AIDS

DOI: 10.1097/QAD.0000000000001066

TITLE PAGE

Full title: **Zidovudine treatment in HIV-infected pregnant women is associated with fetal cardiac remodelling.**

Short title: **Zidovudine and fetal heart.**

Laura GARCÍA-OTERO[a], Marta LÓPEZ[a], Olga GÓMEZ[a], Ana GONCÉ[a], Mar BENNASAR[a], Josep María MARTÍNEZ[a], Brenda VALENZUELA-ALCARAZ[a], Mérida RODRIGUEZ-LOPEZ[a], Marta SITGES[b], Montserrat LONCÀ[c], Bart BIJNENS[d], Fàtima CRISPI[a], Eduard GRATACÓS[a].

[a]BCNatal - Barcelona Center for Maternal-Fetal and Neonatal Medicine (Hospital Clínic and Hospital Sant Joan de Deu), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, and Centre for Biomedical Research on Rare Diseases (CIBER-ER), Barcelona, Spain.

[b]Department of Cardiology, Thorax Clinic Institute, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain

[c]Infectious Diseases Department, Hospital Clinic, Fundació Clínic per a la Recerca Biomèdica (FCRB), University of Barcelona, Barcelona, Spain.

[d]ICREA, Universitat Pompeu Fabra, Barcelona, Spain

Disclosures: None of the authors has any financial, consultant, institutional and other relationship that might lead to bias or a conflict of interest for the present manuscript. The present manuscript has not been submitted elsewhere.

Address for correspondence and reprints: Eduard Gratacós, MD PhD Head & Professor. Department of Maternal-Fetal Medicine BCNatal - Hospital Clínic. University of Barcelona, Spain. C/ Sabino de Arana,1 08028. Barcelona. Spain. Telephone number. +34 932279904. FAX number: +34 932275605. e-mail: gratacos@clinic.ub.es

ABSTRACT

Objective: To evaluate the cardiac structure and function of the fetuses of pregnant women with Human Immunodeficiency Virus (HIV) infection on combined antiretroviral treatment (cART) and the HIV related and non-related determinants of abnormal findings.

Design: A prospective cohort study including 42 non-infected fetuses from HIV pregnant women on cART and 84 fetuses from non HIV-infected women.

Methods: Fetal echocardiography was performed at 26-32 weeks of pregnancy to assess cardiac structure and function. The impact of maternal and perinatal factors on fetal cardiac remodelling was evaluated by multivariate regression analysis.

Results: Fetuses from HIV pregnant women on cART presented larger hearts and pericardial effusion together with thicker myocardial septal walls (mean 3.56 mm (SD 0.88) vs. non-HIV mean 2.75 mm (SD 0.77); p=0.002) and smaller left ventricular cavities (10.81 mm (SD 2.28) vs. 12.3 mm (SD 2.54); p=0.033). Fetuses from HIV women also presented signs of systolic (mitral systolic annular peak velocity 5.85 cm/s (SD 0.77) vs. non-HIV 6.25 cm/s (SD 0.97); p=0.007) and diastolic (isovolumic relaxation time 52 ms (SD 8.91) vs. non-HIV 45 ms (SD 7.98); p<0.001) dysfunction.

In the multivariate analysis, maternal treatment with zidovudine was the only factor significantly associated with fetal cardiac changes (p=0.014).

Conclusions: Fetuses from HIV-infected mothers on cART have cardiac remodelling and dysfunction, which might explain the cardiovascular changes described in childhood. Fetal cardiac remodelling was essentially associated with maternal treatment with zidovudine which challenges its use during pregnancy.

Keywords: HIV, pregnancy, antiretroviral therapy, zidovudine, fetal heart.

MANUSCRIPT

Introduction

Perinatal transmission of Human Immunodeficiency Virus (HIV) is almost negligible if the current preventive strategies are applied (mainly the use of combined antiretroviral treatment -cART- during pregnancy) [1, 2]. HIV-exposed to maternal cART but uninfected (HEU) children are generally considered healthy, although several studies have reported long-term differences in neurodevelopmental, immunological and cardiac parameters as compared with controls [3, 4]. In relation with the latter, consistent evidence has demonstrated subclinical changes in the cardiac structure and function of HEU patients from birth up to adolescence [5-7].

The underlying mechanism of cardiac changes in the offspring of HIV-infected mothers remains to be elucidated, but toxicity from antiretroviral drugs during fetal life is suggested as a pathogenic pathway [7, 8]. Evidence from previous studies is inconclusive. A large study including 173 fetuses reported significant changes in fetal cardiac structure and function, but only one third of the mothers received prenatal

monotherapy with zidovudine (ZDV) [9]. A more recent study with 29 HIV-infected pregnancies on cART reported only mild changes in fetal cardiac function [10]. Aside from prenatal cART, HIV-infected pregnancies are more commonly exposed to perinatal complications including fetal growth restriction [11], prematurity [12], and preeclampsia [13], which could directly affect fetal cardiac structure and function [14-17], but the impact of these factors has not been assessed.

In this study, we comprehensively evaluate fetal cardiac structure and function in a cohort of 42 uninfected fetuses from HIV mothers under cART, which were compared with 84 controls. Our aim was to describe the pattern of fetal structural and/or functional cardiac changes during fetal life and to evaluate the independent and combined association with antiretroviral treatment, maternal and perinatal factors.

Methods

Study populations

The study design was a prospective cohort including non-infected fetuses from HIV-infected pregnant women on cART followed up in the Maternal-Fetal Medicine Department at BCNatal in Barcelona (Spain) from May 2010 to December 2014. The control group included consecutive non HIV-infected pregnancies from the same Department accepting to participate in the study. Controls were frequency paired (2:1) with HIV-infected pregnancies by gestational age at scan (±1 week). Twin pregnancies, diagnosis of fetal malformations (including congenital heart disease) or chromosomal anomalies, delivery before 24 weeks of gestational age as well as perinatal transmission of HIV were considered exclusion criteria. Both groups underwent the same study protocol including collection of baseline and perinatal characteristics and third trimester fetal echocardiography. The study protocol was approved by the local Ethical Committee and all pregnant women participating signed a written consent form.

Study protocol

Baseline characteristics and perinatal outcome

Maternal epidemiological and obstetric parameters were collected by interview and review of medical records including maternal age, body mass index, ethnicity, socioeconomic status (illiterate or only primary educational level were considered low socioeconomic status), smoking status and illicit substance abuse (heroin, cocaine or cannabis) during pregnancy and maternal hepatitis C infection. Maternal comorbidity was defined as the presence of chronic hypertension, pregestational diabetes or autoimmune disorder.

Upon delivery, pregnancy and perinatal outcomes were recorded including the presence of gestational diabetes, preeclampsia, preterm delivery (<37 weeks of gestation), gestational age at delivery, mode of delivery, birthweight, small for gestational age, Apgar score, umbilical artery pH, neonatal admission to intensive unit care and perinatal morbidity and mortality. Small-for-gestational age was defined as birthweight below 10^{th} centile according to local standards [18]. Gestational age was calculated according to first trimester crown-rump length (CRL) [19]. Preeclampsia was defined by new onset of hypertension of >140 mmHg systolic or >90 diastolic pressure together with >300 mg proteins in 24 hours urine [20]. Major neonatal morbidity was defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular haemorrhage, periventricular leukomalacia, retinopathy, persistent ductus arteriosus or sepsis. Perinatal mortality was defined by either intrauterine fetal death after 22 weeks of pregnancy or neonatal death within the first 28 days of life [21].

HIV infection parameters

Immunovirological parameters for HIV-infected women were also recorded including mode of HIV transmission, presence of previous opportunistic infections, diagnosis of HIV during pregnancy, months of HIV infection at delivery, $CD4^+$ T cell count (by flow-cytometry) and plasmatic viral load by HIV RNA copy quantification (Amplicor HIV Monitor; Roche Diagnostic Systems, Branchburg, New Jersey, USA) at first trimester and at delivery. We also documented the haemoglobin in the third trimester blood test in order to detect maternal anaemia. All HIV-infected pregnant women were treated with cART. The specific cART regimen during pregnancy was decided by the practitioner following local and international guidelines [22, 23]. Type and duration of antiretroviral treatment before and during pregnancy were recorded.

Fetal standard ultrasound and echocardiography

All pregnancies underwent ultrasonographic examination at 26-32 weeks of gestation using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) with 6-4 MHz linear curved-array and 2-10 MHz phased-array probes including estimated fetal weight, feto-placental Doppler and fetal echocardiography. Ultrasounds were performed by maternal-fetal medicine specialists skilled in fetal echocardiography who were blinded to the particular antiretroviral therapy but not to the HIV status. Fetal weight was estimated measuring the abdominal circumference, head circumference, biparietal diameter and femur length, following the Hadlock formula [24]. Feto-placental Doppler assessment included the measurement of pulsatility index of uterine arteries, umbilical artery and middle cerebral artery as well as the peak velocity of systolic blood flow in the middle cerebral artery according to the previously published methodology [25-28].

A comprehensive two-dimensional, M-mode and Doppler echocardiographic examination was performed initially to assess structural heart integrity and to evaluate cardiac morphometry and function. The cardio-thoracic ratio was calculated as the ratio of the area of the heart to that of the thorax on 2D images from an apical or basal four-chamber view [29]. The presence of pericardial effusion was evaluated at the midventricular level from a transverse four-chamber view and was considered present when it exceeded 2 mm. Ventricular base-to-apex lengths and basal diameters were measured on 2D images from an apical or basal four-chamber view at end-diastole [30]. Left and right ventricular sphericity indices were calculated as base-to-apex length / basal diameter. Myocardial wall thicknesses (septum and left and right free wall) were measured on 2D images from a transverse four-chamber view. Relative wall thickness was calculated as septal wall thickness plus posterior wall thickness divided by left ventricular end diastolic diameter [31]. Left and right atrial areas were measured at maximum distension from a four-chamber view. Left and right ventricular ejection fraction (%) were obtained from M-mode transverse four chamber views using the Teichholz‘s formula. Mitral/tricuspid annular displacement (MAPSE/TAPSE) were assessed by M-mode from an apical or basal four chamber view [32]. Tissue Doppler Imaging was applied in the spectral Doppler mode to record systolic (S’) and early diastolic (E’) peak velocities at mitral (septal) and tricuspid annuli from an apical or basal four-chamber view and measured in real time [33]. Atrioventricular flows were obtained from a basal or apical four-chamber view, placing the pulsed Doppler sample volume at the tip of atrioventricular valve leaflets. Right and left E/A ratios were calculated by dividing early ventricular filling (E wave) by late ventricular filling (A-wave). Left isovolumic and ejection times were measured from a single Doppler spectrum including mitral inflow and aortic outflow as previously described [34, 35].

Left isovolumic contraction time (ICT) was measured from the closure of the mitral valve to the opening of the aortic valve. Left ejection time (ET) was measured from the opening to the closure of the aortic valve. Left isovolumic relaxation time (IRT) was measured from the closure of the aortic valve to the opening of the mitral valve [36].

Statistical analysis

Data are presented as mean (standard deviation), median (interquartile range) or number (percentage) as appropriate. Normal distribution of variables was checked using Kolmogorov-Smirnov test. Values of annular peak velocity measured by TDI were chosen to calculate sample size as a representative parameter of cardiac dysfunction previously described in fetuses [33, 37]. On this basis, sample size was calculated to allow observation of a difference of 0,55 cm/s in systolic peak velocity (S') at mitral (septal) valve as compared to controls. For a power of 80% and an α risk of 0.05, a minimum of 40 subjects per study group (HIV-infected pregnancies) and 80 per control group (HIV-uninfected pregnancies) were required. We included 42 and 84 fetuses respectively.

Differences on baseline and perinatal characteristics between the study groups were assessed using chi-square, t-test or Mann-Whitney as appropriate. Differences on cardiovascular parameters were assessed by using multivariate regression analysis adjusting by the potential baseline and perinatal confounders detected in the univariate analysis. In order to assess the potential factors associated with fetal cardiac changes, the exposed group was subdivided taking into account the most relevant cardiac findings selected from a principal component analysis (details in Supplementary material), and comparisons among these two groups were assessed by chi-square, t-test or Mann-Whitney as appropriate. SPSS Statistics 19 (IBM) was used for the statistical

analysis. All reported P-values are two-sided. The level of significance was set at 0.05 for all the statistical tests.

Results

Baseline and perinatal characteristics

A total of 42 HIV-infected pregnant women and 84 non HIV-infected pregnant women were included (the acceptance rates to participate in the study were 100% and 92% respectively). Baseline and perinatal characteristics of the study groups are shown in Table 1. Baseline characteristics where similar among groups, with the exception of higher prevalence of black ethnicity, toxics exposure (smoking and illicit substance use during pregnancy) and hepatitis C among the HIV-infected women as compared to non-infected women.

As expected, HIV pregnancies presented a worse perinatal outcome including higher prevalence of preterm delivery, small-for-gestational age, caesarean section and admission to neonatal intensive care unit. No perinatal transmission of HIV occurred.

HIV infection parameters

HIV infection characteristics of the HIV-infected pregnancies are described in table 2. Most HIV infections were diagnosed and were receiving cART before pregnancy (88.1% and 76.2%, respectively), and all of them received combined antiretroviral treatment during pregnancy. The main cART regimens -decided according to the practitioner practice- included two nucleoside reverse transcriptase inhibitor (NRTI) and one non-nucleoside reverse transcriptase inhibitors (NNRTI) or one boosted protease inhibitor (PI). Regarding NRTI, the most common combinations during pregnancy were ZDV+Lamivudine (47.6%); Emtricitabine+Tenofovir (35.7%), and Abacavir+Lamivudine (21.4%). The mean CD4 cell count throughout pregnancy was

>500 cells/µl, and 73.8% of patients at first trimester and 95.2% at third trimester had undetectable viral load.

Fetal standard ultrasound and echocardiography

Gestational age at scan, estimated fetal weight and feto-placental Doppler were similar between the study groups (Table 3). Fetuses from HIV mothers showed larger hearts (increased cardio-thoracic ratio) with thicker myocardial walls and more spherical and smaller left ventricular cavities and atria as compared to controls. In addition, 35% of fetuses from HIV-infected pregnancies presented pericardial effusion. All cases of pericardial effussion were mild (2-5 mm). Fetuses from HIV mothers also showed signs of systolic and diastolic dysfunction with decreased mitral S' and increased left isovolumic contraction and relaxation time as compared to controls. Most fetal cardiac changes remained significant after adjustment by maternal black ethnicity, smoking and other toxics during pregnancy, preterm delivery and small for gestational age. Fetal echocardiographic results were similar among male and female offsprings (data not shown).

Association of fetal echocardiography with baseline and perinatal characteristics

Principal component analysis identified myocardial septal wall thickness as the most representative cardiac morphometric parameter among HIV pregnancies (see Supplementary material). Then, the HIV group was subdivided into fetal cardiac hypertrophic (septal wall thickness $\geq$ 4.5 mm that corresponds to the 95th centile reported in normal fetuses [38] and the 75th centile of our cases) and non-hypertrophic (< 4.5 mm) phenotype in order to evaluate its association with baseline and perinatal factors. Characteristics of these fetal cardiac phenotypic groups are described in table 4.

The use of ZDV during pregnancy, mainly from the first trimester was significantly associated with the hypertrophic fetal heart (Figure 1). No other maternal baseline characteristic, pregnancy and perinatal outcome or HIV infection parameter neither the use of other cART regimens were related to the fetal cardiac hypertrophic phenotype.

Discussion

Fetuses from HIV-infected pregnant women presented signs of cardiac concentric hypertrophy *in utero* which was significantly associated to maternal treatment with ZDV. This is the first study in the cART era to assess the association of antiretroviral treatment with fetal cardiac remodelling.

Fetuses exposed to maternal HIV and cART presented larger hearts, smaller and more spherical left cavities and increased wall thickness which is consistent with a pattern of concentric hypertrophy. In addition, 35% of the cases presented mild pericardial effusion and most of them showed signs of subclinical systolic and diastolic dysfunction. These results are partially in agreement with previously reported data in HIV-exposed fetuses. Hornberger *et al.* reported reduced left ventricular cavities and increased wall thickness but with preserved cardiac function in HIV-exposed fetuses [9]. However, this study was conducted in the pre cART era, including infected and non-infected fetuses with only one third of mothers receiving ZDV. A more recent study evaluated cardiac function in 29 fetuses from HIV-infected pregnant women under cART showing prolonged diastolic time with preserved shortening fraction and E/A ratios [10]. They also evaluated cardiac morphometry by means of mitral, tricuspid, aortic and pulmonary valves diameters, and no changes were observed, which is in line with our findings. In the present study, we further evaluated ventricular sphericity and

wall thickness, cardio-thoracic ratio and highly sensitive myocardial imaging techniques such as tissue Doppler which allowed us to ascertain with higher accuracy a pattern of cardiac concentric hypertrophy with systolic and diastolic dysfunction in non HIV-infected fetuses under cART.

From a pathophysiological point of view, the cause of cardiac remodelling in HIV exposed non-infected fetuses is unclear. Although fetal cardiac remodelling has been previously described in other obstetrical conditions such as small-for-gestational age fetuses, prematurity or preeclampsia [14-17] in the present study, we could not demonstrate any association between fetal cardiac changes and maternal or perinatal factors in our population. The only significant association was found with the use of ZDV -above all and from the first trimester-. This finding may suggest direct fetal cardiac toxicity. The role of ZDV in cardiac function among HEU children has been previously evaluated with conflicting results. The P^2C^2 HIV study stated that prophylaxis with ZDV monotherapy is not related to increased risk of cardiac abnormalities in these children [39]. Conversely, a secondary analysis of 50 children from the PRIMEVA study demonstrated mild but significant cardiac changes in 1 year-old girls exposed to cART containing ZDV from 26 weeks of pregnancy. Finally, Lipshultz et al. recently described different associations between specific antiretroviral exposures and different echocardiographic findings among 3-5 year-old HEU children from the PHACS, and exposure to ZDV was found to be related to ventricular wall thickness [7]. These controversial results could be explained by the use of ZDV monotherapy or the late initiation of ZDV in the third trimester, together with the fact that cardiac assessment was conducted postnatally with a considerable time lapse between the exposure and the echocardiography. In contrast with previous studies, our

study was set up in a single centre modern cohort, mostly treated with cART from the first trimester, and the impact of antiretroviral treatment in cardiac function has been assessed directly in the fetus, concurrently with the maternal exposure to the presumptive toxic drugs.

From a clinical point of view, Spanish and international guidelines [22, 23] consider ZDV as one of the preferred NRTI when starting or continuing cART during pregnancy due to its long experience of use, while ZDV is no longer considered a first choice treatment in adult due to its toxic profile [40]. It is unquestionable that the benefits of cART in controlling perinatal transmission outweigh the risks. Nevertheless, were our results to be confirmed, they support the possibility of considering safer alternative cART regimens during pregnancy, mainly during the first trimester in order to guarantee the prevention of perinatal transmission with the fewest adverse effects.

The clinical relevance of cardiac remodelling in the offspring of HIV women remains to be elucidated. Concerning childhood, previous studies suggested cardiac dysfunction and reduced ventricular cavities with controversial results on myocardial thickness in HEU children [6, 7]. Some authors have proposed that the observed cardiac changes in HEU children are probably not clinically relevant [7, 10]. However, for several conditions such as obesity and hypertension [41, 42], cardiac remodelling in childhood is considered a precursor of clinical heart failure [43] associated with incident cardiovascular disease and mortality later in life [44-46]. Therefore, we believe that the evidence of cardiac remodelling in HIV non-infected exposed fetuses and children warrants the need for long-term follow-up studies to assess the long-term clinical impact of these changes.

Some strengths and limitations have to be acknowledged in this study. In the present work we have succeeded in conducting a comprehensive fetal cardiac evaluation in a well-described cohort. This fact has made it possible to identify the factors associated with fetal cardiac changes. This is the first study in the cART era to assess the impact of antiretroviral treatment on cardiac function during fetal life. The fact of being closer to the insult by studying the fetuses in cART exposed HIV-infected mothers has enabled us to find stronger associations with ZDV exposure during pregnancy. On the other hand, we acknowledge the small sample size may have underestimated other factors potentially associated with fetal cardiac changes in exposed fetuses and might limit the interpretation of the multiple comparisons performed in this study. We also acknowledge that our study might be underpowered to detect differences among the subgroups and the results should be confirmed in larger studies. Secondly, although we tried to adjust our results according to confounders, we acknowledge the existence of residual potential confounders and recognize that the influence of maternal factors associated to the HIV infection (such as black ethnicity or comorbidities) could not be completely ruled out. Due to the fact that all HIV-infected pregnant women were treated with cART makes it difficult to discriminate between potential cardiac effects of maternal HIV infection and fetal exposure to antiretroviral agents. Future studies are warranted to try to elucidate the still unexplained issues and describe the intrinsic mechanisms related to cardiac remodelling in fetuses and children from HIV-infected mothers.

In conclusion, our data demonstrate the presence of fetal cardiac remodelling in HIV pregnancies leading to an impaired systolic and diastolic function already *in utero*. These changes were significantly associated with maternal treatment with ZDV. The

existent evidence of cardiac remodelling in non HIV-infected fetuses under cART warrants future studies to evaluate potential long-term cardiovascular consequences and risks later in life.

ACKNOWLEDGEMENTS

This study was supported by grants from Instituto de Salud Carlos III y Ministerio de Economia y Competitividad (PI12/00851, PI14/00226) cofinanciado por el Fondo Europeo de Desarrollo Regional de la Unión Europea "Una manera de hacer Europa", Spain; Obra Social la Caixa; Cerebra Foundation for the Brain Injured Children, Carmarthen Wales, UK. Laura García-Otero was supported by a grant (Premi Emili Letang 2014) from Hospital Clinic, Barcelona, Spain.

REFERENCES

1. Townsend CL, Byrne L, Cortina-Borja M, Thorne C, de Ruiter A, Lyall H, *et al.* **Earlier initiation of ART and further decline in mother-to-child HIV transmission rates, 2000-2011**. *AIDS* 2014,28:1049-1057.
2. Govender T, Coovadia H. **Eliminating mother to child transmission of HIV-1 and keeping mothers alive: recent progress**. *J Infect* 2014,68 Suppl 1:S57-62.
3. Kerr SJ, Puthanakit T, Vibol U, Aurpibul L, Vonthanak S, Kosalaraksa P, *et al.* **Neurodevelopmental outcomes in HIV-exposed-uninfected children versus those not exposed to HIV**. *AIDS Care* 2014,26:1327-1335.
4. Afran L, Garcia Knight M, Nduati E, Urban BC, Heyderman RS, Rowland-Jones SL. **HIV-exposed uninfected children: a growing population with a vulnerable immune system?** *Clin Exp Immunol* 2013,176:11-22.
5. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starc TJ, Bricker JT, *et al.* **Cardiac dysfunction and mortality in HIV-infected children: The Prospective P2C2 HIV Multicenter Study. Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV Infection (P2C2 HIV) Study Group**. *Circulation* 2000,102:1542-1548.
6. Lipshultz SE, Shearer WT, Thompson B, Rich KC, Cheng I, Orav EJ, *et al.* **Cardiac effects of antiretroviral therapy in HIV-negative infants born to HIV-positive mothers: NHLBI CHAART-1 (National Heart, Lung, and Blood Institute Cardiovascular Status of HAART Therapy in HIV-Exposed Infants and Children cohort study)**. *J Am Coll Cardiol* 2011,57:76-85.
7. Lipshultz SE, Williams PL, Zeldow B, Wilkinson JD, Rich KC, van Dyke RB, *et al.* **Cardiac effects of in-utero exposure to antiretroviral therapy in HIV-uninfected children born to HIV-infected mothers**. *AIDS* 2015,29:91-100.
8. Sibiude J, Le Chenadec J, Bonnet D, Tubiana R, Faye A, Dollfus C, *et al.* **In Utero Exposure to Zidovudine and Heart Anomalies in the ANRS French Perinatal Cohort and the Nested PRIMEVA Randomized Trial**. *Clin Infect Dis* 2015,61:270-280.
9. Hornberger LK, Lipshultz SE, Easley KA, Colan SD, Schwartz M, Kaplan S, *et al.* **Cardiac structure and function in fetuses of mothers infected with HIV: the prospective PCHIV multicenter study**. *Am Heart J* 2000,140:575-584.
10. De la Calle M, Rodriguez R, Deiros L, Bartha JL. **Fetal cardiac biometry and function in HIV-infected pregnant women exposed to HAART therapy**. *Prenat Diagn* 2014.
11. Lopez M, Palacio M, Gonce A, Hernandez S, Barranco FJ, Garcia L, *et al.* **Risk of intrauterine growth restriction among HIV-infected pregnant women: a cohort study**. *Eur J Clin Microbiol Infect Dis* 2015,34:223-230.
12. Lopez M, Figueras F, Hernandez S, Lonca M, Garcia R, Palacio M, Coll O. **Association of HIV infection with spontaneous and iatrogenic preterm delivery: effect of HAART**. *AIDS* 2012,26:37-43.
13. Suy A, Martinez E, Coll O, Lonca M, Palacio M, de Lazzari E, *et al.* **Increased risk of pre-eclampsia and fetal death in HIV-infected pregnant women receiving highly active antiretroviral therapy**. *AIDS* 2006,20:59-66.
14. Crispi F, Bijnens B, Figueras F, Bartrons J, Eixarch E, Le Noble F, *et al.* **Fetal growth restriction results in remodeled and less efficient hearts in children**. *Circulation* 2010,121:2427-2436.

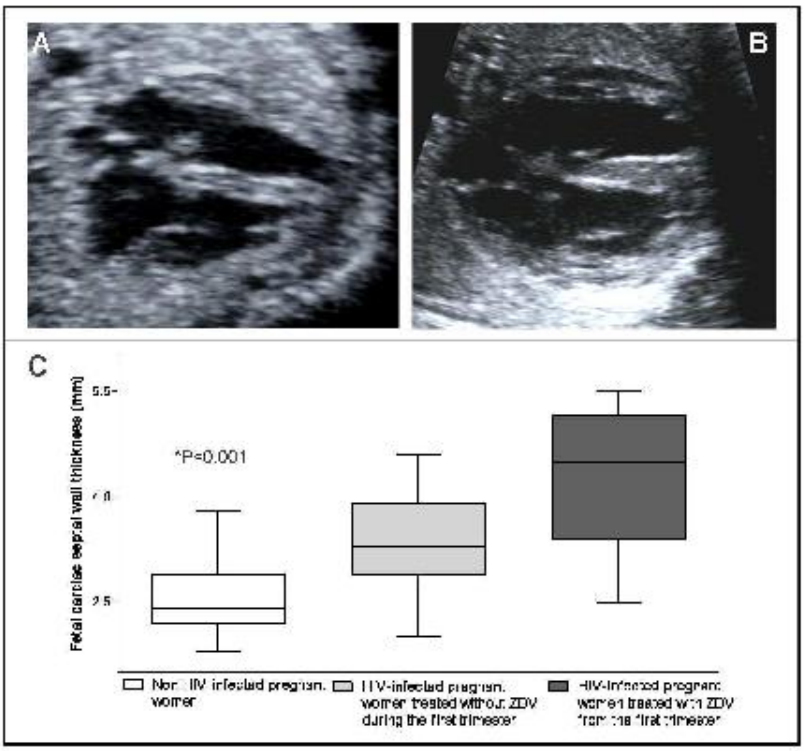
15. Demicheva E, Crispi F. **Long-term follow-up of intrauterine growth restriction: cardiovascular disorders**. *Fetal Diagn Ther* 2014,36:143-153.
16. Romero R, Espinoza J, Goncalves LF, Gomez R, Medina L, Silva M, *et al.* **Fetal cardiac dysfunction in preterm premature rupture of membranes**. *J Matern Fetal Neonatal Med* 2004,16:146-157.
17. Balli S, Kibar AE, Ece I, Oflaz MB, Yilmaz O. **Assessment of fetal cardiac function in mild preeclampsia**. *Pediatr Cardiol* 2013,34:1674-1679.
18. Figueras F, Meler E, Iraola A, Eixarch E, Coll O, Figueras J, *et al.* **Customized birthweight standards for a Spanish population**. *Eur J Obstet Gynecol Reprod Biol* 2008,136:20-24.
19. Robinson HP. **Sonar measurement of fetal crown-rump length as means of assessing maturity in first trimester of pregnancy**. *Br Med J* 1973,4:28-31.
20. Walker JJ. **Pre-eclampsia**. *Lancet* 2000,356:1260-1265.
21. **World Health Organization. International Statistical Classification of Diseases and Related Health Problems10th Revision (ICD-10)**. 2015.
22. **Guidelines for HIV control during pregnancy, delivery and prevention of Mother to Child Transmission. GESIDA/Spanish National AIDS study group. http://www.gesida-seimc.org/guias_clinicas.php?mn_MP=406&mn_MS=407. (Last updated March 22, 2013)**.
23. **Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health and Interventions to Reduce Perinatal HIV Transmission in the United States. http://aidsinfo.nih.gov/guidelines/html/3/perinatal-guidelines/0/ (Last updated March 28, 2014)**.
24. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. **Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements**. *Radiology* 1984,150:535-540.
25. Gomez O, Figueras F, Fernandez S, Bennasar M, Martinez JM, Puerto B, Gratacos E. **Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation**. *Ultrasound Obstet Gynecol* 2008,32:128-132.
26. Arduini D, Rizzo G. **Normal values of Pulsatility Index from fetal vessels: a cross-sectional study on 1556 healthy fetuses**. *J Perinat Med* 1990,18:165-172.
27. Hecher K, Campbell S, Snijders R, Nicolaides K. **Reference ranges for fetal venous and atrioventricular blood flow parameters**. *Ultrasound Obstet Gynecol* 1994,4:381-390.
28. Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ, Jr., *et al.* **Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses**. *N Engl J Med* 2000,342:9-14.
29. Paladini D, Chita SK, Allan LD. **Prenatal measurement of cardiothoracic ratio in evaluation of heart disease**. *Arch Dis Child* 1990,65:20-23.
30. Rychik J, Ayres N, Cuneo B, Gotteiner N, Hornberger L, Spevak PJ, Van Der Veld M. **American Society of Echocardiography guidelines and standards for performance of the fetal echocardiogram**. *J Am Soc Echocardiogr* 2004,17:803-810.

31. Foppa M, Duncan BB, Rohde LE. **Echocardiography-based left ventricular mass estimation. How should we define hypertrophy?** *Cardiovasc Ultrasound* 2005,3:17.
32. Germanakis I, Pepes S, Sifakis S, Gardiner H. **Fetal longitudinal myocardial function assessment by anatomic M-mode**. *Fetal Diagn Ther* 2012,32:65-71.
33. Comas M, Crispi F, Gomez O, Puerto B, Figueras F, Gratacos E. **Gestational age- and estimated fetal weight-adjusted reference ranges for myocardial tissue Doppler indices at 24-41 weeks' gestation**. *Ultrasound Obstet Gynecol* 2011,37:57-64.
34. Cruz-Martinez R, Figueras F, Bennasar M, Garcia-Posadas R, Crispi F, Hernandez-Andrade E, Gratacos E. **Normal reference ranges from 11 to 41 weeks' gestation of fetal left modified myocardial performance index by conventional Doppler with the use of stringent criteria for delimitation of the time periods**. *Fetal Diagn Ther* 2012,32:79-86.
35. Hernandez-Andrade E, Figueroa-Diesel H, Kottman C, Illanes S, Arraztoa J, Acosta-Rojas R, Gratacos E. **Gestational-age-adjusted reference values for the modified myocardial performance index for evaluation of fetal left cardiac function**. *Ultrasound Obstet Gynecol* 2007,29:321-325.
36. Mahajan A, Henry A, Meriki N, Hernandez-Andrade E, Crispi F, Wu L, Welsh AW. **The (Pulsed-Wave) Doppler Fetal Myocardial Performance Index: Technical Challenges, Clinical Applications and Future Research**. *Fetal Diagn Ther* 2015,38:1-13.
37. Comas M, Crispi F, Cruz-Martinez R, Figueras F, Gratacos E. **Tissue Doppler echocardiographic markers of cardiac dysfunction in small-for-gestational age fetuses**. *Am J Obstet Gynecol* 2011,205:57 e51-56.
38. Patchakapat L, Uerpairojkit B, Wacharaprechanont T, Manotaya S, Tanawattanacharoen S, Charoenvidhya D. **Interventricular septal thickness of Thai fetuses: at 32 to 35 weeks' gestation**. *J Med Assoc Thai* 2006,89:748-754.
39. Lipshultz SE, Easley KA, Orav EJ, Kaplan S, Starc TJ, Bricker JT, *et al.* **Absence of cardiac toxicity of zidovudine in infants. Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV Infection Study Group**. *N Engl J Med* 2000,343:759-766.
40. **Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/AdultandAdolescentGL.pdf. Section accessed [July 2015]**.
41. Lai CC, Sun D, Cen R, Wang J, Li S, Fernandez-Alonso C, *et al.* **Impact of long-term burden of excessive adiposity and elevated blood pressure from childhood on adulthood left ventricular remodeling patterns: the Bogalusa Heart Study**. *J Am Coll Cardiol* 2014,64:1580-1587.
42. Hietalampi H, Pahkala K, Jokinen E, Ronnemaa T, Viikari JS, Niinikoski H, *et al.* **Left ventricular mass and geometry in adolescence: early childhood determinants**. *Hypertension* 2012,60:1266-1272.
43. Gjesdal O, Bluemke DA, Lima JA. **Cardiac remodeling at the population level--risk factors, screening, and outcomes**. *Nat Rev Cardiol* 2011,8:673-685.
44. Bluemke DA, Kronmal RA, Lima JA, Liu K, Olson J, Burke GL, Folsom AR. **The relationship of left ventricular mass and geometry to incident cardiovascular events: the MESA (Multi-Ethnic Study of Atherosclerosis) study**. *J Am Coll Cardiol* 2008,52:2148-2155.

45. Krumholz HM, Larson M, Levy D. **Prognosis of left ventricular geometric patterns in the Framingham Heart Study**. *J Am Coll Cardiol* 1995,25:879-884.
46. Lorell BH, Carabello BA. **Left ventricular hypertrophy: pathogenesis, detection, and prognosis**. *Circulation* 2000,102:470-479.

Figure 1: Relationship between fetal cardiac remodelling and maternal use of zidovudine (ZDV).

Figure 1: Relationship between fetal cardiac remodelling and maternal use of zidovudine (ZDV).



Upper panel: Fetal echocardiographic images illustrating the concentric hypertrophic phenotype in HIV-infected pregnancies (B) as compared to non HIV-infected pregnancies (A).
Lower panel (C): Box and whiskers graph of fetal cardiac septal wall thickness among non HIV-infected and HIV-infected subdivided according to the use of ZDV during the first trimester. A significant trend of thicker septal wall among the categories was observed. HIV-infected women treated with ZDV from the first trimester presented significantly thicker septal myocardial walls compared to HIV-infected women treated without ZDV during the first trimester or non HIV-infected women. *P-value calculated by ANOVA test among the three categories. Boxes in the graphic represent 25th, 50th (band inside the box) and 75th centile values. Whiskers express dispersion (1.5 x interquartilic range).

Table 1. Baseline characteristics and perinatal outcome of the study populations.

	HIV-infected pregnancies (N=42)	Non HIV-infected pregnancies (N=84)	P-Value
Maternal characteristics			
Age (yr); mean ± SD	33.1 ± 6.3	33.4 ± 4.7	0.786
BMI (kg/m^2) mean ± SD	23.8 ± 4.4	22.7 ± 3.7	0.178
Black ethnicity; n (%)	11 (26.2)	1 (1.2)	<0.001
Low socioeconomic status; n (%)	7 (16.7)	16 (19)	0.744
Smoking during pregnancy; n (%)	16 (38.1)	17 (20.2)	0.032
Illicit substance use during pregnancy; n (%)	3 (7.1)	0	0.013
Hepatitis C infection; n (%)	7 (16.6)	0	<0.001
Maternal comorbidity; n (%)	2 (4.76)	0	0.109
Maternal haemoglobin concentration during pregnancy (g/dl); mean ± SD	11.3 ± 1.1	11.5 ± 1.1	0.334
Pregnancy and perinatal outcome			
Gestational diabetes; n (%)	3 (7.1)	1 (1.2)	0.072
Preeclampsia; n (%)	1 (2.4)	1 (1.2)	0.614
Small-for-gestational age; n (%)	6 (14.3)	1 (1.2)	0.002
Preterm delivery; n (%)	6 (14.3)	1 (1.2)	0.002
Gestational age at delivery (weeks); mean ± SD	38.3 ± 3.1	39.9 ± 1.5	<0.001
Cesarean section; n (%)	25 (59.5)	15 (17.9)	<0.001
Birthweight (g); mean ± SD	3054 ± 694	3352 ± 424	0.040
5 minutes Apgar score; median (range)	10 (4-10)	10 (8-10)	0.067
Hospitalization in NICU; n (%)	9 (21.4)	5 (5.9)	0.008
Major neonatal morbidity; n (%)	1 (2.4)	0	0.336
Perinatal mortality; n (%)	0	0	-

Data are mean ± standard deviation (SD), median (range) or n (percentage) as indicated.
BMI = body mass index. NICU=Neonatal Intensive Unit Care

Table 2. HIV infection parameters among the HIV-infected pregnancies.

	n=42
HIV transmission	
Heterosexual; n (%)	33 (78.6)
Injection drug use; n (%)	5 (11.9)
Vertical n (%)	4 (9.5)
Previous opportunistic infection; n (%)	17 (40.5)
HIV diagnosis in pregnancy; n (%)	5 (11.9)
Months of HIV infection at delivery; median (range)	78 (6-324)
Infection parameters during pregnancy	
CD4 cell count at first trimester (cells/µl) ; mean ± SD	546 ± 204
CD4 cell count at delivery (cells/µl) ; mean ± SD	576 ± 220
Viral load <50 copies/ml at first trimester; n (%)	31 (73.8)
Viral load <50 copies/ml at delivery; n (%)	40 (95.2)
Antiretroviral treatment characteristics	
cART before pregnancy; n (%)	32 (76.2)
Weeks of cART before pregnancy; median (range)	38 (0-240)
cART during pregnancy; n (%)	42 (100)
Weeks of cART during pregnancy; median (range)	38.1 (4-41.1)
NRTI during pregnancy; n (%)	42 (100)
Weeks of NRTI during pregnancy; median (range)	36.2 (4.0-40.6)
ZDV during pregnancy; n (%)	19 (45.2)
ZDV first trimester; n (%)	11 (26.8)
Weeks of ZDV during pregnancy; median (range)	29.3 (4-40.1)
Lamivudine during pregnancy; n (%)	30 (71.4)
NNRTI during pregnancy; n (%)	10 (23.8)
Weeks of NNRTI during pregnancy; median (range)	36.2 (4-40.6)
PI during pregnancy; n (%)	33 (78.6)
Weeks of PI during pregnancy; median (range)	36.2 (10-40.3)

Data are mean ± standard deviation (SD); median (range) or n (percentage) as indicated.
HIV= Human Immunodeficiency Virus. cART: Combined antiretroviral treatment; NRTI: Nucleoside reverse transcriptase inhibitors; ZDV: Zidovudine; NNRTI: Non nucleoside reverse transcriptase inhibitors; PI: Protease Inhibitors.

Table 3. Fetal standard ultrasound and echocardiography among the study groups.

Characteristic	HIV-infected pregnancies (N=42)	Non HIV-infected pregnancies (N=84)	Adjusted P-Value*
Gestational age at scan (weeks)	31.3 ± 3.3	31.2 ± 3.5	0.604
Fetal standard ultrasound			
Estimated fetal weight at scan (g)	1787 ± 650	1763 ± 679	0.440
Mean uterine artery pulsatility index	0.82 (0.08)	0.88 (0.23)	0.347
Umbilical artery pulsatility index	1.07 ± 0.24	1.03 ± 0.18	0.340
Middle cerebral artery pulsatility index	2.07 ± 0.47	2.11 ± 0.35	0.698
Middle cerebral artery peak systolic velocity (cm/s)	47.89 ± 14.03	40.42 ± 11.41	0.126
Fetal echocardiography			
Cardio-thoracic ratio	0.29 ± 0.04	0.27 ± 0.04	0.049
Pericardial effusion; n (%)	15 (35.7)	0	<0.001
Cardiac morphometry			
Left ventricular sphericity index	1.72 ± 0.32	1.83 ± 0.33	0.043
Right ventricular sphericity index	1.47 ± 0.42	1.62 ± 0.32	0.058
Left ventricular transverse diameter (mm)	10.8 ± 2.28	12.3 ± 2.54	0.033
Right ventricular transverse diameter(mm)	12.4 ± 2.66	12.4 ± 2.45	0.520
Left free wall thickness (mm)	3.55 ± 0.61	2.89 ± 0.77	<0.001
Septal wall thickness (mm)	3.56 ± 0.88	2.75 ± 0.77	0.002
Right free wall thickness (mm)	3.60 ± 1.09	2.96 ± 0.55	0.025
Relative wall thickness	3.78 ± 0.81	2.99 ± 0.82	0.001
Left atrial area (cm^2)	1.53 ± 0.62	1.70 ± 0.67	0.015
Right atrial area (cm^2)	1.02 ± 0.49	1.51 ± 0.57	0.632
Systolic function			
Left ejection fraction (%)	73 ± 10.39	73.5 ± 9.47	0.759
Right ejection fraction (%)	71.2 ± 10.29	71.5 ± 8.41	0.230
Mitral ring displacement (mm)	5.52 ± 1.29	5.41 ± 1.18	0.947
Tricuspid ring displacement (mm)	7.01 ± 1.42	7.10 ± 1.31	0.874
Mitral S' (cm/s)	5.85 ± 0.77	6.25 ± 0.97	0.007
Tricuspid S' (cm/s)	7.96 ± 1.18	7.68 ± 1.01	0.126
Diastolic function			
Mitral E/A ratio	0.76 ± 0.15	0.73 ± 0.09	0.538
Tricuspid E/A ratio	0.72 ± 0.09	0.73 ± 0.10	0.408
Mitral E' (cm/s)	6.08 ± 0.86	6.37 ± 1.09	0.202
Tricuspid E' (cm/s)	7.96 ± 1.18	7.68 ± 1.01	0.194
Heart rate and timing			
Heart rate (bpm)	138 ± 9	139 ± 9	0.972
Left isovolumic contraction time (ms)	37.1 ± 8.13	30.2 ± 6.87	<0.001
Left isovolumic relaxation time (ms)	52.2 ± 8.91	45.5 ± 7.98	<0.001
Left ejection time (ms)	162 ± 17.71	172 ± 9.73	0.018

Data are mean ± standard deviation (SD) or n (percentage)

HIV= Human Immunodeficiency Virus. bpm=beats per minute. S' = systolic annular peak velocity. E = ventricular inflow in early diastole. A = ventricular inflow during atrial contraction. E' = annular peak velocity in early diastole.

* P-value calculated by linear regression adjusted by black ethnicity, smoking, toxics during pregnancy, preterm delivery and small-for-gestational age.

Table 4. Baseline and perinatal characteristics in HIV-infected pregnancies subclassified by the presence of fetal cardiac hypertrophy.

Characteristic	Hypertrophic fetal heart (n=12)	Non-hypertrophic fetal heart (n=30)	P-value*
Maternal characteristics			
Age (yr) ; mean ± SD	33.58 ± 6.58	32.97 ± 6.40	0.781
BMI (kg/m^2) ; mean ± SD	23.21 ± 2.68	23.99 ± 4.89	0.652
Black ethnicity; n (%)	4 (33.3)	7 (23.3)	0.505
Low socioeconomic status; n (%)	2 (16.5)	5 (16.7)	0.879
Smoking during pregnancy; n (%)	3 (25)	13 (43.3)	0.269
Illicit substance abuse during pregnancy; n (%)	0	3 (10)	0.852
Injection drug use; n (%)	1 (8.3)	4 (13.3)	0.651
Maternal comorbidity; n (%)	1 (8.3)	1 (3.3)	0.066
Pregnancy and perinatal characteristics			
Gestational diabetes; n (%)	0	3 (10)	0.256
Preeclampsia; n (%)	0	1 (3.3)	0.522
Preterm delivery; n (%)	1 (8.3)	5 (16.7)	0.655
Small-for-gestational age; n (%)	2 (16.7)	4 (13.3)	0.780
HIV infection parameters			
Previous opportunistic infection; n (%)	7 (58.3)	10 (33.3)	0.174
Months of HIV infection at delivery; median (range)	93 (21-273)	60 (6-324)	0.427
HIV diagnosis in pregnancy; n (%)	0	5 (16.7)	0.132
CD4 cell count at first trimester (cells/µl); mean ± SD	499.22 ± 173.92	563.68 ± 214.75	0.425
CD4 cell count at delivery (cells/µl); mean ± SD	578.20 ± 209.69	563.71 ± 231.58	0.866
Viral load <50 copies/ml at first trimester; n (%)	10 (83.3)	22 (81.5)	0.889
Viral load <50 copies/ml at delivery; n (%)	12 (100)	28 (93.3)	0.359
HIV treatment characteristics			
cART before pregnancy; n (%)	10 (83.3)	22 (73.3)	0.492
cART during pregnancy; n (%)	12 (100)	30 (100)	-
Weeks of cART during pregnancy; median (range)	39 (4-40.4)	37.6 (12.1-41.1)	0.365
NRTI during pregnancy; n (%)	12 (100)	30(100)	-
ZDV during pregnancy; n (%)	9 (75)	10 (33.3)	0.014
ZDV first trimester; n (%)	6 (50)	5 (16.7)	0.049
Weeks of ZDV during pregnancy; median (range)	31.7 (0-40.1)	0 (0-39.4)	0.004
Lamivudine; n (%)	9 (75)	21 (70)	0.746
ZDV + Lamivudine; n (%)	9 (75)	10 (33.3)	0.025
Abacavir + Lamivudine; n (%)	0 (0)	9 (30)	0.041
Tenofovir + Emtricitabine; n (%)	3 (25)	11 (36.6)	0.485
NNRTI during pregnancy; n (%)	4 (33.3)	5 (16.7)	0.433
PI during pregnancy; n (%)	8 (66.7)	25 (83.3)	0.234

HIV= Human Immunodeficiency Virus.
Data are mean ± standard deviation (SD); median (range) or n (percentage) as indicated.
cART= Combined antiretroviral treatment. NRTI= Nucleoside reverse transcriptase inhibitors. ZDV= Zidovudine. NNRTI= Non nucleoside reverse transcriptase inhibitors. PI= Protease Inhibitors.
* P-value calculated by linear regression.