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Cardiac Remodeling and Hypertension in HIV-Uninfected Infants Exposed in utero to Antiretroviral Therapy

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Background. We aimed to assess the postnatal pattern of cardiovascular remodeling associated with intrauterine exposure to maternal HIV and antiretroviral treatment (ART).

Methods. Prospective cohort including 34 HIV-exposed uninfected (HEU) infants and 53 non-HIV-exposed infants were evaluated from fetal life up to 6 months postnatally. A cardiovascular evaluation was performed including echocardiography, blood pressure, and carotid intima media thickness (cIMT) measurement.

Results. ART regimens during pregnancy included 2 nucleoside reverse transcriptase inhibitors (Abacavir + Lamivudine (32.4%), Emtricitabine + Tenofovir (41.2%), and Zidovudine + Lamivudine (20.6%)). At 6 months of age, HIV-exposed uninfected infants showed thicker myocardial walls (septal wall thickness mean 5.02 mm (SD 0.85) vs 3.98 mm (0.86); $P < .001$), relative systolic dysfunction with decreased mitral ring displacement (8.57 mm (2.03) vs 10.34 mm (1.84); $P = .002$), and decreased tricuspid S' (9.71 cm/s (1.94) vs 11.54 cm/s (2.07); $P = .003$) together with relative diastolic dysfunction showed by prolonged left isovolumic relaxation time (58.57 ms (13.79) vs 47.94 (7.39); $P < .001$). Vascular assessment showed significantly higher systolic and diastolic blood pressure (102 mmHg (16.1) vs 80 mmHg (13.9); $P < .001$ and 64 mmHg (14.4) vs 55 mmHg (10.2); $P = .045$ respectively), with 50% of HIV-exposed children meeting criteria for hypertension vs 3.77% of the non-HIV-exposed group ($P < .001$) and thicker mean cIMT in the HIV-exposed group (0.62 µm (0.09) vs 0.51 µm (0.09); $P = .015$).

Conclusions. Subclinical cardiac impairment together with higher blood pressure and thicker cIMT were observed in HIV-exposed infants at 6 months of age. Half of them presented hypertension. Our findings support a possible increased cardiovascular risk in HIV uninfected infants exposed *in utero* to ART.

Keywords. HIV; cardiovascular remodeling; cardiovascular risk; ART exposure; infant hypertension.

Human Immunodeficiency Virus (HIV) affects 38 million people worldwide [1]. More than half of them are women, mostly of childbearing age [2]. Mother-to-child transmission of the virus can be prevented if the appropriate measures are applied, mainly the use of combined antiretroviral treatment (ART) [3]. As a result, one of the main concerns nowadays in HIV pregnancies is the potential consequences of in utero exposure to maternal HIV and ART on the increasing number of HIV-exposed but uninfected (HEU) infants [4].

Regarding cardiovascular health, previous studies showed that HEU children present mild functional cardiac changes already in utero [5]. Importantly, further studies showed that the fetal cardiac concentric hypertrophy identified was significantly associated with maternal treatment with zidovudine (ZDV) [6, 7]. On the other hand, postnatal cardiac studies performed in HEU children showed inconsistent data. While some studies showed that HEU infants and preadolescents exposed to ART presented reduced left ventricular (LV) mass and increased contractility [8, 9], others failed to show a difference in echocardiographic parameters [10], or even conclude that intrauterine exposure to specific ART regimens was associated with an increased posterior wall thickness [11, 12] and a higher left ventricular shortening fraction [11]. The most recent study also showed increased left ventricular wall thickness and diastolic dysfunction in HEU children aged 2–7 years [12]. Moreover, previous studies assessing the vascular system in HEU children were performed in children with other cardiovascular risk factors such as obesity [13, 14] or pediatric HIV-infection [15].

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Nonstandard Abbreviations: ART, antiretroviral treatment; c-IMT, carotid intima-media thickness; E', diastolic annular peak velocity; E/A ratio; early diastolic and atrial contraction; GA, gestational age; HEV, HIV-exposed uninfected infants; INI, integrase inhibitor; IRT, left isovolumic relaxation time; LV, left ventricular mass; MAPSE, mitral annular plane systolic excursion; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; SGA, small for gestational age; TAPSE, tricuspid annular plane systolic excursion; ZDV, zidovudine.

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We aimed to assess the cardiovascular profile in infants exposed in utero to maternal HIV and ART, compared with nonexposed infants. Therefore, we designed a prospective cohort to follow-up until infancy our previous cohort of uninfected fetuses, in which we had observed cardiac concentric hypertrophy associated with maternal ZDV treatment [6].

METHODS

Study Population and Protocol

A prospective cohort study was conducted in the Department of Maternal–Fetal Medicine at BCNatal (Hospital Clinic and Hospital Sant Joan de Déu) in Barcelona, (Spain) from 2014 to 2017, including 34 fetuses from HIV-infected pregnant women. The unexposed group was frequency paired (2:1) by gestational age at fetal scan (± 1 week) and included 53 consecutively recruited non-HIV-infected pregnancies from the same Department accepting to participate. Both groups were recruited during fetal life and followed up after birth up to 6 months of age. Multiple pregnancies, malformations, chromosomal anomalies, and perinatal transmission of HIV infection were considered exclusion criteria. Both groups underwent the same study protocol including fetal echocardiography, collection of maternal and perinatal characteristics, and postnatal evaluation at 6 months of age. The study protocol was approved by the Hospital Ethics Committee (Reg HCB/2014/0401) and written parental consent was obtained for all study participants.

Maternal, Fetal and Perinatal Characteristics

Maternal baseline characteristics were recorded. Information regarding obstetric and perinatal outcomes included: preeclampsia [16] (new onset of hypertension of >140 mmHg systolic or >90 mmHg diastolic pressure and >300 mg proteins/24 h of urine), gestational diabetes, prematurity (<37 weeks), gestational age (GA) at delivery, Caesarean section, birth weight, birth weight centile, small for gestational age (SGA) (<10th percentile) [17], 5-minute Apgar score, neonatal admission to intensive care unit ,and major neonatal morbidity.

Maternal immunovirological parameters for HIV-infected women included registering mode of transmission, time from diagnosis of HIV infection to delivery, and measuring CD4+ T-cell count (by flow cytometry) and plasmatic viral load (by HIV RNA copy quantification through Amplicor HIV Monitor; Roche Diagnostic Systems, Branchburg, New Jersey, USA) at first and third trimester. All HIV-infected pregnant women were treated with ART. The specific ART regimen during pregnancy was decided by the infectious diseases' specialist following international guidelines [18, 19]. The type and duration of ART during pregnancy were recorded.

A conventional fetal ultrasound and echocardiography were performed at 28–32 weeks of gestational age (Methods described in Supplementary Material A).

After birth, all newborns perinatally exposed to HIV received antiretroviral treatment to reduce the risk of perinatal transmission of HIV [18]. For newborns whose mothers had received ART during pregnancy with viral suppression near delivery and adequate treatment adherence, a 4-week Zidovudine prophylaxis regimen was used. Newborns at higher risk of HIV acquisition received an ART regimen including a 6-week Zidovudine and Lamivudine prophylaxis and 3 doses of Nevirapine [18]. In order to rule out perinatal or postnatal HIV infection, plasmatic viral load (by HIV RNA copy quantification through Amplicor HIV Monitor; Roche Diagnostic Systems) was performed in all HIV-exposed newborns at 0–48 hours of life and at 6 weeks (2 weeks after having finished prophylactic treatment) and 4 months of age [18]. The type of postnatal and infant feeding was also recorded. All HIV-exposed infants were formula fed [18].

Postnatal Assessment at 6 Months of Age

Postnatal follow-up evaluation was scheduled at 6 months of age and included anthropometric data, echocardiography, and vascular assessment. Anthropometric data included the child's height, weight, body surface area, and body mass index.

Echocardiography

Echocardiography was performed while infants rested quietly following a standardized protocol [20] using a Vivid q (General Electric Healthcare, Horten, Norway) with 2–10 MHz phased-array transducer. Ultrasounds were performed by specialists skilled in echocardiography, and measurements were carried out blinded to the HIV status. A complete two-dimensional M-mode and Doppler echocardiographic examination was performed to assess initially structural heart integrity and morphometry [21].

Systolic function was evaluated in both ventricles using shortening fraction, tricuspid and mitral annular plane systolic excursion (TAPSE and MAPSE) and annular systolic peak velocities (S') [22, 23].

Diastolic function of both ventricles was evaluated by atrioventricular peak velocities at early diastole and atrial contraction (E/A ratios), E deceleration time, diastolic annular peak velocity (E') and left isovolumic relaxation time (IRT) [23].

Complete postnatal echocardiographic methods are described in Supplementary Material B. All cardiac parameters are also reported in Supplementary Material B as normalized z scores by previously published reference values when available [24–28].

Vascular Assessment

A vascular assessment was performed including blood pressure and carotid wall thickness measured by ultrasound.

Systolic and diastolic blood pressures were obtained at the beginning of the medical evaluation from the brachial artery

using a validated ambulatory automated Omron 5 Series device, while the child was resting. Each infant's blood pressure was evaluated twice, and the average was calculated. According to current pediatric guidelines, elevated blood pressure was defined as blood pressure over the 90th centile and hypertension when blood pressure was over the 95th centile [29].

Carotid ultrasound was performed using a Vivid q (General Electric Healthcare). Longitudinal clips of the far wall of both carotid arteries were obtained approximately 1 cm proximal to the bifurcation using a 3.33–10.0 MHz linear-array transducer. Carotid intima-media thickness (cIMT) was measured offline according to a standardized protocol based on a trace method assisted by a computerized program EchoPAC Software Only. To obtain cIMT, 3 end-diastolic still frames were selected across a length of 10 mm and analyzed for mean and maximum cIMT, and the average was calculated [30].

Statistical Analysis

First, uninfected infants born to HIV-infected women were compared to infants born to non-HIV-infected women. Data are presented as mean ± SD, median [interquartile range], or number (percentage) as appropriate. Baseline characteristics and perinatal outcome were compared between groups. HIV infection data was described for the exposed group.

The Student's *t*-test was used for continuous variables and the Chi-square test for proportions. The Mann–Whitney test was used for nonparametric variables. Simple comparisons used a 2-sided level of 0.05. Differences on cardiovascular parameters were assessed by using multivariate regression analysis adjusting by the potential baseline and perinatal confounders detected in the univariate analysis (black ethnicity, low education level, GA at birth and birthweight). A subanalysis according to gender was also performed.

Second, the association and predictive value of maternal and perinatal data and fetal echocardiography for infant hypertension was assessed. Data were analyzed by logistic regression to obtain odds ratios (ORs) for the cardiovascular parameters that were associated with the infant vascular outcome.

Stata IC version 14 (StataCorp. LP, College Station, TX) was used for the statistical analysis.

RESULTS

Maternal, Fetal, and Perinatal Characteristics

Maternal baseline and perinatal characteristics of the study populations are shown in Table 1. Maternal baseline characteristics were similar between the study groups, with the exception of higher prevalence of black ethnicity and low education level in the group of HIV-infected pregnant women. Fetal echocardiography was performed at median 30.3 weeks [28.4–32.6]. Concordantly with previous data [6, 7], HIV-exposed fetuses presented thicker myocardial walls (septal wall thickness z-score 0.59 [-0.26–2.03] vs -1.34 [-2.07 to -0.56]; *P* value < .001) and systolic and diastolic impairment (tricuspid ring displacement z-score -0.29 [-0.64–0.28] vs -0.68

Table 1. Baseline, Fetal and Perinatal Characteristics of the Study Populations

	HIV-exposed (n = 34)	Non-HIV-exposed (n = 53)	*P* value
Maternal characteristics			
Maternal age (years)	31.9 (5.4)	33.1 (5.0)	.297
Body mass index (kg/m^2)	23.9 (4.4)	22.5 (3.7)	.130
Smoking habit	7 (20.6)	11 (20.8)	.999
Black ethnicity	5 (14.7)	1 (1.89)	.028
Primiparity	16 (47.1)	33 (62.3)	.259
Low education level[a]	9 (26.5)	3 (5.66)	.008
Pregnancy complications			
Preeclampsia	3 (8.82)	2 (3.77)	.364
Gestational diabetes	1 (2.94)	1 (1.89)	.926
Prematurity	5 (14.7)	1 (1.89)	.028
Small-for-gestational age	4 (11.8)	1 (1.89)	.062
Perinatal data			
Gestational age at delivery (weeks)	37.6 (2.6)	39.6 (1.5)	<.001
Cesarean section	22 (64.7)	10 (18.9)	<.001
Birthweight (g)	2914 (605)	3371 (420)	<.001
Birthweight centile	37 [21–55]	48 [34–77]	.050
5 minutes Apgar score	10 [8–10]	10 [9–10]	.845
Admission to neonatal intensive care unit	5 (14.7)	2 (3.77)	.289
Major neonatal morbidity[b]	1 (2.94)	0 (0)	.411

Data are mean (SD), median [range] or n (percentage). HIV = Human Immunodeficiency Virus.

[a]Education level considered low when illiterate or primary educational level.

[b]Major neonatal morbidity defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy, persistent ductus arteriosus, or sepsis.

[-1.25–0.15]; *P* value = .019, mitral S' z-score 0.19 [-0.35–0.72] vs 0.80 [0.17–1.25]; *P* value = .011, isovolumic relaxation time z-score 1.56 [0.39–2.01] vs 0.19 [-0.31–0.79]; *P* value < .001). Maternal treatment with ZDV was significantly associated with fetal septal wall thickness OR 1.67 [1.24–2.10]; *P* value < .001 when compared with non- HIV-exposed fetuses. The complete fetal ultrasonographic results are included in Supplementary Material A. The HIV-infected group showed a worse perinatal outcome with higher incidence of prematurity, lower birthweight, and a higher rate of cesarean section.

Maternal immunovirological parameters for HIV-infected women are shown in Table 2. The majority of women were diagnosed of HIV and receiving ART before pregnancy (88.2% and 79.4%, respectively). During pregnancy, all HIV women except for one patient (lacking treatment adherence) were receiving ART. The mean CD4 cell count was more than 500 cells/mL and most women had viral load suppression at first trimester and at delivery (79.4% and 91.4%, respectively). Regarding ART regimens, all pregnant women were receiving 2 nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitors (NNRTI) or one boosted protease inhibitor (PI) or one integrase inhibitor (INI). Seven patients (20.6%) received Zidovudine as one of the NRTIs. The most common NRTIs combinations ART regimens during pregnancy were Abacavir + Lamivudine (n = 11; 32.4%), Emtricitabine + Tenofovir (n = 14; 41.2%), and ZDV + Lamivudine (n = 7; 20.6%).

Table 2. Immunovirological and Therapeutic Characteristics of HIV Pregnancies (n = 34)

HIV transmission	
Heterosexual	28 (82.4)
Vertical	5 (14.7)
Parenteral drug use	1 (2.9)
Previous opportunistic infection	8 (23.5)
HIV diagnosis in pregnancy	4 (11.8)
Months of HIV infection at delivery	56.5 [18.8–108]
Infection parameters during pregnancy	
CD4 cell count at first trimester (cells/μl)	522 ± 173
CD4 cell count at delivery (cells/μl)	572 ± 238
Viral load <50 copies/mL at first trimester	27 (79.4)
Viral load <50 copies/mL at delivery	31 (91.2)
Antiretroviral treatment characteristics	
ART before pregnancy	27 (79.4)
Weeks of ART before pregnancy	40.5 [5.3–86.3]
ART during pregnancy	33 (97.1)
Weeks of ART during pregnancy	38 [32–39.2]
ART during first trimester pregnancy	24 (70.6)
NRTI during pregnancy	33 (97.1)
Zidovudine during pregnancy	7 (20.6)
Weeks of zidovudine during pregnancy	36 [25.1–39.4]
Zidovudine during first trimester	5 (14.7)
NNRTI during pregnancy	7 (20.6)
PI during pregnancy	22 (64.7)
Integrase inhibitors during pregnancy	5 (14.7)

Data are mean ± standard deviation (SD); median [range] or n (percentage) as indicated. HIV, Human Immunodeficiency Virus; ART, Combined antiretroviral treatment; NRTI, Nucleoside reverse transcriptase inhibitors; NNRTI, Non-nucleoside reverse transcriptase inhibitors; PI, Protease inhibitors.

All newborns perinatally exposed to HIV received antiretroviral treatment, 33 (97.1%) received 4 weeks of ZDV, and one (2.9%) received combination prophylaxis with ZDV and Lamivudine for 6 weeks and 3 doses of Nevirapine. All infants had undetectable viral load at the 3 time points previously described (0–48 hours of life, 6 weeks, and 4 months of age). All HIV-exposed infants were formula fed while 8 (15.1%) of the non-HIV-exposed infants were formula fed; *P* value < .001.

Anthropometric and Cardiovascular Profile at 6 Months of Age

Anthropometric and cardiovascular results in the study groups are shown in Tables 3 and 4. Both groups showed similar age and anthropometric characteristics at the time of the evaluation.

HIV-exposed uninfected infants showed thicker myocardial septal walls as compared to non-HIV-exposed infants. Moreover, HIV-exposed uninfected infants showed lower systolic values with decreased shortening fraction, MAPSE, and systolic myocardial velocities. They also had worse diastolic values with decreased E/A ratios and diastolic myocardial velocities, together with prolonged mitral E deceleration time and IRT.

In addition, uninfected infants prenatally exposed to HIV showed significantly increased systolic and diastolic blood pressure (58.8% presented elevated blood pressure vs 7.55%; *P* < .001) as well as thicker cIMT (Figure 1) with half of them meeting criteria for hypertension.

Most cardiovascular changes remained statistically significant after adjustment for potential confounding factors (black ethnicity, low education level, gestational age at birth, and birthweight).

Prediction of Hypertension

We analyzed the association of the different maternal, perinatal characteristics, and fetal echocardiographic parameters for the prediction of infant hypertension, which was assessed by univariate regression analysis. Maternal HIV status was significantly associated with infant hypertension (OR 25.18 [5.13–123.66]; *P* value < .001 together with maternal treatment with Zidovudine during pregnancy (OR 30.67 [3.91–240.69]; *P* value: 0.001) and black ethnicity (OR 7.25 [1.10–48.19]; *P* value = .040). Among the fetal echocardiographic parameters, septal wall thickness (mm) showed the strongest association (OR 10.06 [2.87–35.18]; *P* value < .001). Other echocardiographic parameters significantly associated with infant hypertension were ICT (OR 1.13 [1.04–1.23]; *P* value = .005) and left S' (OR 0.54 [0.29–0.98]; *P* value = .044).

To assess the potential confounding effect among the different parameters, a multivariate analysis was performed including the parameters that showed the strongest association

Table 3. Infants' Anthropometric and Echocardiographic Results

Characteristic	HIV-exposed (n = 34)	Non-HIV-exposed (n = 53)	*Crude P-value*	*Adjusted P-valuea*
Age at evaluation (months)	6.9 (2.6)	6.4 (1.3)	.244	.897
Anthropometric data				
Height (cm)	68.1 (5.75)	67.6 (2.23)	.573	.414
Weight (Kg)	7.96 (1.59)	7.69 (0.71)	.303	.677
Body mass index (kg/m^2)	16.97 (2.14)	16.84 (1.53)	.753	.539
Body surface area (m^2)	0.37 (0.05)	0.36 (0.02)	.404	.628
Echocardiography				
Cardiac morphometry				
Left atrial area (cm^2)	3.34 (0.92)	3.48 (0.79)	.463	.524
LV longitudinal diameter (mm)	36.03 (5.26)	33.77 (4.22)	.030	.087
LV transverse diameter (mm)	19.85 (3.86)	18.14 (2.72)	.403	.205
LV sphericity index	1.69 (0.26)	1.76 (0.27)	.135	.301
LV free wall thickness (mm)	4.61 (0.79)	4.44 (0.69)	.288	.482
Septal wall thickness (mm)	5.02 (0.85)	3.98 (0.86)	<.001	<.001
Relative wall thickness (mm)	0.40 (0.09)	0.35 (0.09)	.024	.605
Right atrial area (cm^2)	3.29 (0.96)	2.83 (0.49)	.068	.208
RV longitudinal diameter (mm)	29.32 (3.45)	28.07 (3.76)	.233	.553
RV transverse diameter (mm)	15.78 (2.97)	13.63 (2.11)	.085	.132
RV sphericity index	1.55 (0.24)	1.69 (0.33)	.319	.409
Systolic function				
LV shortening fraction (%)	30.09 (4.47)	32.43 (6.94)	.088	.054
Mitral ring displacement (mm)	8.57 (2.03)	10.34 (1.84)	<.001	.002
Tricuspid ring displacement (mm)	14.42 (2.44)	15.54 (2.35)	.034	.278
Mitral S' peak velocity (cm/s)	6.79 (1.07)	7.69 (1.59)	.006	.085
Tricuspid S' peak velocity (cm/s)	9.71 (1.94)	11.54 (2.07)	<.001	.003
Heart rate (bpm)	125 (17)	128 (13)	.603	.460
Diastolic function				
Mitral E/A ratio	1.17 (0.22)	1.39 (0.25)	<.001	.004
Tricuspid E/A ratio	1.22 (0.28)	1.09 (0.28)	.063	.026
Mitral E deceleration time (ms)	117 (32.7)	97 (25.4)	.002	.025
Tricuspid E deceleration time (ms)	127 (55.3)	103 (41.6)	.026	.227
Mitral E' (cm/s)	10.1 (2.46)	11.6 (2.89)	.013	.019
Tricuspid E' (cm/s)	12.8 (2.96)	14.6 (3.19)	.012	.025
LV isovolumic relaxation time (ms)	58.57 (13.79)	47.94 (7.39)	<.001	<.001

Data are mean (standard deviation). HIV, Human Immunodeficiency Virus; LV, Left ventricle; S', systolic annular peak velocity; E, ventricular inflow in early diastole; A, ventricular inflow during atrial contraction; E', annular peak velocity in early diastole; cIMT,carotid intima media thickness.

[a]Adjusted *P* value calculated by linear or logistic regression adjusted by black ethnicity, low education level, gestational age at birth, and birth weight.

with hypertension (intrauterine exposure to Zidovudine and fetal septal wall thickness) and potential confounders (black ethnicity, birthweight, and gestational age at birth). In both cases, the independent predictive value remained statistically significant in the multivariate analysis: OR 25.07 [2.81–223.41]; *P* value = .004 for maternal treatment with Zidovudine when

Table 4. Infants' Vascular Results

Characteristic	HIV-exposed (n = 34)	Non-HIV-exposed (n = 53)	*Crude P-value*	*Adjusted P-value*[a]
Blood pressure				
Systolic blood pressure (mmHg)	102 (16.1)	80 (13.9)	<.001	<.001
Diastolic blood pressure (mmHg)	64 (14.4)	55 (10.2)	<.001	.045
Mean blood pressure (mmHg)	83 (13.8)	64 (11.4)	<.001	.001
Elevated blood pressure (%)	20 (58.8)	4 (7.55)	<.001	.001
Hypertension (%)	17 (50)	2 (3.77)	<.001	.001
Carotid ultrasound				
Mean cIMT (mm)	0.42 (0.08)	0.33 (0.08)	.001	.015
Maximum cIMT (mm)	0.82 (0.12)	0.51 (0.09)	<.001	.005

Data are mean (standard deviation) or percentage. HIV, Human Immunodeficiency Virus; cIMT, carotid intima media thickness.

[a]Adjusted *P* value calculated by linear or logistic regression adjusted by black ethnicity, low education level, gestational age at birth and birth weight.

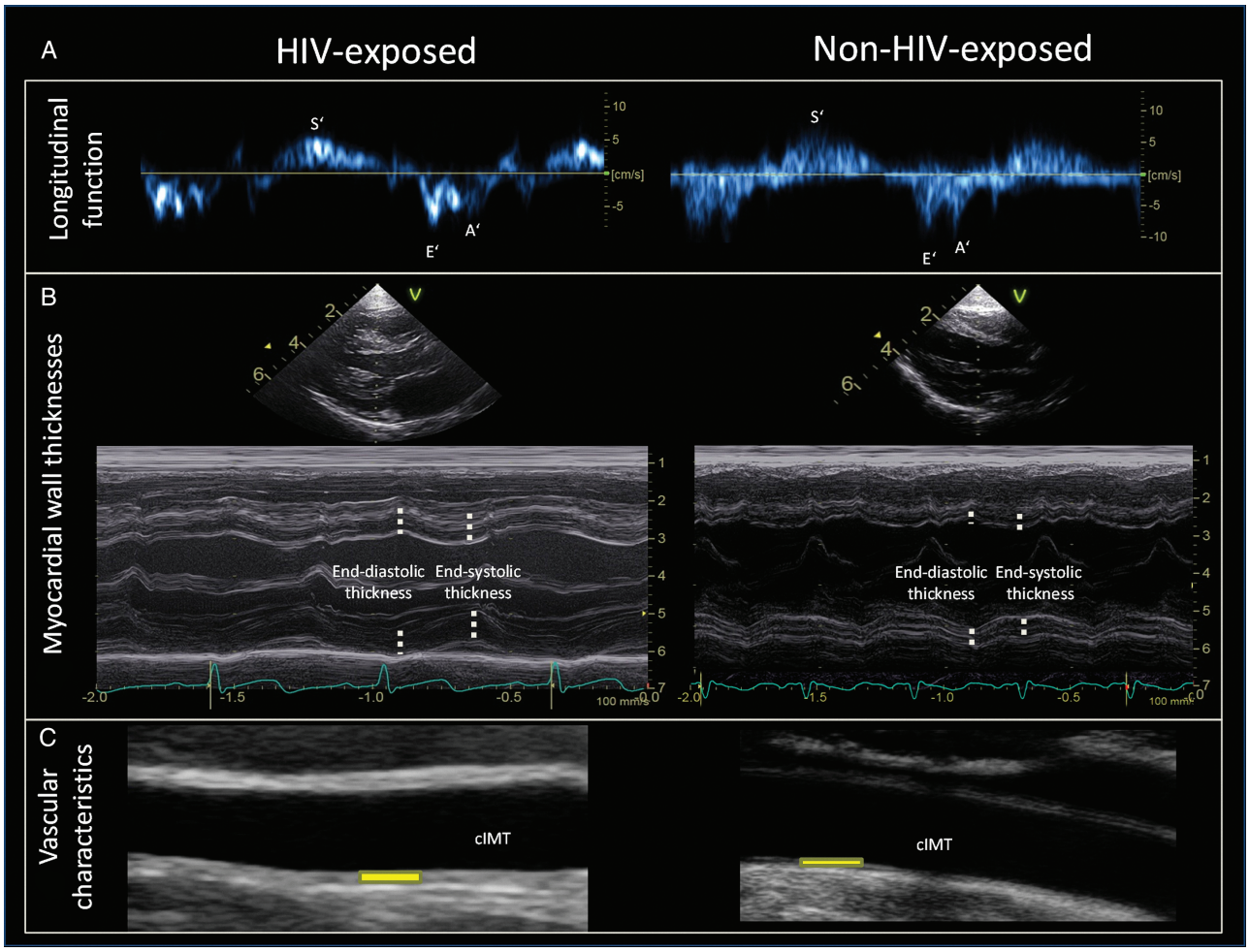

Figure 1. Echocardiographic images in HIV-exposed infants and non-HIV-exposed infants at 6 months of age illustrating (*A*) impaired longitudinal motion by lower annular peak velocities (S', E', A'); (*B*) thicker myocardial wall thicknesses; (*C*) thicker carotid intima media thickness (cIMT) in HIV-exposed infants.

compared with non-HIV-exposed children, and OR 6.65 [1.20–36.74]; *P* value = .030 for fetal septal wall thickness.

DISCUSSION

The present study demonstrated relevant cardiovascular changes in HIV-uninfected infants exposed in utero to ART, including a significant proportion of infants with hypertension. Prenatal factors such as intrauterine ZDV exposure and fetal echocardiographic parameters may predict hypertension in HEU infants.

Our data on cardiovascular changes observed in HEU infants during fetal life, concordantly with previous data [6], are mainly relative cardiac hypertrophy significantly associated with maternal use of ZDV, as well as subclinical systolic and diastolic impairment. In the present study, we assess the same cohort of fetuses prenatally and after birth. We showed that HIV uninfected infants exposed in utero to ART present an asymmetric relative hypertrophy (thicker septal myocardial walls with similar left and right myocardial walls as well as similar LV cavities) and more pronounced functional changes (decreased longitudinal function and impaired relaxation as longer diastolic times). Our infant data are consistent with previous reports demonstrating cardiac dysfunction with controversial results on myocardial wall thicknesses [8, 10]. The first study assessing the cardiovascular impact of perinatal exposure to ART concluded that ZDV monotherapy was not associated with cardiac abnormalities [31]. However, further studies by the same group found cardiovascular changes in children and preadolescents associated with intrauterine ART exposure, including decreased LV mass and thickness of interventricular septum, increased contractility together with diastolic impairment. More recent studies differed with these results, concluding that exposure to ART was associated with thicker left ventricular posterior wall thickness [10–12]. Specifically, they conclude that first trimester ZDV exposure was associated with congenital heart defects [11]. Zidovudine exposure was also associated with higher wall stress scores and higher heart size factor scores [32]. The controversial results may be explained by the use of historical cohorts where the ART exposed group included both

HIV-infected and non-infected children and the exposure to different ART regimens over time.

Moreover, HEU infants presented significantly higher systolic and diastolic blood pressures and thicker cIMT. Half of the HIV-exposed infants presented hypertension [29, 33], which is strongly predictive of adult blood pressure [34]. Our data show a higher prevalence of elevated blood pressure and hypertension than previously reported. The recent studies assessing blood pressure in HEU children were performed at 6–18 years in obese HEU children [13] and at 9.3 years (range 7–11) in South Africa, where, contrary to our setting, part of the HIV-exposed infants were breastfed [14]. As previous studies indicate, breastfeeding may have a beneficial influence on cardiac structure and function in infants and adults being born preterm [35] or growth restricted [36].

Interestingly, our study shows for the first time that infant hypertension could be predicted from fetal life by assessing maternal ART regimens containing ZDV and/or by measuring fetal septal wall thickness by fetal echocardiography.

Concordantly with our echocardiographic results, increased afterload conditions, such as hypertension, have been associated with cardiac asymmetric hypertrophy [37, 38]. Hypertension during infancy and childhood is strongly predictive of adult blood pressure [34]. Furthermore, even though thicker cIMT has been described in HIV-infected children [15], to our knowledge, this is the first study assessing cIMT in HEU population. Increased cIMT (an indicator of early or structural atherosclerosis) is a strong predictor of future cardiovascular events in adulthood. A recent study performed in infants showed that increased cIMT was also predictive of elevated blood pressure that persisted into adulthood [39]. Therefore, our findings may represent an increased cardiovascular risk later in life in HIV-uninfected infants exposed in utero to ART.

An important strength of our study was the recruiting of specific prospective cohort with a comprehensive echocardiography in fetal life using well-defined and strict methodology and subsequent follow-up into childhood. This allowed us to examine prospectively with optimal accuracy and reproducibility the cardiovascular effects of intrauterine exposure to maternal HIV and ART while controlling for confounders previously associated with cardiovascular changes such as black ethnicity [40, 41], birth weight [42], and gestational age at birth [43]. Furthermore, this is the first study assessing cIMT in HEU population, with relevant results which may indicate an increased further cardiovascular risk. Examinators performed the measurements blinded to the HIV status which minimizes potential biases.

Some limitations have to be acknowledged. Even though we adjusted the results for relevant potential confounders, unaccounted residual confounders may be present and ethnic differences should be acknowledged. Postnatal factors such as prophylaxis with ZDV for 4–6 weeks in HIV-exposed newborns and differences in infant feeding between the study groups may have influenced the results. Moreover, in spite of defined protocols, we recognize that blood pressure and cIMT measurement at 6 months of age may be challenging and highly variable, therefore data to define nomograms for these parameters are lacking [29].

In conclusion, this study showed cardiovascular changes in HEU infants at 6 months of age and a significantly higher incidence of hypertension. These changes could allow us to target them as a population at risk even from fetal life, establishing a strict and longer follow-up. As demonstrated in other pathologies such as fetal growth restriction [36] and prematurity [43], infants would benefit from an early control of the cardiovascular risk factors and from primary and secondary preventive measures. Our data support the current trend of considering ZDV-containing ART regimens during pregnancy as alternative due to complex dosing [44] and association with higher rates of mild-to-moderate adverse effects [45]. Avoiding ART regimens containing ZDV during pregnancy, promoting healthy lifestyle habits, and avoiding other cardiovascular risks from the childhood, could potentially prevent cardiovascular events later in life.

Our results highlight the relevance of further studies evaluating the potential long-term impact of different ART regimens on the constantly evolving HEU infants.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

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