with inhibition of cardiac DNA synthesis, impaired vascular smooth muscle structure and function, and structural cardiac defects [36].

The second study aim was to determine if elevated serum cardiac biomarker levels were associated with echocardiographic parameters in HEU infants and children. Higher hsCRP levels were associated with significantly lower ED septal thickness and LV contractility. The NHLBI-funded Cardiovascular Status of HAART Therapy in HIV-Exposed Infants and Children (CHAART-1) study of HEU children found that decreased septal thickness and LV mass were associated with *in utero* exposure to ARV [4]. The same study found an association between specific cardiac biomarkers and LV echocardiographic parameter changes [37]. The CHAART-1 study found that *in utero* ARV exposure was associated with increased LV contractility at age 2 years. We found that HEU children in the SMARTT cohort had significantly decreased LV contractility in those with elevated hsCRP who were aged 3-5 years, suggesting the possibility that perhaps HEU children with ongoing generalized inflammation, as indicated by elevated hsCRP, are at risk for decreased LV function as they age. This has implications for recommendations for cardiac screening and follow-up of this group. It has been reported that generalized inflammation in the neonatal period is associated with myocardial injury as measured by cTnT [10]. If such generalized inflammation persists, the expected consequence in later childhood may be decreased septal thickness and perhaps decreased LV contractility. We have previously found that generalized inflammation is associated with echocardiographic abnormalities, myocardial injury, and poorer clinical status in children with heart failure [13, 14]. Ongoing generalized inflammation has been associated with restrictive cardiomyopathy in anthracycline-exposed long-term survivors of childhood cancer [38-40].

Detectable serum cTnT suggests the possibility of active myocardial injury or cardiomyocyte apoptosis [7,41-49]. In this study we found that 12% of SMARTT HEU children had detectable serum cTnT raising concerns that active myocardial injury occurred. We are concerned that these low level cTnT elevations are significantly associated with a higher LV thickness-to-dimension ratio suggesting that low-level cTnT elevations may relate to subclinical changes in LV structure. Although 2 of 338 (0.6%) of SMARTT children had cTnT levels >10 pg/mL, none of them had cTnT levels at the higher levels seen with unstable angina (~100 pg/mL) or myocardial infarction (200 pg/mL) in adults. In a previous study of apparently HIV or ART unexposed pediatric outpatients [9], we reported elevated levels of cTnI >0.05 ng/mL in 7.8% of 193 acutely ill children and 4.4% of well children. Four of five children with elevated cTnI, who also had cTnT measured (level of detection=0.01 ng/mL or 10 pg/mL), had highly elevated cTnT levels in the range seen in adults with unstable angina or myocardial infarction. Previously, it had been shown that cTnI accurately shows myocardial injury similar to cTnT [50,51]. Compared to the previous report of cardiac troponin levels in non-HIV or ART exposed children in an outpatient setting [9], the HEU children described in this current report had reassuringly lower rates of active myocardial injury as defined by serum cardiac troponin concentrations. Based on our results, we cannot determine whether elevated cTnT levels in our HEU population are related to HIV or ART exposure, acute febrile illnesses, or other conditions. We do not

consider evidence of dead or dying cardiomyocytes, as indicated by measureable cardiac troponin blood concentrations, in healthy children in this age range to be normal.

In active myocardial injury with myocytolysis related to inflammatory heart disease, such as myocarditis, cardiac biopsy specimens often demonstrate a mononuclear intramyocardial infiltrate [52]. This infiltrate is accompanied by interstitial edema with a thicker LV wall by cardiac imaging [53,54]. Therefore, for the subset of children in this study with a measureable cTnT, the association with a thicker LV wall could be consistent with active myocardial inflammation, although similar levels have been reported in 4% of healthy children in one study. Further investigation would be needed to establish the significance of this finding [9].

The association of a greater LV dimension with increased NT-proBNP is consistent with LV neurohormonal activation due to LV stress (cardiomyocyte release of NT-pro BNP in response to increased LV volume or pressure) [55]. The finding of a significant correlation between LV dimension and NT-proBNP, coupled with overall mean decrease in LV thickness-to-dimension ratio and septal thickness for the 94 children with both echocardiographic and biomarker measurements noted above, could be suggestive of early LV remodeling (LV structural changes of the LV wall thickness and dimension in response to a cardiac injury or insult). We have previously reported that in children with heart failure that a 10-fold increase in NT-proBNP serum levels was associated with cardiac symptoms and indices of LV systolic dysfunction and remodeling [13].

Our results suggest that there may be several pathophysiologic processes occurring in some of these patients. An acute and active myocardial injury process may explain the detectable serum cTnT. Acute myocardial hypertrophy may be the results of an inflammatory cardiac process such as myocarditis with an infiltrate and interstitial edema. There also may be a chronic inflammatory process resulting in decreased septal thicknesses and a decrease in the LV thickness-to-dimension ratio consistent with LV remodeling even at 3-5 years of age. The significantly reduced LV thickness-to-dimension ratio overall is consistent with active LV remodeling being present in this population.

Study Limitations

The first limitation of this study is that only a subset (28%) of the children with biomarker measurements had temporally matched cardiac echocardiographic measurements. This affects the power of the study to detect significant associations between these parameters. Secondly, using only a single time point for both cardiac biomarker measurements and echocardiography limits the ability to assess temporal trends and evaluate causal associations. Next, the large number of comparisons between echocardiographic parameters, cardiac biomarker levels, and maternal ARV use means that statistically significant associations could be a result of chance. Analyses were not adjusted for multiple comparisons in order to detect safety signals.

However, the findings of LV structural and functional abnormalities are consistent with other studies and the associations with cardiac biomarkers are biologically plausible. Finally, the clinical significance of these findings is unclear; as is the optimal postnatal time to

conduct these measurements to best identify those HEU infants and children who should receive additional longitudinal cardiac follow-up.

Conclusions

We observed no potentially adverse significant associations between specific intrauterine ARV exposures and elevated cardiac biomarkers of abnormal cardiac structure or function, with the exception of a positive association between maternal abacavir use and measureable serum cTnT. Our results highlight the need for further evaluation of the potential for *in utero* exposure to abacavir to cause adverse cardiac effects in HEU children. HIV strains that are resistant to zidovudine or lamivudine are generally sensitive to abacavir. The largest maternal risk from abacavir use is a hypersensitivity reaction in less than 10% of patients [56]. Hypersensitivity testing has been determined to be cost-effective [57]. The maternal or child health effects of removing abacavir from HAART regimens for pregnant mothers are unknown. The child health cardiac effects of pre-natal abacavir exposures need to be further studied.

We observed associations between elevations of specific biomarkers and echocardiographic parameters consistent with an acute and/or chronic cardiac inflammatory response. The results of this study suggest that there may be a role for cardiac biomarkers to monitor HEU children for increased risk of deleterious cardiac effects that merit echocardiographic evaluation. The cost of these three biomarkers, or a similar panel, is less than $100 while the cost of an echocardiogram is $1000 or more. Future longitudinal studies of these and other, possibly more sensitive and specific, cardiac biomarkers or biomarker combinations in a larger population of HEU infants with echocardiograms could better define the utility of cardiac biomarkers to screen for cardiac changes in this population.

Acknowledgments

Abbreviation List

ARV	antiretroviral drugs
HEU	HIV-exposed, uninfected
ED	end-diastolic
LV	left ventricular
HIV	human immunodeficiency virus
HAART	highly active antiretroviral therapy
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
PI	protease inhibitor

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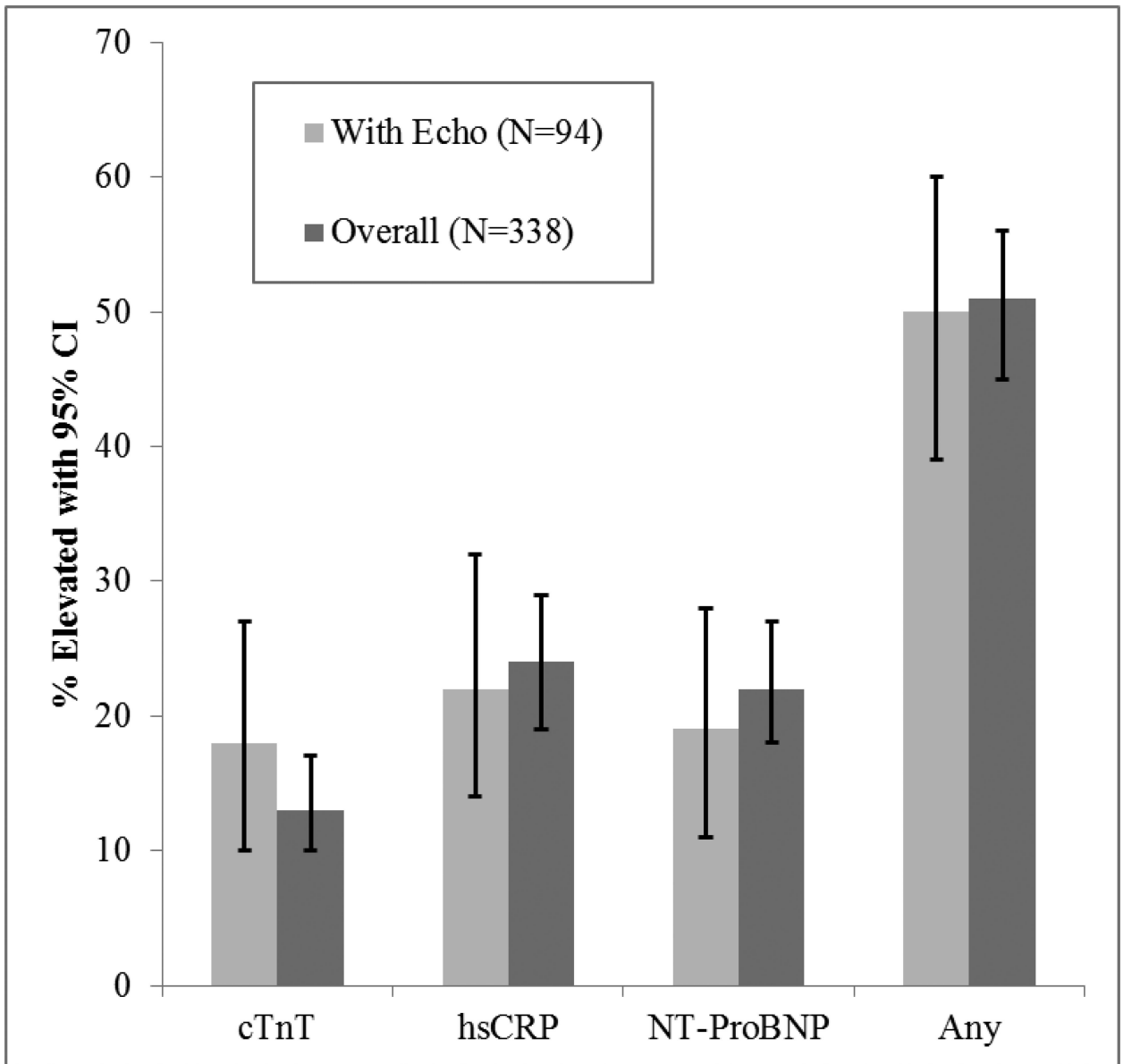
Figure 1. A comparison of all SMARTT children with elevated biomarkers compared to those with an echocardiogram within 3 months of the biomarker assays

The percent (%) of study children with elevated cardiac biomarker levels, for those with an accompanying echocardiogram within 3 months of a biomarker assay (*n*=94) versus the entire study cohort (*n*=338). cTnT=cardiac troponin T; hsCRP=high-sensitivity C-reactive protein; NT-proBNP=N-terminal pro-brain natriuretic peptide.

Table 1

Child and Maternal Characteristics of HIV-Exposed Uninfected Children with and without an Echocardiogram within 3 months of the Biomarker Measurement.

Characteristic	Echocardiogram within 3 months of biomarker		Total (n = 338)	P Value
	No (n = 244)	Yes (n = 94)		
Child Demographic and Birth Characteristics				
Age at biomarker, Mean (SD)	4.28 (0.82)	4.22 (0.85)	4.26 (0.83)	0.58
BMI Z-score, Mean (SD)	0.42 (1.39)	0.38 (1.34)	0.40 (1.38)	0.63
Female sex	122 (50%)	47 (50%)	169 (50%)	1.00
Non-White	146 (65%)	60 (70%)	206 (66%)	0.50
Latino/Hispanic	108 (44%)	36 (38%)	144 (43%)	0.33
Gestational age <37 weeks	43 (18%)	21 (22%)	64 (19%)	0.44
Low birth weight (<2500 grams)	46 (19%)	21 (22%)	67 (20%)	0.54
Neonatal ARV prophylaxis with zidovudine only	235 (96%)	89 (95%)	324 (96%)	0.73
Maternal Factors/Exposures during Pregnancy				
Age <25 years at birth of child	76 (32%)	28 (30%)	104 (31%)	0.79
Household income <$20,000	162 (70%)	61 (71%)	223 (71%)	1.00
Caregiver <HS education	85 (36%)	27 (29%)	112 (34%)	0.30
Maternal RNA >1000 copies/mL	35 (16%)	19 (22%)	54 (17%)	0.24
Maternal CD4 <250 cells/mm^3 prior to labor and delivery	22 (10%)	9 (11%)	31 (10%)	0.83
Maternal Substance Use During Pregnancy				
Illicit drug use	19 (9%)	10 (12%)	29 (10%)	0.52
Hard drug use (cocaine/opiate)	6 (3%)	2 (2%)	8 (3%)	1.00
Alcohol use	17 (8%)	7 (8%)	24 (8%)	1.00
Tobacco use	43 (20%)	18 (21%)	61 (21%)	1.00

BMI=Body mass index; HS=High school; CD4=CD4+ T-cell count; RNA=HIV-1 RNA copies; SD=standard deviation. Percentages are based on non-missing observations, P value by t-test for continuous covariates and by Fisher's Exact test for categorical measures.

Table 2

Adjusted Logistic Regression Models for Association of Elevated Biomarker Levels with *in utero* Antiretroviral Exposure

Exposure	Percent Exposed	hsCRP[*]		NT-proBNP[†]		cTnT[‡]	
		aOR (95% CI)	P Value	aOR (95% CI)	P Value	aOR (95% CI)	P Value
Exposure at any time during pregnancy							
		n = 323		*n* = 272		*n* = 289	
HAART	84%	0.91 (0.46, 1.81)	0.79	1.00 (0.43, 2.33)	0.99	1.83 (0.58, 5.70)	0.30
NRTI	97%	---	-	0.08 (0.01, 0.85)	0.04	0.62 (0.06, 5.91)	0.67
NNRTI	23%	0.73 (0.39, 1.37)	0.33	1.06 (0.53, 2.15)	0.87	1.27 (0.56, 2.87)	0.57
PI	69%	1.09 (0.62, 1.91)	0.77	0.93 (0.48, 1.80)	0.83	1.07 (0.49, 2.32)	0.87
Exposure During the 1st Trimester of Pregnancy							
HAART	42%	1.55 (0.93, 2.60)	0.09	0.79 (0.43, 1.46)	0.45	1.22 (0.62, 2.43)	0.57
NRTI	50%	1.44 (0.86, 2.41)	0.16	0.60 (0.33, 1.11)	0.10	0.97 (0.49, 1.93)	0.93
NNRTI	13%	0.93 (0.44, 1.99)	0.86	0.65 (0.25, 1.69)	0.38	1.00 (0.35, 2.83)	1.00
PI	32%	1.57 (0.91, 2.70)	0.10	0.92 (0.48, 1.76)	0.80	1.02 (0.49, 2.09)	0.97
Exposure to Individual ARV Agents (restricted to children with *in utero* HAART exposure)							
		n = 270		*n* = 231		*n* = 243	
Zidovudine	85%	1.20 (0.52, 2.80)	0.67	1.39 (0.52, 3.72)	0.51	0.41 (0.17, 1.01)	0.05
Lamivudine	59%	1.27 (0.40, 4.00)	0.69	0.60 (0.21, 1.77)	0.36	0.71 (0.23, 2.15)	0.54
Abacavir	25%	1.08 (0.53, 2.20)	0.84	0.64 (0.26, 1.60)	0.34	2.33 (1.03, 5.26)	0.04
Didanosine	7%	0.54 (0.17, 1.65)	0.28	0.89 (0.24, 3.37)	0.87	0.98 (0.21, 4.64)	0.98
Stavudine	8%	0.45 (0.15, 1.39)	0.17	0.13 (0.02, 0.99)	0.05	0.89 (0.24, 3.31)	0.86
Tenofovir	17%	1.14 (0.56, 2.32)	0.73	1.11 (0.50, 2.46)	0.79	1.37 (0.58, 3.24)	0.47
Emtricitabine	4%	1.22 (0.31, 4.88)	0.78	2.05 (0.55, 7.65)	0.29	0.81 (0.16, 4.10)	0.79
Efavirenz	4%	1.97 (0.53, 7.32)	0.31	1.28 (0.24, 6.78)	0.77	1.51 (0.30, 7.73)	0.62
Nevirapine	20%	0.63 (0.31, 1.29)	0.21	1.06 (0.50, 2.25)	0.89	1.13 (0.46, 2.77)	0.80
Nelfinavir	47%	1.52 (0.85, 2.70)	0.16	0.87 (0.46, 1.68)	0.69	0.56 (0.26, 1.18)	0.13
Indinavir[§]	3%	---	-	---	-	0.67 (0.07, 6.57)	0.73
Saquinavir	4%	0.49 (0.10, 2.29)	0.37	0.69 (0.13, 3.59)	0.66	0.62 (0.07, 5.09)	0.65
Lopinavir/Ritonavir	20%	0.79 (0.40, 1.55)	0.49	1.10 (0.53, 2.31)	0.80	1.23 (0.56, 2.74)	0.60

aOR=adjusted odds ratio, CI=confidence interval, HAART=highly active antiretroviral therapy, NRTI=nucleoside reverse transcriptase inhibitor, NNRTI=non-nucleoside reverse transcriptase inhibitor, PI=protease inhibitor. NT-proBNP=N-terminal pro-brain natriuretic peptide; hsCRP=high-sensitivity C-reactive protein; cTnT=cardiac troponin T.

Logistic regression models adjusted for:

*
(hsCRP) - age at biomarker draw and gestational age at birth <37 weeks

†
(NT-proBNP) - sex, ethnicity, birth weight <2500g, maternal viral load during pregnancy, and maternal tobacco use during pregnancy

‡
(cTnT) - age at biomarker draw, mother's age at delivery, and maternal alcohol use during pregnancy

§
Unable to estimate odds ratio of elevated biomarker due to small cell counts.

Table 3

Association of Cardiac Biomarkers with Echocardiographic *Z*-scores for 94 Children in SMARTT having an Echocardiogram within 3 months of Biomarker Assay

Echocardiographic *Z*-score	Comparison of Adjusted* Mean Echocardiographic *Z*-score between Children with Elevated versus Normal Cardiac Biomarker Levels			Correlation of *Z*-scores with Continuous Biomarker Level	
	Elevated Biomarker Mean (SE)	Normal Biomarker Mean (SE)	*P* Value	Spearman Correlation Coefficient	*P* Value
hsCRP					
Contractility	−0.37 (0.24)	0.21 (0.12)	0.04	−0.09	0.41
ED Septal Thickness	−0.41 (0.19)	−0.18 (0.10)	0.30	−0.22	0.03
NT-proBNP					
LV ED Dimension	0.29 (0.22)	−0.11 (0.10)	0.12	0.20	0.05
ES Septal Thickness	−0.89 (0.20)	−0.39 (0.09)	0.03	−0.05	0.65
cTnT					
LV ED Posterior Wall Thickness	0.33 (0.24)	−0.21 (0.11)	0.04	0.20	0.06
LV Thickness-to-Dimension Ratio	−0.10 (0.22)	−0.61 (0.10)	0.04	0.21	0.04

NT-proBNP=N-terminal pro-brain natriuretic peptide; hsCRP=high-sensitivity C-reactive protein; cTnT=cardiac troponin T, LV=Left ventricular; ED=End-diastolic.