Cognitive and language outcomes in HIV-uninfected infants exposed to combined antiretroviral therapy *in utero* and through extended breast-feeding

Mary S. Ngoma[a,b], Jennifer A. Hunter[c], Jessica A. Harper[c], Paige T. Church[c], Scholastica Mumba[b], Mulapati Chandwe[b], Hélène C.F. Côté[d,e,f], Arianne Y.K. Albert[e], Mary-Lou Smith[c,g], Chisomo Selemani[a], Paul A. Sandstrom[h], Lucas Bandenduck[c], Utsile Ndlovu[c], Sara Khan[c], Lina Roa[c] and Michael S. Silverman[c,i]

Objective: To determine whether there is a higher risk for cognitive or language delay among HIV-exposed uninfected (HEU) children exposed to cART (zidovudine/lamivudine/lopinavir/ritonavir) *in utero* and through 1 year of breast-feeding (World health Organization Option B+), compared with the control children born to HIV-uninfected mothers.

Design: This is a double cohort study from Lusaka, Zambia.

Methods: HEU ($n = 97$) and control (n = 103) children aged 15–36 months were assessed on their early nonverbal problem-solving and language skills using the standardized Capute Scales. A score of less than 85 on the Capute Full-Scale Developmental Quotient (FSDQ) was considered indicative of developmental delay and was the primary outcome of interest.

Results: An FSDQ of less than 85 was found in eight (8.3%) of HEU participants and 15 (14.6%) of controls. In univariate logistic regressions, lower income [odds ratio (OR) = 0.93, $P = 0.02$], older infant age (OR = 1.08, $P = 0.03$), lower birth weight (OR = 0.16, $P < 0.001$), and less maternal education (OR = 0.41, $P = 0.047$) were associated with the probability of FSDQ less than 85, whereas Group (control/HEU) was not (OR = 1.88, $P = 0.16$). In the multivariable analysis, only lower birth weight (OR = 0.15, $P < 0.001$) remained associated with FSDQ less than 85.

Conclusions: Our study did not support the presence of an adverse effect on cognitive and language development with prolonged antepartum and postpartum cART e/xposure. Larger studies and studies of older HEU children will be required to confirm these reassuring findings.

AIDS 2014, **28** (Suppl 3):S323–S330

Keywords: breast-feeding, Capute Full Scale Developmental Quotient, combination antiretroviral therapy exposure, developmental delay, HIV-exposed uninfected

Introduction

Antiretroviral therapies taken by pregnant HIV-positive mothers are very effective for the prevention of mother-to-child transmission (PMTCT) of HIV [1–3]. Many nations have recently scaled up provision of antiretroviral therapy to HIV-infected pregnant women in accordance with the Millennium Development Goals

[a]University of Zambia School of Medicine, [b]Department of Pediatrics, University Teaching Hospital, Lusaka, Zambia, [c]University of Toronto, Toronto, [d]University of British Columbia, [e]Women's Health Research Institute, [f]Centre for Blood Research, Vancouver, [g]The Hospital for Sick Children, Toronto, [h]Public Health Agency of Canada, Ottawa, and [i]Western University, London, Canada.

Correspondence to Dr Michael Silverman MD, FRCP, St Joseph's Health Care, 268 Grosvenor St, London, Ontario, Canada N6A 4V2.

Tel: (416)8777641; e-mail mikesilverman@rogers.com

DOI:10.1097/QAD.0000000000000357

[4] and the 2011 United Nations Political Declaration on HIV and AIDS [5]. The 2013 World Health Organization (WHO) guidelines on PMTCT of HIV recommend combination antiretroviral therapy (cART) during pregnancy to reduce maternal viral load and prevent viral drug resistance [6]. In countries where no acceptable, feasible, affordable, sustainable, or well tolerated alternative to breast-feeding exists, it is recommended that women continue cART during exclusive breast-feeding, regardless of CD4 cell count, until the infant is 6 months' old. This was subsequently extended to include continued breast-feeding with the addition of complementary foods until weaning at 12 months of age [6]. For cART in pregnancy, the US Department of Health and Human Services guidelines [7] recommend the use of two nucleoside reverse transcriptase inhibitors (NRTIs) with either a nonnucleoside reverse transcriptase inhibitor (NNRTI) or one of two boosted protease inhibitor(s) At the time of this study, the preferred regimen in pregnancy was zidovudine (ZDV)/lamivudine (3TC) and lopinavir/ritonavir [7].

Despite the substantial benefit related to the PMTCT of HIV, exposure to cART has been associated with several undesirable pregnancy outcomes including an increased risk of premature delivery [8–11]. Published results are mixed with respect to low birth weight and still births in cART-treated pregnancies [8,11]. Perinatal exposure to ZDV has been associated with transient anemia in the neonate [12]. A link with mitochondrial dysfunction is suggested by some but not all cohorts [13–15].

Several studies have reported developmental delay in HIV-infected infants treated with cART when compared with HIV-exposed uninfected (HEU) infants [16–18]; however, it has been difficult to clarify how much of this effect may be related to HIV infection of the brain versus a potential adverse drug effect of cart [17]. In fact, early cART may be protective for the neurological development of HIV-infected infants [19].

Although a small Canadian study suggested that HEU infants exposed to perinatal cART ($n = 39$) had lower development scores than nonexposed infants born to mothers infected with hepatitis C virus ($n = 24$), this difference became nonsignificant after controlling for maternal substance use [20]. Other studies suggest that the neurocognitive development of HEU infants is not negatively impacted by antepartum antiretroviral exposure [18,21–23], although two studies questioned the delayed development of language with in-utero Atazanavir exposure [24,25]. The impact of a combination of antepartum cART exposure followed by prolonged postpartum exposure during breast-feeding is unknown, yet the number of exposed infants is large and rising. Rapid and essential brain development occurs during the first year of life [26]. As ZDV and 3TC (but not lopinavir/ritonavir) are well secreted into breast milk [27–29] and cross the blood-brain barrier, the impact of long-term exposure to NRTIs on the developing infant's brain is of significant concern.

To better understand the potential neurodevelopmental effects of such exposure, we conducted neurocognitive assessments on HEU infants born to HIV-infected women previously enrolled in the Aluvia Study in Lusaka, Zambia [30] who were treated with cART throughout pregnancy and breast-feeding, as well as on infants born to HIV-uninfected women from the same community.

Methods

Between June 2011 and August 2013, 200 children aged 15–36 months were recruited to participate in this double-cohort study of cognitive and language outcomes. All participants lived within a single district of Lusaka, which includes the neighborhoods of Chelstone, Kamanga, and Avondale. Each mother answered a baseline demographic questionnaire and their infant underwent an assessment of cognitive and language development, anthropometric measurements, and biological sampling of finger capillary blood. Cognitive and language development was assessed using the standardized Capute Scales Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS)[31]. Gestational age at birth was determined by recollection of the date of the last menstrual period, as screening ultrasounds were not generally available.

The total time required by the mother and child to perform all of the above assessments was approximately 30–60 min. A stipend to cover the cost of a meal while delayed for testing was offered to all mothers (equivalent to US$6).

HIV-exposed uninfected participants

The Aluvia Study was an open-label, single-arm trial that enrolled cART-naive, HIV seropositive pregnant women with the intent to breast-feed. Detailed inclusion and exclusion criteria for women enrolled in the Aluvia Study can be found in the supplemental materials. HEU children of women enrolled in the Aluvia Study based at Chelstone Clinic were recruited for this study. Mothers received ZDV 300 mg/3TC 150 mg twice daily and lopinavir 200 mg/ritonavir 50 mg (two tablets) twice daily, to start between 14 and 30 weeks of gestation until weaning was complete. A single dose of nevirapine (sdNVP) was given to all mothers in labor and to infants within 48 h postpartum. Infants also received ZDV liquid for 7 days postpartum. Maternal cART was continued during 6 months of exclusive breast-feeding and then 6 months of complementary feeding, with weaning

between 12 and 13 months of age. Mothers remained on cART indefinitely thereafter as per WHO Option B+.

Control participants

Control infants were children of HIV-seronegative mothers living in the same community as the HEU participants. Zambian child health guidelines recommend that children attend under-5 clinics monthly from birth to 5 years for vaccinations and health promotion campaigns. Asymptomatic children were recruited from the local 'under-5' clinics.

Inclusion and exclusion criteria

The inclusion criteria were being between 15 and 36 months for the infant, a minimum maternal age of 15 years, the ability to give informed consent, and to attend follow-up visits with the child if any abnormality was found during testing. Children were included in the HEU group if the infant was documented to be HIV-seronegative, with negative RNA PCR heel prick dried blood spot (DBS) testing. Children were included in the control group if the mother had a screening HIV test during pregnancy with a known negative result. Children were excluded from the study if they had a preexisting known major chronic illness likely to influence neurodevelopment such as congenital anomalies (chromosomal disorders, hydrocephalus, spina bifida, among others), chronic severe hematological or metabolic disorders (sickle cell disease, heart/lung/liver disease, juvenile diabetes, among others), or active tuberculosis. As well, children were excluded if they had an active acute illness (fever, pneumonia, malaria, among others), or were HIV DBS RNA PCR-positive (HEU children), or HIV-seropositive (controls).

Procedure for cognitive assessment

Given the study population's age, a measure of early childhood development was indicated, to assess early developmental skills that tend to be species specific and less impacted by environment [32]. Cognition and language were assessed with the Capute CAT/CLAMS [31]. The CAT measures early nonlanguage-based problem-solving abilities and the CLAMS measures early language-based problem-solving abilities and language comprehension/expression. Items require demonstration and/or parental report [33]. The scale has been well correlated with the cognitive and language aspects of the Bayley Scales of Infant Development-2nd Edition (BSID-II) [34,35] [CAT Development Quotient ($r = 0.582$, $P = 0.0001$), CLAMS Development Quotient ($r = 0.718$, $P = 0.0001$), Full-Scale Developmental Quotient (FSDQ) ($r = 0.742$, $P = 0.0001$)] [35] as well as a neurodevelopmental assessment for risk of neurological complications of AIDS [36]. The Capute Scales was also selected as the primary outcome because of the need for a transportable, time-efficient assessment that could be administered by medical students and/or allied health workers [31], for its established generalizability [34–36], and use in the developing world setting [37–39].

Assessors were allied health professionals or medical students with standardized training in the CAT/CLAMS in a university developmental program. All assessments were administered by a pair of assessors, to enhance standardization of administration. The child's parent(s)/caregiver(s) were permitted to be present for the assessment, which was administered and scored in a standardized fashion [31]. A single local bilingual pediatric nurse provided verbatim translation of the assessor's instructions. Results of all assessments were then reviewed by a Developmental Behavioral Pediatrician based in a university progra. The CAT and CLAMS are scored independently resulting in a CAT Developmental Quotient and a CLAMS Development Quotient, averaged to yield the FSDQ. The Capute Scales are primarily used as an indicator of developmental delay, and a Development Quotient less than 85 is consistent with an abnormal finding indicative of mild to moderate delays in development [31]. All infants who scored less than 85 on the FSDQ were referred for a pediatric consultation and thereafter referral to appropriate specialty services.

Procedure for biological sampling and testing

Following cognitive assessment, all children had finger prick DBS collected and stored for HIV testing. Briefly for HEU children, HIV RNA PCR testing was done using the Gen-Probe Aptima HIV RNA Qualitative Assay (Hologic Inc, Bedford, Massachusetts, USA), whereas in controls, HIV serology was done on the DBS using the Avioq HIV-1 Microelisa system (Avioq Inc, Durham, North Carolina, USA) according to the manufacturer-'s protocol.

Analysis

An FSDQ less than 85 [−1 standard deviation (SD)] was the prespecified primary outcome. Detection of a difference using this approach would reflect subtle, potentially nondisabling differences in development. Covariates analyzed were maternal age (years), maternal education (>primary vs. ≤primary), family income per month (units of 100 000 kwacha), marital status (married vs. single/separated/divorced/widowed), duration of breast-feeding (weeks), infant age (months), infant birth weight (kg), and gestational age at birth (weeks).

Univariate logistic regressions of FSDQ less than 85 on all of the covariates listed above were performed, followed by a multivariable logistic regression including group (HEU vs. controls) and all variables significantly associated with FSDQ less than 85 in the univariate analyses: monthly income, maternal education, infant age, and birth weight. Significance of variables in logistic regressions was determined by a likelihood-ratio test comparing a model containing the variable vs. one with it removed. A P value less than 0.05 suggests that inclusion

of the variable significantly increased the fit of the model. All analyses were carried out in *R* [40].

The logistic regression analyses were repeated for CAT Development Quotient and CLAMS Development Quotient less than 85 individually, using the same covariates in the multivariable models as for FSDQ. Given that lower birth weight may be causally linked to both the group variables (HEU children are more likely to be born preterm) and to the neurodevelopmental outcome, we also conducted sensitivity analyses that excluded birth weight in the multivariable models.

Ethical approval for the study was obtained from the research ethics board of the University of Zambia. All mothers provided informed written consent.

Results

Study participants

A total of 116 women who were enrolled in the Aluvia study were approached to participate in the cognitive assessment substudy and all consented. Among their children, 97 met the inclusion criteria. Nineteen (19) children were excluded because of incomplete testing ($n=6$), not being within age requirements ($n=10$), or unknowingly repeating the assessment ($n=2$). A total of 172 HIV-uninfected women were approached to participate in the study. Twenty refused because of inadequate time available for their participation. Among the 152 HIV-uninfected women consented to participate in the study, four had infants who were not within age requirement and were excluded. A further 45 children were excluded postassessment when it was realized that they had received improper testing by a single assessor. None of the HEU infants were HIV RNA PCR-positive and none of the controls were HIV-seropositive. Therefore, 200 children were included in the analyses (HEU, $n=97$; controls, $n=103$). The maternal and child demographic information is provided in Table 1.

Cognitive outcomes

The CAT, CLAMS, and FSDQ scores are presented in Table 2.

Full-Scale Developmental Quotient

Eight (8.3%) HEU participants and 15 (14.6%) control infants had FSDQ scores of less than 85. In the univariate logistic regressions, lower family income [odds ratio (OR) = 0.93, 95% confidence interval (CI) = 0.82–0.99, $P=0.02$], less maternal education (OR = 0.41, 95% CI = 0.16–0.99, $P=0.047$), older infant age (OR = 1.08, 95% CI = 1.01–1.17, $P=0.03$), and lower birth weight (OR = 0.16, 95% CI = 0.05–0.44, $P<0.001$) were associated with greater odds of FSDQ less than 85, whereas Group (control/HEU) was not (OR = 1.88, 95% CI = 0.77–4.87, $P=0.16$). Gestational age at birth ($P=0.10$), duration of breast-feeding ($P=0.12$), infant sex ($P=0.49$), maternal age ($P=0.5$), and marital status ($P=0.11$) were not significantly associated with the odds of FSDQ less than 85. The ORs of the significant variables suggest that for every increase of 100 000 kwacha in monthly income, the odds of having FSDQ less than 85 are reduced by approximately 7%, that mothers having a secondary education or above reduce the odds of her infants having FSDQ less than 85 by about 59%, that for

Table 1. Maternal and child demographic information.

Variable	HEU, $n=97$	Controls, $n=103$	P value[b] HEU vs. control	P value and univariate OR for FSDQ <85
Infant age at assessment (months)	22.4 (5.0)	24.1 (6.1)	0.03	0.03 OR = 1.08 (per month)
Infant sex, male (reference)	52 (53.6)	49 (47.6)	0.39	0.49
Infant birth weight (kg)	2.9 (0.4)	3.0 (0.6)	0.07	<0.001 OR = 0.16 (per kg)
Gestational age at birth (weeks)	38.6 (2.7)	37.8 (3.2)	0.09	0.10
Duration of breast-feeding (weeks)	50.0 (9.0)	71.6 (24.6)	<0.0001	0.12
Maternal age (years)	29.9 (5.5)	26.7 (6.1)	0.0002	0.50
Maternal education			0.05	0.047 OR = 0.41
≤Primary (reference)	37 (38.5%)	53 (53.5%)		
Secondary/college/university	59 (61.4%)	46 (46.5%)		
Family income per month (Kwacha)[a] median (IQR)	500 000 (300 000–1 000 000)	507 600 (286 000–1 015 000)	0.86	0.02 OR = 0.93 (per 100 000/month increase)
Marital status			0.03	0.06
Single/divorced/widowed/ separated (reference)	15 (15.4%)	29 (29.3%)		
Married	82 (84.5%)	70 (70.7%)		

Variables are reported for the groups separately, as well as tests for differences between the groups, and the odds ratio of scoring a FSDQ <85. Results are expressed as mean (SD) or *n* (%) unless otherwise indicated. IQR, interquartile range; FSDQ, Full-Scale Development Quotient; HEU, HIV-exposed uninfected.

[a]For the logistic regression, monthly income was divided by 100 000 Kwacha to ease interpretation of the odds ratio, but is reported as raw numbers in the table. Monthly income was also multiplied by 0.920 and 0.846 for 2012 and 2013, respectively, to account for yearly inflation of ~8%.

[b]Tests for differences were *t*-tests for normally distributed continuous variables, chi-square tests for categorical variables, and Wilcoxon rank-sum test for income.

Table 2. Comparison of participant's Development Quotient scores on the Capute Scales (CAT/CLAMS).

Outcomes	All participants ($n=200$)	HEU ($n=97$)	Controls ($n=103$)
CAT Development Quotient			
Score, mean (SD)	100.6 (14.0)	103.2 (15.2)	98.0 (12.3)
<85%, *n* (%) (95% CI)	21 (10.5) (6.8–15.8)	10 (10.3) (5.3–18.6)	11 (10.7) (5.7–18.7)
CLAMS Development Quotient			
Score, mean (SD)	96.9 (14.0)	99.0 (14.6)	94.9 (13.1)
<85%, *n* (%) (95% CI)	29 (14.6) (10.1–20.4)	9 (9.4) (4.6–17.5)	20 (19.4) (12.5–28.6)
FSDQ			
Score, mean (SD)	98.7 (12.2)	101.2 (13.0)	96.5 (11.0)
<85%, *n* (%) (95% CI)	23 (11.6) (7.6–17.3)	8 (8.3) (3.9–16.2)	15 (14.6) (8.6–23.2)

Shown are means (SD) and *n* (%) with 95% confidence intervals of those scoring <85. CAT, Clinical Adaptive Test; CI, confidence interval; CLAMS, Clinical Linguistic and Auditory Milestone Scale; FSDQ, Full-Scale Development Quotient; HEU, HIV-exposed uninfected; SD, standard deviation.

every month increase in infant age, the odds of having FSDQ less than 85 are increased by about 8%, and that for every increase of 1 kg in birth weight, the odds of having FSDQ less than 85 are decreased by approximately 84%. Only one HEU child and two controls scored less than 70 on the FSDQ.

In the multivariable analysis including group, income, maternal education, infant age, and birth weight, only lower birth weight (OR = 0.15, 95% CI = 0.04–0.45, $P<0.001$) remained independently associated with FSDQ less than 85. Group was not significantly associated with the odds of FSDQ less than 85 (OR control/HEU = 1.07, 95% CI = 0.35–3.25, $P=0.90$; Table 3). Missing data on some variables reduced the number of infants in the analysis to 185, with 19 infants having FSDQ less than 85. A sensitivity analysis with birth weight removed from the multivariable model gave qualitatively similar results with no significant difference between the groups in the odds of FSDQ less than 85 once infant age, monthly income, and maternal education were taken into account (Table S1 in supplementary material).

Clinical Adaptive Test Development Quotient

For CAT Development Quotient, lower income (OR = 0.91, 95% CI = 0.79–0.99, $P=0.014$), older infant age (OR = 1.13, 95% CI = 1.05–1.23, $P=0.001$), and lower infant birth weight (OR = 0.20, 95% CI = 0.07–0.54, $P=0.001$) were significantly univariately associated with greater odds of scoring less than 85. Group was not significantly associated with the odds of scoring less than 85 (OR controls/HEU = 1.04, 95% CI = 0.42–2.62, $P=0.93$). After including income, maternal education, infant age, infant birth weight, and group (as for the FSDQ) in a multivariable logistic regression, older infant age (OR = 1.17, 95% CI = 1.05–1.31, $P=0.003$) and lower birth weight (OR = 0.16, 95% CI = 0.04–0.56, $P=0.004$) remained significantly associated with the odds of scoring less than 85. Group (OR control/HEU = 0.31, 95% CI = 0.08–1.06, $P=0.06$) was not significantly associated with the odds of scoring less than 85 in the multivariable model, although a trend may be present. A sensitivity analysis with birth weight removed gave qualitatively similar results with no significant difference in CAT Development Quotient less than 85 between the groups ($P=0.07$, Table S1).

Table 3. Results of the multivariable logistic regressions for all three outcome variables.

Variable	OR[a]	95%CI	*P* value
CAT Development Quotient	0.31	0.08–1.06	0.06
CLAMS Development Quotient	1.90	0.75–5.03	0.18
FSDQ	1.07	0.32–3.25	0.90

Shown are the OR and 95% CIs for scoring <85 (control/HEU) adjusted for the other variables in the models.CAT, Clinical Adaptive Test; CI, confidence interval; CLAMS, Clinical Linguistic and Auditory Milestone Scale; FSDQ, Full-Scale Development Quotient; OR, odds ratio.

[a]OR adjusted for birth weight, infant age, maternal education, and monthly income. *P* values are derived from likelihood ratio tests comparing the model with the term included vs. one with it removed.

Clinical Linguistic and Auditory Milestone Scale Development Quotient

For CLAMS Development Quotient, lower income (OR = 0.88, 95% CI = 0.77–0.96, $P<0.001$), less maternal education (OR = 0.39, 95% CI = 0.16–0.87, $P=0.02$), lower infant birth weight (OR = 0.27, 95% CI = 0.10–0.64, $P=0.002$), and Group (OR Control/HEU = 2.33, 95% CI = 1.03–5.65, $P=0.04$) were significantly univariately associated with the odds of scoring less than 85.

After including the income, maternal education, infant age, and birth weight in a multivariable logistic regression, lower income (OR = 0.91, 95% CI = 0.80–0.99, $P=0.046$) and lower birth weight (OR = 0.29, 95% CI = 0.11–0.73, $P=0.008$) remained significantly associated with CLAMS Development Quotient less than 85. Group (OR control/HEU = 1.90, 95% CI = 0.75–5.03) had a nonsignificant *P* value of 0.18. Again, a sensitivity analysis with birth weight removed gave qualitatively similar results with no significant difference in CLAMS Development Quotient less than 85 between the groups ($P=0.20$) and monthly income retaining significance (Table S1).

Discussion

This study is the first to assess the cognitive and language outcomes in HEU infants exposed to both antenatal and 1 year of postnatal cART exposures as per the new WHO guidelines. Our study did not support the presence of increased cognitive or language delay for HEU children compared with uninfected unexposed controls from the same community. This is reassuring, especially in light of the long exposure and the fact that ZDV and 3TC are well secreted in breast milk [27]. This finding is important in terms of the uptake of cART regimens around the world, as countries are increasing cART provision. For example, Zambia has reached its target goal of providing 90% of HIV-infected pregnant women with antiretroviral medications [5]. With this increased cART coverage to pregnant women throughout pregnancy and longer infant exposure to antiretrovirals through breast-feeding, an unprecedented number of children are being exposed to long-term cART. Thus, it is important to follow-up on the development of these children.

Analyses of our data showed that lower birth weight was significantly associated with an FSDQ score less than 85, after controlling for other important variables, a score that is indicative of developmental delay. Additionally, lower income, less maternal education, and lower birth weight were significantly associated with a CLAMS Development Quotient less than 85, which is indicative of a language delay, and lower birth weight and older infant age were associated with a CAT Development Quotient less than 85, which is indicative of a cognitive delay. The association between older infant age and CAT Development Quotient scores less than 85 could reflect a limitation of the assessment tool, which may have reduced sensitivity for developmental delay at lower ages. It is also possible that as children get older, deficits are apparent in more complex skills that are not seen in earlier stages of development [41]. These possibilities imply that longer-term follow-up studies of older children may be required to assess whether deficits emerge with further development in HEU children.

Lower income was also significantly associated with lower language score, something expected based on current child development literature [42–44], and presumably related to the possibility that lower-income families have lower literacy levels and provide less language stimulation. Another possible explanation is that families of lower income in Lusaka were less likely to speak English, and therefore children may have had less reliable scores because of difficulties in translation or other examiner miscommunication.

Finally, lower birth weight was also associated with an increased risk of scoring less than 85 on CAT Development Quotient, CLAMS Development Quotient, and FSDQ, as could be expected based on literature of low-birth-weight children [45–47]. Despite the fact that our HEU group had lower birth weight, this did not translate into significantly lower development scores in that group.

Limitations

A limitation to the study was that there was more than one assessor in the study, potentially introducing inter-rater variability. The use of a standardized assessment administered in pairs with oversight by a Developmental Pediatrician mitigates this risk. The forty-five control infants were excluded because of an error in testing by a single assessor, following an attempt to include local staff. There was no evidence of significant variation in scores by assessor once this single assessor was removed.

A further limitation was the choice of the Capute Scales itself. As a screening tool, it does not have the ability or the depth to diagnose specific cognitive or language delays in children. However, given the limited resource setting in which the study was conducted, the lack of a validated local measure, and the correlation to the BSID II [34–36], this assessment proved the most feasible. Furthermore, measurement of skills at this age tends to reflect species-specific skills that are basic and less influenced by cultural exposures. Indeed, the CAPUTE has been useful in other developing world studies [37–39]. The control infant scores were generally similar to those of US infants wherein the tools were developed [31], which suggests that this is a useful test in this context. This approach to tool assessment has also recently been used in other African contexts [48]. The choice of a score cutoff of less than 85 (1 SD) as outcome was a conservative choice, as this would reflect only mild to moderate delays. Detection of a difference using this approach would reflect subtle, potentially nondisabling differences in development.

Owing to the limited sample size and therefore wide CIs, this study cannot rule out the possibility of an increased relative risk of delay in the HEU group particularly related to nonverbal skills. Future assessment of the population at an older age with functional and culturally sensitive measures is indicated.

Conclusions

Our study did not support the presence of an adverse effect on cognitive or language development with prolonged antepartum and postpartum cART exposure. Larger studies and studies of older HEU children will be required to confirm these findings.

Acknowledgements

We would like to thank Malembe Mumba, for contributing to this project by performing several

neurocognitive assessments, and Barry Picov for coordinating Aluvia study support. We would also like to thank the mothers and infants of the Chelstone clinic who participated in this project.

Funding: This study was partially supported by Abbvie, Rotary International, The Stephen Lewis Foundation, The Mount Sinai Foundation, ViiV, and The University of Toronto CREMS program for the Aluvia study; and by the Canadian Institutes for Health Research (Team grant in Cellular Aging and HIV comorbidities in Women and Children TCO-125269 to HCFC and MSS).

Conflicts of interest

There are no conflicts of interest.

References

1. Shapiro RL, Hughes MD, Ogwu A, Lockman S, Moffat C, Makhema J, *et al*. **Antiretroviral regimens in pregnancy and breast-feeding in Botswana.** *N Engl J Med* 2010; **362**:2282–2294.
2. Chasela CS, Hudgens MG, Jamieson DJ, Kayira D, Hosseinipour MC, Kourtis AP, *et al*. **Maternal or infant antiretroviral drugs to reduce HIV-1 transmission.** *N Engl J Med* 2010; **362**:2271–2281.
3. Jamieson DJ, Chasela CS, Hudgens MG, King CC, Kourtis AP, Kayira D, *et al*. **Maternal and infant antiretroviral regimens to prevent postnatal HIV-1 transmission: 48-week follow-up of the BAN randomised controlled trial.** *Lancet* 2012; **379**:2449–2458.
4. United Nations. The Millennium Development Goals Report 2013. New York: United Nations (Copyright June 2013). Sales No. E.13.I.9, ISBN 978-92-1–101284-2.
5. Joint United Nations Program on HIV/AIDS (UNAIDS). Global report: UNAIDS report on the global AIDS epidemic 2013. (Copyright 2013) UNAIDS /JC2502/1/E, ISBN 978-92-9253-032-7.
6. World Health Organization. Consolidated Guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: Recommendations for a public health approach. (June 2013) ISBN 978 92 4 150572 7.
7. 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. 2012. 2013. http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.
8. Cotter AM, Garcia AG, Duthley ML, Luke B, O'Sullivan MJ. **Is antiretroviral therapy during pregnancy associated with an increased risk of preterm delivery, low birth weight, or stillbirth?** *J Infect Dis* 2006; **193**:1195–1201.
9. van der Merwe K, Hoffman R, Black V, Chersich M, Coovadia A, Rees H. **Birth outcomes in South African women receiving highly active antiretroviral therapy: a retrospective observational study.** *J Int AIDS Soc* 2011; **14**:42.
10. Lopez M, Figueras F, Hernandaz S, Lonca M, Garcia R, Palacio M, *et al*. **Association of HIV infection with spontaneous and iatrogenic preterm delivery: effect of HAART.** *AIDS* 2012; **26**:36–43.
11. Chen JY, Ribaudo HJ, Souda S, Parekh N, Ogwu A, Lockman S, *et al*. **Highly active antiretroviral therapy and adverse birth outcomes among HIV-infected women in Botswana.** *J Infect Dis* 2012; **206**:1695–1705.
12. Hankin C, Thorne C, Peckham C, Newell M-L. **European Collaborative Study. Exposure to antiretroviral therapy in utero or early life: the health of uninfected children born to HIV-infected women.** *J Acquir Immune Defic Syndr* 2003; **32**:380–387.
13. Barret B, Tardieu M, Rustin P, Lacroix C, Chabrol B, Desguerre I, *et al*. **French Perinatal Cohort Study Group. Persistent mitochondrial dysfunction in HIV-1-exposed but uninfected infants: clinical screening in a large prospective cohort.** *AIDS* 2003; **17**:1769–1785.
14. Brogly SB, Ylitalo N, Mofenson LM, Oleske J, Van Dyke R, Crain MJ, *et al*. **In utero nucleoside reverse transcriptase inhibitor exposure and signs of possible mitochondrial dysfunction in HIV-uninfected children.** *AIDS* 2007; **21**:929–938.
15. Brogly SB, DiMauro S, Van Dyke RB, Williams PL, Naini A, Libutti DE, *et al*. **Transplacental nucleoside analogue exposure and mitochondrial parameters in HIV-uninfected children.** *AIDS Res Hum Retroviruses* 2011; **27**:777–783.
16. Whitehead N, Potterton J, Coovadia A. **The neurodevelopment of HIV-infected infants on HAART compared to HIV-exposed but uninfected infants.** *AIDS Care* 2014; **26**:497–504.
17. Puthanakit T, Ananwornanich J, Vonthanak S, Kosalarska P, Hansudewechakul R, van der Lugt J, *et al*. **Neurodevelopmetal outcome among HIV+ children ≥1 year of age randomized to immediate vs deferred ART: the Predict Neurodevelopmental Study (NCT00234091).** *Pediatr Infect Dis J* 2013; **32**:501–508.
18. Hutchings J, Potterton J. **Developmental delay in HIV exposed infants in Harare, Zimbabwe.** *Vulnerable Children and Youth Studies* 2014; **9**:43–55http://dx.doi.org/10.1080/17450128.2013.778440.
19. Laughton B, Cornell M, Grove D, Kidd M, Springer PE, Dobbels E, *et al*. **Early antiretroviral therapy improves neurodevelopmental outcomes in infants.** *AIDS* 2012; **26**:1685–1690.
20. Alimenti A, Forbes J, Oberlander T, Money D, Grunau R, Papsdorf M, *et al*. **A prospective controlled study of neurodevelopment in HIV-uninfected children exposed to combination antiretroviral drugs in pregnancy.** *Pediatrics* 2006; **118**:e1139.
21. Culnane M, Fowler M, Lee A, McSherry G, Brady M, O'Donnell K, *et al*. **Lack of long-term effects of in utero exposure to Zidovudine among uninfected children born to HIV-infected women.** *JAMA* 1999; **281**:151–157.
22. Gómez C, Archila ME, Rugeles C, Carrizosa J, Rugeles MT, Cornejo JW. **A prospective study of neurodevelopment of uninfected children born to human immunodeficiency virus type 1 positive mothers.** *Rev Neurol* 2009; **48**:287–291.
23. Williams PL, Marino M, Malee K, Brogly S, Hughes MD, Mofenson LM. **Neurodevelopment and in utero antiretroviral exposure of HIV-exposed uninfected infants.** *Pediatrics* 2010; **125**:e250–e260.
24. Rice ML, Zeldow B, Siberry GK, Purswani M, Malee K, Hoffman HJ, *et al*. **Pediatric HIVAIDS Cohort Study (PHACS). Evaluation of risk for late language emergence after in utero antiretroviral drug exposure in HIV-exposed uninfected infants.** *Pediatr Infect Dis J* 2013; **32**:e406–e413.
25. Sirois PA, Huo Y, Williams PL, Malee K, Garvie PA, Kammerer B, *et al*. **Pediatric HIVAIDS Cohort Study. Safety of perinatal exposure to antiretroviral medications: developmental outcomes in infants.** *Pediatr Infect Dis J* 2013; **32**:648–655.
26. Capone GT, Kaufman WE. **Human brain development.** In: Accardo PJ. editor. *Capute & Accardo's neurodevelopmental disabilities in infancy and childhood* Brookes publishing co.; 2008. pp. 27–57.
27. Shapiro RL, Holland DT, Capparelli E, Lockman S, Thior I, Wester C, *et al*. **Antiretroviral concentrations in breast-feeding infants of women in Botswana receiving antiretroviral treatment.** *J Infect Dis* 2005; **192**:720–727.
28. Giuliano M, Guidotti G, Andreotti M, Pirillo MF, Villani P, Liotta G, *et al*. **Triple antiretroviral prophylaxis administered during pregnancy and after delivery significantly reduces breast milk viral load study within the Drug Resource Enhancement Against AIDS and Malnutrition Program.** *J Acquir Immune Defic Syndr* 2006; **14**:459–460.
29. Gandhi M, Mwesigwa J, Aweeka F, Plenty A, Charlebois E, *et al*. **Hair and plasma data show that lopinavir, ritonavir and efavirenz all transfer from mother to infant in utero, but only efavirenz transfers via breastfeeding.** *J Acquir Immune Defic Syndr* 2013; **63**:578–584.

30. Ngoma M, Raha A, Elong A, Pilon R, Mwansa J, Mutale W *et al*. Preliminary results of HIV Transmission Rates Using a Lopinavir/ritonavir (LPV/r, Aluvia) based regimen and the New WHO Breast Feeding Guidelines for PMTCT of HIV. 51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2011). Chicago, September 17–20, 2011. Available at http://www.abstractsonline.com/Plan/ViewAbstract.aspx?sKey=ef930ee8-6a2a-4f2d-93b1-07459a413394&cKey=fe9f7030-9d09-4718-b8c3-ab70f8feb9ef&mKey=%7b0C918954-D607-46A7-8073-44F4B537A439%7d Abstract H1-1153.
31. Accardo P, Capute A. *The Capute Scales: Cognitive Adaptive Test/Clinical Linguistic & Auditory Milestone Scale (CAT/CLAMS)*. Baltimore: Paul H. Brookes Publishing; 2005.
32. Alyward GP. **Developmental screening and assessment: what were we thinking?** *J Dev Behav Pediatr* 2009; **30**:169–173.
33. Wachtel RC, Shapiro BK, Palmer FB, Allen MC, Capute AJ. **CAT/CLAMS: a tool for the pediatric evaluation of infants and young children with developmental delay.** *Clin Pediatr* 1994; **33**:410–415.
34. O'Connor Leppert ML, Shank TP, Shapiro BK, Capute AJ. **The Capute Scales: CAT/CLAMS—A pediatric assessment tool for the early detection of mental retardation and communicative disorders.** *MRDD Res Rev* 1998; **4**:14–19.
35. Voigt RG, Brown FR, Fraley JK, Llorente AM, Rozelle J, Turcich M, *et al*. **Concurrent and predictive validity of the cognitive adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS) and the Mental Developmental Index of the Bayley Scales of Infant Development.** *Clin Pediatr* 2003; **42**:427–432.
36. Wachtel RC, Tepper VJ, Houck D, McGrath CJ, Thompson C. **Neurodevelopment in pediatric HIV infection.** *Clin Pediatr* 1994; **33**:416–420.
37. Bruck I, Tahan TT, Cruz CR, Martins LT, Antoniuk SA, Rodrigues M, *et al*. **Developmental milestones of vertically HIV infected and seroreverters children: follow up of 83 children.** *Arq Neuropsiquiatr* 2001; **59 (3–B)**:691–695.
38. Thepsuthammarat K. **Association between types of play materials and cognitive development among 12-month-old Thai infants: the prospective cohort study of Thai children.** *Asian Biomed* 2012; **6**:703–711.
39. Yanuarti HP, Rusmil K, Effendi SH. **Environment as risk factors in delay development in premature, LBW and mild asphyxia children.** *Pediatr Int* 2014doi: 10.1111/ped.12333. [Epub ahead of print].
40. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
41. Luciana M. **Cognitive development in children born preterm: Implications for theories of brain plasticity following early injury.** *Dev Psychopathol* 2003; **15**:1017–1047.
42. Hoff E. **How social contexts support and shape language development.** *Dev Rev* 2006; **26**:55–88.
43. Perkins SC, Finegood Eric D, Swain JE. **Poverty and language development: roles of parenting and stress.** *Innov Clin Neurosci* 2013; **10**:10–19.
44. Fernald A, Marchman VA, Weisleder A. **SES differences in language processing skill and vocabulary are evident at 18 months.** *Dev Sci* 2013; **16**:234–248.
45. Vohr BR, Wright LL, Dusick AM, Mele L, Verter J, Steichen JJ, *et al*. **Neurodevelopmental and functional outcomes of extremely low birth weight infants in the National Institute of Child Health and Human Development Neonatal Research Network, 1993-1994.** *Pediatrics* 2000; **105**:1216–1226.
46. Sutton PS, Darmstadt GL. **Preterm birth and neurodevelopment: a review of outcomes and recommendations for early identification and cost-effective interventions.** *J Trop Pediatr* 2013; **59**:258–265.
47. Aarnoudse-Moens CS, Weisglas-Kuperus N, van Goudoever JB, Oosterlaan J. **Meta-analysis of neurobehavioral outcomes in very preterm and/or very low birth weight children.** *Pediatrics* 2009; **124**:717–728.
48. Rademeyer L, Jacklin A. **Study to evaluate the performance of black South African urban infants on the Bayley Scales of Infant Development III.** *South African J Child Health* 2013; **7**:54–59.