Neurodevelopmental effects of ante-partum and post-partum antiretroviral exposure in HIV-exposed and uninfected children versus HIV-unexposed and uninfected children in Uganda and Malawi: a prospective cohort study

Michael J Boivin, Limbika Maliwichi-Senganimalunje, Lillian W Ogwang, Rachel Kawalazira, Alla Sikorskii, Itziar Familiar-Lopez, Agatha Kuteesa, Mary Nyakato, Alex Mutebe, Jackie L Namukooli, MacPherson Mallewa, Horacio Ruiseñor-Escudero, Jim Aizire, Taha E Taha, Mary G Fowler

Lancet HIV 2019; 6: e518–30

Published Online May 20, 2019 http://dx.doi.org/10.1016/S2352-3018(19)30083-9

See Comment page e487

Department of Psychiatry (Prof M J Boivin PhD, Prof A Sikorskii PhD, I Familiar-Lopez PhD, H Ruiseñor-Escudero PhD), **Department of Neurology & Ophthalmology** (Prof M J Boivin), **and Department of Statistics & Probability** (Prof A Sikorskii), **Michigan State University, East Lansing, MI, USA; Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA** (Prof M J Boivin); **Malawi College of Medicine, Johns Hopkins University Research Project, Blantyre, Malawi** (L Maliwichi-Senganimalunje MS, R Kawalazira MMed, M Mallewa PhD); **Department of Psychology, Chancellor College, University of Malawi, Zomba, Malawi** (L Maliwichi-Senganimalunje, L Wambuzi Ogwang MMed); **Makerere University-Johns Hopkins University, Kampala, Uganda** (A Kuteesa BS, M Nyakato MS, A Mutebe MS, J L Namukooli MPH); **Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA** (J Aizire PhD, Prof T E Taha PhD); **and Department of Pathology, Johns Hopkins University Medical Institute, Baltimore, MD, USA** (Prof M G Fowler MD)

Correspondence to: Prof Michael J Boivin, Department of Psychiatry, Research Division, Michigan State University, East Lansing, MI 48824, USA **boivin@msu.edu**

Summary

Background Antiretroviral medication during pregnancy and breastfeeding substantially decreases the risk of HIV transmission from mothers to infants, but its effects on the child's neurodevelopment are unknown. This study compared neurodevelopmental outcomes of ante-partum and post-partum antiretroviral exposure in HIV-exposed and uninfected children with HIV-unexposed and uninfected children at ages 12, 24, 48, and 60 months.

Methods For this study, a prospective cohort of HIV-exposed and uninfected children was identified from two research sites in the PROMISE-BF trial (at Blantyre, Malawi, and Kampala, Uganda), in which pregnant HIV-infected mothers were randomly assigned to triple antiretroviral prophylaxis (lopinavir–ritonavir plus either lamivudine and zidovudine or emtricitabine and tenofovir), versus zidovudine alone. Post partum, the mother–infant pairs were randomly assigned to maternal triple antiretroviral treatment or infant nevirapine during breastfeeding. HIV-unexposed and uninfected children matched for age, sex, and socioeconomic background were enrolled at vaccination and well-child clinics at the study sites. We included only children without a history of documented brain infection or injury or substantial malnutrition, and whose mothers were randomly assigned and maintained within their assigned ante-partum and post-partum phases throughout their treatment arm periods. Primary outcomes were the Mullen Scales of Early Learning (MSEL) cognitive composite score at age 12 months, 24 months, and 48 months; and the mental processing index for the Kaufman Assessment Battery for Children, second edition (KABC-II) global score at 48 months and 60 months. Repeated measures were analysed using a linear mixed-effects model controlling for data collection site.

Findings Between Aug 23, 2013, and Dec 17, 2014, we co-enrolled 861 children. For MSEL assessments, 738 were eligible for inclusion at age 12 months, 790 at age 24 months, and 692 at age 48 months. For KABC-II assessments, 685 were eligible for inclusion at age 48 months and 445 at age 60 months. There were no differences in MSEL cognitive composite scores according to exposure at age 12 and 24 months (p=0·19 and 0·24, respectively, for comparison of all groups). At 48 months, MSEL cognitive composite scores were worse for children of mothers who did not remain on triple antiretroviral treatment throughout both the ante-partum and post-partum treatment phases (adjusted means 80·64 [95% CI 77·74–83·54] and 81·34 [78·19–84·48], respectively), compared with those who did remain on triple treatment (adjusted mean 85·93, 95% CI 83·05–88·80; p=0·0486 for the comparison of all groups). The KABC-II composite scores (mental processing index) did not differ at 48 or 60 months according to exposure (p=0·81 and 0·89, respectively, for comparison of all groups). Scores for MSEL and KABC-II for children of mothers on triple antiretrovirals in both the ante-partum and post-partum treatment phases were similar to those for children in the HIV-unexposed and uninfected reference group at all timepoints.

Interpretation Maternal triple antiretroviral exposure during both the ante-partum and post-partum phases did not result in greater developmental risks for the mothers' HIV-exposed and uninfected children through age 60 months, compared with children who were HIV-unexposed and uninfected. This might be because ante-partum triple antiretroviral protection of the health of mothers with HIV during pregnancy might be neuroprotective for the child, and when continued post partum, could enhance the quality of caregiving for the child through better clinical care for the mother.

Funding National Institutes of Health and Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Research in context

Evidence before this study

We linked the search term "child development" with "antepartum" and "postpartum ARV exposure", "prevention of mother-to-child transmission", "HIV", and "maternal" in a search of English-language articles published in PubMed and MEDLINE before June 30, 2018. There was only one previously published study assessing maternal antiretroviral therapy versus zidovudine ante-partum exposure along with infant post-partum antiretroviral exposure through breastfeeding using neurodevelopmental outcomes in sub-Saharan Africa. Some neurodevelopmental outcome studies of prevention of mother-to-child transmission had been published from multisite clinical trials in the USA, but did not include post-partum exposure to HIV-exposed and uninfected infants because they were not breastfeeding as a standard of care in such high-resource settings. This was also a limiting factor in a clinical prospective observational study included within publications from 2017 (comparison of developmental outcomes between HIV-exposed and uninfected children and HIV-unexposed and uninfected children) and 2018 (comparison of triple antiretroviral therapy versus zidovudine maternal ante-partum treatment on child developmental outcomes) in children in Botswana.

Added value of this study

Our unadjusted and adjusted longitudinal Mullen Scales of Early Learning outcome findings showed that both ante-partum and post-partum maternal antiretroviral therapy exposure did not result in greater developmental risks for HIV-exposed and uninfected children through age 48 months compared with HIV-unexposed and uninfected children. Overall, findings from both cohorts of children were similar, as were those from the PROMISE 1077-BF study treatment arms in HIV-exposed and uninfected children at both country sites in Uganda and Malawi. Adding to the importance of these conclusions is that our findings are the first to be based on developmental outcomes from a rigorous randomised trial with well powered sample sizes among maternal or child treatment groups with prolonged post-partum exposure from breastfeeding. The findings were also based on a longitudinal comparison with a well matched HIV-unexposed and uninfected control group for reference at both country sites. This is the first time such neurodevelopmental outcome findings are available for what has become the standard of care across sub-Saharan Africa, and they are reassuring because prevention of mother-to-child transmission programmes using new maternal triple antiretrovirals continue to be widely rolled out in sub-Saharan Africa and globally.

Implications of all the available evidence

Consistent clinical management of HIV disease with ART in mothers during both the ante-partum and post-partum phase can result in better health outcomes in African children because mothers can be encouraged to breastfeed their infants for longer without fear of neurotoxic effects to the infant from prolonged ART exposure. These findings are crucial additions to the minimal knowledge on growth of HIV-exposed and uninfected children in resource-limited African settings, where prenatal and postnatal infant exposure to triple antiretrovirals is common because of prolonged breastfeeding. These findings are also important because they are the most rigorous neurodevelopmental assessments to date of children exposed to WHO-recommended maternal HIV test-and-treat initiation of lifetime ART (option B+strategy). This study documents the importance of monitoring neurodevelopmental and neuropsychological outcomes throughout infancy and early childhood for children exposed to these drugs, and is also important as a new generation of antiretroviral drugs is incorporated into prevention of mother-to-child transmission in resource-constrained settings globally.

Introduction

In resource-rich settings such as the USA and Europe, HIV transmission rates of less than 1–2% have been achieved with use of triple antiretroviral regimens during pregnancy and labour or delivery along with infant zidovudine prophylaxis and avoidance of breastfeeding.[1] WHO recommendations as encompassed within test-and-treat harmonised strategies for all HIV-infected individuals, including pregnant women in Africa, have resulted in some HIV-exposed infants receiving antiretrovirals for up to 24 months (ie, for 6 months in utero plus 12–18 months of breastfeeding) during a crucial period of rapid brain development.[2] This length of antiretroviral exposure among uninfected infants has never occurred on a large scale before. In the USA and Europe, antiretroviral exposure only occurs during pregnancy since infants of HIV-infected individuals are recommended not to be breastfed.

With the continuing roll-out of WHO guidelines, prolonged fetal and infant triple antiretroviral exposure could cause mitochondrial dysfunction or other adverse events affecting the developing human brain and other organs among breastfed HIV-exposed infants.[3] The PROMISE 1077-BF study from which children in this study were co-enrolled was the first, multisite, randomised, clinical trial based in Africa comparing the safety and efficacy of use of maternal triple antiretrovirals with antenatal maternal zidovudine alone; and postnatal maternal triple antiretrovirals with infant nevirapine throughout breastfeeding.[2,4,5] In reporting the results of this trial done in 15 International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) sites, the investigators concluded that triple antiretroviral ante-partum regimens showed significantly increased efficacy (0·5%) in preventing early HIV transmission through age 14 days compared with ante-partum zidovudine alone (1·8%).

However, triple antiretroviral regimens also resulted in a greater risk of adverse pregnancy outcomes including infant low birthweight (<2500 g), preterm delivery, and

For more on the **PROMISE 1077-BF study** see https://clinicaltrials.gov

a lower rate of HIV-free survival than a zidovudine-alone antiretroviral regimen.[5] These adverse pregnancy outcomes could affect later physical growth development. A subsequent study[4] of these children assessed the effects of prolonged infant antiretroviral exposure throughout the breastfeeding period on adverse outcomes for mother and infant. Both triple antiretroviral therapy and infant nevirapine prophylaxis strategies were safe and associated with minimal breastfeeding HIV-1 transmission, minimal adverse health events, and high infant HIV-1-free survival at 24 months. However, these studies did not assess neurodevelopmental outcomes throughout infancy and early childhood, which we set out to do in this study.

Methods

Study design and participants

HIV-exposed and uninfected children were identified from the IMPAACT Promoting Maternal and Infant Survival Everywhere (PROMISE) breastfeeding study.[4,5] We included those who had been randomly assigned and maintained on their assigned PROMISE treatment arm throughout both the ante-partum and post-partum periods. An HIV-unexposed and uninfected cohort of age-matched and sex-matched reference or control children were enrolled at two international research sites: Malawi College of Medicine-Johns Hopkins Project, in Blantyre, Malawi, and Makerere University-Johns Hopkins University research project, in Kampala, Uganda. Originally, there were 944 children in the study population at the two sites; however, HIV-exposed and uninfected children from PROMISE of mothers not randomly assigned to either the zidovudine alone or triple antiretroviral antenatal treatment arms (enrolled in PROMISE very late in pregnancy) were not included in this analysis. Likewise, children of mothers not randomly assigned to one of the two post-partum treatment arms (maternal triple antiretroviral treatment or infant nevirapine only) were not included. This would have happened for mothers in the study who were switched to triple antiretroviral treatment during pregnancy or shortly after the child's birth out of clinical necessity because of their HIV disease status.

We recruited HIV-unexposed and uninfected children with similar socioeconomic backgrounds via child-wellness or immunisation clinics within the study sites. The eligibility criteria were: age 6–12 months at enrolment (subsequently revised to age 6–24 months on Nov 9, 2012) and controls matched for sex and age (plus or minus 4 months); birthweight at least 2000 g; confirmed child HIV-uninfected and maternal HIV-infected status (exposed group); confirmed maternal HIV-uninfected (control group); no known serious chronic condition; and obtained written informed consent from the mother. Exclusion criteria were serious pre-existing clinical conditions, residence outside the site catchment area, or not willing to be home visited. Institutional review boards and other relevant regulatory bodies in Malawi and Uganda, and the Johns Hopkins Medical Institute review boards, approved the study.

Procedures

Medical history, breastfeeding and nutrition, socioeconomic status, and anthropometric measures (weight, length, and head circumference) were collected at baseline (age 12 months) and updated at age 24, 48, and 60 months study visits. We abstracted local trial records for data on maternal HIV infection confirmation and viral load, antiretroviral exposures, and infant HIV-negative status based on nucleic acid testing at age 12 and 24 months. Maternal viral load (log RNA) based on the Roche assay was summarised for the last 60 days of pregnancy, first 30 days after childbirth, and within 60 days of the child turning 1 year of age, as described previously.[5] Standardised Z scores for length or height and weight by age and sex were calculated using the WHO Anthro macro software tools.[6] HIV-infected women from the original trial co-enrolled in our study had been randomly assigned during pregnancy to either triple antiretroviral prophylaxis (lopinavir–ritonavir plus either lamivudine and zidovudine or emtricitabine and tenofovir), or zidovudine alone. Post partum, the mother–newborn pairs were randomly assigned to either maternal triple antiretrovirals or infant nevirapine during breastfeeding.[4]

Outcomes

Mullen Scales of Early Learning (MSEL)[7] is a comprehensive test assessing visual reception, gross motor skills, fine motor skills, receptive language, and expressive language. A cognitive composite score was derived from standardised T scores of the four domains (excluding gross motor) providing a general measure of fluid intelligence thought to underlie general cognitive ability. The MSEL has previously been adapted for use with young HIV-exposed and uninfected children and HIV-unexposed and uninfected children in Uganda and showed high sensitivity when used in this population.[8] Spoken instructions were provided to the children or their caregiver in the local language.

The Kaufman Assessment Battery for Children, second edition (KABC-II)[9] was the principal test for cognitive ability outcomes from a neuropsychological perspective for the 48–60 month age range.[9] This test has been validated in the sub-Saharan African context in our study settings.[10–13] Using the Luria model for neuropsychological assessment within the KABC-II, the primary outcome variables were the global scores for children at age 48 and 60 months. These were sequential processing (memory), simultaneous processing (visual-spatial processing and problem solving), learning (immediate and delayed memory), non-verbal index (subtests not dependent on the understanding of instructions in English), and mental processing index (a composite of simultaneous processing, sequential processing, and learning).

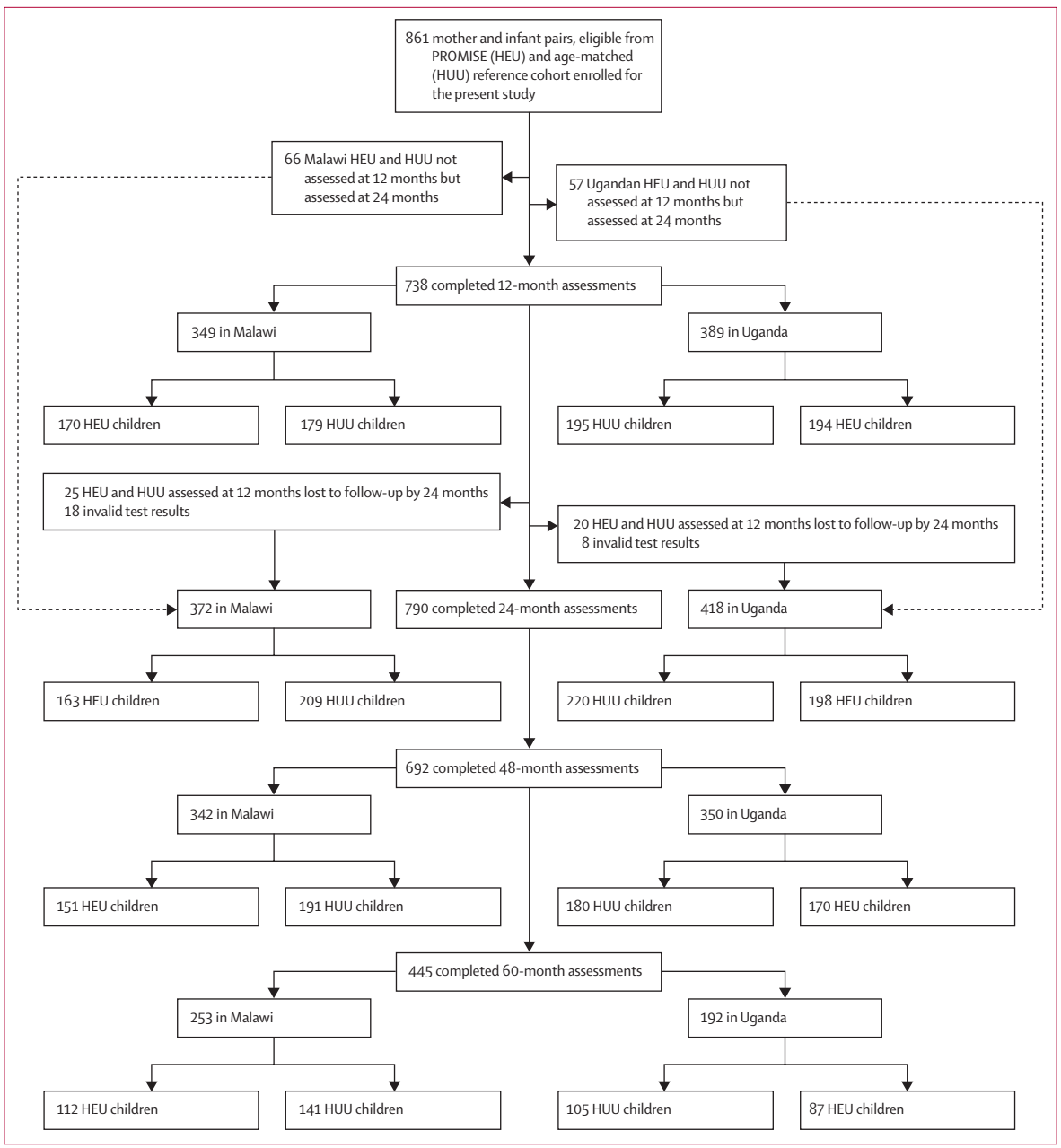

Figure 1: **Trial profile**
HEU=HIV-exposed uninfected. HUU=HIV-unexposed uninfected.

Home Observation for the Measurement of the Environment (HOME)[14] is a composite measure designed to assess the quality and quantity of stimulation that the child is exposed to in their home environment. The version for infants and toddlers includes 45 yes or no items. Higher HOME scores indicate higher quality interactions. Acceptable, Feasible, Affordable, Sustainable and Safe (AFASS)[15] is a questionnaire administered as part of the original clinical trial protocol to assess the socioeconomic environment of the mother–infant pair, as well as whether any infant feeding practices alternative to breastfeeding were identified by the AFASS.[15] Because of the potential effect of breastfeeding on neurodevelopmental outcomes in HIV-exposed and uninfected children, most mothers breastfed their children for longer than 12 months, but we only assessed the importance of breastfeeding on developmental outcomes of the study children until age 12 months.

Statistical analysis

Data were entered and validated using FileMaker 12 and Stata version 14, and analysed using SAS version 9.4.

	HIV-exposed and uninfected children				HIV-unexposed and uninfected children (controls)	p value for comparison of all groups
	Triple ARV plus infant nevirapine	Triple ARV plus maternal triple ARV	Zidovudine plus infant nevirapine	Zidovudine plus maternal triple ARV		
12 months						
Female	54 (52%)	43 (46%)	38 (48%)	39 (44%)	185 (49%)	0·81
Male	49 (48%)	50 (54%)	42 (52%)	49 (56%)	189 (51%)	..
24 months						
Female	48 (49%)	47 (49%)	36 (46%)	38 (42%)	207 (48%)	0·85
Male	49 (51%)	49 (51%)	42 (54%)	52 (58%)	222 (52%)	..
48 months						
Female	45 (52%)	44 (50%)	32 (44%)	34 (47%)	181 (49%)	0·88
Male	42 (48%)	44 (50%)	41 (56%)	39 (53%)	190 (51%)	..
60 months						
Female	20 (42%)	23 (42%)	19 (40%)	23 (47%)	120 (49%)	0·71
Male	28 (58%)	32 (58%)	28 (60%)	26 (53%)	126 (51%)	..
12 months						
Malawi	47 (46%)	40 (43%)	41 (51%)	42 (48%)	179 (48%)	0·85
Uganda	56 (54%)	53 (57%)	39 (49%)	46 (52%)	195 (52%)	..
24 months						
Malawi	43 (44%)	39 (41%)	36 (46%)	45 (50%)	209 (49%)	0·61
Uganda	54 (56%)	57 (59%)	42 (54%)	45 (50%)	220 (51%)	..
48 months						
Malawi	44 (51%)	36 (41%)	34 (47%)	37 (51%)	191 (51%)	0·48
Uganda	43 (49%)	52 (59%)	39 (53%)	36 (49%)	180 (49%)	..
60 months						
Malawi	28 (58%)	28 (51%)	26 (55%)	30 (61%)	141 (57%)	0·86
Uganda	20 (42%)	27 (49%)	21 (45%)	19 (39%)	105 (43%)	..
Age						
12 months	14·24 (2·60)	14·21 (2·73)	14·12 (2·29)	15·02 (2·88)	14·33 (2·70)	0·16
24 months	24·85 (2·04)	25·15 (2·08)	25·05 (1·96)	25·11 (2·14)	25·12 (2·21)	0·84
48 months	48·48 (1·28)	48·79 (1·25)	48·36 (1·22)	48·48 (1·06)	48·54 (1·46)	0·33
60 months	58·62 (2·68)	59·27 (1·78)	58·66 (2·58)	59·11 (1·77)	58·04 (2·62)	0·01
Breastfeeding at 12 months*	83 (80%)	71 (73%)	73 (83%)	67 (73%)	421 (95%)	0·01
Breastfeeding at 24 months	4 (5%)	3 (4%)	6 (9%)	2 (3%)	79 (23%)	0·01
Duration of breastfeeding in months if stopped before 24 months	15·28 (3·71)	14·64 (3·67)	14·69 (4·30)	14·60 (4·39)	20·04 (4·97)	0·01
Maternal viral load (log RNA)†						
Last 60 days of pregnancy	3·18 (1·05)	3·13 (1·14)	3·53 (0·92)	3·95 (1·02)	NA	0·01
Within 30 days of childbirth	2·25 (0·80)	2·32 (0·96)	3·14 (0·87)	2·98 (0·92)	NA	0·01
12 months after childbirth	3·79 (1·06)	1·84 (0·91)	3·63 (1·04)	1·82 (0·83)	NA	0·01
HOME score‡ at enrolment	24·16 (4·03)	25·02 (3·98)	24·73 (3·98)	23·99 (3·95)	25·50 (4·08)	0·01
WHO WAZ						
12 months	−0·54 (1·00)	−0·38 (1·05)	−0·54 (1·05)	−0·48 (1/06)	−0·32 (1·09)	0·19
24 months	−0·59 (0·91)	−0·54 (1·06)	−0·70 (0·96)	−0·53 (1·10)	−0·46 (0·99)	0·31
48 months	−0·70 (0·81)	−0·53 (0·89)	−0·72 (0·76)	−0·63 (0·85)	−0·54 (0·80)	0·27
60 months	−0·80 (0·83)	−0·73 (0·81)	−1·05 (0·77)	−0·94 (0·87)	−0·80 (0·78)	0·24
WHO LAZ						
12 months	−1·57 (1·12)	−1·44 (1·08)	−1·48 (1·02)	−1·61 (1·21)	−1·33 (1·26)	0·18
24 months	−1·84 (1·19)	−1·57 (1·24)	−1·87 (1·05)	−1·81 (1·15)	−1·51 (1·16)	0·01
48 months	−1·49 (0·92)	−1·23 (0·87)	−1·59 (0·91)	−1·50 (0·83)	−1·30 (0·97)	0·03
60 months	−1·59 (0·81)	−1·58 (0·74)	−1·85 (0·88)	−1·80 (1·03)	−1·55 (0·92)	0·17

(Table 1 continues on next page)

	HIV-exposed and uninfected children				HIV-unexposed and uninfected children (controls)	p value for comparison of all groups
	Triple ARV plus infant nevirapine	Triple ARV plus maternal triple ARV	Zidovudine plus infant nevirapine	Zidovudine plus maternal triple ARV		
(Continued from previous page)						
MSEL						
Standardised composite						
12 months	94·21 (15·00)	92·80 (13·15)	92·84 (14·28)	91·95 (14·08)	95·20 (14·54)	0·23
24 months	88·16 (11·94)	88·27 (12·64)	91·04 (14·77)	89·84 (12·15)	91·07 (12·91)	0·14
48 months	80·64 (15·00)	85·81 (14·91)	84·92 (13·41)	81·15 (12·88)	83·97 (14·23)	0·06
Gross motor scale T score						
12 months	49·87 (9·09)	47·87 (9·35)	49·09 (11·98)	46·84 (9·71)	47·94 (10·69)	0·28
24 months	45·91 (10·11)	47·25 (10·70)	48·01 (10·02)	47·09 (10·79)	48·23 (10·31)	0·33
48 months	NA	NA	NA	NA	NA	NA
Fine motor scale T score						
12 months	48·45 (9·64)	48·47 (9·39)	49·69 (9·47)	49·20 (9·33)	50·64 (8·98)	0·11
24 months	41·91 (8·57)	41·62 (9·26)	41·17 (7·62)	42·83 (9·06)	43·00 (9·43)	0·36
48 months	41·30 (14·14)	45·53 (13·36)	43·62 (13·17)	40·84 (10·33)	43·83 (13·39)	0·11
Visual reception scale T score						
12 months	48·45 (10·24)	47·31 (9·85)	48·23 (10·19)	46·25 (11·17)	49·23 (10·04)	0·11
24 months	41·49 (8·97)	40·34 (7·69)	42·03 (7·91)	41·61 (9·51)	42·50 (8·35)	0·22
48 months	38·64 (10·55)	40·92 (9·97)	40·85 (9·14)	38·86 (9·25)	40·64 (10·67)	0·33
Expressive language scale T score						
12 months	44·10 (10·81)	44·00 (9·29)	44·03 (10·50)	44·38 (9·87)	44·65 (10·11)	0·97
24 months	42·62 (7·80)	43·56 (7·84)	44·99 (7·64)	43·06 (7·75)	44·51 (8·52)	0·14
48 months	43·65 (9·40)	46·00 (10·28)	46·21 (8·68)	44·43 (9·99)	45·03 (9·41)	0·39
Receptive language scale T score						
12 months	44·29 (10·61)	45·35 (9·24)	43·20 (10·12)	42·74 (10·35)	45·04 (9·26)	0·18
24 months	49·46 (9·37)	49·38 (10·79)	50·56 (7·83)	50·26 (11·15)	50·57 (8·94)	0·72
48 months	35·16 (7·98)	37·67 (8·55)	37·25 (7·94)	35·48 (7·62)	36·50 (8·20)	0·21
KABC						
Mental processing index						
48 months	77·48 (10·49)	79·11 (11·39)	79·65 (10·83)	77·42 (9·44)	78·22 (10·52)	0·60
60 months	72·63 (11·60)	75·56 (11·25)	72·91 (9·68)	73·43 (10·72)	74·68 (10·81)	0·51
Non-verbal index						
48 months	72·31 (12·52)	73·82 (13·70)	74·40 (11·74)	73·59 (11·02)	73·11 (12·74)	0·85
60 months	69·52 (13·22)	71·53 (12·70)	70·23 (12·10)	71·73 (13·28)	72·80 (12·97)	0·45
Sequential processing						
48 months	80·15 (17·28)	84·57 (12·49)	83·75 (10·51)	82·44 (14·84)	82·18 (14·55)	0·30
60 months	78·00 (10·97)	82·56 (11·11)	79·10 (9·72)	80·25 (11·33)	81·40 (13·05)	0·26
Simultaneous processing						
48 months	72·33 (14·42)	74·33 (12·20)	76·00 (9·47)	72·45 (12·81)	73·50 (11·68)	0·31
60 months	70·63 (12·68)	71·55 (14·31)	71·23 (11·49)	71·79 (12·87)	72·47 (12·87)	0·89
Learning						
48 months	84·21 (18·99)	86·93 (14·97)	89·08 (11·51)	83·63 (18·57)	87·96 (15·73)	0·08
60 months	82·33 (11·97)	85·03 (12·93)	82·11 (10·15)	81·42 (10·80)	82·17 (12·69)	0·56

Data are n (%) or mean (SD). Data for HIV-exposed and uninfected children organised by PROMISE 1077-BF ante-partum and post-partum intervention arm. p values are for ANOVA differences between five groups (four intervention groups with HIV-exposed and uninfected children and one reference group with HIV-unexposed and uninfected children). ARV=antiretrovirals. NA=not applicable. HOME=Home Observation for the Measurement of the Environment. WAZ=weight-for-age Z scores. LAZ=length-for-age Z scores. MSEL=Mullen Scales of Early Learning. KABC=Kaufman Assessment Battery for Children. ··=data not available. *Breastfeeding data available for 825 women. †Viral load data available for 168 women during pregnancy, 346 at childbirth, and 388 at 12 months after childbirth. ‡HOME score data available for 723 children.

Table 1: **Descriptive measures for the study cohorts**

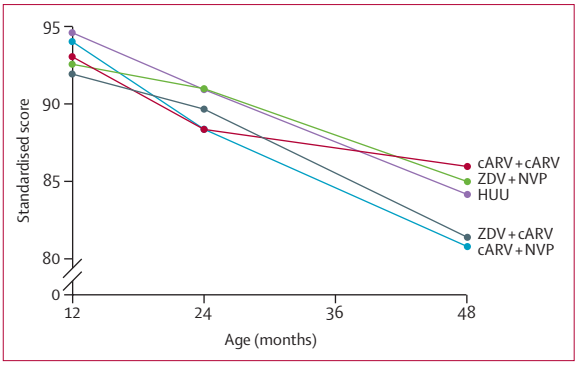

Figure 2: **Mullen Scales of Early Learning standardised composite cognitive performance scores**

Data are scores using American norms, adjusted for site, plotted for the three assessment timepoints (age 12, 24, and 48 months). Plots are colour-coded according to the HIV-exposed and uninfected children for ante-partum and post-partum antiretroviral exposure; and for the HIV-unexposed and uninfected reference control group. The treatment arms (from top to bottom) were ante-partum plus post-partum maternal triple antiretrovirals (cARV + cARV; red), ante-partum maternal zidovudine plus post-partum infant nevirapine (ZDV + NVP; green), HIV-unexposed and uninfected (HUU; purple), ante-partum maternal zidovudine plus post-partum maternal triple antiretrovirals (ZDV + cARV; grey), and ante-partum maternal triple antiretrovirals plus post-partum infant nevirapine (cARV + NVP; blue).

These analyses were restricted to children with a negative HIV result through age 60 months. To address the principal study goal of comparisons of children's neurodevelopmental outcomes according to HIV and antiretroviral exposure at age 12, 24, 48, and 60 months, analysis of variance or χ^2 tests were used for unadjusted comparisons of five groups: four based on ante-partum and post-partum treatment arm combinations, and the fifth comprised of HIV unexposed and uninfected children (reference group). These groups were compared cross-sectionally at each assessment timepoint.

To address the study goal in a longitudinal context, linear mixed-effects (LME) models were used to analyse the MSEL and KABC testing outcomes. These outcomes were assessed while adjusting for data collection site as a fixed effect. Random effect in the LME models was by individual participant, to account for nesting of repeated measures within each child, with the compound symmetry covariance structure. The LME modelling generalises classic analysis of repeated measures and allows for a missing at random mechanism, so that all children with one or more non-missing assessment data were included in the analysis. Modelling was done using the MIXED procedure in SAS 9.4. To model potentially non-linear longitudinal patterns, the follow-up timepoint was entered as a categorical variable. Time-by-intervention interaction was included to capture potential changes in differences by intervention arm over time. The least-squares (adjusted) means for each timepoint and group were obtained from the LME models and compared across the five groups. If there were significant differences among the groups, follow-up pairwise comparisons were done to determine which of the five groups differed. 95% CIs and p values were reported for each domain and summary score of the MSEL and KABC at each timepoint. Additionally, to control for the false discovery rate, we applied the Benjamini-Hochberg adjustment for the analysis of the domains of pre-school and school-age outcomes.[16]

Breastfeeding, maternal viral load, HOME, and length or height-for-age growth measures were also considered in separate analyses in assessing the effects of antiretroviral treatment on MSEL and KABC outcomes. Because of the differences in distributions of the HOME scores and breastfeeding prevalence by country and HIV exposure, these analyses were stratified by country.

Role of the funding source

The funder of the study had no role in study design, study management, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (MJB) and study statistician (AS) had full access to all the data in the study, and with the study co-principal investigator (MGF), had final responsibility for the decision to submit for publication.

Results

Between Aug 23, 2013, and Dec 17, 2014, 861 mother–infant pairs were enrolled. 405 (47%) of 461 were HIV-exposed uninfected children, and the remaining 456 (53%) of 461 were HIV unexposed and uninfected matched case or control pairs (figure 1). Of these, 4156 (48%) of 861 were enrolled in Malawi and 446 (52%) of 861 in Uganda. None of the AFASS questionnaire measures differed significantly for those pairs assessed at 12 months in this study compared with those from the original PROMISE clinical trial sample at our study sites who were not assessed at 12 months. Because the socioeconomic-related indicators in the AFASS were strongly correlated with the physical dwelling and safety domains of the Caldwell HOME scale for quality of caregiving (p=0·0082), we only used breastfeeding at 12 months (yes or no) in the present analyses in terms of our neurodevelopmental outcomes. This was because the other socioeconomic status indicators from the AFASS might compromise the present analyses by introducing additional covariate measures in the analyses that were redundant and possibly co-linear with the HOME scale.

In total, 100 (59%) of 170 children lost to follow-up (LTFU) were HIV-unexposed and uninfected and 70 (41%) of 170 were HIV-exposed and uninfected, so HIV-unexposed and uninfected children were at greater risk of being LTFU (p=0·01). However, children LTFU did not differ from those assessed at 48 months on any of descriptive measures (table 1). 445 children (65%) of 692 assessed at 48 months were assessed at 60 months with the KABC (figure 1), primarily because study funding for follow-up was exhausted during this period. Assessment findings at age 60 months, therefore, are only preliminary in nature because of the missing portion of the original longitudinal sample.

At each assessment point, a little less than half of the children were from Malawi, about half were female, and less than half were HIV-exposed and uninfected rather than HIV-unexposed and uninfected (table 1). As would be expected, viral load differed by treatment arm, with mothers on triple antiretrovirals having a lower mean

	HIV-exposed and uninfected children				HIV-unexposed and uninfected children (controls)	p value for comparison of all groups
	Triple ARV plus infant nevirapine	Triple ARV plus maternal triple ARV	Zidovudine plus infant nevirapine	Zidovudine plus maternal triple ARV		
MSEL						
Standardised composite						
12 months	94·12 (91·47–96·78)	93·11 (90·32–95·91)	92·65 (89·63–95·66)	92·06 (89·19–94·93)	95·31 (93·92–96·70)	0·19
24 months	88·43 (85·69–91·17)	88·35 (85·59–91·11)	90·97 (87·92–94·02)	89·75 (86·91–92·59)	91·10 (89·80–92·41)	0·24
48 months	80·64 (77·74–83·54)*†	85·93 (83·05–88·80)	84·96 (81·81–88·11)	81·34 (78·19–84·48)†	84·03 (82·62–85·43)	0·0486
Gross motor scale T score						
12 months	49·92 (47·93–51·90)	48·19 (46·09–50·29)	48·89 (46·64–51·14)	47·05 (44·90· 49·19)	47·97 (46·94–49·01)	0·34
24 months	46·26 (44·22–48·31)	47·50 (45·46–49·55)	48·18 (45·90–50·45)	47·06 (44·94–49·19)	48·28 (47·31–49·26)	0·44
48 months	NA	NA	NA	NA	NA	NA
Fine motor scale T score						
12 months	48·19 (46·18–50·20)*	48·35 (46·23–50·46)	49·62 (47·34–51·90)	49·19 (47·02–51·36)	50·52 (49·46–51·57)	0·18
24 months	41·79 (39·72–43·86)	41·31 (39·23–43·38)	40·92 (38·61–43·22)	42·81 (40·66–44·96)	42·95 (41·97–43·94)	0·36
48 months	41·34 (39·14–43·53)†	45·21 (43·04–47·39)	43·47 (41·09–45·86)	40·79 (38·41–43·17)*†	43·93 (42·87–44·98)	0·0236
Visual reception scale T score						
12 months	48·45 (46·60–50·31)	47·46 (45·50–49·41)	48·14 (46·03–50·24)	46·31 (44·31–48·32)	49·31 (48·34–50·28)	0·08
24 months	41·64 (39·73–43·56)	40·46 (38·55–42·38)	42·06 (39·93–44·19)	41·57 (39·58–43·55)	42·53 (41·62–43·44)	0·39
48 months	38·63 (36·59–40·65)	41·07 (39·07–43·07)	40·91 (38·71–43·11)	38·97 (36·77–41·17)	40·64 (39·66–41·62)	0·25
Expressive language scale T score						
12 months	44·29 (42·57–46·03)	44·41 (42·59–46·23)	43·96 (42·00–45·92)	44·48 (42·61–46·35)	44·76 (43·86–45·67)	0·95
24 months	42·92 (41·14–44·71)	43·99 (42·20–45·79)	45·12 (43·13–47·11)	45·12 (43·13–47·11)	44·56 (43·72–45·41)	0·28
48 months	43·64 (41·75–45·53)	46·44 (44·57–48·31)	46·41 (44·35–48·46)	44·52 (42·46–46·57)	44·94 (44·03–45·85)	0·18
Receptive language scale T score						
12 months	44·25 (42·49–46·02)	45·45 (43·60–47·31)	43·16 (41·16–45·16)	42·80 (40·89–44·71)*†	45·11 (44·18–46·03)	0·11
24 months	49·58 (47·76–51·40)	49·36 (47·53–51·19)	50·56 (48·53–52·58)	50·21 (48·33–52·10)	50·58 (49·71–51·44)	0·72
48 months	35·20 (33·27–37·13)	37·72 (35·81–39·62)	37·28 (35·18–39·37)	35·50 (33·41–37·59)	36·50 (35·57–37·43)	0·32
KABC						
Mental processing index						
48 months	77·62 (75·37–79·87)	78·90 (76·68–81·12)	79·42 (76·96–81·87)	77·77 (75·33–80·20)	78·26 (77·17–79·35)	0·81
60 months	73·54 (70·67–76·42)	74·53 (71·82–77·23)	73·24 (70·31–76·17)	73·84 (70·96–76·72)	74·63 (73·34–75·92)	0·89
Non-verbal index						
48 months	72·39 (69·70–75·08)	73·83 (71·16–76·50)	74·38 (71·44–77·32)	73·93 (71·02–76·85)	73·09 (71·79–74·39)	0·84
60 months	69·98 (66·52–73·44)	70·27 (67·02–73·52)	70·37 (66·84–73·89)	71·52 (68·06–74·97)	72·23 (70·69–73·78)	0·63
Sequential processing						
48 months	80·16 (77·51–82·82)	83·69 (81·07–86·32)	83·29 (80·39–86·19)	82·58 (79·70–85·46)	82·33 (81·04–83·62)	0·40
60 months	79·67 (76·15–83·20)	82·32 (79·03–85·61)	79·54 (75·97–83·10)	81·41 (77·88–84·93)	82·13 (80·57–83·69)	0·55
Simultaneous processing						
48 months	72·36 (69·74–74·98)	74·18 (71·58–76·77)	75·94 (73·07–78·80)	72·60 (69·75–75·44)	73·51 (72·24–74·78)	0·39
60 months	71·08 (67·61–74·56)	71·29 (68·04–74·54)	71·52 (68·01–75·04)	72·01 (68·52–75·49)	72·46 (70·91–74·00)	0·93
Learning						
48 months	84·21 (81·11–87·31)*	86·79 (83·73–89·86)	89·00 (85·61–92·39)	83·67 (80·31–87·04)*	87·98 (86·47–89·49)	0·0437
60 months	82·66 (78·52–86·79)	84·90 (81·03–88·76)	82·16 (77·98–86·34)	81·70 (77·56–85·84)	82·27 (80·43–84·09)	0·79

Data are least-square means and 95% CIs for outcomes by four ante-partum and post-partum treatment arm combinations, and HIV-unexposed and uninfected children at each timepoint, adjusted for country. p values are for ANCOVA differences among all five groups (four intervention arms, and the HIV-unexposed and uninfected reference group). ARV=antiretrovirals. MSEL=Mullen Scales of Early Learning. NA=not applicable. KABC=Kaufman Assessment Battery for Children. *Significant (p<0·05) difference of ARV treatments from HIV unexposed controls. †Significant (p<0·05) difference of ARV treatments from triple ARV plus maternal triple ARV exposure.

Table 2: **Longitudinal analysis of neurodevelopmental and cognitive outcomes by exposure group**

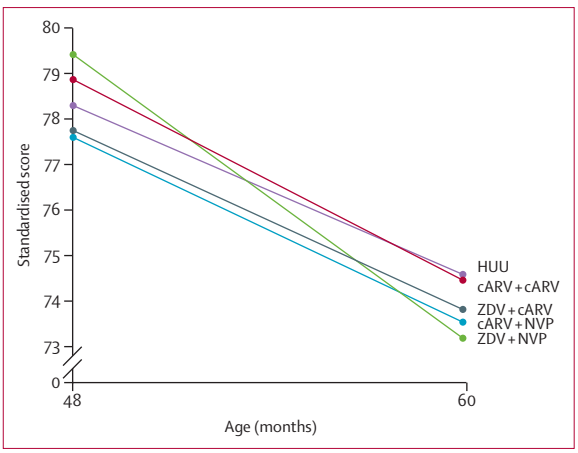

Figure 3: **Kaufman Assessment Battery for Children standardised mental processing index scores**

Data are scores using US norms, adjusted for site, plotted for the two assessment timepoints (48 and 60 months). Plots are colour-coded according to the HIV-exposed and uninfected PROMISE 1077-BF clinical trial study arm children for ante-partum and post-partum antiretroviral exposure; and for the HIV-unexposed and uninfected reference control group. The treatment arms (from top to bottom) are HIV-unexposed and uninfected (HUU; purple), ante-partum plus post-partum maternal triple antiretrovirals (cARV + cARV; red), ante-partum maternal zidovudine plus post-partum maternal triple antiretrovirals (ZDV + cARV; grey), ante-partum maternal triple antiretrovirals plus post-partum infant nevirapine (cARV + NVP; blue), and ante-partum maternal zidovudine plus post-partum infant nevirapine (ZDV + NVP; green).

viral load than women on zidovudine during pregnancy, or whose infants received nevirapine prophylaxis during breastfeeding, when available (table 1). Infant length-for-age differed by exposure at 24 and 48 months. For the unadjusted MSEL or KABC outcomes there were no differences based on child antiretroviral exposure at any of the timepoints (table 1).

In longitudinal analysis, controlling for data collection site, there were no differences in the MSEL scores based on antiretroviral exposure at age 12 and 24 months (figure 2). At 48 months, the MSEL composite scores were slightly higher for children with mothers assigned to triple antiretrovirals at both the ante-partum and post-partum periods, compared with the children of mothers not consistently maintained on triple antiretrovirals (table 2, figure 2). Additionally, children of mothers consistently maintained on triple antiretrovirals throughout performed at a similar level to HIV-unexposed and uninfected children. There were no differences by exposure in the KABC mental processing index scores; among the domains of the KABC, only learning was lower in the maternal triple antiretroviral group followed by infant nevirapine, and in zidovudine followed by maternal triple antiretrovirals at 48 months, compared with unexposed controls (table 2). None of the differences among groups remained significant after applying the Benjamini-Hochberg adjustment to control for the false discovery rate. These findings were consistent when analyses were stratified by country. The overall performance means appear to be decreasing for all groups across all timepoints in figure 3. The MSEL composite cognitive scores and the KABC-II mental processing index scores can only be calculated with scale measures standardised using American norms. Their standardised scores decreased over time as they fell further behind American norms on these measures, although their raw score performance did improve over time.

A country-specific analysis was done because of the difficulty in interpreting predictor-by-country significant interaction effects for predictors that differed significantly between countries (eg, proportion breastfeeding, HOME scores). The PROMISE treatment arm as well as maternal viral load were not predictive of MSEL or KABC scores for the Ugandan HIV-exposed and uninfected children (table 3). However, lower maternal viral load at 12 months was associated with the KABC mental processing index in Malawi (table 4).

Length-for-age was positively associated with the MSEL composite and KABC mental processing index in both countries, although significance was not reached for the KABC mental processing index in HIV-unexposed and uninfected children in Uganda. The HOME score was strongly positively associated with the MSEL and KABC outcomes of both HIV-exposed and uninfected children and HIV-unexposed and uninfected children in Uganda (table 3), and with the MSEL composite for HIV-unexposed and uninfected children in Malawi (table 4). Among HIV-unexposed and uninfected children, breastfeeding at 12 months was positively associated with MSEL scores in Uganda (table 3). Significance was not reached in Malawi because of the high prevalence of breastfeeding at 12 months among HIV-unexposed and uninfected children. Most mothers breastfed their children for more than 12 months (table 1), potentially extending the duration of antiretroviral exposure for some children for the subsequent neurodevelopmental outcome measures (eg, age 24 months).

Discussion

We concluded from our unadjusted and adjusted longitudinal neurodevelopmental (MSEL) and neuropsychological (KABC) outcome analyses that various combinations of both ante-partum and post-partum maternal antiretroviral exposure (at least for the first 12 months after birth) did not result in greater neurocognitive risk for their HIV-exposed and uninfected children through age 60 months compared with HIV-unexposed and uninfected children. Overall, neurodevelopmental results for both HIV-exposed and uninfected children and HIV-unexposed and uninfected children were similar, as were the PROMISE treatment arms among the HIV-exposed and uninfected children at both country sites. There were few adverse neurodevelopmental effects potentially related to maternal or infant antiretroviral exposure compared with age-matched and sex-matched HIV-unexposed and uninfected children from similar socioeconomic backgrounds. This held true even though most mothers breastfed their children beyond the

12-month timepoint, resulting in prolonged exposure to antiretrovirals in early childhood. In fact, Ugandan HIV-exposed and uninfected children still breastfeeding at 12 months had a significant neurodevelopmental advantage on the MSEL cognitive composite score at both 12 and 24 months. The Malawian HIV-exposed and

	MSEL composite cognitive score		KABC mental processing index	
	HIV-exposed and uninfected children (PROMISE 1077-BF)	HIV-unexposed and uninfected children (controls)	HIV-exposed and uninfected children (PROMISE 1077-BF)	HIV-unexposed and uninfected children (controls)
Age				
12 months	0	0	NA	NA
24 months	–5·87 (1·33), <0·0001	–4·35 (1·41), 0·0022	NA	NA
48 months	–6·03 (1·38), <0·0001	–8·54 (1·46), <0·0001	0	0
60 months	NA	NA	–1·75 (1·08), 0·11	0·31 (1·37), 0·82
Length-for-age *Z* score	1·98 (0·67), 0·0037	1·59 (0·69), 0·0211	1·63 (0·82), 0·0497	1·48 (0·90), 0·11
Breastfeeding at 12 months post partum	–4·38 (1·54), 0·0051	5·96 (2·34), 0·0119	–2·16 (1·60), 0·18	1·49 (2·76), 0·59
No breastfeeding at 12 months post partum	0	0	0	0
HOME score	0·39 (0·18), 0·0311	0·50 (0·16), 0·0019	0·60 (0·19), 0·0021	0·70 (0·21), 0·0003
Maternal viral load at 12 months post partum	–0·19 (0·84), 0·82	NA	0·82 (0·86), 0·34	NA
Trial arm				
Ante-partum maternal triple ARV plus post-partum infant nevirapine	2·98 (2·96), 0·32	NA	–2·10 (2·12), 0·51	NA
Ante-partum maternal triple ARV plus post-partum maternal triple ARV	0·98 (2·02), 0·62	NA	1·60 (2·12), 0·45	NA
Ante-partum maternal zidovudine plus post-partum infant nevirapine	2·66 (2·89), 0·36	NA	0·03 (2·95), 0·99	NA
Ante-partum maternal zidovudine plus post-partum maternal triple ARV	0	NA	0	NA

Data are coefficients for the variables (SE), p values for the HIV-exposed and uninfected (PROMISE 1077-BF Protocol) and HIV-unexposed and uninfected cohorts of children. 0 signifies reference values. MSEL=Mullen Scales of Early Learning. KABC=Kaufman Assessment Battery for Children. NA=not applicable. HOME=Home Observation for the Measurement of the Environment. ARV=antiretrovirals.

Table 3: **Predictors of the MSEL composite cognitive score and the KABC mental processing index for the Ugandan study cohorts**

	MSEL composite cognitive score		KABC mental processing index	
	HIV-exposed and uninfected children (PROMISE 1077-BF)	HIV-unexposed and uninfected children (controls)	HIV-exposed and uninfected children (PROMISE 1077-BF)	HIV-unexposed and uninfected children (controls)
Age				
12 months	0	0	NA	NA
24 months	0·10 (1·66), 0·95	–4·43 (1·46), 0·0027	NA	NA
48 months	–14·01 (1·62), <0·0001	–14·93 (1·49), <0·0001	0	0
60 months	NA	NA	–7·14 (1·15), <0·0001	–5·85 (0·92), <0·0001
Length-for-age *Z* score	1·46 (0·67), 0·0303	0·88 (0·55), 0·11	1·66 (1·03), 0·11	1·82 (0·73), 0·0147
Breastfeeding at 12 months post partum	–3·99 (2·64), 0·13	4·73 (9·32), 0·61	–7·39 (3·13), 0·0203	–6·82 (7·98), 0·39
No breastfeeding at 12 months post partum	0	0	0	0
HOME score	0·12 (0·21), 0·55	0·73 (0·20), 0·0003	0·39 (0·24), 0·12	0·28 (0·18), 0·12
Maternal viral load at 12 months post partum	–1·45 (0·88), 0·10	NA	–2·24 (1·06), 0·0364	NA
Trial arm				
Ante-partum maternal triple ARV plus post-partum infant nevirapine	1·17 (2·45), 0·63	NA	4·68 (2·97), 0·12	NA
Ante-partum maternal triple ARV plus post-partum maternal triple ARV	1·85 (2·25), 0·41	NA	–2·38 (2·72), 0·38	NA
Ante-partum maternal zidovudine plus post-partum infant nevirapine	3·64 (2·66), 0·17	NA	5·37 (3·23), 0·08	NA
Ante-partum maternal zidovudine plus post-partum maternal triple ARV	0	NA	0	NA

Data are coefficients for the variables (SE), p values for the HIV-exposed and uninfected (PROMISE 1077-BF Protocol) and HIV-unexposed and uninfected cohorts of children. 0 signifies reference values. MSEL=Mullen Scales of Early Learning. KABC=Kaufman Assessment Battery for Children. NA=not applicable. HOME=Home Observation for the Measurement of the Environment. ARV=antiretrovirals.

Table 4: **Predictors of the MSEL composite cognitive score and the KABC mental processing index for the Malawian study cohorts**

uninfected breastfeeding children had less of an advantage, although they still had an advantage on the KABC mental processing index score at 48 months.

However, a clinically meaningful difference on the MSEL cognitive composite score is usually considered about half an SD for the standardised score distribution, based on previous studies in the sub-Saharan African context (around six points).[17] Therefore, the significant relationship noted for breastfeeding and MSEL or KABC global cognitive performance outcomes in our findings are probably not clinically meaningful.

To increase the methodological rigour of our assessment of neurodevelopmental outcomes in response to prolonged antiretroviral exposure, we restricted our cohort of HIV-exposed and uninfected children to those whose mothers had been randomly assigned to a PROMISE trial treatment arm at both the ante-partum and post-partum stages within the source trial. By doing so, our selection criteria were less likely to bias the study in any systematic way and did not remove severely affected children from the population. Because of these inclusion criteria, our findings are the first to be based on developmental outcomes from a rigorous trial with well powered sample sizes in maternal or child treatment arms with prolonged post-partum exposure from breastfeeding.[4,5] These findings are also based on a longitudinal comparison with a well matched HIV-unexposed and uninfected control group for reference, for two different resource-constrained African country sites.

Furthermore, our findings are unique in that they are based on both longitudinal developmental (MSEL at 12, 24, and 48 months) and neuropsychological (KABC-II at 48, 60 months) assessments. This is the first time such neurodevelopmental outcome findings are available for HIV-exposed and uninfected children who were exposed to antiretrovirals both ante partum and post partum through prolonged breastfeeding. These are important findings given that such exposures are now common because of what has become the standard of HIV prevention of mother-to-child treatment care across sub-Saharan Africa. The similarity of these longitudinal neurodevelopmental outcomes in the HIV-exposed and uninfected children with the HIV-unexposed and uninfected reference group in the prevention of mother-to-child treatment care ante-partum and post-partum treatment arms should be highly reassuring for policy makers and ministries of health. This is especially true as triple antiretroviral drugs continue to be widely rolled out in sub-Saharan Africa and globally.[3]

A study in 2017[18] assessed neurodevelopmental outcomes at age 24 months in prospectively observed HIV-exposed and uninfected and HIV-unexposed and uninfected children in Botswana.[18] The HIV-exposed and uninfected children achieved similar scores on the Bayley-III and Developmental Milestones Checklist personal-social domain measures as the HIV-unexposed and uninfected children, suggesting no adverse effect on developmental milestones of in-utero HIV and antiretroviral exposure.[18] In a subsequent study[19] of HIV-exposed and uninfected children of mothers who took triple antiretrovirals during pregnancy compared with those on zidovudine alone, neurodevelopmental outcomes at 24 months were similar in both groups. This was the case even though the mothers on triple antiretrovirals continued to breastfeed their children, thereby prolonging exposure of their infants to triple antiretroviral exposure for up to 24 months. However, this study was a combination of two prospective observational studies, in which antenatal exposure to triple antiretrovirals or zidovudine alone was not randomly assigned. Likewise, postnatal antiretroviral exposure through breastfeeding was not randomly assigned, but determined by whether the mother continued on triple antiretrovirals or not after the child's birth. As such, the assessment of neurodevelopmental outcomes was not framed within an randomised source trial, unlike this study. Nonetheless the findings, along with ours, are crucial additions to the limited knowledge on growth of HIV-exposed and uninfected children in resource-limited African settings where prolonged breastfeeding is common. Our findings are also important throughout sub-Saharan Africa and low-income and middle-income countries globally where WHO-recommended maternal HIV test and initiation of lifetime antiretroviral therapy (eg, test and treat, and the option B+ strategy) have been rolled out.

Previous studies attempting to address these issues had design limitations. Low weight-for-age Z scores and length-for-age Z scores were documented among HIV-exposed and uninfected children with in-utero exposures to triple antiretrovirals compared with those with in-utero exposure to zidovudine alone. This poorer growth persisted from birth through age 24 months. However, neurodevelopmental performance and psychosocial adjustment at age 24 months were similar across the two groups.[17] HIV-exposed and uninfected children were also similar to an HIV-unexposed and uninfected comparison group on neurodevelopmental outcomes at age 2 years,[18] even though breastfeeding cessation was mostly encouraged as early as 6 months of age per standard of care for the mothers in Botswana living with HIV. Our study did not impose this restriction.

If antiretroviral or HIV exposure (not infection) does not impair the development of African children in the early years of life, what factors do affect their early development? Quality of caregiving is one factor that might be important in the early years for these children.[20] Some clinical trials[8,21] have been done with early childhood development interventions that enriched the developmental milieu for Ugandan infants and very young children of mothers living with HIV. The investigators found that rather than the child's exposure to antiretrovirals as the predominant risk factor in development, it is the pervasive poverty and nutritional hardship (eg, low height for age or stunting) conducive to poor caregiving that place these children

at greatest neurodevelopmental risk throughout early childhood. Therefore, keeping mothers with HIV physically and emotionally healthy so that they can provide good-quality caregiving in such resource-constrained settings should be a major goal for policy makers.[20,22–24] In such settings, the health, vitality, and emotional wellbeing of the caregiver are important for the early neurodevelopmental wellbeing and health of HIV-exposed and uninfected children.[20] Access to lifetime triple antiretroviral regimens for safe management of mothers' HIV disease and good nutritional and psychosocial support are important factors to help maintain their health and vitality.[22,23]

The mothers with HIV in the PROMISE combination antiretroviral treatment ante-partum and post-partum arm had lower viral loads than the other antiretroviral treatment combination arms.[2,4,5] However, in our findings, maternal viral load at baseline was not predictive of MSEL or KABC performance for the Ugandan HIV-exposed and uninfected children. Lower maternal viral load at 12 months post partum was associated with the KABC mental processing index in Malawi. Although not conclusive, these findings suggest that enhancing the health of the mothers through good clinical management of their HIV disease can have neurodevelopmental benefits for their HIV-exposed and uninfected children. However, a more systematic study with additional immunological biomarkers (eg, CD4 cell counts) is needed to more fully elucidate these neurodevelopmental benefits, since this was not a primary objective of the present study.

There were some limitations to our neurodevelopmental study. The study was done in only two of the sites participating in the PROMISE trial, so our results cannot necessarily be generalised to other resource-limited settings. Additionally, risk factors for adverse neurodevelopmental outcomes are multifactorial and difficult to determine for HIV-exposed and uninfected children. These factors include the effect of maternal disease progression over time, effects of maternal antiretroviral drugs on adverse pregnancy outcomes including low birthweight and prematurity, adequacy of nutrition and child physical growth, potential ameliorating effects of breastfeeding, and maternal disease compromising the quality of caregiving and other positive psychosocial factors in the home environment. However, we adjusted for these factors as much as possible in the analyses.

Likewise, there were strengths to this study. Our findings were based on closely monitored cohorts of HIV-exposed and uninfected children and age-matched and sex-matched HIV-unexposed and uninfected children assessed at age 12, 24, 48, and 60 months. We used standardised neurodevelopmental assessment procedures calibrated with a quality assurance programme for all testers across two international research-experienced sites. The neurodevelopmental and neuropsychological assessments were previously proven with HIV-affected cohorts in our country sites.[25] Other assessments[25,26] have also shown no neuropsychological differences in KABC II between HIV-exposed and uninfected children and HIV-unexposed and uninfected children across six sub-Saharan African study sites in four different countries.

Maternal viral load differences between the study treatment arms supported the fidelity of antiretroviral intervention for mothers in this study. By contrast with previous studies in Botswana,[18,19] our study design was not actively confounded by a standard of care, which actively discouraged mothers living with HIV from breastfeeding their children after birth. As such, we provide the most conclusive and rigorous neurodevelopmental outcome evidence to date, based on randomised triple antiretroviral regimens in utero and breastfeeding exposure, regarding the safety of antiretroviral exposure for infants born to mothers with HIV. The safety of such prevention of mother-to-child treatment care programmes should be assessed, especially when children are subjected to prolonged exposure throughout gestation and breastfeeding (potentially from 24 to 30 months of triple antiretroviral exposure in some cases). Therefore, our findings generalise to other breastfeeding HIV-exposed and uninfected paediatric populations in sub-Saharan Africa, home to more than 80% of the global prevention of mother-to-child treatment need, and where scale-up of maternal antiretrovirals is ongoing.

Developmental surveillance of HIV-exposed and uninfected and antiretroviral-exposed children needs to continue throughout childhood and adolescence. This is because of their reported heightened vulnerability to neurocognitive and psychiatric risk in middle childhood through adolescence.[27] There is presently a longer-term follow-up (PROMOTE cohort study) of former PROMISE children at seven sites, which will assess their later neurocognitive performance during their early school years. Although our present neurodevelopmental findings among HIV-exposed and uninfected children through age 5 years are reassuring, longer-term follow-up is still needed to gauge how these children progress on neurocognitive executive functioning, psychosocial adjustment, and academic school-based performance.[26]

Contributors

MJB designed the developmental assessment protocol, trained the assessment team, participated in the analysis and interpretation of findings, and completed the initial complete draft and revisions of the manuscript as corresponding author. LM-S supervised the assessment team at the Malawi site and participated in the manuscript writing. LWO supervised the assessment team at the Uganda site, coordinated participant scheduling and follow-up, and participated in the manuscript writing. RK supervised the study team at the Malawi site, led participant scheduling and follow-up, participated in data manuscript and assessment quality assurance at the Malawi site, and participated in the manuscript writing. AS was lead clinical methodologist and biostatistician, verified data integrity, completed all data analyses, tables, and figures, and co-authored the complete first draft of the manuscript. IF-L provided scientific oversight for the Uganda site, helped to lead the quality assurance for testing and data management, and helped to co-author the complete first draft of the manuscript. AK helped to lead the quality assurance of assessment for the study, did neurodevelopmental assessments at the Uganda site,

and worked on data management. MN supervised the quality assurance of assessment for both study sites, led the assessment team at the Uganda site, developed all study standard operating procedures, and participated in the manuscript writing. AM supervised the data management teams at both sites and worked with AS and IF-L on data integrity and management. JLN coordinated all testing quality assurance at both sites and participated in developmental assessments at the Uganda site. MM participated in scientific conceptualising for the study, institutional review board coordination for the Malawi site, and in the manuscript writing. HR-E co-led the quality assurance process for the neurodevelopmental assessment at both sites and participated in the manuscript writing. JA supported institutional review board approvals by coordinating study protocols, coordinated standard operating procedures, and coordinated monthly conference call meetings for the study, led the data sharing process with the IMPAACT PROMISE 1077-BF study, and participated in drafting the manuscript. TET provided scientific leadership of the Malawi site, participated in interpretation of principal findings, and participated in writing of the manuscript. MGF was study co-primary investigator, and participated in all phases of study conceptualisation, study design, proposal writing for funding, analysis plan, study implementation of protocols, interpretation of findings, results dissemination, and manuscript writing.

Declaration of interests

We declare no competing interests.

Acknowledgments

This work was presented in part at the 10th International Workshop on HIV Pediatrics (abstract P_87) and at the 22nd International AIDS Conference (AIDS 2018); Abstract Oral Presentation Session Code WEAB0201) in Amsterdam, Netherlands, July, 2018. This study was funded by the National Institutes of Health (NIH), grant number R01-HD073296 to principal investigators MJB and MGF. The source clinical trial for this study was sponsored by the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) with funding from the National Institute of Allergy and Infectious Diseases and co-funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health, all components of the NIH (award numbers UM1AI068632 [IMPAACT LOC], UM1AI068616 [IMPAACT SDMC] and UM1AI106716 [IMPAACT LC], and NICHD [contract number HHSN275201800001I]). The content of this report is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

1 Cooper ER, Charurat M, Mofenson L, et al. Combination antiretroviral strategies for the treatment of pregnant HIV-1-infected women and prevention of perinatal HIV-1 transmission. *J Acquir Immune Defic Syndr* 2002; **29:** 484–94.

2 Fowler MG, Mofenson LM, Taha TE. Antiretroviral therapy for perinatal HIV prevention. *N Engl J Med* 2017; **376:** 699–700.

3 Fowler MG, Flynn P, Aizire J. What is new in perinatal HIV prevention? *Curr Opin Pediatr* 2018; **30:** 144–51.

4 Flynn PM, Taha TE, Cababasay M, et al. Prevention of HIV-1 transmission through breastfeeding: efficacy and safety of maternal antiretroviral therapy versus infant nevirapine prophylaxis for duration of breastfeeding in HIV-1-infected women with high CD4 cell count (IMPAACT PROMISE): a randomized, open-label, clinical trial. *J Acquir Immune Defic Syndr* 2018; **77:** 383–92.

5 Fowler MG, Qin M, Fiscus SA, et al. Benefits and risks of antiretroviral therapy for perinatal HIV prevention. *N Engl J Med* 2016; **375:** 1726–37.

6 WHO. WHO Anthro Jan 2011, version 3.2.2. https://www.who.int/childgrowth/software/en/ (accessed Aug 17, 2017).

7 Mullen EM. Mullen scales of early learning: AGS edition. Minneapolis, MN, USA: American Guidance Service Publishing/Pearson products, 1995.

8 Boivin MJ, Bangirana P, Nakasuja N, et al. A year-long caregiver training program to improve neurocognition in preschool Ugandan HIV-exposed children. *J Dev Behav Pediatr* 2013; **34:** 269–78.

9 Kaufman AS, Kaufman NL. Manual for the Kaufman assessment battery for children, 2nd edition. Circle Pines: American Guidance Service Publishing/Pearson Products, 2004.

10 Bangirana P, Musisi S, Allebeck P, et al. A preliminary investigation of the construct validity of the KABC-II in Ugandan children with prior cerebral insult. *Afr Health Sci* 2009; **9:** 186–92.

11 Giordani B, Boivin MJ, Opel B, Dia Nseyila D, Diawaku N, Lauer RE. Use of the K-ABC with children in Zaire, Africa: an evaluation of the sequential-simultaneous processing distinction within an intercultural context. *Int J Disabil Dev Educ* 1996; **43:** 5–24.

12 Jansen P, Greenop K. Factor analysis of the Kaufman assessment battery for children assessed at 5 and 10 years. *S Afr J Psychol* 2008; **38:** 355–65.

13 Ochieng CO. Meta-analysis of the validation studies of the Kaufman assessment battery for children. *Int J Test* 2003; **3:** 77–93.

14 Caldwell BM, Bradley RH. Home observation for measurement of the environment. Little Rock, AR, USA: University of Arkansas Press, 1979.

15 Adegbehingbe SM, Paul-Ebhohimhen V, Marais D. Development of an AFASS assessment and screening tool towards the prevention of mother-to-child HIV transmission (PMTCT) in sub-Saharan Africa—a Delphi survey. *BMC Public Health* 2012; **12:** 402.

16 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B* 1995; **57:** 289–300.

17 Boivin MJ, Sikorskii A, Familiar-Lopez I, et al. Malaria illness mediated by anaemia lessens cognitive development in younger Ugandan children. *Malar J* 2016; **15:** 210.

18 Chaudhury S, Williams PL, Mayondi GK, et al. Neurodevelopment of HIV-exposed and HIV-unexposed uninfected children at 24 months. *Pediatrics* 2017; **140:** e20170988.

19 Chaudhury S, Mayondi GK, Williams PL, et al. In-utero exposure to antiretrovirals and neurodevelopment among HIV-exposed-uninfected children in Botswana. *AIDS* 2018; **32:** 1173–83.

20 Bass JK, Nakasujja N, Familiar-Lopez I, et al. Association of caregiver quality of care with neurocognitive outcomes in HIV-affected children aged 2–5 years in Uganda. *AIDS Care* 2016; **28** (suppl 1)**:** 76–83.

21 Boivin MJ, Nakasujja N, Familiar-Lopez I, et al. Effect of caregiver training on the neurodevelopment of HIV-exposed uninfected children and caregiver mental health: a Ugandan cluster-randomized controlled trial. *J Dev Behav Pediatr* 2017; **38:** 753–64.

22 Familiar I, Collins SM, Sikorskii A, et al. Quality of caregiving is positively associated with neurodevelopment during the first year of life among HIV-exposed uninfected children in Uganda. *J Acquir Immune Defic Syndr* 2018; **77:** 235–42.

23 Murray SM, Familiar I, Nakasujja N, et al. Caregiver mental health and HIV-infected child wellness: perspectives from Ugandan caregivers. *AIDS Care* 2017; **29:** 793–99.

24 Seffren V, Familiar I, Murray SM, et al. Association between coping strategies, social support, and depression and anxiety symptoms among rural Ugandan women living with HIV/AIDS. *AIDS Care* 2018; **30:** 888–95.

25 Chernoff MC, Laughton B, Ratswana M, et al. Validity of neuropsychological testing in young African children affected by HIV. *J Pediatr Infect Dis* 2018; **13:** 185–201.

26 Boivin MJ, Barlow-Mosha L, Chernoff MC, et al. Neuropsychological performance in African children with HIV enrolled in a multisite antiretroviral clinical trial. *AIDS* 2018; **32:** 189–204.

27 Malee KM, Tassiopoulos K, Huo Y, et al. Mental health functioning among children and adolescents with perinatal HIV infection and perinatal HIV exposure. *AIDS Care* 2011; **23:** 1533–44.